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(Fluoro)quinolones and quinolone resistance genes in the aquatic environment: a river catchment perspective

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Abstract

This study provides an insight into the prevalence of (fluoro)quinolones (FQs) and their specific quinolone *qnrS r*esistance gene in the aquatic environment from the Avon river catchment area receiving treated wastewater from 5 wastewater treatment plants (WWTPs), serving 1.5 million people and accounting for 75% of inhabitants living in the catchment area in the South West of England. FQs were analysed by stereoselective chiral chromatography and tandem mass spectrometry and their specific *qnrS* resistance gene was measured with digital PCR, which allowed for spatiotemporal evaluation of the prevalence of FQs and *qnrS* across the catchment. Ofloxacin, ciprofloxacin, nalidixic acid and norfloxacin were found to be ubiquitous with daily loads reaching a few hundred g/day in wastewater influent and tens of g/day in receiving waters. This was in contrast to other FQs analysed: flumequine, nadifloxacin, lomefloxacin, ulifloxacin, prulifloxacin, besifloxacin and moxifloxacin, which were hardly quantified. Enantiomeric profiling revealed that ofloxacin was enriched with the *S*-(-)-enantiomer, likely deriving from its prescription as the more potent enantiomerically pure levofloxacin, alongside racemic ofloxacin. While ofloxacin's enantiomeric fraction (EF) remained constant, high stereoselectivity was observed in the case of its metabolite ofloxacin-*N*-oxide. The removal efficiency of quinolones during wastewater treatment at 5 WWTPs utilising either trickling filters (TF) or activated sludge (AS), was compound and wastewater treatment process dependent, with AS providing better efficiency than TF. The *qnrS* resistance gene was ubiquitous in wastewater. Its removal was WWTP treatment process dependent with TF performing best and resulting in significant removal of the gene (from 28 to 75%). AS underperformed with only 9% removal in the case of activated sludge and actual increase in the gene copy number within sequencing batch reactors (SBRs). Interestingly, the data suggests that higher removal of antibiotics could be linked with high prevalence of the gene (SBR and WWTP E) and vice versa low removal of antibiotic is correlated with lower prevalence of the gene in wastewater effluent (TF, WWTP B and D). This is especially prominent in the case of ofloxacin and could indicate that AS might be facilitating antimicrobial resistance (AMR) prevalence to higher extent than TF. Wastewater-based epidemiology (WBE) was also applied to monitor any potential misuse (*e.g.* direct disposal) of FQs in the catchment. In most cases higher use of antibiotics with respect to official statistics (*i.e.* ciprofloxacin, ofloxacin) was observed, which suggests that FQs management practice require further attention.

Key words: fluoroquinolones, AMR, resistance genes, wastewater, environment

1. Introduction

Antimicrobial resistance (AMR) is considered to be one of the most significant threats worldwide. Defined as the ability of a population of microorganisms to neutralise the effect of an antimicrobial drug, AMR is a natural process that has been greatly accelerated by misuse of available antimicrobials. With limited innovation in drug discovery for new antibiotics, current strategies are directed to monitor the usage of antibiotics. A key factor that plays a fundamental role in AMR is microbial exposure to antibiotics (Rizzo et al. 2013). Indeed, such exposure at sub-lethal concentrations could lead to selective advantages for certain resistant strains, in particular those containing antibiotic resistant genes (ARGs), and enhance the possibilities of their survival, development and spread. A number of resistance mechanisms are acknowledged, such as mutation of existing DNA, DNA exchange by vertical transmission or by horizontal gene transfer that can occur by (i) transformation, (ii) transduction and (iii) exchange of conjugative plasmids between bacteria that are physically connected. The latter mechanism is the most common in nature (Grohmann, Muth, and Espinosa 2003).

Along with hospitals, well-known hotspots for the spread of AMR are wastewater treatment plants (WWTPs) (Rizzo et al. 2013). This results from the exposure of microbial communities living in the reservoir of the WWTP to sub-inhibitory concentrations of antibiotics contained in the sewage from households, pharmaceutical plants and hospitals. Therefore, the chances for a microorganism to gain such exposure and survive are likely to encourage horizontal gene transfer and the development of AMR. Some studies have reported evidence that these environmental hotspots coincide with an increased level of antibiotic resistance genes (ARGs), like in the case of waters receiving effluent from pharmaceutical plants. Others have found that biocides and metals are also fundamental AMR drivers (Singer et al. 2016).

Antibiotics are often chiral molecules and, in such cases, are frequently marketed as racemates (as 1:1 ratio of enantiomers in each enantiomeric pair) or as enantiomerically pure eutomers. Enantiomers of the same drug, despite having the same physicochemical properties, differ in the spatial arrangement of enantiomers, which results in diverse interactions not only at the molecular level, but also at the biological level, where differences in pharmacodynamic and pharmacokinetic responses could occur. The fact that enantiomers stereoselectively react with biological systems, that are chiral themselves (*e.g.* enzymes), is carefully monitored in pharmaceutical legislation and policy. Unfortunately, the awareness that such differing interactions could occur in the environment is limited. This is mainly due to lack of research and unavailability of analytical methods allowing for analysis at the enantiomeric level. Indeed, during its environmental life-cycle, a chiral antibiotic could alter its stereoisomeric composition during WWTP processes and in the environment. The impact of stereochemistry on the environmental fate and effects of several chiral contaminants is well demonstrated. Examples include illicit drugs, beta-blockers and antidepressants (Castrignano et al. 2017, Evans, Bagnall, and Kasprzyk-Hordern 2017, Kasprzyk-Hordern and Baker 2012). It has been proven that stereoselective transformation together with enantiomer-dependent ecotoxicity frequently occur in the environment (Rice et al. 2018). Moreover, the formation of enantiomers not believed to exist in nature, such as *1S,2R*-(+)-ephedrine, was also reported during wastewater treatment (Kasprzyk-Hordern and Baker 2012). Despite these findings, there is a gap in the knowledge of the environmental fate of chiral antibiotics. A pioneering study highlighted alterations in the enantiomeric composition of ofloxacin in receiving waters after the wastewater effluent discharge point, enriched with *S*-(-)-enantiomer, with respect to the initial racemic composition in the upstream waters (Castrignano et al. 2018). Hence, such an effect could influence activity and toxicity of the chiral antibiotic in the environment. As a result, tackling issues of stereoisomerism of chiral antibiotics in the urban water cycle and in the surrounding environment is of utmost relevance as it could also affect the interactions with microbes living in the WWTP and receiving waters with possible effects on AMR evolution and spread.

This paper aimed to:

(i) verify occurrence and (stereoselective) bio-physicochemical transformation of FQs during wastewater treatment and in receiving waters;
(ii) verify occurrence and fate of fluoroquinolone (FQN) resistance *qnrS* gene during wastewater treatment and in receiving water;
(iii) estimate public exposure to FQs and *qnrS* using wastewater-based epidemiology (WBE).

Quinolones were selected as the target compounds as they satisfy a number of criteria to first attempt the realisation of the above objectives: (i) they are extensively used globally in the treatment of a wide range of illness, including urinary tract, respiratory and gastrointestinal infections; (ii) previous studies have detected quinolones in urban wastewater with concentrations up to microgram per litre (Rizzo et al. 2013); (iii) due to their wide use, the quinolone resistance rate has increased since 1990s (Aldred, Kerns, and Osheroff 2014); (iv) the World Health Organization (WHO) has included them in the list of “Highest Priority Critically Important Antimicrobials“ for human medicine (Organization 2016); and (v) many quinolones exist as enantiomers.

2. Experimental

2.1.Chemicals and materials

The following quinolones were selected for the study: (a) chiral: (±)-ofloxacin, (±)-ofloxacin-*N*-oxide, (±)-desmethyl-ofloxacin, (±)-lomefloxacin, (±)-moxifloxacin, *S,S*-moxifloxacin-*N*-sulfate, *R*-(+)-besifloxacin, (±)-prulifloxacin, (±)-ulifloxacin, (±)-flumequine and (±)-nadifloxacin; (b) achiral: ciprofloxacin, desethylene-ciprofloxacin, norfloxacin and nalidixic acid. Their chemical structure, properties, chirality, marketing, use, metabolic and excretion patterns, and stereoselective metabolism are presented in Figure S1, Table S1-S2.

The elution order of the following analytes was determined as reported in Castrignanò *et al.* 2018 by using stereoisomerically pure standard solutions: *S*-(-)-ofloxacin, also known as levofloxacin, *R,R*-moxifloxacin, *S,S*-moxifloxacin and *S,S*-moxifloxacin-*N*-sulfate. The following deuterated and isotopic analogues of target analytes were used as isotopically-labelled internal standards: ciprofloxacin-D_8, (±)-ofloxacin-D_3, (±)-desmethyl-ofloxacin-D_8 and (±)-flumequine-$^{13}C_3$.

Standard stock solutions were prepared at 1 mg mL^{-1} in methanol for all the analytes, with exception of (±)-prulifloxacin, (±)-ulifloxacin, (±)-ofloxacin-D_3 and (±)-flumequine-$^{13}C_3$ that were dissolved in acetonitrile, and (±)-lomefloxacin, desethylene-ciprofloxacin, ciprofloxacin-D_8 and (±)-desmethyl-ofloxacin-D_8 that were dissolved in water. Mixed working solutions containing all analytes were prepared from stock solutions by dilution with mobile phase. They were used for the preparation of the aqueous standard calibration solutions and for spiking samples. Stock and working solutions of standards were stored at -20° C.

HPLC-grade methanol, acetonitrile, ammonium formate and formic acid (≥96%) were purchased from Sigma Aldrich (UK). Ultrapure water was obtained from a MilliQ system, UK. All glassware was deactivated in order to prevent the adsorption of polar compounds to the hydroxyl sites on the glass surface. The deactivation process consisted of rinsing cycles with 5% dimethyldichlorosilane in toluene once, with toluene twice and with methanol thrice.

2.2.The study area and sampling points

Wastewater influent and effluent were collected for 7 consecutive days running from Wednesday to Tuesday between June and October 2015 from five major WWTPs (Figure 1, Table 1, sites A-E) contributing to one river catchment in the South-West UK and covering an area of approximately

2,000 km^2 and the population of ~1.5 million (this constitutes >75% of the overall population in the catchment). All WWTPs use conventional sedimentation following secondary treatment (except for sequencing batch reactors (SBRs) that used decantation following settling *in-situ*). Respective wastewater and river water samples were collected on the same days. Selected WWTPs utilise different treatment technologies: activated sludge (AS) and trickling filters (TF). Influent wastewater samples were collected between screening and primary sedimentation. Digested sludge was also collected at WWTP B and E over three consecutive days. River water was collected from upstream and downstream of the effluent discharge point at varying distances depending on accessibility (Table 1). River water was not collected for Site E as the WWTP discharges directly to the estuary.

Influent wastewater was collected as volume proportional 24 h composites with average sub-sample collection frequencies of approximately 15 minutes using an ISCO 3700 autosampler. Sub-samples (80 mL) were cooled to 4˚C (samplers were packed with ice) during collection to limit biological activity and pooled after 24 h (Petrie *et al.*, 2017). This sampling mode should provide unbiased sampling error distributions and be ≤20 % for quinolones with ≥50 'pulses' (p, number of toilet flushes containing the micropollutant of interest) per day. Effluent wastewater samples were collected using time proportional approach due to the limited variation of this matrix over 15-minute intervals as discussed elsewhere (Petrie et al. 2016). River waters (8 L) were collected as grab samples. All samples were transported to the laboratory on ice for further processing. It is important to mention here that one-week monitoring study did not account for several variables including seasonality, including rainfall, sunlight, microbial activity, season dependent pharmaceutical prescription.

2.3. Sample preparation and analysis

2.3.1. Antibiotic analysis using chiral liquid chromatography coupled with tandem mass spectrometry

Once in the laboratory, wastewater samples were filtered through GF/F 0.7 μm glass fibre filter (Whatman, UK) and 50 mL of filtered wastewater was spiked with 50 μL of a mixture of isotopically-labelled internal standards at 1 mg L^{-1}. Analytes were extracted using SPE and Oasis HLB cartridges (60 mg, Waters, UK), previously conditioned with 3 mL of methanol and equilibrated with 3 mL of ultrapure water. 50 mL of spiked environmental samples were loaded on HLB cartridges that were then washed with 1 mL of ultrapure water. The elution was carried out with 4 mL of methanol into 5 mL silanised glass tubes. The extracts were transferred to the TurboVap evaporator (Caliper, UK) and completely evaporated to dryness under nitrogen flow (5-10 psi). Samples were reconstituted with 0.5 mL of 10 mM ammonium formate/methanol 1:99 v/v with 0.05% formic acid and filtered through 0.2 µm PTFE filters. The filtered samples were transferred to polypropylene plastic vials bonded pre-slit PTFE/Silicone septa (Waters, UK) and then 20 µL were directly injected into a chiral HPLC-MS/MS system. Samples were prepared and analysed in duplicate.

Wastewater suspended particulate matter (SPM) was filtered from the wastewater samples using GF/F 0.7 µm glass fibre filters. The SPM collected was frozen, before being freeze-dried, homogenised, weighed to 0.25 g and spiked with 50 µL of a mixture of isotopically-labelled internal standards at 1 mg L^{-1}. Microwave assisted extraction was carried out with 30 mL of 50:50 methanol: acidified ultrapure water (pH 2) at 110 °C for 30 minutes using 800 W MARS 6 microwave (CEM, UK). Samples were then filtered with GF/F 0.7 µm glass fibre filters and diluted with acidified ultrapure water (pH 2) to < 5% methanol. SPE was then carried out on the filtrate using Oasis MCX (60mg, Waters, UK). The cartridges were conditioned with 2 mL methanol and equilibrated with 2 mL acidified ultrapure water (pH 2). The entire filtrate (300 mL) was loaded onto the cartridge and then cartridge then dried under vacuum. The samples were then eluted in two fractions. An acidic fraction with 2 mL acidified methanol (2 mL 0.6% formic acid in methanol) and a basic fraction with 3 mL 7% ammonium hydroxide in methanol. These were evaporated to dryness with TurboVap evaporator at 40°C under nitrogen and then reconstituted with 0.5 mL 80:20 ultrapure water:methanol and filtered 0.2 µm PTFE filters. The filtered samples were transferred to polypropylene plastic vials bonded pre-slit PTFE/Silicone septa and then 20 µL were directly injected into a chiral HPLC-MS/MS system. Samples were prepared and analysed in duplicate. This was the same procedure used in the preparation of digested solids. Quantity of SPM per litre of wastewater was carried out by filtering 100 mL of wastewater through a pre-weighed GF/F 0.7 µm glass fibre filter, this was then dried and reweighed.

Samples were analysed using a Waters ACQUITY UPLC® system (Waters, Manchester, UK). Chromatographic separation of all the analytes was carried out using a chiral CHIRALCEL® OZ-RH column (5 µm particle size, L × I.D. 15 cm × 2.1 mm, Chiral Technologies, France) with a 2.0 mm ×

2.0 mm guard filter (Chiral Technologies, France). The column temperature was set at 30°C. The autosampler was kept at 4°C. A mobile phase consisting of 10 mM ammonium formate/methanol 1:99 v/v with 0.05% formic acid was used at a flow rate of 0.1 mL min^{-1} under isocratic conditions.

The MS system was a triple quadrupole mass spectrometer (Xevo TQD, Waters, Manchester, UK) equipped with an electrospray ionisation source. Analyses were performed in positive mode with an optimised capillary voltage of 3 kV, source temperature of 350°C, desolvation temperature of 350°C and desolvation gas flow of 650 l h^{-1}. Nitrogen, supplied by a high purity nitrogen generator (Peak Scientific, UK), was used as a nebulising and desolvation gas. Argon (99.999%) was used as a collision gas. MassLynx 4.1 (Waters, UK) was used to control the Waters ACQUITY system and the Xevo TQD. Data processing was carried out on TargetLynx software (Waters, Manchester, UK).

The mass spectrometer acquired data using MRM mode. Two MRM transitions were selected for each compound. The most abundant transition product ion was typically used for quantification, whilst the second ion was used for confirmation purposes. The MRM transitions, CV and CE values of the studied compounds are presented in Table S3. The method was fully validated as described elsewhere (Castrignano et al. 2018) and (Proctor et al. 2019) (Figure S2, Table S4-S7).

2.3.2. *qnrS* gene quantification using dPCR

2.3.2.1. DNA extraction and quantification

1 mL of unfiltered wastewater samples were centrifuged in sterilised micro-centrifuge tubes for 5 minutes at 3000 g. The supernatant was discarded and the remaining cell pellet was re-suspended in 200 µL phosphate buffered saline (PBS). 5 µL lysozyme were then added, followed by an incubation at 37 °C for 15 minutes. 200 µL of binding buffer and 40 µL proteinase K were added and incubated at 70 °C for 10 minutes. DNA extraction was performed in accordance with manufacturer’s instructions (High Pure PCR Template Preparation Kit, Roche, Germany). Briefly, 100 µL of isopropanol alcohol was added. The samples were then transferred to a filter tube assembled inside a collecting tube and centrifuged for a minute at 8000 g. The supernatant was discarded and the filter tube assembled in a new collecting tube. 500 µL of inhibitor buffer and 500 µL of washing buffer were respectively added after cycles of centrifugation at 8000 g. The supernatant was finally discarded before centrifugation for 10 min at 9000 g. The filter tube was then assembled into a

sterilised micro-centrifuge tube. 200 µL of elution buffer pre-warmed to 60 °C were used. Samples were centrifuged at 8000 g for a minute. The resulting DNA samples in the micro-centrifuge tubes were stored at -20 °C. To determine the success of the DNA extraction method, DNA was quantified by using a Thermofisher Nanodrop instrument, that was first calibrated and blanked using pure water.

2.3.2.2. *qnrS* gene quantification using dPCR

A QuantStudio 3D Digital PCR system was used with a QuantStudio 3D PCR V2 kit (Life Technologies, Thermo Fisher Scientific). PCR reaction mixtures were prepared with 7.3 µL Master Mix V2, 0.7 µL *qnrS* TaqMan Assay (20 X primer/ probe mix), 1.5 µL nuclease free water and 6.0 µl DNA sample. 14.5 µL of this mixture were loaded onto the digital PCR load blades and distributed in high density nanofluidic PCR chips that were loaded onto a GeneAmp PCR 9700 system.

The program was run using thermal cycling conditions. Temperature was first ramped to 95 °C and held for 10 min. It was then lowered to 60 °C for 2 min before increasing to 98 °C for 30 seconds. This cycle between 60 °C and 98 °C was repeated 40 times to allow for efficient gene amplification. The system was then lowered being to 60 °C and held for 2 min, before cooling to room temperature. After cooling, each chip was processed using the QuantStudio 3D Digital PCR system. AnalysisSuite™ software was used to get quantification of the targeted gene and statistical analysis of the results.

2.4.Calculations

In order to obtain daily mass loads, the concentrations of analytes expressed in ng L^{-1} (Tables S8-S12) were multiplied by the flow rate (L day^{-1}) and then normalised by the population size of the catchment area. FQs' and *qnrS* gene removal during wastewater treatment, expressed as a percentage, was calculated by considering hydraulic retention time and the difference between the influent load and the effluent load in relation to the influent load.

Results from digital PCR analysis were given as copies μL^{-1} (Table S13). In order to obtain daily copy loads, *qnrS* copies expressed in copies day^{-1} were multiplied by the flow rate (L day^{-1}) and then normalised by the population size of the catchment area.

Concentrations of the analytes in SPM from influent wastewater during the monitoring week are gathered in (Tables S14-S18)

3. Results and Discussion

3.1. FQs during wastewater treatment: activated sludge vs trickling filters technology

3.1.1. Occurrence of FQs in wastewater

Ofloxacin and its metabolites. In the investigated catchment area, the highest loads among chiral quinolones were found for ofloxacin in influent wastewater from WWTP E. Indeed, the total average load was found at 53±14 g day^{-1}, ten times higher than in other WWTPs (Figure 2). To assess whether stereoselective enrichment or depletion of the enantiomeric composition of the drug occurred, the enantiomeric fraction (EF) was used as a dimensionless indicator of (i) the equal amount of two enantiomers in the case of EF = 0.5 or (ii) the predominance of one enantiomer with respect to the other in the case of EF <0.5 (predominance of *S*-(-)-ofloxacin) or >0.5 (predominance of *R*-(+)-enantiomer) (Figure 2). $EF_{ofloxacin}$ was found with an average value of 0.13±0.07 in influent samples from WWTP A, 0.26±0.03 in WWTP E, 0.28±0.05 in WWTP C, 0.36±0.02 in WWTP D and 0.40±0.03 in WWTP B. This signifies that a high proportion of ofloxacin was present as the *S*-(-)-enantiomer, likely deriving from the prescription of enantiomerically pure isomer, levofloxacin, that is more potent than *R*-(+)-ofloxacin (Al-Omar 2009). Average effluent loads were found to be considerably lower than influent in the majority of the sites with expected high levels observed at 21±4 g day^{-1} for WWTP E. No significant change in EF was observed across all WWTPs ($EF_{WWTP\ A,\ E}$ unvaried, $EF_{WWTP\ B}$ 0.32±0.03, $EF_{WWTP\ C}$ 0.32±0.04 and $EF_{WWTP\ D}$ 0.34±0.01), signifying that the wastewater treatment process had no impact in altering the enantiomeric composition.

