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# Photocatalytic degradation of the antibiotic chloramphenicol and effluent toxicity effects

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ABSTRACT

Chloramphenicol sodium succinate (CAP, $C_{15}H_{15}Cl_2N_2\ Na_2O_8$) is a broad-spectrum antibiotic exhibiting activity against both Gram-positive and Gram-negative bacteria as well as other groups of microorganisms only partially removed by conventional activated sludge wastewater treatment plants. Thus, CAP and its metabolites can be found in effluents. The present work deals with the photocatalytic degradation of CAP using $TiO_2$ as photocatalyst. We investigated the optimization of reaction contact time and concentration of $TiO_2$ considering CAP and its by-products removal as well as effluent ecotoxicity elimination. Considering a CAP real concentration of 25 mg $L^{-1}$, kinetic degradation curves were determined at 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 g $L^{-1}$ $TiO_2$ after 5, 10, 30, 60 and 120 min reaction time. Treated samples were checked for the presence of by-products and residual toxicity (*V. fischeri*, *P. subcapitata*, *L. sativum* and *D. magna*). Results evidenced that the best combination for CAP and its by-products removal could be set at 1.6 g $L^{-1}$ of $TiO_2$ for 120 min with an average residual toxicity of approximately 10%, that is the threshold set for negative controls in most toxicity tests for blank and general toxicity test acceptability.



## 1. Introduction

Emerging contaminants are continually discharged into the aquatic environment without any restriction posing potential risks for public health and the environment. Antibiotics have been increasingly detected in sewage water, natural water, surface water and groundwater (Chatzitakis et al., 2008; Fatta-Kassinos et al., 2011, Van Doorslaer et al., 2015). Antibiotics are readily available on the market being used to treat diseases in humans and in animals, promote animal growth and improve nutritional efficiency of feed (Sarmah et al., 2006). Despite their low environmental concentrations (from ng $L^{-1}$ to μg $L^{-1}$), the continuous input and persistence into the aquatic ecosystem make antibiotics one of the most urgent environmental issue, primarily due to the potential for the development of antimicrobial resistance (Dunlop et al., 2015).

The limitations of conventional wastewater treatment plants (WWTPs) in removing these bio-recalcitrant molecules point toward the urgent need for improved wastewater treatments such as Advanced Oxidation Processes (AOPs), a special class of oxidation techniques characterized by production of •OH radicals. Amongst several AOPs, heterogeneous photocatalysis has proven its potential in degrading antibiotics from aqueous matrices (Zhang et al., 2010; Lofrano et al., 2014, Van Doorslaer et al., 2015). The elimination of mother compounds does not necessarily result in toxicity removal, since the photocatalytic degradation can produce intermediate by-products, which can still exert adverse biological effects. Therefore to evaluate the overall behavior and efficiency of the process, it is worth to assess not only the removal of a specific compound, but also of the whole ecotoxicity potential (Rizzo et al., 2009; Libralato et al., 2010a, 2016; Carotenuto et al., 2014; Lofrano et al., 2014). So far, ecotoxicity data for AOPs treated solutions of antibiotics are scarce or missing, making their environmental risk assessment difficult.

In the present study, the photocatalytic degradation of chloramphenicol sodium succinate (CAP, $C_{15}H_{15}Cl_2N_2\ Na_2O_8$), which is a

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representative antibiotic applied to inhibit Gram-positive and Gram-negative bacteria, was investigated at various aqueous suspensions of $TiO_2$. Its photo-degradation by-products as well as the toxicity of the final treated effluent were assessed as well. CAP has been widely used due to its low cost and high efficiency in the treatment of various infectious diseases. Due to its carcinogenic effects and other serious adverse reactions, such as bone marrow depression, aplastic anemia and severe blood disorders, CAP has been banned from China, Japan, Canada, United States, Australia and European Union in animals used for human consumption, even if it is still legally used in Brazil and other countries, or illegally, in livestock, due to the easy access, low price and steady antibacterial effectiveness (Andrade et al., 2006). As consequence CAP has been found in concentrations between 0.001 and 0.031 $\mu g\,L^{-1}$ in surface waters in Singapore and Korea, respectively. Average CAP concentrations between 2.08 and 26.6 $\mu g\,L^{-1}$ were found in effluents of sewage treatment plants in China (Choi et al., 2008; Liu et al., 2009; Peng et al., 2006; Xu et al., 2011). The degradation of CAP has been evaluated by several AOPs such as $UV/H_2O_2$ (Baeza et al., 2007), photo-Fenton (Trovó et al., 2013), photoelectron Fenton (Garcia-Segura et al., 2014), photocatalysis (Chatzitakis et al., 2008; Zhang et al., 2010), electrochemical degradation (Rezende et al., 2010). None of these carried out ecotoxicity tests on the $TiO_2$ photo-catalytically treated effluent. Data on CAP ecotoxicological effects are available only for single species like for *Vibrio fischeri* ($EC50 = 20.68\ mg\,L^{-1}$) (Choi et al., 2008) and *Daphnia magna* ($EC50 = 1086\ mg\,L^{-1}$ (Calleja et al., 1994); $EC50 = 227–600\ mg\,L^{-1}$ (Müller, 1982); $EC50 = 542.86\ mg\,L^{-1}$ (Lilius et al., 1994)). Currently, no toxicity data are available for widely used photosynthetic biological models like the microalga *Pseudokirchneriella subcapitata* and dicotyledonous macrophyte *Lepidium sativum*.

