Environmental Sciences and Engineering

Dynamics of antibiotics concentrations in the environment at different time scales

Master Thesis

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Abstract

The aim of this study was to assess the most relevant time scales of antibiotics concentration variations at the sewage treatment plant (STP) inlet and to create a model for the simulation of short-term concentration variations.

During one year six antibiotics (ciprofloxacin, clindamycin, metronidazole, norfloxacin, ofloxacin, and trimethoprim) were being successfully and repeatedly measured at the inlet of the STP of Vidy in Lausanne. The campaigns were arranged that way that seasonal, daily and hourly mass flow rate variations of the antibiotics could be analysed. The measurement campaigns confirmed the occurrence of seasonal antibiotics variations. The hourly variations showed about the same order of magnitude as the seasonal ones. The day-to-day variations were in general found to be not as heavy as seasonal and hourly variations.

A model simulating the antibiotics concentrations at the STP inlet on an hourly basis has been developed. It is applied for three antibiotics (ciprofloxacin, norfloxacin and trimethoprim) in the catchment area of the STP of Vidy in Lausanne. The modelled antibiotics concentrations have in some cases another order of magnitude compared to the measurements. This could be due to seasonal effects that cannot be adjusted by the calibration such as the antibiotics losses during sewer transit that are not known a priori. It could also be due to hypotheses made on the amount of consumed antibiotics. The modelled detailed hourly dynamics are limited in their validity because of the random noise due to the Monte Carlo modelling approach. Moreover, the uncertainties concerning the toilet use attitudes are limiting for these short-term dynamics. The view on the general modelled dynamic throughout the day reveals a first antibiotic peak in the morning between 07:00 and 10:00 and a second one in towards evening between 17:00 and 21:00 which corresponds perfectly to the findings of the measurement analysis.

The development of the model allowed identifying the most important parameters contributing to short-term antibiotics dynamics namely the amount of consumed antibiotic and the number of persons administering the medicine, the behaviour of the antibiotics' accumulation in the urine and the toilet use habits.

Résumé

L'objective de ce projet était d'évaluer les échelles de temps les plus importantes des variations de concentrations d'antibiotiques à l'entrée de la station d'épuration des eaux usées (STEP) et de créer un modèle pour la simulation des variations de concentrations à court-terme.

Pendant une année, six antibiotiques (ciprofloxacine, clindamycine, métronidazole, norfloxacine, ofloxacine et triméthoprime) ont été mesurés à succès et de façon répétée à l'entrée de la STEP de Vidy à Lausanne. Les campagnes de mesures ont été faites de manière à ce que les variations du flux de masse des antibiotiques puissent être analysées aux échelles saisonnières, journalières et d'heure en heure. La présence des variations saisonnières des antibiotiques a été confirmée. Les variations durant la journée ont montrées avoir unefréquence du même ordre de grandeur que les variations saisonnières. Les variations d'une journée à l'autre ont généralement été obsérvées d'être moins importantes que les variations saisonnières ainsi que celles d'heure en heure.

Un modèle qui simule les concentrations d'antibiotiques à la résolution d'une heure a été développé. Il est appliqué pour trois substances (ciprofloxacine, norfloxacine et triméthoprime) dans le réseau d'assainissement de la STEP de Vidy à Lausanne. Dans certains cas les concentrations d'antibiotiques modélisées ont des différents ordres de grandeur par rapport aux mesures. Cela pourrait être dû à des effets saisonniers non ajustés par la calibration comme des pertes d'antibiotiques pendant leur passage à la STEP qui ne sont pas connues a priori. En outre cela pourrait être dû à des hypothèses faites sur la masse d'antibiotiques consommée. Les dynamiques détaillées d'une heure à l'autre générées par le modèle sont limitées dans leur force d'expression en raison du bruit du caractère fortuit de l'approche Monte Carlo du modèle. De plus, les incertitudes concernant les attitudes d'utilisation des toilettes limitent ces dynamiques à court-terme. La vue sur la dynamique modélisée en général pendant la journée montre un premier pic d'antibiotiques le matin entre 7 heures et 10 heures et un deuxième le soir entre 17 heures et 21 heures. Cela correspond exactement aux résultats de l'analyse des mesures.

Le développement du modèle a permis d'identifier les paramètres contribuant le plus aux dynamiques des antibiotiques à court-terme. Ceux-ci sont la masse d'antibiotiques consommés et le nombre de personnes qui en ingèrent, le comportement de l'accumulation des antibiotiques dans l'urine et les habitudes d'utilisation des toilettes.

Zusammenfassung

Das Ziel dieser Arbeit war es Schwankungen von Antibiotikakonzentrationen im Zufluss der Abwasserreinigungsanlage (ARA) auf deren bedeutendsten Zeiträume zu evaluieren sowie ein Modell zu schaffen, das kurzfristige Konzentrationsschwankungen simuliert.

Während eines Jahres wurden sechs Antibiotika (Ciprofloxacin, Clindamycin, Metronidazol, Norfloxacin, Ofloxacin und Trimethoprim) wiederholt und erfolgreich im Zufluss zur ARA von Vidy in Lausanne gemessen. Die Messkampagnen waren so angeordnet, dass der Antibiotikamassenfluss auf saisonale, tägliche und stündliche Schwankungen analysiert werden konnte. Die Messkampagnen bestätigten das Auftreten von saisonalen Antibiotikaschwankungen. Die stündlichen Schwankungen zeigten sich in ungefähr denselben Grössenordnungen wie die saisonalen Schwankungen. Die Schwankungen der Tagesmittel von einem Tag zum nächsten, wurden im Allgemeinen als nicht so bedeutend befunden wie die saisonalen und stündlichen Konzentrationsschwankungen.

Es wurde ein Modell entwickelt, das mit stündlicher Auflösung Antibiotikakonzentrationen im ARA-Zufluss simuliert. Das Modell wurde auf drei Antibiotika (Ciprofloxacin, Norfloxacin und Trimethoprim) im Einzugsgebiet der ARA von Vidy in Lausanne angewendet. Die simulierte Antibiotikakonzentrationen haben verglichen mit den gemessenen teilweise andere Grössenordnungen. Dies könnte mit saisonalen Effekten zu tun haben, die in der Kalibration nicht ausgeglichen werden können wie zum Beispiel mögliche Antibiotikaverluste während des Transportes durch die Kanalisation. Es könnte aber auch wegen Annahmen zur konsumierten Antibiotikamenge sein. Die simulierte stündliche Dynamik hat im Detail etwas beschränkte Aussagekraft wegen des Zufallsrauschens des Monte Carlo Modellansatzes. Zudem sind auch Unsicherheiten rund um die Verhaltensweisen der Toilettenbenützung limitierend für diese kurzfristigen Dynamiken. Die Ansicht der simulierten Tagesdynamik im Allgemeinen bringt zwischen 7 Uhr und 10 Uhr einen ersten Spitzenwert der Antibiotikakonzentration hervor und einen zweiten zwischen 17 Uhr und 21 Uhr. Dies entspricht genau den Befunden der Analyse der Antibiotikamessungen.

Das Entwickeln des Modells erlaubte die wichtigsten Parameter zu erkennen, die zu den beachtlichen kurzzeitlichen Dynamiken beitragen. Dies sind nämlich die konsumierte Antibiotikamenge sowie die Anzahl der Konsumentinnen und Konsumenten, das Akkumulationsverhalten der Antibiotika im Urin und die Gewohnheiten der WC-Benützung.

Abbreviations

ARA	Abwasserreinigungsanlage
CSO	Combined sewer overflow
LOD	Limit of detection
LOQ	Limit of quantification
MFR	Mass flow rate
STEP	Station d'épuration des eaux usées
STP	Sewage treatment plant
WTP	Wastewater treatment plant

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1 Introduction

1.1 Antibiotics in the aquatic environment

The increasing worldwide contamination of freshwater is a crucial environmental problem asking for sustainable global strategies and solutions. Micropollutants are an important matter in the contamination of surface and ground waters [1, 2]. Considering freshwater as a source of food, drinking water and recreation this concerns not only environmental aspects but also economic and social interests [1, 3].

Antibiotics are part of the micropollutants occurring only in low concentrations but having still an ecotoxicological hazard potential [1, 4]. The antibiotics originate mainly from the medical treatment of humans as well as from agriculture. Antibiotics used for the treatment of human infections are excreted by urine and faeces and reach the surface water by incomplete extraction from the wastewater at the STP or by the direct release into the environment during rain events through combined sewer overflows (CSO) [2, 4, 5]. One of the main problems related to antibiotics in the aquatic environment is the emergence of bacterial resistance [1, 6, 7].

In the review of Schwarzenbach et al. [1] three broad scientific challenges concerning micropollutants in the aquatic environment are discussed addressing this issue not only in the frame of environmental protection but also in the context of human health in a sustainable sense. Firstly, the micropollutants' impact on the aquatic environment is to be assessed. Secondly, their impact is to be reduced and the water contamination by micropollutants should be avoided by appropriate treatment technologies, and thirdly a preventive management of water quality is to be achieved by a fewer introduction of micropollutants through more environmentally friendly usage and disposal strategies. To assess the ecotoxicological impact of antibiotics in the aquatic environment and to find good strategies to treat wastewater and contaminated sites it is important to have thorough knowledge about the distribution and fate of the antibiotics and at what concentration they occur in the aquatic environment [1, 3].

In that context, antibiotics measurements can be very contributively. Models are against this less suitable for predicting concentrations for real world situations. If however, good information on consumption and excretion is available there can be good agreement between modelled and measured values. Models can be very cost-effective compared to measurement campaigns for example when it comes to investigations in larger areas or if the concentration of a given pollutant is very low and difficult to measure [3]. So, both, measurements and modeling are helpful methods for a better and detailed understanding and quantification of antibiotics in the environment but they are not equally suitable for all applications.

They are also the tools chosen in this study to investigate the antibiotics dynamics in wastewater. The measurements are used to analyse the antibiotic occurrence at the hourly, daily and monthly time scale. The model concentrates on short-term variations within a day.

1.2 Antibiotics measurements

There are a lot of studies having effectuated micropollutant measurements in wastewater quantifying them in the wastewater, activated sludge or STP effluent [8], [5]. They either explore the fate of the micropollutants in the wastewater like also the present study, or they investigate on sampling methods of micropollutants [9], [10]. Table 1 shows some examples in more details.

Depending on the purposes of a measurement campaign a certain sampling mode is chosen. When for example the occurrence of a substance is to be proved the grab sampling mode can be applied. In this case one or a number of samples are taken independent of time, the wastewater flow rate or to each other. When the development of the concentration of a micropollutant in wastewater for a certain time period (e.g. during 24 hours) is being analysed, the continuous flow-proportional sampling mode or the discrete flow-proportional sampling mode with a high sampling frequency should be chosen. Since the flow rate in a sewer is very probable to vary significantly in the course of the day it is continuously measured and the wastewater is collected depending on the measured flow rate. In the case of discrete flow-proportional sampling a certain number of subsamples containing each a flow-proportional amount of wastewater are put together to obtain a composite sample. Composite samples then represent the average micropollutant concentration of the time period during which their subsamples have been collected [9].

Table 1: Studies in which micropollutant concentrations in wastewater were being measured to investigate sampling methods or the origin and fate of micropollutants in the wastewater, STP or in the environment

References	Goal / Substances	Sampling mode	Sampling frequency	Sampling period	Sampling location	Measuring unit
Ort, Lawrence, Reungoat et al. (2010) [10]	Comparison of different sampling modes and optimisation Substance: Gadolinium	Diverse, for example: • grab sampling • continuous flow-proportional sampling • discrete volume proportional sampling • discrete time-proportional sampling	• 2 min • - • 1 per 400 m^3 • 1 per hour	• 4 hours • 5 days • 5 days • 5 days	Influent of STP (gravity-fed, 100'000 inhabitants, separate sewer system)	• Load [g/min] • Concentration [µg/l] • Concentration [µg/l] • Concentration [µg/l]
Managaki et al. (2008) [5]	Diurnal behaviour of pharmaceuticals and gross organic pollutants Diverse Substances, among others: • Sulfamethoxazole • Clarithromycin	• No declaration • No declaration	• 30 minutes • 1 hour	• 24 hours • 24 hours	Influent of STP (5500 inhabitants, combined sewer system (± dry weather conditions))	• Load [µg/s]
Plósz et al. (2010) [8]	Diurnal variations and fate of hormones and antibiotics Hormones: • Estrone • Estriol Antibiotics:	8 hours flow-proportional composite sampling	• 15 minutes	• 3 days	• Effluent of primary clarifier (≈ pre-clarified influent of STP) • Effluent of Anoxic and aerobic treatment tank (≈ effluent of STP	• Load [g/8h] • Concentration [ng/l]

	• Ciprofloxacin • Sulfamethoxazole • Trimethoprim • Tetracycline					
Heberer et al. (2005) [11]	Contribution of hospital and household effluents to total load of • Carbamazepine • Diclofenac	24 h composite sampling, no declaration about time-, volume- or flow-proportional sampling but flow rate was measured continuously	• No declaration	• 7 days	• Hospital effluent • Sewage pumping station(96'000 inhabitants, 4 additional hospitals • Influent and effluent of STP (1 Mio inhabitants + 12060 hospital beds)	• Load [g/week]
Göbel et al. (2007) [12]	Fate of antibiotics in different wastewater treatment technologies, among others: • Azithromycin • Clarithromycin • Trimethoprim • Sulfamethoxazole	• 24 h composite flow-proportional sampling • Ditto • Ditto • Ditto • Ditto • Grab samples	• No declaration	• 1 week	• raw influent of STP • effluent of primary clarifier of STP • effluent of secondary clarifier of STP • effluent of sand filter • other places in STP (55'000 population equivalents, combined sewer system)	• Concentration [µg/l]

1.3 Modelling of antibiotics

There are also some examples of models predicting the concentration of different micropollutants at the STP inlet, effluent or in the environment (see Table 2) [13], [14], [15], [11]. According to Johnson et al. [3] a model should contain information about consumption and excretion to obtain reliable results. Some of the models presented in Table 2 go even more in detail by taking into account also the mode of application (oral, dermal etc.) and the transformations or losses during sewer transit [11, 13].Nevertheless, none of the models predicts the micropollutants load on a temporal resolution of less than one day as it can be seen in Table 2.

Table 2: Studies in which micropollutant concentrations in wastewater were being modelled on different temporal resolutions

References	Temporal resolution	Substances	Result / remarks
Johnson et al. (2004) [13]	1 day	Steroid estrogens: • Estradiol • Estrone • Ethinylestradiol	Concentration in the STP influent and effluent
Rowney et al. (2009) [14]	1 day	Diverse; among others: • Azithromycin • Ciprofloxacin • Clarithromycin • Trimethoprim	Mass flow rate (MFR) in the STP influent, concentration in the effluent and in the environment as well as drinking water exposure
Ort et al. (2010) [15]	1 day	• Carbamazepine • Diclofenac	MFR in STP influent and effluent and in the environment Consumption data assumed to be evenly distributed throughout the year
Heberer et al. (2005) [11]	1 week	• Carbamazepine • Diclofenac	Mass flow rate in the effluents of military hospital, sewage pumping station (= MFR in the waste water before STP) and municipal STP

1.4 Objectives

There are studies pointing out that antibiotics concentrations in wastewater show seasonal variations [7] [16] [17]. In contrast to these long-term variations, short-term variations are not

very properly known yet and as seen before, they are not predicted by the models considered in Table 2, nor has there been found any other model predicting micropollutants concentration on a high temporal resolution in the course of this study. However, they are important for the accuracy of research. A concrete example is the choice of the sampling frequency for antibiotics measurements which depends on the frequency of concentration variations [9]. And also in the context of environmental impacts a proper knowledge of short-term variations is important be it for example for the optimisation of modern removal techniques of micropollutants as well as in the context of the direct inflow into surface waters by CSOs [18].

The measurements used in this study allow analysing the antibiotics dynamics at the monthly, daily and hourly time scale. The objective of the analysis of the measurements is to

identify the dominant time scale of antibiotics concentration variations in the wastewater.

Considering the antibiotics short-term variations in the wastewater being still quite unknown and that there has not been found a model predicting and investigating them the second objective of this study is to

create a model simulating the short-term concentration variations of several antibiotics in the wastewater that is to say at the STP inlet.

In the next chapter the measurement campaigns that were being carried out are explained. Then, it is presented how the model is designed. In the chapter about the results, first, the outcomes of the analyses of the measurements are presented and afterwards the model results are shown. The conclusion finally resumes the main findings of this study.

2 Experimental design

2.1 Local situation and campaigns

Between December 2010 and February 2012 several antibiotics measurement campaigns were carried out at the inlet of the STP of Vidy in Lausanne in order to analyse monthly, daily and hourly mass fluxes. The STP of Vidy treats around 110'000 m^3 every day which corresponds to about a 220'000 population equivalent. The wastewater arises from the households of Lausanne and 15 surrounding communities and from several hospitals. The sewer network is partially separated and so the wastewater contains occasionally also storm water and water from the two rivers le Flon and la Louve. Figure 1 shows the catchment basin of the STP of Vidy whose area is about 74 km^2 [19].

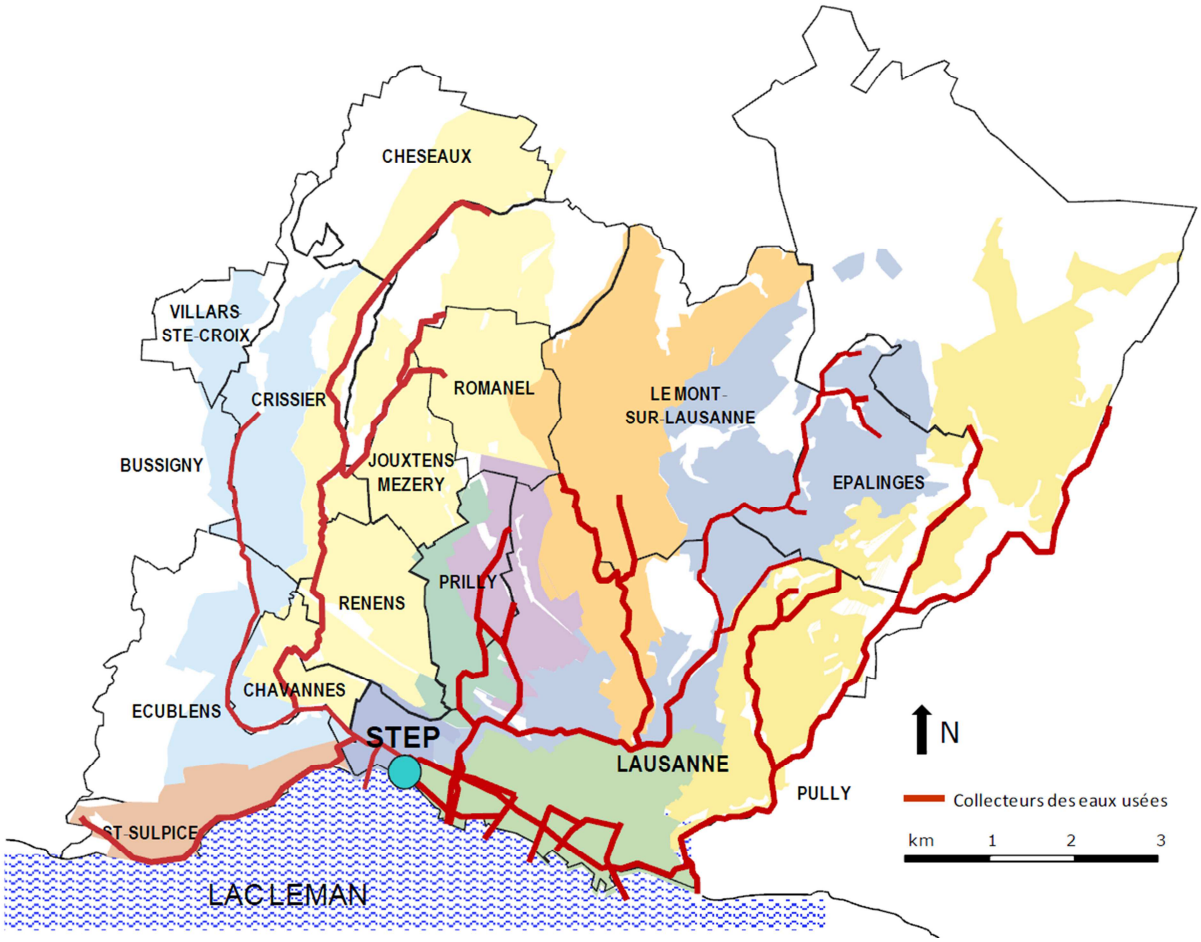


Figure 1: Catchment basin (colored areas) and communities (bordered areas) connected to the STP (STEP) of Vidy [19]

There are two types of campaigns which were being carried out. They are explained in the following section from Coutu et al. [18]:

- A yearlong field campaign was conducted at the wastewater treatment plant (WTP) entrance, following the recommendations of Ort et al. [20]. Starting in March 2011, 12 (i.e., once each month) week-long sampling campaigns were conducted. A week-long experimental campaign consists of seven daily-composite samples, for the seven consecutive days of the week. A daily-composite sample was obtained by mixing (flow proportionally) 24 hourly samples of 200 ml, collected with an automatic sampling device (6712FR Teledyne ISCO). Collected samples were stored on-site in plastic bottles inside the refrigerated sampling device at a temperature below 4°C before collection for laboratory analysis. Samples were analyzed or frozen at -20°C within 24 hours after collection. Eighty-four samples were collected during this first campaign.
- Alongside this yearlong campaign, four 24-h campaigns were conducted to evaluate the dynamics of antibiotics mass flux at hourly scale. The four campaigns were conducted during the four seasons: winter (December), spring (May), summer (September), autumn (November). A 24-h campaign provided 24 samples, one per hour, starting at 7:00am. Each hourly sample was composed from four 200 ml wastewater samples collected every 15 minutes. Ninety-six samples were collected within this campaign.

