

Publishing research results

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Framework

- Bibliometric indexes and journals databases;
- Manuscript preparation;
- Manuscript publication;
- Manuscript revision;
- •Reply to reviewers.

Bibliometric indexes

The impact factor

- The impact factor (IF) of an academic journal is an index which accounts for the average number of citations to recent articles published in that journal.
- It is frequently used as an index for the relative importance of a journal within its field (the higher the impact factor the more important the journal).
- ✓IFs are calculated yearly for those journals that are indexed in the Journal Citation Reports (Thomson Reuters).
- The IF of a journal is the average number of citations received per paper published in that journal during the two preceding years.

Bibliometric indexes

The impact factor

For example, if a journal has an IF of 5 in 2013, then its papers published in 2011 and 2012 received 5 citations each on average in 2013.

✓ Example: calculation of IF for a Journal in 2013:

- Citations in 2013 (in all indexed journals) of papers published in the journal in the 2 preceding years: e.g., 230 in 2012 and 198 in 2011, total1=428;
- Number of papers published in the journal in the 2 preceding years: e.g., 98 in 2012 and 82 in 2011, total2=180;

✓ IF=total1/total2=428/180= 2,377

✓IFs are published yearly in Journal Citation Reports (JCR). IF is calculated for thousands scientific journals indexed in citation Thomson Reuters database.

Bibliometric indexes

H-index or Hirsch index

- ✓ It was originally proposed by Jorge E. Hirsch from University of California San Diego in 2005 to quantify the impact of scientists' work according to the number of their publications and citations;
- According to its definition, a scientist has an H-index n if she/he published at least n manuscripts, each one was cited at least n times.
- H-index not only quantifies the scientific production but also evaluate the influence of the scientist by distinguishing her/him from highly prolific scientists which published manuscripts of poor interest.
- Moreover, the H-index is not affected so much by highly succesful single papers.

Journals databases

- Science Citation Index (SCI) is a citation index originally produced by the Institute for Scientific Information (ISI) and created by Eugene Garfield in 1960, and presently owned by Thomson Reuters.
- It allows the access to bibliographic informations and citations, as well as the analysis of trend, journals and scientists.
- The most expanded version (Science Citation Index Expanded) include more than 8,500 journals from 150 scientific and technological areas (2013), since 1900.
- SCI is available on-line through "Web of Science" database, which is part of "Web of Knowledge" database.



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Journals databases

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- Scopus database was created in 2004 by Elsevier publisher;
- Scopus database allows (i) to access to paper abstracts and full papers (just for subscribers) and (ii) to sign in for alerts to keep updated about some information (e.g., paper citations).

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4 Urban wastewater treatment plants as hotspots for antibiotic,	2013					16	47	28	91		91
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6 Degradation of diclofenac by TiO2 photocatalysis: UV absorba	2009		12	12	14	11	21	5	63		75
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- Original research paper;
 - ✓Title page
 - ✓Abstract
 - ✓Keywords
 - ✓Introduction
 - Material and methods
 - ✓ Results and discussion
 - ✓Conclusions
 - Acknowledgements
 - ✓ References

Original research paper: title page

✓Title

- As short as possible
- The use of acronyms should be avoided
- To be arranged according to the journal type
- Emphasize the novelty of the manuscript
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Original research paper: title page



Original research paper: introduction

- Overview and relevance of the (environmental) issue according to international literature;
- State of art with regard to approaches, processes, technologies used to address the target issue/problem;
- Explanation of possible drawbacks/limitations of the approaches, processes, technologies available;
- Explanation of the proposed solution and the potential advantages compared to the state of art (novelty);
- Description of the objectives;
- Short description of methods/methodologies/approach.

Original research paper: introduction

Overview of the (environmental) issue

State of art

Novelty

Objectives and approach

Chemosphere Journal homepage: www.elsevier.com/locate/chemosphere

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Advanced treatment of urban wastewater by UV radiation: Effect on antibiotics and antibiotic-resistant *E. coli* strains

Luigi Rizzo ^{a,*}, Antonino Fiorentino^a, Antonella Anselmo^b

2003; Baquero et al., 2008). At least 18 000 Americans and 25000 people in the European Union countries die every year because of drug-resistant infections (Associated Press, 2007; ECDC/EMEA, 2009).