The presence of ofloxacin metabolites had a more scattered profile in the studied WWTPs (Figures 3, 5). Ofloxacin-*N*-oxide was present at an average load of 0.2 and 3.8 g day^{-1} in influent wastewater from WWTP A and E, most likely due to human metabolism (Figure 4). This was confirmed by WBE estimated ofloxacin-*N*-oxide load (0.2 – 4.0 g day^{-1}) based on measured daily loads of ofloxacin in wastewater (Table 2). In effluent, ofloxacin-*N*-oxide was found at quantifiable average load of 0.9 g day^{-1} only in WWTP E (Figure 4). In WWTP B, C and D ofloxacin-*N*-oxide was present in both matrices but still below the method limit of quantification (MQL), whilst it was not detected in effluent samples from WWTP A. The enantiomeric composition favoured the *S*-(-)-enantiomer in influent wastewater at WWTP A (EF=0) and WWTP E (EF=0.22). It is important to highlight that $EF_{ofloxacin\text{-}N\text{-}oxide}$ from effluent WWTP E was 0.45±0.03, showing that the enantiomeric composition was likely altered during the wastewater treatment process. *S*-(-)-Desmethyl-ofloxacin was detected but was not quantifiable in all influent and effluent samples from WWTP A. *R*-(+)-desmethyl-ofloxacin was at <MQL in a couple of influent and effluent samples (Figure 3). In WWTP B desmethyl-ofloxacin was found at 0.33 g day^{-1} only in one influent sample with a slight predominance of the *S*-(-)-enantiomer. In WWTP C, *S*-(-)-desmethyl-ofloxacin reached the average load of 0.7 g day^{-1} in influent and 0.4 g day^{-1} in effluent, whilst *R*-(+)-desmethyl-ofloxacin was <MQL. In WWTP D, *S*-(-)-desmethyl-ofloxacin reached the average load of 0.1 g day^{-1} in influent and 0.05 g day^{-1} in effluent, whilst *R*-(+)-desmethyl-ofloxacin was found at 0.03 g day^{-1} in influent and in the same amount from one day in effluent wastewater. In WWTP E, desmethyl-ofloxacin was not detected in any analysed samples.

Interestingly, the analysis of the SPM from all the sites indicated ofloxacin's partitioning to solids with higher levels recorded for the *S*-(-)-enantiomer (Figure 5). Ofloxacin's metabolites were not detected in SPM due to their high polarity.

Ciprofloxacin and its metabolites. Ciprofloxacin, a non-chiral fluoroquinolone, was detected in all collected samples (Figure 2). Its metabolite, desethylene-ciprofloxacin, was also present in most analysed samples. The average influent concentration of ciprofloxacin was 427±86 ng L^{-1} that corresponded to a load of 65±8 g day^{-1}. There was a significant decrease in load from influent to effluent (i.e. average effluent load was 22±3 g day^{-1}) (Figure 2). Wastewater influent derived SPM average daily loads were much lower and spanned from 0.01 (WWTP B) to 0.3 (WWTP D) g day^{-1} (Figure 5).

Nalidixic acid. The highest loads of nalidixic acid were recorded in WWTP E (4.9±1.3 g day^{-1}) followed by WWTP C (0.8±0.0 g day^{-1}) and then WWTP B, A, D with loads 0.09, 0.07 and 0.01 g day^{-1} respectively. The removal efficiency of nalidixic acid was site dependent and spanned between 38 to 82%. Due to varying removal of nalidixic acid from wastewater, effluent loads varied from 1.8±0.5 g day^{-1} at WWTP D, 2.4±0.5 g day-1 at WWTPC and 3.1±0.2 g day^{-1} at WWTP A and reached the highest levels at WWTP E 12.6±5.1 g day^{-1} (Figure. 2). Nalidixic acid was quantified in influent wastewater SPM from WWTP A and E only and spanned from 0.001 to 0.1 g day^{-1} in WWTP A and E respectively (Figure 5).

Norfloxacin. Norfloxacin was quantified in wastewater influent at three sites only: WWTP D, B and E with average daily loads spanning from 0.6±0.4 g day^{-1} at WWTP D, through 1.4±0.4 g day^{-1} at WWTP B to 98.4±60 g day^{-1} at WWTP E. WWTP removal was in the range 19% (WWTP E) to 75% (WWTP C), which lead to daily loads in effluent denoting 0.4±0.1 g day^{-1} at WWTP D, 0.9±0.3 g day^{-1} at WWTP B to 29±5.3 g day^{-1} at WWTP E (Figure 2). Norfloxacin was quantified in influent wastewater SPM from WWTP B only with daily loads at 0.11±0.05 g day^{-1} (Figure 5).

Flumequine. (±)-Flumequine was found in all sites, interestingly, with a different enantiomeric signature for each site. In WWTP A and B, the E1-enantiomer was at <MQL in influent (liquid phase), but quantifiable in effluent samples with a load average of 0.1 g day^{-1} for both sites. E2-enantiomer was barely detected in both matrices. In WWTP C E1-enantiomer was detected in influent and quantified more frequently than E2 with a load average of 0.3 g day^{-1}. Both enantiomers were at <MQL in all effluent samples. In WWTP D, E1-enantiomer was quantified in all influent and effluent wastewater samples with an average load of 0.03 g day^{-1}, while E2 was mostly <MQL. Hence, EF could not be calculated. In WWTP E, E1-enantiomer was quantified only in two influent samples, and it was present at an average load of 1.1 g day $^{-1}$ in effluent samples, while E2 was not found in any of the two matrices (Tabs S10-14).

Interestingly, flumequine was the most prevalent FQN in SPM derived from influent wastewater. This is likely due to its relatively high hydrophobicity (Table S2). Flumequine was quantified at average daily loads of 7 g day^{-1}. Average EF across four WWTPs denoted 0.8 indicating a significant enrichment of flumequine with E1 enantiomer, likely due to stereoselective human metabolism as flumequine is marketed as a racemate. (Figure 5).

Other FQs. *S,S*-Moxifloxacin and moxifloxacin-*N*-sulfate were detected only in WWTP D in a few samples from influent and effluent. (±)-Nadifloxacin was found at <MQL only in influent samples from WWTP B. In WWTP C, the first-eluting enantiomer was detected more frequently than the second one in influent samples, whilst it was more consistently detected in effluent samples. An

analogue profile was seen in WWTP E. (±)-Lomefloxacin was only found in WWTP C at quantifiable amounts in three effluent samples, whilst it was not detected in influent samples (Tables S10-14). (±)-Prulifloxacin, (±)-ulifloxacin and *R*-(+)-besifloxacin have not been detected in the catchment area investigated.

3.1.2. Fate of FQs during wastewater treatment

The following WWTP treatment technologies are used in studied WWTPs: TF (WWTP B, C and D) or AS (WWTP A), and SBR (WWTP E) (Table 1). The sewer residence time along with features of the treatment process, such as solid retention time and hydraulic retention time, are also included in Table 1. Hydraulic retention time varied from 11h (WWTP E) to 46h (WWTP A) and solid retention time was from 4h (WWTP E) to 19h (WWTP A). These two parameters are widely regarded as being of primary importance for the FQs removal (Batt, Kim, and Aga 2007, Gao et al. 2012, Li et al. 2013). Figure 6 summarises the removal efficiency of target quinolones in this study. The sorption on AS is the main mechanism involved in the removal process of FQs from wastewater (Conkle et al. 2010, Golet et al. 2003, Jia et al. 2012) followed by biodegradation, whose roles in the prevalence and dissemination of AMR are not yet fully understood (Van Doorslaer et al. 2014). Sorption is more highly influenced and driven by electrostatic interactions rather than hydrophobic partitioning (Golet et al. 2003, Lindberg et al. 2006, Tolls and technology 2001). Due to the amphoterism of FQs such as (±)-ofloxacin, norfloxacin and (±)-moxifloxacin, partitioning is also pH-dependent (Langlois et al. 2005, Takács-Novák et al. 1992, Van Doorslaer et al. 2014, Kümmerer 2008) and influenced by the salinity of the water phase (Van Doorslaer *et al.* 2014). The results of this study indicate that the removal efficiency of FQs is compound and wastewater treatment process dependent (Figure 6). Ciprofloxacin showed the highest removal efficiency during trickling filters treatment (38-73% in WWTPs B-D vs 15-64% in WWTPs A and E), while ofloxacin showed the highest removal during AS treatment treatment (22-62% in WWTPs B-D vs 57-75% in WWTPs A and E). Nalidixic acid and norfloxacin showed better removal during AS than TF treatment. No clear stereoselectivity was observed in the case of chiral ofloxacin.

3.1.3. Occurrence of *qnrS* gene during wastewater treatment

As highlighted by Van Doorslaer *et al.* (2014), FQs can induce the AMR phenomenon in microbial communities present in the environment, as these drugs are excreted unchanged (up to 70%) with only a small proportion being metabolised. Three mechanisms for the development of resistance have been described in literature. These are caused by mutations leading to (i) target-site alterations and (ii) decreased drug accumulations due to a change in the membrane permeability, and (iii) by horizontal gene transfer carrying *qnr* gene, like in the case of plasmids, that are mobile quinolone resistance elements (Ruiz 2003, Jacoby 2005). The latter mechanism of resistance to FQs results from the binding between the *qnr* protein and the target topoisomerase, that avoids the action of the antibiotic on the targeted enzyme (Redgrave et al. 2014). Examples of plasmid mediated quinolone resistance genes are from (i) the families of *qnr* genes (*qnrB*, *qnrS, etc*.), (ii) a variant of an aminoglycoside acetyl transferase, *aac(6)-Ib-cr* and (iii) the efflux systems that can remove the drug through the usage of transporters (i.e. oqxAB and qepA) (Redgrave et al. 2014). In this study, the targeted resistance gene was *qnrS* because of (i) its reduced susceptibility to fluoroquinolones as stated elsewhere (Rodriguez-Mozaz et al. 2015) (Marti, Variatza, and Balcázar 2014) and (ii) its prevalence in environmental matrices as reported in previous studies (Castrignano et al. 2018, Marti et al. 2016).

In this study, in order to verify whether the level of resistance gene detected in the different areas of the catchment corresponds with estimated quinolone loads, the *qnrS* gene extracted from the DNA contained in the wastewater samples was quantified through the use of digital PCR (Table S13). Figure 7 shows the concentration of the gene *qnrS* in influent and effluent wastewater collected at sites A-E during one monitoring week. The results confirm previous published findings indicating that resistant genes are present in wastewater (Marti et al. 2016). Overall, a higher absolute copy number of *qnrS* gene was observed in this study with respect to findings in Rodriguez-Mozaz *et al.* (Rodriguez-Mozaz et al. 2015). Interestingly, the fate of the *qnrS* gene was different in different WWTPs, with TFs (WWTP B, C and D) performing best and resulting in significant removal of the gene (from 28 to 75%). AS and SBRs underperformed with only 9% removal of *qnrS* gene in the case of AS and actual increase of the number of copies of the gene during SBR.

Interestingly, the data suggests that higher removal of antibiotic is linked with low removal of the gene (SBR and WWTP E) and *vice versa*, low removal of antibiotic is correlated with lower prevalence of the gene in wastewater effluent (TF, WWTP B and D). This is especially prominent in the case of ofloxacin and could indicate that AS might be facilitating AMR prevalence to higher extent than TF.

3.2.The catchment perspective

The potential contamination of receiving waters by antibiotics and ARGs is highly influenced by a number of variables dependent on (i) the nature and the physico-chemical properties of the compounds, (ii) the environmental conditions such as the temperature and the effect of sunlight and (iii) the loads of pharmaceuticals, and therefore their dilution due to rainfall, discharge by WWTPs and the technology of treatment used for their removal (Baker and Kasprzyk-Hordern 2013). In this study a clear trend of increasing antibiotic concentration levels (and corresponding antibiotic loads) was observed with an increase of treated communal wastewater discharge, especially for samples collected downstream from wastewater discharge points (Figure 8). Interestingly, the *qnrS* gene was not quantifiable in receiving water samples with the method used. There are several possible reasons including analytical constraints as well dilution of wastewater effluent with receiving waters.

As discussed in section 3.1, *qnrS* gene concentration levels remained fairly constant in all WWTPs. Quinolone concentrations varied across WWTPs (i.e. the highest total concentration levels were observed at WWTP E and the lowest in WWTP B (Figure 7)) but no clear pattern was observed when comparing antibiotic and gene concentration levels. However, normalisation of data to account for water flows revealed a strong correlation between daily loads of antibiotics present in each WWTP and corresponding loads of resistance genes. The highest loads of both FQs and ARG were observed in wastewater influent from site E followed by C, A, B, and D. Interestingly, this coincides with the size of a population served by individual WWTPs. It is therefore evident that the higher the population, the larger the load of both FQs and ARGs. The efficiency of wastewater treatment is another key variable influencing environmental FQs and ARG loads. Our study has shown that TF although are not as effective in the removal of FQs, they do remove ARGs. In contrast, AS process (also in SBR configuration) effectively removes FQs but also contributes to higher levels of ARGs.

3.3.Wastewater based epidemiology

WBE was applied to estimate usage of antibiotics across the catchment. Antibiotic usage data obtained via wastewater analysis were then analysed against prescription data to highlight any misuse of quinolones. We have also applied this approach in a European study (Castrignanò et al. 2020) where spatiotemporal changes in quinolone usage across different European cities were observed.

This study covers 75% of the population (~1.5 million people) residing in five urban areas (cities A-E) served by the selected five major WWTPs which allows for comprehensive understanding of the quinolones usage in the study area (Table 2). Where possible, metabolites were considered as biomarkers of antibiotic consumption.

Ofloxacin. Indeed, (±)-ofloxacin is mostly excreted unchanged in urine (90%), but it also undergoes metabolism in humans to form (±)-ofloxacin-*N*-oxide and (±)-desmethyl-ofloxacin. Therefore, these two metabolites were selected, alongside (±)-ofloxacin, as biomarkers. This is despite their low excretion rate, i.e. 1-5% as ofloxacin-*N*-oxide and 3-6% as desmethyl-ofloxacin. Ofloxacin is a chiral fluoroquinolone in which the *S*-(-)-enantiomer is significantly more potent as an antibiotic. In 2015, 212 kg of (±)-ofloxacin and 120 kg of *S*-(-)-ofloxacin were prescribed in England according to PCA data (http://www.nhsbsa.nhs.uk/PrescriptionServices/3494.aspx). Taking into account the urinary excretion, the annual excreted amounts of *R-(+)*-ofloxacin and *S-(-)*-ofloxacin were calculated as 87.5 kg and 186.5 kg, respectively. In particular, the latter calculation considered the excreted contribution from the racemate formulation (i.e. 87.5 kg) and the one from the pure *S-(-)*-drug (i.e. 99 kg). Hence, the consumption estimates from PCA data were 4 mg day^{-1} 1000 people^{-1} as *R*-(+)-ofloxacin and 8 mg day^{-1} 1000 people^{-1} as *S*-(-)-ofloxacin. The estimates from wastewater analysis were fully in agreement with the NHS data only in city D served by WWTP D (5 mg day^{-1} 1000 people^{-1} as *R-(+)*-ofloxacin and 8 mg day^{-1} 1000 people^{-1} as *S*-(-)-ofloxacin). Estimates were lower in the case of city B (WWTP B) with 2 mg day^{-1} 1000 people^{-1} as *R-(+)*-ofloxacin and 3 mg day^{-1} 1000 people^{-1} as *S*-(-)-ofloxacin, whilst they were much higher in city E (WWTP E) (18 mg day^{-1} 1000 people^{-1} as *R-(+)*-ofloxacin and 51 mg day^{-1} 1000 people^{-1} as *S*-(-)-ofloxacin). In the cities served by WWTPs A and C the estimates showed that *S*-(-)-ofloxacin usage was much higher (i.e. 42 and 24 mg day^{-1} 1000 people^{-1}, respectively) than the R-(+)-ofloxacin (i.e. 6 and 10 mg day^{-1} 1000 people^{-1} as *R-(+)*-ofloxacin). The analysis of the data indicates that the ratio of the two enantiomers (*R*:*S*) is 1:2 in most sites investigated, which indicates similar prescription habits. Only one site, city C (WWTP C), revealed the dominance of the *S*-(-)-enantiomer, which demonstrates that the enantiopure formulation (levofloxacin) was used and it was seven times higher when compared to other sites. With regard to the metabolic pattern, 2 and 4 kg were respectively excreted as *R*-(+)- and *S*-(-)-form of the metabolites. The official national estimates were in agreement with estimates from ofloxacin-*N*-oxide as drug target residue (DTR) for the site served by WWTP B and slightly lower for WWTPs C and D. The estimates were higher for WWTP E (i.e. 43 mg day^{-1} 1000 people^{-1} as *R-(+)*-ofloxacin and 177 mg day^{-1} 1000 people^{-1} as *S*-(-)-ofloxacin) and WWTP A (91 mg day^{-1} 1000 people^{-1} as *S*-(-)-ofloxacin). The estimates from desmethyl-ofloxacin used as DTR were above in four sites over five (with exception for WWTP E, in which there was no detection of the metabolite). It could be concluded from the metabolic profiling data, that levofloxacin was highly consumed with respect to national prescription data and that the estimation of ofloxacin usage with WBE benefits from the metabolite estimates when they are both used as ofloxacin DTRs.

Ciprofloxacin. As previously mentioned, ciprofloxacin was found at the highest loads in wastewater. The biomarkers used were ciprofloxacin itself and its metabolite desethylene-ciprofloxacin. In 2015, 5782 kg of ciprofloxacin were prescribed in England according to PCA (http://www.nhsbsa.nhs.uk/PrescriptionServices/3494.aspx). As a result of human metabolism, ciprofloxacin is excreted as unchanged (40-50%) and as desethylene-ciprofloxacin (2-3%). Therefore, 2602 kg of ciprofloxacin and 116 kg of desethylene-ciprofloxacin were calculated as excreted quantities. Hence, ciprofloxacin consumption was estimated at 115 mg day^{-1} 1000 people^{-1}. The estimates calculated from wastewater analysis were below this estimate only in city B served by WWTP B (77 mg day^{-1} 1000 people^{-1}) and were much higher in cities D and E: 160 mg day^{-1} 1000 people^{-1} in city E (WWTP E), 256 mg day^{-1} 1000 people^{-1} in city D (WWTP D), using ciprofloxacin as DTR. By using desethylene-ciprofloxacin, data were in agreement with consumption of ciprofloxacin (Table S19). Therefore, as estimates from wastewater data were higher than those from official prescription sources, veterinary usage needed to be accounted for as another source of ciprofloxacin. Indeed, enrofloxacin, a veterinary synthetic fluoroquinolone, is metabolised to ciprofloxacin and therefore it could considerably enhance ciprofloxacin levels in the environment.

Ciprofloxacin in conjunction with desethylene-ciprofloxacin were therefore considered suitable for biomarkers of ciprofloxacin use.

Other FQs. WBE highlighted spatial differences in (i) norfloxacin and (ii) moxifloxacin uses in the same catchment area.

Norfloxacin is a FQ that was selected as a biomarker for its usage. 25-40% of its dose is excreted in urine and 5-10% as several metabolites within 24-48 hours, whilst 30% is excreted in faeces within 48 hours. 1.1 kg of norfloxacin were prescribed in England in 2015 and national consumption was estimated in the range of 0.1 mg day^{-1} 1000 people^{-1}. The estimates from wastewater analysis were much higher in cities B, D and E and denoted: 61, 101 and 172 mg day^{-1} 1000 people^{-1} respectively, whilst in cities A, C norfloxacin was not detected at all.

The biomarkers chosen for moxifloxacin were the parent compound (*S,S*-moxifloxacin) and its sulfate metabolite. *R,R*-moxifloxacin is not prescribed and was monitored in order to ensure that no chiral inversion occurs in the environment, therefore it was not used for WBE calculations. From PCA, 39.6 kg were prescribed and the relative consumption was estimated at 3 mg day^{-1} 1000 people^{-1}, by considering excretion of 20% and 25% as unchanged in urine and in faeces respectively and 35% as sulfate in faeces. Similar estimates from wastewater analysis were 16 and 17 mg day^{-1} 1000 people^{-1} in two sites only corresponding to WWTPs D and E, respectively.

