Universitat de Girona

# INTEGRATED ASSESSMENT OF WASTEWATER TREATMENT PLANTS AND THEIR RECEIVING RIVER SYSTEMS IN A GLOBAL CHANGE CONTEXT

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*Doctoral Thesis*

# Integrated assessment of wastewater treatment plants and their receiving river systems in a global change context

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**Certifiquen**

Que el llicenciat en Enginyeria Industrial **Ignasi Aymerich Blazquez** ha realitzat, sota la direcció de **Lluís Corominas**, el treball que amb el títol "**Integrated assessment of wastewater treatment plants and their receiving river systems in a global change context**", es presenta en aquesta memòria la qual constitueix la seva Tesi per optar al Grau de Doctor amb menció Internacional per la Universitat de Girona.

I perquè en prengueu coneixement i tingui els efectes que corresponguin, presentem davant la Facultat de Ciències de la Universitat de Girona l'esmentada Tesi, signant aquesta certificació a

Girona, 31 de octubre de 2018

Supervisor: Dr. Lluís Corominas Tabares

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Tutor: Dr. Ignasi Rodríguez-Roda Layret

A la meva **mare**, **pare** i **germà**,

Y en especial a ti, **iaia**.

# Agraïments

# List of Publications

The research work presented in this thesis (chapters 4 to 6) has been redrafted from a group of scientific publications listed below:

**Aymerich, I**., Acuña, V., VonSchiller, D., Rodriguez-Roda, I., Corominas, L., in preparation. Attenuation of organic matter and nutrients in a WWTP and its receiving river ecosystem through an integrated sampling and modelling approach: analysing the urgency for adaptation to global change. In preparation.

***Author's contribution:*** Design and execution of the different sampling campaigns, modelling and data analysis of the results. Writing the paper, with contributions from other authors.

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***Author's contribution:*** Modelling work and data analysis of the results. Writing the paper, with contributions from other authors.

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#BEWATER.

# Summary

For a long time, there has been a need and an ambition to better understand the behavior of integrated systems by considering the whole urban water cycle, including wastewater transportation, wastewater treatment and the receiving water. Fragmented environmental policies on wastewater sanitation, global change, and emerging contaminants are increasingly threatening freshwater ecosystems and human health. Given this background, an integrated approach to the management of the artificial and natural elements of the urban wastewater system is needed, where a better understanding of the interplay between a wastewater treatment and the receiving water system is previously required.

Within this context, this thesis embeds a series of research studies aiming to improve our comprehension of the functioning of urban wastewater systems (UWWS), considering both natural and artificial elements, and with a special emphasis on the occurrence of global change and on the fate of emerging contaminants. In the thesis, an integrated model for a UWWS in NE Iberian Peninsula has been developed and calibrated using data from an intensive and integrated survey, not only combining early developed mathematical models for the different sub-processes, but also verifying the model parameters with full-scale and dynamical measurements. More specifically, the work developed in this thesis was divided into three parts. First, we investigated how an UWWS perform together in the removal of conventional contaminants and evaluated the impact of future global change scenarios. Second, we investigated the occurrence and fate of pharmaceuticals and their transformation products in the UWWS. Third, and as a continuation of this second work, we assessed the influence of the sampling strategy when estimating the loads and attenuation of emerging contaminants in UWWS.

In the first chapter, the detailed assessment of the integrated system of Puigcerdà showed that the coupled WWTP-river system contributed to the overall removal of carbon, nitrogen and phosphorus, where we can clearly see that the river is influenced by the WWTP dynamics. Whereas the WWTP could not remove nitrogen, the river had the capacity to nitrify 80% of the ammonia load coming from the catchment within just five kilometers. An integrated model was developed and calibrated by connecting the ASM3-bioP and the RWQM nº1 models, contributing in the better understanding of the processes occurring in the different systems, as well as allowing for the generation of different global change scenarios. The simulations allowed us to conclude that under future foreseen population growth and decrease in the river flow (leading to increased loads from the catchment discharged to the river and decreased dilution capacity) the chemical status of the system will turn into bad conditions as well for the last 3 km studied. Hence, actions will be needed to adapt to changing conditions.

In the second chapter, and with regards to pharmaceuticals and their transformation products, the results showed that these compounds are highly present in both the wastewater treatment and their receiving water bodies. For the period under study, the results showed that only 5 out of the 19 pharmaceuticals were reduced by more than 90% at the WWTP, while the rest were partially or non-attenuated (or released) and discharged into the receiving river. The study showed that higher attenuation efficiencies were obtained in the river compared to the WWTP, while load reductions were higher in the WWTP. The consideration of transformation products allowed to identify the routing between some pharmaceuticals and their transformation products, knowledge that is still scarce (especially in full-scale studies). Finally, the followed model-based approach showed that dynamic attenuation could be successfully predicted with simple first order attenuation kinetics, as well as providing some insights on how uncertainty can be addressed.

In the last chapter, and as a continuation of the second chapter, we could demonstrate that sampling matters when investigating attenuation micro-contaminants in WWTPs and rivers, showing that it highly affects the uncertainty associated in the estimates of attenuation. The study was conducted after the model validation and extended for the generation of different sampling scenarios. The study showed that different levels of uncertainty are obtained in the estimation of loads (influent and effluent of the WWTP, upstream and downstream of the river) and in the estimation of attenuation depending on the consumption rate and degradability the compound, and the sampling strategy applied. We identified that WWTP influent sampling is especially critical when designing sampling strategies and that compound degradability plays a significant role, showing that short sampling intervals and longer sampling durations are needed to obtain attenuation estimations with low uncertainty.

Overall, this thesis highlights the need for integrated approaches to better understand the performance of WWTPs and their receiving rivers, against the increase of micro-contaminants concentrations and the effects of global change.

# Resum

La necessitat i ambició per entendre millor el comportament dels sistemes de sanejament d'aigües residuals de manera integrada és una de les prioritats en la gestió de l'aigua, considerant el cicle urbà de l'aigua al complet, des de el transport, tractamet i descarrega als seus medis receptors. Per altra banda, les actual polítiques segmentades en la gestió dels sistemes de sanejament d'aigües residuals urbanes, el canvi global i l'ocurrència de contaminants emergents en els mitjans receptors, amenacen cada vegada més els ecosistemes d'aigua dolça i salut humana. En aquest context, es requereix un enfoc més integrat en la gestió dels elements artificials i naturals involucrats en els sistemes d'aigües residuals urbanes, on una millor coneixement de les interaccions entre els diferents sistemes és necessaria.

En aquest sentit, aquesta tesi integra una sèrie d'estudis d'investigació que apunten a millorar la nostra comprensió en el funcionament dels sistemes d'aigües residuals urbanes (UWWS), considerant el conjunt d'elements naturals i artificials, i amb un èmfasi especial en els canvis globals i ocurrència dels contaminants emergents. En aquesta tesi, s'ha desenvolupat i calibrat un model integrat per a un UWWS al NE de la Península Ibèrica, utilitzant dades recollides durant intensa campanya integrada de monitorització, no només combinant avançats models per els diferents sub-processos, sinó també verificant els paràmetres de modelització amd dades dinàmiques i a escala. Més específicament, el treball desenvolupat en aquesta tesi s'estructura en tres parts. Primer, investiguem com funcionen els UWWS en l'eliminació de contaminants convencionals i avaluem l'impacte dels futurs escenaris de canvi global. Segon, investiguem l'ocurrència i la destinació dels productes farmacèutics i els seus productes de transformació en el UWWS. En tercer lloc, i com a continuació d'aquest segon treball, s'estudia la influència de diferents escenaris de mostreig en l'estimació de càrregues i atenuació dels micro-contaminants en UWWS.

En el primer capítol, l'análisi detallat del sistema integrat mostra com el sistema depuradora-riu contribueix a l'eliminació total de carboni, nitrogen i fòsfor, on es veu clarament com les dinàmiques de l'EDAR es reflexen en els següents trams de riu després de la descàrrega de la EDAR. Els resultats mostren com l'EDAR no és capaç d'eliminar el nitrogen, mentre que al riu es capaç de nitrificar el 80% de la càrrega d'amoníac provinent de la conca en només cinc km. En aquest primer estudi, el desenvolupament i calibració d'un model integrat connectant els models ASM3-bioP i RWQM nº1 a partir de les dades recollides durant la campanya, ha contribuït significativament a una millor comprensió dels diferents procesos que es produeixen en cada un dels sistemes analitzats, i al mateix temps generar i analitzar una sèrie d'escenaris dins el context de canvis globals. Les simulacions ens han permés concloure que, sota el futur creixement demogràfic previst, i la disminució en el flux del riu, l'estat químic del sistema també passarà a estar en males condicions per als últims 3 km estudiats. Per tant, es necessitaran accions per adaptar-se a les condicions canviants.

Pel que fa als productes farmacèutics i els seus productes de transformació, els resultats mostren que aquests compostos estan molt presents tan a l'entrada de l'EDAR com en el seu medi receptor. Durant el període d'estudi, els resultats mostren que només 5 dels 19 productes farmacèutics es van reduir en més del 90% en la EDAR, mentre que la resta es redueixen de manera pacial o no (o fins hi tot es generen), amb la posterior descàrrega en el medi receptor. L'estudi mostra que majors eficiències d'atenuació en el medi receptor en comparació a la EDAR, tot i que les reduccions de càrrega eren majors en l'EDAR. La consideració dels productes de transformació també ha permés identificar la ruta entre alguns productes farmacèutics i els seus productes de transformació, coneixement relativament escàs quan mirem estudis a escala real. Finalment, la metodologia utilitzada en la modelització dels sistemes ha permés veure que l'atenuació dinàmica es pot predir de manera satisfactòria amb una simple cinètica de primer ordre per a la majoria de contaminants i sistemes modelitzats.

Finalment, en l'últim capítol es demostra la importància del mostreig quan s'investiguen micro-contaminants en EDAR i rius. L'estudi s'ha desenvolupat a partir del model desenvolupat i validat en l'anterior capítol, el qual s'ha adaptat per la generació de diferents escenaris de mostreig. L'estudi mostra que la estratègia de mostreig a utilitzar es veu molt afectada per la incertesa en les estimacions d'atenuació, obtenint diferents nivells d'incertesa en l'estimació de les càrregues (entrada i sortida de l'EDAR, aigües amunt i aigües avall del riu) i atenuació (EDAR i riu), depenen del consump d'aquell compost, la seva degradació i finalment l'estratègia de mostreig utilitzada. Aquest mostreig serà especialment crític a l'entrada de l'EDAR el mostreig és especialment crític, on l'estratègia de mostreig i degradació del compost juga un paper important, necessitan intervals de mostreig curts i campanyes de mostreig més llargues per obtenir baixes incerteses en les estimacions.

En general, aquesta tesi destaca la necessitat d'enfocaments integrats per comprendre millor el rendiment de les EDAR i els seus rius receptors, per prendre mesures contra l'augment de les concentracions de micro-contaminants i els efectes del canvi global.

# Resumen

La necesidad y ambición por entender mejor el comportamiento de los sistemes de saneamiento de manera integrada es una de las prioridades en la gestión del agua, considerando el cicló urbano del agua por completo, desde el transporte, tratamiento y descarga en sus medios receptores. Por otra parte, las políticas fragmentadas en la gestión de los sistemas de saneamiento de aguas residuales urbanas, el cambio global y la ocurrencia de contaminantes emergentes en los medios receptores amenazan cada vez más los ecosistemas de agua dulce y la salud humana. En este contexto, se requiere un enfoque integrado en la gestión de los elementos artificiales y naturales involucrados en los sistemas de aguas residuales urbanas, donde una major comprensión de las interacciones entre los diferentes sistemas involucrados es necesaria.