The aim of this study was to elucidate the photocatalytic degradation kinetics of CAP (increasing contact times and photocatalytic agent concentrations, *i.e.* $TiO_2$) and to assess the efficiency of degradation processes through the removal of ecotoxicological effects related to the potential by-product residues applying the principles of the whole effluent assessment (WEA) (OSPAR Commission, 2007; Libralato et al., 2010b) also in order to meet the goal of the best available technology (BAT). A battery of acute (A) and chronic (C) toxicity tests was used including biological models belonging to various trophic levels like *V. fischeri* (A), *P. subcapitata* (C), *L. sativum* (A) and *D. magna* (A). The toxicity of CAP as a pure substance was investigated on *P. subcapitata* and *L. sativum* due to missing data.

## 2. Materials and methods

### 2.1. Reagents and analytical procedures

All reagents were of analytical grade. Photocatalytic degradation experiments were carried out using gravimetrically measured aliquots of $TiO_2$ Degussa P25. The decay of CAP dispersed in ultrapure water was followed by HPLC-UV (Finnigan Surveyer) equipped with a reversed phase C18 analytical column (Vydac, 5 μm, 150 mm × 3.0 mm). The injection volume was 10 μL and the wavelength set for the quantification was 275 nm according to the maximum light absorption of CAP. The limit of quantification (LOQ) was 0.5 $\mu g\,mL^{-1}$.

HPLC grade water and methanol were supplied from Sigma Aldrich. The compounds were separated using as mobile phase a mixture of methanol/ultrapure water (30%/70%) at flow rate of 1 $mL\,min^{-1}$. The UV–Vis spectra were recorded using a spectrophotometer (Varian Cary 50). Chlorides and nitrates were determined by ion chromatography (Dionex 2000).

Electrospray ionization mass spectrometry (ESI-MS, Micromass Quattro *micro*™) was used to detect CAP by-products. Samples were introduced into the electrospray (ESI) source by continuous flow injection. The following conditions were found to provide the optimum signal: ion source temperature 100 °C, desolvation temperature 250 °C, desolvation gas 500 $L\,h^{-1}$, cone voltage 30 V, and capillary voltage 3.5 KV. The instrument was run in the negative ion mode.

### 2.2. Experimental design

Preliminary investigations took into consideration the effects of $TiO_2$ in dark conditions to set the background level of CAP removal and potential adsorption. Photolysis experiments were carried out at 20 ± 2 °C in a 250 mL magnetic stirred cylindrical Pyrex vessel filled with 200 mL ultra-pure aqueous solution (25 $mg\,L^{-1}$ of CAP). In photocatalysis experiments various $TiO_2$ concentrations (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 $g\,L^{-1}$) were added to the solution at natural pH of 5.5.

The CAP concentration was selected after an initial screening assessment not reported here and allowed to clearly follow CAP degradation kinetics. The reaction vessel was placed in a chamber and illuminated for 5, 10, 30, 60, 120 min with a xenon arc lamp (450 W, LotOriel Group, Italy) equipped with special glass filters restricting the transmission of wavelengths below 300 nm. The light intensity determined by the potassium ferrioxalate actinometry (Hatchard and Parker, 1956) was $4.5 \times 10^{-7}$ Einstein $s^{-1}$. After the photocatalysis process, samples were slowly filtered through 0.45 μm polymer membrane filters (Whatman) to remove the catalyst.

### 2.3. Ecotoxicity

Toxicity tests were carried out on untreated 25 $mg\,L^{-1}$ CAP solution (pure substance, only for *P. subcapitata* and *L. sativum*) and after the photocatalytic treatment with various $TiO_2$ concentrations (0.1, 0.2, 0.4 and 0.6 $mg\,L^{-1}$) according to various photo-oxidation times (5, 10, 30, 60 and 120 min). For *V. fischeri*, the toxicity of treated CAP solutions was investigated only after 120 min. Treated CAP solutions were assessed for ecotoxicity collecting samples' aliquots after each treatment interval. All toxicity tests included the assessment of negative and positive controls in accordance with the specific reference method.

The acute bioluminescence inhibition assay was carried out using *V. fischeri* (NRRL-B-11177) according to ISO (2007). The luminescence was measured with a Microtox® analyzer (Model 500, AZUR Environmental) after 5 and 15 min at 15 °C. Tests were carried out in duplicate. Data were analyzed with Microtox Omni software and the result expressed as percentage of bioluminescence inhibition (%).

The chronic growth inhibition test with *P. subcapitata* was carried out according to ISO (2012). Cultures were kept in Erlenmeyer flasks. The initial inoculum contained $10^4$ cells $mL^{-1}$. The specific growth inhibition rate was calculated considering 6 replicates exposed at 20 ± 1 °C for 72 h under continuous illumination (6000 lx). Effect data were expressed as percentage of growth inhibition.

The acute bioassay with *L. sativum* evaluated the potential toxicity considering the root elongation according to OECD (2006). Experiments were conducted in triplicate at 25 ± 1 °C for 72 h in aqueous solutions. The root elongation inhibition normalized on negative control data were expressed as percentage of effect.