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Antibiotics for human use and their metabolites, as excreted by humans, reach urban wastewater treatment plants (UWWTPs) via sewage system, Conventional UWWTPs are typically based on biological processes which only partially remove antibiotics, thus making their effluents possible sources of antibiotics and antibiotic-resistant bacteria (ARB) spread into the environment (Watkinson et al., 2007; Behera et al., 2011). In particular, biological processes are suspected to contribute to ARB selection as well as resistance transfer among bacteria (Ferreira da Silva et al., 2006). Advanced treatment technologies (e.g., adsorption, advanced oxidation processes, membranes) have been investigated to control antibiotics' release into the effluent (Putra et al., 2009; Rizzo, 2011; Sahar et al., 2011). UV irradiation has also been investigated in the removal of antibiotics from wastewater effluents. In particular, sulfonamide (sulfamethoxazole (SMZ) and sulfadimethoxine) and guinolone (norfloxacin and nalidixic acid) antibiotics showed quite high removal efficiency (86-100%), compared to macrolide (clarithromycin, erythromycin and azithromycin) and tetracycline which were only poorly removed (24-34% and 15%, respectively) (Kim et al., 2009), Photodegradation reactions can take place in two different ways: direct and indirect (Lam and Mabury, 2005; Fatta-Kassinos et al., 2011). In direct photolysis, a molecule absorbing radiation may become unstable and subsequently decompose, while indirect photolysis involves naturally occurring molecules which generate strong reactive species (such as singlet oxygen, hydroxyl radicals or alkyl peroxyl radicals), that can react with organic compounds. UV radiation has also been increasingly used as disinfection process since conventional disinfection by chlorine was found to promote the formation of potentially carcinogenic byproducts (Rook, 1974). However, poor information and pon-conclusive data are available on the effect of disinfection processes on ARB (Iwane et al., 2001; Munir et al., 2011), Moreover, the effect of disinfection processes on the changes of bacterial resistance to antibiotics (evaluated in terms of minimum inhibiting concentration (MIC)) has not yet been investigated to our knowledge, therefore it should be of interest to understand if the colonies surviving a disinfection treatment show any change in their resistance to antibiotics. Finally, since the occurrence of antibiotics at sub-lethal concentrations in wastewater may promote the development of resistance among bacteria in receiving water (Gullberg et al, 2011), the simultaneous effect of UV radiation process on both antibiotics and ARB is worthy of investigation to advance the knowledge and consequently to plan the strategies to control the risk of antibiotic resistance spread into the environ ment

To address these tasks, in the present work, preliminary UV radiation tests were carried out on actual wastewater samples taken from the effluent of the secondary treatment of an UWWTP. The wastewater samples were used as received to evaluate the effect of UV radiation on indigenous *Escherichia coli* (*E. coli*), Gramnegative bacteria typically occurring in wastewater and used to evaluate antibiotic resistance changes in our study. In the subsequent tests, real wastewater samples were first autoclaved (to inactivate all naturally occurring bacteria) and then inoculated independently with two *E. coli* strains selected from the same wastewater samples, according to their resistance to a mixture of three antibiotics amoxicillin (AMX), ciprofloxacin (CPX), and SMZ. The inactivation of the inoculated *E. coli* strains by UV radiation as well as the effect on their resistance to the target antibiotics was investigated and compared to chlorination process. Finally,

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Original research paper: material and methods

- It should be organized in sub-paragraphs (sometime this is a requirement of the journal);
- Experimental procedure/design should be clearly explained;
- The characteristics of the environmental matrices investigated should be explained;
- Materials and equipment (including producers) should be explained;
- (Analytical) methods should be explained (or eventually quoted if official/well established methods);

2.1. Chemicals

2.5. UV radiation tests

rial count was detected.

1 mg L⁻¹, respectively.

2.6. Chlorination test

duplicated

2.7. Bacterial count

2.8. Antibiotic resistance assay

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Amoxicillin, CPX and SMZ were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as obtained. Milli-Q water system (Millipore, Billerica, MA, USA) wantsed for the preparation of the freshly prepared solutions. Sodium hypotologice: obucon (aviiable chlorine 10%) was purchased from Sigma and ic. (St. Louis, MO, USA) and used as obtained.

22. Wastewater samples

Wastewater samples for *E*, coli strain selection and disinfection experiments were taken from an UWWTP located in the province Salemo, Italy. In particular, the samples were taken from the eluphic fielding on process (a) and a proceeding of the contract of the sample statement of the sample statement of the sample statement of the sample statement of BOD₅, 460 mg L⁻¹ of COD, 44 mg L⁻¹ of TSS, 4.0 × 10⁶ CFU 100 mL⁻¹ of *E*, coli, in the influent stream and pH 6.7, 25 mg L⁻¹ of BOD₅, 64 mg L⁻¹ of COD, 18 mg L⁻¹ of TSS, 3.4 × 10³ CFU 100 mL⁻¹ of *E*, coli, in the effluent stream.

2,3. Selection and identification of antibiotic-resistant E, coli strains

E coli strains' resistant to the target antibiotics were selected according to the procedure described in our previous work (Rizzo et al., 2012). Briefly, 50 mL of wastewater sample was filtered through 0.45 µm membrane filters (Millipore, Billerica, MA, USA) and then cultivated (24 h incubation time at 44 °C) in tryptone bile X-glucuronide (TBX) agar medium (Oxoid, Basingstoke, UK), a selective, chromogenic medium for the detection and enumeration of E. coli, including tryptone (20.0 g L⁻¹), bile salts (1.5 g L⁻¹), agar (15.0 gL⁻¹), X-glucuronide (0.075 gL⁻¹) and pH 7.2 ±0.2. Ten colonies were randomly collected from TBX agar medium after incubation period and used in the subsequent step for the selection of resistant strains, Each colony was cultivated (24 h incubation time at 37 °C) in four different tryptone soya agar (TSA) media (Oxoid, Basingstoke, UK) prepared with antibiotic concentrations of 2 mg L-1 for CPX, 16 mg L-1 for AMX, 64 mg L-1 for SMZ (and half antibiotic concentrations) and their mixture, respectively. Tryptone soya agar is a general purpose medium for the growth of a wide variety of organisms, including pancreatic digest of casein (15.0 g L⁻¹), enzymatic digest of soya bean (5.0 g L⁻¹), sodium chloride (5.0 g L⁻¹), agar (15.0 g L⁻¹) and pH 7.3 ±0.2. The full antibiotic concentrations were chosen according to the respective MICs for E coli listed in "Clinical and Laboratory Standards Institute" documentation (CLSI, 2011). The E colistrains used for subsequent UV radiation and chlorination experiments, were selected from the two Petri dishes which showed the lower number of colonies (presumably, the higher resistance strains), (transferred in 15% glycerol tryptic soya broth (TSB) from Oxoid, Basingstoke, UK) and frozen at -20 °C. Tryptic soya broth is a highly nutritious general purpose medium for the growth of bacteria and fungi, including pancreatic digest of casein (17.0 gL⁻¹), enzymatic digest of soya bean (3.0 gL⁻¹), sodium chloride (5.0 gL⁻¹), dipotassium hydrogen phosphate (2.5 g L-1), glu cose (2.5 g L-1) and pH 7.3 ±0.2. The selected strains were identified by Rapid One System method (Remel, Len exa, KS, USA).