En este sentido, esta tesis integra una serie de estudios de investigación que apuntan a mejorar nuestra comprensión del funcionamiento de los sistemas de aguas residuales urbanas (UWWS), considerando el conjunto de elementos naturales y artificiales, con especial énfasis en los cambios globales y ocurrencia de los contaminantes emergentes. En esta tesis, se ha desarrollado y calibrado un modelo integrado para un UWWS en el NE de la Península Ibérica, utilizando datos recogidos durante intensa campaña de monitorización e integrada, no solo combinando modelos avanzados para los diferentes sub-procesos, sinó tambien verificando los parámetros de modelización a través de datos en dinámico y escala real. Más específicamente, el trabajo desarrollado en esta tesis se estructura en tres partes. Primero, investigamos cómo funcionan los UWWS en la eliminación de contaminantes convencionales y evaluamos el impacto de los futuros escenarios de cambio global. Segundo, investigamos la ocurrencia y el destino de los productos farmacéuticos y sus productos de transformación en el UWWS. En tercer lugar, y como continuación de este segundo trabajo, estimamos la influencia de la estrategia de muestreo al estimar las cargas y atenuación de los contaminantes emergentes en UWWS.

En el primer capítulo, la evaluación detallada del sistema integrado muestra como el sistema integrado depuradora-rio contribuye en la eliminación total de carbono, nitrógeno y fósforo, donde el río está influenciado por la dinámica de la PTAR. La EDAR no es capaz de eliminar el nitrógeno, mientras que en el rio es capaç de nitrificar el 80% de la carga de amoníaco proveniente de la cuenca en tan solo cinco km. En este primer estudio, se desarrolló y calibró un modelo integrado al conectar los modelos ASM3-bioP y RWQM nº1 en base a los datos recogidos durante la campaña, ha contribuido de manera significativa en una mejor comprensión de los diferentes procesos que se producen en cada uno de los sistemas analizados, y al mismo tiempo generar y analizar una série de escenarios en el contexto de cambio global. Las simulaciones nos han permitido concluir que, bajo el futuro crecimiento demográfico previsto, y la disminución en el flujo del río (el estado líder de la cuenca se elevará al río y disminuirá la capacidad de dilución), el estado químico del sistema también pasará a estar en malas condiciones para los últimos 3 km estudiados. Por lo tanto, se necesitarán acciones para adaptarse a las condiciones cambiantes.

Con respecto a los productos farmacéuticos y sus productos de transformación, los resultados mostraron que estos compuestos están muy presentes en ambos sistemas de saneamiento y medios de agua receptores. Durante el periodo de estudio, solamente 5 de los 19 productos farmacéuticos se redujeron en más del 90% en la EDAR, mientras que el resto se eliminarion de manera parcial o no (o se generaron), con la posterior descarga en el medio receptor. El estudio mostró mayores eficiencias de atenuación en el medio receptor comparado con la EDAR, aunque que las reducciones de carga de contaminantes eran mayores en la EDAR. La consideración de los productos de transformación también nos ha permitido identificar la ruta entre algunos productos farmacéuticos y sus productos de transformación. Finalmente, la metodología utilitzada en la modelización de sistemas ha permitido ver que la atenuación dinámica se puede predecir de manera satisfactoria con una simple cinética de primer orden para la mayoría de contaminantes en ambos sistemas estudiados.

Finalmente, en el último capítulo demostramos la importante del muestreo cuando se investigan micro-contaminantes en EDAR y ríos. Este trabajo se ha realizado a partir del modelo desarrollado en el anterior capítulo, y extendido con la generación de diferentes escenarios de mostreo. Esta se ve muy afectado por la incertidumbre en las estimaciones de atenuación, obteniendo diferentes niveles de incertidumbre en la estimación de las cargas (influente y efluente de la EDAR, aguas arriba y aguas abajo del río) y atenuación. En la entrada de la EDAR el muestreo es especialmente crítico, donde la estrategia de muestreo y degradación del compuesto juega un papel importante. Por esto, se necesitan intervalos de muestreo cortos y duraciones de muestreo más largas para obtener bajas incertidumbres en las estimaciones.

En general, esta tesis destaca la necesidad de enfoques integrados para comprender mejor el rendimiento de las EDAR y sus ríos receptores, para tomar medidas contra el aumento de las concentraciones de micro-contaminantes y los efectos del cambio global.

# SECTION I

# BACKGROUND, AIMS AND RESEARCH APPROACH

# Chapter 1

# General Introduction

Urban wastewater systems (UWWS) are designed to collect and treat wastewater before its discharge to freshwater or marine ecosystems. Sanitation systems have been implemented since Indian civilizations (3,200-2,800 BC) times to reduce environmental impacts and risks on human health derived from wastewater. Nowadays, current environmental policies (e.g., EC 91/271) regulate the quality of the treated wastewater, setting caps on the concentration of different compounds such as total nitrogen. However, current legislation ignores the effects of emerging contaminants, as well as the low and variable dilution capacity of freshwater ecosystems in arid and semi-arid regions. In fact, upcoming environmental policies in the European Union argue for the inclusion of some emerging contaminants, as well as for an immission-based management of UWWS, so that caps are set on the receiving ecosystem rather than on the effluents of UWWS. This shift from emission to immission-based management, as well as the need to comprehend patterns of occurrence of emerging contaminants requires of an integrated approach to the study of UWWS, including all the artificial and natural elements, and through the combination of state-of-the art experimental and modelling tools.

The need of studying the coupling between UWWS and receiving freshwater ecosystems is growing because of the effects of global change on sanitation systems. Thus, growing urban populations and increasing usage of some emerging contaminants leads to increasing loads of these compounds to the wastewater treatment plants (WWTP), and these increasing loads at the WWTP influents are often translated to increasing loads at the WWTP effluents because of the low attenuation efficiencies that most sanitation systems have. These low attenuation efficiencies often lead to the continuous presence of pharmaceuticals, illicit drugs and personal care products (among others) in receiving freshwater ecosystems, which may cause toxic effects (Petrie et al 2014). Pharmaceuticals are one of the most important concerns due to the high consumption and variability of these compounds, where knowledge on transformations, attenuation and toxicity is still incomplete (Acuña et al. 2015). It is expected that the combination of increased urbanization (higher loads generated) and global change (lower river flows) implications will lead to higher concentrations of micro-contaminants in receiving freshwater ecosystems.

So far, the majority of existing studies focus on either the artificial or the natural elements of the UWWS, thus limiting the comprehension of the coupling between them. In this context, integrated modelling tools offer great opportunities to face important challenges related with the design and the management of WWTP. Basically, integrated modelling of UWWS allows the possibility to analyze the system as a whole, understanding the patterns observed at the different elements within a broader context. In the last decades, we have seen great advances in the application of these models (Benedetti et al 2013), however, the application of these models into practice is still scarce (Langeveld 2013; Bach 2014). In general, these limitations are related with institutional barriers, expensive data requirements, and classical modelling approaches by connecting independent models, consequently suffering from poor performance and unwieldy models (Batch et al. 2014). Although these approaches can help to better understand the performance of these systems as a whole; they are not enough to describe the interactions and processes occurring in the real system.

To better understand the interactions between WWTP and their receiving freshwater ecosystems, efforts should be made in the introduction of integrated concepts in monitoring programs, so that sufficient data to develop and validate these models is gathered (Langeveld

2013, Batch et al 2014). Needless to say, this will change depending on the goal and system under study, the modelling complexity and the difficulties related with the development and integration of the different models; as well as other barriers such as costs, resources, data availability, etc. When designing integrated models of UWWS, we should also account for global change and emerging contaminants. In regards to global change, we will need to deal with proper modelling of different processes occurring in the different systems, as well as developing realistic scenarios (Batch et al., 2014) and incorporating immision-based criteria to proper evaluate the river chemical status (UPM2, 1998). In regards to emerging contaminants, we will need to deal with transformation products and unknown processes (Petri et al., 2015), as well as other issues such as sampling uncertainty and how addressing these uncertainties for scenario analysis and decision-making. Overall, the integration of the natural and artificial elements in the analysis and modelling of the UWWS, in the framework of global change, emerging contaminants and uncertainty, is the main contribution of this PhD thesis.

# Chapter 2

# Objectives and structure of the thesis

The main objective of this thesis is to improve our comprehension of the functioning of UWWS, considering both natural and artificial elements, and with a special emphasis on the occurrence and fate of emerging contaminants, as well as the impact of global change scenarios. The specific objectives of this thesis are:

1) To quantify, model and understand the dynamics of organic matter and nutrients in a UWWS, and to assess the impact of global change on the UWWS functioning.
2) To quantify and model the occurrence and fate of pharmaceuticals and their transformation products in a UWWS.
3) To assess the influence of the sampling strategy on the uncertainty of the load and attenuation estimates in WWTP and freshwater ecosystems, to define guidelines on most cost-effective sampling strategies in UWWS.

In order to achieve these objectives, an integrated model for a UWWS in NE Iberian Peninsula has been developed and calibrated using data from an intensive and integrated survey including both artificial and natural elements of the UWWS. Given the initial knowledge we had on the processes governing the occurrence and fate of traditional (organic matter and nutrients) and emerging contaminants (pharmaceuticals), different modelling approaches were followed. Thus, a mechanistic model was developed for the conventional contaminants (**Chapter 4**), whereas a less complex model was used for emerging contaminants (**Chapter 5** and **6**). Finally, the effects of the sampling strategy were assessed by extending the previously used model with a sewage pattern generator (**Chapter 6**).

# Chapter 3

# Methodology

## 3.1 System description

The monitoring site chosen consists of a semi-rural sewer catchment drained into one WWTP and discharging into one receiving river (Segre) situated in the North-East of Spain, near the town of Puigcerdà (**Figure 3.1A**). The receiving river system is a fresh-water ecosystem with no previous WWTP discharge, and thus a good ecological and chemical status is occurring upstream the WWTP discharge. The WWTP under study is an important contributor of loads and flows in the river, where preliminary studies showed that the WWTP is an important pollutant source in the river in terms of conventional and emerging contaminants (Acuña et al., 2015). Other studies showed that the impact of these discharges where affecting aquatic organisms through bioaccumulation and causing structural changes in the community (Corcoll et al., 2014; and Ruhí et al., 2016). On the other hand, the WWTP is currently operating at the limit of its capacity and with some limitations in the removal of nitrogen and phosphorus contaminants, where concerns such as climate change, water scarcity and the presence of emerging contaminants is requiring an urgent analysis.

### 3.1.1 Catchment

The catchment under study is a combined sewer gravity system that serves 16,000 PE as a result of 17 municipals distributed in Spain and France regions (**Figure 3.1A**). The town of Puigcerdà is the major contributor (8,000 PE) followed by the towns of Sallagouse (1,045 PE), Llívia (900 PE) and Bourge-Madame (800 PE). The catchment is sparsely populated covering a later urban and agriculture area of approximately 1,000 km$^2$. The WWTP is located close to the largest town in the area (Puigcerdà, see **Figure 3.1A**), therefore the majority of flow and pollutant loads received by the WWTP (60%) are expected to originate from nearby (<1.5km). In dry-weather conditions, the 28% of the wastewater arriving in the WWTP is assumed to originate from households, and the remaining 72% is due to irrigation from groundwater infiltration (due to the large catchment area and poor quality of the sewer pipes), while industrial activity is expected to be negligible (Snip et al., 2016).The catchment is characterized with high seasonal load variation with fluctuating average flows due to the touristic activities in the area during summer and winter times. There is also an increase in population during weekends as many people living in large cities (e.g. Barcelona) have their second house located in the catchment. The average monthly temperature in the city of Puigcerdà is around 9ºC and varies between 2ºC and 18ºC in winter and summer, respectively; while mean annual precipitation is 940 mm and varies between 50 mm (Winter periods) and 100 mm (Spring season).