An overall agreement of estimates between official PCA data and wastewater analysis was observed in the case of nalidixic acid consumption. The parent compound was used as DTR. This choice was also supported by the hypothesis that in wastewater faecal bacteria might hydrolyse the glucuronide conjugates highly formed during the metabolism and thus release the nalidixic acid. Its metabolism produces also glucuronides of 7-hydroxynalidixic acid (2-3% as unchanged), but they were not taken into account in this study. National official consumption estimates for nalidixic acid were 0.3 mg day^{-1} 1000 people^{-1}. In the majority of the sites similar estimates were calculated (average load of 3 mg day^{-1} 1000 people^{-1}) with exception for the site served by WWTP E (average load of 14 mg day^{-1} 1000 people^{-1}).

The prodrug (±)-prulifloxacin was not prescribed according to PCA data and, as confirmed by wastewater analysis, neither (±)-prulifloxacin nor its active compound ulifloxacin were detected in the wastewater samples. (±)-Lomefloxacin was also not prescribed and data from wastewater analysis were overall in agreement in all the sites (in WWTP B it was detected <MQL only in one day, therefore estimates were not considered).

4. Conclusions

This study focussed on understanding stereoselective spatiotemporal speciation of FQs and the corresponding quinolone *qnrS* resistance gene in a river catchment in SW England. The conclusions are as follows:

1. Ofloxacin, ciprofloxacin, nalidixic acid and norfloxacin were ubiquitous in the studied catchment with daily loads in the river reaching tens of g day^{-1} in receiving water and a few hundred g day^{-1} in wastewater influent. This is in contrast to other FQs studied, which were undetected: flumequine, nadifloxacin, lomefloxacin, ulifloxacin, prulifloxacin, besifloxacin and moxifloxacin.
2. Ofloxacin was present in the catchment as the *S*-(-)-enantiomer, likely deriving from the prescription of enantiomerically pure and much more potent levofloxacin, alongside racemic ofloxacin. While $EF_{ofloxacin}$ remained constant, high stereoselectivity was observed for its metabolite ofloxacin-*N*-oxide.
3. The removal efficiency of quinolones during wastewater treatment is compound and wastewater treatment process dependent. Ciprofloxacin showed the highest removal efficiency during TF treatment, while ofloxacin showed the highest removal during AS treatment. No clear stereoselectivity was observed.
4. The fluoroquinolone *qnrS* resistance gene was ubiquitous in wastewater. Its removal was WWTP treatment process dependent with TF performing best and resulting in significant removal of the gene (from 28 to 75%). Activated sludge and SBRs underperformed with only 9% removal in the case of activated sludge and actual enrichment of the gene during SBR. Interestingly, the data suggests that higher removal of antibiotic is linked with low removal of the gene (SBR and WWTP E) and *vice versa*, low removal of antibiotic is correlated with

lower prevalence of the gene in wastewater effluent (TF, WWTP B and D). This is especially prominent in the case of ofloxacin and could indicate that AS might be facilitating AMR prevalence to higher extent than TF.

5. Exceeding the prescribed use of quinolones is also considered as an AMR driver for enhancing quinolone resistance. For this reason, an eventual misuse of such class of antibiotics was evaluated by applying WBE to wastewater analysis data *versus* official prescription data. Higher use of *S*-(-)-ofloxacin was confirmed by the predominance of the *S*-(-)-enantiomer of ofloxacin's metabolites in wastewater. Hence, these quantities in the environment were interpreted as resulting from consumption (and not as direct disposal). Ciprofloxacin that was found with the highest load among quinolones was present in higher amounts with respect to official statistics. Despite the usage of its metabolite as DTR for ciprofloxacin consumption, the contribution from veterinary usage needed to be included for accounting for another ciprofloxacin source.

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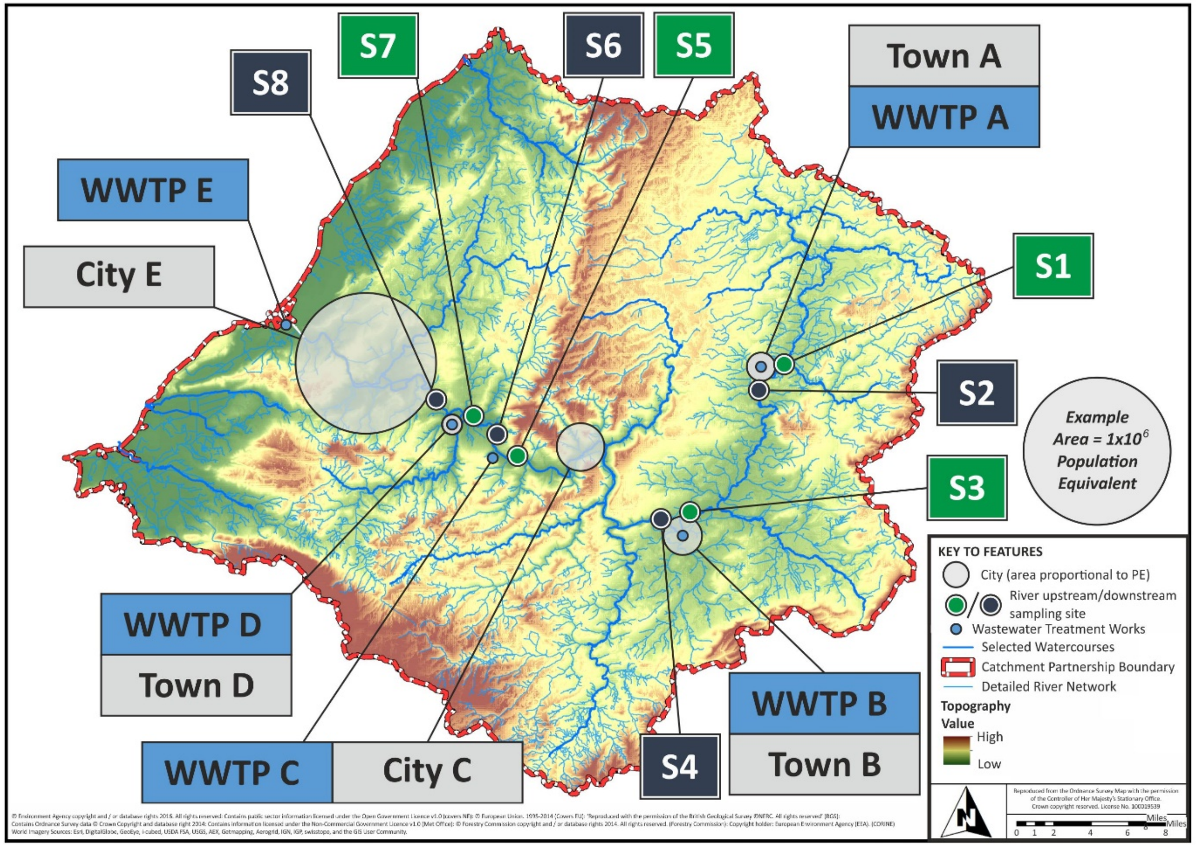
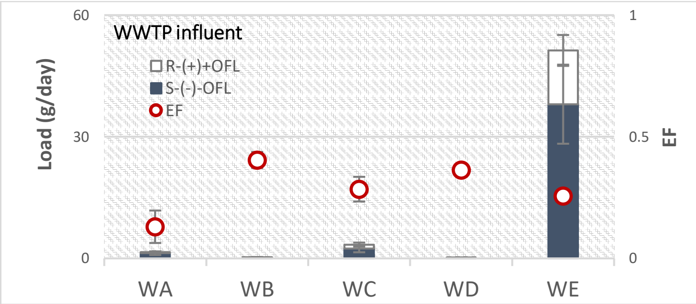
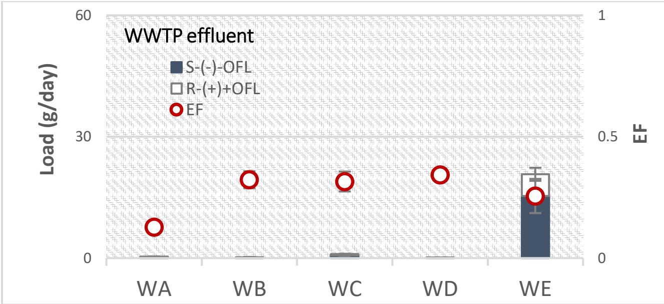


Figure 1 Site information of studied WWTPs and corresponding river locations (*S* refers to the sampling site, R1-8 in the manuscript), WWTP means wastewater treatment plant, W1-8 in the manuscript).

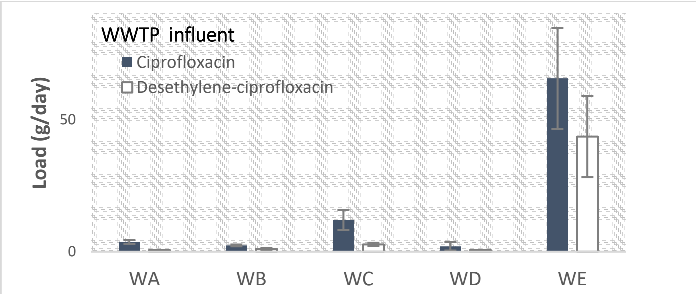
WWTP INFLUENT (liquid phase)) **WWTP EFFLUENT (liquid phase)**

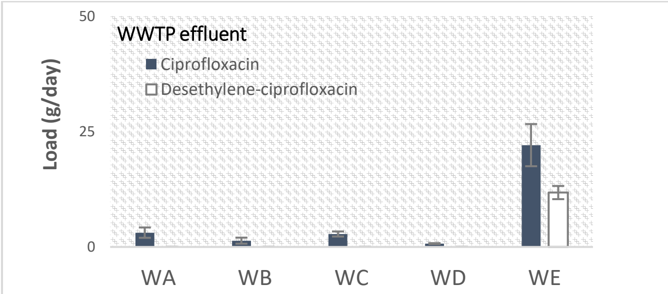
Ofloxacin



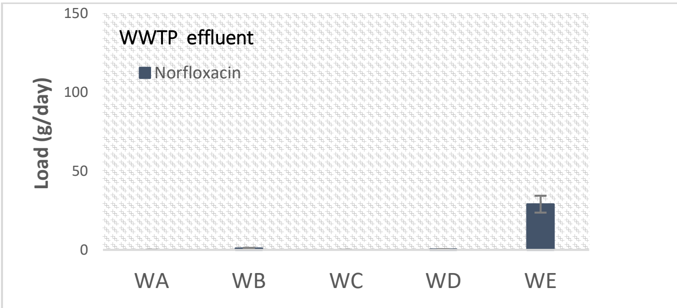


Ciprofloxacin





Norfloxacin



Nalidixic acid

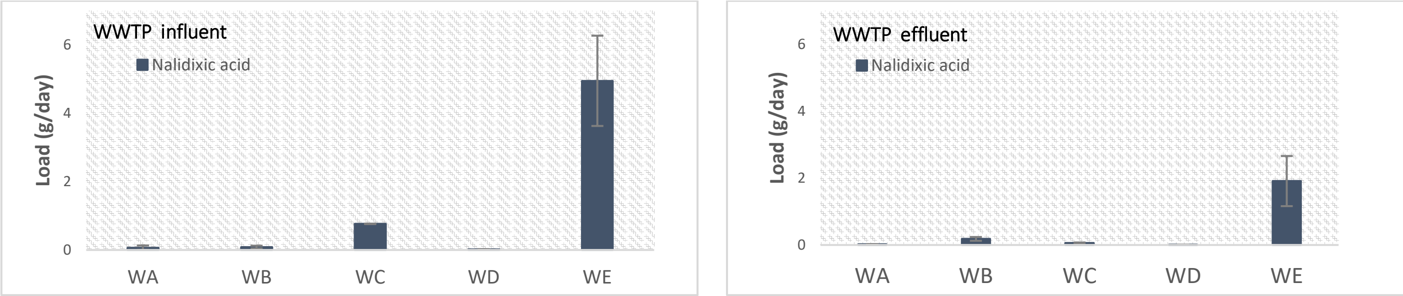


Figure 2 Average daily loads of ofloxacin, ciprofloxacin, norfloxacin and nalidixic acid in wastewater (liquid phase) in the investigated catchment area (sites A-E) during the monitoring week

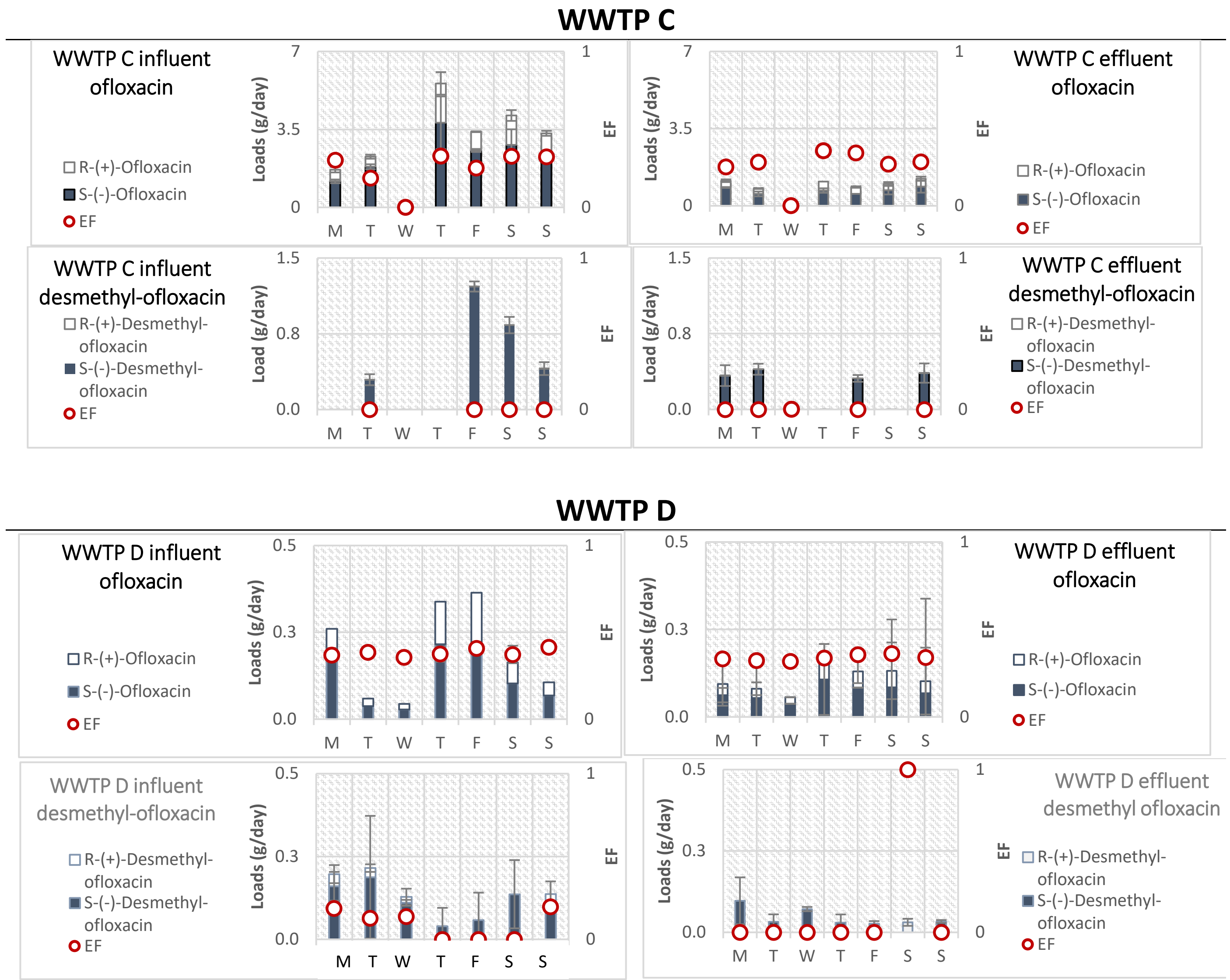


Figure 3 Average daily loads of ofloxacin and its metabolite desmethylofloxacin in wastewater (liquid phase) during the monitoring week (M -Monday, T – Tuesday, W – Wednesday, T – Thursday, F – Friday, S – Saturday and S – Sunday).

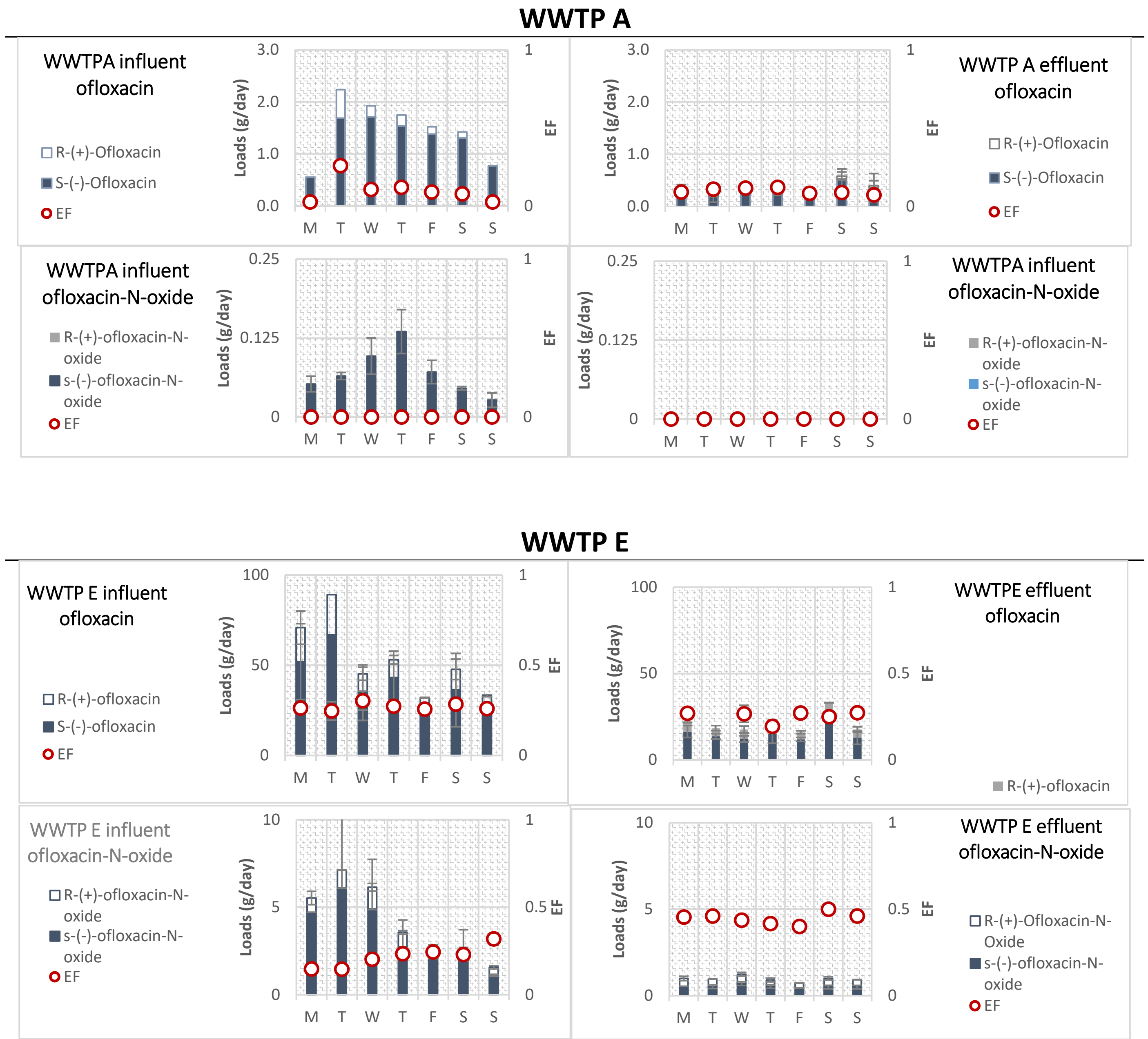


Figure 4 Daily loads of ofloxacin and its metabolites in wastewater (liquid phase): desmethylofloxacin in WWTPs C and D and ofloxacin-N-oxide in WWTPs A and E during the monitoring week