  The different field campaigns are summarized in Table 3. All samples were collected at the same location using the same sampler. Water samples were analysed using online SPE UPLC-MS/MS, and concentrations were obtained for six antibiotics – trimethoprim, norfloxacin, ciprofloxacin, ofloxacin, clindamycin, metronidazole (>1000 analyses).
  In summary, the sampling program allows the comparison of mass flux fluctuations month-to-month, day-to-day and hour-to-hour, thereby providing information on the time scales that control antibiotic fluxes in wastewater.

Table 3: Description of the methodology used to obtain the monthly, daily and hourly samples in the field campaign. Samples were all collected at the same location of the WTP entrance.

type of sample	sample aggregation	number of samples	frequency
monthly samples	Flow proportional average of seven consecutive daily samples	12	one per month
daily samples	200 ml of sample collected every hours during a day. The 24 samples are mixed flow proportionally to get daily average.	84	7 days (1 week) per month
hourly samples	200 ml of sample collected every 15 min during an hour. Each hour, the four 200 ml samples are aggregated to form a hourly sample.	96	four 24 hours campaign (spring, summer, autumn, winter)

Apart from the measurement campaign of the 06.12.2010 the measurement campaigns were carried out under dry weather flow conditions.

In the following sections from Coutu et al. [18] the flow measurements, the sample treatment and analysis of active compounds as well as the data analysis are presented:

2.2 Flow measurements

An ultrasonic sensor was installed next to the automatic sampler device to record the wastewater levels at 5 min time intervals. The water level was later converted into flow by use of the appropriate calibration[1].

2.3 Sample treatment and analysis of active compounds

A method proposed by Morasch et al. [21] was adapted to measure antibiotic concentrations in the WTP influent samples. The methodology has proven in the past its ability to quantify micropollutants in environmental matrices [21, 22]. As a first step samples were acidified approximately to pH 2 to 2.5 with hydrochloric acid (concentrated 25%). Then, samples were filtered, first, through 2.7 µm glass fibre pre-filters (type GF/D, Whatman). The pre-filtered samples were filtered again through 0.45 µm membrane filters (type ME 25, mixed cellulose ester, Whatman). The samples were stored at -20◦C until analysis.

The analytical method involves online solid-phase extraction (SPE) and Ultra Performance Liquid Chromatography coupled with tandem mass spectrometer (Xevo UPLC-MS/MS, Waters). Prior to analysis, frozen samples were brought back to room temperature a few hours. 8 mL of each sample was filtered through 0.2 µm syringe filters (type GMF, BGB-analytik) directly into the injection vials. Then, a mixture of deuterated antibiotic surrogates was added with gas-tight syringe into all injection vials (samples as well as standards). Targeted compounds were first extracted in the online SPE system, which consists in a 2.1×20 mm SPE column (type Oasis HLB 25 µm, Waters). Extracted compounds were separated in a 2:1×50 mm chromatographic analytical column (type Acquity UPLC BEH C18 1.7 µm, Waters) with an organic mobile phase in gradient mode. All the targeted compounds were identified and quantified in tandem mass spectrometry according to their masses of precursor and product ions as well as their mass-to-charge ratio.

The analytical limit of quantification (LOQ) was defined as the concentration of the lowest standard with a signal-to-noise ratio greater than 10 [22]. The antibiotic concentration in the samples was calculated based on calibration curves using seven calibration points closest to the sample concentration. Correlation coefficients for the calibration curves were typically set to 0.99 at least. In the calculation of sample concentration, recovery rates of deuterated surrogates and exact sample mass weighed during sample preparation were taken into account for each associated antibiotic compound.

[1] http://www.isco.com, site last accessed in June 2012

2.4 Data analysis

Only mass fluxes of antibiotics are discussed. As some sampling was performed in rainy periods, dilution would have produced low concentration measurements. Hereafter, we use the term "measured mass flux" to indicate the mass flux obtained by multiplying, for each sample, the measured flow and measured concentration.

Statistical techniques were used to assess potential temporal patterns in mass flux fluctuation and the relative importance of the different time scales (m, d, h) in antibiotics mass flux dynamics at WTP inlet. They were carried out using Matlab (2009b, The Mathworks, Natick, MA).

3 Modelling

3.1 Design of the model of antibiotics concentration at STP inlet

The objective is to simulate the antibiotics concentration at the STP inlet with a temporal resolution of one hour. In a first step a model for short-term antibiotics discharge into the sewer is composed. Afterwards, it is coupled with an existing model simulating the flow rate in the sewer network to get the antibiotic concentration in the wastewater or at the STP inlet. This basic idea can be expressed a little more analytically the following way:

$$C_{AB\ STPinlet} = \frac{r_{ABdischarge} * \frac{100 - k}{100}}{Q_{sewer}} \quad (1)$$

$C_{AB\ STPinlet}$: Antibiotic concentration in the wastewater at the STP inlet on an hourly basis in [mass/volume]

$r_{ABdischarge}$: Hourly antibiotic discharge rate into the sewer in [mass/time(hour)]

k: Transit loss fraction [%]

Q_{sewer}: Waste water flow rate in the sewer [volume/time] resolved in time steps of ten minutes

The **antibiotic discharge rate** describes the mass of antibiotic flowing every hour into the sewer network. The models briefly described in section 1.3 estimate this amount based on data on consumption and excretion of the medicament. Since this model concentrates on short-term variations, the model inputs about consumption and excretion have to be in a high temporal resolution. Therefore, the excretion is subdivided into the physiological excretion rate into the urine and the time when the toilet is used so that the loaded urine enters then the sewer system.
Thus, the hourly antibiotic discharge rate is finally a function of the amount and time of intake of the antibiotic, its accumulation in the urine and the time of toilet use.

The **transit loss fraction** gives an estimate of the fraction of antibiotics getting lost during sewer transit for example by sorption to the biofilm in the sewers or biodegradation [17, 23, 24].

The **waste water flow rate** is predicted using the already existing hydrological model created by Dario Del Giudice in his master thesis in 2011 (see [25]). One part of his master thesis consisted in the development of a hydrological model predicting the flow rate in the sewer system and its application on the sewer catchment of Lausanne. He described the model among other things as being lumped, deterministic and continuous. This means it treats the catchment area as one unit not considering spatial variations of variables and parameters. As a deterministic model it does not account for random variables with probability distributions and gives as output only one solution. Furthermore it simulates the hydrosystem's behaviour over a certain time period and hence, it is a continuous model [25].

Borrowing the idea of this classification the present antibiotics discharge model can be described as a lumped, stochastic and continuous model. In contrary to his model this one is stochastic because a Monte Carlo approach is used combining different probabilistic curves: The probability distribution of the time of antibiotics intake, of the number of toilet uses a day and the probability distribution of the time of toilet use. A Monte Carlo method uses deliberately random numbers in calculations with the structure of stochastic processes [26]. In this case it means that for example the time when somebody goes to the toilet is picked randomly but weighted following a certain probability distribution. The time of the antibiotic intake, the number of toilet uses a day and the hours when the toilet is used are determined this way and separately for the estimated number of persons consuming the medicament at a certain day. Then, the amount of antibiotic discharged is calculated for the considered day. This way, a nonprobabilistic problem can be solved by use of probabilistic methods [26].

The following subchapters describe first, how the antibiotic discharge model is constructed, then how it is coupled with the transport model and finally how the whole model is calibrated and validated. The entire labour is effectuated by using Matlab[2].

3.2 The antibiotics discharge model

3.2.1 Antibiotics consumption data

The consumed amount of antibiotics is an essential input for the model. However, direct consumption information does not exist. For that reason it is assumed that the consumed amount corresponds to the sold amount of the prescribed antibiotics. The model created is applied for the catchment area of the STP of Vidy in Lausanne. For this area there are no data available that comprise the amounts of prescribed antibiotics from all private and public health centres. Hence, this information is deduced from the monthly sold amounts of prescribed antibiotics. The interpolation of the data provided by the Professional Cooperative of Swiss Pharmacist (OFAC) allowed getting an estimate for the ambulatory antibiotics sales. Data provided by the pharmacy of the university hospital centre of the canton of Vaud (CHUV, centre hospitalier universitaire Vaudois) enabled the estimation of the antibiotics amounts used in the hospitals.

The model is applied for three antibiotics: ciprofloxacin, norfloxacin and trimethoprim. For ciprofloxacin and norfloxacin it is assumed that tablets of 400 mg and 500 mg respectively are administred two times a day [8, 27, 28]. Trimethoprim is assumed to be administered by four tablets of 160 mg (960 mg Bactrim) a day. Especially for trimethoprim this assumptions include uncertainties because the antibiotics are prescribed in different amounts depending on the disease and the age of the patient [28, 29].

[2] Matlab versions 7.9.0.529 (R2009b) and 7.10.0.499 (R2010a)

3.2.2 Time of antibiotics intake

For the consumption time a probability distribution throughout the day has to be found. No general information is found on the medicament intake habits of patients. There is information however on the (ideal) frequency of the medicine administration such as the administration instructions that can often be found on the packages of prescribed medicaments. So, the intake probability distribution is estimated based on such information. However, they just indicate the daytimes when (e.g. morning – evening) or the number of times a day a medicament should be administered and not precise hours.

The first antibiotics intake a day is assumed to happen in the same hour as the first toilet use. The succeeding intakes are picked randomly but weighted after a normal distribution with their mean values as many hours later as a perfectly regular administration would allow for (see example given in Figure 2). The standard deviation of the normal distribution is unknown. It is arbitrary assumed to be between one and seven hours and determined through the calibration.

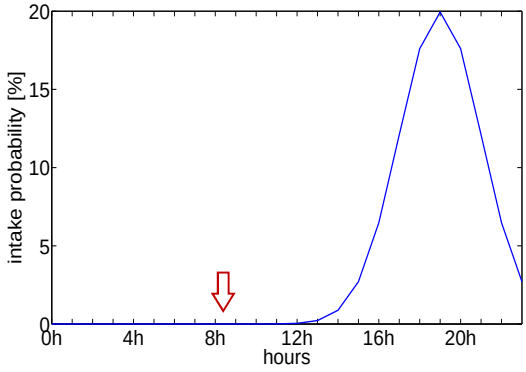


Figure 2: Example for the intake probability distribution of the second administration having had the first one at 8 am (red arrow) in the case of 2 administrations a day (= 12 h-interval in-between); the standard deviation is here assumed to be 2

3.2.3 Accumulation in the urine

After the intake of an antibiotic such as norfloxacin or ciprofloxacin it is partially absorbed, distributed and metabolised in the body [31]. The elimination of the metabolites as well as of the unchanged agent happens via urine or faeces but the excretion in urine is predominant for a lot of pharmaceuticals [4] [32]. In the case of ciprofloxacin about 40 to 50% of the oral dose is excreted unchanged in the urine and about 20-30% are excreted not metabolized in faeces [4, 30, 31, 33]. Of Trimethoprim about 50 to 75% of a dose is excreted unchanged in the urine (Kasamen et al., 1978 cited in [34]) and less than 4% are present in faeces (Schwartz et al., 1969 cited in [34]). Norfloxacin on the contrary is in higher amounts excreted by faeces than by urine. About 25 to 30 % of the oral dose is excreted unchanged in the urine and 30 to 40% are excreted unchanged via faeces [4, 30, 31, 33]. Figure 3 shows exemplarily the cumulative urinary excretion of unmetabolised norfloxacin after single oral doses. For the model such curves are found and extrapolated for the considered antibiotics with the corresponding pill masses. The model concentrates on unmetabolised antibiotics because metabolites were not being measured in the measurement campaigns. In addition, for most drugs the toxicity is reduced for metabolites compared to their parent drug [4] which makes them more important in an environmental point of view.

The excretion or accumulation curves found in literature that were viable for the model are the curves of ciprofloxacin, norfloxacin and trimethoprim and they all concern only the urinary accumulation. For that reason the accumulation in faeces is not considered in the model. The found urinary accumulation curves are all originating from studies investigating among others the renal excretion after single doses only. However, for disease treatment patients might often be advised to take the antibiotic for several days. Based on the study of Ledergerber et al. [35] it can be said that the urinary accumulation of ciprofloxacin does not change strongly with multi-doses prescriptions. Norfloxacin has about the same half-life time as ciprofloxacin [35], [36] and shows only little accumulation in the human body [31]. Thus, it can be assumed that just as for ciprofloxacin the urinary accumulation of norfloxacin does not change considerably with multiple doses compared to a single application.

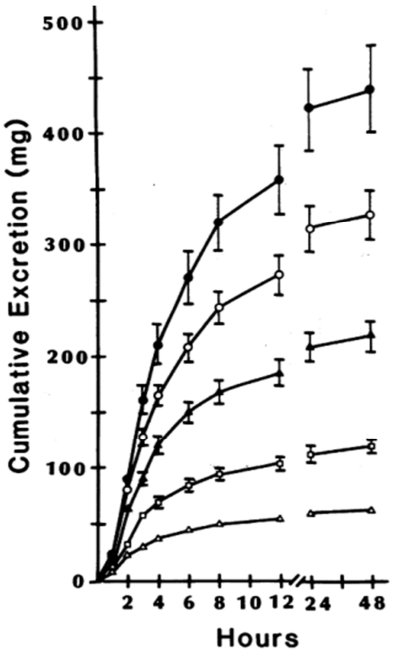


Figure 3: Cumulative urinary excretion of unmetabolized norfloxacin after various single oral doses: 200 mg (△), 400 mg (□), 800 mg (▲), 1200 mg (○) and 1600 mg (●). Points and bars indicate means ± standard errors of the means. [30]

The information found on the urinary accumulation of ciprofloxacin is not as precise as for norfloxacin. The standard errors of the means of norfloxacin are not exceeding 10% of the means as it can be seen in Figure 3. In the case of ciprofloxacin these variances are up to around 60% of the measured mean. If accumulation mass values are assumed to be 60% higher compared to the average, then the concentration modelled at the STP inlet increases also to about 60%. This is accounted for during the model calibration by applying a substance-specific parameter accounting for such variations.

In literature the urinary accumulation after oral administration as well as after intravenous administration is documented. Depending on such an application mode the dynamic of the urinary accumulation can be slightly different [33]. For the model the intravenous administration is not considered. According to the amount of prescribed antibiotics in the Canton of Vaud there were no sales from 2006 to 2010 of norfloxacin and trimethoprim medicaments for intravenous application. In contrast, ciprofloxacin was being sold in the form of intravenous medicaments. The mass of ciprofloxacin intravenously administered accounts for about 15 to 20% of the total mass sold. Considering this rather not so high amount and the hypotheses that must be made to account for the slight differences of this application mode in the urinary accumulation (there is for example no reliable way to find a probability distribution for the time of injection) it is decided not to treat it separately. Instead, the amount of ciprofloxacin intravenously administered is assumed to be orally administered.

3.2.4 Time and mean number of daily toilet uses

The time when the toilet is used is always the time when antibiotics are discharged into the sewer. In that sense, the time of toilet use plays an important role for the model.

Based on Rauch et al. [37], Friedler et al. [38] and Rossi et al. [39] a distribution of toilet use throughout the day is assembled. These studies investigated among others the diurnal urine load entering the sewer and the (domestic) toilet use by surveys or by measurements. According to Friedler et al. [38] and Rossi et al. [39] not all but the majority of the flushes were related to urinal use. This is important because the model only considers urinary antibiotics discharge.

The frequency diagrams of the three mentioned studies are shown in Figure 4a)-c).

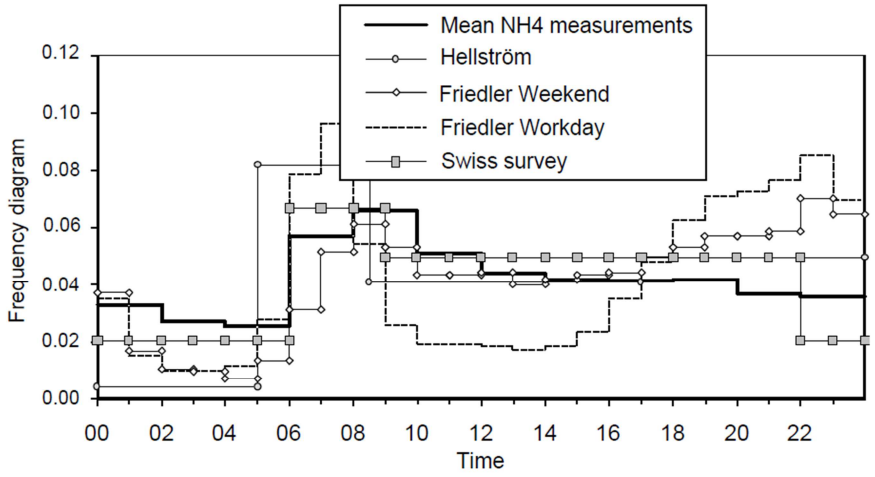


Figure 4a): Frequency diagram of diurnal toilet use by Rauch et al. [37]

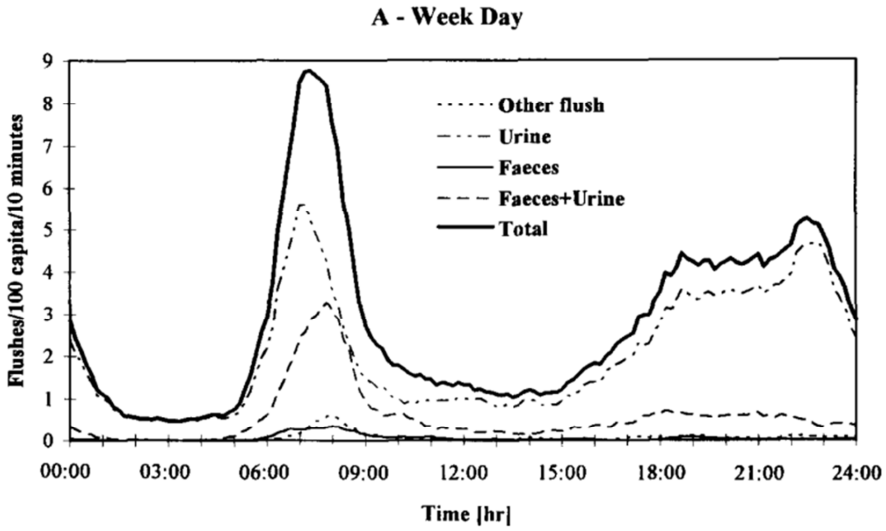


Figure 4b): Diurnal pattern of toilet flushes by Friedler et al. [38]

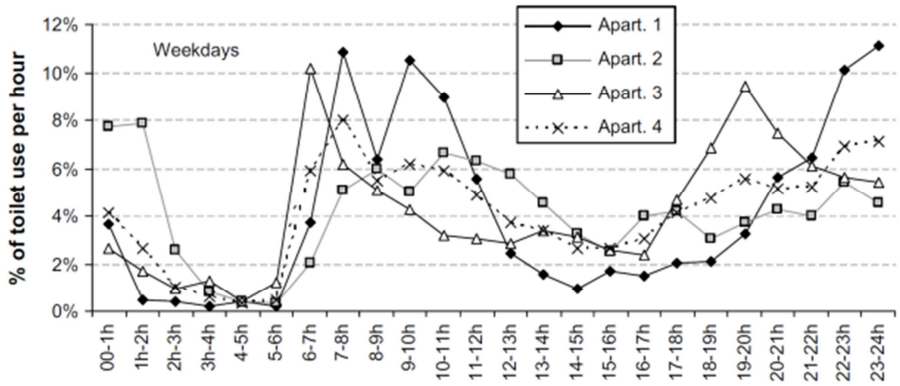


Figure 4c): Toilet use profile for four different apartments during weekdays by Rossi et al. [39]

The assembled toilet use distribution is presented in Figure 5.

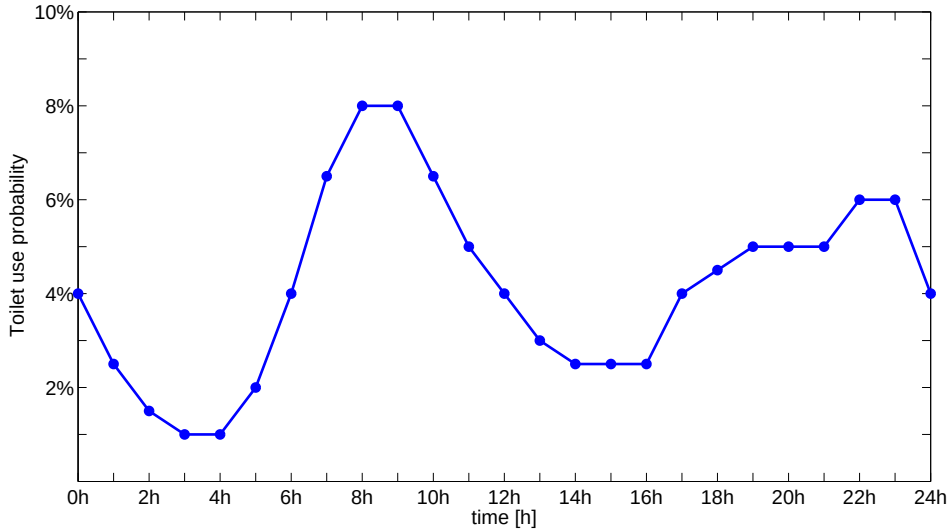


Figure 5: Assembled toilet use probability distribution. The sum over 24 hours amounts to 100%

Indications about the number of toilet uses per day can be found in the studies of Friedler et al. [38] and Rossi et al. [39] (see Table 4). Taking into account that not all flushes are due to urinal use and that these numbers refer to domestic toilet use suggesting a probable underestimation since people might use toilets also outside of their households, the mean number of toilet uses per day and person could be estimated to around 6. The standard deviation is assumed to be around ±2. Since the two values are quite uncertain they are not directly introduced into the model as they are. Instead, the mean number of toilet uses a day and its standard deviation are

Table 4: Literature values on average number of toilet uses per capita and day and its standard deviation

	Friedler et al. [38]	Rossi et al. [39]
Weekdays	3.98 ± 3.30	5.2 ± 1.2
Weekend days	4.75 ± 2.79	5.6 ± 1.4

calibrated. The two values found in literature served as reference points for the definition of the range in which the calibration should take place (see section 3.4).