### 3.1.2 WWTP

The WWTP under study is a conventional activated sludge system designed for the removal of organic matter, nitrogen and solids compounds. The WWTP is composed of a pre-treatment (PT), biological treatment (BT), secondary treatment (ST) and sludge treatment (SLT) and disposal (**Figure 3.1B**). The pre-treatment contains screen, pumps and grid removal units for the removal of heavy particles. The biological treatment is an activated sludge system with an oxidation ditch configuration designed for the removal of organic matter and nitrogen compounds. The biological treatment is composed of two biological reactors with a total volume of 4,860 m$^3$, 2,430 m$^3$ for each biological treatment line. Each biological reactor consists of two anoxic zones (1,710 m$^3$ in total, approximately) and two aerobic zones (720 m$^3$ in total, approximately). Aeration system in the biological treatment is equipped with four surface aerators (two for each biological reactor), operated simultaneously with a table schedule defined by the plant operator (10-minswitch on, 50-min switch off), and with a total aeration capacity installed of 4,550 $O_2$ $m^3$ $d^{-1}$ (1,140 $O_2$ $m^3$ $d^{-1}$ for each aeration line). The secondary treatment is composed of two secondary clarifiers of 1,103 $m^3$ to separate water from solids before discharging into the river. The sludge treatment line is composed of sludge thickener, centrifuges and a sludge disposal tank. Centrifuges are operating only during weekdays, whereas the reject water from centrifuges is discharged in a buffer tank and then recycled back to the pumping station before the pre-treatment. Legislation requires the removal of organic matter and total suspended solids which is well achieved. The WWTP discharges into a non-sensitive area, meaning that there are no requirements to remove nitrogen and phosphorus. There are evidences of combined sewer overflows to the river under wet weather conditions, but these did not happen during the period of study.

#### 3.1.3 River

The river Segre drains an area of 287 $km^2$ with a rain-snow fed flow regime at an altitude of 1,108 m. The river has it source close to the town of Lló in France, crosses Catalonia (Spain) from Puigcerdà to Lleida, and flows in to the river Ebro in the frontier between Aragon and Catalonia (Spain) (**Figure 3.1A**). The river site under study is located close to the river source (<20 km), and runs through a gravel-bed meandering channel across a broad valley primarily covered with native forest, where some pastures and small agricultures fields are also present. The average base flow discharge at the study site amounts to approximately 30,000 $m^3$ $d^{-1}$from October to March, which can significantly increase up 250,000-300,000 $m^3$ $d^{-1}$ under wet weather periods (from April to September)([http://aca-web.gencat.cat](http://aca-web.gencat.cat)).From the point of source to the study site before the WWTP discharge, there is no previous discharge of any other WWTP (the WWTP under study is the first point of source of discharge), and therefore, the chemical and ecological status can be considered as very good until the WWTP discharge. During the period under study, the river flow before 500 metres the WWTP discharge was approximately 27,000 $m^3d^{-1}$, while the WWTP accounts approximately 18-27% of the river flow. Groundwater contribution in the following 4,500 metres was quite significant with a segment dilution of approximately 55% (after tracer test analysis).

### 3.2 Measuring campaign

A measuring campaign was conducted within the frame of the EU projects EcoMaWat (project ID 293535) and Globaqua (project ID 603629). The aim of this campaign was to collect information to understand the performance of the WWTP and its receiving water ecosystem in terms of macro and micro-contaminants. An intensive campaign was conducted from the 8$^{th}$ of October 2012 at 8am until the 11$^{th}$ of October 2012 at 8am in the integrated system formed by the WWTP and the receiving river system under study (**Figure 3.1B**). During this period, physical information was collected from the built and natural systems. Also, operational conditions were obtained. A tracer test was conducted for the integrated system and online sensors were installed at the influent of the biological reactor and at the effluent of the WWTP. Grab and composite samples were taken from different points from the WWTP and the river, which were analysed for organic matter, nutrients and pharmaceutical products. A detailed description of the measuring campaigns will be found in each of the chapters.

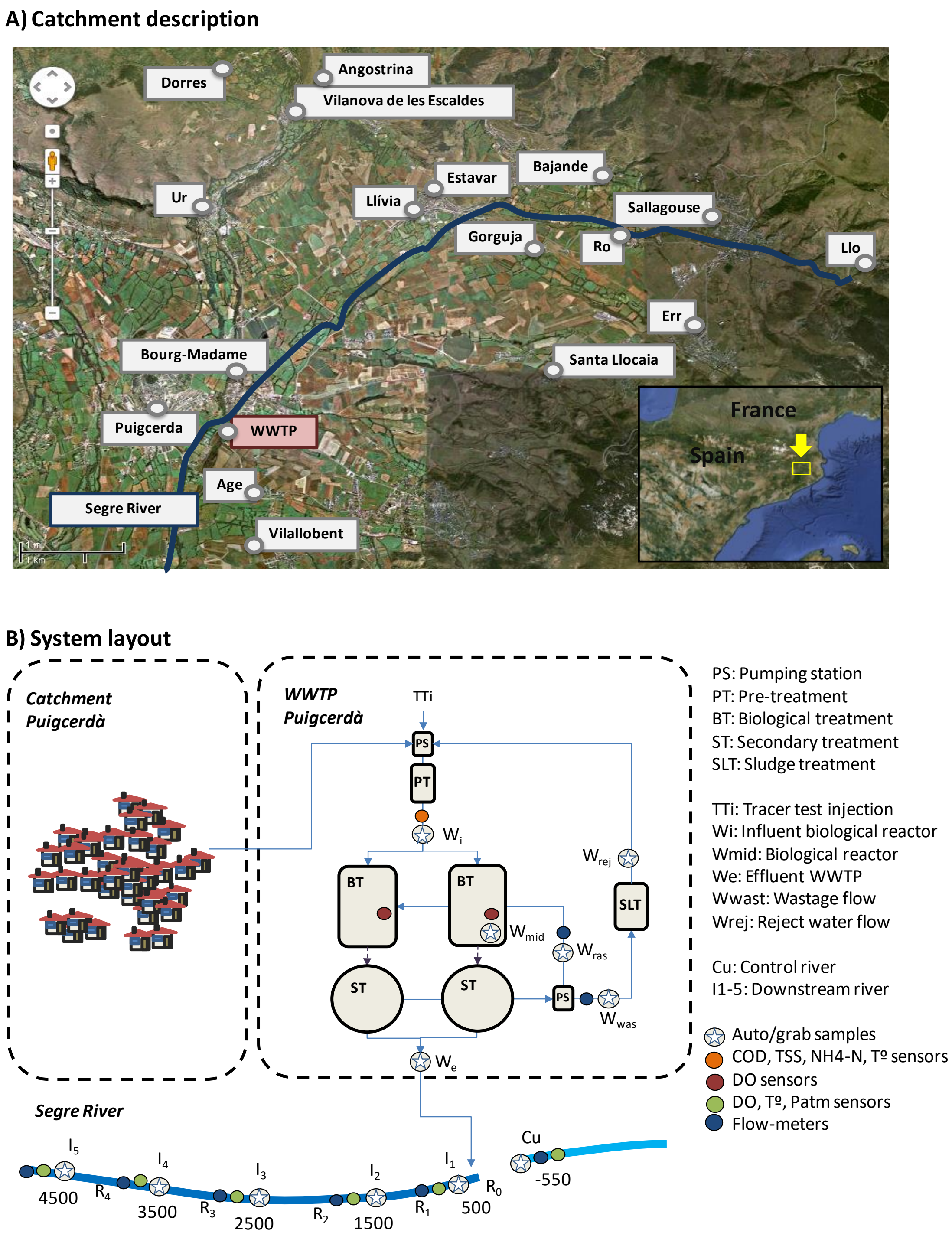


**Figure 3.1.** A) Catchment description and B) system layout of the system under study.

**Table 3.1.** Design and operational information of the WWTP and environmental characteristics of the river segment under study. Mean and standard deviations are provided for the period under study.

| WWTP and river information | |
|---|---|
| ***Design and operational information WWTP*** | ***Value*** |
| PE (inhabitants) | 16,000 |
| Flow-rate WWTP ($m^3$ $d^{-1}$) | 6,417 ± 862 |
| Organic (mg COD $L^{-1}$) / Nitrogen (mg N $L^{-1}$) / Phosphorus (mg P $L^{-1}$) concentrations at the influent of the WWTP | 318 ± 161/ 28 ± 8/ 6 ± 2 |
| Configuration | Oxidation ditch |
| Volume biological treatment ($m^3$) | 4,860 |
| Volume secondary clarifier ($m^3$) | 1,624 |
| Temperature (ºC) | 15.5 ± 2.5 |
| HRT (h) | 21 |
| MLSS (mg SS $L^{-1}$) | 1,453 |
| SRT | 3-4 |
| Organic / Nitrogen / Phosphorus removals WWTP (%) | 82 / 45 / 63 |
| ***Environmental information river*** | ***Value*** |
| Riv. Slope(m $m^{-1}$) | 0,0078 |
| Riv. Width (m) | 13 ± 3,6 |
| Riv. Depth (m) | 0,22 ± 0,06 |
| Elevation (m a.s.l) | 1,108 |
| Drainage area (km2) | 287 |
| Segment length (m) | 4,500 |
| WWTP effluent discharge CV (%) | 18 |
| Segment dilution (%) | 55 |
| Segment travel time (h) | 4.6 |

## 3.3 Modelling tools

In the different studies of the thesis several modelling approaches with varying levels of complexity were applied to fulfil the particular goals. A short description of the modelling platforms used in the thesis is given below together with a general modelling approach overview. More detailed information can be found in each of the chapters:

**MATLAB ® - SIMBA:** The Matlab® mathematical software (The Mathworks, Inc.) was used as the main platform for data analysis, modelling and simulation of the different results presented in this thesis. The SIMBA simulation platform (version 6, IFAK, Germany) was used to model, calibrate and simulate the biological wastewater treatment plant and the receiving river ecosystem, which was communicating to other platforms to exchange files (such as the influent generator from the benchmarking community or the sewage pattern generator developed at EAWAG). SIMBA is a dedicated software package which includes hydraulic and biochemical models allowing for a proper modelling and simulation of the integrated urban wastewater system as a whole (e.g. sewer, wastewater treatment plant and the receiving river). For the WWTP, the software includes special libraries for the Activated Sludge Models (e.g. ASM1, ASM2, ASM3 and ASM3-bioP), which allows the description of chemical and biological processes for the organic, nitrogen and phosphorus elimination. SIMBA also includes primary and secondary clarification models, distribution and connection elements or reactor and storage tanks in order to proper build the WWTP model according to the reality. For the river, the SIMBA platform also includes Lagrangian models and different versions of the RWQM no. 1, which allows for a proper hydraulic a biochemical process descriptions such as microorganisms, algae function and oxygen balances.

**MATLAB ® - BSM Influent Generator:** The BSM influent generator is an integrated model library programmed in Matlab® that can be used to simulate the dynamics of an UWS on a single simulation platform. It defines a hypothetical UWS using the model library, so that future users can use the pre-defined layout to study multiple control strategies, scenarios and system modifications. The existing influent generator has been extended in the last years with new model blocks to describe catchment (Flores-Alsina et al. 2014b), as well as to describe the transport and transformation of micro-pollutants (Snip et al., 2016), which was successfully tested with data of micro-pollutants collected in this thesis (Snip et al., 2016). This tool was used in **Chapter 4** for the generation of long-term influent series data by adapting the model developed from Snip et al., 2016. Further information can be found in **Chapter 4**.

**R ® - Sewage Pattern Generator (SPG):** SPG is an R-package that provides functions to i) simulate flow and substance patterns at high temporal resolution (complex sewer networks including pump stations) and ii) evaluate and optimize sampling setups to facilitate the collection of representative composite samples. The main aim of SPG is to efficiently model realistic short-term variations of flows and substances in sewers and to evaluate the suitability of different sampling setups, with special focus on the down-the-drain chemicals, e.g. pharmaceuticals or illicit drugs that usually enter the sewer system through toilet flushes. For this thesis, the model was adjusted to the characteristics of the case-study and calibrated with experimental hydraulic and concentrations patterns data. Additional information can be found in **Chapter 6**.