ter samples used as received to evaluate the effect of UV radiation on indigenous 5 coli trains speically occurring in watewater to the tubbiquent tysts. The a tual was ewater samples were first puticlay diso invitis ate all caturally occurring baseria) in the efficiency of the steratation brocess was tensed (in bacteria surviving autocaving step as seen by the subsequent bacteria) count.

Two sets of experiments with UV radiation were carried out,

Preliminary UV radiation tests were carried out on actual wastewa-

The autoclaved wastewater samples were then inoculated with the selected *E*, *coli* strains. The selected *E* co*li* strains were unfrozen and transferred to 10 mL physiological solution to achieve 1.5×10^8 CFU (0.5 McFarland). Suitable dilutions were made to achieve the desired initial bacterial density before inoculating *E* co*li* strains to wastewater samples

UV radiation experiments were carried out in a 2.2 L cylindrical

glass reactor (14.5 cm in diameter) filled in with 1 L wastewater

sample (6.0 cm water height). The reactor was placed in a water

bath to keep the temperature constant (roughly 30 °C) during

experiments and continuously stirred. Experiments were con-

ducted in a box equipped with a wide spectrum 250 W lamp (Proc-

omat, Florence, Italy) fixed at 33 cm from the upper water level in

the reactor. In disinfection experiments, the wastewater samples

were prepared according to the procedure described in Section 2.4.

The samples were exposed to a range of UV doses (0-2.5 × 10⁴-

µW s cm⁻²) by varying the exposure time from 0 to 120 min. The

corresponding UV dose was calculated by multiplying the irradia-

tion time for the intensity of UV lamp measured at the bactericidal

wavelength (i.e., 254 nm), UV radiation experiments were dupli-

cated. Control tests using inoculated wastewater sample under

dark condition were also carried out in parallel to UV radiation

experiments and no significant change compared to initial bacte-

dissolved in 1L wastewater sample to achieve a mixture of

In photodegradation experiments, 1 mg of each antibiotic was

Sodium hypochlorite solution was added to the inoculated

wastewater sample (1 L) to simulate the chlorination process.

Chlorine was dosed (2 mgL⁻¹) to achieve roughly 0.2 mgL⁻¹ of

residual chlorine after 60 min of contact time to meet the Italian

standard for residual disinfectant in UWWIP effluent. Bacterial

inactivation, MIC and residual chlorine were monitored up to

120 min contact time, After sampling, 0,1 mL of sodium thiosulfate

solution (10%) was added to each 100 mL sample to remove resid-

ual chlorine before bacterial count. Chlorination experiments were

Bacterial count was performed by the membrane filtration

method (APHA, 1998), Briefly, samples were filtered through

0.45 µm pore size cellulose nitrate membranes (Millipore, Billerica,

MA, USA), placed onto TBX agar and incubated at 44 °C for 24 h.

Measurements were carried out in triplicates and average values and standard deviation were plotted as CFU 100 mL⁻¹.

Minimum inhibiting concentration was evaluated by E-test, a well-established method for antimicrobial resistance testing using AMX, CPX and SMZ antibiotic strips (Biomérieux, Marcy l'Etoile, ing to the procedure described in our previous work E foli A TO 259 2 and Pseudomonas aeruginosa 278-3 ven the strans used or the quality control test. In commany UV reservon ests with indigenous E, coli strains, 278 three colonies were randomly selected among those surviving after 60 min treatment and were placed in a physiological solution until a turbidity as high as 0.5 McFarland was achieved. Afterward, a sterile tampon was firstly dipped in the respective suspension and then uniformly spread on three different 150 mm Mueller Hinton agar plates (Biomérieux, Marcy l'Etoile, France), in three differaces of each plate respectively. AMD CPX and SMZ strips appled on the lates on the the direct places, respec-, The MC value was read from the scale where the ellipse edge intersected the strip, after 24 h of incubation at 37 °C. The average value for MIC calculated on the three different readings was reported (Table 1).

In the disinfection experiments with selected E, coli strains, the procedure for MIC assessment was the same as described above for the tests with indigenous E, coli strains, but one colony was taken for each time (according to the procedure explained in Section 2.4). The procedure was repeated and the average value for MIC was reported. Isolates were considered multidrug resistant (MDR) when simultaneous resistance to two or three antibiotics was observed.