3.2.5 Model procedure

As briefly explained in section 3.1 the time of antibiotic intake as well as the mean number of toilet uses a day and the corresponding time is found randomly but weighted depending on the probabilities presented in sections 3.2.2 3.2and 3.2.4. Once having obtained these parameters for one day, the antibiotics' mass entering the sewer system can be found. For every hour when the toilet is used the antibiotics mass released is found by calculating the difference of the accumulated antibiotics in the urine since the previous toilet use (see Figure 6).

This random parameter determination and antibiotic discharge calculation is repeated for the number of days of the considered time period and the estimated number of persons a day consuming the antibiotic. This latter number could be estimated by means of the data base of sold antibiotics in the Canton of Vaud. The available data on the antibiotics consumption reaches from 2005 to 2010 and is indicated as mass per month. For every month the average consumption of these five years is calculated. By assuming that the consumption is evenly distributed throughout the month the daily consumed mass is found. This assumption is likely to have little impact on the final model results since seasonal variations as well as the variations within the day are generally more important than the variations from one day to another as it is explained in section 4.1.

Finally, the hourly mass distribution of antibiotics discharged by a person a day is summed up for the estimated number of consuming persons a day. This way, the hourly antibiotic mass entering the sewer system over the considered time period is found (see again Figure 6).

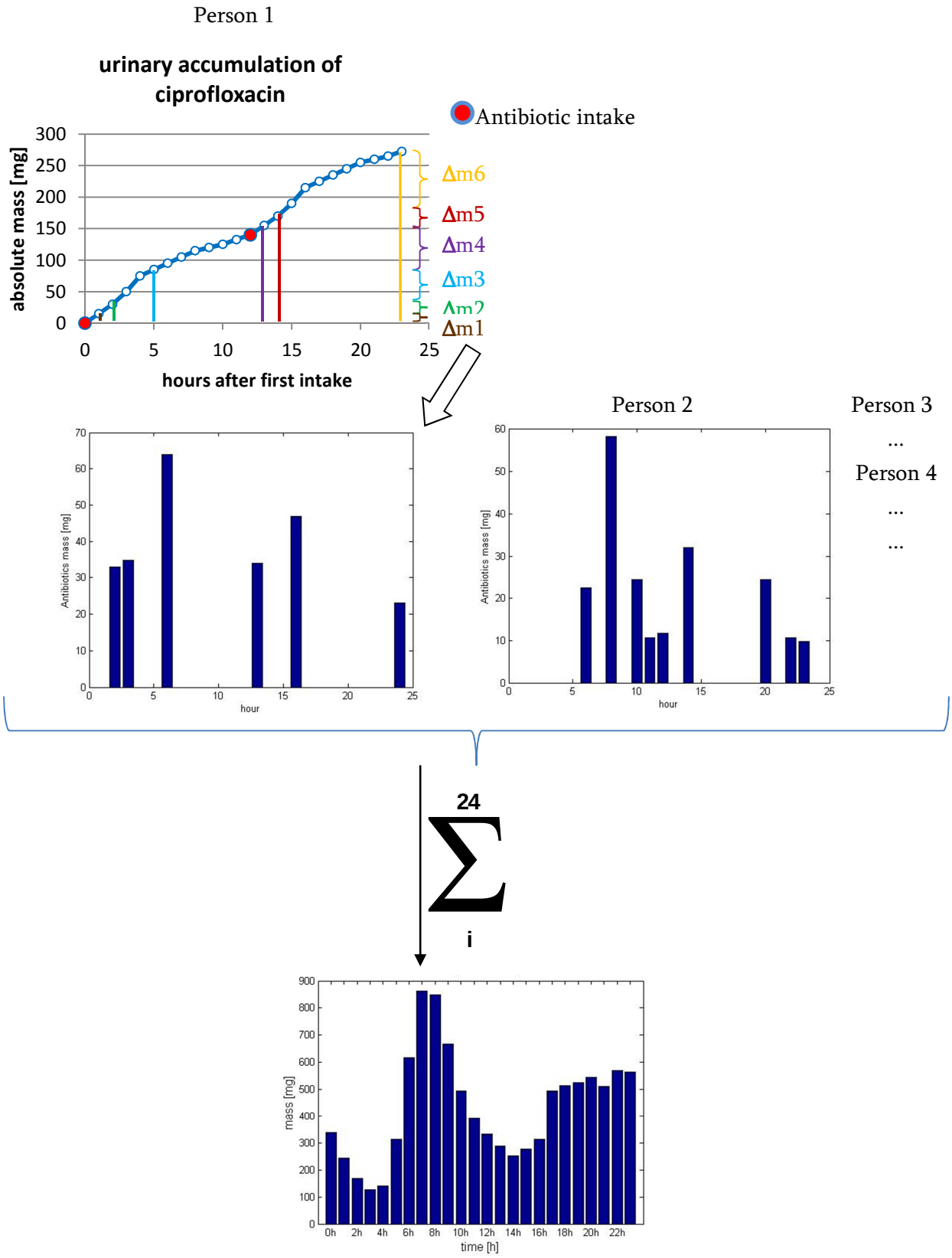


Figure 6: Illustration of the calculation of the hourly antibiotics discharge out of the urinary accumulation and the random discharges of single persons

3.3 Antibiotics transport to STP and coupling with transport model

3.3.1 Transit loss fraction

During the transport from the toilet to the STP parts of the antibiotics in the wastewater probably get lost. A process likely to happen is the sorption of the antibiotics to biofilms in the sewer system. In laboratory conditions the sorption rates of different substances are quite diverse [23]. There is no detailed information available about sorption rates of antibiotics in sewers or other processes impacting their concentration at the STP inlet. To account for such losses anyhow, the transit loss fraction k is introduced. Its value is a priori unknown and can vary between 0% and 100%. Later on, by calibrating the model a value is found for k.

3.3.2 Travel time

The travel time is the time it takes the wastewater that is to say also the antibiotics to go from the toilet to the STP. It leads to a delay of a certain antibiotic wave from its discharge to its arrival at the STP. In their study Ort, Lawrence Reungoat et al.[10] estimated the travel time of a certain renal excreted micropollutant from the toilet to the STP inlet to get an idea about the time when the peak of the micropollutant could be expected at the STP inlet. Instead of estimating the wastewater travel time in the catchment of Lausanne's STP it is introduced into the model as an a priori unknown constant, just as the transit loss fraction. Then, it is determined by the calibration. Since the catchment area is inclined which suggests a rather short travel time it is assumed to be two hours at most and zero if it is less than one hour.

3.3.3 Transport model coupling

For modelling the flow rate in the sewer network the transport model needs as input the temperature and rain data for the considered period. These data are obtained from the weather station in Pully kept by MeteoSwiss. Where data are missing (rain data from the 13.07.2011 to the 20.07.2011) they are replaced by the data of the weather station in Bière which has a linear distance to Lausanne of around 20 km.

The wastewater flow rate (Q) resulting of the transport model is given in cubic meter per seconds in time steps of ten minutes. So, the modelled hourly antibiotic discharge rate is converted as well in mass per second and time steps of ten minutes and then divided by the flow rate (see equation 2). This way, the antibiotic concentration (C) in ten minute time steps at the STP inlet is received and averaged afterwards to the mean hourly concentration.

$$\frac{MFR\ [mg/s]}{Q\ [m^3/s]} * 1000[ng/mg] = C\ [ng/l] \tag{2}$$

3.4 Model calibration and validation

In accordance with Dario Del Giudice's procedure with the transport model, here too, a model calibration by means of the Nash-Sutcliffe efficiency coefficient (*NS*) and the normalized bias (*NB*) is applied. These are helpful tools to evaluate the accuracy of data simulated by watershed models. They both compare modelled data with observed values [40].

The Nash-Sutcliffe efficiency is calculated as follows [25, 40]:

$$NS = 1 - \frac{\sum_{i=1}^{n}(Y_i^{obs} - Y_i^{sim})^2}{\sum_{i=1}^{n}(Y_i^{obs} - \overline{Y^{obs}})^2} \quad (3)$$

n: number of values in considered calibration period
Y_i^{obs}: observed value at time i
Y_i^{sim}: simulated value at time i
$\overline{Y^{obs}}$: mean observed values over the period from i=1 to n

The resulting values range from -∞ up to 1.0 whereby NS = 1.0 is the optimal value.

The normalized bias is defined as follows [25, 40]:

$$NB = \frac{\sum_{i=1}^{n}(Y_i^{obs} - Y_i^{sim})}{n * \overline{Y_{obs}}} \quad (4)$$

Values can be positive or negative indicating under- or overestimation of the modelled values. The optimal value is 0.0.

During the calibration these two coefficients are combined in a way that a single optimal value (V_{opt}) is found which is evaluating the simulation, (based on [25]):

$$V_{opt} = MIN\{(1 - NS) + |NB|\} \quad (5)$$

The model is run several times, every time with different parameter values randomly chosen. Hence, again a Monte Carlo approach is applied. After every simulation V_{opt} is calculated and compared to the V_{opt} of the best previous simulation. If it is higher than this previous best V_{opt} it is rejected. Otherwise the applied parameter set will show a better fit of modelled to measured values and so it is saved.

For calibrating the model of the present work the measurement campaign of autumn 2011 is chosen. This means that the 24 measured concentration values of the 6th September 2011 (n=24)

are compared in every model run to the 24 values simulated for the same day. The measurement campaign of autumn is chosen because it was effectuated in dry-weather conditions and shows generally a regular journal concentration pattern (see section 4.1.2).

The model is run 10'000 times for every substance. The parameters that are varied in every run are parameters within the model that are fraught with substantial uncertainties such as the transit loss fraction. Table 5 resumes the parameters that are considered as uncertain and are varied during the model calibration. For each of them a range of possible values is chosen where possible based on literature values. Out of the determined range the parameter values are chosen randomly. The discretisation step of each parameter stands for the interval between two values a parameter could have and hence decides also how many possible values a certain parameter could take on.

The parameter determining the order of magnitude of the urinary accumulation curve can in all cases be higher than 100%. The accumulation curves found in literature are not strict curves. They have bars showing standard errors pointing to analytical uncertainties or bars illustrating the standard deviation of the samples which are due to differences in the metabolism of individuals. This is tried to account for by submitting this parameter to the calibration.

As mentioned, the calibration is conducted for all substances separately since the variations of the urinary accumulation as well as the sorption to the biofilms and the biodegradation resumed in the transit loss fraction are not the same for all substances.

Table 5: Calibrated parameters with ranges of plausible values

Parameters	Literature values	Value ranges	Discretisation steps
Urinary accumulation		% of values on accumulation curve	
Ciprofloxacin	See appendix	3% – 150%	1%
Norfloxacin	See appendix	85% – 115%	1%
Trimethoprim	See appendix	70% – 130%	1%
Mean number of toilet uses a day	6	3 – 12	1
Standard deviation of number of toilet uses a day	2	1 – 5	0.1
Standard deviation of time of the subsequent intake(s)	-	1 – 7 hours	0.1 hour
Transit loss fraction	-	0% – 100%	1%
Delay due to transit	-	0 – 2 hours	1 hour

In the model validation the model is run with the parameter set which is found to be best fitting in the calibration. The resulting values are then compared with the measurements of the three remaining seasons winter 2010, spring 2011 and summer 2011.

4 Results

4.1 Experimental results

The presentation of the measurement results is again extracted from Coutu et al. [18]:

4.1.1 Seasonality of mass flux

The experimental campaign was designed to identify seasonality in the mass flux of antibiotics in wastewater. For this purpose, mass fluxes measured monthly at the WTP inlet are compared throughout the year. Seven consecutive daily samples are aggregated flow proportionally to form a monthly sample (see Table 3). The median mass flux obtained is presented for each month and all substances in Figure 7. The minimum monthly mass fluxes are generally found during the period running from June to September. Mass fluxes measured in December and January have systematically higher values except for ciprofloxacin. High values are also observed in March for ciprofloxacin, ofloxacin and clindamycin. Curiously, a drop in mass fluxes is observed for all substances (except for ciprofloxacin) in February. We suspect that a problem occurred in either the sampling campaign or the analytical procedure during this month. Indeed, we could not retrieve concentrations for norfloxacin and metronidazole for this month, which supports the

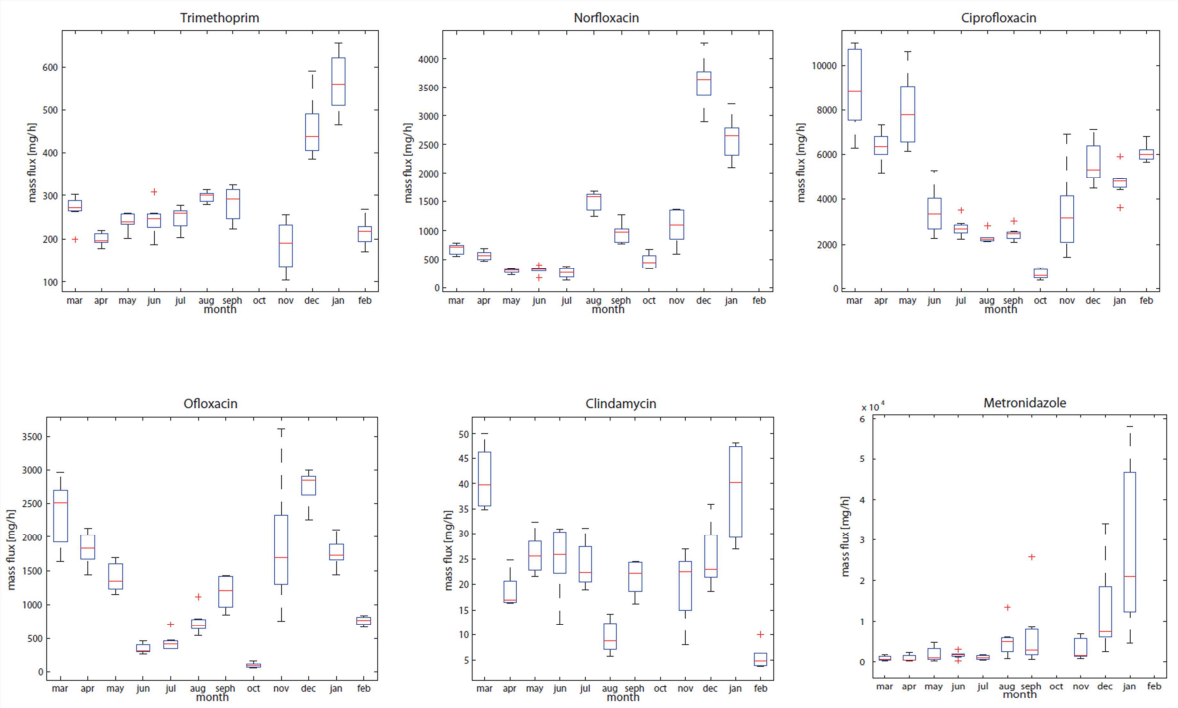


Figure 7: Boxplot of mass flux for the 6 antibiotics investigated. One box per month. Each box is obtained from seven daily samples. The red line shows the median. Upper and lower box limits correspond to the 75th and 25th percentiles respectively. Upper and lower whiskers correspond to the last datum being respectively still within 1.5IQR of the higher and lower quartile. IQR is the interquartile range i.e., the difference between the upper and lower quartiles.

hypothesis of a failure in either the field sampling campaign or the laboratory analytical

procedure.

The monthly mass flux normalized by the annual mean is also helpful to evaluate the seasonality of antibiotics, as well as the range of the fluctuation. This relative fluctuation is presented in Figure 9a) for the six antibiotics considered. Mean monthly mass fluxes appraise between a quarter and double of the mean annual mass flux measured at WTP inlet. Ciprofloxacin, ofloxacin and clindamycin present an obvious seasonality of their mass flux, with values in winter being up to eight times greater than values in the summer. Trimethoprim, on the other hand, shows a less variable monthly mass fluxes over the year, although a higher mass flux is still observed in January and December. The behaviour of norfloxacin and metronidazole is somewhat curious; values of monthly mass fluxes are located broadly between a quarter and half of the mean, with a sudden rise in December and January. A general observation for all substances is that the highest mass flux is measured during the (winter) months of December and January.

4.1.2 Periodicity in hourly mass flux

The four 24-h campaigns described in Table 3 were used to evaluate periodicity in the mass flux of antibiotics reaching the WTP during dry weather. For this, the measured hourly mass flux at each hour was first normalized to the mean for that day (Figure 9c)). This procedure removes any seasonal effect that can exist in the dynamics of antibiotics and thus allows the comparison of the 24-h campaigns, which were conducted once per season. After normalization, the four 24-h campaigns were combined sequentially into a series of 96 experimental values. The periodogram of ciprofloxacin is shown in Figure 8.

For all substances considered, two peaks clearly appear in the computed periodogram, which indicate that the mass flux of antibiotics has a 12-h return period. According to Figure 9c), the first peak occurs between 07:00 and 09:00. This morning peak is up to double the average daily flux reaching the WTP. It corresponds to the peak in toilet flushes observed by Friedler et al. [38] and Rauch et al.[37]. The second peak occurs 12 h later between 19:00 and 21:00. We observe that this peak is less pronounced than the morning peak, and occurs over a broader period, between 17:00 and 21:00. Its value reaches only up to around 1.2 times the daily mean, except for metonidazole. Gain, the more diffuse aspect of this second peak in mass flux corresponds to the one observed by Friedler et al. [38] and Rauch et al. [37] in the cycle of toilet flushes.

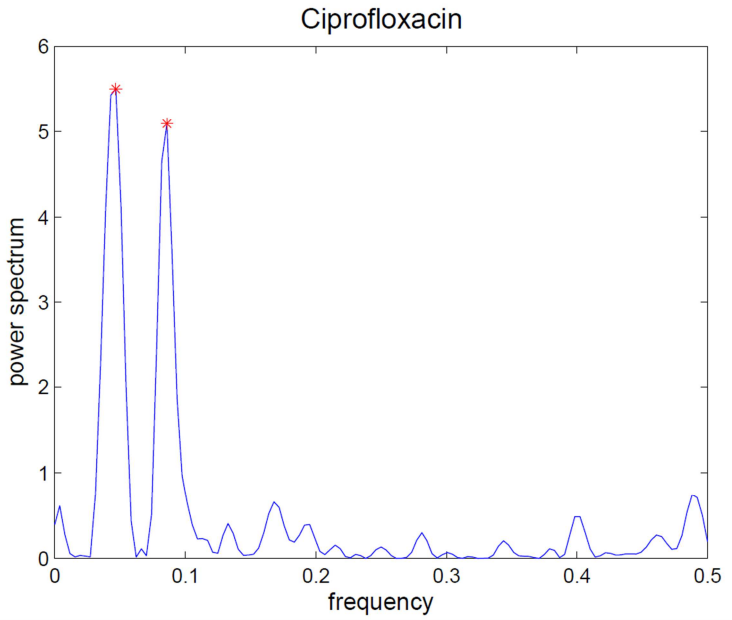


Figure 8: Periodogram of the measured hourly mass flux. Peaks in power spectrum observed at 0.042 Hz and 0.083 Hz correspond to periods of 24 h and 12 h, respectively. The same periods were observed for the other substances.

4.1.3 Dominant time scale

We used mean normalized mass fluxes to compare the temporal dynamics of antibiotics at different time scales. All the results are summarized in Figure 9. We immediately observe that whereas the monthly and hourly mean concentration can fluctuate between 0.25 and 2 times the reference mean — i.e. the annual mean and daily mean mass fluxes —, very little fluctuation is observed at the daily scale. Indeed, all daily measured mass fluxes remain located between ±20% of the weekly average, the only exception being metronidazole. This indicates that from one day to the next, the load of antibiotic load to the WTP is constant, throughout the week. Nevertheless, the total load from one month to another can change on the seasonal time scale. Similarly, strong discrepancies exist between the hourly loads measured at WTP entrance within a given day.

The incoming flow at the WTP inlet influences mass flux. In Figure 9, we present the flow measured during the campaign at the sampling location. The flow is normalized to the mean flow to highlight its relative fluctuation. We can discuss the influence of flow on mass flux for the hourly and daily time scale only, as monthly samples are affected by rainwater input that does not contain antibiotics.