## 3.4 Modelling approach

By combining the above mentioned modelling tools, different modelling approaches were followed to achieve the particular goals proposed in each of the results chapters. Data analysis and preparation was all done with the Matlab® platform, which was used as main platform to conduct the data preparation, reconciliation, execution of simulations and finally the different results analysis. The basis of the modelling approach was the hydraulic model of the WWTP-river system implemented in SIMBA and calibrated based on the data collected during the tracer test. This model was then extended in the different chapters of this thesis, where different chemical and biological models where implemented with varying levels of complexity according to the particular goals.

In **Chapter 4**, a detailed layout of the WWTP-river system model was implemented in order properly describe the processes occurring in the different sub-systems, such as aeration and anoxic zones, treatment lines, aeration transfer and schedules, as well as in the different hydraulic and biochemical processes occurring along the river sections under study. For the WWTP, the tracer test showed the system could be modeled as a combination of 2 CSTRs. Still, we used 8 CSTRs to properly describe concentrations in different zones of the reactor where samples were taken. In the river, one CSTR between each of the sampling locations was used to describe well the hydraulics. For the biochemical processes, the ASM3-bioP and RWQM no 1 models were implemented and then calibrated according to the data available (see **Chapter 4**). Then, an influent generator was integrated in order to generate realistic global change scenarios by modifying the catchment parameters. The execution of the model is sequential (but still automated), first generating a .mat on the influent characteristics, then loading that file into SIMBA to run the WWTP and river simulations and afterwards evaluating the results. An example of the model layout is shown in **Figure 3.2**. Further information can be found in the chapter.

In **Chapter 5** and **6**, the hydraulic model was extended with a simple attenuation first-order model describing attenuation of micro-contaminants in the integrated WWTP-river system. For the WWTP we only used 2 CSTRs (confirmed with the tracer test), as we assumed micro-contaminants rates were not dependent on electron acceptor. In **Chapter 5**, attenuation rates were estimated from mass balancing and loads calculation, which were then simulated and evaluated under dynamic conditions. In **Chapter 6**, and after validation of the first-order modelling approach, the model was extended with the integration of the SPG R-package in order to generate different influent patterns under different number of pulses. Then, the generated patterns were simulated in the WWTP-river model, and the outputs of the patterns were finally evaluated under different sampling strategies to analyse the impact of sampling duration, sampling frequency and compounds degradation under different scenarios. In that case, the execution of the model is sequential and semi-automated), first generating a .mat from the SPG R-package, then loading that file into SIMBA to run the WWTP and river simulations, and afterwards evaluating the results again in the SPG R-package in order to evaluate the different sampling strategies tested and the uncertainty associated. An example of the model layout is shown in **Figure 3.3**. Additional information can be found in each of the chapters.

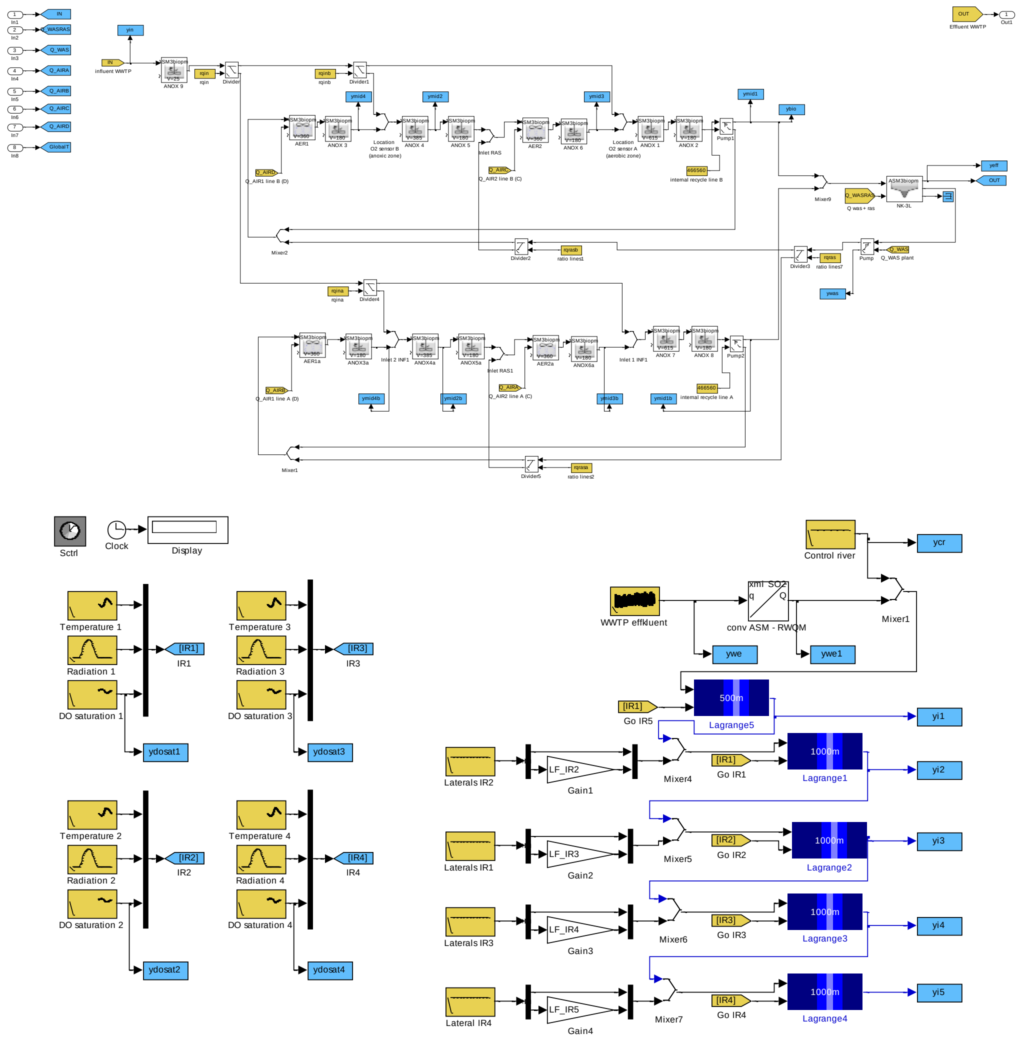


**Figure 3.2.** Layout WWTP-river model used in the Chapter 4.

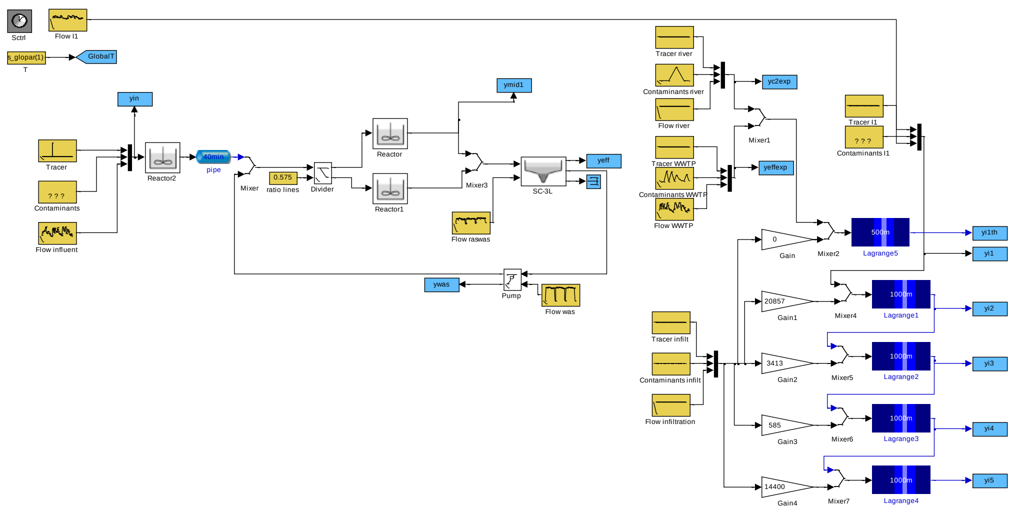


**Figure 3.3**. Layout WWTP-river model used in the Chapter 5 and 6.

# SECTION II

# RESULTS

# Chapter 4

# Attenuation of organic matter and nutrients in a WWTP and its receiving river ecosystem through an integrated sampling and modelling approach: analyzing the urgency for adaptation to global change

ARTICLE IN PREPARATION. EMBARGO UNTIL PUBLICATION DATE

# Chapter 5

# Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river ecosystem

The content of this chapter has been published as:

*Aymerich, I., Acuña, V., Barceló, D., Garcia, M.J., Petrovic, M., Poch, M., Sabater, S., Rodriguez-Mozaz, S., Rodríguez-Roda, I., von Schiller, D., Corominas, L., 2016. Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river system. Water Research 100, 126-136.*

## 5.1 Introduction

Despite pharmaceutical products are designed to improve human and animal health, they may pose a threat to freshwater ecosystems because of their effects on non-target organisms when entering the environment from urban or wastewater treatment plant (WWTP) effluents (Daughton and Ternes 1999). Once administered, pharmaceuticals are metabolized to varying degrees, and their excreted transformation products and unaltered parent compounds are transported through sewer systems to WWTPs. There, some pharmaceuticals are totally removed through biodegradation or adsorption into sludge, whereas others remain unaltered or partially degraded, yielding intermediate transformation products (Luo et al., 2014; Verlicchi et al., 2012). When entering freshwater ecosystems, pharmaceuticals levels can also be attenuated through biodegradation, adsorption to sediments, and photodegradation (Kunkel and Radke, 2011; Kunkel and Radke, 2012; Kadke et al., 2010; Writer et al., 2013; Ying et al., 2013). The incomplete removal of pharmaceuticals and the appearance of transformation compounds leads to a continuous input into the aquatic environment that results in their widespread and ubiquitous presence, which has raised environmental and human health concerns (Hughes et al., 2013; Kümmerer, 2009; Osorio et al., 2012; Pal et al., 2010). For instance, the anti-inflammatory diclofenac, the synthetic hormone ethynilestradiol (EE2) and the antibiotics (Erythromycin, Clarithromycin y Azithromycin) have been recently included in the 'watch list' of priority substances under the Water Framework Directive (Decision 2015/495/EU). In addition, in 2015 the U.S. Environmental Protection Agency included pharmaceuticals such as erythromycin and EE2 in the Water Contaminant Candidate List 4, a list of contaminants that are currently not subject to any proposed or promulgated national primary drinking water regulations but are known or anticipated to exist in public water systems.

Overall, knowledge on the transformation of pharmaceuticals in WWTP and freshwater ecosystems is rather fragmented. In fact, several studies have assessed attenuation (net balance between removal and release from and to the water column) of pharmaceuticals in either WWTPs or freshwater ecosystems, but none of them have assessed attenuation of pharmaceuticals and their transformation products including both systems within the studied system boundaries and using the same approach. The most common method to assess the removal of pharmaceuticals in WWTPs requires taking a one-day composite sample from the influent and a one-day composite sample from the effluent after delaying the start of the effluent composite sample one time the hydraulic residence time (HRT) (Majewsky et al., 2013); attenuation is thus commonly expressed as a percentage of loads removal. In contrast, the attenuation of pharmaceuticals in rivers is assessed using a variety of calculation approaches, including intrinsic tracers, dye or conservative tracer studies, and Lagrangian sampling (Writer et al., 2013); and the results are commonly expressed as half-life times of the compounds. Attenuation mechanisms may include physical processes (adsorption, photolysis) as well as biological (uptake, accumulation) (Daughton and Ternes 1999, Ruhí et al. 2016). Because of the differences in the numerical approaches to estimate attenuation rates, results cannot be compared and integrated in a general framework on the fate of pharmaceuticals and their transformation products in engineered and natural systems. In addition, sampling methods (flow-based composite, time-based, etc.) can substantially affect the reliability of the load estimation (Ort et al., 2010) and in turn compromise the calculations of attenuation rates (Majewsky et al., 2013). Hence, the incorporation of the different sources of uncertainty (e.g.

sampling, chemical analysis, flow measurements, etc) is essential as well and should be properly addressed in the calculations.