2.9. Analytical measurements

Free residual chlorine was measured by a portable spectrophotometer (Pocket colorimeter Chlorine; Hach, Loveland, CO, USA). Residual concentration of each antibiotic was measured by LC-MS. The chromatographic system used consisted of a Waters 2695 separation module (Milford, MA, USA) equipped with an automatic injector, a degasser system and a binary pump with four solvent channels. The separation module is connected to a triple quadrupole mass spectrometer Quattro micro™ API detector with electrospray ionization, A C18 (Ascentis Express, Sigma-Aldrich, St. Louis, MO, USA) reversed phase column (2.1 mm × 150 mm, 3 µm) was used for the separation of target analytes. Sample aliquots of 10 µL were injected into the column. A binary mobile phase with gradient elution was used. Ultra-pure water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as solvents A and B, respectively. The gradient was started with 20% B, increased up to 45% in 8 min, and then returned to the initial mobile phase composition in 10min. The flow-rate was set at 0.2 mL min⁻¹. The column temperature was set at 25 °C.

UV absorbance spectra of antibiotics were detected in the range of 200–500 nm by a lambda 12 UV–Vis spectrophotometer from Perkin Elmer (Waltham, MA, USA), equipped with 1 cm optic pathway quartz cells.

A spectrometer from Ocean Optics (HR-2000; Dunedin, FL, USA), equipped with cosine corrector with Spectralon diffusing material, was used to measure the spectral radiant flux of the UV lamp.

Table 1

Comparison between MIC values (mg L⁻¹) detected for AMX, CFX and SMZ after 60 min irradiation time of indigenous E coll colonies and corresponding WT, S and R values from EUCAST (2012).

Antibiotic	MIC	WT	S	R
AMX	8	<8	-	>8
CPX	0.016	≤0.064	<05	>1
SM7	24	≤64	-	-

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Original research paper: results and discussion

- Depending on the journal, these sections can be separated or eventually merged in one section;
- The results must be explained and duplication of information/data between text and figures/tables should be avoided;
- The discussion of the results is a fundamental part of a scientific paper; the results should be compared and discussed according to the relevant and updated international scientific literature;
- In particular, the authors are expected to explain the differences with the results available in scientific literature.



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3. Results and discussion

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3.1. Preliminary UV radiation tests on indigenous E coli

10

Preliminary UV radiation tests were performed to evaluate the effect of disinfection process on the inactivation of indigenous E coli colonies. Accordingly, wastewater samples taken from the effluent of the biological process were used for UV radiation tests as received. The results in Fig. 1 show that E. coli inactivation ist after 5 min of irradiation ti was as his as (UV)

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up to 120 mil of an

diation time (UV dose = 2.5 10⁴ µW s cm⁻²) surviving 60 min. Antibiotic resistance (measured as MIC) of E coli colonies, randomly selected from the colonies survived to 60 min irradiation time, was found to be as high as 8, 24, 0.016 mg L⁻¹ for AMX. SMZ and CPX, respectively, the same values for all selected colonies. In Table 1, the MIC values calculated for AMX, CPX and SMZ are compared to epidemiological cut-off (WT), clinical susceptibility (S) and clinical resistance (R) values, according to the definitions of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2012).

Minimum inhibiting concentration value for AMX matched the corresponding value of WT and R, but differences were observed for CPX and SMZ.

3.2. Inactivation of selected antibiotic-resistant E. coll strains: comparison between chlorination and UV radiation processes

E. coll strains' colonies were randomly selected from the wastewater sample according to their resistance to the target antibiotics. In particular, the MDR strain which was capable of growing on the mixture of CPX, AMX and SMZ at 2, 16 and 64 mg L⁻¹, respectively (MDR1), was identified as E. coli with a 99.9% probability. The second MDR strain (1 mg L⁻¹ of CPX, 8 mg L⁻¹ of AMX and 32 mg L⁻¹ of SMZ) (MDR2) was also identified as E, coll with a 99.9% probability. These two strains were subsequently used for both chlorination and UV radiation experiments.

Disinfection of UWWIP effluent was simulated to evaluate the effect of chlorine on the inactivation of the E coli strains and their resistance to the target antibiotics. The disinfectant contact time of 45-60 min is applied for chlorine. Chlorine dose is determined based on C-t values. The log inactivation and residual chlorine concentration results are plotted in Fig. 2 for both MDR strains.

Inactivation rate was different depending on E. coll strain. In particular, roughly 5 log unit decrease was observed for MDR1



Fig. 1. UV radiation but on indiremous E coll strains in the UWWIP effluent.



Fig. 2. Chlorination bettron the UWWTP effluent inoculated with MDRI and MDR2 E coll strains: bacteria inactivation and consumed chlorine.

strain under typical full scale conditions (60 min contact time). but only a 2.5 log unit decrease was observed for MDR2 strain. MDR1 strain was totally inactivated after 120 min of contact time. but MDR2 was found to be resistant to oblogination process, because total inactivation was not observed after 120 min of contact time (99.97% inactivation). Moreover, the wastewater sample was characterized by a high chlorine demand, resulting in a drastic decrease in chlorine residual in the early 5 min of treatment (0.8 and 0.16 mg L⁻¹ for MDR1 and MDR2 wastewater suspensions, respectively). Higher inactivation rate in a shorter contact time was observed in a previous study, but different E coll strains (i.e., trimethoprim and ampicillin-resistant) and aqueous matrices were investigated (Templeton et al., 2009). In particular, solids and dissolved constituents of wastewater which affect chlorine demand can decrease inactivation rate compared to particle-free water used in the previously quoted study. When the effect of chlorination process on ARB was investigated under experimental conditions similar to those inspected in our work (real secondary effluent of an UWWTP. 2.0 mg L⁻¹ of initial chlorine concentration. 10 min contact time (Huang et al. 2011)), the inactivation rates (2-3 log units) were in agreement with our results at the same contact time (Fig. 2).