Concerning the daily time scale (Figure 9b)), no large fluctuations are present in either the water flow or the antibiotic mass fluxes. This observation is reasonable, considering that (i) antibiotics consumption does not depend on a specific day of the week as consumers must stick to the posology of the substance and, (ii) water use in terms of daily average is nearly constant on the weekly timescale, even though a small drop is expected during the weekend, where less industrial water is consumed. On the other hand, we see on Figure 9c) that a pattern exists in hourly flow to

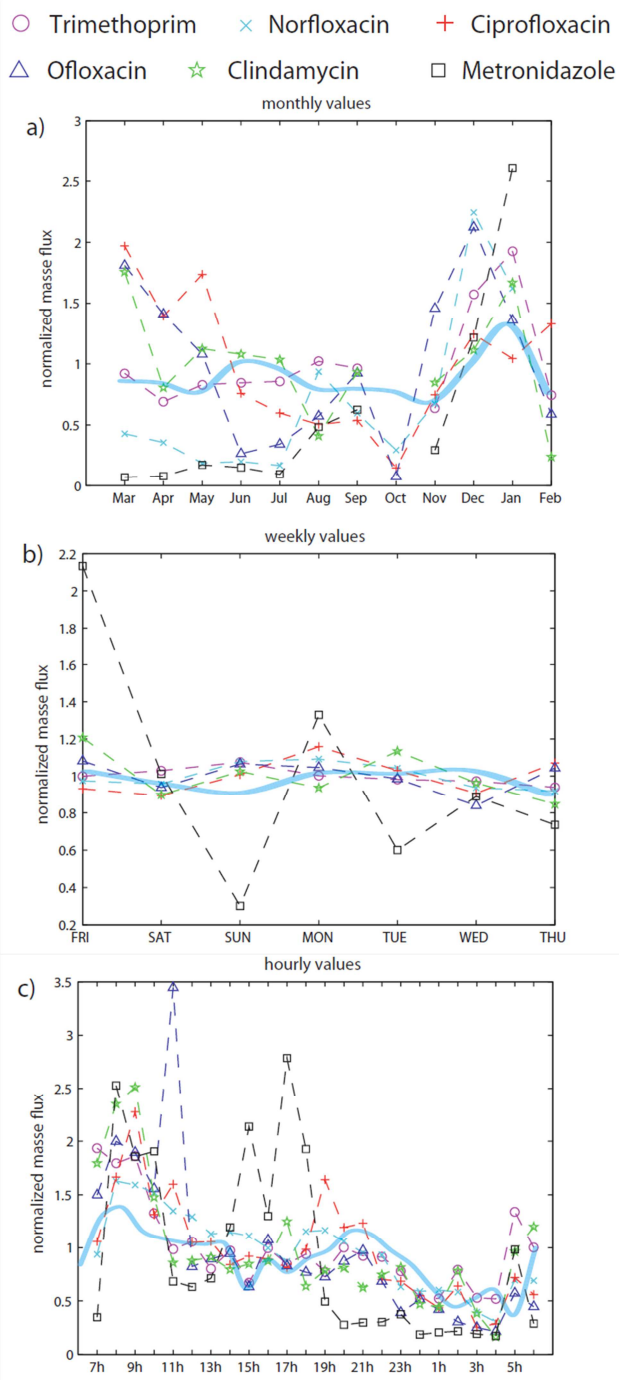


Figure 9: Fluctuations of mass flux at different time scales: month a), day b) and hour c). In a), each symbol corresponds to the average mass flux of the seven daily samples collected in the corresponding month. In b), each symbol corresponds to the average value of the twelve times (one per month) the corresponding day was sampled. In c), each point corresponds to the average of the four times (four 24-h campaigns) the corresponding hour was sampled. The mass flux is normalized to the total mean mass flux over the considered time scale to remove seasonal effects. For example, in a) the monthly mass flux is normalized by the mean monthly mass flux measured over one year. Clear variability is observed at the monthly and hourly time scales for all substances. No variability is observed at the daily time scale, except for metronidazole. The corresponding flow—normalized to the mean flow over the considered time scale — is on each graph shown by the blue line. The monthly mean flow includes rainwater peaks in addition to the baseflow. Daily and hourly mean flows were selected to include only dry periods. i.e., their fluctuation is not affected by any rain input.

the WTP. This is due to the toilet flush use cycle identified in Friedler et al. [38] and Rauch et al. [37] in addition to other domestic usage. However, hourly flow lies between 0.6 and 1.4 times the

mean hourly flow. This means that flow into the WTP varies less than the mass flux of antibiotics, which is usually between 0.2 and 2.5 times the mean hourly mass flux.

The dominance of monthly and hourly time scales over the daily time scale in the dynamics of antibiotic mass fluxes at the WTP inlet is clearly illustrated by Figure 10. The length of the box plot computed for each time scale reveals the dispersion of the measured data. The dispersion of the daily mass flux along the week is systematically lower than the dispersion of the monthly mass flux considered over the year or hourly mass flux over a day. Daily values are typically distributed between $\pm 20\%$ around the weekly mean for all substances, with an exception for metronidazole, whose daily variations are of the same order of hourly and monthly variations. For all other antibiotics, monthly and hourly variations of mass fluxes exceed daily variations. Measured fluxes for these time scales are broadly distributed between 0.2 and 2.5 times the mean. This shows the importance of the design of the sampling campaign when analyzing antibiotics in environmental samples. Depending on the month or the hour of the field campaign a factor higher than 12 can exist between the measured mass fluxes. This is due to two factors. First, the seasonality of antibiotic mass fluxes reaching the WTP entrance results from seasonality in antibiotics consumption [41]. Second, there are concentration fluctuations on the hourly time scale that can be explained partially through the toilet use frequency.

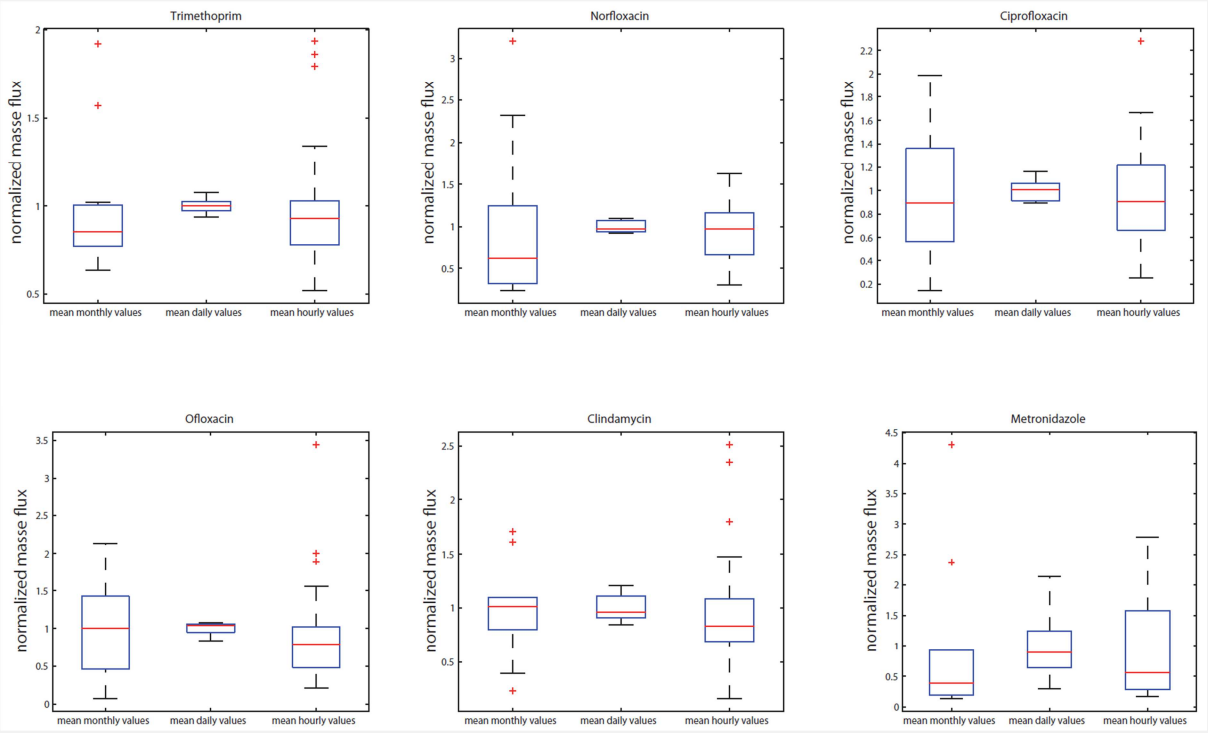


Figure 10: Boxplots illustrating the dispersion of the measured mass fluxes for the three different time scales (m, d, h). Similar to Figure 9, the mass flux is normalized by the mean mass flux over the considered time scale. For each substance, the box on the left is obtained from the 12 mean monthly values (twelve values in Figure 9a)), the box in the middle from the seven mean daily values (seven values in Figure 9b)), and the box in the right-hand side from the 24 mean hourly values (twenty-four values in Figure 9b)). Boxes are computed following the same rule described in Figure 7.

4.2 Model results

4.2.1 Flow rates of transport model

The transport model is run for the whole year when the antibiotic measurements took place that is to say from the 1st of December 2010 to the 30th of November 2011. Figure 11 shows the development of the wastewater flow rates in the sewer network leading to the STP of Vidy in December 2010. The precipitation as one important factor influencing the wastewater flow rate is also inserted. When there is no precipitation the diurnal flow rate pattern is clearly visible. The figures for the other seasons can be found in the appendix.

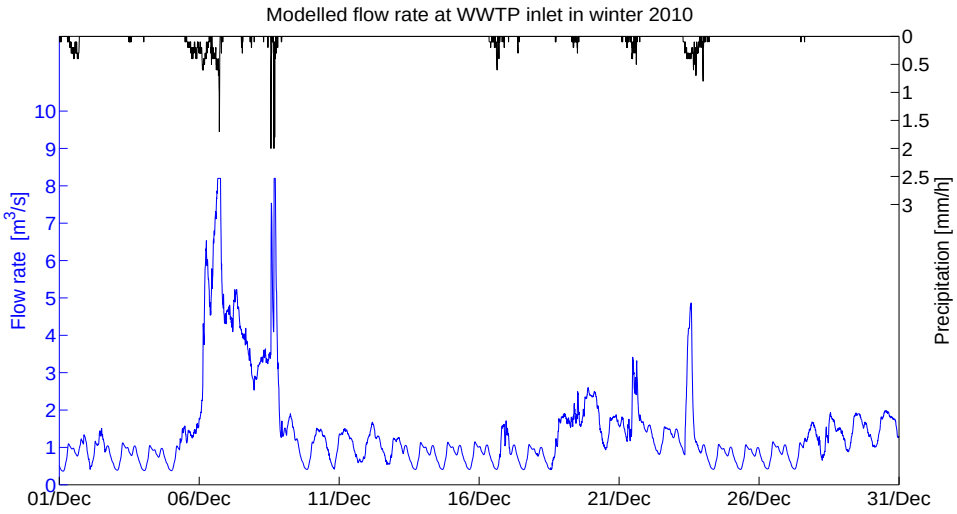


Figure 11: Flow rate in winter (blue) resulting from the transport model with the precipitation (in black) which is a predominant factor in the model

4.2.2 Calibration

This modelled wastewater flow rate is then coupled with the antibiotic discharge model as described in section 3.3.3. Afterwards, the calibration is carried out (see section 3.4) determining the one parameter set out of 10'000 which has the best fit to the measurements of autumn. The resulting parameters are presented in Table 6.

As explained in section 3.4 the values of the parameter calibrating the urinary accumulation curve aims to compensate analytical uncertainties in the determination of the curve's values and pharmacokinetic differences of individuals. In case of an underestimation of the antibiotic amount a priori set in the model inputs this parameter gets higher than 100% as it is the case for ciprofloxacin and norfloxacin. The mean number of toilet uses determined by the calibration is rather high. This refers to uncertainties in the determination of the time when the toilet is used as a whole. This aspect will be discussed later in section 4.2.5. The standard deviation of the time of the subsequent antibiotic intake(s) is higher for ciprofloxacin and norfloxacin. The two substances are assumed to be administered every twelve hours while trimethoprim is assumed to be ingested

every six hours. From this point of view it is reasonable that the standard deviation for the subsequent intake(s) is smaller for trimethoprim. The transit loss fraction rather high for trimethoprim compared to the other substances. This parameter seems in that case to reduce the trimethoprim amount a priori set in the model inputs. Also this aspect is discussed later in section 3.4.

Table 6: Model parameters found through the calibration

Parameters	Ciprofloxacin	Norfloxacin	Trimethoprim
Urinary accumulation	109%	115%	81%
Mean number of toilet uses a day	12	11	12
Standard deviation of number of toilet uses a day	2	3.6	4.2
Standard deviation of the time of the subsequent intake(s)	6.9	6.2	1.1
Transit loss fraction	10%	11%	55%
Delay due to transit	0 hours	0 hours	0 hours

Figure 12a)-c) show the graph resulting from the calibration comparing antibiotics measurements with the modelled concentrations. The order of magnitude is in all three cases at about the same level. However, the detailed temporal dynamic of the modelled and the measured concentrations do not correspond very properly with each other. On the one hand this has to do with the random character of the model with its Monte Carlo approach. On the other hand a certain mismatch is expectable with only 24 measured values to compare during the calibration.

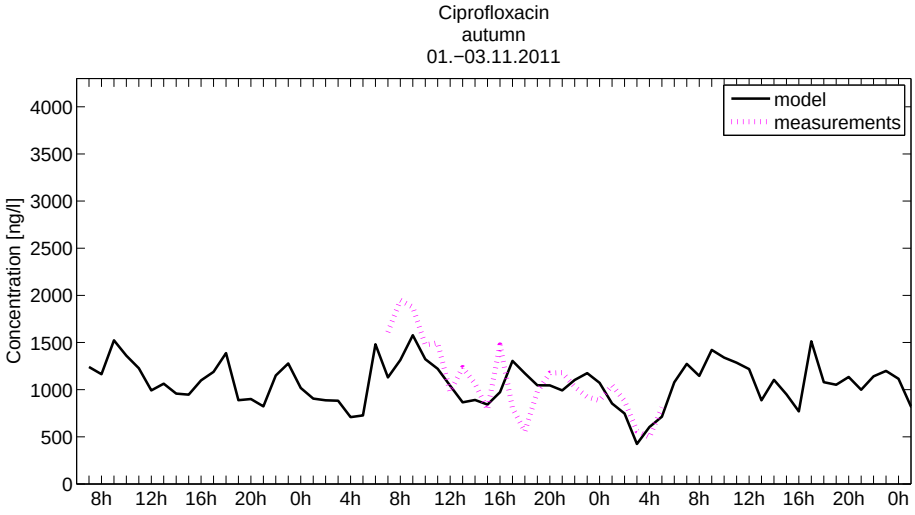


Figure 12a): Comparison of the calibrated ciprofloxacin concentrations with the measurements

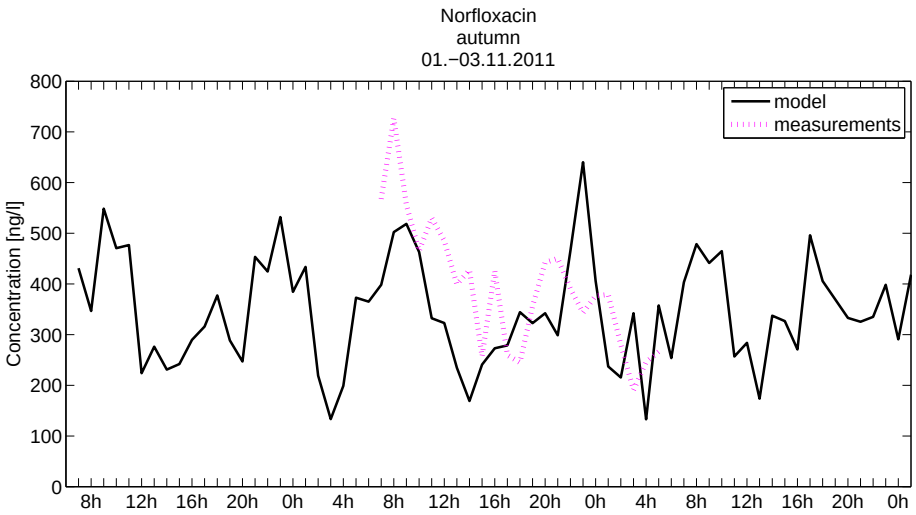


Figure 12b): Comparison of the calibrated norfloxacin concentrations with the measurements

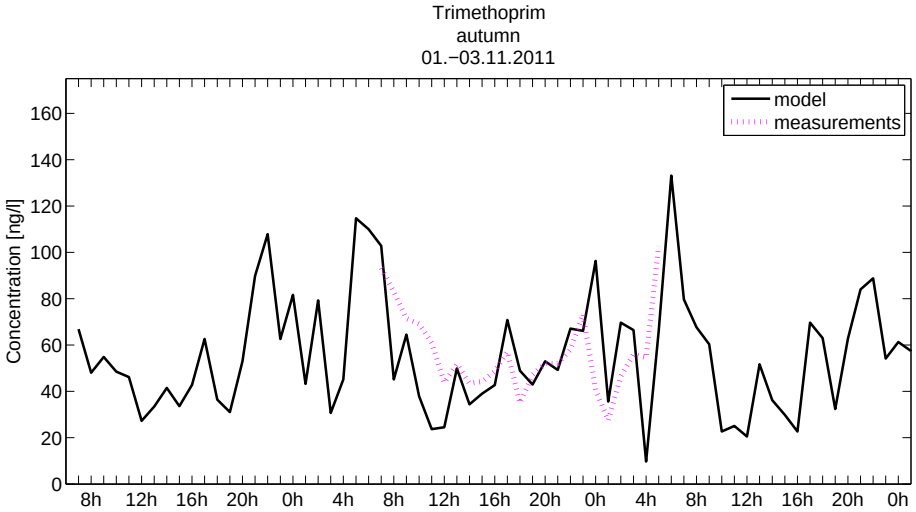


Figure 12c): Comparison of the calibrated trimethoprim concentrations with the measurements

Figure 13 allows a closer look at these deviations of the measurements. There, for every hour the average and standard deviation over the 10'000 runs are calculated. The standard deviation is added and subtracted to and from the average value and then they are plotted as grey areas around the mean (dark grey) to make it visible if the best fit remains in this variations section. The minimum and maximum values are found and plotted as light grey areas, too.

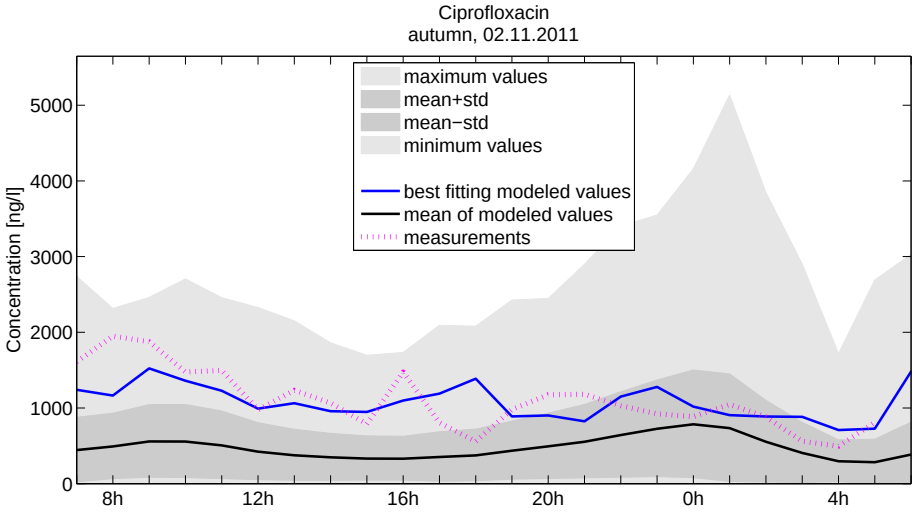


Figure 13a): Comparison of measured ciprofloxacin concentrations (pink dashed line) and best fitting curve (blue line) of the calibration combined with hourly average out of the 10'000 calibration runs (black line). The dark grey zone shows the average + and – the standard deviation of the 10'000 runs calculated separately for every hour. The light grey zones show the range within hourly maximum and minimum values of the 10'000 calibration runs

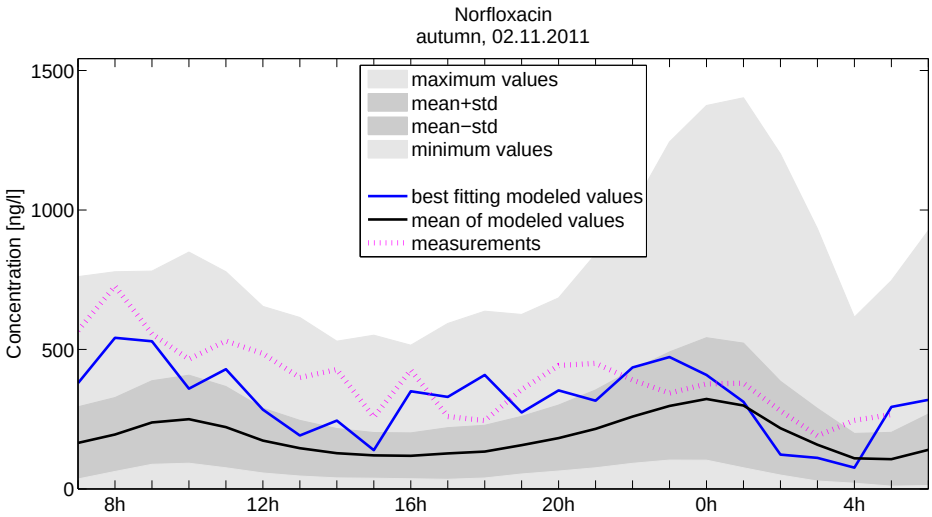


Figure 13b): The same setting as in Figure 13a) shown for norfloxacin.

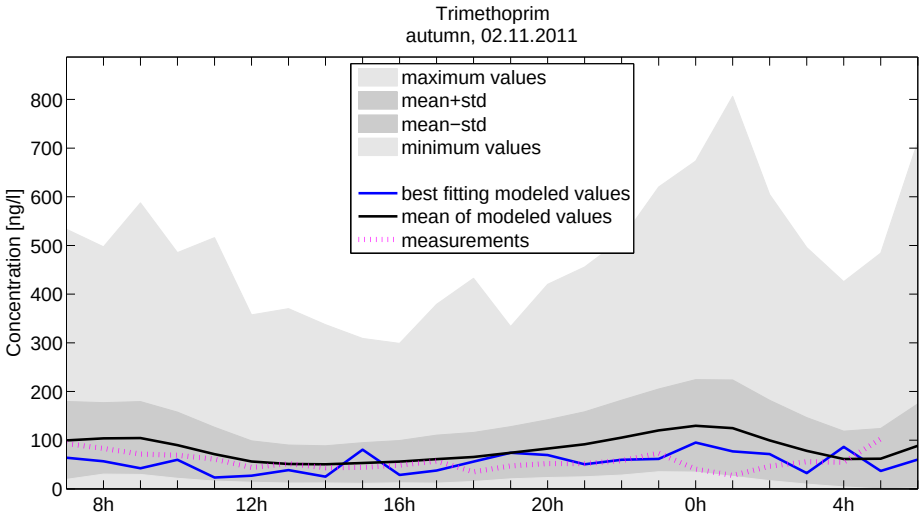


Figure 13c): Figure settings of Figure 13a) shown for trimethoprim.