Given this background, our goal was to assess attenuation of pharmaceuticals and some of their transformation products in a WWTP and its receiving river ecosystem by using a comparable approach, and thus tracking these chemicals along their route through the WWTP and the river. Specifically, the dynamics of 8 pharmaceuticals and 11 of their transformation products were assessed in terms of occurrence and fate based on different attenuation metrics (load reductions, attenuation rates, and half-life times) by combining experimental and modelling approaches and considering different sources of uncertainty. Finally, attenuation rates obtained were used to model pharmaceuticals attenuation in the coupled system under dynamic conditions, showing that attenuations estimated could be successfully predicted with simple first order attenuation kinetics in both engineered and natural systems and for most modelled compounds.

## 5.2 Materials and methods

### 5.2.1 Sampling points

Sampling stations were located at the WWTP influent ($W_i$), effluent ($W_e$), effluent biological reactor ($W_{mid}$), wastage (e.g. excess sludge) ($W_{was}$), and reject water from centrifuges (Wrej) (Figure 1). At the receiving river system, one sampling site was located 50 m upstream of the WWTP effluent ($C_u$) to characterize the water chemistry upstream of the discharge of the WWTP effluent (**Figure 3.1**), whereas two sampling sites were located at 500 ($I_1$, $I_u$ from now on) and 4 500 ($I_5$, $I_d$ from now on) m downstream of the discharge of the WWTP effluent (**Figure 3.1**).

### 5.2.2 Sample collection for pharmaceuticals analysis

At the $W_i$, 24 grab samples were collected over 72 hours, 1 sample every 4 hours and extra samples at the morning and evening peaks, resulting in 8 sampling intervals per day. This sampling strategy was defined after analyzing influent variability from the installation of an on-line ammonium sensor (ammolyzer from S::CAN, scan Messtechnik GmbH, Austria). At the $W_e$ and the sampling sites $I_u$ and $I_d$ in the river, we collected 13 samples over 48 hours (one sample every 4 hours; 6 sampling intervals per day) by applying a delay corresponding to 1 time the HRT (which was estimated through the tracer test) (Majewsky et al., 2013). Furthermore, water samples at $C_u$ were collected over 48 hours (one sample every 12 hours) to characterize the background concentrations of pharmaceuticals. Samples were filtered on-site through 0.45 µm Nylon filters (Whatman, Maidstone, UK) to eliminate suspended solid matter and were stored at -20ºC prior to analysis. Uncertainties due to the selected sampling strategy are considered in the calculations according to Ort et al. (2010), and its calculation and propagation are described below.

### 5.2.3 Analysis of pharmaceuticals

Analysis of the pharmaceuticals was performed following a fully automated method, based on on-line solid-phase extraction-liquid chromatography-electrospray tandem mass spectrometry (García-Galán et al., submitted) using a Thermo Scientific EQuanTM system consisting of two quaternary pumps: a loading pump (AccelaTM 600 pump) and an elution pump (Accela 1250 pump) from Thermo Scientific (Franklin, US). Two HypersilGoldTM (Thermo Scientific) liquid chromatography (LC) columns were used: the first for pre-concentration of the sample (20x2.1

mm, 12 µm particle size), and the second for chromatographic separation (50x2.1 mm, 1.9 µm particle size). Different volumes were used: 5 mL for surface water, 1 mL for Wi, and 2 mL for We samples. Mass spectrometry detection was carried out on a TSQ Vantage triple quadrupole (QqQ) mass spectrometer (Thermo Scientific, Franklin, US), equipped with an ESI turbo spray ionization source. Two selected reaction monitoring (SRM) transitions per compound were recorded -one for quantitation and the other for confirmation. Time-specific SRM windows were adjusted to the chromatographic retention times of each target compound to improve the sensitivity performance of the QqQ. Quantification was performed by the internal standard approach using isotopically labelled compounds in order to compensate matrix effects. The recovery rates (see **Table SI 5.1**) ranged between 55% and 150% for the three types of water matrices, whereas the limits of detection (LOD) of the method ranged from 0.05 to 50.98 $ng \cdot L^{-1}$ for surface water ($C_u$, $I_u$ and $I_d$), 0.03 to 338.37 $ng \cdot L^{-1}$ for $W_e$, and 0.21 to 635.97 $ng \cdot L^{-1}$ for $W_i$ (see **Table SI 5.2**).

In this study, initially 33 compounds were measured at the different sampling sites, but only 19 compounds with concentrations with significant presence in influent wastewaters and well above the detection limits were selected. Accordingly, target compounds in this study were the parental compounds acetaminophen, sulfapyridine, sulfamethoxazole, carbamazepine, venlafaxine, ibuprofen, diclofenac, and diazepam; and the studied transformation products include N4-acetylsulfapyridine, N4-acetylsulfamethoxazole, 2-hydroxy-carbamazepine, 10,11-epoxycarbamazepine, D,L-N-desmethylvenlafaxine (N-desmethylvenlafaxine), D,L-O-desmethylvenlafaxine (O-desmethylvenlafaxine), ibuprofen carboxylic acid (carboxy-ibuprofen), 1-hydroxy-ibuprofen, 2-hydroxy-ibuprofen, 4'-hydroxy-diclofenac, and nordiazepam.

### 5.2.4 Load reduction calculation

Pharmaceuticals load reduction was calculated in both WWTP and river. The loads at the influent ($M_{in}$) and effluent ($M_{out}$) of the systems ($W_i$ and $W_e$ at the WWTP, and $I_u$ and $I_d$ at the river) were calculated over 3 consecutive days as the product of flow and the concentrations obtained at each sampling interval. Wastage loads ($W_{was}$) at the WWTP and upstream the WWTP effluent ($C_u$) were also estimated and considered in this study. The loads estimated were then used to estimate attenuation in terms of load reduction. Furthermore, loads of $I_u$ with the sum of $C_u$ and $W_e$ were also compared to assure that the mass balances in each compartment were correct.

### 5.2.5 Attenuation rates.

In order to obtain a measure of attenuation comparable among the WWTP and the river, attenuation rates were calculated using the same numerical approach both WWTP and river systems. Pharmaceuticals attenuation can be modelled by using different attenuation kinetic expressions (Pomies et al., 2013), and basic forms of equations for attenuation modelling includes generally one or two parameters: i) based on the pharmaceutical concentration (the substance concentration; C) and formalized as a first order kinetic, or ii) based on pharmaceutical concentration and biomass concentration (which is able to convert this substrate) and formalized as a pseudo-first order. In this study, attenuation (net balance between removal and release) was modelled assuming a first order attenuation kinetic at the WWTP and river in order to compare attenuation in comparable way. In particular, the first order attenuation kinetic expression is given by **Eq 5.1**, and refers to the direct proportionality of the attenuation constant rate k ($d^{-1}$) to the soluble substance concentration C (ng $L^{-1}$) under study:

$$\frac{dC}{dt} = k \cdot C \qquad \textbf{(Eq 5.1)}$$

According to that, k values were calculated through the formulation of different model equations after applying mass balances and assuming steady-state conditions for both WWTP and river systems (Joss et al., 2006). For the WWTP, the biological treatment was modelled as one completely stirred tank reactor (CSTR) according to the tracer test results (see Results section). For one CSTR, the mass balance is given by **Eq 5.2**:

$$V \cdot \frac{dC}{dt} = Qin \cdot Cin - Qout \cdot C - V \cdot (k \cdot C) \qquad \textbf{(Eq 5.2)}$$

Where V is the reactor volume ($m^3$), Qin is the influent flow-rate ($m^3$/d), Qout the effluent flow-rate ($m^3d^{-1}$), Cin the influent concentration (ng $L^{-1}$) and C the effluent concentration (ng $L^{-1}$). From **Eq 5.2** and assuming steady-state conditions (dC/dt=0), k and half-life times (HLT) at the WWTP can be estimated using **Eq 5.3** and **Eq 5.4**, respectively.

$$\frac{Cout}{Cin} = \frac{1}{k \cdot \theta_h + 1} \qquad \textbf{(Eq 5.3)}$$

$$HLT = 1/k \qquad \textbf{(Eq 5.4)}$$

For the river, the system was modelled as a plug-flow system also according to the tracer test results (see 5.3 Results section). For a plug-flow river system and by including lateral flows, the mass balance can be expressed as **Eq 5.5**:

$$(A \cdot \Delta x)\frac{\partial C}{\partial t} = -Qout \cdot \Delta C - (k \cdot C) \cdot (A \cdot \Delta x) \qquad \textbf{(Eq 5.5)}$$

Where A is the river area ($m^2$), Δx is the river distance (m), Qout is the river flow ($m^3d^{-1}$), and ΔC is the difference of concentrations (ng $L^{-1}$) between upstream and downstream the river segment under study by considering lateral flows. Hence, from **Eq 5.5** and assuming steady-state conditions, k and HLT at the river can be estimated from **Eq 5.6** and **Eq 5.7**:

$$\frac{Cout}{Cin} = \frac{e^{(-k \cdot \tau)}}{(1+\beta)} \qquad \textbf{(Eq 5.6)}$$

$$HLT = \ln(2)\frac{1}{\tau} \qquad \textbf{(Eq 5.7)}$$

Where τ is the hydraulic residence time (d) and β (-) is the dilution factor correction due to flow increases from lateral flows.

### 5.2.6 Uncertainty propagation

Uncertainties from the sampling procedure and the chemical analyses of pharmaceuticals (lumped into a percentage of variation applied to the concentrations) and the flow measurements for each sampling point ($W_i$, $W_e$, $I_u$ and $I_d$) were incorporated into the attenuation rates calculations. A range of uncertainty was assigned to the concentrations of pharmaceuticals at each sampling point ($C_j$, $W_i$; $C_j$, $W_e$; $C_j$, $I_u$; $C_j$, $I_d$) (where j is each of the studied pharmaceuticals) by including chemical analysis and sampling uncertainties. These uncertainties were estimated based on the inter-daily variability of the measurements by overlapping the measurements from the three sampling days over a 1 day scale at each sampling point, and using the median of the observed deviations as uncertainty. The resulting range of uncertainties applied to each compound and sampling point is presented in **Table SI 5.3**. Uncertainties from flow measurements were also considered in the calculation, 10% uncertainty for the WWTP and a 13% uncertainty for the river flow (Harremöes et al., 1993). Afterwards, we defined uniform probability distribution functions for the concentrations and flows at each sampling point. These sources of uncertainty were propagated through Equation 3 and 6 by running Monte Carlo iterations (10,000) and changing concentrations at each sampling point and hydraulic residence time for each run. Median and the deviation bounds (5$^{th}$ and 95$^{th}$ percentiles) for the attenuation rates obtained were used in the results analysis. This modelling approach was implemented in Matlab® (The MathWorks, Inc.).

### 5.2.7 Dynamic simulations

The estimated attenuation constant rates were simulated and evaluated under dynamic conditions in order to first evaluate whether the first-order modelling approach describing attenuation of pharmaceuticals was enough to describe the observed dynamics, and second to ensure that the model-based approach used in the estimation of attenuation rates (by considering uncertainty) was enough to describe the observed dynamics. Hence, a dynamic integrated model for the WWTP and river systems was built based on ordinary differential equations which included first order attenuation kinetic and on top of a hydraulic model constructed and calibrated based on the tracer test data (see Chapter 4). At the WWTP, inputs to the model were the dynamic flow and the influent concentrations of the pharmaceuticals at the $W_i$ (covering a period of 72 hours), and simulations were run to obtain WWTP effluent concentration profiles at the $W_e$. For each compound we run three simulations, each one with a different k value. One simulation corresponds to the non-attenuation case of the compound, in which k is equal to zero. The other two simulations correspond to the lower and upper bounds (5$^{th}$ and 95$^{th}$ percentiles) of the attenuation rates obtained from the steady-state analysis, after the propagation of uncertainty. The same approach was followed for the river, using flows and concentrations at $I_u$ as inputs to the hydraulic model and running simulations to obtain the concentration temporal patterns at $I_d$. This modelling approach was built in SIMBA® (version 6, IFAK, Germany).