Differences between the two investigated MDR strains were also observed in UV radiation trials (Fig. 3). In particular, a lower resistance to UV radiation was observed for MDR1 strain, which was totally inactivated after 60min of irradiation time (UV dose = $1.25 \times 10^4 \,\mu\text{W} \,\text{s}\,\text{cm}^{-2}$). On the other side, total inactivation was not observed for MDR2 after 120 min of irradiation time (UV dose = 2.5 × 10⁴ µW s cm⁻²). According to chlorination trials, the inactivation curves showed a tailing dose-response behavior: higher levels of bacterial irractivation in the early min of the treatment and a subsequent less marked decreasing trend to the end of the UV radiation treatment. The tailing of the UV dose-response profiles was not observed in Templeton et al. (2009) trials with UV radiation, probably because of the lower UV dose range investigated (0.1-0.5 $\times 10^4 \mu$ Ws cm⁻²); but a comparable inactivation rate was observed at 0.5 × 104 µW s cm⁻² UV dose.

Compared to UV disinfection tests on indigenous E, coli (Fig. 1) we could observe a higher inactivation rate (4-6 log units for MDR E coll strains compared to 3 log units for indigenous E coll).

Furthermore, disinfection by chlorine (Fig. 2) resulted in a lower inactivation rate of the antibiotic-resistant E coll strains (5 log units for MDR1 and 2 log units for MDR2, after 60 min contact time) compared to UV radiation process (7 log units for MDRI and 5 log units for MDR2) under typical UV dose (2.0 × 104µWs cm⁻²) (Templeton et al., 2009; Keen et al., 2012).



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Fig. 3. UV radiation tests on the UWWTP offluent inoculated with MDR1 and MDR2 E coll strains.

3.3. Effect on antibiotic resistance: comparison between UV radiation and chloringtion processes

Antibiotic resistance of E. colistrains MDR1 and MDR2 surviving to disinfection processes was affected in a different way depending on the target antibiotic, disinfectant agent and contact time. Despite chlorination process decreased bacterial count (Fig. 2), the surviving colonies were found to be resistant to AMX (MIC > 256 mg L⁻¹), SMZ (MIC > 1024 mg L⁻¹) and CPX (MIC = 12 mgL⁻¹) after 120 min of chlorination treatment. No detectable changes in resistance level to AMX and SMZ could be observed after UV radiation, while for CPX MIC was found to change for MDR1 strain (no change was observed for MDR2 strain). In particular, UV radiation process did not affect MIC up to 30 min treatment (UV dose = 0.62 × 10⁴ µW s cm⁻²), but after 60 min (UV dose = $1.25 \times 10^4 \mu W \text{ s cm}^{-2}$) MIC started to decrease (8 mg L⁻¹) to reach 6 mg L⁻¹ value at 120 min of irradiation (UV dose = 2.5 × 10⁴ µW s cm⁻²) (Table 2). However, it is worthy of mention that possible mutation of E. coli population survived to disinfection process cannot be excluded.

The effect of disinfection process on antibiotic resistance in wastewater has been morely investigated. Iwane et al. (2001) observed that the percent resistance to one or more antibiotics (from 147% to 14.0%) in E coli strains randomly isolated from wastewater samples in an UWWTP in Tokyo Metropolitan Prefecture was not significantly affected by chlorination. Munir et al. (2011) found that chlorimation process did not significantly reduce the occurrence of antibiotic resistance genes (quantified by Real-time Polymerase Chain reaction) and ARB (counted by heterotrophic plate count (HPC) method by plating samples on antibiotics amended media) in full scale UWWTPs. Templeton et al. (2009) investigated the effect of chlodnation on E coll strains resistant to antibiotics ampicillin and trimethoprim, compared to an antibiotic-sensitive E coll strain isolated from wastewater; trimethoprim-resistant E coll strain was found to be more resistant to chlorine than the antibiotic sensitive isolate

Table 2 MIC values (mg.L-1) detected for AMX, CPX and SMZ in UV radiation tests of wastewater sampler inoculated with MDR1 strain.

Antibiotic	radiation time (min)						
	0	5	10	30	60	120	
AMOX	>256	>256	>256	>256	>256	>256	
CTX C	12	12	12	12		G	
9MZ	>1024	>1024	>1024	>1024	>1024	>1024	

astewater by UV radiation

tion data fit quite well a pseudo first order kinetic model (Table 3). The tig values calculated for the photolysis of CPX, AMX and SMZ were 14, 20 and 25 min, respectively.

The different photodegradation rates observed for the investigated antibiotics can be explained by comparing their absorbance prectra with the intensity of the UV lamp (Fig. 4).

econdary peak us explaining the successful photodegradation by direct photolysis mechanism. On the opposite, the main SMZ (250 nm) and AMX (220 nm) peaks are either only marginally or not overlapped at all to UV lamp emission spectrum, thus resulting in a lower photodegradation rate by the direct photolysis mechanism.