Acute toxicity tests with *D. magna* were carried out according to ISO (2013). Newborn daphnids (< 24 h old) were exposed in four replicates for 24 and 48 h at 20 ± 1 °C under continuous illumination (1000 lx). Before starting the test they were fed with *P.*

*subcapitata* (300,000 cells $mL^{-1}$) *ad libitum*. Toxicity was expressed as the percentage of dead organism.

The significance of differences between average values of different experimental treatments and controls was assessed by the analysis of variance (ANOVA) considering a significance threshold level always set at 5%. When ANOVA revealed significant differences among treatments, *post-hoc* tests were carried out with Tukey's test. Statistical analyses were performed using Microsoft® Excel 2013/XLSTAT©-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA).

## 3. Results and discussion

### 3.1. Kinetic studies

Experiments on CAP at 25 mg $L^{-1}$ carried out under dark at various (0.1, 0.2, 0.4, 1.6, 3.2 g $L^{-1}$) $TiO_2$ concentrations proved that adsorption did not influence significantly the CAP degradation. As shown in Fig. 1A, the CAP plotted as function of time showed a very slight removal after 120 min as an effect of the potential adsorption.

As for most of the organic compounds, the photolysis of CAP results strongly influenced by both the wavelength and intensity of UV source. Previous studies proved that a medium pressure mercury lamp (majority of photons emitted between 300 and 400 nm) provided superior results in the degradation of aromatic compounds compared to those obtained using a low-pressure mercury lamp (254 nm) (Oliveros et al., 1997). Chatzitakis et al. (2008) irradiated a CAP solution of 50 mg $L^{-1}$ using a lamp emitting between 300 and 400 nm with a maximum at 365 nm and a light intensity of $1.12 \times 10^{-7}$ Einstein $s^{-1}$ observing no CAP removal. da Rocha et al. (2013) reported a 75% and 6% of CAP removal starting from an initial concentration of 20 mg $L^{-1}$ after 12 h of photolysis using UV-C radiation and solar radiation, respectively. In our study, after 120 min of irradiation the photolysis removed about the 40% of CAP. This result was not unexpected, considering that the intensity of the lamp used in our study was higher compared to Chatzitakis et al. (2008). The loss of CAP by photocatalysis in presence of various $TiO_2$ loads can be described by pseudo first order kinetics ($R^2 > 0.99$) (Fig. 1A). A similar pathway of decay was followed in both electro-Fenton (EF) and solar photo-electro-Fenton (SPEF) treatments of 245 mg $L^{-1}$ CAP ($k = 1.4 \times 10^{-3}$ $s^{-1}$ ($R^2 = 0.994$) and $1.6 \times 10^{-3}$ $s^{-1}$ ($R^2 = 0.994$)), respectively (Garcia-Segura et al., 2014).

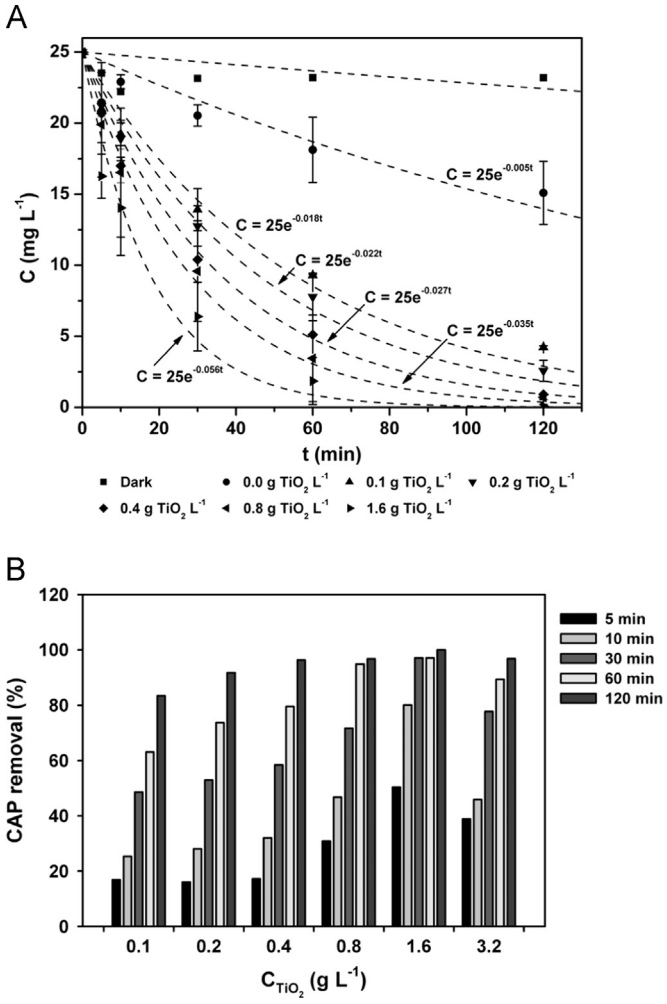


**Fig. 1.** Photocatalytic kinetic curves (A) and removal percentage (B) of CAP (25 mg $L^{-1}$) after 5, 10, 30, 60 and 120 min at 0.0, 0.1, 0.2, 0.4 and 1.6 g $L^{-1}$ of $TiO_2$ at pH=5.5. Dark experiments at 1.6 g $L^{-1}$ of $TiO_2$ (only Fig. 1A – No significant differences could be observed in dark experiments at various $TiO_2$ concentrations investigated); 3.2 g $L^{-1}$ of $TiO_2$ kinetic curve was not reported due to a removal efficiency decrease (Fig. 1B) compared to 1.6 g $L^{-1}$ of $TiO_2$.