For all three substances the areas and the curve of the mean modelled values show a dynamic which recalls in principal the toilet use distribution. In the morning and in the evening the modelled concentration tends to be higher than in the afternoon and at night. It is not completely clear why the increase of the maximum values in the afternoon is stronger than the one in the morning. What can be said is that the maximum values generally occur when there is no toilet use during quite a long time before. Like this the antibiotics have more time to accumulate in the urine and resulting load is then rather high. Considering that the first intake is defined to happen at the same hour as the first toilet use and the second one being picked randomly it seems possible that the urinary accumulation of these administrations will happen in the course of the day and higher loads will get discharged towards the evening.

The measurements of all three substances do not surpass the maximum values. But only the measured trimethoprim concentration values remain within the range of standard deviation of the modelled values. As it can be expected the best fitting values are in general closer to the measurements than the curve with mean modelled values of all calibration runs.

4.2.3 Validation

With the parameters found through the calibration the model is run for the same time period as the transport model that is to say from the 1st of December 2010 to the 30th November 2011. Out of this period those days are extracted when the measurements were done and compared to the measurements.

The modelled trimethoprim concentrations agree generally fine with the measurements (see Figure 14a)-c)).

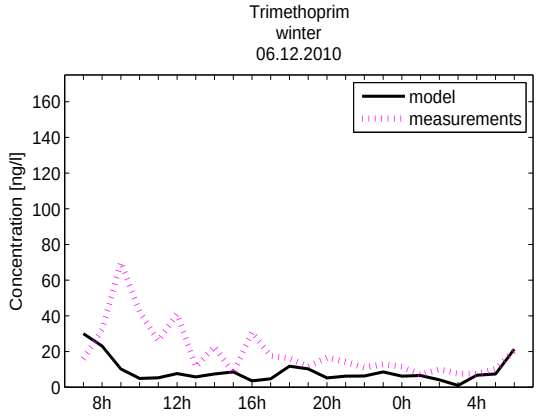


Figure 14a): Comparison of modelled and measured trimethoprim concentrations in winter

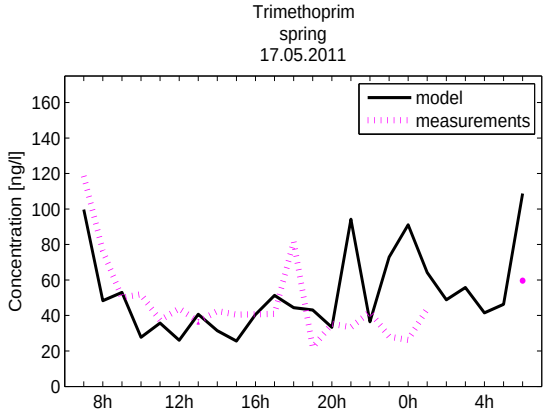


Figure 14b): Comparison of modelled and measured trimethoprim concentrations in spring

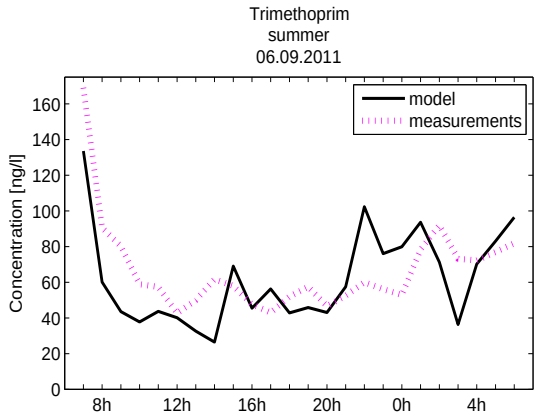


Figure 14c): Comparison of modelled and measured trimethoprim concentrations in summer

The other substances show sometimes much more disagreement between measured and modelled values (see Figure 15, e.g. ciprofloxacin in winter, ciprofloxacin and norfloxacin in spring). In summer however, for all antibiotics the modelled concentrations are to some extent consistent with the measured values (see Figure 15e) and f)). The good agreement in summer might be due to the fact that the measurement campaigns of autumn (used for calibration) and summer took place in quick succession (less than two months).

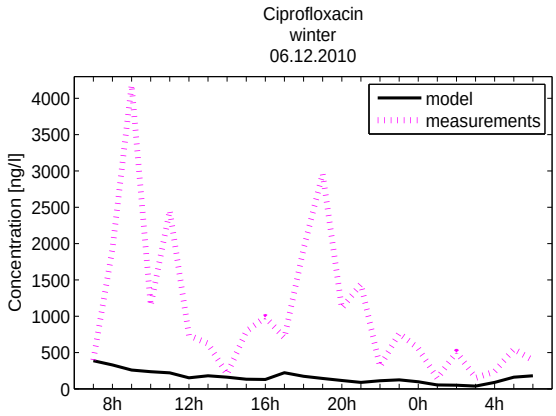


Figure 15a): Comparison of modelled and measured ciprofloxacin concentrations in winter

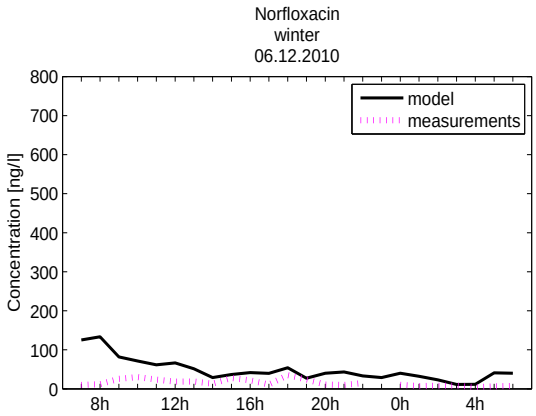


Figure 15b): Comparison of modelled and measured norfloxacin concentrations in winter

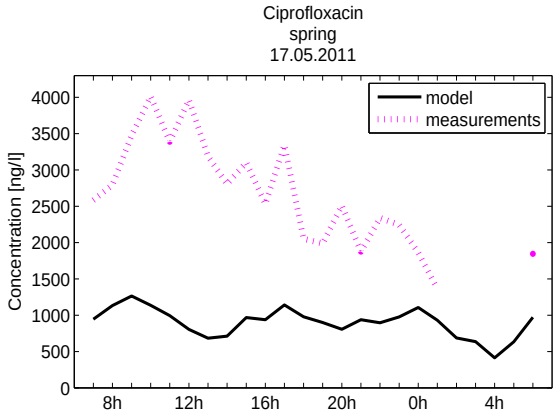


Figure 15c): Comparison of modelled and measured

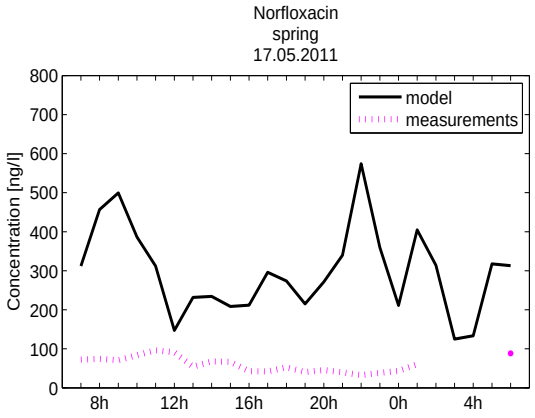


Figure 15d): Comparison of modelled and measured

ciprofloxacin concentrations in spring

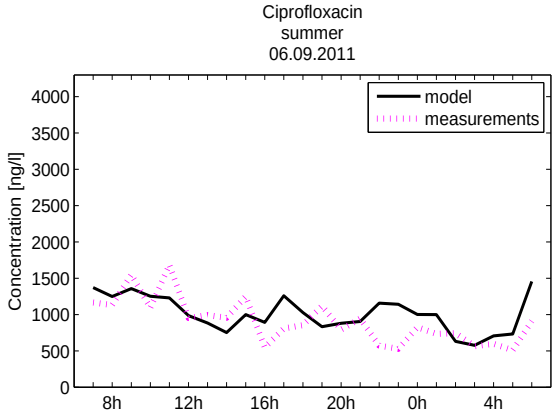


Figure 15e): Comparison of modelled and measured ciprofloxacin concentrations in summer

norfloxacin concentrations in spring

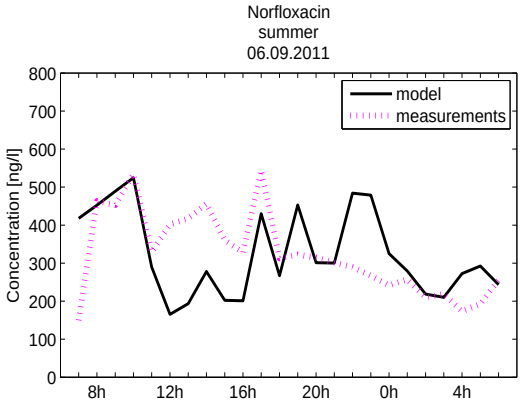


Figure 15f): Comparison of modelled and measured norfloxacin concentrations in summer

For none of the substances and seasons a perfect fit of the very short-term dynamic of the modelled and measured values is found. This is however not a big surprise in consideration of the calibration plots (Figure 12 and Figure 13) showing an exact reproduction of the hourly concentration dynamic neither.

4.2.4 Modelled dynamics

Short-term dynamics of the antibiotics discharge

The distribution of the time of toilet uses is quite an important component of the model as mentioned in section 3.2.4. The daily antibiotic discharge pattern on a monthly average follows quite strongly the diurnal pattern of the toilet use distribution. This is illustrated in Figure 16.

By comparing the mean diurnal patterns of the three modelled substances ciprofloxacin, norfloxacin and trimethoprim with each other slight differences from one substance to another can be discovered.

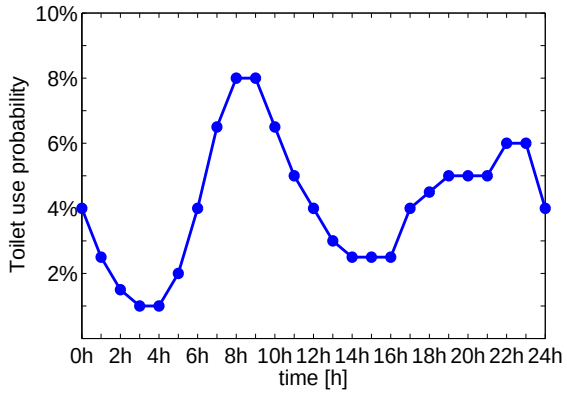


Figure 16a): Toilet use distribution during one day which is a predominant component of the discharge model

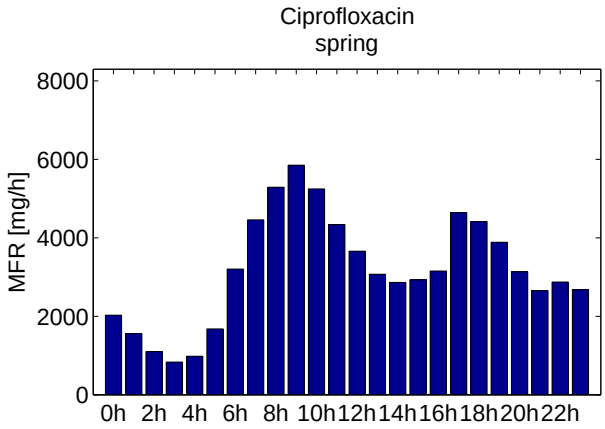


Figure 16b): Mean diurnal pattern of the ciprofloxacin mass flow rate (MFR) discharged into the sewer shown on the example of spring. It shows a similar dynamic to the toilet use distribution in 16a)

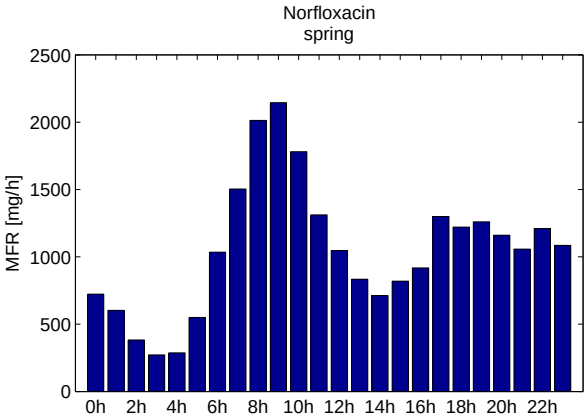


Figure 16c): Mean diurnal pattern of the norfloxacin discharge into the sewer in spring

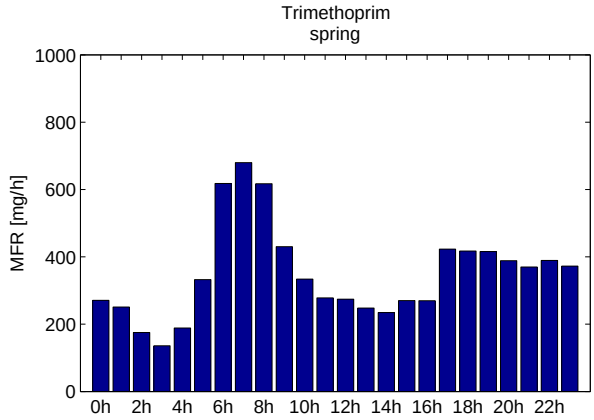


Figure 16d): Mean diurnal pattern of the trimethoprim discharge into the sewer in spring

This modelled average diurnal dynamic of the antibiotics shows some similarity with the measured dynamic explained in section 4.1.2 (Figure 9c)). They both show a first peak in the morning and a second one in the afternoon. The morning peak is in both cases clearer and takes place within a shorter time frame than the afternoon peak. The measured peak in the morning takes place between 07:00 and 09:00. The modelled morning peak arrives rather between 07:00 and 10:00. The peak in the afternoon can in both cases be located between 17:00 and 21:00. Figure 17 shows by way of example the quite similar daily pattern of measured and modelled hourly norfloxacin load in autumn.

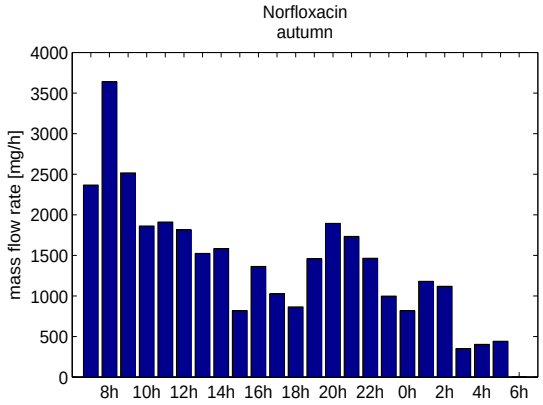


Figure 17a): Measured norfloxacin mass flow rate from the 02.to the 03. November 2011

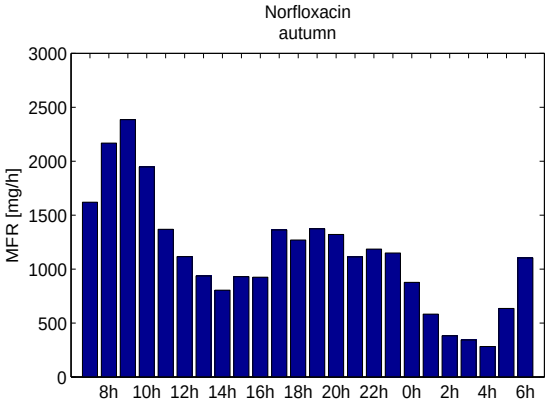


Figure 17b): Average of the modelled norfloxacin mass flow rate in autumn (10.10.2011 – 09.11.2011) showing a very similar diurnal pattern as the measurements (see 17a))

The model reproduces also seasonal differences of the amount of antibiotic sold, that is to say consumed (see Figure 18). Ciprofloxacin for example is sold in higher amounts during winter than during spring. Equally, the modelled daily ciprofloxacin discharge in winter is slightly higher compared to the one of spring and summer.

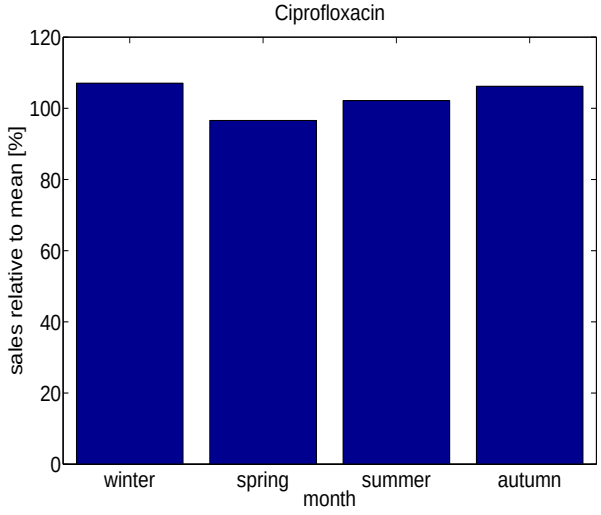


Figure 18a): Relative seasonal differences of sold antibiotic in the case of ciprofloxacin. 100% correspond to the yearly mean of the amount of sold antibiotic.
The seasons are here represented by one month each: winter = 01.-31.12.2010, spring = 01.-31.05.2011, summer = 10.08.-09.09.2011, autumn = 10.10.-09.11.2011

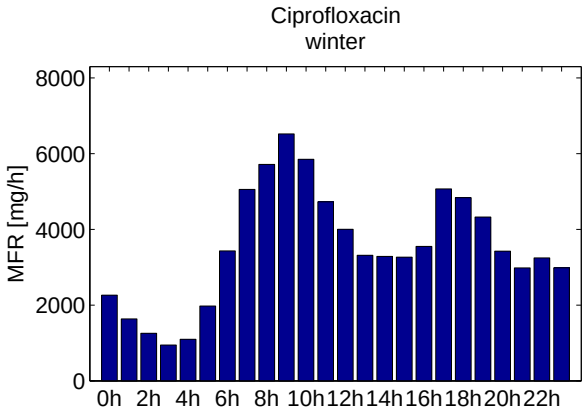


Figure 18b): Modelled monthly average of the ciprofloxacin discharge in winter (01.-31.12.2010) demonstrating the discharge model's precise reproduction of the slight seasonal differences in sold antibiotic amounts. The values in winter are slightly higher than the values in spring (see 18c))

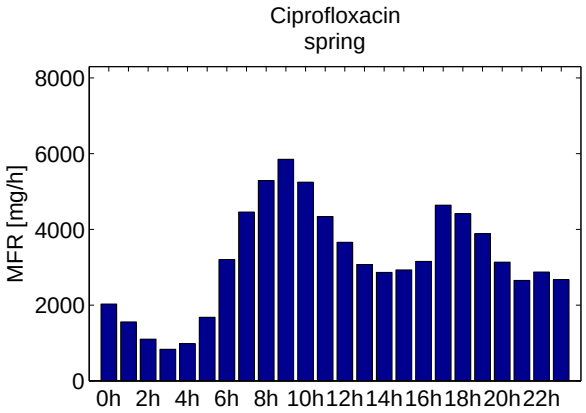


Figure 18c): Modelled monthly average of the ciprofloxacin discharge in spring (01.-31.05.2011). These values are generally a little lower than the values of winter 18b).

Short-term dynamics of the antibiotics occurrence at STP inlet

In previous section the modelled dynamic of antibiotics going into the sewer are discussed. By comparing this dynamic with the modelled concentration dynamic at the STP inlet that is to say after the coupling with the transport model it can be stated that the main pattern persists but it is much more vaguely. Figure 19 presents this on the example of the ciprofloxacin occurrence at the STP inlet but it holds true also for norfloxacin and trimethoprim. Again, there is a clear increase in the morning hours and another one in the evening while in the afternoon and at night there is only little contribution. Comparing the two dynamics with each other (only the dynamic because the ciprofloxacin discharge is given as mass flow rate in mg/h, the flow rate in m^3/s and the ciprofloxacin occurrence at the STP as concentration in ng/l) the modelled wastewater flow rate seems to blur the very regular pattern of the discharge model. The small peak in the afternoon seen in Figure 19a) is delayed a little towards the evening and it gets more accentuated by the coupling with the flow rate.

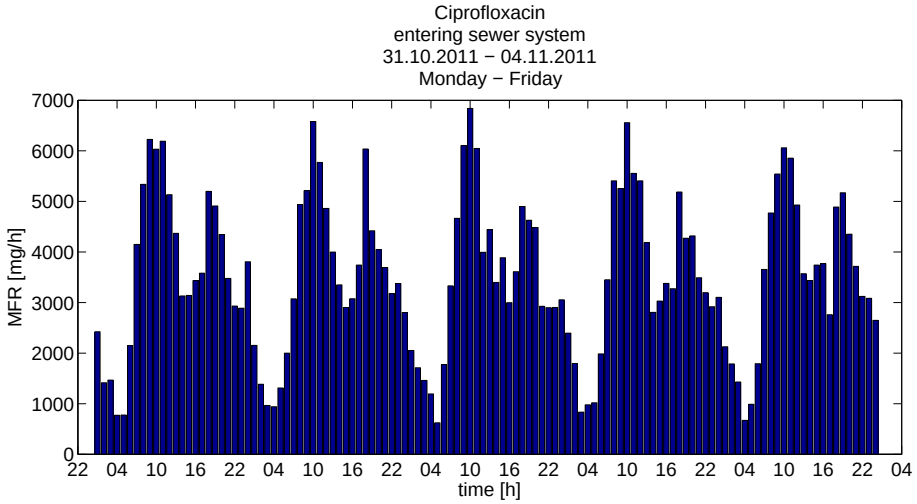


Figure 19a): Repeated presentation of the development of the modelled ciprofloxacin mass flow rate entering the sewer system.