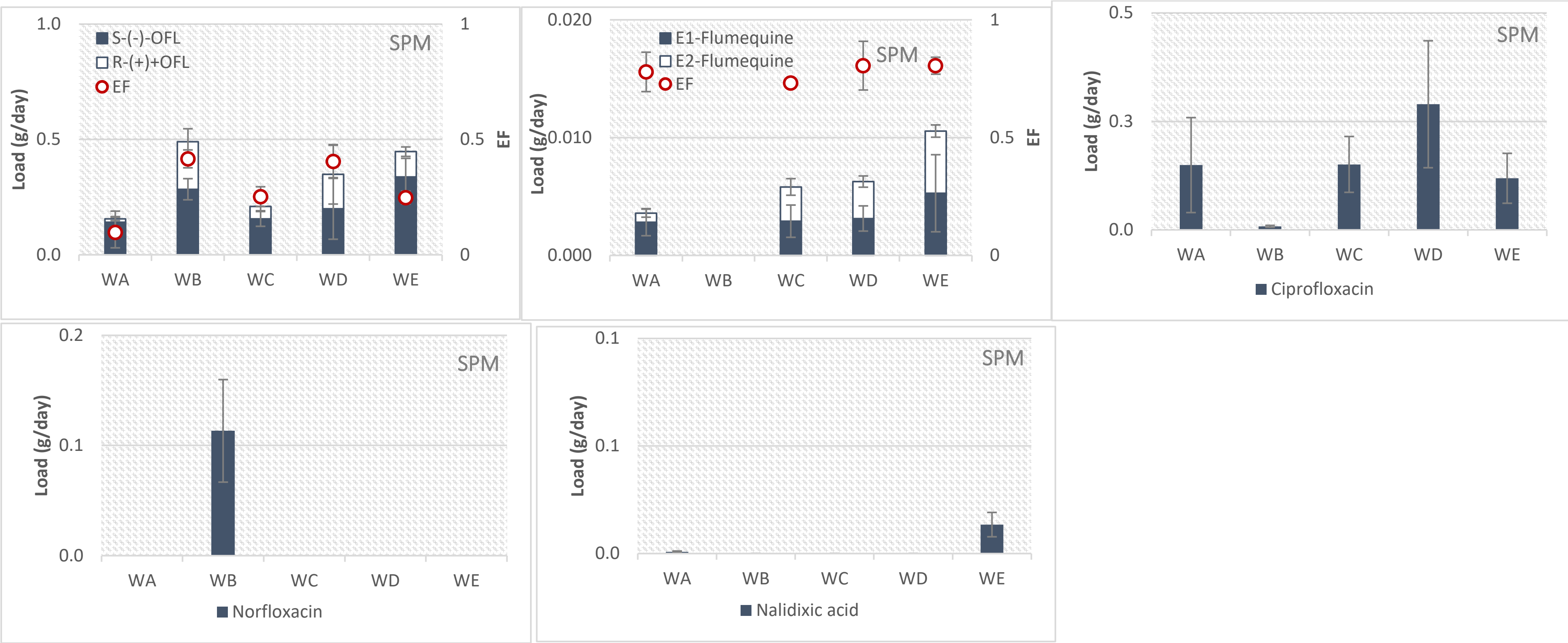


Figure 5 Average daily loads of ofloxacin, ciprofloxacin, norfloxacin and nalidixic acid in SPM from wastewater influent in the investigated catchment area (sites A-E) during the monitoring week

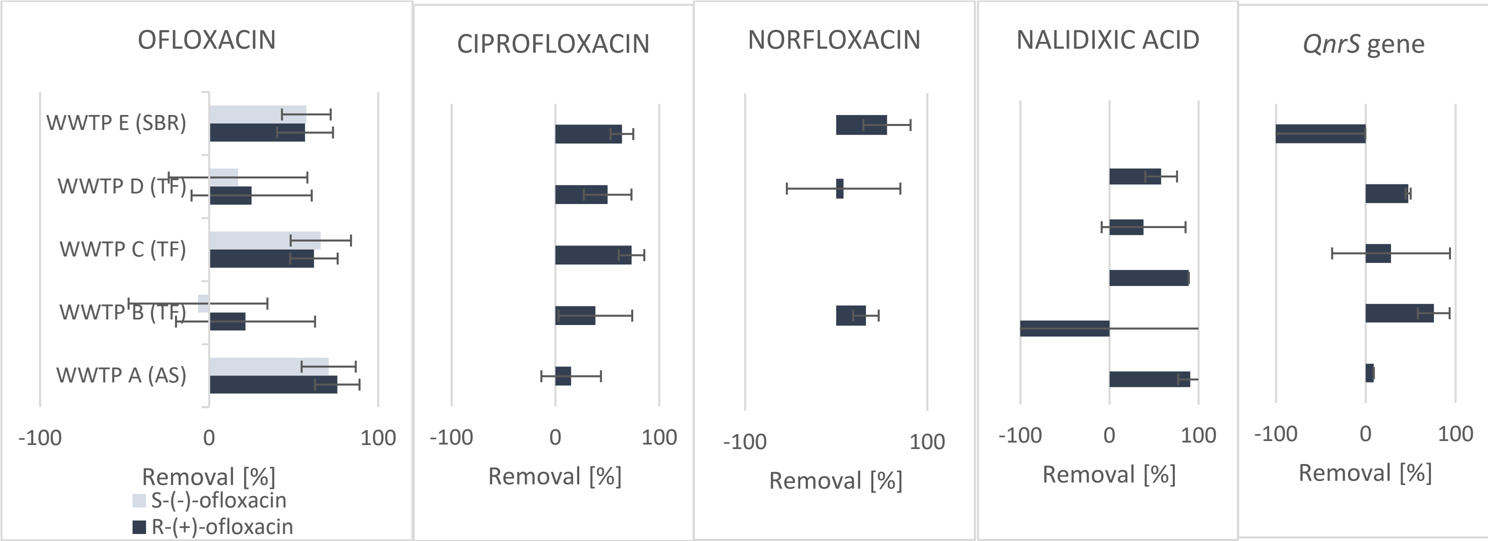
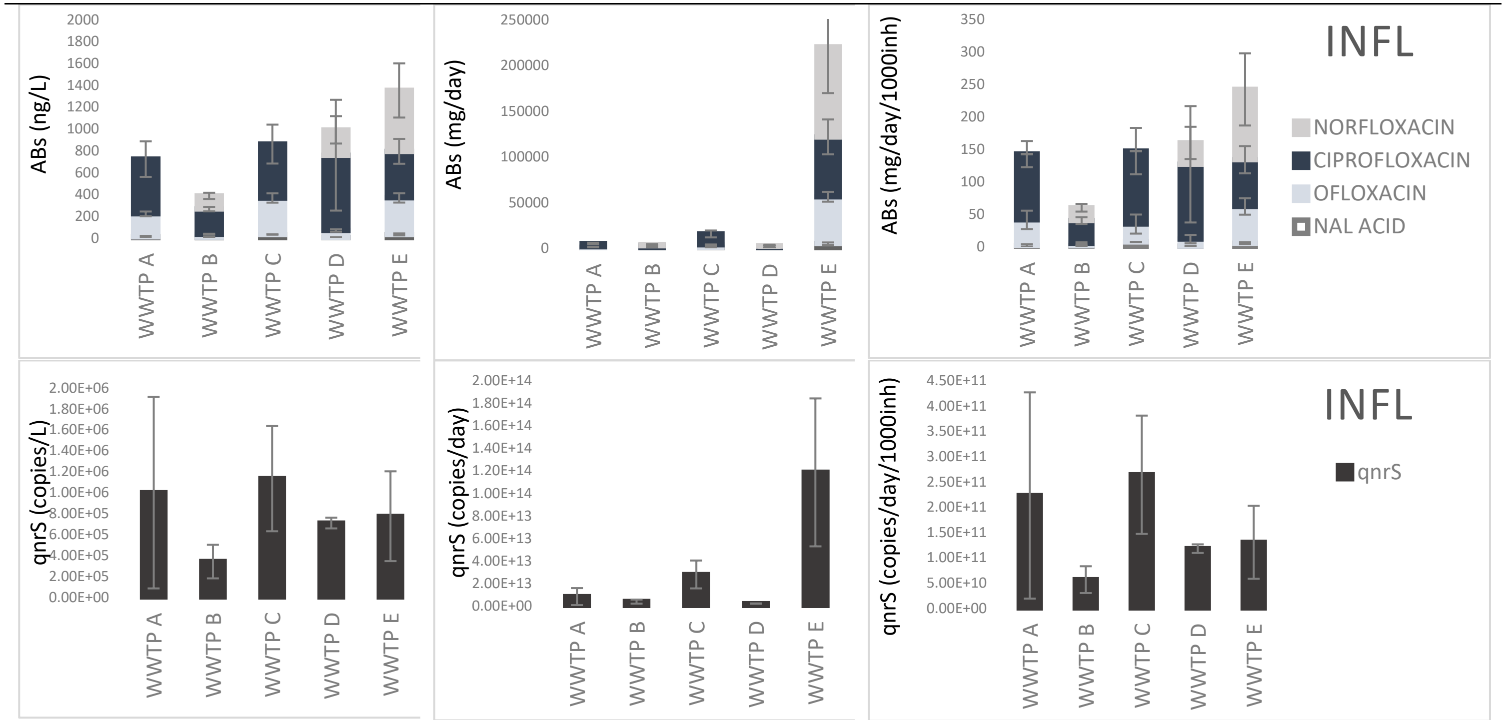


Figure 6 Percentage removal of ABs and ARG during wastewater treatment in five studied wastewater treatment plants (SBR – Sequencing Batch Reactor; TF – Trickling Filters, AS – Activated Sludge).

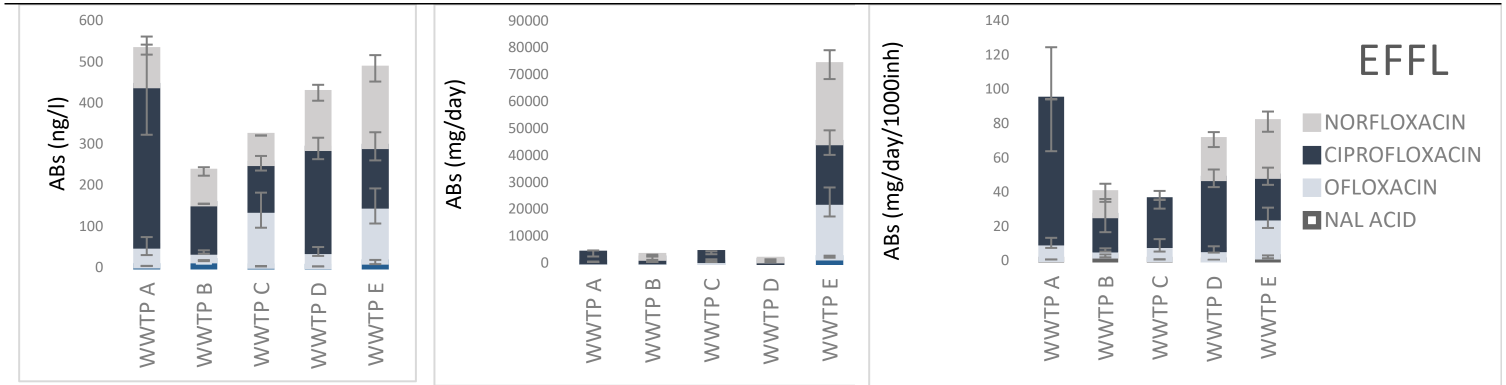
WWTP INFLUENT

CONCENTRATION | **DAILY LOADS** | **POPULATION NORMALISED LOADS**



WWTP EFFLUENT

CONCENTRATION | **DAILY LOADS** | **POPULATION NORMALISED LOADS**



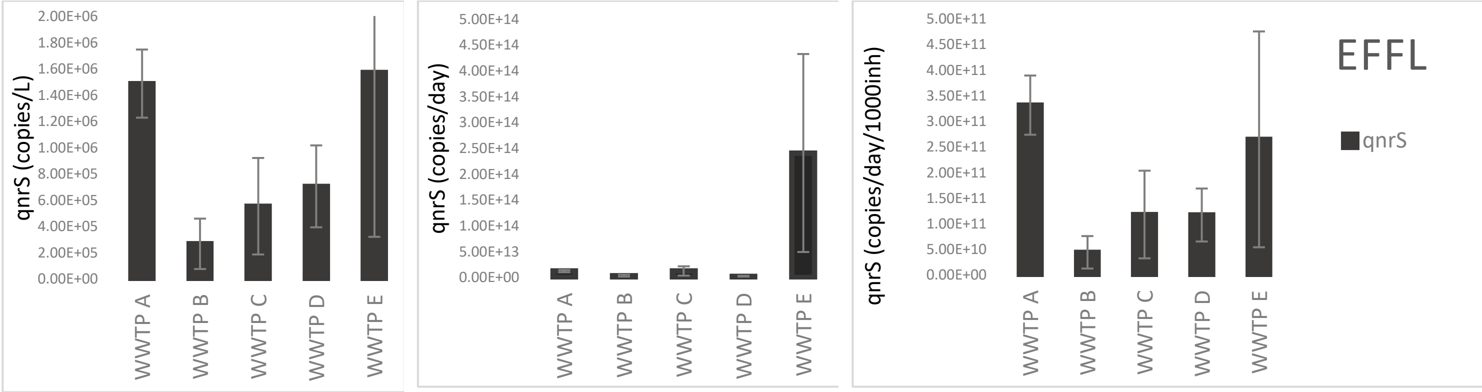


Figure 7 Daily loads and population normalised daily loads of Quinolones (ABs: ciprofloxacin, ofloxacin, norfloxacin and nalidixic acid) and *qnrS* gene in wastewater in the studied catchment

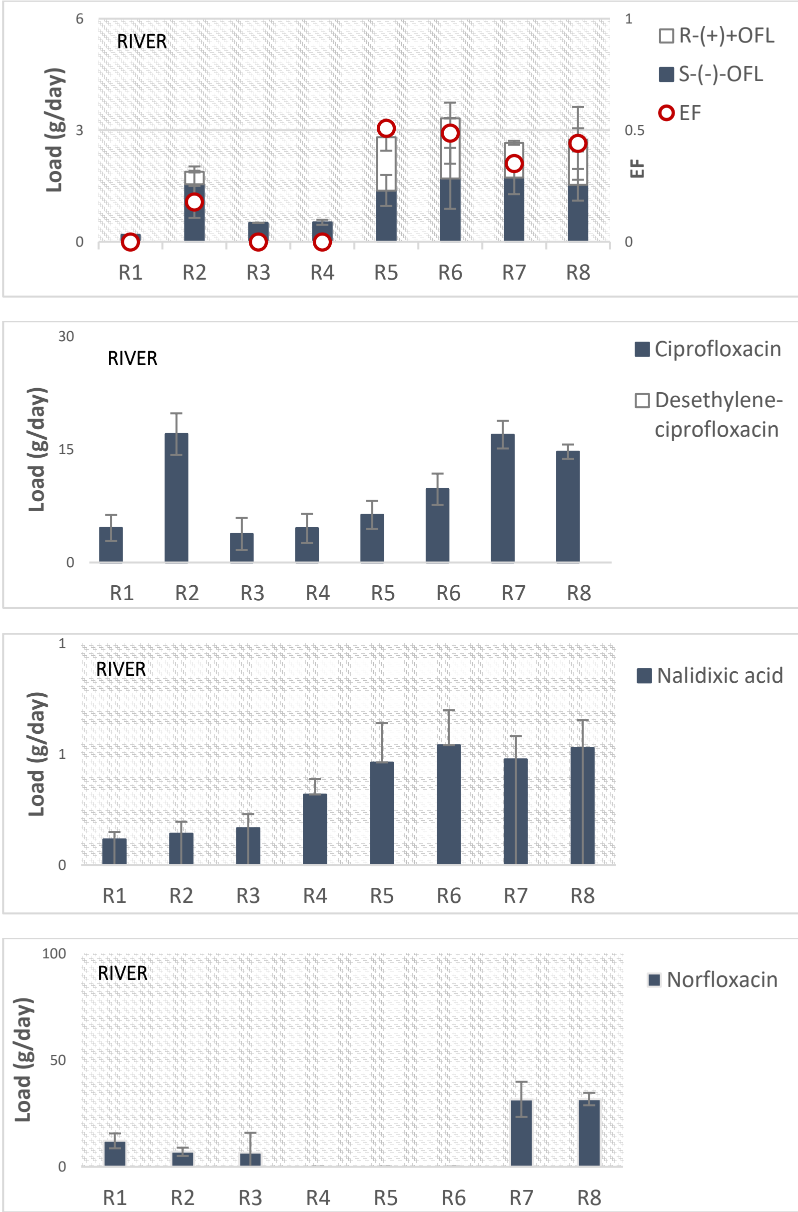


Figure 8 Average daily loads of ofloxacin, ciprofloxacin, norfloxacin and nalidixic acid in the investigated catchment area (sites A-E) during the monitoring week.

Table 1. Characteristics of the studied WWTPs contributing to one river catchment area in the South-West UK (a) and wastewater/river water flow rates (b) (n.a. means not available).

(a) Characteristics

WWTP	Population served by WWTP	Industrial contribution (%)	Sewer residence time (h)	Wastewater treatment secondary process	Solid retention time (d)	Hydraulic retention time (h)	River sampling: distance to discharge point (km)	
							Upstream	Downstream
A	37000	<1	<0.5–4	Activated sludge	19	46.2	0.5	n.a.
B	67870	19	<0.5–4	Trickling filter	n.a.	24.5	0.5	0.5
C	105847	1	<0.5–9	Trickling filter	n.a.	13.9	2	2
D	17638	<1	<0.5–2	Trickling filter	n.a.	17.6	1	1
E	909617	5	<1-24	90% Sequencing batch reactor 10% Activated sludge	4 8	10.9 25.8	–	–

(b) Flow rates [m^3/day]

WWTP	A			B			C			D			E
	Waste water	River up	River down	Waste water	River up	River down	Waste water	River up	River down	Waste water	River up	River down	Waste water
Mon	15386.1	125625.9	141012.0	13212.0	169092.0	182304.0	29163.4	400032.0	422560.9	3080.0	355363.2	358443.2	181229.0
Tue	9409.8	132431.2	141841.0	18275.0	328189.0	346464.0	25694.6	384480.0	413643.4	2661.2	321840.0	324501.2	148587.0
Wed	6386.8	71118.2	77505.0	9527.0	141673.0	151200.0	N.A.	355968.0	381662.6	2604.3	318297.6	320901.9	155494.0
Thu	6203.9	62145.1	68349.0	9979.0	137765.0	147744.0	23891.2	513216.0	539499.6	2940.5	305164.8	308105.3	151767.0
Fri	6601.6	110857.4	117459.0	9364.0	130604.0	139968.0	23651.5	454464.0	478355.2	2981.4	340416.0	343397.4	148678.0
Sat	7017.4	79175.6	86193.0	9558.0	118314.0	127872.0	22914.6	427680.0	451331.5	3184.9	315100.8	318285.7	143128.0
Sun	6689.2	78846.8	85536.0	8496.0	128880.0	137376.0	22528.9	405216.0	428130.6	3018.4	348192.0	351210.4	142542.0

Table 2. Comparison of consumption estimates between prescriptions data and wastewater (WW) analysis.