## 5.3 Results

### 5.3.1 Occurrence of pharmaceuticals

Pharmaceuticals concentrations were well above the detection limits for most compounds (**Table SI 5.2**). For the samples that were below the LOD, the concentration was set to zero. For samples below the limit of quantification (LOQ), we used the value that corresponded to LOQ/2. Mean concentrations with their standard deviations obtained from the measuring campaign at $W_i$, $W_{wast}$, $W_e$, $I_u$ and $I_d$ are shown in **Table 5.1**. Carboxy-ibuprofen was the compound with the highest concentrations at the $W_i$ (20,240 ng $L^{-1}$), followed by acetaminophen (18,518 ng $L^{-1}$), ibuprofen (12,312 ng $L^{-1}$) and its reminding transformation products (2-hydroxy-ibuprofen, 1-hydroxy-ibuprofen). The other analyzed compounds showed concentrations below 1,000 ng $L^{-1}$. At $W_{was}$, diclofenac, sulfapyridine and carbamazepine were the parent compounds showing the highest concentrations in the sludge with 20, 18 and 16 ng g $SS^{-1}$ in the sediments, respectively. Concerning their transformation products, highest concentrations in the sludge were observed for 4-hydroxy-diclofenac (190 ± 102 ng g $SS^{-1}$) and 2-hydroxy-carbamazepine (18 ± 7 ng g $SS^{-1}$). At $W_e$, ibuprofen was now the parent compound showing the highest concentrations (636 ng $L^{-1}$), followed by diclofenac, sulfamethoxazole, venlafaxine and carbamazepine (**Table 5.1**), while the other compounds showed $W_e$ concentrations below 100 ng·$L^{-1}$. Regarding their transformation products, carboxy-ibuprofen, 2-hydroxy-ibuprofen, 2-hydroxycarbamazepine and O-desmethylvenlafaxine were the compounds showing the highest concentrations at the $W_e$ (**Table 5.1**). At the receiving river system ($I_u$), Ibuprofen was again the compound showing the highest concentration (254 ± 101 ng $L^{-1}$), followed by diclofenac, carbamazepine, venlafaxine, sulfamethoxazole and sulfapyridine with concentrations ranging from 12 to 51 ng $L^{-1}$ (**Table 5.1**). Note that at sampling site $C_u$ in the river, the concentrations for all compounds were below the LOD except for carbamazepine, which nonetheless showed concentrations 10 times lower than in $I_d$.

### 5.3.2 Load reductions

The loads estimated in the different sampling points for the analyzed parent compounds are shown in **Figure 5.1**. Acetaminophen and ibuprofen were the parent compounds showing highest loads in $W_i$ (113 ± 33 and 77 ± 14 g $d^{-1}$). Loads in the wastage ($W_{was}$) were lower than 10% of the mass entering the WWTP for all compounds, except for diazepam, which wastage load represented 16% of the one at $W_i$. Loads estimated in the river upstream of the WWTP effluent ($C_u$) were lower than 0.1 g $d^{-1}$ for all compounds, except for ibuprofen (0.5 g $d^{-1}$). The sum of $W_e$ and $C_u$ loads was within load ranges observed in $I_u$ for ibuprofen, sulfapyridine, carbamazepine and venlafaxine, and differences in that balance for sulfamethoxazole and diazepam. This imbalance in the pharmaceuticals load could be most likely caused by the different analytical procedures used for the WWTP and river samples. These imbalances did however not affect the estimated attenuation rates, which were separately calculated for WWTP and river.

**Table 5.1.** Pharmaceuticals occurrence for influent and effluent wastewaters (WW), sludge wastage, and surface waters in the studied system (SS), and compared with those reported in Petrie et al. (2015)(PET). SS values are presented as min - max (mean) measured values during the period under study. N.D.: not detected; A "-" is used when pharmaceutical values were not reported.

| | Influent WW, $W_i$ (ng $L^{-1}$) | | Sludge Wastage, $W_{was}$ (ng g $SS^{-1}$) | | Effluent WW, $W_e$ (ng $L^{-1}$) | | Surface water, $I_u$ (ng $L^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | PET | SS | PET | SS | PET | SS | PET | SS |
| **ACETAMINOPHEN** | <96,924 - 492,340 | 800 - 60,176 (18,518) | N.D. | 3 - 6 (5) | <20 - 11,733 | 0 - 174 (31) | <1.5 - 1,388 | N.D. |
| **SULFAPYRIDINE** | <914 - 4,971 | 16 - 394 (108) | N.D. | 10 - 24 (18) | <277 - 455 | 38 - 102 (67) | <2 - 28 | 6 - 22 (12) |
| N4-ACETYLSULFAPYRIDINE | - | 11 - 1,251 (229) | - | N.D. | | 5 - 25 (10) | - | 0 - 25 (7) |
| **SULFAMETHOXAZOLE** | <3 - 115 | 45 - 1,094 (360) | N.D. | 5 - 20 (13) | <10 - 19 | 131 - 283 (183) | <0.5 - 2 | 12 - 27 (19) |
| N4-ACETYLSULFAMETHOXAZOLE | - | 0 - 1,044 (193) | - | N.D. | - | N.D. | - | N.D. |
| **CARBAMAZEPINE** | <950 - 2,593 | 105 - 435 (230) | N.D. | 11 - 24 (16) | <826 - 3,117 | 124 - 232 (166) | <0.5 - 251 | 25 - 40 (32) |
| 2-HYDROXYCARBAMAZEPINE | - | 195 - 1,337 (508) | - | 18 ± 7 | - | 461 - 974 (622) | - | 43 - 78 (59) |
| EPOXYCARBAMAZEPINE | - | 22 - 149 (55) | - | N.D. | - | 30 - 62 (40) | - | - |
| **VENLAFAXINE** | <120 - 249 | 45 - 364 (213) | N.D. | 3 - 15 (11) | <95 - 188 | 129 - 240 (174) | <1.1 - 35 | 21 - 36 (28) |
| N-DESMETHYLVENLAFAXINE | - | 0 - 83 (21) | - | N.D. | - | 33 - 57 (44) | - | 11 - 17 (13) |
| O-DESMETHYLVENLAFAXINE | - | 112 - 787 (398) | - | N.D. | - | 349 - 672 (468) | - | 28 - 48 (38) |
| **IBUPROFEN** | <1,681 - 33,764 | 3,623 - 22,078 (12,312) | 0,38 | N.D. | <143 - 4,239 | 282 - 1,173 (636) | <1 - 2,370 | 98 - 392 (254) |
| CARBOXY-IBUPROFEN | - | 1,501 - 30,160 (20,240) | - | N.D. | - | 659 - 3,132 (1,548) | - | 0 - 562 (196) |
| 1-HYDROXY-IBUPROFEN | - | 0 - 2,944 (1,091) | - | N.D. | - | 98 - 558 (270) | - | 46 - 86 (63) |
| 2-HYDROXY-IBUPROFEN | - | 2,292 - 13,074 (7,168) | - | N.D. | - | 389 – 1,311 (771) | - | 146 - 303 (53) |
| **DICLOFENAC** | <69 - 1,500 | 63 - 945 (379) | 0.07 | 24 - 34 (20) | <58 - 599 | 275 - 575 (383) | <0.5 - 154 | 34 - 65 (51) |
| 4-HYDROXY-DICLOFENAC | - | 252 - 366 (205) | | 12 - 54 (37) | - | 117 - 261 (157) | - | 10 - 22 (17) |
| **DIAZEPAM** | <0.9 - 7.6 | 1 -23 (4) | N.D. | 1 - 3 (2) | <1.6 - 5.1 | 1.5 - 3 (2) | <0.6 - 0.9 | 0 - 0.4 (0.1) |
| NORDIAZEPAM | - | 2 - 32 (6) | | 3 - 5 (4) | | 5 - 10 (7) | - | - |

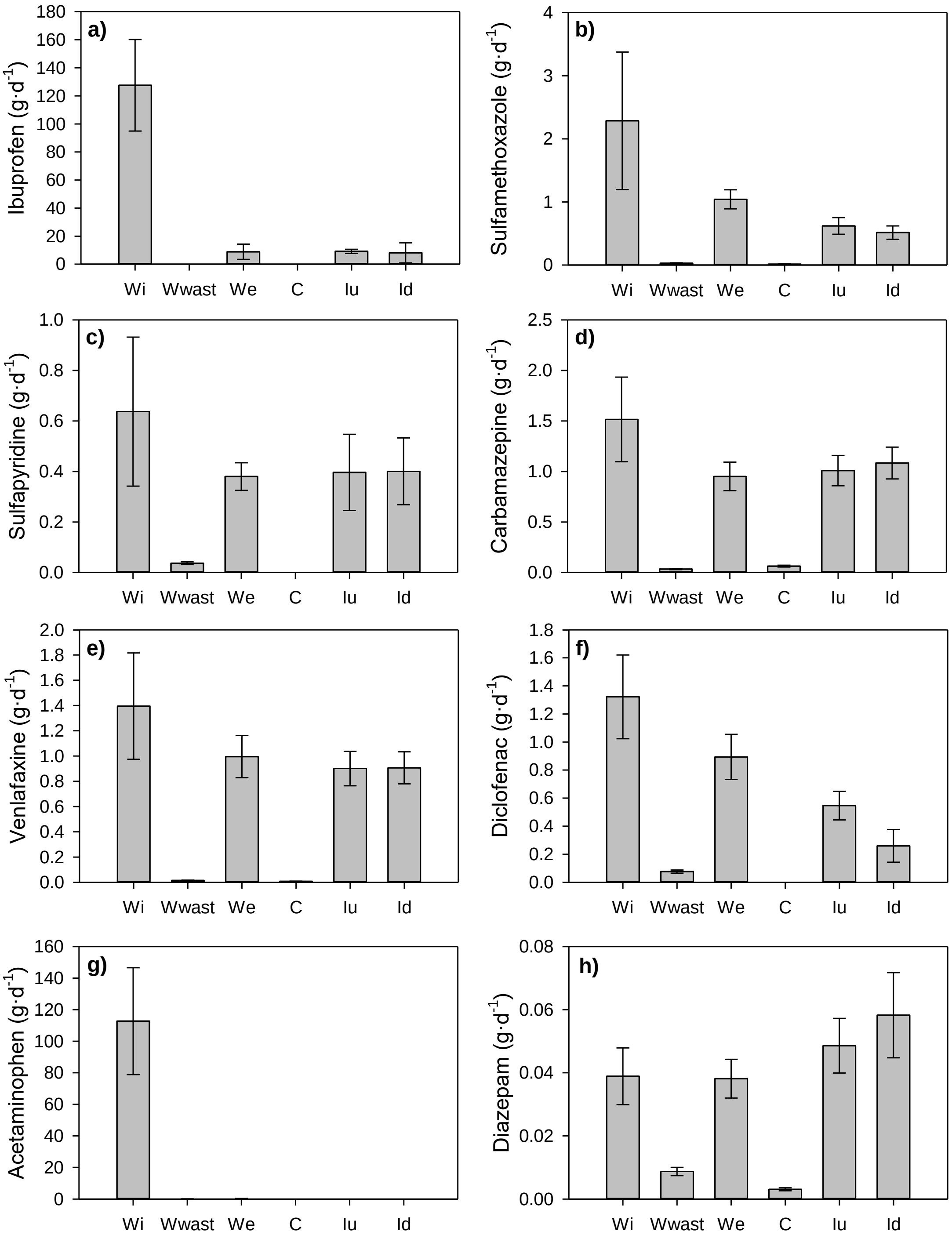


**Figure 5.1.** Loads with uncertainty in the different sampling points from the studied system for some of the pharmaceuticals selected in this study. $W_i$ = WWTP influent; $W_{was}$ = WWTP wastage; $W_e$ = WWTP effluent; $C_u$ = upstream river before WWTP discharge; $I_u$ = upstream end of river impact reach; $I_d$ = downstream end of the river impact reach.