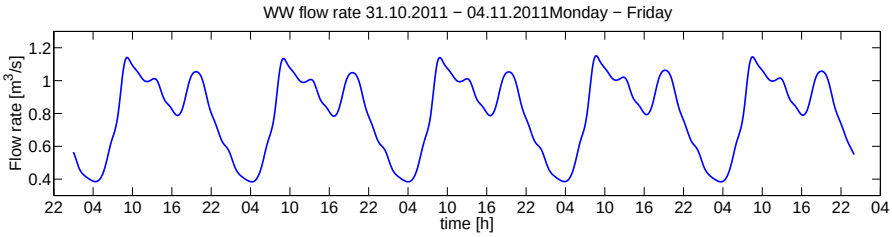


Figure 19b): Modelled wastewater flow rate showing as well a daily cycle which is however not perfectly synchronous with the modelled cycle of antibiotics discharge leading to a diffusion of the peaks at the STP inlet

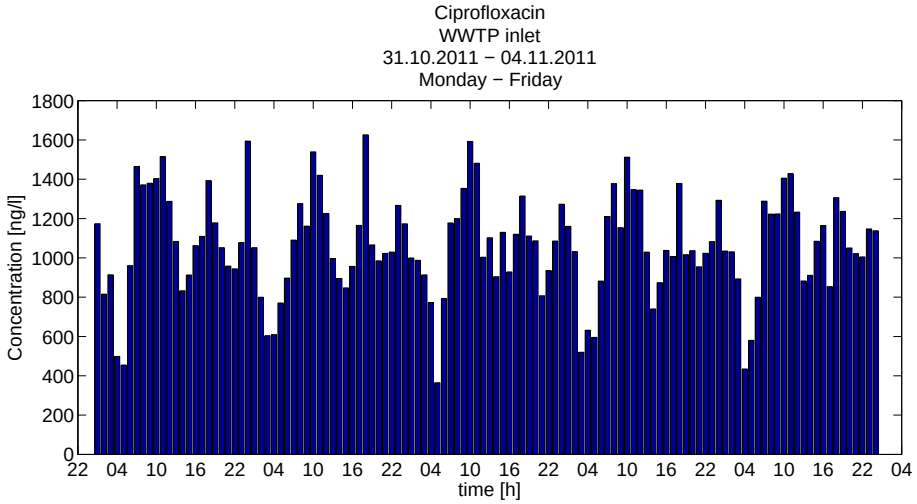


Figure 19c): Development of the modelled ciprofloxacin concentrations at the STP inlet over the same five days as above (19a)) showing again a diurnal pattern which is however more diffuse because of the coupling with the wastewater flow rate.

Long-term dynamics of the antibiotics concentration at the STP inlet

The created model concentrates on short-term dynamics of the antibiotics occurrence in the wastewater. But with the available data it can be run for the whole year from the 1st of December 2010 to the 30th of November 2011. The measurement campaign concentrating on long-term antibiotics concentration variations was being carried out from March 2011 to February 2012. So, the overlapping period lasts from March 2011 to November 2011 that allows having a look at the long-term performance of the model.

Figure 20 shows in black and grey lines the monthly mean values with the standard deviation added and subtracted in grey. The measurements consist of seven daily average values every month. Their monthly average is presented in pink dashed lines and their added and subtracted standard deviation in light pink dashed lines.

In general the order of magnitude of the modelled values is more or less consistent with the measurements. November shows the best agreement. The measurements used for the calibration are not from the same campaign as the measurements presented here. Even so the calibration is conducted for November which is the reason for this almost perfect accordance of the average values.

The modelled values show less dynamic than the measurements. This is probably due to the fact that not the real antibiotic consumption of the considered time period is given as antibiotic consumption input but the monthly average antibiotics sales between 2006 and 2010 instead. This aspect is further discussed in the section about the hypotheses of the model design in subchapter 4.2.5. Generally the ciprofloxacin and norfloxacin concentrations seem to be overestimated by the model while the trimethoprim concentration is rather underestimated by the model.

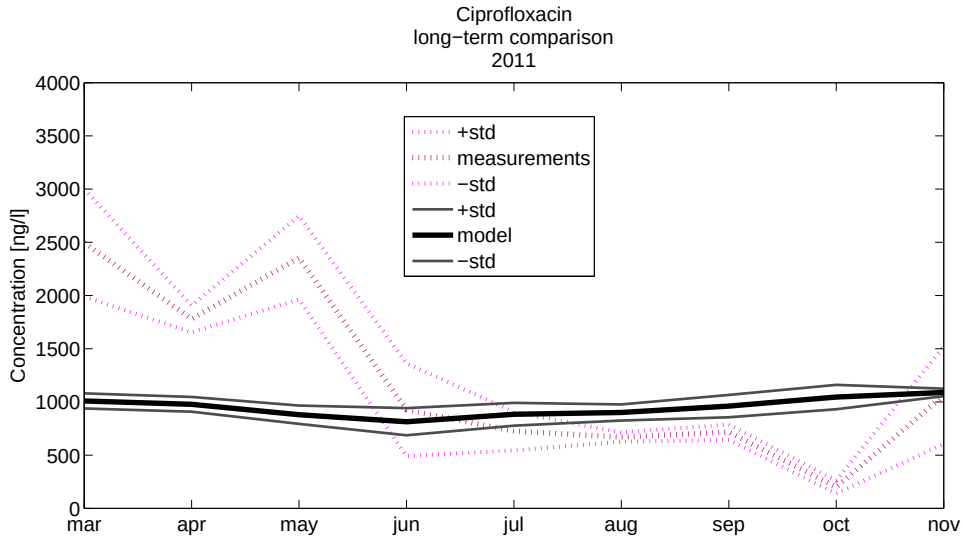


Figure 20a): Comparison of measured (pink dashed lines) and modelled (black and grey lines) ciprofloxacin values on the long-term. The pink dashed lines show the measured monthly mean consisting of seven daily means per month. The light pink dashed lines are the added and subtracted standard deviations. In black the monthly mean of the modelled values and in grey their added and subtracted standard deviations are shown.

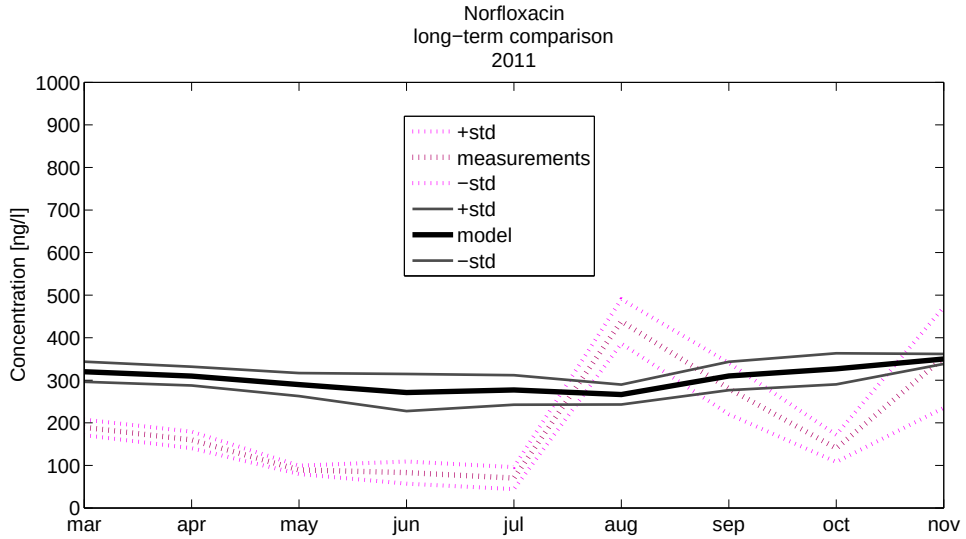


Figure 20b): The same setting as in 19a) for norfloxacin

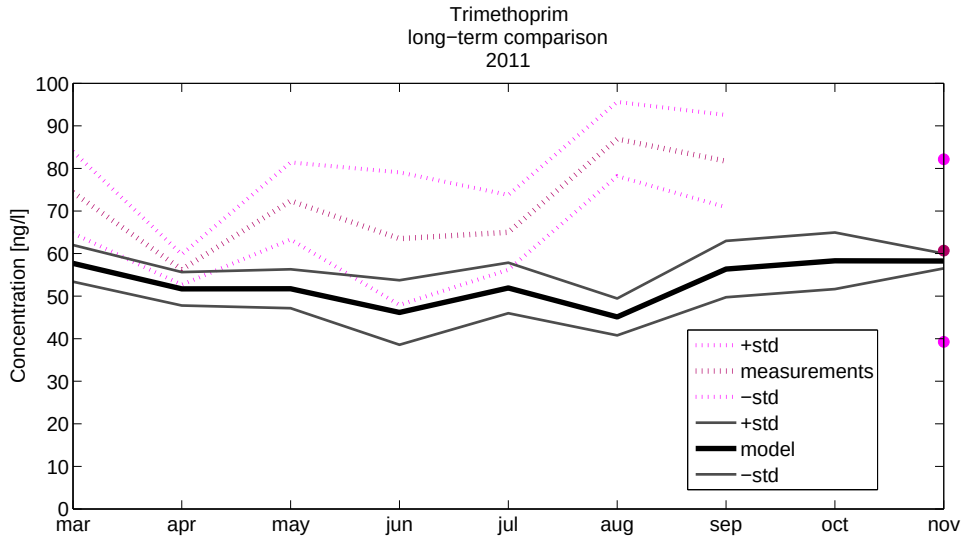


Figure 20c): The same setting as in 19a) for trimethoprim

Dividing the nine modelled monthly values from March to November 2011 by the nine measured values the percentage of the modelled to the measured values is found. The average of the percentages over these nine months is calculated for all three substances. The values are presented in Table 7 together with the range of these percentages indicating minimum and maximum deviations between measured and modelled values. Furthermore in Table 7 the long-term performance of the model is compared to the model results of other models. The comparison is employed by calculating again the percentage of the modelled to the measured values of two of the models presented at the beginning in section 1.3. The first one is the model of Heberer and Feldmann [11] predicting diclofenac and carbamazepine loads and the second one is the model of Johnson and Williams [13] estimating estradiol estrone and ethinylestradiol concentrations. The first of the two models predicts the micropollutant load on a weekly basis. Johnson and Williams [13] based their model on daily excretion values (e.g. µg/d). The values consulted here for the comparison are given as concentrations (ng/L). In both models the micropollutants were predicted at different sites. For the comparison only the predicted and measured values at the STP inlet are

considered here. Johnson and Williams [13] additionally applied their model on seven different catchment areas. The average values and the range given in Table 7 refer to the values of the different areas taken all together.

Table 7: Comparison of the model performance of the present short-term model (a)) with existing models with lower temporal resolution (b) and c)). The percentage of modelled to measured values is calculated and averaged in the case of the present short-term model as well as of the model of Johnson and Williams. A mean percentage of 100% signifies a perfect agreement of modelled with measured values. The ranges give minimum and maximum percentages of the modelled to the measured values.

a) Present short-term model		Range	Mean percentage over the 9 monthly values
	Ciprofloxacin	37-520%	137%
	Norfloxacin	99-390%	212%
	Trimethoprim	53-96%	76%

b) Heberer and Feldmann [11]		Range	Percentage
	Carbamezine	65-104%	85%
	Diclofenac	135-157%	146%

c) Johnson and Williams [13]		Range	Mean percentage over the 7 different catchment areas
	Estrone	60-95%	79%
	17β-estradiol	56-111%	84%
	17αethinylestradiol	50-120%	81%

Table 7 shows that the model developed in the present master thesis works overall less precise on the long-term than the two models found in literature. For ciprofloxacin and norfloxacin the ranges are larger than for the other models. As already hinted before, the hypotheses made on the consumed antibiotic amounts might be an important factor contributing to these deviations. Beside it, the transit loss fraction could play an important role, too.

The model results of the two models found in literature show on the other hand average deviations of at least 15% to the measurements. Considering the model of the present work as a first approach to simulate short-term micropollutants concentration variations the large mean percentage deviations seem acceptable.

4.2.5 Discussion of deviations and uncertainties

The comparison of modelled and measured values in section 4.2.3 reveals two types of deviations: deviations in the order of magnitude of the resulting values and dynamical deviations.

These deviations are very likely to arise from the already mentioned random character of the model, from uncertainties in the calibrated parameters or an imperfect calibration and the hypotheses made in the design of the model. They all act in different ways on the result.

The random noise

Based on the data on medicament prescription from 2006 to 2010 in the Canton of Vaud it is estimated that roughly 270 persons consume ciprofloxacin twice a day. For norfloxacin they are estimated to be about 100 persons and trimethoprim assumed to be administered 4 times a day by about 50 persons every day. These numbers are not big enough to eliminate a certain randomness of the cumulated discharge from one day to another. Figure 21 shows by way of example the modelled hourly ciprofloxacin from the 31st October to the 4th November 2011. Only workdays are considered here because the toilet use distribution of weekend days is likely to differ from the one used here in the model [38]. Again, the diurnal pattern of the ciprofloxacin discharge can be recognised but the mass entering the sewer at a certain hour is not the same every day. These detailed dynamical differences are due to the random character of the model.

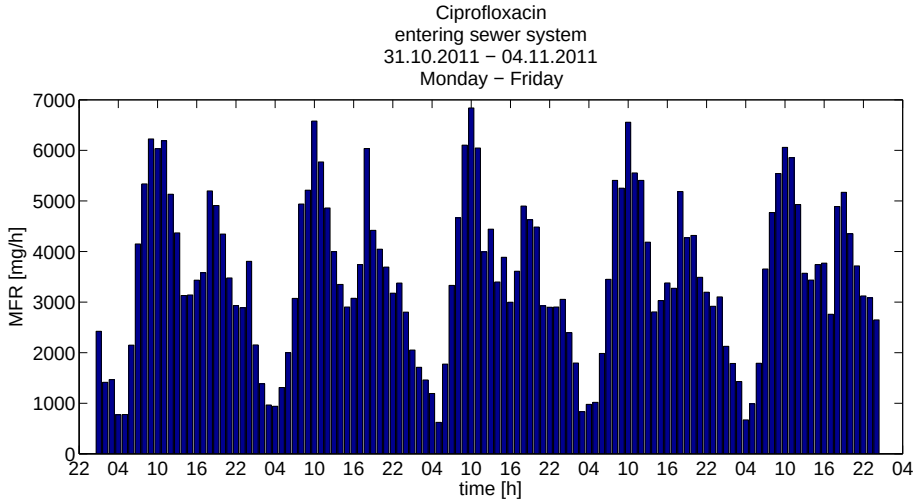


Figure 21: Development of the modelled ciprofloxacin mass flow rate entering the sewer. During these days there was almost no rain and they correspond to workdays only (the underlying toilet use distribution is mainly based on workdays). It shows beside the diurnal pattern also differences in the hourly dynamics from one day to another that are only due to the random character of the antibiotic discharge model.

Figure 22 gives a more precise idea about the extent of this random variability or noise shown on the example of December. It is plotted with the parameter set that resulted from the calibration. The black line shows the average modelled ciprofloxacin mass discharged into the sewer in December. The grey areas around that curve stand for the random noise in terms of the standard deviation added to and subtracted from the mean values. The blue bars indicate the absolute value of the standard deviation of the corresponding hour showing that during night between 0 and 5 o'clock the standard deviation is a little bit smaller than for the rest of the day.

There are fewer persons consuming trimethoprim than ciprofloxacin and norfloxacin. This means that the monthly average is more "exposed" to the randomness of the model approach. Therefore its standard deviation is relatively higher. On a daily average in this example of December it counts to about 28% of the mean mass (black line) while it counts only 22% for norfloxacin and 10% for ciprofloxacin which is consumed by the highest number of persons.

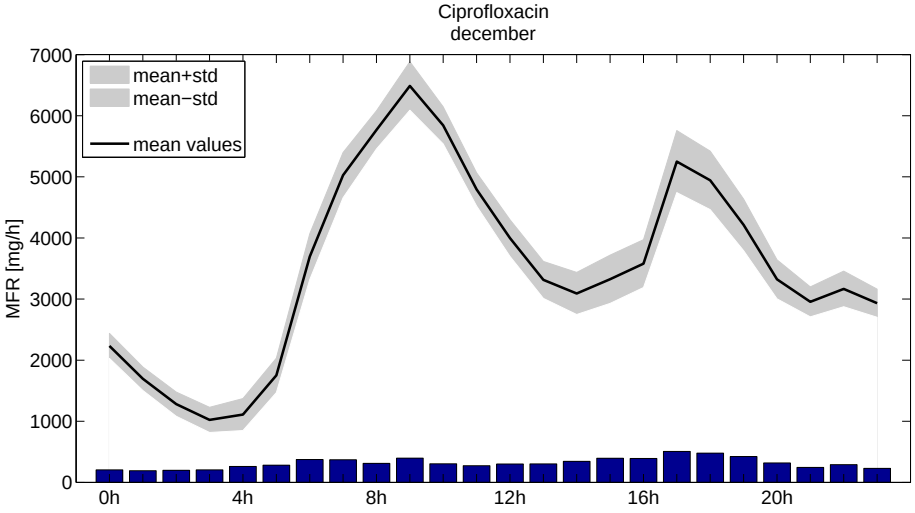


Figure 22a): Mean development of the discharged ciprofloxacin mass flow rate in December 2010 (black line) + and – the standard deviation (grey areas). The blue bars show the absolute values of the standard deviation. The number of persons estimated to consume ciprofloxacin every day is about 270.

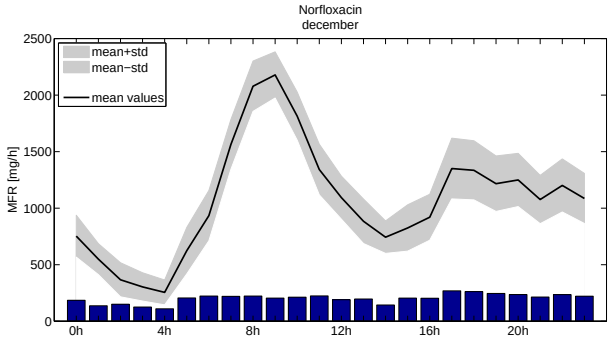


Figure 22b): Same setting as described in 20a) but this time with norfloxacin. The number of persons estimated to consume norfloxacin every day is around 100.

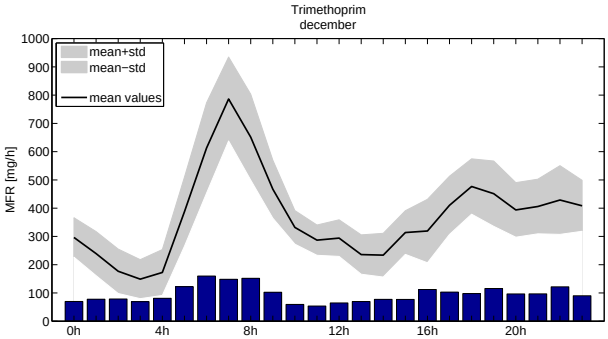


Figure 22c): Same setting as described in 20a) but this time with trimethoprim. The number of persons estimated to consume trimethoprim every day is around 50.

Figure 23 shows again the comparison between measured (pink dashed lines) and modelled (black line) values but with introducing this time the standard deviation which is due to the random noise. It is shown on the examples of norfloxacin and trimethoprim in summer because these substances are stronger influenced by the random model character. By considering these graphs it gets clear that the Monte Carlo approach limits the validity of the model results but only on the very short-term dynamic. The randomness causes different model results from one run to another depending on the parameters randomly chosen in every run. The norfloxacin concentration at 08:00 for example can be 400 ng/l or 500 ng/l or in-between (see Figure 23). This holds true also for 09:00 and 10:00. So, in this time frame the exact concentration dynamic is not clear. The principal dynamic throughout the day however is thereby not affected.

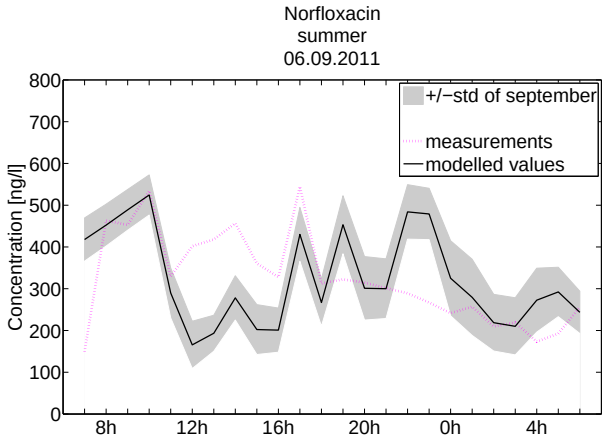
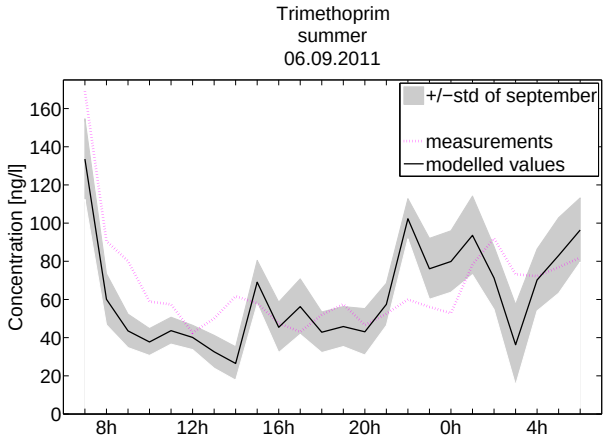


Figure 23: Measured and modelled values of norfloxacin in summer with addition and deduction of the standard deviation resulting exclusively of the random character of the model.