Pharmaceuticals	Total consumption (kg/year) in England	DTR	CF	Consumption (intake) estimates (mg day^{-1} 1000 people^{-1}) in England					
				NHS data (2015)	WW analysis (2015)				
					Site A	Site B	Site C	Site D	Site E
Ciprofloxacin	5782.0	Ciprofloxacin	2.22	115.0	235	77	249	256	160
		Desethylene-ciprofloxacin	54.2		915	788	1223	1548	2595
Ofloxacin	212 as (±)-OFL 120 as *S*-(−)-OFL	Ofloxacin	1.21	4 as (±)-OFL 8 as *S*-(−)-OFL	6 as (±)-OFL 42 as *S*-(−)-OFL	2 as (±)-OFL 3 as *S*-(−)-OFL	10 as (±)-OFL 24 as *S*-(−)-OFL	5 as (±)-OFL 8 as *S*-(−)-OFL	18 as (±)-OFL 51 as *S*-(−)-OFL
		Ofloxacin-*N*-oxide	47.9		91 as *S*-(−)-OFL	N.d.	N.d.	N.d.	43 as (±)-OFL 177 as *S*-(−)-OFL
		Desmethyl-ofloxacin	52.0		42 as *S*-(−)-OFL	16 as (±)-OFL 19 as *S*-(−)-OFL	193 *S*-(−)-OFL	46 as (±)-OFL 338 as *S*-(−)-OFL	N.d.
Norfloxacin	1.1	Norfloxacin	3.07	0.1	N.d.	61	N.d.	101	172
Nalidixic acid	–	Nalidixic acid	2.50	0.3	3	3	3	2	14
Lomefloxacin	–	Lomefloxacin	1.35	–	–	–	–	–	–
Moxifloxacin	39.6	Moxifloxacin	2.94	3	N.d.	N.d.	N.d.	16	17
		Moxifloxacin-*N*-sulfate	2.04		N.d.	N.d.	N.d.	N.d.	N.d.
Prulifloxacin	–	Prulifloxacin	–	–	–	–	–	–	–
		Ulifloxacin	5.00		–	–	–	–	–

Supplementary Information

(Fluoro)quinolones and quinolone resistance genes in the aquatic environment: a river catchment perspective

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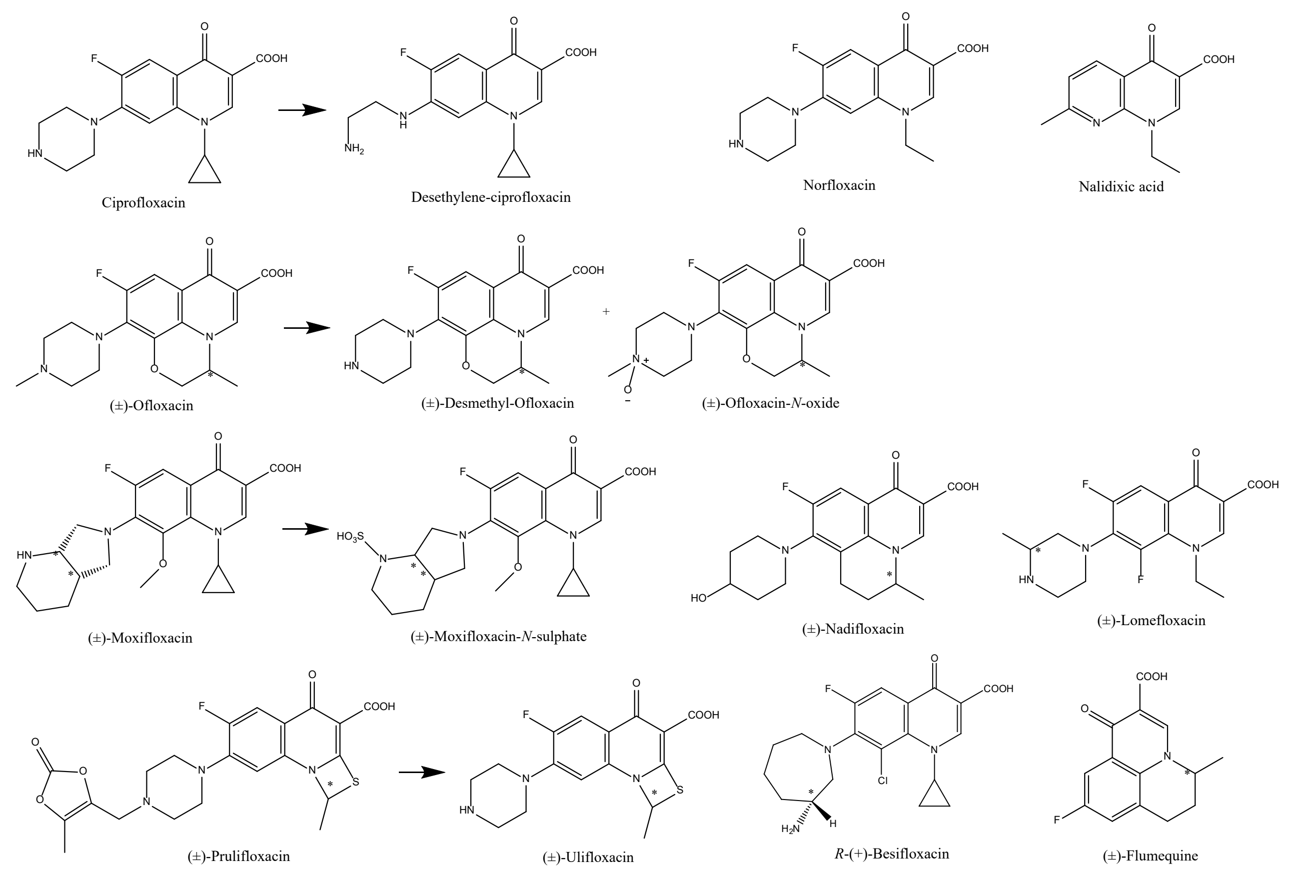

Figure S1 All (fluoro)quinolones selected in the study (* indicates the position of the chiral center). The arrow indicates that the produced analyte is a metabolite (to be noticed that not all the metabolites were included in the figure).

Table S1 Quinolones-info on their chirality and the marketed form use (n.a. not available).

	Compound	Chirality	Marketed form	Restrictions in marketing areas
Quinolones listed by WHO Collaborating Centre for Drug Statistics Methodology among antibacterials for systemic use	Ciprofloxacin	N	-	-
	Ofloxacin	Y	Racemate	-
	L-Ofloxacin	Y	Single enantiomer	-
	Pefloxacin	N	-	Not approved in the USA
	Enoxacin	N	-	Withdrawn in the USA
	Temafloxacin	Y	Racemate	Not marketed in Europe
	Norfloxacin	N	-	-
	Lomefloxacin	Y	Racemate*	-
	Fleroxacin	N	-	Withdrawn
	Sparfloxacin	Y	Single enantiomer	Withdrawn
	Rufloxacin	N	-	-
	Grepafloxacin	Y	Racemate	Withdrawn
	Trovafloxacin	Y	n.a.	Withdrawn
	Moxifloxacin	Y	Single enantiomer	-
	Gemifloxacin	Y	Racemate	Not approved in Europe (Korea only)
	Gatifloxacin	Y	Racemate	Available only in the US and Canada
	Prulifloxacin	Y	Racemate	-
	Pazufloxacin	Y	Single enantiomer	Marketed only in Japan
	Garenoxacin	Y	Single enantiomer	Available in Korea, Japan and China
	Sitafloxacin	Y	Single enantiomer	Marketed only in Japan
	Rosoxacin	N	-	Not available in theUSA
	Nalidixic acid	N	-	-
	Piromidic acid	N	-	n.a.
	Pipemidic acid	N	-	-
	Oxolinic acid	N	-	n.a.
	Cinoxacin	N	-	-
	Flumequine	Y	Racemate	-
	Nemonoxacin	Y	n.a.	
Other quinolones	Nadifloxacin	Y	Racemate	-
	Besifloxacin	Y	Single enantiomer	-
	Danofloxacin	Y	Maybe single enantiomer	Not marketed in Europe
	Orbifloxacin**	Y		Not marketed in Europe
	Ibafloxacin	Y	Racemate	Not marketed in Europe
	Pradofloxacin	Y	Single enantiomer	-
	Balofloxacin	Y	n.a.	Available only in Korea
	Tosufloxacin	Y	Racemate	Marketed only in Japan
	Marbofloxacin	N	-	-
	Difloxacin	N	-	-
	Enrofloxacin	N	-	-

* A patent of *R*-lomefloxacin is also available

** It is a *meso*-compound as *R,S* and *S,R* isomers are equivalent [1]

Table S2 Selected analytes and their properties (MW=molecular weight).

Compound	CAS	Formula	MW	LogP	pKa		Supplier
					Strongest acidic	Strongest basic	
Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.3	-0.81[a]	5.76[a]	8.68[a]	Fluka
Desethylene-ciprofloxacin	528851-31-2	$C_{15}H_{17}ClFN_3O_3$	341.8	-	-	-	TRC
S-(-)-Ofloxacin (*L*-Ofloxacin)	100986-85-4	$C_{18}H_{20}FN_3O_4$		0.65[a]	5.45[a]	6.20[a]	Sigma Aldrich
(±)-Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.4	0.65[a]	5.45[a]	6.20[a]	Sigma Aldrich
Norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.3	-0.92[a]	5.77[a]	8.68[a]	Fluka
(±)-Ofloxacin-*N*-oxide	104721-52-0	$C_{20}H_{24}FN_3O_7$	437.4	-	-	-	TRC
(±)-Desmethyl-ofloxacin	82419-52-1	$C_{17}H_{18}FN_3O_4$	347.3	-	-	-	TRC
Nalidixic acid	3374-05-8	$C_{12}H_{11}N_2NaO_3$	254.2	1.01[a]	5.95[a]	4.68[a]	Sigma Aldrich
(±)-Lomefloxacin	98079-52-8	$C_{17}H_{19}F_2N_3O_3$		-0.30[e]	5.64[e]	8.70[e]	Sigma Aldrich
R,R-(+)-Moxifloxacin	1346603-25-5	$C_{21}H_{25}ClFN_3O_4$	437.9	2.9[e]	5.69[a]	9.42[a]	TRC
S,S-(-)-Moxifloxacin	192927-63-2	$C_{21}H_{27}ClFN_3O_5$	455.9	2.9[e]	5.69[a]	9.42[a]	TRC
Moxifloxacin-*N*-sulphate	n.a.	$C_{21}H_{22}FN_3Na_2O_7S$	525.5	-	-	-	TRC
(±)-Prulifloxacin	123447-62-1	$C_{21}H_{20}FN_3O_6S$	461.5	2.49[b] 3.27[c]	5.85[d]	6.25[d]	Sigma Aldrich
(±)-Ulifloxacin	112984-60-8	$C_{16}H_{16}FN_3O_3S$	349.4	-0.56[d]	5.85[d]	8.69[d]	TRC
(±)-Flumequine	42835-25-6	$C_{14}H_{12}FNO_3$		2.42[a]	6.00[e]	-4.30[e]	Sigma Aldrich
(±)-Nadifloxacin	124858-35-1	$C_{19}H_{21}FN_2O_4$	360.4	1.87[d]	5.55[d]	1.27[d]	TRC
R-(+)-Besifloxacin	405165-61-9	$C_{19}H_{22}Cl_2FN_3O_3$	430.3	0.54[a]	5.64[e]	9.67[e]	TRC

n.a.-not available
[a] ChemAxon
[b] Chemicalize.org
[c] ChemSpider
[d] ChEMBL (www.ebi.ac.uk/chembldb)
[e] DrugBank

Table S3 MRM transitions selected for studied analytes and internal standards.

Compound	CV/CE[a]	MRM1 (quantification)	CV/CE[a]	MRM2 (confirmation)	MRM1/MRM2 ratio ± SD	Internal standard
Ciprofloxacin	42/40	332.2 > 231.1	42/32	332.2 > 245.1	8.9 ± 2.2	Ciprofloxacin –D_8
Desethylene-ciprofloxacin	40/34	306.3 > 217.1	40/26	306.3 > 268.0	1.4 ± 0.4	Ciprofloxacin –D_8
S-(-)-Ofloxacin (*L*-Ofloxacin)	20/32	362.2 > 261.2	20/32	362.2 > 318.7	29.6 ± 3.4	*S*-(-)-Ofloxacin-D_3
R-(+)-Ofloxacin	20/32	362.2 > 261.2	20/32	362.2 > 318.7	30.0 ± 3.0	*R*-(+)-Ofloxacin-D_3
Norfloxacin	58/26	320.2 > 233.1	58/38	320.2 > 204.9	2.6 ± 0.5	Ciprofloxacin –D_8
S-(-)-Ofloxacin-*N*-oxide	28/18	378.3 > 316.7	28/44	378.3 > 246.9	2.7 ± 0.2	*S*-(-)-Ofloxacin-D_3
R-(+)-Ofloxacin-*N*-oxide	28/18	378.3 > 316.7	28/44	378.3 > 246.9	2.9 ± 0.4	*R*-(+)-Ofloxacin-D_3
S-(-)-Desmethyl-ofloxacin	50/26	348.2 > 261.0	50/33	348.2 > 221.0	7.1 ± 0.6	*S*-(-)-Desmethyl-ofloxacin-D_8
R-(+)-Desmethyl-ofloxacin	50/26	348.2 > 261.0	50/33	348.2 > 221.0	7.2 ± 0.7	*R*-(+)-Desmethyl-ofloxacin-D_8
Nalidixic acid	30/28	233.2 > 187.0	30/28	233.2 > 215.1	5.6 ± 0.3	Ciprofloxacin –D_8
(±)-Lomefloxacin	22/24	352.0 > 265.0	22/22	352.0 > 308.0	3.0 ± 0.2	Ciprofloxacin –D_8
R,R-(+)-Moxifloxacin	54/27	402.2 > 364.0	54/23	402.2 > 261.0		*S*-(-)-Desmethyl-ofloxacin-D_8
S,S-(-)-Moxifloxacin	54/27	402.2 > 364.0	54/23	402.2 > 261.0		*R*-(+)-Desmethyl-ofloxacin-D_8
Moxifloxacin-*N*-sulphate	54/27	402.2 > 364.0	54/28	402.2 > 341.0	2.8 ± 0.8	*S*-(-)-Desmethyl-ofloxacin-D_8
Prulifloxacin-E1	42/22	462.2 > 444.1	42/32	462.2 > 360.1	1.2 ± 0.1	*S*-(-)-Ofloxacin-D_3
Prulifloxacin-E2	42/22	462.2 > 444.1	42/32	462.2 > 360.1	1.2 ± 0.2	*R*-(+)-Ofloxacin-D_3
Ulifloxacin-E1	42/22	350.2 > 306.4	42/26	350.2 > 263.0	1.2 ± 0.3	*S*-(-)-Desmethyl-ofloxacin-D_8
Ulifloxacin-E2	42/22	350.2 > 306.4	42/26	350.2 > 263.0	1.2 ± 0.2	*R*-(+)-Desmethyl-ofloxacin-D_8
Flumequine-E1	28/34	262.2 > 201.9	28/26	262.2 > 244.5	1.7 ± 0.1	Flumequine-$^{13}C_3$-E1
Flumequine-E2	28/34	262.2 > 201.9	28/26	262.2 > 244.5	1.8 ± 0.2	Flumequine-$^{13}C_3$-E2
Nadifloxacin-E1	40/38	361.3 > 282.9	40/44	361.3 > 256.8	1.6 ± 0.1	Flumequine-$^{13}C_3$-E1
Nadifloxacin-E2	40/38	361.3 > 282.9	40/44	361.3 > 256.8	1.6 ± 0.2	Flumequine-$^{13}C_3$-E2
R-(+)-Besifloxacin	34/14	394.1 > 376.4	34/24	394.1 > 356.0	3.4 ± 0.4	Ciprofloxacin –D_8
Internal Standard	**CV/CE[a]**	**MRM1 (quantification)**				
Ciprofloxacin –D_8	30/26	340.1 > 296.1				
S-(-)-Ofloxacin-D_3	47/28	365.2 > 261.0				
R-(+)-Ofloxacin-D_3	47/28	365.2 > 261.0				
S-(-)-Desmethyl-ofloxacin-D_8	64/32	356.6 > 265.1				
R-(+)-Desmethyl-ofloxacin-D_8	64/32	356.6 > 265.1				
Flumequine-$^{13}C_3$-E1	40/24	265.1 > 246.9				
Flumequine-$^{13}C_3$-E2	40/24	265.1 > 246.9				

[a]CV, cone voltage (V); CE, collision energy (eV)

SOLID PHASE EXTRACTION

Cartridge: Waters Oasis HLB
Conditioning: 3 ml methanol followed by 3 ml ultrapure water (3 ml min^{-1})
Loading: 50 ml samples (8 ml min^{-1})
Elution: 4 ml methanol (3 ml min^{-1})

LIQUID CHROMATOGRAPHY

Waters ACQUITY UPLC® system (Waters, Manchester, UK)
Column: chiral CHIRALCEL® OZ-RH column (5 μm particle size, L × I.D. 15 cm × 2.1 mm, Chiral Technologies, France)
Column temperature: 30°C
Autosampler temperature: 4°C
Mobile phase: Isocratic. 10 mM ammonium formate/methanol 1:99 v/v with 0.05% formic acid
Flow rate: 0.1 ml min^{-1}
Injection volume: 20 μl

MASS SPECTROMETRY

Xevo TQD (Triple quadrupole mass spectrometer, Waters, Manchester, UK)
Source: Electrospray ionisation (ESI)
Mode: POS
Capillary voltage: 3 kV
Source temperature: 350°C
Desolvation temperature: 350°C
Desolvation gas flow: 650 L h^{-1}
Nebulising and desolvation gas: Nitrogen (Peak Scientific, UK)
Collision gas: Argon

Figure S2: Conditions for sample preparation via SPE and analysis via UPLC-MS/MS.

Table S4 Validation parameters - enantiomeric fraction (EF) of compounds, which stereoisomers were separated under studied conditions, at three concentrations.

Compounds	**EF (n=9)**		
	5 µg/L	**50 µg/L**	**500 µg/L**
(±)-Ofloxacin	0.53±0.01	0.49±0.01	0.49±0.00
(±)-Ofloxacin-*N*-oxide	0.49±0.01	0.48±0.01	0.50±0.01
(±)-Desmethyl-ofloxacin	0.50±0.04	0.51±0.02	0.51±0.01
(±)-Prulifloxacin	0.49±0.04	0.41±0.01	0.47±0.02
(±)-Ulifloxacin	0.51±0.01	0.49±0.01	0.49±0.01
(±)-Flumequine	0.51±0.03	0.50±0.02	0.49±0.02
(±)-Nadifloxacin	0.51±0.02	0.52±0.01	0.50±0.02
(±)-Moxifloxacin	0.53±0.03	0.50±0.04	0.51±0.01

Table S5 Validation parameters - retention time, relative retention time, linearity range, correlation coefficient obtained from calibration curve and instrumental and method limits of detection and instrumental and method limits of quantification.

Compound	R_t (min)	Rel. R_t	Sample diluent				WW influent		WW effluent		River		SPM	
			Linearity range (µg/L)	R^2	$IDL_{S/N}$ (µg/L)	$IQL_{S/N}$ (µg/L)	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)	MDL (ng/g)	MQL (ng/g)
Ciprofloxacin	8.7 ± 0.1	2.5	0.05-1000	0.9945	0.050	0.100	0.6	1.1	0.5	1.1	0.6	1.2	0.04	0.07
Desethylene-ciprofloxacin	6.6 ± 1.1	1.3	5.0-1000	0.9906	5.000	5.000	54.3	54.3	70.3	70.3	81.4	81.4	9.06	9.06
S-(-)-Ofloxacin	13.1 ± 0.1	0.2	0.25-1000	0.9983	0.250	0.250	2.2	2.2	2.6	2.6	2.7	2.7	0.08	0.08
R-(+)-Ofloxacin	18.3 ± 0.5	2.5	0.25-1000	0.9973	0.250	0.250	2.3	2.3	2.6	2.6	2.6	2.6	0.09	0.09
Norfloxacin	9.0 ± 0.3	4.1	0.25-1000	0.9900	0.250	5.000	3.1	62.6	2.9	58.7	2.8	55.5	0.16	3.22
S-(-)-Ofloxacin-N-oxide	20.3 ± 0.2	0.5	0.5-1000	0.9981	0.500	1.000	4.8	9.6	6.4	12.9	5.9	11.7	4.90	9.80
R-(+)-Ofloxacin-N-oxide	29.2 ± 0.5	1.8	0.5-1000	0.9974	0.500	1.000	5.5	10.9	6.4	12.8	6.1	12.2	22.73	45.45
S-(-)-Desmethyl-ofloxacin	7.8 ± 0.1	0.4	0.125-1000	0.9985	0.125	0.500	1.2	5.0	1.6	6.6	1.7	6.7	0.28	1.13
R-(+)-Desmethyl-ofloxacin	9.9 ± 0.1	0.4	0.125-1000	0.9982	0.125	0.500	1.3	5.1	1. 7	6.7	1.7	6.7	0.31	1.24
Nalidixic acid	14.5 ± 0.1	2.9	0.01-2000	0.9940	0.010	0.025	0.1	0.3	0.1	0.3	0.1	0.2	0.01	0.02
(±)-Lomefloxacin	8.8 ± 0.1	2.6	0.25-2000	0.9981	0.250	0.250	2.6	2.6	3.1	3.1	2.7	2.7	0.18	0.18
R,R-(+)-Moxifloxacin	8.3 ± 0.1	0.7	0.5-1000	0.9902	0.500	0.500	4.2	4.2	5.8	5.8	4.7	4.7	0.40	0.40
S,S-(-)-Moxifloxacin	9.0 ± 0.1	0.6	0.5-1000	0.9914	0.500	0.500	6.4	6.4	6.6	6.6	5.1	5.1	0.31	0.31
Moxifloxacin-*N*-sulphate	13.6 ± 0.2	1.7	0.5-2000	0.9941	0.500	1.000	5.7	11.3	5.2	10.4	5.2	10.3	1.13	2.25
Prulifloxacin-E1	23.4 ± 0.9	2.4	0.5-1000	0.9969	0.500	0.500	5.8	5.8	5.7	5.7	7.0	7.0	0.28	0.28
Prulifloxacin-E2	26.5 ± 0.5	2.5	0.5-1000	0.9966	0.500	0.500	5.1	5.1	6.2	6.2	7.0	7.0	0.27	0.27
Ulifloxacin-E1	9.0 ± 0.6	6.1	2.5-1000	0.9981	2.500	2.500	23.5	23.5	35.1	35.1	33.9	33.9	1.19	1.19
Ulifloxacin-E2	11.2 ± 0.9	7.8	2.5-1000	0.9950	2.500	2.500	33.9	33.9	36.2	36.2	28.7	28.7	1.16	1.16
Flumequine-E1	12.9 ± 0.1	0.2	0.025-1000	0.9991	0.025	0.500	0.3	5.3	0.3	5.4	0.3	5.3	0.01	0.26
Flumequine-E2	17.5 ± 0.1	0.1	0.025-1000	0.9978	0.025	0.500	0.3	5.3	0.3	5.3	0.3	5.3	0.01	0.25
Nadifloxacin-E1	15.2 ± 0.1	0.3	0.025-1000	0.9989	0.025	0.500	0.2	4.3	0.3	5.6	0.3	5.4	0.01	0.11
Nadifloxacin-E2	22.4 ± 0.2	0.2	0.025-1000	0.9978	0.025	0.500	0.2	5.0	0.3	5.6	0.3	5.4	0.01	0.12
R-(+)-Besifloxacin	6.4 ± 0.2	3.6	1.0-1000	0.9916	1.000	1.000	11.9	11.9	12.8	12.8	11.7	11.7	0.22	0.22

Table S6 Validation parameters – average enantiomeric fraction (EF), enantiomeric resolution (Rs) of compounds, which stereoisomers were separated under studied conditions, in mobile phase (MP) and in wastewater (WW), precision (RSD %) and accuracy (%).