Both the illumination and the catalyst are necessary for effectively degrade CAP. The dosage of $TiO_2$ in slurry photocatalytic processes generally represents a key factor that can strongly influence the degradation of organic compounds (Chatzitakis et al., 2008, da Rocha et al., 2013, Lofrano et al., 2014). As shown in Fig. 1B, the degradation rate of CAP increased when the concentration of $TiO_2$ increased up to 1.6 g $TiO_2$ $L^{-1}$. Beyond this value, the removal efficiency decreased. Approximately the total removal of CAP was already achieved at 0.8 g $TiO_2$ $L^{-1}$ after 60 min of irradiation. The best solution should be to balance the hydroxyl radical produced from irradiation keeping $TiO_2$ as low as possible to avoid aggregation or shading phenomena and thus limiting the photo-reaction efficiency.

Chatzitakis et al. (2008) observed that during the photocatalytic degradation of 50 mg $L^{-1}$ CAP rising the $TiO_2$ concentration from 0.25 to 4 g $L^{-1}$ the initial reaction rate increased by a factor of 2 showing a plateau after 1 g $L^{-1}$ meaning that the photo-oxidation reached the saturation. Such an optimum $TiO_2$ concentration must be determined time-by-time to avoid the use of excess reactive agents as well as to ensure that the absorption of radiation photons is maximized for an efficient degradation.

Our results were in agreement with previous studies where a central composite methodology was applied for modeling and optimizing the operation parameters on $TiO_2$ photocatalytic degradation of CAP (Zhang et al., 2010). According to these authors the degradation rate of CAP gradually increased with increasing $TiO_2$ concentrations and decreasing CAP ones. On the other hand, the trend of CAP degradation rate declined under both the higher level of $TiO_2$ concentration and CAP initial concentration. The optimum region of CAP degradation rate was obtained keeping $TiO_2$ concentration between 0.9 and 1.1 g $L^{-1}$ and CAP initial concentration between 12 and 16 mg $L^{-1}$, respectively.

### 3.2. Photo-oxidation by-products

The formation of photo-oxidation by-products is of great concern in water treatment because they can generate more toxic effects than their parent compounds. In our study, the formation of aromatic organic intermediates at wavelengths close to 280 nm was observed during the photocatalytic treatment (Fig. 2). After 120 min, the absorbance peak was only slightly removed by photocatalysis at 0.1, 0.2, 0.4, 0.8 g $TiO_2$ $L^{-1}$, which indicated that the oxidation of by-products required further time to be completely oxidized. Only at 1.6 g $TiO_2$ $L^{-1}$ they were completely removed.