Measured and modelled values of trimethoprim in summer with addition and deduction of the standard deviation resulting exclusively of the random character of the model.

The calibrated parameters

Among the calibrated parameters there are some acting on the order of magnitude of the model results and some influencing the dynamic.

- A given **urinary accumulation curve** as for example seen in Figure 3 results in a certain order of magnitude of the resulting antibiotic concentration in the urine. By the calibration a rate is determined which changes the accumulation curve as a whole accounting for the given uncertainties in this curve. The detailed dynamic within the curve however remains the same.

- The **transit loss fraction** is a reduction rate accounting for antibiotic losses during the transport from the toilet to the STP. It is determined through the calibration and it

concerns all hourly values the same. This means that altering the transit loss fraction influences only the order of magnitude of the resulting antibiotic concentration.

Both, the transit loss fraction and the rate of magnitude of the urinary accumulation curve have influence only in the order of magnitude of the results. The rates determining the urinary accumulation of ciprofloxacin and norfloxacin are both estimated to be slightly higher than 100% (109% and 115% respectively). Also their transit loss fractions are estimated to be around 10% which tends to compensate the additional urinary accumulation. In the case of trimethoprim both calibrated parameters tend to reduce the originally given amount of trimethoprim (81% for urinary accumulation and 55% for the transit loss fraction). This could perhaps be due to smaller trimethoprim consumption in autumn 2011 compared to the mean amount consumed in autumn from 2006 to2010. The calibration is conducted just to account for such uncertainties. The plots of the calibration (Figure 13 and Figure 14) indicate that the two parameters are likely to be correctly calibrated for all substances.

However, the validation plot of winter and spring of ciprofloxacin and spring of norfloxacin show a deviation in the order of magnitude. If there is not another (not yet considered) reason for these deviations perhaps a separate calibration of these two parameters for these seasons would be needed. It is conceivable that the transit loss fraction varies from one season to another. Reason for this could be for example the diffusion of antibiotics in the biofilms since it is occasionally dependent on the temperature [42].

In contrary the other calibrated parameters, namely the mean number of toilet uses a day and its standard deviation as well as the standard deviation of the time of the 2nd medicine intake and the travel time, they have an impact on the dynamic of the hourly antibiotic occurrence at the STP. They are directly coupled to the random choice of certain parameters within the model. That is why their influence on the final model results is assessed here in dependence of the variability due to the random model approach.

- An inaccurate determination of the **standard deviation of the time of the subsequent antibiotic intake(s)** has negligible influence in the final result. When changing this parameter by adding or subtracting 50% of its number found in the calibration the resulting random noise does not noticeably change.

- In the calibration the **mean number of toilet uses a day** is determined to be twelve or eleven depending on the substance. According to the numbers found in literature (see Table 4) this corresponds to very high numbers. The best fit of model and measurements seems to be achieved with a rather high frequency of toilet uses which implies that the amount of antibiotic introduced each time is smaller but on the other hand the antibiotics are introduced more continuously than with less toilet uses a day.

Varying the mean number of toilet uses in the model shows that the more the mean number of toilet uses is reduced, the more the random noise is increased. This is reasonable because a smaller number of toilet uses ends up in the more sporadic introduction of higher antibiotics amounts which is indicative for a higher variability.
It seems that with a more realistic mean number of toilet uses as for example 6 times a day the random noise gets that high (on average 38% to the mean values in December instead of 28% as seen in the previous section) that stronger differences to the measured values occur. In the calibration a better fit seems to be fulfilled with a smoother curve which is achieved with a smaller random noise by a higher mean number of toilet uses a day.

- The analysis of the **standard deviation of the number of toilet uses** reveals that an overestimation of this parameter leads to a bigger random noise while an underestimation reduces the random noise of the final results. The influence of uncertainties in the determination of this parameter however is not as big as in the case of the mean number of toilet uses a day. This is explained also in the following example: If the validation is run for trimethoprim with only 6 instead of 12 toilet uses a day on average (-50%) the resulting random noise is about 38% instead of 28% as seen before. If the simulation is run again with 12 average toilet uses a day and a standard deviation of the number of toilet uses of 6.3 instead of 4.2 (+50%) the resulting random noise is about 32% instead of 28%. So the increase of the random noise is smaller if the standard deviation of the number of toilet uses is changed by 50% of its original value than it is the case with the average number of toilet uses. If the same simulation is run with 2.1 as standard deviation of the number of toilet uses (-50%) the final random noise is about 23%. So, the random noise gets smaller when the standard deviation is smaller.
  The expected standard deviation based on literature (see Table 4) is around 2. For ciprofloxacin the calibration determined it to be 2 for norfloxacin it is 3.6 and for trimethoprim it is 4.2. Compared to the mean number of toilet uses a day it seems that the random noise is increased by the calibration of this parameter but clearly less than it is decreased by the calibration of the mean number of toilet uses.

- The **travel time** is the parameter accounting for the time it takes the antibiotics to be transported from toilet to the STP. The calibration determined it to be zero. This is reasonable given the short travel time expected in this catchment area and considering also the fact that the modelled mass flow rate peak in the morning is already a little late compared to the measured one. If this however was wrong the hourly dynamic would be kept as it is but the whole concentration curve would be shifted by one hour or by at most two hours. Altogether it seems that this parameter is rather not or even not at all contributing considerably to the deviations between modelled and measured values.

The hypotheses of the model design

For the design of the model there are also some hypotheses made which could contribute to a certain imprecision of the results:

- On the **consumed antibiotic amounts** several hypotheses were made: The amount of prescribed antibiotics is assumed to equal the amount consumed. This hypothesis includes that patients consume always the whole package they bought and that the medicaments bought outside but consumed inside the perimeter and the medicaments bought inside and consumed outside balance each other.
  Since sold antibiotic amounts are not directly known for the considered area, they had to be deduced from data of the pharmacy of the CHUV and from the pharmacy of the whole canton of Vaud.
  Additionally for the modelled period from December 2010 to November 2011 the antibiotics amount consumed or rather prescribed is assumed to be equal the monthly average of prescribed antibiotics from 2006 and 2010. The rate of the standard deviation to the mean for these 5 years counts about 7% for ciprofloxacin 16% for norfloxacin and 12% for trimethoprim.
  Deviations between the estimated and the real monthly consumption could lead to a wrong order of magnitude of the modelled results. As seen in section 4.2.4, the model reproduces differences of the amounts of consumed antibiotics quite precisely. This however does not impact the daily dynamic.
  There is no way to prevent this problem through the calibration because these data have a temporal resolution of one month and the calibration is run for a time period of 24 hours. To avoid deviations of this kind the data of the actual considered time period are needed.

- The **time of the first antibiotic administration** a day is assumed to happen in the same hour as the toilet is used the first time. If this does not hold true the urinary accumulation would be temporally shifted which finally would impact the short-term dynamic. This hypothesis might lose some importance by the fact that the urinary accumulation is calibrated in the model although it is only the magnitude of the urinary accumulation curve which is calibrated.

- The **intravenous administration** is not considered in the model. It concerns only ciprofloxacin with a contribution of 15 to 20% of the total consumed mass. The intravenous application of ciprofloxacin is expected to alter the short-term dynamic of its occurrence at the STP inlet since the total mass input to the model remains the same. In addition, it would concern all seasons because the frequency of intravenous administration is not following a clear seasonality.

- In the model **only the urinary excretion** is considered. As explained in section 3.2.3, a part of the administered antibiotics is excreted by faeces. This is in the majority of the cases a minor part but for norfloxacin this does not hold true [4]. Since the excretion via faeces

also happens via the toilet it can be regarded as being temporally related with the urinary excretion [38]. So this hypothesis concerns mainly the order of magnitude of the antibiotics concentration at the STP and does rather not impact its temporal dynamic. The urinary accumulation curve is calibrated as a whole which is assumed to have compensated also for this aspect. Indeed, the calibration determined the urinary accumulation parameter of norfloxacin to be as high as the proposed maximum value of 115% (see Table 5 and Table 6).

- The **toilet use distribution** is based on three studies which have investigated in this field but primarily in the domain of the domestic toilet use. The toilet use distribution applied in this model is finally something like a patchwork of the information given in these studies. It is also assumed that the toilet use distribution curves of these studies are all related to urinary use. As discussed and shown by Friedler et al. [38]this hypothesis itself is not very critical. However, given the strong influence of the toilet use distribution on the dynamic of the antibiotic occurrence at the STP inlet the uncertainties existing in this model component as a whole might be substantial.

4.2.6 Improvements and extensions

The created model can be regarded as a first attempt to simulate antibiotics concentration in high temporal resolution. The results show sometimes considerable deviations compared to the measurements in the detailed dynamic as well as in the predicted order of magnitude. The analysis of the uncertainties contained in the model showed that a better knowledge about certain aspects could lead to a better model performance.

The model reproduces the entered amount of consumed antibiotic rather precisely. Thus, precise consumption data is needed for the time period that is to be modelled.

A sound knowledge about the local toilet use attitudes would help to reduce uncertainties which have a substantial impact on the model results. Using a tracer which is specific for urinary excretion such as the ammonium nitrogen loads [39] might be a promising approach to find a toilet use distribution which is reliable in particular also for the considered region.

Furthermore, the good agreement of the modelled and measured values in summer which concern a day relatively short before the calibration suggest that one calibration per season instead of one per year could improve the model performance. For this, additional short-term measurements would be needed. With more measurements also the validation would not be so selective. The transit loss fraction is suspected to be seasonal. Even if the calibration would be conducted separately for the seasons a more precise determination of the transit loss fraction could promote the model performance. For this, better knowledge of the biofilm sorption and biodegradation of the antibiotics in the wastewater is needed.

The applicability of the model could be extended on other substances. To do so information on the accumulation of the substance in the urine and perhaps also in faeces is needed. An important precondition is that the substance is mainly excreted via the urine. Another condition would be that it is consumed by a large number of persons. Otherwise the random noise will be too large for reliable short-term dynamic predictions. To completely eliminate this randomness in the model which is presently limiting the validity of the predicted short-term dynamic from one hour to another a model approach without the Monte Carlo method would be necessary.

It could be interesting to extend the model's applicability on metabolites of the antibiotics. Although metabolites of most drugs have a lower ecotoxicological risk they matter and should be considered if the model one day should assist to estimate the ecotoxicological risk of antibiotics [4].

5 Conclusions

The behaviour of antibiotics concentration on the long-term have already been investigated and modelled multiply and seasonal antibiotics concentration variations have been discovered [7, 15-17, 41]. Short-term variations are by contrast not very well known yet [9]. They are important in the methodical context of antibiotics measurements as well as in concern of the environmental impact of antibiotics [1, 6, 9]. For that reason in this project the antibiotics concentration variations at the STP inlet at different time scales were analysed and a model is developed simulating short-term antibiotics concentration variations at the STP inlet.

Measurements of six antibiotics (ciprofloxacin, clindamycin, metronidazole, norfloxacin, ofloxacin and trimethoprim) were being carried out successfully at the inlet of the STP of Vidy in Lausanne for one year. They are arranged in two types of measurement campaigns: one consisting of daily measurements for one week every month and the other consisting of hourly measurements for one day per season. With the first dataset the expected seasonal variations could be confirmed. The measured mass flow rates were in general higher in winter and spring than in summer and autumn. The second dataset was used to analyse short-term variations. They revealed a daily cycle with a first peak in the morning and a second a little less accentuated increase towards the evening. During night the mass flow rates are generally low. This cycle was analytically confirmed by the analysis of the periodicity of the measured antibiotics mass flow rate which showed clear return periods of 24 and 12 hours for the mass flow rate peaks in the morning and the evening respectively.

The variations of the different time scales (monthly, daily and hourly time scale) were compared with each other. The hourly variations within the day were found to reach about the same extent as the monthly or seasonal ones. The day-to-day variations however are in general relatively small. The seasonal variations are comprehensible in consideration of the seasonality in antibiotics consumption [41]. Given the relatively important magnitude of the short-term variations it matters to have a good understanding of their origin.

In that context a model was created focusing on the simulation of antibiotics short-term concentration variations. It is designed in a first step by the constitution of a model for the antibiotic discharge into the sewer on an hourly basis. Parts of the modeol rest upon on a Monte Carlo approach and take into account information on the time of the medicament intake, the antibiotic accumulation in the urine and the time when the toilet is used. The discharge model is coupled with a transport model that simulates the wastewater flow rate in the sewer system. It is applied for the catchment area of the STP of Vidy in Lausanne and for three antibiotics (ciprofloxacin, norfloxacin and trimethoprim). The calibration was executed with the 24 hourly

measurement values of autumn. The comparison of the modelled and measured values was done for the 3×24 hours of the remaining seasons. This is both quite limiting for the validity of this direct comparison. However, it is probably the best possible way to calibrate and validate the model.

The predicted concentrations in summer are in good agreement with the measurements. This is possibly due to the short time difference (less than two months) to the time frame of the calibration. Some of the validation graphs of the remaining seasons show certain deviations in the order of magnitude between measured and modelled values. The results of the calibration suggest that the deciding parameters acting on the result's order of magnitude are in principal correctly calibrated. It is possible however that the transit loss fraction changes in the course of the year and a separate calibration for the different seasons would be necessary to account for that. In addition, the model calculations are based on the hypothesis that the monthly average of sold antibiotics from 2006 to 2010 corresponds to the consumption of the modelled time period from December 2010 to November 2011. It is likely that more precise results could be achieved with the exact consumption data of the modelled time period.

The very short-term dynamic from one hour to another shows for all three substances some discrepancies. This holds true for the calibration results, too. The detailed hourly antibiotics dynamic cannot perfectly be predicted because of the noise caused by the randomness of the Monte Carlo approach. The random noise is the more reduced the more random samples are taken. So, ciprofloxacin showed the smallest random noise because it is consumed by a higher number of persons than the other two antibiotics. Important peaks or dynamics throughout the day are however not affected by the random noise.

The modelled short-term dynamic of the antibiotics is additionally influenced by uncertainties in the determination of the hours when the toilet is used. The moment the toilet is used there will be antibiotics entering the sewer which demonstrates the relevance of this aspect. The toilet use distribution and the mean number of toilet uses a day can be assumed to be the most powerful factors concerning their influence on the short-term antibiotics dynamics. The calibration revealed rather high values for the mean number of toilet uses a day (around 12) which indicates certain uncertainties in this aspect. The toilet use distribution curve is based on literature values on domestic toilet use and contains some hypotheses incorporated during its assemblage.

By comparing in a more general sense the diurnal dynamics of the modelled and the measured mass flow rates side by side a certain resemblance appears. In both cases a strong peak in the morning between 07:00 and 10:00 was found and a second one towards the evening between 17:00 and 21:00. The latter is a little less accentuated in both cases.

The comparison of the modelled values with the measured concentration on the long-term over months revealed that the modelled values agree not too bad with the measurements. Again, the hypotheses on the antibiotics consumption and the lack of knowledge about losses during the transit from toilet to the STP could be cited here for the observed deviations.

The created model is a first attempt to simulate antibiotics concentration on an hourly basis. There are still a lot of improvements that could be done and possibilities to extend the model like for example the application on other substances. Despite the discussed deviations between the model results and the measurements the development of the model allowed identifying the most important parameters contributing to the short-term dynamic: Firstly, the amount of consumed antibiotic and the number of persons administering the medicine are very important model inputs. Secondly, it is essential to have good information about the pharmacokinetics of the considered substances. This means more precisely that the amount and dynamic of the accumulation of the antibiotics in the urine and faeces in unchanged as well as in metabolised form are basic requirements for reliable model calculations. And thirdly, the time when the toilet is used is very deciding because it is the moment when the antibiotics are released into the sewer.

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Appendix

Flow rates

Table 8 : Flow rates during monthly campaigns at the STP inlet in m^3/s

Date			MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	1.111	0.916	0.997	0.997	0.997	0.997	1.005				
	08.-15.	April					1.111	0.916	0.950	0.997	1.005	0.971	0.988
	06.-13.	May					0.908	0.857	0.854	0.957	0.939	0.924	1.080
	17.-24.	June					1.398	1.407	0.878	0.880	0.890	1.367	1.027
	08.-15.	July					0.838	0.956	1.126	1.137	1.189	1.519	0.850
	19.-26.	August					1.062	0.938	0.958	0.987	1.004	0.856	0.869
	09.-16	September					1.062	0.938	0.958	0.987	1.004	0.856	0.869
	07.-14.	October					0.866	1.119	1.052	1.028	0.817	0.831	0.847
	11.-18.	November					0.834	0.876	0.779	0.861	0.813	0.851	1.072
	06.-13.	December		1.014	1.679	1.173	0.958	0.754	0.786	1.404			
2012	06.-13.	January					1.666	1.544	1.511	1.326	1.233	1.162	1.119
	03.-10.	February					0.981	0.901	0.846	0.970	0.966	0.986	0.834

Table 9: Flow rates during seasonal campaigns at the STP inlet in m^3/s

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				-
7.00	6.019	1.080	1.043	0.565
8.00	8.271	1.354	1.247	1.157
9.00	8.988	1.201	1.403	1.391
10.00	8.988	1.136	1.048	1.258
11.00	8.988	1.223	0.932	1.112
12.00	8.988	1.142	1.038	1.001
13.00	8.988	1.085	1.214	0.853
14.00	11.156	0.980	1.127	1.060
15.00	12.484	0.890	0.891	0.375
16.00	12.832	0.912	0.855	1.028
17.00	12.832	0.771	0.869	0.885
18.00	12.660	0.915	0.815	1.098
19.00	14.866	1.068	0.972	0.981
20.00	16.033	1.244	1.054	1.137
21.00	16.454	1.186	1.002	1.189
22.00	16.095	0.914	1.035	1.070
23.00	14.621	0.830	0.817	1.042
24.00	13.897	0.667	0.697	0.808
1.00	14.146	0.559	0.600	0.602
2.00	13.828	0.491	0.579	0.864
3.00	13.065	0.475	0.560	1.109
4.00	13.065	0.469	0.612	0.513
5.00	13.065	0.472	2.211	0.456
6.00	13.065	0.586	0.855	

Antibiotics

Ciprofloxacin

Table 10 : Measured ciprofloxacin concentrations of monthly campaigns at the STP inlet in ng/l

Date			MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	2729.95	3072.7	3069.8	1752.7	2413.05	1990.8	2436.4				
	08.-15.	April					1828.1	1805.7	1516.3	1921.6	1818.7	1781.7	1788.1
	06.-13.	May					1995.1	1997.3	2172.3	2454.8	3142.9	2339	2386.8
	17.-24.	June					634.8	448.6	1052.8	1662.9	1317.3	692.8	679.6
	08.-15.	July					908.4	843.9	616.9	854.6	628.7	406.5	818.7
	19.-26.	August					738.5	645.15	625.95	642.5	639.4	718.5	678.7
	09.-16	September					643.7	759.05	645.05	848.8	692.95	678.95	741.2
	07.-14.	October					158.1	97.65	249.3	226.9	202.65	185.45	284.7
	11.-18.	November					632	845.8	1207.1	1411.6	1077.5	471.7	1794.7
	06.-13.	December		1224.4	878.9	1507.5	1854.75	1895.15	2517	968.45			
2012	06.-13.	January					602.45	794.3	894.3	1239.4	1110.45	1146.75	1190.45
	03.-10.	February					1702.05	1745.45	1878.05	1942.6	1801.25	1693.6	1993.8

Table 11: Measured ciprofloxacin concentrations of seasonal campaigns at the STP inlet in ng/l

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				1529.4
7.00	397.6	2584.7	1166.1	1609.8
8.00	1922.4	2792.7	1130.7	1949.5
9.00	4186.1	3475.2	1550.3	1875.4
10.00	1147.7	4013.2	1093.2	1474.8
11.00	2455.8	3387.5	1685.1	1496.2
12.00	738.3	3966.0	951.9	976.2
13.00	621.5	3187.4	992.4	1235.3
14.00	201.1	2806.4	956.4	1063.8
15.00	790.5	3104.4	1250.6	788.3
16.00	989.7	2531.2	545.9	1475
17.00	700.9	3343.7	809	805.1
18.00	1927.2	2052.6	853.7	557.4
19.00	2963.1	1986.8	1118.5	979.9
20.00	1110.4	2510.0	792.5	1174.7
21.00	1443.2	1875.9	944.6	1181.2
22.00	305.4	2340.2	573.5	1030.8
23.00	766.5	2243.6	527.4	922
24.00	557.9	1863.2	825.2	885.1
1.00	143.2	1365.2	733.7	1046.9
2.00	510.7		743.2	881
3.00	150.1		563.6	560.8
4.00	237.5		597.3	495.1
5.00	548.4		507.6	810.2
6.00	392.7	1845.6	910.4	

Clindamycin

Table 12 : Measured clindamycin concentrations of monthly campaigns at the STP inlet in ng/l. Numbers in red are between the limit of detection (LOD) and the limit of quantification (LOQ)

Date			MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	12	10.75	11.1	9.7	13.95	10	11.4				
	08.-15.	April					4.1	5.3	4.9	4.6	4.7	7.1	6.1
	06.-13.	May					9.9	7	7.1	7.4	8.7	7.7	6.7
	17.-24.	June					4.9	2.4	9.8	8.2	9.6	5.9	5.8
	08.-15.	July					7.4	7.6	5.1	5	6.5	5.7	6.2
	19.-26.	August					3.7	2.1	< LOQ	1.6	3.4	3.25	2.45
	09.-16	September					6.35	7.2	6.4	6.25	6.8	5.25	5.6
	07.-14.	October											
	11.-18.	November					7.9	5.5	8.8	8.7	7.7	4.6	2.1
	06.-13.	December		8.2	4.85	8.5	6.1	8.3	6.6	4.55			
2012	06.-13.	January					8.05	5.95	7.4	9.8	10.7	6.7	6.7
	03.-10.	February					2.85	1.55	1.3	1.05	< LOD	< LOD	1.6