Compound	Rs		$EF_{average}$	Intra-day instrument performance		Intra-day method performance	
	Mobile phase	Wastewater		Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)
Ciprofloxacin	-	-	-	5.0	89.8	8.8	110.0
Desethylene-ciprofloxacin	-	-	-	8.7	90.9	7.8	83.8
S-(-)-Ofloxacin (*L*-Ofloxacin)	1.25	0.89	0.50±0.00	4.5	103.6	4.5	118.3
R-(+)-Ofloxacin				4.3	101.7	5.7	105.3
Norfloxacin	-	-	-	8.8	111.3	11.5	83.3
S-(-)-Ofloxacin-N-oxide	1.71	1.07	0.49±0.00	4.0	100.9	6.2	104.3
R-(+)-Ofloxacin-N-oxide				9.0	96.0	5.6	91.4
S-(-)-Desmethyl-ofloxacin	0.97	0.56	0.50±0.01	2.9	102.5	3.9	100.7
R-(+)-Desmethyl-ofloxacin				6.3	102.8	5.1	97.5
Nalidixic acid	-	-	-	4.4	96.5	8.0	92.1
(±)-Lomefloxacin	-	-	-	3.9	94.6	6.7	97.0
R,R-(+)-Moxifloxacin	0.84	0.21	0.51±0.01	7.7	102.0	9.8	122.4
S,S-(-)-Moxifloxacin				6.6	93.3	5.3	77.6
Moxifloxacin-*N*-sulphate	-	-	-	7.9	101.0	6.0	81.7
Prulifloxacin-E1	1.06	0.46	0.46±0.01	6.8	88.4	7.2	116.6
Prulifloxacin-E2				4.7	106.4	7.9	72.4
Ulifloxacin-E1	0.67	0.41	0.50±0.01	5.8	93.6	10.6	118.0
Ulifloxacin-E2				7.8	113.8	6.6	101.3
Flumequine-E1	1.91	1.10	0.50±0.00	2.8	101.8	1.5	94.4
Flumequine-E2				5.5	102.3	2.9	95.5
Nadifloxacin-E1	2.86	1.44	0.51±0.01	3.8	107.8	3.4	118.9
Nadifloxacin-E2				6.7	96.4	6.0	100.2
R-(+)-Besifloxacin	-	-	-	6.2	97.4	7.7	73.1

Table S7. Matrix effect, absolute and relative SPE recoveries (n=3) for the studied analytes (rec=recovery).

Analyte	%ME		Abs rec %	Solid rec.	SPE relative rec in influent WW%			SPE relative rec in effluent WW%			SPE relative rec in river%		
	With ILIS	Without ILIS			25 ng/L*	250 ng/L*	2500 ng/L*	25 ng/L*	250 ng/L*	2500 ng/L*	25 ng/L*	250 ng/L*	2500 ng/L*
Ciprofloxacin	117.0	46.8	68.2	68.9	84.3 ± 5.7	83.8 ± 1.7	101.7 ± 31.7	78.3 ± 3.5	71.0 ± 1.1	126.4 ± 0.2	72.2 ± 4.8	94.8 ± 2.5	82.9 ± 3.4
Desethylene-ciprofloxacin	74.6	23.1	40.3	27.6	83.8 ± 8.9	107.7 ± 3.5	84.9 ± 4.5	87.1 ± 2.1	73.7 ± 8.0	52.7 ± 4.1	82.7 ± 1.5	59.0 ± 5.4	42.6 ± 2.9
S-(-)-Ofloxacin	111.6	81.2	114.8	149.7	110.8 ± 9.3	113.6 ± 1.5	111.9 ± 1.1	91.9 ± 3.8	92.2 ± 4.0	107.5 ± 4.3	109.5 ± 10.3	87.6 ± 0.6	81.4 ± 3.5
R-(+)-Ofloxacin	107.6	78.3	141.5	135.9	113.8 ± 1.9	98.9 ± 1.8	106.4 ± 2.5	93.8 ± 6.1	89.8 ± 6.7	106.3 ± 0.5	117.8 ± 9.1	85.5 ± 5.0	80.6 ± 3.2
Norfloxacin	79.2	15.4	271.7	77.7	73.0 ± 3.7	82.0 ± 1.0	84.7 ± 2.6	87.1 ± 3.7	86.3 ± 1.0	82.2 ± 5.6	110.0 ± 2.0	90.4 ± 10.2	70.1 ± 7.2
S-(-)-Ofloxacin-*N*-oxide	108.7	79.0	124.3	5.1	103.1 ± 5.1	106.7 ± 6.2	103.0 ± 2.1	72.8 ± 3.4	84.3 ± 4.1	76.2 ± 2.6	98.6 ± 8.8	80.4 ± 3.0	77.1 ± 1.0
R-(+)-Ofloxacin-*N*-oxide	102.9	75.1	168.0	1.1	82.2 ± 11.9	95.2 ± 3.0	96.7 ± 2.0	72.8 ± 7.3	83.3 ± 3.7	78.5 ± 7.2	95.4 ± 4.2	76.7 ± 7.6	74.1 ± 2.1
S-(-)-Desmethyl-ofloxacin	96.3	37.6	67.8	22.1	97.1 ± 9.1	103.1 ± 3.5	101.8 ± 3.9	75.0 ± 1.4	82.3 ± 1.5	70.4 ± 0.9	74.7 ± 1.4	76.8 ± 2.1	73.4 ± 0.7
R-(+)-Desmethyl-ofloxacin	95.6	32.0	129.8	20.2	111.3 ± 11.7	88.8 ± 2.9	92.4 ± 2.2	72.3 ± 4.1	79.3 ± 5.3	73.2 ± 1.0	76.4 ± 2.8	75.6 ± 3.3	72.1 ± 2.8
Nalidixic acid	98.6	63.8	112.6	61.5	89.8 ± 7.9	89.3 ± 10.8	90.1 ± 12.7	90.3 ± 2.3	103.1 ± 4.3	94.0 ± 1.7	105.6 ± 5.4	109.2 ± 4.7	98.7 ± 7.5
(±)-Lomefloxacin	90.8	36.1	99.6	68.9	102.9 ± 6.0	90.4 ± 1.0	97.6 ± 1.2	78.4 ± 5.3	87.0 ± 4.3	76.4 ± 4.3	101.4 ± 2.4	98.9 ± 4.5	80.4 ± 4.5
R,R-(+)-Moxifloxacin	104.0	86.3	70.5	61.8	118.0 ± 0.8	118.7 ± 1.4	117.3 ± 0.9	81.6 ± 4.5	94.5 ± 5.0	84.4 ± 2.9	118.5 ± 12.4	104.7 ± 5.5	94.2 ± 9.5
S,S-(-)-Moxifloxacin	90.0	61.1	87.9	80.7	78.4 ± 5.6	71.7 ± 6.9	85.7 ± 7.5	75.9 ± 7.5	78.3 ± 4.4	72.6 ± 2.8	113.3 ± 17.0	91.9 ± 3.0	89.5 ± 2.8
Moxifloxacin-*N*-sulphate	116.4	45.5	88.8	22.2	85.2 ± 4.0	82.5 ± 2.8	96.9 ± 3.6	92.4 ± 2.6	108.9 ± 2.0	88.0 ± 7.5	101.3 ± 12.7	105.6 ± 14.0	83.2 ± 9.3
Prulifloxacin-E1	109.0	132.4	73.5	89.3	73.3 ± 2.8	81.9 ± 8.2	105.1 ± 5.7	63.6 ± 4.5	81.9 ± 8.2	119.7 ± 4.9	73.3 ± 2.8	81.9 ± 8.2	105.1 ± 5.7
Prulifloxacin-E2	102.2	74.6	144.4	91.2	97.8 ± 20.4	96.8 ± 3.8	100.7 ± 3.4	66.5 ± 6.8	82.4 ± 1.7	91.9 ± 4.1	97.8 ± 20.4	96.8 ± 3.8	100.7 ± 3.4
Ulifloxacin-E1	119.1	47.8	92.5	105.0	119.0 ± 0.5	98.6 ± 3.5	101.1 ± 4.5	72.9 ± 3.8	73.8 ± 1.1	67.1 ± 1.0	71.9 ± 2.6	71.5 ± 3.6	77.7 ± 0.9
Ulifloxacin-E2	73.8	20.8	274.1	108.0	80.7 ± 9.3	70.3 ± 0.3	70.5 ± 0.5	80.4 ± 9.5	60.1 ± 3.8	66.9 ± 4.7	98.2 ± 16.0	83.9 ± 10.1	79.5 ± 4.1
Flumequine-E1	98.3	66.5	136.8	96.3	90.2 ± 2.7	95.0 ± 1.1	95.9 ± 0.1	90.1 ± 1.6	99.2 ± 1.3	86.3 ± 1.7	101.3 ± 1.3	93.0 ± 3.1	86.2 ± 0.7
Flumequine-E2	97.8	70.8	155.6	101.4	89.3 ± 1.8	96.4 ± 0.3	98.4 ± 0.9	90.4 ± 10.5	105.2 ± 1.5	85.9 ± 5.0	102.4 ± 4.5	94.5 ± 14.2	86.3 ± 3.1
Nadifloxacin-E1	112.3	75.9	119.3	232.8	118.3 ± 0.6	115.6 ± 4.5	116.8 ± 3.0	87.5 ± 3.9	94.5 ± 2.5	86.6 ± 1.6	96.9 ± 2.7	94.9 ± 3.0	83.5 ± 4.0
Nadifloxacin-E2	111.7	80.9	136.0	206.2	94.9 ± 6.8	98.6 ± 4.7	107.0 ± 4.6	84.2 ± 2.1	97.8 ± 2.6	83.8 ± 5.1	97.3 ± 12.6	95.9 ± 7.4	82.9 ± 8.0
R-(+)-Besifloxacin	85.6	17.4	221.6	232.1	106.8 ± 2.7	73.5 ± 5.7	72.0 ± 1.6	94.6 ± 2.5	73.0 ± 3.0	66.3 ± 6.8	114.3 ± 6.3	72.6 ± 2.0	70.5 ± 0.1

*- the following concentrations were used: 50, 500 and 5000 ng L^{-1} in the case of compounds that were not enantioseparated

Table S8 Concentrations of the analytes in liquid environmental matrices (influent, effluent, river upstream and river downstream) during the monitoring week in WWTP A.

	Ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	198.3	22.5	249.8	22.3	52.0	3.2	109.7	16.5
Tues	508.4	30.5	505.4	11.9	52.9	3.8	98.7	13.7
Wed	728.3	109.5	300.3	57.6	42.5	2.6	271.6	8.6
Thu	579.5	14.7	359.0	41.2	45.5	3.3	209.6	38.6
Fri	528.8	58.0	308.4	55.5	49.8	0.3	169.9	3.1
Sat	546.2	30.7	578.0	101.4	42.8	1.1	187.4	2.0
Sun	421.7	82.0	428.9	124.4	48.9	1.0	213.1	57.0

	Desethylene-ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	<MQL	-	<MQL	-	<MQL	-	<MQL	-
Tues	53.4	2.0	<MQL	-	<MQL	-	<MQL	-
Wed	80.8	6.3	<MQL	-	<MQL	-	<MQL	-
Thu	115.2	1.6	<MQL	-	<MQL	-	<MQL	-
Fri	91.6	6.7	<MQL	-	<MQL	-	<MQL	-
Sat	99.4	5.7	<MQL	-	<MQL	-	<MQL	-
Sun	74.8	20.9	<MQL	-	<MQL	-	<MQL	-

		(±)-Ofloxacin							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	3.7	0.7	<MQL	-	<MQL	-
	Tues	58.6	3.1	3.8	0.5	<MQL	-	<MQL	-
	Wed	33.2	18.3	7.4	1.8	<MQL	-	4.5	0.8
	Thu	34.0	7.6	8.0	1.9	<MQL	-	4.5	0.0
	Fri	21.1	2.0	4.6	1.0	<MQL	-	<MQL	-
	Sat	16.5	4.5	7.8	0.8	<MQL	-	4.1	1.5
	Sun	n.d.	-	5.6	3.0	<MQL	-	<MQL	-
***S*-(-)-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	36.2	7.8	19.7	3.2	<MQL	-	8.6	1.8
	Tues	179.3	8.5	20.3	0.0	<MQL	-	6.1	1.8
	Wed	268.2	91.1	47.0	2.0	<MQL	-	30.9	0.9
	Thu	247.9	2.4	50.5	2.8	2.9	0.4	24.9	3.4
	Fri	209.8	10.8	31.2	6.0	<MQL	-	12.2	0.5
	Sat	186.3	10.3	74.8	12.9	<MQL	-	22.8	0.2
	Sun	115.5	32.6	53.8	31.3	<MQL	-	14.9	5.1

		(±)-Ofloxacin-*N*-Oxide							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	<MQL	-	<MQL	-
	Thu	n.d.	-	n.d.	-	<MQL	-	<MQL	-
	Fri	n.d.	-	n.d.	-	n.d.	-	<MQL	-
	Sat	n.d.	-	n.d.	-	n.d.	-	<MQL	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
***S*-(-)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	3.4	0.8	n.d.	-	n.d.	-	n.d.	-
	Tues	6.9	0.6	n.d.	-	n.d.	-	n.d.	-
	Wed	15.1	4.5	n.d.	-	<MQL	-	<MQL	-
	Thu	21.8	5.6	n.d.	-	<MQL	-	<MQL	-
	Fri	10.8	2.8	n.d.	-	<MQL	-	<MQL	-
	Sat	6.5	0.4	n.d.	-	n.d.	-	<MQL	-
	Sun	4.0	1.7	n.d.	-	n.d.	-	n.d.	-

		(±)-Desmethyl-ofloxacin							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Tues	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	<MQL	-
	Thu	n.d.	-	<MQL	-	n.d.	-	<MQL	-
	Fri	n.d.	-	<MQL	-	n.d.	-	<MQL	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	<MQL	-	n.d.	-	n.d.	-	n.d.	-
***S*-(-)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Tues	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Wed	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Thu	<MQL	-	<MQL	-	n.d.	-	0.0	0.0
	Fri	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Sat	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Sun	<MQL	-	<MQL	-	n.d.	-	<MQL	-

		(±)-Flumequine							
		Influent		Effluent		Upstream		Downstream	
Flumequine-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	5.6	0.8	<MQL	-	<MQL	-
	Tues	<MQL	-	8.2	0.7	<MQL	-	<MQL	-
	Wed	8.4	3.4	7.9	0.5	<MQL	-	7.9	2.4
	Thu	<MQL	-	6.6	0.1	<MQL	-	5.9	1.7
	Fri	<MQL	-	5.5	0.6	<MQL	-	5.5	0.9
	Sat	<MQL	-	11.2	1.4	<MQL	-	<MQL	-
	Sun	<MQL	-	8.0	2.4	<MQL	-	5.4	2.0
Flumequine-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Tues	<MQL	-	n.d.	-	<MQL	-	<MQL	-
	Wed	n.d.	-	<MQL	-	<MQL	-	n.d.	-
	Thu	n.d.	-	n.d.	-	<MQL	-	n.d.	-
	Fri	n.d.	-	n.d.	-	<MQL	-	n.d.	-
	Sat	n.d.	-	n.d.	-	<MQL	-	n.d.	-
	Sun	n.d.	-	<MQL	-	<MQL	-	n.d.	-

	Nalidixic acid							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	1.1	0.5	n.d.	-
Tues	0.8	1.3	n.d.	-	0.9	0.3	n.d.	-
Wed	30.4	25.0	3.5	0.2	1.7	0.0	2.0	0.3
Thurs	n.d.	-	2.9	0.5	1.0	0.0	1.3	0.3
Fri	7.7	3.0	n.d.	-	1.6	0.3	1.9	0.4
Sat	8.3	1.7	2.9	0.3	1.1	0.0	1.9	0.3
Sun	4.4	0.4	n.d.	-	1.2	0.2	0.9	0.1

	Norfloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	91.9	34.0	134.4	30.6	74.2	20.3
Tues	n.d.	-	<MQL	-	113.9	0.2	59.5	2.6
Wed	n.d.	-	73.5	28.1	117.7	12.5	64.8	24.4
Thurs	n.d.	-	74.2	15.2	113.5	13.4	86.5	16.6
Fri	n.d.	-	77.9	10.3	137.0	36.4	71.9	10.2
Sat	n.d.	-	103.0	35.8	127.5	3.4	59.6	30.3
Sun	n.d.	-	105.1	24.7	158.2	43.6	67.8	2.5

	Lomefloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Nadifloxacin							
		Influent		Effluent		Upstream		Downstream	
Nadifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Nadifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

	Moxifloxacin-*N*-sulphate							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Moxifloxacin							
		Influent		Effluent		Upstream		Downstream	
***R,R*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
***S,S*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	8.7	2.5	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Prulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Pr uli flo		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD

	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Prulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Ulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Ulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

	Besifloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table S9 Concentrations of the analytes in liquid environmental matrices (influent, effluent, river upstream and river downstream) during the monitoring week in WWTP B.

	Ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	147.8	4.3	152.6	31.1	31.4	10.4	29.0	5.4
Tues	108.3	17.3	140.0	3.3	24.5	4.1	24.8	4.3
Wed	269.0	0.5	116.7	23.8	18.0	1.3	28.3	1.4
Thu	212.8	16.2	98.1	1.8	24.8	3.1	22.5	5.1
Fri	303.1	15.7	106.9	2.2	16.0	1.1	27.5	3.2
Sat	247.6	21.3	105.9	20.0	21.8	6.7	24.9	0.1
Sun	316.2	6.1	99.8	1.3	19.9	2.9	23.6	0.4

	Desethylene-ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	131.3	5.8	74.3	1.8	<MQL	-	<MQL	-
Tues	70.9	1.0	<MQL	-	<MQL	-	<MQL	-
Wed	72.8	6.2	<MQL	-	<MQL	-	<MQL	-
Thu	85.3	2.8	<MQL	-	<MQL	-	<MQL	-
Fri	80.2	0.2	<MQL	-	<MQL	-	<MQL	-
Sat	83.0	4.7	<MQL	-	<MQL	-	<MQL	-
Sun	91.7	1.8	<MQL	-	<MQL	-	<MQL	-

		(±)-Ofloxacin							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	5.3	0.7	8.3	2.2	<MQL	-	<MQL	-
	Tues	6.3	0.7	6.0	0.4	<MQL	-	<MQL	-
	Wed	12.1	0.9	7.8	1.6	<MQL	-	<MQL	-
	Thu	16.2	0.5	6.3	0.6	<MQL	-	<MQL	-
	Fri	18.7	0.3	6.7	0.3	<MQL	-	<MQL	-
	Sat	5.4	0.4	5.9	0.8	<MQL	-	<MQL	-
	Sun	12.1	1.3	5.9	0.9	<MQL	-	<MQL	-
***S*-(-)-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	9.3	1.9	13.7	2.4	<MQL	-	<MQL	-
	Tues	8.0	0.9	12.6	0.9	<MQL	-	<MQL	-
	Wed	15.4	5.0	19.9	0.9	<MQL	-	3.0	0.1
	Thu	24.0	1.2	13.7	0.8	3.7	0.3	4.0	2.8
	Fri	25.9	0.6	16.6	2.9	<MQL	-	<MQL	-
	Sat	9.6	0.8	12.7	6.3	<MQL	-	<MQL	-
	Sun	17.8	1.2	10.6	1.5	<MQL	-	<MQL	-

		(±)-Ofloxacin-*N*-Oxide							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Tues	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Wed	n.d.	-	<MQL	-	n.d.	-	n.d.	-
	Thu	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Fri	n.d.	-	<MQL	-	n.d.	-	<MQL	-
	Sat	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Sun	<MQL	-	<MQL	-	n.d.	-	<MQL	-
***S*-(-)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Tues	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Wed	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Thu	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Fri	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Sat	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Sun	<MQL	-	<MQL	-	n.d.	-	n.d.	-

		(±)-Desmethyl-ofloxacin							
		Influent		Effluent		Upstream		Downstream	
_R_-(+)-Desmethyl-Ofloxacin		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	15.6	11.0	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	<MQL	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	5.5	2.0	n.d.	-	n.d.	-	n.d.	-
_S_-(-)-Desmethyl-Ofloxacin		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	18.5	13.1	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Flumequine							
		Influent		Effluent		Upstream		Downstream	
Flumequine-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	10.3	2.2	<MQL	-	<MQL	-
	Tues	<MQL	-	9.6	1.6	<MQL	-	<MQL	-
	Wed	<MQL	-	7.1	0.8	<MQL	-	<MQL	-
	Thu	<MQL	-	8.8	1.7	<MQL	-	<MQL	-
	Fri	<MQL	-	9.6	0.9	<MQL	-	<MQL	-
	Sat	<MQL	-	11.0	1.8	<MQL	-	<MQL	-
	Sun	<MQL	-	10.2	0.4	<MQL	-	<MQL	-
Flumequine-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Tues	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Wed	<MQL	-	<MQL	-	<MQL	-	n.d.	-
	Thu	n.d.	-	n.d.	-	<MQL	-	n.d.	-
	Fri	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Sat	<MQL	-	n.d.	-	n.d.	-	<MQL	-
	Sun	<MQL	-	<MQL	-	n.d.	-	n.d.	-

	Nalidixic acid							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	8.4	1.9	16.5	2.7	1.2	0.6	1.3	0.6
Tues	1.0	0.1	16.5	2.0	0.9	0.1	1.3	0.3
Wed	5.0	0.9	17.7	4.0	1.2	0.4	2.2	0.0
Thurs	14.4	0.9	15.6	2.4	1.2	0.0	1.5	0.0
Fri	7.9	0.3	17.9	0.8	0.9	0.1	2.2	0.7
Sat	8.7	0.2	14.4	3.5	1.0	0.3	2.7	0.1
Sun	14.3	1.5	14.1	0.7	0.7	0.2	2.4	0.0

	Norfloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	139.4	85.6	84.6	7.4	67.3	28.0	<MQL	-
Tues	94.0	10.7	90.9	10.4	81.8	11.7	<MQL	-
Wed	171.1	40.7	85.1	21.5	<MQL	-	<MQL	-
Thurs	150.1	68.7	86.0	2.1	<MQL	-	<MQL	-
Fri	99.9	47.3	63.7	22.7	<MQL	-	<MQL	-
Sat	112.5	11.9	79.8	49.2	<MQL	-	<MQL	-
Sun	82.8	53.4	60.2	9.3	62.3	13.8	<MQL	-

	Lomefloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	<MQL	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Nadifloxacin							
		Influent		Effluent		Upstream		Downstream	
Nadifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	<MQL	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	<MQL	-
	Thu	<MQL	-	n.d.	-	<MQL	-	n.d.	-
	Fri	<MQL	-	n.d.	-	n.d.	-	<MQL	-
	Sat	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Sun	<MQL	-	n.d.	-	<MQL	-	<MQL	-
Nadifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	<MQL	-	<MQL	-	<MQL	-	n.d.	-
	Thu	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Fri	<MQL	-	n.d.	-	n.d.	-	<MQL	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	<MQL	-	n.d.	-	<MQL	-	<MQL	-

	Moxifloxacin-*N*-sulphate							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Moxifloxacin							
		Influent		Effluent		Upstream		Downstream	
***R,R*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
***S,S*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Prulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Prulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Prulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Ulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Ulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

	Besifloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table S10 Concentrations of the analytes in liquid environmental matrices (influent, effluent, river upstream and river downstream) during the monitoring week in WWTP C.

	Ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	248.0	70.7	128.0	12.7	13.5	0.0	21.3	3.2
Tues	370.5	6.4	99.0	9.9	19.3	11.7	19.0	1.4
Wed	-	-	-	-	12.3	0.4	21.8	7.4
Thu	570.0	199.4	110.5	0.7	17.5	3.5	24.5	0.7
Fri	771.0	297.0	89.5	2.1	18.3	9.5	23.5	2.8
Sat	511.5	16.3	121.5	26.2	12.3	0.4	19.5	1.4
Sun	490.0	21.2	136.0	31.1	11.5	0.0	19.3	5.3

	Desethylene-ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	114.0	22.63	-	-	-	-	-	-
Tues	112.5	3.54	-	-	-	-	-	-
Wed	-	-	-	-	-	-	-	-
Thu	86.5	10.61	-	-	-	-	-	-
Fri	85.0	120.21	-	-	-	-	-	-
Sat	131.5	17.68	-	-	-	-	-	-
Sun	150.0	19.80	-	-	-	-	-	-

		(±)-Ofloxacin							
		Influent		Effluent		Upstream		Downstream	
		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
***R*-(+)-Ofloxacin**	Mon	18.0	4.2	10.5	2.1	<MQL	-	<MQL	-
	Tues	17.5	3.5	9.5	0.7	<MQL	-	<MQL	-
	Wed	-	-	-	-	<MQL	-	<MQL	-
	Thu	74.0	21.2	17.0	0.0	3.5	1.4	3.0	0.7
	Fri	35.5	0.7	13.0	1.4	<MQL	-	<MQL	-
	Sat	57.5	10.6	12.5	3.5	2.5	1.4	<MQL	-
	Sun	46.5	4.9	16.0	4.2	<MQL	-	<MQL	-
		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
***S*-(-)-Ofloxacin**	Mon	40.0	2.8	28.5	0.7	<MQL	-	3.0	1.4
	Tues	71.0	4.2	21.0	4.2	<MQL	-	<MQL	-
	Wed	-	-	-	-	<MQL	0.0	4.3	1.1
	Thu	159.0	49.5	29.0	4.2	3.5	2.8	5.8	1.1
	Fri	108.0	2.8	23.0	0.0	<MQL	-	3.0	0.0
	Sat	122.5	30.4	31.0	8.5	2.3	1.8	2.3	0.4
	Sun	101.0	1.4	38.5	12.0	<MQL	-	<MQL	-

		(±)-Ofloxacin-*N*-Oxide							
		Influent		Effluent		Upstream		Downstream	
		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
***R*-(+)-Ofloxacin-*N*-Oxide**	Mon	0	0.0	<MQL	-	<MQL	-	0.0	0.0
	Tues	0	0.0	0.0	0.00	0.0	0.00	0.0	0.0
	Wed	-	-	-	-	0.0	0.00	0.0	0.0
	Thu	<MQL		<MQL	-	0.0	0.00	0.0	0.0
	Fri	0	0.0	<MQL	-	<MQL	-	0.0	0.0
	Sat	<MQL	-	<MQL	-	0.0	0.00	<MQL	-
	Sun	<MQL	-	<MQL	-	<MQL	-	0.0	0.0
		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
***S*-(-)-Ofloxacin-*N*-Oxide**	Mon	<MQL	-	<MQL	-	0	0.00	<MQL	-
	Tues	0	0.0	<MQL	-	<MQL	-	0	0.0
	Wed	-	-	-	-	<MQL	-	0	0.0
	Thu	0	0.0	<MQL	-	<MQL	-	0	0.0
	Fri	<MQL	-	<MQL	-	<MQL	-	0	0.0
	Sat	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Sun	<MQL	-	<MQL	-	<MQL	-	<MQL	-

		(±)-Desmethyl-ofloxacin							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Tues	<MQL	-	<MQL	-	<MQL	-	-	-
	Wed	-	-	-	-	<MQL	-	-	-
	Thu	<MQL	-	<MQL	-	3.50	1.4	<MQL	-
	Fri	<MQL	-	<MQL	-	<MQL	-	-	-
	Sat	<MQL	-	<MQL	-	2.50	1.4	-	-
	Sun	<MQL	-	<MQL	-	<MQL	-	-	-
***S*-(-)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	-	-	11.5	3.5	-	-	-	-
	Tues	11.5	2.1	15.5	2.1	-	-	-	-
	Wed	-	-	-	-	-	-	-	-
	Thu	-	-	<MQL	-	-	-	-	-
	Fri	51.5	2.1	13	1.4	-	-	-	-
	Sat	36.5	3.5	<MQL	-	-	-	-	-
	Sun	18	2.8	16	4.2	-	-	-	-

		(±)-Flumequine							
		Influent		Effluent		Upstream		Downstream	
Flumequine-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	8	1.4	<MQL	-	<MQL	-	<MQL	-
	Tues	17	1.4	<MQL	-	<MQL	-	<MQL	-
	Wed	-	-	-	-	<MQL	-	<MQL	-
	Thu	28	5.7	<MQL	-	<MQL	-	<MQL	-
	Fri	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Sat	7	1.4	<MQL	-	<MQL	-	<MQL	-
	Sun	8.5	3.5	<MQL	-	<MQL	-	<MQL	-
Flumequine-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	-	-	<MQL	-	<MQL	-	<MQL	-
	Tues	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Wed	-	-	-	-	<MQL	-	<MQL	-
	Thu	13.5	3.5	<MQL	-	<MQL	-	<MQL	-
	Fri	-	-	<MQL	-	<MQL	-	<MQL	-
	Sat	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Sun	<MQL	-	<MQL	-	<MQL	-	<MQL	-

	Nalidixic acid							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	< MQL	-	2.5	0.7	1.0	0.0	1.5	0
Tues	-	-	2.5	0.7	1.5	1.4	1.0	0
Wed	-	-	-	-	1.0	0.0	0.7	0.3
Thurs	32.0	17.0	3.5	0.7	1.5	0.7	1.2	0.3
Fri	-	-	2.0	0.0	1.3	0.3	1.5	0
Sat	-	-	2.0	0.0	0.8	0.3	1.5	0
Sun	-	-	2.0	0.0	0.5	0.0	1.0	0

	Norfloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	-	-	<MQL	-	<MQL	-	-	-
Tues	-	-	68.0	29.7	<MQL	-	-	-
Wed	-	-	-	-	<MQL	-	-	-
Thurs	-	-	<MQL	-	<MQL	-	-	-
Fri	-	-	<MQL	-	<MQL	-	-	-
Sat	-	-	<MQL	-	<MQL	-	-	-
Sun	-	-	67.0	2.8	<MQL	-	-	-

	Lomefloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	-	-	3.5	0.707	<MQL	-	<MQL	-
Tues	-	-	<MQL	-	<MQL	-	<MQL	-
Wed	-	-	-	-	<MQL	-	<MQL	-
Thurs	-	-	4	0.0	2.8	-	3	0.00
Fri	-	-	<MQL	-	<MQL	-	<MQL	-
Sat	-	-	<MQL	-	<MQL	-	<MQL	-
Sun	-	-	3	1.4	<MQL	-	<MQL	-

		(±)-Nadifloxacin							
		Influent		Effluent		Upstream		Downstream	
Nadifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	<MQL	-	-	-	-	-	-	-
	Tues	<MQL	-	<MQL	-	<MQL	-	-	-
	Wed	-	-	-	-	<MQL	-	-	-
	Thu	36.0	33.9	<MQL	-	<MQL	-	<MQL	-
	Fri	<MQL	-	<MQL	-	-	-	-	-
	Sat	<MQL	-	<MQL	-	-	-	-	-
	Sun	<MQL	-	<MQL	-	<MQL	-	-	-
Nadifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	-	-	-	-	-	-	-	-
	Tues	-	-	-	-	<MQL	-	-	-
	Wed	-	-	-	-	-	-	-	-
	Thu	15.5	10.6	<MQL	-	<MQL	-	-	-
	Fri	<MQL	-	<MQL	-	-	-	<MQL	-
	Sat	-	-	<MQL	-	-	-	<MQL	-
	Sun	-	-	<MQL	-	-	-	-	-

	Moxifloxacin-*N*-sulphate							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Moxifloxacin							
		Influent		Effluent		Upstream		Downstream	
***R,R*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
***S,S*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Prulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Prulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Prulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Ulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Ulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

	Besifloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table S11 Concentrations of the analytes in liquid environmental matrices (influent, effluent, river upstream and river downstream) during the monitoring week in WWTP D.

	Ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	1763.8	10.0	255.5	32.0	52.9	1.2	45.3	0.9
Tues	739.5	162.3	241.2	33.0	48.4	3.5	44.1	0.4
Wed	502.5	107.9	282.1	23.1	45.5	2.4	41.8	0.3
Thu	439.2	163.3	257.1	95.3	57.2	3.8	45.1	1.0
Fri	264.9	43.4	212.7	28.7	57.4	5.9	43.7	0.4
Sat	366.2	60.9	279.6	109.5	55.1	14.1	48.7	0.6
Sun	749.7	33.6	225.1	11.6	44.8	6.9	41.3	0.8

	Desethylene-ciprofloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	298.2	63.4	<MQL	-	n.d.	-	n.d.	-
Tues	135.6	24.5	<MQL	-	n.d.	-	n.d.	-
Wed	111.7	37.4	<MQL	-	n.d.	-	n.d.	-
Thu	196.5	47.7	<MQL	-	n.d.	-	n.d.	-
Fri	105.7	16.2	<MQL	-	n.d.	-	n.d.	-
Sat	148.4	26.2	<MQL	-	n.d.	-	n.d.	-
Sun	193.4	5.4	<MQL	-	n.d.	-	n.d.	-

		(±)-Ofloxacin							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	27.5	1.4	10.2	3.7	<MQL	-	<MQL	-
	Tues	8.1	0.0	10.0	0.5	<MQL	-	<MQL	-
	Wed	6.1	0.7	7.2	0.3	<MQL	-	<MQL	-
	Thu	41.8	17.9	17.8	3.3	3.2	1.0	4.9	0.4
	Fri	48.8	1.1	15.5	2.1	2.8	0.7	2.6	0.3
	Sat	18.9	3.7	15.0	6.9	2.7	1.1	<MQL	-
	Sun	12.6	0.8	11.6	1.7	<MQL	-	<MQL	-
***S*-(-)-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	57.0	5.2	20.2	7.0	3.7	0.2	4.3	0.4
	Tues	14.2	0.5	20.3	0.1	3.9	0.7	4.7	1.0
	Wed	10.8	3.4	14.4	2.6	4.3	1.1	3.6	0.5
	Thu	73.4	22.4	36.4	10.4	7.8	1.1	7.8	0.3
	Fri	73.3	2.0	28.1	4.6	5.8	0.6	5.3	0.7
	Sat	32.3	1.6	26.4	11.1	7.3	4.3	3.8	0.4
	Sun	22.5	2.0	22.3	2.3	4.3	2.6	3.2	0.1

		(±)-Ofloxacin-*N*-Oxide							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	<MQL	-	n.d.	-	n.d.	-
	Fri	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
***S*-(-)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	<MQL	-	n.d.	-	<MQL	-
	Tues	n.d.	-	<MQL	-	n.d.	-	<MQL	-
	Wed	n.d.	-	<MQL	-	n.d.	-	n.d.	-
	Thu	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Fri	<MQL	-	<MQL	-	n.d.	-	<MQL	-
	Sat	n.d.	-	<MQL	-	<MQL	-	<MQL	-
	Sun	<MQL	-	<MQL	-	<MQL	-	<MQL	-

		(±)-Desmethyl-ofloxacin							
		Influent		Effluent		Upstream		Downstream	
***R*-(+)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	11.9	8.9	<MQL	-	n.d.	-	n.d.	-
	Tues	10.3	4.3	<MQL	-	n.d.	-	n.d.	-
	Wed	6.8	9.6	<MQL	-	n.d.	-	n.d.	-
	Thu	<MQL	-	n.d.	-	n.d.	-	n.d.	-
	Fri	<MQL	-	<MQL	-	n.d.	-	n.d.	-
	Sat	<MQL	-	9.7	3.4	n.d.	-	n.d.	-
	Sun	9.0	12.7	<MQL	-	n.d.	-	n.d.	-
***S*-(-)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	52.0	14.1	31.4	23.4	n.d.	-	n.d.	-
	Tues	70.5	69.6	12.3	8.5	n.d.	-	n.d.	-
	Wed	42.3	3.5	27.4	2.5	n.d.	-	n.d.	-
	Thu	13.4	18.9	10.1	8.6	n.d.	-	n.d.	-
	Fri	19.6	27.7	8.8	2.8	n.d.	-	n.d.	-
	Sat	42.8	32.3	<MQL	-	n.d.	-	n.d.	-
	Sun	36.3	2.0	11.1	1.4	n.d.	-	n.d.	-

		(±)-Flumequine							
		Influent		Effluent		Upstream		Downstream	
Flumequine-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	6.9	9.7	11.6	3.5	<MQL	-	<MQL	-
	Tues	13.5	5.5	10.1	1.2	<MQL	-	<MQL	-
	Wed	14.5	6.6	9.0	1.8	<MQL	-	<MQL	-
	Thu	13.2	3.7	11.7	4.2	<MQL	-	<MQL	-
	Fri	6.0	0.4	11.8	2.0	<MQL	-	<MQL	-
	Sat	7.9	3.3	13.6	6.7	<MQL	-	<MQL	-
	Sun	8.6	2.5	14.6	6.1	<MQL	-	<MQL	-
Flumequine-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	5.6	1.3	<MQL	-	<MQL	-	<MQL	-
	Tues	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Wed	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Thu	6.6	2.1	<MQL	-	<MQL	-	<MQL	-
	Fri	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Sat	<MQL	-	<MQL	-	<MQL	-	<MQL	-
	Sun	<MQL	-	<MQL	-	<MQL	-	<MQL	-

	Nalidixic acid							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	12.5	10.9	,MQL	-	0.9	0.4	1.5	0.0
Tues	5.9	2.6	1.2	1.7	1.3	0.4	1.9	0.2
Wed	5.5	1.0	2.1	0.7	1.3	0.1	1.3	0.3
Thurs	2.3	3.2	2.5	1.1	1.8	1.0	1.6	0.5
Fri	3.0	1.3	0.9	1.2	1.9	0.6	1.0	0.1
Sat	1.8	0.0	2.0	2.9	1.8	1.0	2.4	0.6
Sun	1.7	2.4	2.1	1.7	1.2	1.0	1.5	0.3

	Norfloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	336.1	27.4	154.3	29.9	94.7	5.0	96.0	1.7
Tues	419.4	24.7	86.7	25.5	65.1	19.0	108.4	8.9
Wed	127.0	35.9	137.6	35.3	103.1	8.4	93.2	11.1
Thurs	99.7	5.5	135.9	17.3	65.4	47.2	86.3	17.8
Fri	<MQL	-	144.4	20.0	122.7	21.3	94.3	0.2
Sat	335.1	42.9	141.0	30.3	134.1	61.7	93.4	54.4
Sun	74.4	35.4	147.6	9.2	86.2	4.0	97.4	12.1

	Lomefloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Nadifloxacin							
		Influent		Effluent		Upstream		Downstream	
Nadifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	<MQL	-	<MQL	-
	Fri	n.d.	-	n.d.	-	n.d.	-	<MQL	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	<MQL	-	n.d.	-	n.d.	-
Nadifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

	Moxifloxacin-*N*-sulphate							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	<MQL	-	<MQL	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Moxifloxacin							
		Influent		Effluent		Upstream		Downstream	
***R,R*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
***S,S*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	44.1	13.1	<MQL	-	n.d.	-	n.d.	-
	Fri	23.4	4.1	<MQL	-	n.d.	-	n.d.	-
	Sat	41.1	25.4	<MQL	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Prulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Prulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Prulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

		(±)-Ulifloxacin							
		Influent		Effluent		Upstream		Downstream	
Ulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Ulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

	Besifloxacin							
	Influent		Effluent		River Upstream		River Downstream	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-	n.d.	-	n.d.	-

Table S12 Concentrations of the analytes in liquid environmental matrices (influent, effluent, river upstream and river downstream) during the monitoring week in WWTP E.

