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# Degradation of a fluoroquinolone antibiotic in an urbanized stretch of the River Tiber

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ABSTRACT

The widespread detection of antibiotics in terrestrial and aquatic systems has engendered significant scientific and regulatory concern. Overall, knowledge concerning the ecotoxicology and sub-lethal effects in water is scarce, but some experimental studies show that antibiotics can induce pathogen resistance and they can also have detrimental effects on natural microbial communities and their key functions.
The main aim of this study was to investigate the occurrence of the biodegradation and photodegradation processes of the fluoroquinolone ciprofloxacin (CIP) in the River Tiber waters, in a stretch highly impacted from human pressure. Two set of microcosms consisting of river water containing the natural microbial community and treated with 500 μg/L of CIP in absence or presence of UV-light were performed. Moreover, some microcosms were filled with river water previously sterilized and then treated with the antibiotic. The combined experimental set made it possible to evaluate if the antibiotic CIP could be photodegraded and/or biodegraded.
CIP residual concentrations were measured over time by using HPLC coupled to fluorescence detection (FLD) and the effects of the antibiotic on the natural microbial community were assessed in terms of live cell abundance. The key role of light in CIP disappearance was confirmed, but also its biodegradation in natural river water was demonstrated. In fact, differently from other experiments we found a higher degradation rate ($DT_{50}$ = 10.4 d), in presence of both light and the natural river bacterial populations than in the same sterilized river water ($DT_{50}$ = 18.4 d). Moreover, even in the dark, a partial CIP biodegradation was also observed ($DT_{50}$ = 177 d). The overall results were supported by the increase in live cell numbers with the decrease of CIP concentrations both in the dark and light condition.



## 1. Introduction

Antibiotics are a class of emerging micro-pollutants found commonly in wastewater [1] water and soil ecosystems [2]. Antibiotics are partially metabolized in treated organisms and, through excretions, reach wastewater treatment plants (WWTPs) [3]. Most WWTPs are not able to remove them efficiently and through their effluents they reach surface water. Moreover, the use of sewage sludge (biosolids) and manure as a crop fertilizer and irrigation with reclaimed water transports human and veterinarian antibiotics into agricultural soil [4,2].

The widespread detection of pharmaceuticals in terrestrial and aquatic systems has engendered significant scientific and regulatory concern [5,6].

Ciprofloxacin (CIP) is among the antibiotics most frequently detected in European surface water ecosystems in concentrations ranging from a few ng to >100 ng per liter (8–119 ng/L), [7,8]. In Italy CIP has been found as a micro-contaminant of several river water ecosystems in concentrations comparable to those found in other European rivers [9,10].

CIP is a fluoroquinolone antibiotic, broad-spectrum antibacterial agent widely used for the treatment of both Gram-positive and Gram-negative bacterial infections in both human and animals [11]. It is an amphoteric molecule obtained by modification of the quinolone core structure by insertion of the fluorine atom and this enhances its biological activity and contributes to its recalcitrance. CIP acts through inhibition of the key bacterial enzymes involved in DNA replication [12]. Fluoroquinolones target the enzymes DNA gyrase and topoisomerase IV with varying efficiency in different bacteria and inhibit their control of supercoiling within the cell, resulting in impaired DNA replication (at lower concentrations) and cell death (at lethal concentrations) [13]. The targeting of either DNA gyrase or topoisomerase IV as the primary target by fluoroquinolones varies with bacterial species and the specific fluoroquinolone; however, as a broad generalisation, the key

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target in Gram-negative bacteria is DNA gyrase, whereas in Gram-positive microorganisms topoisomerase IV is preferentially targeted [14].

CIP is effective against Gram-negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and *Haemophilus* spp., but also some Gram-positive bacteria such as *Staphylococcus aureus* [15,16]. Owing to its chemical structure and intrinsic biocide property, it can be considered recalcitrant to biodegradation [17]. Some studies report CIP to be not readily biodegradable, with a disappearance time of 50% of its initial concentration ($DT_{50}$) higher than 100 days [18]. Similarly another biodegradability test (OECD 301B) showed CIP to be recalcitrant to degradation in water; however CIP has been found (test OECD 307) to be less persistent in soil with a $DT_{50}$ of 32 days and also a slight mineralization at day 93. In the same experiments, CIP was able to inhibit microbial activity with higher effects in water than in soil. These results were ascribable to soil adsorption phenomena, which made this molecule less bioavailable and consequently less toxic to microorganisms. Moreover, CIP was found to inhibit soil respiration in the first 20 days of the experiment [8]. The toxicity of CIP was tested using as an endpoint the growth inhibition of the bacterium *Pseudomonas putida*, which is known to be sensitive to this antibiotic. The antibiotic CIP inhibits *P. putida* at concentrations ranging from 2 to 8000 μg/L; moreover, CIP was also found to be genotoxic [18]. Although CIP is generally considered not biodegradable, just a few studies report some microorganisms able to metabolize it [18,19,20] as in the case of a bacterial strain of *Labrys portucalensis* able to degrade CIP alone or together with ofloxacin and norfloxacin [21].