Percentage of load reductions for the analysed pharmaceuticals (parent and transformation products) at the WWTP and river are presented in **Table 5.2**. Results show that the load of only 2 out of 8 parent compounds was reduced by more than 90% at the WWTP (acetaminophen and ibuprofen), while 4 parent compounds were only partially attenuated with load reductions ranging from 20 to 90% (sulfapyridine, sulfamethoxazole, carbamazepine and diazepam), and 2 were slightly or non-attenuated with load reductions ranging from -20 to 20% (venlafaxine and diclofenac). Concerning their transformation products, loads from 3 out of 11 compounds were reduced by more than 90% (N4-acetylsulfapyridine, N4-acetylsulfamethoxazole, carboxy-ibuprofen), while the rest were only partially attenuated (epoxy-carbamazepine, 1-hydroxy-ibuprofen, 2-hydroxy-ibuprofen, 4'-hydroxy-diclofenac and diazepam) or slightly or non-attenuated (O-demesthylvenlafaxine and nordiazepam). Some compounds were even released, with a load increase of more than 20% (2-hydroxycarbamazepine, N-desmethylvenlafaxine). In the river, only ibuprofen load (out of the 6 parent compounds that were present in Iu) decreased more than 50% (see **Table 5.2**), while only a slight or insignificant load reduction (or release) was observed for diclofenac (12 ± 14%), sulfamethoxazole (16 ± 15%), venlafaxine (-1 ± 12%), sulfapyridine (-6 ± 33%), carbamazepine (-8 ± 13%), respectively. Regarding the transformation products, loads from 3 transformation products out of 9 present in the river partially decreased by percentages ranging from 20% to 70% (1-hydroxy-ibuprofen, 4'-hydroxy-diclofenac and 2-hydroxy-carbamazepine), while 3 compounds were slightly or non-attenuated with load reductions ranging from -20 to 20% (carboxy-ibuprofen, N4-acetylsulfapyridine and N-desmetylvenlafaxine), and the load of 3 transformation products were released or generated with an increase of more than 20% (O-desmethylvenlafaxine, 2-hydroxy-ibuprofen and nordiazepam).

### 5.3.3 Attenuation rates

Attenuation rate metrics (k and HLT) of pharmaceuticals and transformation products were highly variable (**Table 5.2**). In terms of k, positive k's were obtained for the compounds that showed larger removal than generation, and negative rates for compounds with larger generation than removal. Attenuation constant rates were higher at the WWTP (median k of 0.6 $d^{-1}$) compared to the river (median k of 0.35 $d^{-1}$), while the range of k's obtained for the different compounds at the WWTP was also wider (from -0.6 ± 0.1 $d^{-1}$ to > 23.3 ± 8 $d^{-1}$) compared to the river (-1.8 ± 0.8 $d^{-1}$ to 8.3 ± 6 $d^{-1}$). In terms of HLT, larger values were obtained at the WWTP (median at 23 hours; range from 0 to 437 hours) compared to the river (median at 10 hours; range from 2 to 25 hours). For both the WWTP and the river, attenuation rates of parent compounds were smaller than those of the transformation products. Excluding acetaminophen, N4-acetylsulfamethoxazole and diazepam from the comparison, the average k for parent compounds at the WWTP was 4.0 $d^{-1}$, whereas for metabolites it was 5.3 $d^{-1}$. In the river, the average of absolute value of k was 1.2 $d^{-1}$ for the parent compounds and 2.3 $d^{-1}$ for the metabolites.

**Table 5.2.** Average load reduction at the WWTP and the river (%) with the coefficient of variance resulting from the MonteCarlo simulations, average attenuation rates *k* (1/d) with their CV and HLT (h) obtained from the average *k*, for the analyzed pharmaceuticals in the studied system. HLT were only calculated for compounds with positive k's. N.D.: not detected; N.Q.: not quantifiable.

| | **WWTP** | | | **River** | | |
|---|---|---|---|---|---|---|
| **Compound** | Load reduction (%) (SD) | k ($d^{-1}$) (SD) | HLT (h) | Load reduction (%) (SD) | k ($d^{-1}$) (SD) | HLT (h) |
| **ACETAMINOPHEN** | 100 (0) | N.Q. | N.Q. | N.D. | N.D. | N.D. |
| **SULFAPYRIDINE** | 28 (22) | 0,6 (0,5) | 40,6 | -6 (33) | -0,1 (1,7) | N.Q. |
| N4-ACETYLSULFAPYRIDINE | 95 (2) | 23,3 (8) | 1,0 | 7 (28) | 0,7 (1,8) | 25,1 |
| **SULFAMETHOXAZOLE** | 45 (18) | 1,1 (0,7) | 21,3 | 16 (15) | 1,1 (1) | 15,5 |
| N4-ACETYLSULFAMETHOXAZOLE | 100 (0) | N.Q. | N.Q. | N.D. | N.D. | N.D. |
| **CARBAMAZEPINE** | 28 (13) | 0,5 (0,3) | 47,3 | -8 (13) | -0,4 (0,7) | N.Q. |
| 2-HYDROXYCARBAMAZEPINE | -23 (22) | -0,2 (0,2) | N.Q. | 23 (11) | 1,5 (0,8) | 10,9 |
| EPOXYCARBAMAZEPINE | 25 (15) | 0,5 (0,3) | 53,3 | N.D. | N.D. | N.D. |
| **VENLAFAXINE** | 18 (17) | 0,3 (0,3) | 77,2 | -1 (12) | 0,04 (0,7) | N.Q. |
| N-DESMETHYLVENLAFAXINE | -128 (32) | -0,6 (0,1) | N.Q. | -9 (16) | -0,5 (0,8) | N.Q. |
| O-DESMETHYLVENLAFAXINE | -19 (21) | -0,2 (0,2) | N.Q. | -23 (16) | -1,1 (0,8) | N.Q. |
| **IBUPROFEN** | 95 (1) | 21,8 (5) | 1,1 | 58 (8) | 5,0 (1) | 3,3 |
| CARBOXY-IBUPROFEN | 92 (3) | 16,2 (8) | 1,5 | 12 (47) | 1,8 (4) | 9,2 |
| 1-HYDROXY-IBUPROFEN | 70 (14) | 3,5 (2) | 6,8 | 68 (19) | 8,3 (6) | 2,0 |
| 2-HYDROXY-IBUPROFEN | 89 (2) | 9,8 (2) | 2,5 | -39 (19) | -1,8 (0,8) | N.Q. |
| **DICLOFENAC** | 0,6 (23,4) | 0,1 (0,3) | 437,4 | 12 (14) | 0,8 (0,9) | 20,7 |
| 4-HYDROXY-DICLOFENAC | 23 (13) | 0,4 (0,3) | 61 | 52 (14) | 4,4 (1,7) | 3,7 |
| **DIAZEPAM** | 43 (13) | 1,0 (0,5) | 25,1 | N.Q. | N.Q. | N.Q. |
| NORDIAZEPAM | -11 (19) | -0,1 (0,2) | N.Q. | -21 (21) | -1,0 (1) | N.Q. |

### 5.3.4 Uncertainty assessment

Uncertainties associated to load reductions and attenuation constant rates at the WWTP and river are also shown in **Table 5.2**. At the WWTP, uncertainties associated to the load reductions range from 0% to 32%, with a mean and standard deviation of 13% and 9%, respectively. Interestingly, a high correlation ($r^2 = 0.84$) was observed when comparing the percentage load reduction and the associated uncertainty (**Figure SI 5.1**), thus having lower uncertainties for those compounds that showed higher percentages of load reductions. When looking at uncertainties associated to the estimated k, larger ranges of k can be obtained (from -0.6 to 21.8 $d^{-1}$), and thus higher uncertainty ranges were obtained depending on the compound and the magnitude of k (from 0 $d^{-1}$ to 8 $d^{-1}$). At the river, uncertainties associated to the load reductions are higher compared to WWTP, ranging from 0% to 46% (with mean and standard deviation of 18% and 11% respectively), and showing very low correlation ($r^2 = 0.17$) between the percentage of load reduction and the associated uncertainty (**Figure SI 5.1**). The magnitudes of k in the river ranges from -1.8 to 4.4 $d^{-1}$, while uncertainties of k ranged from 0 $d^{-1}$ to 6$d^{-1}$.

### 5.3.5 Dynamic simulations results

The dynamic simulation results for 5 selected parent compounds at the WWTP and river are shown in **Figure 5.2**. For the presented compounds, we can observe that the simulated concentrations in $W_e$ and $I_d$ matched the dynamics observed with the experimental values, and that general trends observed at We and Id were properly described by the model for most compounds. For example, temporal changes in river concentrations (ibuprofen, sulfamethoxazole and carbamazepine) corresponding to decreased influent loads at the WWTP (see tracer test results in **Figure 4.1**), were properly described by the model (see times 28h and 52h). Results show that uncertainties at the effluent of the WWTP were higher compared to the river, probably related to the high variability occurring at the influent of the WWTP (**Table SI 5.3**). At the WWTP, the simulated $W_e$ concentrations (grey zone in **Figure 5.2**) for sulfamethoxazole, sulfapyridine and carbamazepine contained all observed values, however, in some cases the uncertainty was too large to properly describe the observed dynamics. More specifically, the experimental concentrations of carbamazepine at We showed abrupt changes that could not be explained by the model, probably more related to analytical issues rather than effects of the dynamics of the system. At the river, the simulated Id concentrations for sulfamethoxazole and carbamazepine matched the observed ones and dynamics were also properly described. Uncertainties at the river were too large for sulfapyridine simulated values at Id to describe its dynamics. This is explained due to the changing concentrations upstream river, leading to large uncertainties when the calculation of uncertainty is based on the inter-daily variability of the measurements (**Table SI 5.3**). However, in general the results show that the approach used to estimate uncertainties was valid for most of the compounds, and that the model-based approach and first-order attenuation kinetics was enough to estimate and model attenuation of pharmaceuticals at both WWTP and river.