	Ciprofloxacin			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	420.5	126.6	130.5	19.1
Tues	479.5	154.0	123.0	19.8
Wed	635.5	120.9	133.5	40.3
Thu	394.5	77.1	142.0	56.6
Fri	257.5	84.1	115.5	2.1
Sat	389.0	162.6	217.5	14.8
Sun	408.5	16.3	153.0	4.2

	Desethylene-ciprofloxacin			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	334.1	58.4	82.1	6.8
Tues	368.4	250.3	80.0	1.4
Wed	373.8	18.6	74.6	4.4
Thu	171.9	19.1	73.5	10.1
Fri	171.6	47.7	72.3	1.4
Sat	221.3	138.0	79.9	9.3
Sun	334.1	58.4	75.6	6.8

		(±)-Ofloxacin			
		Influent		Effluent	
_R_-(+)-Ofloxacin		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	104.0	50.9	33.5	4.9
	Tues	150.0		32.0	9.9
	Wed	67.5	23.3	35.0	12.7
	Thu	65.0	15.6	37.5	14.8
	Fri	57.0	1.4	28.0	5.7
	Sat	80.0	39.6	64.5	0.7
	Sun	63.0	4.2	33.0	11.3
_S_-(-)-Ofloxacin		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	287.0	116.0	90.5	19.1
	Tues	450.0		91.5	10.6
	Wed	224.0	99.0	78.0	11.3
	Thu	284.5	96.9	100.0	36.8
	Fri	158.5	6.4	79.5	7.8
	Sat	253.5	142.1	166.5	6.4
	Sun	169.5	3.5	90.0	26.9

		(±)-Ofloxacin-*N*-Oxide			
		Influent		Effluent	
***R*-(+)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	4.5	2.1	2.5	0.7
	Tues	7.0	n.a.	3.0	0.0
	Wed	8.0	1.4	3.5	0.7
	Thu	5.5	0.7	2.5	0.7
	Fri	4.5	0.7	2.0	0.0
	Sat	4.0	1.4	3.5	0.7
	Sun	3.5	0.7	3.0	0.0
***S*-(-)-Ofloxacin-*N*-Oxide**		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	26.0	11.3	3.0	0.0
	Tues	41.0	26.9	3.5	0.7
	Wed	31.5	19.1	4.5	0.7
	Thu	18.0	9.9	3.5	0.7
	Fri	14.0	0.0	3.0	0.0
	Sat	13.5	12.0	3.5	0.7
	Sun	7.5	0.7	3.5	0.7

		(±)-Desmethyl-ofloxacin			
		Influent		Effluent	
***R*-(+)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-
***S*-(-)-Desmethyl-Ofloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-

		(±)-Flumequine			
		Influent		Effluent	
Flumequine-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	6.5	0.7
	Tues	31.0	n.a.	6.0	0.0
	Wed	n.d.	-	21.0	0.0
	Thu	n.d.	-	8.0	2.8
	Fri	n.d.	-	6.5	0.7
	Sat	n.d.	-	16.0	1.4
	Sun	4.0	1.4	0.0	0.0
Flumequine-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-

	Nalidixic acid			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	15.5	2.1	10.0	1.4
Tues	44.5	16.3	8.5	2.1
Wed	42.0	11.3	7.5	2.1
Thurs	26.0	7.1	11.5	4.9
Fri	27.5	17.7	14.5	0.7
Sat	40.0	7.1	25.0	1.4
Sun	34.5	2.1	11.5	0.7

	Norfloxacin			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	673.0	n.a.	183.5	37.5
Tues	n.d.	-	164.0	4.2
Wed	933.5	416.5	230.5	113.8
Thurs	186.0	n.a.	150.5	37.5
Fri	n.d.	-	170.5	21.9
Sat	436.0	264.5	251.5	16.3
Sun	n.d.	-	176.5	6.4

	Lomefloxacin			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-

		(±)-Nadifloxacin			
		Influent		Effluent	
Nadifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	<MQL	-
	Wed	n.d.	-	<MQL	-
	Thu	<MQL	-	<MQL	-
	Fri	n.d.	-	<MQL	-
	Sat	n.d.	-	n.d.	-
	Sun	<MQL	-	n.d.	-
Nadifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	<MQL	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-

	Moxifloxacin-*N*-sulphate			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-

		(±)-Moxifloxacin			
		Influent		Effluent	
***R,R*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-
***S,S*-Moxifloxacin**		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	<MQL	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	<MQL	-
	Sat	n.d.	-	<MQL	-
	Sun	n.d.	-	n.d.	-

		(±)-Prulifloxacin			
		Influent		Effluent	
Prulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-
Prulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-

		(±)-Ulifloxacin			
		Influent		Effluent	
Ulifloxacin-E1		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-
Ulifloxacin-E2		Conc. (ng/L)	SD	Conc. (ng/L)	SD
	Mon	n.d.	-	n.d.	-
	Tues	n.d.	-	n.d.	-
	Wed	n.d.	-	n.d.	-
	Thu	n.d.	-	n.d.	-
	Fri	n.d.	-	n.d.	-
	Sat	n.d.	-	n.d.	-
	Sun	n.d.	-	n.d.	-

	Besifloxacin			
	Influent		Effluent	
	Conc. (ng/L)	SD	Conc. (ng/L)	SD
Mon	n.d.	-	n.d.	-
Tues	n.d.	-	n.d.	-
Wed	n.d.	-	n.d.	-
Thurs	n.d.	-	n.d.	-
Fri	n.d.	-	n.d.	-
Sat	n.d.	-	n.d.	-
Sun	n.d.	-	n.d.	-

Table S13 Concentrations of qnrS gene in influent and effluent wastewater during the monitoring week in all the sites.

qnrS		
Sample	Copies/microliter	CI Copies/microliter
Town B influent Thursday	0.502	0.209 -- 1.205
Town B influent Sunday	0.181	4.52E-2 -- 0.723
Town B effluent Thursday	0.460	0.191 -- 1.105
Town B effluent Sunday	7.61E-02	1.07E-2 -- 0.54
Town D influent Thursday	0.761	0.396 -- 1.462
Town D influent Thursday	0.658	0.314 -- 1.38
Town D influent Sunday	1.994	1.347 -- 2.951
Town D influent Sunday	2.076	1.367 -- 3.152
Town D effluent Thursday	0.394	0.148 -- 1.049
Town D effluent Sunday	1.018	0.382 -- 2.71
Town A influent Friday	8.51E-02	1.20E-2 -- 0.604
Town A influent Sunday	1.915	1.154 -- 3.176
Town A effluent Friday	1.229	0.728 -- 2.076
Town A effluent Sunday	1.748	1.14 -- 2.681
City E SPM day 2 Thursday	20.229	17.635 -- 23.205
City E SPM day 5 Sunday	35.502	32.237 -- 39.098
City E influent day 1 Wednesday	0.951	0.527 -- 1.718
City E influent day 1 Wednesday	1.286	0.775 -- 2.132
City E influent day 2 Thursday	0.983	0.529 -- 1.827
City E influent day 2 Thursday	0.681	0.325 -- 1.429
City E influent day 3 Friday	0.424	0.177 -- 1.019
City E influent day 3 Friday	0.406	0.169 -- 0.975
City E influent day 4 Saturday	0.628	0.3 -- 1.318
City E influent day 4 Saturday	0.225	7.25E-2 -- 0.697
City E influent day 5 Sunday	0.0761	1.07E-2 -- 0.54
City E influent day 5 Sunday	0.550	0.262 -- 1.154
City E influent day 6 Monday	1.742	1.136 -- 2.671
City E influent day 6 Monday	0.791	0.412 -- 1.52
City E influent day 7 Tuesday	1.158	0.672 -- 1.994
City E influent day 7 Tuesday	0.937	0.519 -- 1.693
City E effluent day 2 Thursday	0.498	0.224 -- 1.108
City E effluent day 3 Friday	0.808	0.404 -- 1.616
City E effluent day 4 Saturday	0.703	0.292 -- 1.688
City E effluent day 5 Sunday	1.415	0.853 -- 2.348
City E effluent day 6 Monday	4.184	3.18 -- 5.506
City E effluent day 7 Tuesday	1.833	1.183 -- 2.841
City C influent Thursday	0.614	0.293 -- 1.288
City C influent Thursday	0.653	0.293 -- 1.454
City C influent Sunday	1.559	0.995 -- 2.444
City C influent Sunday	1.706	1.134 -- 2.567
City C effluent Thursday	0.444	0.185 -- 1.068
City C effluent Thursday	1.167	0.663 -- 2.055
City C effluent Sunday	0.188	0.110 – 0.234
City C effluent Sunday	0.419	0.387 – 0.499

Table S14 Concentrations of the analytes in suspended particulate matter from influent wastewater during the monitoring week in WWTP A.

	Concentration ± SD (ng/g)						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Ciprofloxacin	98.3 ± 31.4	55.9 ± 6.2	99.3 ± 7.4	45.8 ± 5.2	73.0 ± 10.0	58.5 ± 10.7	280.0 ± 57.3
S-(-)-Ofloxacin	99.0 ± 18.8	58.9 ± 3.0	79.7 ± 12.9	74.3 ± 7.3	83.9 ± 6.5	140.0 ± 3.2	122.1 ± 32.3
R-(+)-Ofloxacin	6.4 ± 2.8	17.3 ± 2.8	13.0 ± 5.8	7.5 ± 2.7	8.5 ± 1.8	4.2 ± 3.2	5.7 ± 2.9
Norfloxacin	213.7 ± 17.9	92.6 ± 19.6	-	68.8 ± 25.2	-	-	-
Nalidixic acid	1.4 ± 0.4	0.5 ± 0.2	0.8 ± 0.5	0.3 ± 0.1	0.2 ± 0.0	0.5 ± 0.2	0.6 ± 0.1
R,R-Moxifloxacin	-	-	-	-	-	-	-
S,S-Moxifloxacin	-	-	-	-	-	-	-
Moxifloxacin-*N*-sulphate	-	-	-	-	-	-	-
S-(-)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
R-(+)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
S-(-)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
R-(+)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
Desethylene-ciprofloxacin	27.0 ± 1.7	16.6 ± 1.8	14.8 ± 0.6	13.8 ± 1.0	16.2 ± 0.9	17.5 ± 0.8	16.8 ± 0.5
E1-Flumequine	3.9 ± 1.0	1.2 ± 0.8	1.9 ± 0.5	0.9 ± 0.1	0.9 ± 0.8	2.0 ± 0.3	3.3 ± 0.2
E2-Flumequine	1.7 ± 0.5	0.2 ± 0.2	0.4 ± 0.4	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.4	0.6 ± 0.5
E1-Prulifloxacin	-	-	-	-	-	-	-
E2-Prulifloxacin	-	-	-	-	-	-	-
Besifloxacin	-	-	-	-	-	-	-
Nadifloxacin-E1	-	-	-	-	-	-	-
Nadifloxacin-E2	-	-	-	-	-	-	-
Lomefloxacin	-	-	-	-	-	-	-
Ulifloxacin-E1	-	-	-	-	-	-	-
Ulifloxacin-E2	-	-	-	-	-	-	-

Table S15 Concentrations of the analytes in suspended particulate matter from influent wastewater during the monitoring week in WWTP B.

	Concentration ± SD (ng/g)						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Ciprofloxacin	4.5 ± 0.4	5.7 ± 0.3	6.7 ± 0.6	5.3 ± 0.5	4.9 ± 0.4	4.7 ± 0.8	5.7 ± 0.6
S-(-)-Ofloxacin	326.7 ± 188.5	180.5 ± 51.9	128.6 ± 29.4	160.0 ± 29.2	168.6 ± 64.3	160.5 ± 84.9	262.5 ± 69.6
R-(+)-Ofloxacin	232.6 ± 163.8	103.5 ± 25.9	80.9 ± 15.4	99.2 ± 13.9	139.3 ± 76.6	131.1 ± 63.5	223.4 ± 77.8
Norfloxacin	38.9 ± 9.1	73.1 ± 10.9	116.5 ± 22.3	61.0 ± 4.8	75.7 ± 9.5	80.7 ± 20.1	57.1 ± 7.7
Nalidixic acid	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
R,R-Moxifloxacin	-	-	-	-	-	-	-
S,S-Moxifloxacin	-	-	-	-	-	-	-
Moxifloxacin-*N*-sulphate	-	-	-	-	-	-	-
S-(-)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
R-(+)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
S-(-)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
R-(+)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
Desethylene-ciprofloxacin	19.6 ± 0.3	24.8 ± 2.0	24.0 ± 3.3	19.5 ± 2.3	19.7 ± 0.8	17.6 ± 2.5	19.4 ± 1.3
E1-Flumequine	-	-	<MQL	-	<MQL	-	-
E2-Flumequine	<MQL	-	<MQL	-	-	<MQL	<MQL
E1-Prulifloxacin	-	-	-	-	-	-	-
E2-Prulifloxacin	-	-	-	-	-	-	-
Besifloxacin	-	-	-	-	-	-	-
Nadifloxacin-E1	-	-	-	-	-	-	-
Nadifloxacin-E2	-	-	-	-	-	-	-
Lomefloxacin	-	-	-	-	-	-	-
Ulifloxacin-E1	-	-	-	-	-	-	-
Ulifloxacin-E2	-	-	-	-	-	-	-

Table S16 Concentrations of the analytes in suspended particulate matter from influent wastewater during the monitoring week in WWTP C.

	Concentration ± SD (ng/g)						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Ciprofloxacin	116.2 ± 11.6	74 ± 10.33	170.62 ± 39.7	80 ± 2.68	53.8 ± 10.8	104.1 ± 29.3	98.89 ± 12.86
S-(-)-Ofloxacin	109.63 ± 13.77	77.68 ± 8.21	101.94 ± 20.26	100.26 ± 6.55	106.61 ± 6.61	116.73 ± 20.25	107.28 ± 17.77
R-(+)-Ofloxacin	32.43 ± 4.48	18.22 ± 3.20	46.07 ± 1.50	35.42 ± 3.36	29.57 ± 1.60	41.40 ± 1.31	44.72 ± 11.92
Norfloxacin	-	-	-	-	-	-	-
Nalidixic acid	-	-	-	-	-	-	-
R,R-Moxifloxacin	-	-	-	-	-	-	-
S,S-Moxifloxacin	-	-	-	-	-	-	-
Moxifloxacin-*N*-sulphate	-	-	-	-	-	-	-
S-(-)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
R-(+)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
S-(-)-Desmethyl-Ofloxacin	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
R-(+)-Desmethyl-Ofloxacin	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Desethylene-ciprofloxacin	130.85 ± 37.55	87.36 ± 26.13	37.70 ± 2.05	71.77 ± 21.73	52.63 ± 12.20	82.81 ± 3.58	131.07 ± 32.97
E1-Flumequine	2.00 ± 0.5	2.40 ± 0.58	3.45 ± 1.1	1.20 ± 0.43	1.53 ± 0.42	1.35 ± 0.41	1.45 ± 0.34
E2-Flumequine	0.65 ± 0.41	0.80 ± 0.16	1.60 ± 0.99	0.35 ± 0.1	0.47 ± 0.19	0.65 ± 0.3	0.55 ± 0.19
E1-Prulifloxacin	-	-	-	-	-	-	-
E2-Prulifloxacin	-	-	-	-	-	-	-
Besifloxacin	-	-	-	-	-	-	-
Nadifloxacin-E1	-	-	-	-	-	-	-
Nadifloxacin-E2	-	-	-	-	-	-	-
Lomefloxacin	-	-	-	-	-	-	-
Ulifloxacin-E1	-	-	-	-	-	-	-
Ulifloxacin-E2	-	-	-	-	-	-	-

Table S17 Concentrations of the analytes in suspended particulate matter from influent wastewater during the monitoring week in WWTP D.

	Concentration ± SD (ng/g)						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Ciprofloxacin	397.1 ± 282.9	126.4 ± 42.6	236.7 ± 14.2	92.1 ± 31.8	372.6 ± 258.3	87.2 ± 15.3	130.6 ± 58.
S-(-)-Ofloxacin	112.3 ± 55.7	305.1 ± 202.2	112.0 ± 55.1	141.4 ± 81.4	134.6 ± 70.5	48.0 ± 1.7	56.8 ± 6.8
R-(+)-Ofloxacin	69.4 ± 16.1	278.6 ± 202.9	92.4 ± 53.1	56.6 ± 14.5	118.4 ± 80.1	25.6 ± 6.5	41.2 ± 5.0
Norfloxacin	-	-	-	100.1 ± 36.8	299.9 ± 12.3	173.9 ± 60.0	-
Nalidixic acid	-	-	-	-	-	-	-
R,R-Moxifloxacin	-	-	-	-	-	-	-
S,S-Moxifloxacin	-	-	-	-	-	61.4 ± 3.7	-
Moxifloxacin-*N*-sulphate	-	-	-	-	-	-	-
S-(-)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
R-(+)-Ofloxacin-*N*-oxide	-	-	-	-	-	-	-
S-(-)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
R-(+)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
Desethylene-ciprofloxacin	201.4 ± 7.5	71.1 ± 19.8	54.6 ± 8.5	79.4 ± 24.2	58.1 ± 12.9	159.5 ± 31.2	63.0 ± 28.5
E1-Flumequine	1.5 ± 0.7	1.4 ± 0.5	2.0 ± 0.6	1.8 ± 0.8	2.2 ± 0.6	3.0 ± 0.2	2.3 ± 1.0
E2-Flumequine	0.5 ± 0.1	0.5 ± 0.4	<MQL	0.4 ± 0.3	0.4 ± 0.1	0.7 ± 0.1	1.1 ± 2.2
E1-Prulifloxacin	-	-	-	-	-	-	-
E2-Prulifloxacin	-	-	-	-	-	-	-
Besifloxacin	-	-	-	-	-	-	-
Nadifloxacin-E1	-	-	-	-	-	-	-
Nadifloxacin-E2	-	-	-	-	-	-	-
Lomefloxacin	-	-	-	-	-	-	-
Ulifloxacin-E1	-	-	-	-	-	-	-
Ulifloxacin-E2	-	-	-	-	-	-	-

Table S18 Concentrations of the analytes in suspended particulate matter from influent wastewater during the monitoring week in WWTP E.

	Concentration ± SD (ng/g)						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Ciprofloxacin	51.0 ± 15.7	28.7 ± 20.3	71.3 ± 36.3	50.2 ± 13.6	118.5 ± 94.7	118.4 ± 51.9	102.4 ± 36.9
S-(-)-Ofloxacin	289.4 ± 23.9	261.0 ± 60.7	172.6 ± 15.2	276.4 ± 34.1	165.8 ± 24.3	205.3 ± 17.3	208.7 ± 32.4
R-(+)-Ofloxacin	79.0 ± 8.6	76.8 ± 15.0	60.7 ± 4.5	76.4 ± 23.4	63.5 ± 11.1	75.3 ± 12.6	78.9 ± 5.3
Norfloxacin	-	57.8 ± 28.0	40.6 ± 9.4	63.5 ± 2.6	62.7 ± 1.7	-	-
Nalidixic acid	5.5 ± 2.5	4.1 ± 3.4	12.0 ± 5.0	11.0 ± 2.8	11.0 ± 4.6	7.0 ± 5.8	9.7 ± 3.6
R,R-Moxifloxacin	-	-	-	-	-	-	-
S,S-Moxifloxacin	-	-	-	-	-	-	-
Moxifloxacin-*N*-sulphate	-	-	-	-	-	-	-
S-(-)-Ofloxacin-*N*-oxide	-	-	-	-	<MQL	<MQL	<MQL
R-(+)-Ofloxacin-*N*-oxide	-	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
S-(-)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
R-(+)-Desmethyl-Ofloxacin	-	-	-	-	-	-	-
Desethylene-ciprofloxacin	-	-	-	-	-	-	-
E1-Flumequine	3.4 ± 1.5	6.9 ± 1.8	5.6 ± 2.7	2.4 ± 0.8	1.3 ± 0.6	2.6 ± 0.6	1.9 ± 0.4
E2-Flumequine	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.4	0.7 ± 0.3	0.4 ± 0.1	0.6 ± 0.3	0.4 ± 0.2
E1-Prulifloxacin	-	-	-	-	-	-	-
E2-Prulifloxacin	-	-	-	-	-	-	-
Besifloxacin	-	-	-	-	-	-	-
Nadifloxacin-E1	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Nadifloxacin-E2	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Lomefloxacin	-	-	-	-	-	-	-
Ulifloxacin-E1	-	-	-	-	-	-	-
Ulifloxacin-E2	-	-	-	-	-	-	-

Table S19 Ratio between ciprofloxacin and its metabolite in all the sites.

RATIO	Town A	Town B	City C	Town D	City E
Monday		1.1	2.2	5.9	1.3
Tuesday	9.5	1.5	3.3	5.5	1.3
Wednesday	9.0	3.7		4.5	1.7
Thursday	5.0	2.5	6.6	2.2	2.3
Friday	5.8	3.8	9.1	2.5	1.5
Saturday	5.5	3.0	3.9	2.5	1.8
Sunday	5.6	3.4	3.3	3.9	1.2
AV	6.7	2.7	4.7	3.9	1.6
SD	2.0	1.1	2.6	1.5	0.4