Fluoroquinolones are photosensitive molecules, and photodegradation has been extensively reported to be the main transformation pathway of CIP [22], which is resistant to hydrolysis, thermal decomposition and biodegradation [21,17,23–25]. Nevertheless, complete mineralization across photochemistry processes is hard to achieve in water systems under the most common environmental conditions; in fact, it can occur only in presence of direct light and, consequently, in deeper water, sediment or soil or in cloudy water this process is very limited [21,7].

Overall, knowledge of the ecotoxicology and sub-lethal effects in water is still scarce, but experimental studies show that antibiotics can have detrimental effects on both natural microbial communities and their key functions and induce resistance mechanisms [5]. An increase in resistance to fluoroquinolones of pathogen bacteria such as some strains of *E. coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* has been found [26–28] and it is currently considered a major problem in a clinical setting. Resistance is common and can occur via a range of mechanisms (target-site mutation, plasmide-mediated quinolone resistance genes, decrease in membrane permeability, efflux pumps) [13].

Antibiotics are chemicals designed to kill or inhibit the growth of bacteria; consequently, their presence in the environment may inhibit key environmental processes mediated by microorganisms like nutrient cycling and xenobiotic degradation [29]. Microbial communities respond promptly to environmental changes by showing resilience and resistance capabilities towards contaminants. The effects of antibiotics on natural microbial communities can be the disappearance and/or inhibition of sensitive populations, selection of populations resistant to its detrimental effects and development of populations able to degrade them like other contaminants.

The main aim of the present study was to assess the degradation processes (biodegradation and photodegradation) of the antibiotic ciprofloxacin (CIP) in microcosms filled with surface water sampled from the River Tiber. The sampling was performed in a river stretch subjected to a high anthropogenic pressure, downstream from the wastewater treatment plant of Southern Rome and very close to the river mouth. Two set of microcosms consisting of river water containing the natural microbial community and treated with 500 μg/L of CIP in absence or presence of UV-light were performed. Moreover, some microcosms were filled with river water previously sterilized and then treated with the antibiotic. The combined experimental set made it possible to evaluate if the antibiotic CIP could be photodegraded and/or biodegraded. The overall results confirmed not only the key role of the light in CIP disappearance but also demonstrated its biodegradation in natural river water.

## 2. Materials and methods

### 2.1. River water collection and characterization

Water samples were collected from the River Tiber at a site located in its final stretch and downstream from the Southern Rome WWTP. The sampling was performed in November 2015. Some parameters (pH, dissolved $O_2$, temperature) were analyzed on site and others were examined in the laboratory. The samples were collected manually by immersing 1 L sterile glass bottles approximately 10 cm below the water surface and were transported to the laboratory within 2 h in a refrigerated (4 °C) bag. Some subsamples were fixed or treated immediately for the initial chemical and microbiological analysis, other ones were used for the set-up of the microcosms. The ion content ($HCO_3^-$, $F^-$, $Cl^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$, expressed as mg/L) of the river water samples was determined by using an Ion Chromatograph (Dionex DX-120). Aliquots of water samples were acidified and then analyzed for dissolved organic carbon content (DOC mg/L) by high temperature catalytic oxidation (HTCO) using a Shimadzu TOC-5000A analyzer with a detection limit of 0.050 mg/L. Dissolved oxygen was measured with an Oxi 538 microprocessor oximeter and Cellox 325 probe.

Ciprofloxacin (CIP) was analyzed in river water samples using an extraction-purification procedure (solid phase extraction - SPE) followed by the analytical determination in HPLC with fluorescence detection (FLD), as described in detail in Section 2.4.

### 2.2. Reagents

CIP (99.0% purity) was purchased from Sigma-Aldrich (Steinheim, Germany). An individual stock solution (100 mg/L) was prepared by dissolving the adequate quantity of the standard powder in methanol and stored in the dark at −20 °C. The working standard solution was performed by diluting the stock solution with water and stored at 4 °C. In the method validation, we have checked the stock standard solutions during the time and it maintained its stability for a month when stored in freezer. The working standard solution was prepared ex-novo at each sampling time and the peak area was always checked by matching the chromatographic data with the calibration curve.

Ammonium acetate, methanol, acetonitrile of HPLC-grade and hydrochloric acid (37%) were obtained from VWR (Radnor, PA, USA). Water for chromatography was purified (18 MΩ/cm quality) through a Milli-Q system (Millipore, Bedford, MA, USA). Oasis HLB (Hydrophilic-Lipophilic-Balanced) reverse-phase extraction cartridges (200 mg) were purchased from Waters (Waters Corporation, Massachusetts, USA). Moreover, a PHM 240 Model pH-meter (Radiometer, Copenhagen, Denmark) with combined glass electrode was used for the pH adjustment of mobile phase of the samples.

### 2.3. Microcosm set-up

A first experimental set consisting of 50 destructive closed microcosms of 100 mL capacity each was set up. Each microcosm was filled under a sterile cabinet with 50 mL of the river water (natural) and aliquots from antibiotic working standard solutions were spiked in the microcosms to reach a final CIP concentration of about 500 μg/L.

In particular, some microcosms (22 replicates) were filled with the natural river water (Microbiologically Active Water, MAW) and the antibiotic. Other 22 replicates were not treated and filled only with river water (Controls), in order to compare the effects of the antibiotics on the natural microbial community. Finally, the pH and dissolved oxygen concentration were measured at each sampling time in other six treated

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