The different results achieved by De la Cruz et al. (2012) for SMZ. and CPX can be explained according to the different UV lamps low-pressure mercury lamp emitting the primary energy at 254 nm) used as a light source. In particular, they observed a higher photodegradation for SMZ (51%) compared to CPX (48%) after 10 min of irradiation; in our work the observed removal efficiencies were 35% and 85% respectively after the same irradiation time. As previously observed, SMZ has the main absorbance peak at 250 nm, which perfectly overlaps the maximum emission peak of the UV lamp used by De la Cruz et al. (2012). On the opposite, the two main absorbance peaks of CPX are shifted on the right side of the spectrum, therefore the UV lamp emission (peak at 254 nm) does not match these peaks, resulting in a lower CPX photodegradation compared to our results.

Amoxicillin photodegradation rates documented in scientific literature also vary in a wide range according to the light source used: from really poor efficiency (2.9% after 5.0h irradiation time) when UV lamp emitting radiation at 365 nm was used (Elmolla and

Table 3

Preside first order kinetics for the UV photodegradation of antibiotics AMX, CPX and SMZ (1 mg L⁻¹ initial concentration, respectively), kinetic parameters (k and t_{ij2}), correlation coefficient ($\hat{\mathbf{x}}^{i}$) and UV does at t_{10} .

Antibiotic	k (min ⁻¹)	R ²	$t_{ij2}\left(min\right)$	UV dose (t ₁₍₂) (jW scm ⁻²)
AMX	0.0335	0,9891	20	0.42×10^4
CPX	0.0470	0,9948	14	0.29×10^4
SMZ	0.0273	0,9770	25	0.52×10^4



Rg. 4. Comparison between AMK, CPX and SMZ absorbance spectra and UV lamp internative.

ce at 300 and

Original research paper: conclusion

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I. Rizzo et al./Chemaspher

Chaudhuri, 2010), to really high photodegradation rate when a low pressure Hg arc-UV lamp with a monochromatic emission at 254 nm, was used as a light source (Jung et al., 2012).

4. Conclusions

In this work, the effect of UV radiation on antibiotics and antibiotic-resistant *E*, colistrains was investigated. According to the results achieved, the simultaneous release of ARB and antibiotics at sub-lethal concentrations may occur into UWWTP effluent, thus possibly promoting the development of resistance among bacteria in the receiving water body. Due to the observed photodegradation rates, the risk of development of SMZ resistance among bacteria may be higher than AMX. The risk related to the development of CPX resistance is expected to be lower due to both the higher photodegradation rate and the MIC value which is comparable with concentrations typically detected in surface water and wastewater. Summary of the main results

Conclusion

Original research paper: conclusion

650 °C. Additionally, at 850 °C, compressive strength drop. about 18-32%, 25-40%, and 49-64% for HPP, CPP and A respectively.

As a result, fibrous mortans effective on compressive strength up to 450°C compared with the control samples.

3.5. Determining optimum polymeric fiber content from the relationship between flexural and compressive strength under high temperature

As mentioned above with fiber addition, as flexural strength improves, compressive strengths are influenced negatively for each temperature. In this subsection, an attempt is made to determine the optimum fiber ratio that presents better compressive strength and flexural strength for each fiber type separately.

It is understood from the above discussion that each fiber shows the best performance at different ad dition ratios when flexural and compressive strength are taken simultaneously into consideration.

Hg 10 shows that the highest increase in flexural strength and the smallest decrease in compressive strength are obtained at 0.3– 0.9% addition ratio for the sample which has HPP. This situation is valid for nearly every temperature. Especially at 450 °C, this fiber shows good performance compared to non-fibrous mortars.

Although the samples containing CPP present best flexural strength with 05% by volume fiber addition at 100 °C, they also show good performance with 0.3-0.6% CPP addition ratio at othe temperatures.

Hg 10 shows that the optimum fiber addition ratios of the samples containing AR is 0.9% by volume for all temperature conditions.

4. Condusions

This study shows the effects of high temperature on the pechanical properties of cement mortars reinforced with provide ric fibers. The conclusions drawn from this study include the study

an increase in temperature, several changes cour in the matrix. At 450 °C some deteriorations cracks occur than 650 °C, the in cem matrices. In temperatures high matrices h ome weakened, spoiled, and racked. As the temboth fibrous and nonous mortars lose their perature rise masses by abox 3-8% because of mical reactions The flexural stren hs of the mor is reduce under high temperature. However, with ber ad don they increase relatively. The flexural strength of no. ous mortar decreases about 74% at 450 °C, about 85% at 6 and about 86% at 850 °C. However, these decrease for mortars on average at about 31ut 88-923 650°C, and about 95-96% at 51% at 450°C. 850 °C. This 🛛 ans the polymer. bers used in this study con e flexural strength of tribute to ortars under normal dry conditi (100 °C). This effect contin s dearly up to 450 °C and meric fibers show their effect on flexural strength ecially at 450 °C. However at higher mperatures, fibers repared to nonave adverse effects on flexural strength fibrous mortars.