Table 13: Measured clindamycin concentrations of seasonal campaigns at the STP inlet in ng/l

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				9
7.00	1.1	19.5	15	10.5
8.00	5.9	9.0	16.8	14.2
9.00	5.1	21.3	14.4	11.1
10.00	4.8	8.8	8.5	10.1
11.00	2.7	5.3	5.9	5.9
12.00	3.6	4.4	5.1	5.8
13.00	3	5.7	6.4	4.8
14.00	2.4	5.8	5	4.3
15.00	3.2	4.0	7.9	4
16.00	2.5	8.3	5.6	4.9
17.00	5.5	4.8	5.2	7.9
18.00	1.9	7.0	2	3.9
19.00	2.1	2.8	7	4
20.00	2.7	2.7	7	1.6
21.00	1.1	2.8	6.7	3
22.00	1.8	2.3	8.1	3.4
23.00	<LOD	5.6	7.3	7.6
24.00	1.1	4.0	5.8	4.5
1.00	1.3	3.3	5	3.4
2.00	1.4		8.8	5.4
3.00	1		6.8	2.3
4.00	<LOD		2.4	1.8
5.00	1		5.4	11.2
6.00	1.1	23.5	11	

Metronidazole

Table 14 : Measured metronidazole concentrations of monthly campaigns at the STP inlet in ng/l

Date		MON	TUE		WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	402.4	130.5	245.75	399.2	122.3	86.05	53.55				
	08.-15.	April					476.2	100.7	49.7	620	113.7	114.2	127.1
	06.-13.	May					1441.7	206	72.9	777.6	123.9	983.8	246.3
	17.-24.	June					343.9	587.4	58.5	599.6	367.1	305.9	495.4
	08.-15.	July					509.9	103.4	92	380.5	159.5	315	279
	19.-26.	August					3534.95	537.95	229.35	1396.1	1143.6	1657.35	1970
	09.-16	September					6725.35	804.95	171.2	2418.95	1616.45	490.35	661.45
	07.-14.	October											
	11.-18.	November					2258.9	2011.9	230	487.5	526.5	407.9	1045.9
	06.-13.	December		4159.2	5637	1344.85	5662.95	2753.2	882.75	1432.1			
2012	06.-13.	January					9690.3	6332.8	838.95	2304.5	3663.8	12077.8	5198.25
	03.-10.	February					46317.1	11531.15	2488	11068.7	5898.15	11539.15	28265.65

Table 15: Measured metronidazole concentrations of seasonal campaigns at the STP inlet in ng/l

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				316.2
7.00	<LOD		104	208.2
8.00	<LOQ		441.1	1376.6
9.00	9.7		728.9	1162.6
10.00	42		1038.5	296.6
11.00	3.6		956.4	166.5
12.00	6.9		594.1	168.9
13.00	5.9		534.9	280.3
14.00	6.5		624.9	819.7
15.00	10.3		540.9	2342.8
16.00	4.6		1169.5	929.8
17.00	17		3809.8	518.1
18.00	12.3		3117.3	197.9
19.00	4.3		450	95.4
20.00	<LOD		107.6	144
21.00	<LOQ		108.9	181.4
22.00	<LOD		204.4	119.9
23.00	4.7		158.4	185.8
24.00	<LOQ		200.9	116.8
1.00	<LOQ		229.3	104.2
2.00	<LOD		136.8	128.7
3.00	<LOQ		233.8	157.5
4.00	<LOQ		217.6	135.7
5.00	14.7		227.1	486.1
6.00	3.0		175	

Norfloxacin

Table 16 : Measured norfloxacin concentrations of monthly campaigns at the STP inlet in ng/l

Date			MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	197.8	169.4	207.85	162.75	206.85	181.5	200.05				
	08.-15.	April					161.9	156.3	141.7	160.3	139.1	198.2	161.1
	06.-13.	May					81.6	75.5	100.5	90.5	102.7	92.5	83.7
	17.-24.	June					80.6	36.3	104.2	107.7	106.2	67.1	80
	08.-15.	July					82.1	111	68.2	66.5	85	33.5	45.3
	19.-26.	August					325.4	468.8	473.3	447.65	466.5	428.95	458.4
	09.-16	September					202.65	376.75	288.9	274.65	225.7	259.85	334.85
	07.-14.	October					145.6	87.6	181.4	160.5	117.7	125.55	161.8
	11.-18.	November					313.1	350.8	487.2	434.3	462.8	272.6	154.7
	06.-13.	December		960.4	625.55	892.9	1241.6	1214.5	1283.8	572.7			
2012	06.-13.	January					381.4	377.45	439	673.45	598.6	673.45	675.75
	03.-10.	February					2337.05	2400.65	1649.15	1980.1	2278.4	1811.45	1977.9

Table 17: Measured norfloxacin concentrations of seasonal campaigns at the STP inlet in ng/l

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				582.5
7.00	9.9	72.2	149.7	568
8.00	11.9	74.3	463.2	727
9.00	25.5	70.6	452.7	555.9
10.00	30.2	83.8	534.3	464.9
11.00	23.7	96.2	329.4	530.3
12.00	18.4	90.8	401.9	485.1
13.00	18.7	54.2	417.9	399.1
14.00	13.1	67.9	456.7	427.8
15.00	27.9	66.5	360.8	257.2
16.00	21.8	42.5	327.8	426
17.00	11	42.0	544.7	259.6
18.00	35	52.6	312.7	244.9
19.00	24.1	40.4	322.4	356.1
20.00	11	45.4	314.7	442.4
21.00	9.9	39.7	301.4	450.2
22.00	14.7	32.5	289.2	390.6
23.00	<LOQ	38.3	266.9	343.3
24.00	10.4	43.3	241.6	376.6
1.00	7.1	60.5	257	379
2.00	7.9		209.7	280.3
3.00	7.9		219	190.4
4.00	4.3		173.1	245.8
5.00	6.5		192.6	266.5
6.00	7.5	88.4	256.1	

Ofloxacin

Table 18 : Measured ofloxacin concentrations of monthly campaigns at the STP inlet in ng/l

Date			MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	740.55	648	760.1	454.95	700.95	515.25	716.45				
	08.-15.	April					531.9	578.1	536.4	576	452.3	506.2	405.7
	06.-13.	May					444.2	397	373.4	367.1	500.3	495.5	347
	17.-24.	June					90.7	53.3	99	101.2	135.1	60.1	82.5
	08.-15.	July					120.5	120.2	115.3	173.4	106.5	62.6	111.9
	19.-26.	August					292.8	205.05	185.65	224.2	205.75	176.65	207.95
	09.-16	September					374.8	412.05	349.55	324.5	249.1	276.8	456.6
	07.-14.	October					26.55	22.65	43.8	30.55	20.05	18.4	33.2
	11.-18.	November					408.7	537.8	878	617.6	521.2	248.5	936.6
	06.-13.	December		796.1	373.95	672.45	837.35	945.2	984.8	591.85			
2012	06.-13.	January					290.4	258.8	300.05	362.75	474.95	462.9	429.6
	03.-10.	February					232.8	257.85	234.85	219.65	225.65	203.65	222.1

Table 19: Measured ofloxacin concentrations of seasonal campaigns at the STP inlet in ng/l

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				930.9
7.00	13.3	742.3	250.2	1166.7
8.00	74.6	937.5	418.2	858.1
9.00	195.4	684.6	426.4	770.4
10.00	422.9	441.6	310.1	528.3
11.00	1865.3	188.8	351	460.1
12.00	108.8	283.4	248.7	377
13.00	11.4	610.2	282	293.1
14.00	<LOQ	363.1	319.2	257.5
15.00	29.4	455.4	241.7	270
16.00	17.1	1198.8	118.2	690.5
17.00	65.6	406.9	356.3	327.8
18.00	101	695.9	189.5	122.1
19.00	53.8	188.0	288.6	306.8
20.00	35.4	264.2	266.7	459.4
21.00	61.5	329.6	300.8	470
22.00	44.6	383.3	163.8	344.1
23.00	11	265.0	179.6	212.7
24.00	<LOQ	375.3	198.5	318.4
1.00	<LOQ	205.9	233.7	222.8
2.00	10.9		182.5	148
3.00	<LOQ		141.7	148.7
4.00	11.2		140.5	186.2
5.00	15.2		117.5	496
6.00	20	437.5	182.8	

Trimethoprim

Table 20 : Measured trimethoprim concentrations of monthly campaigns at the STP inlet in ng/l

Date			MON	TUE	WED	THU	FRI	SAT	SUN	MON	TUE	WED	THU
2011	14.-21.	March	70.55	80.65	84.35	55.5	73.4	80.8	75.35				
	08.-15.	April					55	58.3	56.1	58.6	49.2	56.3	60.2
	06.-13.	May					79.1	76.3	84.6	74.6	59.9	70.6	61.5
	17.-24.	June					61.2	36.8	81.9	70.4	75.2	50	69
	08.-15.	July					67.6	76.8	64	59.9	62.1	50.7	73.8
	19.-26.	August					73	92.85	87.05	80.05	83.6	93.9	97.9
	09.-16	September					63.8	96.15	89.65	82.1	87.05	72.7	80.55
	07.-14.	October											
	11.-18.	November					60.5	60.8	86.5	82.3	68	39.8	27
	06.-13.	December		119	97.65	118.3	127	142.25	139.6	90.65			
2012	06.-13.	January					86.7	103.25	120.65	133.8	113.9	134	115.15
	03.-10.	February					62.65	66.9	61.65	49.2	66.7	75.45	70.5

Table 21: Measured trimethoprim concentrations of seasonal campaigns at the STP inlet in ng/l

	Winter	Spring	Summer	Autumn
	06.-07.12.2010 MON-TUE	17.-18.05.2011 TUE-WED	06.-07.09.2011 TUE-WED	02.-03.11.2011 WED-THU
6.00				124
7.00	15.9	118.6	169	93.2
8.00	32.1	76.5	90.6	82.9
9.00	70.1	50.0	80.1	71.4
10.00	42.3	52.0	58.9	69.1
11.00	26	37.3	57.4	61
12.00	41.1	44.0	42.6	43.8
13.00	11.4	36.5	50	51.8
14.00	22.8	42.5	61.7	43.5
15.00	7.7	40.5	58	44.2
16.00	30.9	40.7	47.6	48.7
17.00	17.5	40.9	43	57.1
18.00	16.1	81.6	52	35
19.00	11.5	22.0	57.5	47.2
20.00	16.6	35.4	46.6	52.1
21.00	14.2	33.3	52.5	52
22.00	11	42.1	60	58.1
23.00	12.8	28.1	56.1	73.3
24.00	11.4	26.2	52.8	40.6
1.00	6.8	43.8	78.3	27.5
2.00	10		92	46.6
3.00	7.6		73.2	55.9
4.00	7.4		72.2	54.6
5.00	10.1		76.9	102.7
6.00	20.4	59.6	81.9	

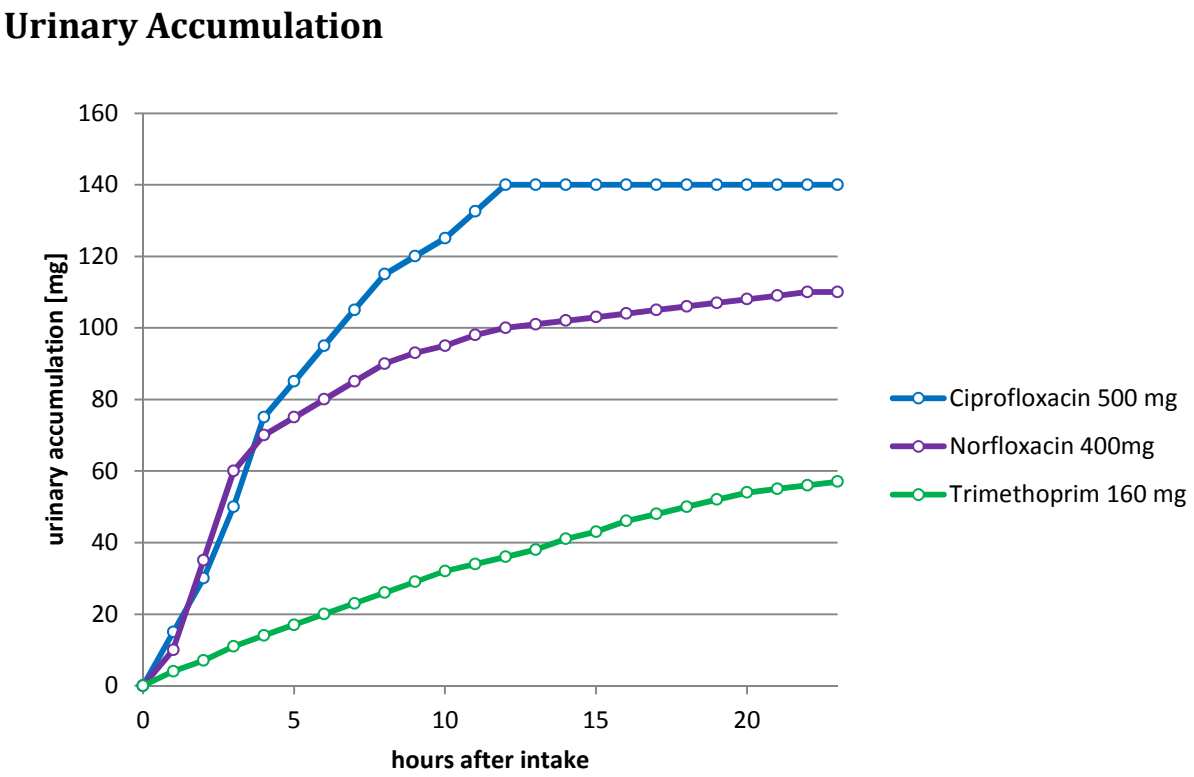

Figure 24: Accumulation of the three modelled antibiotics in the urine after single oral doses, 500 mg for ciprofloxacin, 400 mg for norfloxacin and 160 mg for trimethoprim. The accumulation curves are based on [33], [30] and [43] respectively

Modelled flow rates

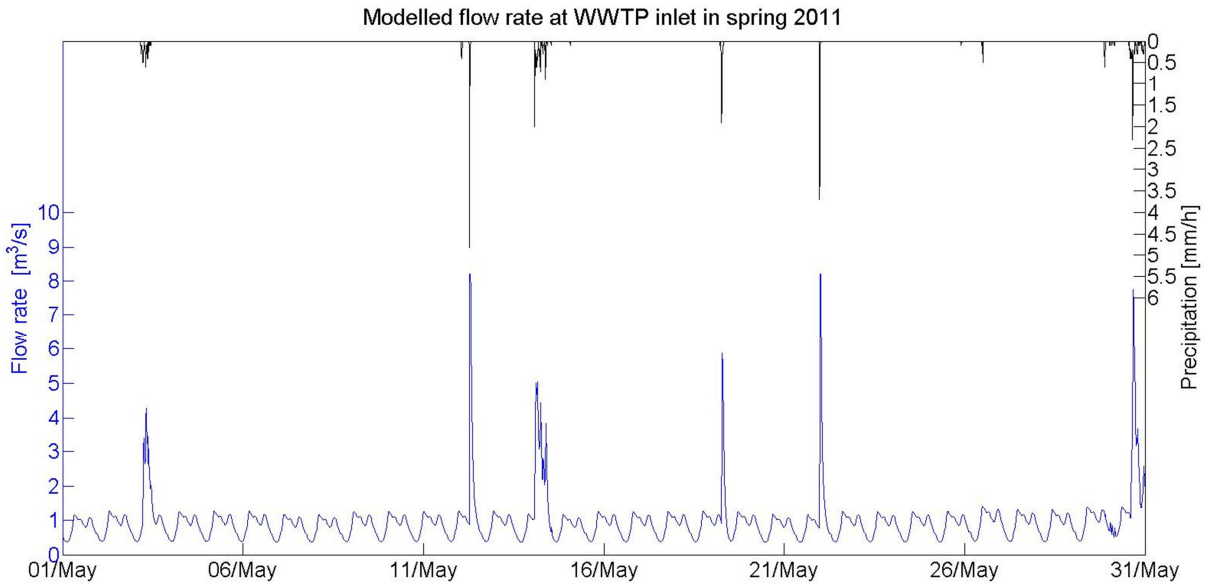

Figure 25a): Flow rate in spring (blue) resulting from the transport model with the precipitation (in black) as a predominant factor in the model

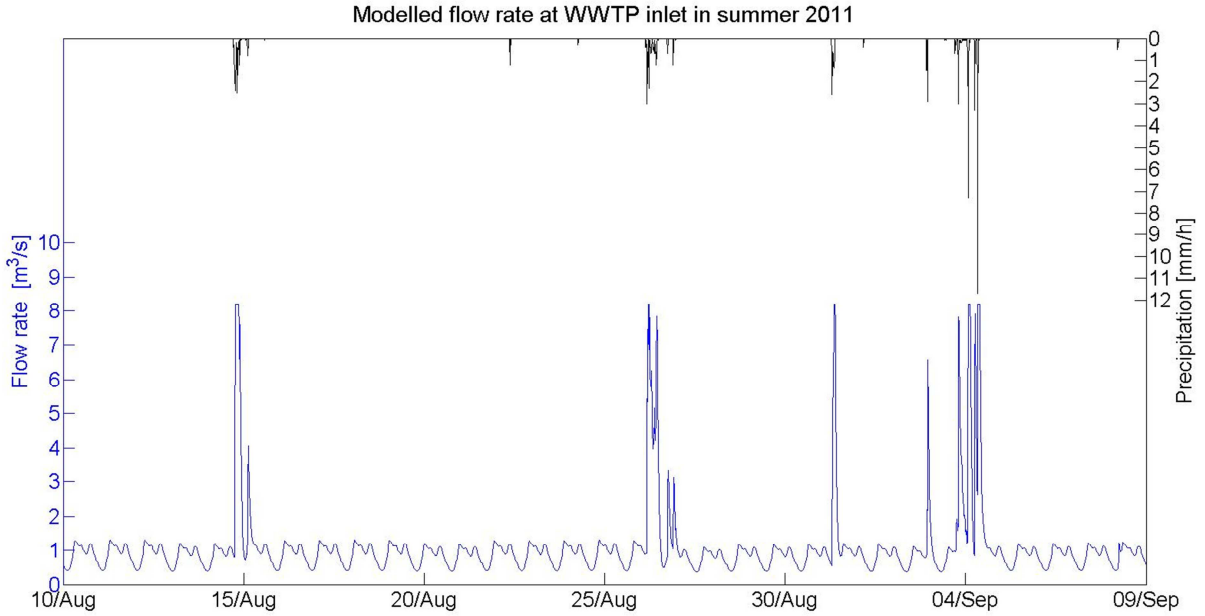

Figure 25b): Flow rate in summer (blue) resulting from the transport model with the precipitation (in black) as a predominant factor in the model

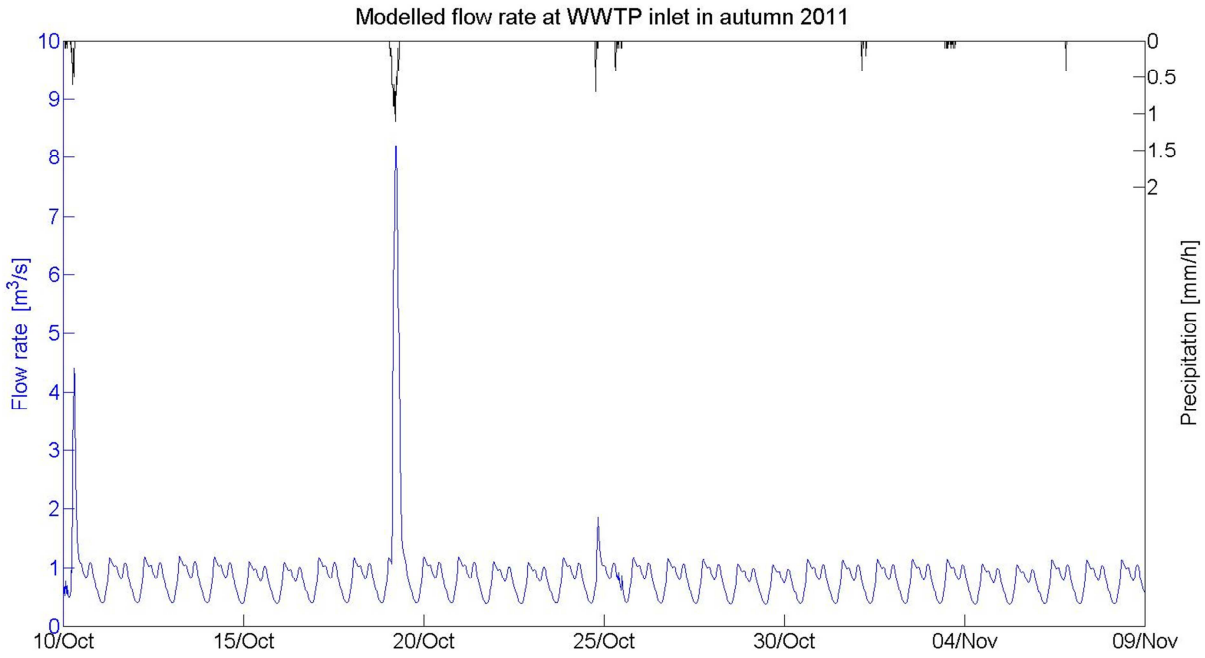

Figure 25c): Flow rate in autumn (in blue, below) resulting from the transport model with the precipitation (in black, above) as a predominant factor in the model