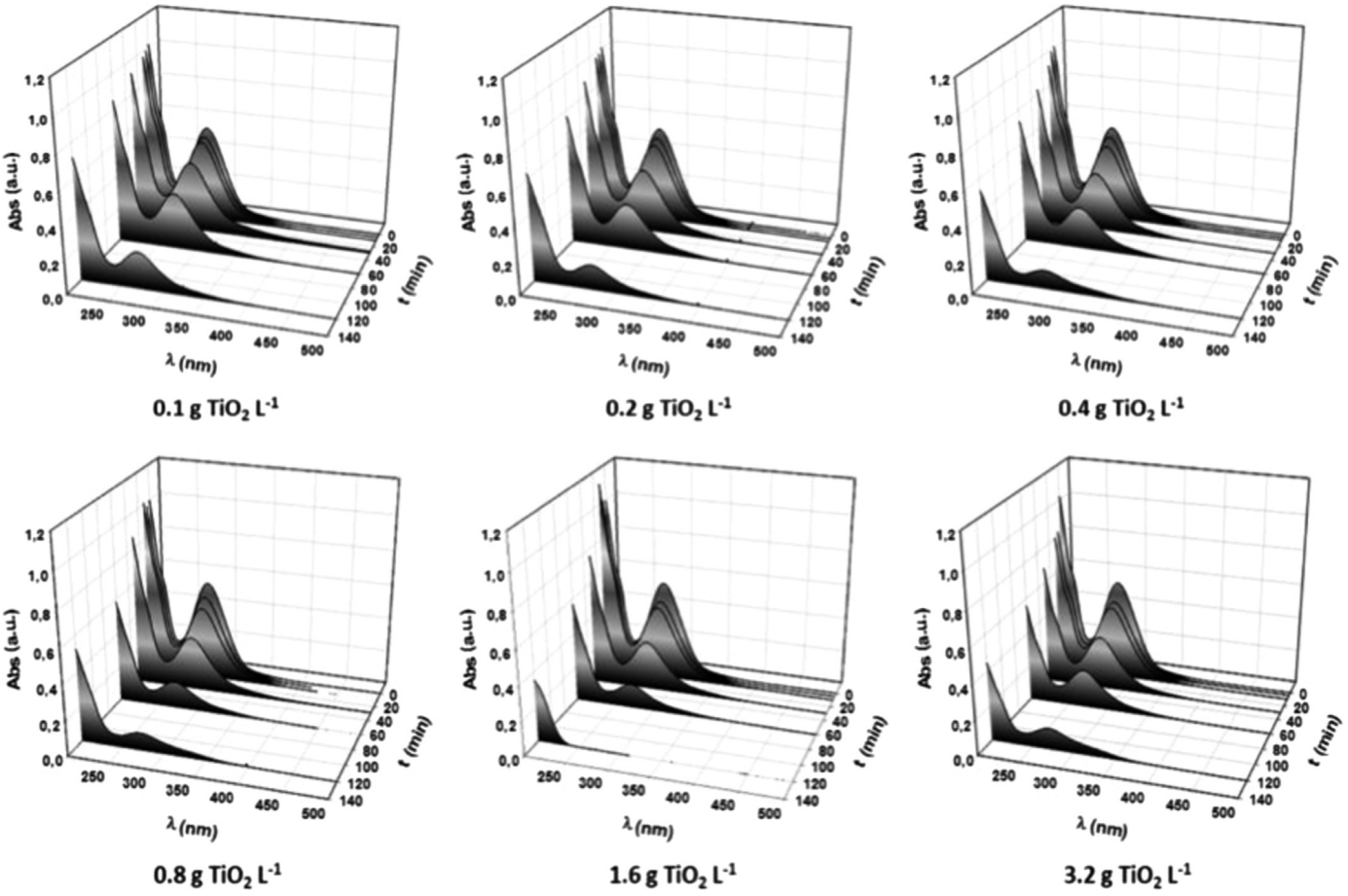


**Fig. 2.** Photo-oxidation by-products of CAP (25 mg $L^{-1}$) formation at various $TiO_2$ concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 g $TiO_2$ $L^{-1}$).

The photo-mineralization progressed with a decreasing efficiency at increasing catalyst dosage. A slight absorbance peak could be still observed after 120 min at 3.2 g $TiO_2$ $L^{-1}$.

In agreement with UV absorbance results, the ESI-MS spectra carried out after 120 min of photocatalysis (Fig. 3) showed a progressive degradation of CAP and its by-products at increasing catalyst concentrations (0.2, 0.4 and 1.6 g $TiO_2$ $L^{-1}$). The ESI-MS spectrum on untreated CAP (25 mg $L^{-1}$) was reported in Fig. 3a. Since hydroxyl radicals are the main oxidant of all AOPs, the same by-products are expected in all them varying concentration and degradation progression versus time. Nine aromatic products and thirteen hydroxylated derivatives along with dichloroacetic acid were found as result of anodic oxidation electro-generated $H_2O_2$ (AO-$H_2O_2$), electro-Fenton, photo-electro Fenton and solar photo-electro-Fenton of 245 mg $L^{-1}$ CAP by Garcia-Segura et al. (2014). The higher oxidation ability of AO-$H_2O_2$ with boron doped diamond anode allowed the faster intermediated degradation with larger accumulation of oxalic, oxamic and formic acid which were also mineralized at long electrolysis time (360 min). According to the studies of Garcia-Segura et al. (2014), the following by-products were detected after 120 min of photolysis and photocatalysis: CAP (321 *m/z*, negative ion with $z=1$), 4-nitrobenzoic acid (166 *m/z*, negative ion with $z=1$) (Fig. 3b); CAP (321 *m/z*, negative ion with $z=1$), 4-nitrobenzoic acid (166 *m/z*, negative ion with $z=1$), dichloroacetic acid (127 *m/z*, negative ion with $z=1$), 4-nitrophenol (138 *m/z*, negative ion with $z=1$), at 0.2 g $TiO_2$ $L^{-1}$ (Fig. 3c); CAP (321 m/z, negative ion with $z=1$), 4-nitrobenzoic acid (166 *m/z*, negative ion with $z=1$) dichloroacetic acid (127 *m/z*, negative ion with $z=1$), 4-nitrophenol (138 *m/z*, negative ion with $z=1$), at 0.4 g $TiO_2$ $L^{-1}$ (Fig. 3d); dichloroacetic acid (166 *m/z*, negative ion with $z=1$) at 1.6 g $TiO_2$ $L^{-1}$.

The conversion of organic chlorine and nitrogen from the CAP molecule to the respective inorganic ions follows the reaction reported in Garcia-Segura et al. (2014).

The maximum values obtained after 120 min at 0.2 g $TiO_2$ $L^{-1}$ for both chloride and nitrate were 0.11 and 0.023 mmol $L^{-1}$, respectively (Fig. 4). In agreement with previous studies, chlorine from antibiotics is easily released as chloride ions (Calza et al., 2006; Lofrano et al., 2014). Up to 87% of the $Cl^-$ stoichiometric amount (0.10 mmol $L^{-1}$) was determined just after 2 h of photocatalysis of 50 mg $L^{-1}$ of vancomycin in presence of 0.2 g $TiO_2$ $L^{-1}$ (Lofrano et al., 2014). In the present study, all $Cl^-$ stoichiometric amount (0.11 mmol $L^{-1}$) was detected after 120 min of photocatalysis at 0.8 g $TiO_2$ $L^{-1}$ (Fig. 4). Whereas the $Cl^-$ is a primary inorganic ion formed as a consequence of CAP mineralization, the conversion of N from CAP into N–$NO_3^-$ was slower according to previous literature studies. Elmolla and Chaudhuri (2010) did not detect nitrate ions during the first 6 h of photocatalytic oxidation of amoxicillin, ampicillin and cloxacillin. Nitrate formation was not observed throughout the photocatalytic oxidation process of 50 mg $L^{-1}$ of vancomycin in presence of 0.2 g $TiO_2$ $L^{-1}$ probably because of the insufficient irradiation time (Lofrano et al., 2014).