3. When fib tous samples are compared with the coursel sample (non-fibrous and at normal conditions), it can be sign that it gives on average 200–236% big deflection at normal conditions and 32–114% high values at 450 °C. These deflectional or ferences are seen negatively at 650 °C as about 42–97%. Just a summary of the main results

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- Why should acknowledgements be included?
- Where in the manuscript?
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4. Conclusions

In this work, the effect of UV radiation on antibiotics and antibiotic-resistant *E. coli* strains was investigated. According to the results achieved, the simultaneous release of ARB and antibiotics at sub-lethal concentrations may occur into UWWTP effluent, thus possibly promoting the development of resistance among bacteria in the receiving water body. Due to the observed photodegradation rates, the risk of development of SMZ resistance among bacteria may be higher than AMX. The risk related to the development of CPX resistance is expected to be lower due to both the higher photodegradation rate and the MIC value which is comparable with concentrations typically detected in surface water and wastewater.

Acknowledgements

The authors are grateful to both University of Salerno for funding the project "Effect of solar photolysis on antibiotic degradation, antibiotic-resist ant bacteria inactivation as well as on their capacity to develop antibiotic resistance in surface water", Ex 60%, anno 2011, and Dr. Patrizia lannece, Department of Chemistry and Biology, University of Salerno, for her technical support in the measurement of antibiotics. The contribution of EU in supporting COST Action TD0803; Detecting evolutionary hot spots of antibiotic resistances in Europe (DARE), was highly appreciated too.

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Problem relevance and novelty

Dear Editor,

we kindly ask you to consider for possible publication in "nome della rivista" journal our research paper entitled:

Effect of solar simulated N-doped TiO₂ photocatalysis on the inactivation and antibiotic resistance of an *E. coli* strain in biologically treated urban wastewater.

The manuscript perfectly matches the aims and scope of "nome della rivista" because it is an original, novel and high-impact expected contribution in relation to some fields relevant for the journal. Specifically, N-doped TiO₂ (NDT) photocatalyst was synthesized and characterized to evaluate the improved capacity, compared to commercially available TiO₂ photocatalysts, in photocatalytic disinfection of biologically treated urban wastewater inoculated with an antibiotic resistant *E. coli* strain.

Urban wastewater treatment plants (UWWTPs) effluents are among the main sources of water pollution by antibiotics. The main concern is related to the development of antibiotic resistant bacteria (ARB), which reduce our therapeutic potential against human and animal pathogens. Accordingly, the role of UWWTPs on the fate of antibiotics and ARB is currently under discussion and consequently attracts a lot of attention among scientists and professionals. In particular, the role of UWWTPs in the spread of antibiotic resistance into the environment has not yet fully investigated, and poor information on the role of disinfection processes is available. Accordingly, our investigation of the effect of photocatalytic disinfection process on (i) environmentally relevant antibiotic resistant bacteria (in our work *E. coli* strains selected from UWWTP effluent) and (ii) antibiotic resistance is really new and timely. Moreover, the possibility to effectively use solar radiation for a more sustainable process was evaluated by ...

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The authors investigated the effect of UV radiation process on antibiotic resistant bacteria (ARB) in the secondary effluent of an urban wastewater treatment plant (UWWTP). The fate of ARB in UWWTP and the effect of disinfection process on ARB is an emerging and relevant scientific problem. Unfortunately, the manuscript is not well organized, some information/data are missing in "material and methods" section, and "results and discussion" paragraph should also be revised because some discussion according to data available in scientific literature is questionable and some is speculative and should be avoided. Finally, conclusion section should be improved (it is just a summary of the main results achieved).

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The authors investigated the effect of Hospital wastewater (HW) on conventional activated sludge process for wastewater treatment. It is particularly interesting the matter the authors addressed the effect of HW on the spread of antibiotic resistance into the environment, which is a timely and scientifically relevant issue and make this contribution novel. The manuscript was quite well organized (in my opinion "Discussion" paragraph should be merged into "Results" paragraph to avoid duplication of information and some supplemental materials moved in the paper) and the results well discussed. In my opinion, the manuscript can be accepted for publication in your journal after moderate revisions according to my comments/suggestions to authors. Because of the nature/type of revisions requested, you can check the authors address/reply to my comments and make your final decision, without send the manuscript back to me for second review.

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General comment

The manuscript deals with a timely and relevant issue, that is how Hospital wastewater can affect conventional activated sludge process for wastewater treatment as well as contribute to antibiotic resistance spread into the environment. The manuscript was quite well organized and the results well discussed. In my opinion, the manuscript is worthy of publication after moderate revisions according to my following comments/suggestions.

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Form revisions

- Syntax/English, units format, tables and/or figures format, match between manuscript and authors guidelines;
- Editorial recommendations about revision submission should be strictly followed (e.g., number and type of files to upload, including reply to reviewers comments).

Reviewer Blind Comments to Author:

General comment

The manuscript was guite well organized, the problem was well introduced, "material and methods" section well organized, the most of the results well explained and discussed according to relevant and updated scientific literature but for nitrate formation. Moreover, in my opinion it is not clear if the results of disinfection process can be attributed to photo-Fenton or rather to solar-H2O2 process. The risk related to the formation of nitrate was not clearly addressed and "Conclusion" section should be improved to explain this risk with the respect to the consumer. Accordingly, revisions are needed before the manuscript may be considered for possible publication. Specific comments/remarks follow.