In the present study, after 120 min of photocatalysis in presence of 1.6 g $TiO_2$ $L^{-1}$ only 20% of the *N* initial concentration (1.57 mg $L^{-1}$) was detected as nitrate. This is indicative of the presence of various aliphatic nitrogen-containing reaction products leading to the conclusion that longer illumination times are needed for the complete conversion to inorganic end-products. Conversely, Chatzitakis et al. (2008) reported a 90% conversion rate of the nitro-group in CAP (50 mg $L^{-1}$) to nitrate after 3 h of

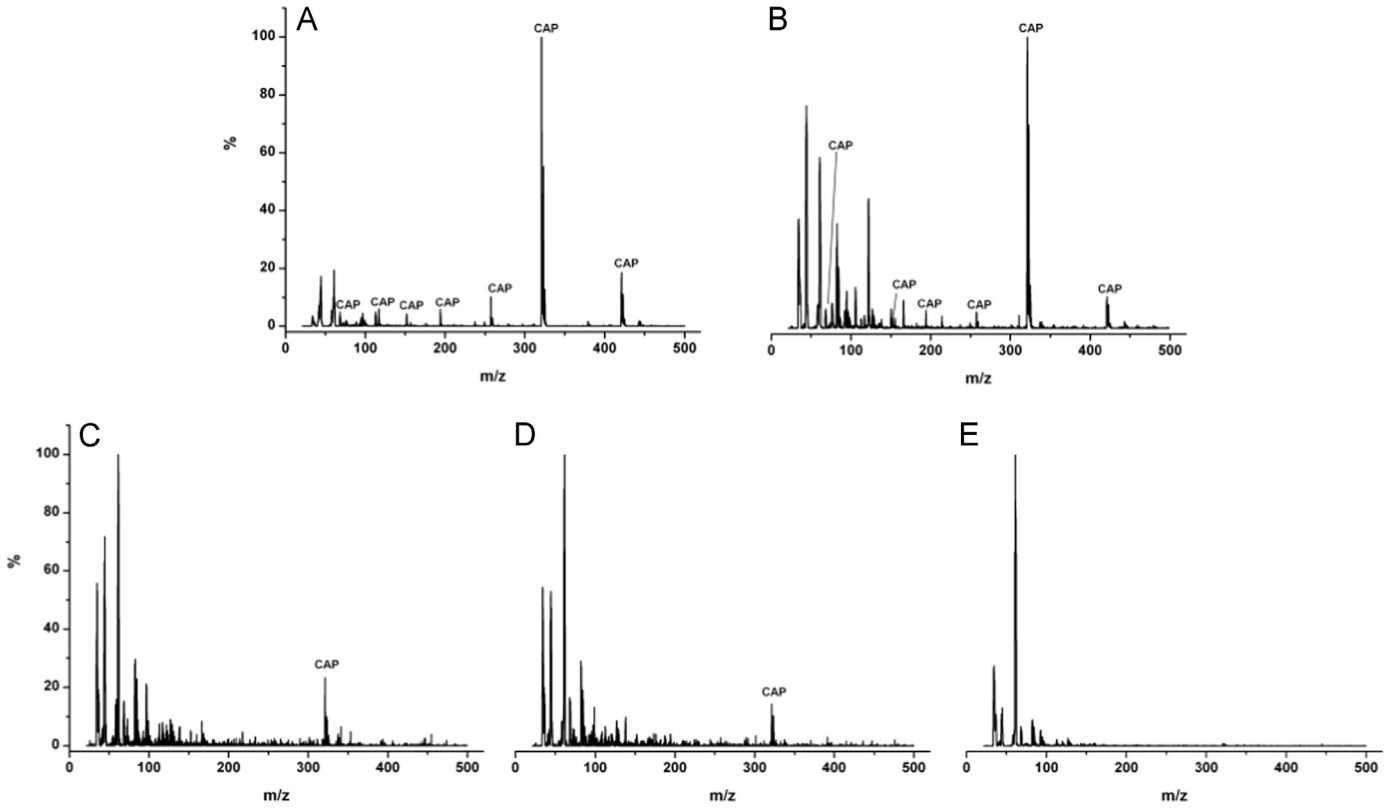


**Fig. 3.** ESI-MS spectra on untreated CAP (25 mg $L^{-1}$) (a), CAP photolysis (25 mg $L^{-1}$) (b) and CAP (25 mg $L^{-1}$) photocatalysis at 0.2 (c), 0.4 (d) and 1.6 (e) g $L^{-1}$ of $TiO_2$ after 120 min.

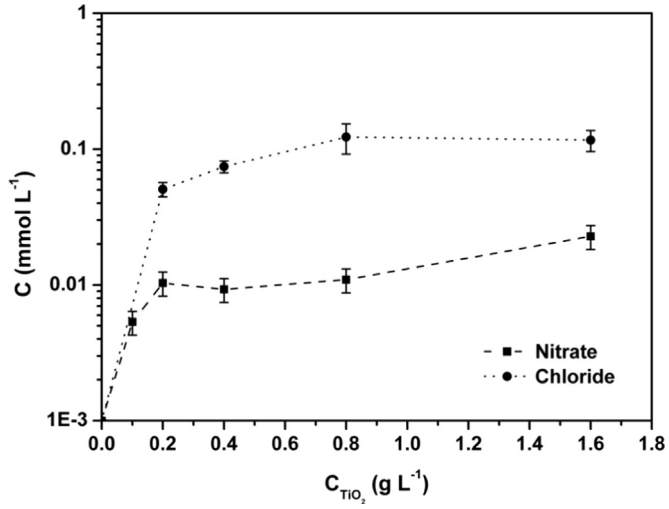


**Fig. 4.** Chloride ($Cl^-$) and nitrate ($NO_3^-$) formation after 120 min CAP (25 mg $L^{-1}$) photocatalysis at 0.1, 0.2, 0.4, 0.8 and 1.6 g/L of $TiO_2$.

photocatalysis in presence of 1 g $TiO_2$ $L^{-1}$. Approximately 59% of the initial *N* of CAP (21.2 mg $L^{-1}$) was detected after 360 min of solar photo-electro-Fenton treatment by Garcia-Segura et al. (2014).

### 3.3. Ecotoxicity

The toxicity trend of *P. subcapitata* and *L. sativum* exposed to CAP was displayed in Fig. S1. Effect data showed similar sensitivities considering the EC50 values: 137 (132, 142) mg $L^{-1}$ for *P. subcapitata* and 140 (130, 150) mg $L^{-1}$ for *L. sativum*. Observing Fig. S1, it is evident that increasing CAP concentration tends to increasingly affect *P. subcapitata*, but for *L. sativum* the toxicity of CAP decreased at higher exposure concentrations suggesting the saturation of sites of actions and/or detoxification phenomena for this model organism. Compared to *V. fischeri* (Choi et al., 2008) and *D. magna* (Müller, 1982; Calleja et al., 1994; Lilius et al., 1994), *P. subcapitata* and *L. sativum* were the most sensitive testing organisms for CAP.

The residual toxicity of treated CAP (25 mg $L^{-1}$) was summarized in Fig. 5 along with statistical data analysis. Results from all bioassays evidenced a decreasing trend in the amount of the residual toxicity at increasing concentrations of $TiO_2$ and times of photo-oxidation. Averagely, the lowest toxicity effects (approximately 10%) were detected after 120 min of treatment with 1.6 g $L^{-1}$ of $TiO_2$. The toxicity of *V. fischeri* after 5 min (Fig. 5A) and 15 min (Fig. 5B) contact time are very similar. They evidenced that after 120 min of photo-oxidation the most significant effect ($p < 0.05$) was obtained at 1.6 g $L^{-1}$ of $TiO_2$ with a residual toxicity of $8 \pm 6\%$ (5 min) and $10 \pm 4\%$ (15 min). Lower $TiO_2$ concentrations showed toxicities ranging between 45–62% (5 min) and 53–76% (15 min).

The effects showed by *P. subcapitata* (Fig. 5C) suggested the same trend observed in Fig. 5A and B. The lowest residual toxicity was observed at both 0.4 and 1.6 g $L^{-1}$ of $TiO_2$ with approximately 4–5% effect. Nevertheless, the toxicity of CAP treated at 0.2, 0.4 and 0.6 g $L^{-1}$ of $TiO_2$ resulted no statistically different ($p < 0.05$) after 5, 10, 30, 60 and 120 min of photo-oxidation mainly due the variability associated to these data.

The effects on *L. sativum* were reported in Fig. 5D. The toxicity showed to be significantly ($p < 0.05$) reduced just after 5 min of photo-oxidation, but mainly when 1.6 g $L^{-1}$ of $TiO_2$ was used. The increase in the photo-oxidation duration did not significantly improve the toxicity removal that ranged between 1% (after 120 min at 1.6 g $L^{-1}$ of $TiO_2$) and 4% (after 5 min at 1.6 g $L^{-1}$ of $TiO_2$). All other $TiO_2$ concentrations presented a residual toxicity