Specific remarks/suggestions

>1. Keywords: just a suggestion, "photo-Fenton" and "disinfection" already appear in the title and may be exchanged with other relevant keywords (e.g., SODIS, developing countries, salmonella...) to enhance the track-ability your manuscript in the main data-base;

Abstract, pag 2, L24: please delete "total coliforms", E coli is enough (please check all the manuscript); 3. Material and methods, pag.6, L130-131: the official methods where these methods are adapted from and corresponding detection limits should be explained;

Material and methods, pag 7, L153-154; was H2O2 removed before regrowth dark experiments? If so, please explain the way. If not, please explain why;

5. Material and methods, pag 7, L151-153: microbiological analysis may be explained in a separate subparagraph;

Material and methods, pag 7, L163: it is not clear what authors mean for time periods of 8-14, 10-16, 12-18, and authors should better explain this part;

7. Results and discussion, pag 9: 3.1.1 is the only-one subparagraph in 3.1 paragraph, therefore I suggest to include it in 3.1 (eventually, you can change the title as "Lab experiments in a solar simulator: influence of H2O2 concentration on bacterial inactivation");

Results and discussion, pag 9, L199-203: can so low iron concentration result in the formation of 8. enough hydroxyl radicals to promote an effective disinfection during solar photo-Fenton process or this efficiency can be rather due to solar-H2O2 process? The authors should clear explain this issue;

Results and discussion, pag.9, L208; symbols are already included in figure captions and can be deleted from the text (please, feel free to accept or decline my suggestion); 10. Results and discussion, Figure 2: the joint use of the figures a and a', b and b', c and c', respectively is

hard to understand and should be better explained;

Results and discussion, pag 14, L314: is "Effective" better than "Efficient" in EDT? Results and discussion, pag 14, L324: table 4 are to detected and discussion.

Results and discussion, pag 14, L324: table 4 can be deleted and data explained in the text;

13 Results and discussion, pag 16, paragraph 3 2 3: the results of dark experiments (Fenton process) confirm my trouble about efficiency of photo-Fenton process (please, see previous comment #8); therefore, the authors are expected to revise this paragraph according to their reply to my previous comment and comparing their results with data available in scientific literature;

Results and discussion, pag 19, L433-435: are nitrite and nitrate concentrations compared to ammonia 14. depletion consistent with a mass balance?

Results and discussion, pag.20, L448-450: initial nitrate concentration is really high and photo-Fenton 15. process further increase this concentration, moreover taking into account that nitrate promote

methemoglobinemia disease, the authors should clearly talk about this result as an adverse effect of the process;

16. Conclusion, pag 21, L480-481: this part should be to improved by explaining the risk related to the increase of nitrate concentration (methemoglobinemia), according to previous comment.

Relevant comments/remarks Form revisions

Journal revision/submission procedure

Da: A: Data invio: lunedì 14 maggio 2012 10.31 Oggetto: - Editor decision - revise Re manuscript: Title: Urban wastewater spreading into the environment. Authors: Schwartz Corresponding author:

Dear

I can now inform you that the reviewers and editor have evaluated your manuscript. As you will see from the comments below and on http://ees.elsevier.com/wr/, publication in its present form is not recommended, and major revision is being requested.

The deadline for revision is 1 month from now, 13 Jun 2012. Please note your paper may be withdrawn if not submitted by the due date.

Please consider the reviews to see if revision would be feasible. For a revised version we require 3 separate items:

 Revision Notes explaining how and where (citing line number) each point of the Editor's/Reviewers' comments has been addressed. Should you disagree with any part of the reviews, please explain why.

A version of the revised manuscript showing the new/changed text using track changes or highlighting (submission item "Revision, changes marked"). To facilitate further review, add line numbers in the text.

3. A clean version of the revised manuscript, also with line numbers.

4. Please remove all files not needed for the new version, but do include all files needed for the new version, and strictly follow the formatting requirements as presented in the Guide for Authors.

Note that for the text source files only are allowed at revision: Word or LaTeX, no PDF. A PDF, however, is allowed for the tracked-changes version.

When submitting the revision, please present any figures, tables etc. as separate files. See the Artwork Guidelines at <u>www.elsevier.com/locate/watres</u> for further file naming conventions, referencing and format issues.

5. Please add a Graphical Abstract to your revised paper, if you had not already done so. This is an illustration summarizing the contents of your paper and able to capture the attention of readers online. See <u>http://www.elsevier.com/graphicalabstracts</u> for examples.

6. Be sure to check that the references cited in the text match those listed in the References section and the other way round, as errors may lead to a significant delay in processing your paper.

and a

To submit a revision, go to <u>http://ees.elsevier.com/wr/</u> and log in as an author. You will find your submission record under Submission(s) Needing Revision.

I hope that you will find the comments to be of use to you.

Kind regards,

Reply to Editor and Reviewers

Useful advices:

- Make wide use of diplomacy! Bearing in mind that...:
 - Sometime authors may not have a "perfect" answer to reviewer question/remark;
 - ✓ Sometime it is better to "agree" about some more <u>form revision</u>...
 - ...to make reviewer more "compliant" about some <u>relevant revision;</u>
 - If possible, avoid to ask explanation to reviewer about her/his comment, this may extend review process (so, it could be better to reply in some way);
 - When you disagree reviewer's comment/remarks, you should support your comment with relevant data/scientific literature.