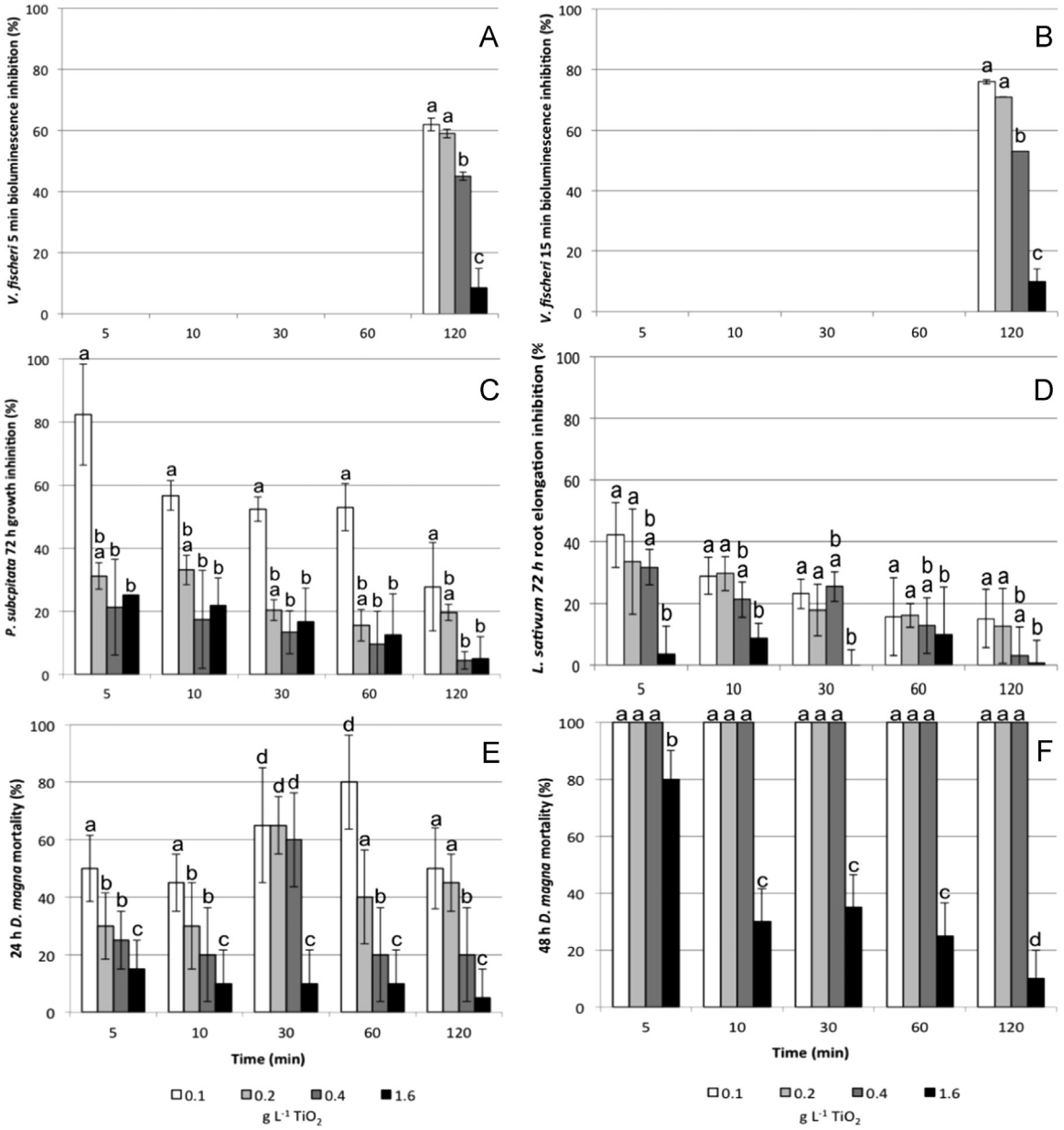


**Fig. 5.** Toxicity results of treated CAP solution (25 mg $L^{-1}$) after 5, 10, 30, 60 and 120 min at 0.1, 0.2, 0.4 and 1.6 g $L^{-1}$ of $TiO_2$ including *V. fischeri* bioluminescence inhibition after 5 min (A) and 15 min (B) contact time, *P. subcapitata* growth inhibition (C), *L. sativum* root elongation inhibition (D), and *D. magna* mortality after 24 h (E) and 48 h (F) contact time; data with different letters (a–d) are significantly different (Tukey's, $p < 0.05$).

$> 10\%$.

The results of 24 h and 48 h mortality test with *D. magna* were displayed in Fig. 5E and F, respectively. Like for Fig. 5D, *D. magna* 24 h results highlighted that the best concentration of $TiO_2$ to reduce toxicity is equal to 1.6 g $L^{-1}$ just after 5 min of photo-oxidation leaving residual effects between 15% (after 5 min at 1.6 g $L^{-1}$ of $TiO_2$) and 5% (after 120 min at 1.6 g $L^{-1}$ of $TiO_2$). It is of great interest to observe how increasing the test duration of 24 h (Fig. 5F), the mortality effects significantly increased for all treatments except for the CAP solution treated for 120 min at 1.6 g $L^{-1}$ of $TiO_2$ that presented a residual toxicity still of 10%.

According to Fig. 4, chlorides and nitrates did not contribute to the definition of the residual toxicity. The residual toxicity cannot be directly attributed to $TiO_2$ as photo-catalytic agent due to its removal by filtration.

According to Tonkes et al. (1999) and Persoone and Wells (1987), the residual toxicity of CAP (25 mg $L^{-1}$) wastewater ($\leq 10\%$) after photo-oxidation (1.6 g $L^{-1}$ for 120) can be classified as not acutely toxic. The amount of the residual toxicity is equivalent to the most frequent threshold set for negative controls in most toxicity tests for blank and general toxicity test acceptability.

## 4. Conclusions

Photocatalysis mediated by $TiO_2$ was very efficient to mineralize, degrade and detoxify water spiked with CAP. After 120 min photo-reaction in presence of 1.6 g $TiO_2$ $L^{-1}$, CAP and its by-products were completely removed. Results obtained by the adsorption in the dark and direct photolysis for 120 min proved that both adsorption and photochemical processes did not significantly influence the observed fast transformations when the solution of CAP was irradiated in presence of $TiO_2$. According to all testing

species (*V. fischeri*, *P. subcapitata*, *L. sativum* and *D. magna*), filtered treated samples after the photo-oxidation process presented a residual toxicity of approximately 10% or lower that is, generally, the accepted toxicity threshold for negative controls in ecotoxicology. This suggests that the photo-oxidation process with $TiO_2$ represents an interesting and eco-safe technology for CAP and its by-products removal.

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## Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2015.07.039.

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