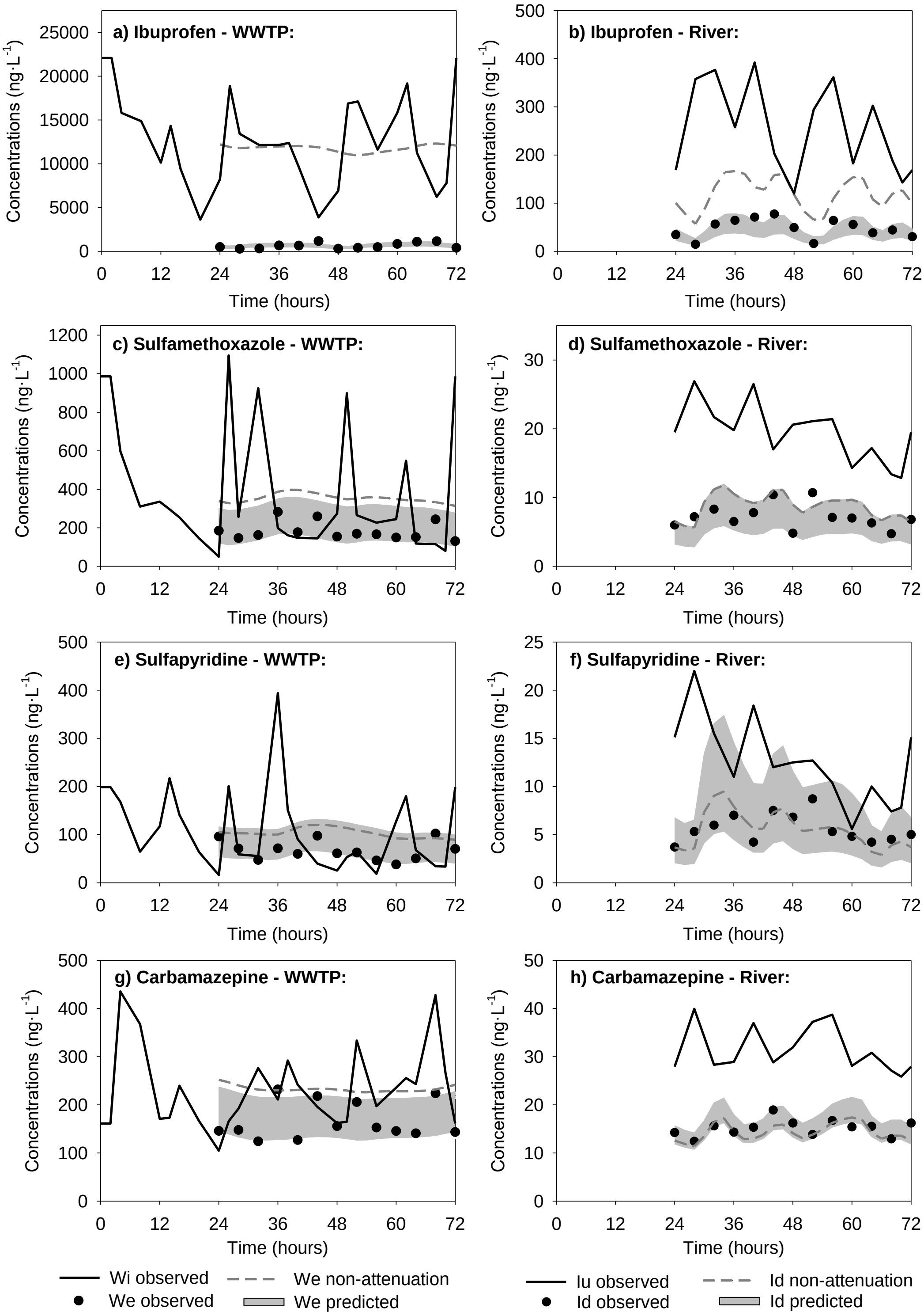


**Figure 5.2.** Simulation of pharmaceuticals dynamics in the WWTP and the river system under study. The grey zone represents the propagation of uncertainties through the model to describe the concentrations at the end-point of the system (We for the WWTP and Id for the river) for the attenuation rates estimated (from the steady-state analysis), and the area between the dashed grey line (simulation corresponding to k=0) and the simulated values (grey zone) corresponds to the mass of compound attenuated.

## 5.4 Discussion

### 5.4.1 Considerations about the approach to estimate attenuation rates

Although multiple approaches have been used to estimate the fate of pharmaceuticals in both WWTPs and rivers, most of them do not consider the two integrally. Our approach was based on the characterization of the residence and travel times in both systems through a slug addition of a conservative tracer and modelling exercise using first order attenuation kinetics for the WWTP and the river. Our approach could not distinguish between the gross removal and release of pharmaceuticals, as only the net balance (i.e., attenuation) was estimated. However, it identified biodegradation as the most important attenuation mechanism in the WWTP, given that the low pharmaceutical loads in the wastage (lower than 10% of the mass entering the WWTP) indicated that adsorption to sludge was not an important mechanism for the most of the analyzed compounds. It is also true that differentiating between attenuation mechanisms in the river compartment would require controlled laboratory experiments such as those designed to quantify sorption to sediments (e.g., Writer et al., 2013) or biodegradation and photodegradation processes (e.g., Fatta-Kassinos et al., 2011).

A novelty of our approach was the study of pharmaceuticals attenuation in a coupled system by the combination of integrated tracer test experiments with hydraulic modelling approaches, together with the consideration and propagation of different uncertainty sources. The results showed the benefits of conducting and combining integrated tracer test experiments with hydraulic modelling, such as i) allowing a proper characterization of the systems, as well as the identification of temporal changes in the observed dynamics; and at the same time ii) reducing hydraulic uncertainty that could affect in our results interpretations (with especial importance in river studies), such as in the loads and attenuation rates estimation. On the other hand, uncertainty from sampling, chemical analysis, and flow measurements was also considered and achieved by combining modelling and MonteCarlo methods, thus obtaining loads and attenuation rates within a confidence range. The consideration of uncertainty is needed when studying the chemical fate of pharmaceuticals in our engineering and natural systems due to the high degrees of uncertainty associated to measured data. Hence, the different degrees of uncertainty should be identified and considered in our results estimation, not only at the influent of the WWTP, but also at the different sampling sites under study. In this study, we present a methodology in which uncertainty has been identified, quantified and propagated in a proper way after the validation through the model-based approach applied. The results showed the importance of reducing such uncertainty when studying the chemical fate of pharmaceuticals, which sometimes limited the analysis of our results (e.g. transformations between parent compounds and their transformation products) due to the high degrees of uncertainties associated with micro-contaminants. Within this context, improving sampling strategies, conducting tracer test studies or ensuring accurate chemical analysis are good alternatives to reduce uncertainty in results, and thus provide more precise conclusions when performing integrated studies.

### 5.4.2 Occurrence of pharmaceuticals

Occurrence of pharmaceuticals and some of their transformation products was evaluated at different sampling sites and compared with a recent review on emerging contaminants in wastewater treatment systems and their receiving water bodies (Petrie et al., 2015) (**Table 5.1**).

For all compounds, the concentrations observed fell within the ranges reported in the literature, except for sulfamethoxazole, which concentration levels in effluent and surface water samples were higher than those reported in the literature (**Table 5.1**). The target pharmaceuticals and their transformation products were continuously entering the WWTP, but most of them were only partially or non-attenuated or even released and consequently discharged into the receiving river. Thus, in the river, we also observed the continuous presence of pharmaceuticals and their transformation products. For the same river and period under study, other studies proved that some of the pharmaceuticals selected in this study (diclofenac, ibuprofen and venlafaxine) were affecting aquatic organisms through bioaccumulation and causing structural changes in the community (Corcoll et al., 2014; and Ruhí et al., 2016).

### 5.4.3 Attenuation rates

The followed approach allowed the direct comparison of attenuation rates across engineered and natural systems. The comparison of both systems revealed that the attenuation in terms of load reduction was higher in the WWTP, but that attenuation in terms of HLT was higher in the river. This difference can be attributed to different residence time of pharmaceuticals in WWTP and river. Thus, the river showed higher attenuation efficiency than the WWTP for a given residence time, but the residence time in the WWTP was 8 times longer than in the considered river reach. These differences between WWTP and river were not the case for all analyzed compounds. Specifically, differences were only observed for sulfamethoxazole, 1-hydroxy-ibuprofen, diclofenac and 4-hydroxy-diclofenac, with shorter HLT in the river than in the WWTP. Despite this apparently higher efficiency in the attenuation of pharmaceuticals in the river respect the WWTP, one should keep in mind that the residence time at the WWTP is much higher than in the river and this results in higher load reductions achieved at the WWTP. In fact, assuming constant hydraulic retention, the residence time of the WWTP would be equaled by the river in a reach of 40 km.

The assessed load reductions, k and HLT values for parent compounds in both WWTP and river were in general consistent with those reported in previous studies (**Table SI 5.4**). For example, acetaminophen was completely attenuated at the WWTP coinciding with results in the literature (**Table SI 5.4**). Sulfapyridine was slightly or non-attenuated (28 ± 22%) at the WWTP, comparable with results reported by Verlicchi et al. (2012) (note that only WWTPs with SRTs lower than 6 days were considered). Ibuprofen load was highly reduced (95 ± 1%) at the WWTP, a percentage similar to those reported in Verlicchi et al. (2012). In the river, ibuprofen HLT was similar to those reported in the literature (Acuña et al., 2015; Lin et al., 2006), but with differences to the ones reported in Kunkel and Radke (2012). Diclofenac showed no attenuation at the WWTP coinciding with other reports (Verlicchiet al.2012; Sun et al., 2014; Fernández et al., 2014), while other studies showed higher reductions (Verlicchi et al.2012; Samaras et al., 2013; Vieno and Sillanpää, 2014). The likely reasons behind the wide range of load reductions observed for diclofenac can be explained due to different process configurations which may influence the attenuation of this compound (Vieno and Sillanpää 2014). In contrast, attenuation of diclofenac in the river was higher, and the observed HLT (20 h) was slightly higher than the maximum value reported in the literature (16h; Kunkel and Radke, 2012).The load reduction of sulfamethoxazole in the WWTP (45 ± 18%) was slightly lower to the average ranges reported (Verlicchi et al., 2012; Urtiaga et al., 2013; Sun et al., 2014: Guerra et al., 2014). In the river, its half-life time (16h) was higher than the range 5.8 ± 4.9 reported in Acuña et al. (2015), but smaller than the values of 29 and

41 hours estimated in Kunkel and Radke (2012). A 18 ± 17% load reduction at the WWTP was estimated for venlafaxine, which falls within the reported ranges of 5% and 39% (Lajeunesse et al., 2012; Subedy& Kannan, 2015). No river attenuation was observed for venlafaxine neither in this study nor in Writer et al. (2013). For carbamazepine, higher attenuation rates were observed in this study (28 ±13%) compared to Lajeunesse et al. (2012) and Verlicchi et al. (2012), ranging from 3 to 18 %. In the river, we did not observe attenuation as in Kunkel and Radke (2012). Still, the study from Acuña et al. (2015) and Writer et al. (2013) reported a HLT of 4.1 ± 2.4 hour and 21 ± 4.5 hours, respectively.

Finally, our study also identified likely causal relationships in the WWTP between positive attenuation rates for some parent compounds with negative attenuation of their respective transformation products (**Table 5.2**). This was obvious in the case of venlafaxine (in the WWTP) and carbamazepine (WWTP and river). Furthermore, in one case, these causal relationships were identified across systems, that is, a positive value of a parent compound at the WWTP and a negative value of its metabolites at the river. For example, the parent compound carbamazepine showed positive attenuation at the WWTP (k=0.5 ± 0.3) and negative at the river (k=-0.4 ± 0.7), whereas its respective metabolite 2-hydroxycarbamazepine showed negative attenuation at the WWTP (k=-0.2 ± 0.2) and positive at the river (k=1.5 ± 0.3). It might be therefore stressed that the transformation product was generated in the WWTP and mostly attenuated in the river. In the case of ibuprofen and diclofenac positive attenuation rates occurred for both parent compounds and metabolites at the WWTP and river. An exception was 2-hydroxy-ibuprofen, which showed negative attenuation in the river, meaning that gross release was higher than removal.

## 5.5 Supporting Information

**Table SI 5.1.** Recovery values (R%) obtained for each of the water matrices investigated, at spike levels of 500 ng $L^{-1}$ for influent wastewater, 100 ng $L^{-1}$ for effluent wastewater and 50 ng $L^{-1}$ for surface water (n=3).

| | | **Influent (Wi)** | | | **Effluent (We)** | | | **Surface water (Iu and Id)** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Therapeutic Family** | **COMPOUND** | **AVERAGE** | **SDV** | **RSD (%)** | **AVERAGE** | **SDV** | **RSD (%)** | **AVERAGE** | **SDV** | **RSD (%)** |
| **Analgesics/ anti-inflammatories** | **ACETAMINOPHEN** | 29.19* | - | - | 25.7* | - | - | 23.8 | 12.4 | 52.1 |
| | **DICLOFENAC** | 88.1 | 19.7 | 22.4 | >150 | 8.6 | 4.9 | 61.1 | 4.0 | 6.5 |
| | 4'-HYDROXY-DICLOFENAC | 66.5 | 11.1 | 16.7 | 152.1 | 36.4 | 23.9 | 143.1 | 7.8 | 5.4 |
| | **IBUPROFEN** | 134.2 | 3.1 | 2.3 | 117.9 | 13.6 | 11.5 | 79.9 | 9.1 | 11.3 |
| | 1-HYDROXY-IBUPROFEN | 95.8 | 10.2 | 10.6 | >150 | 14.8 | 6.7 | 65.9 | 3.6 | 5.5 |
| | 2-HYDROXY-IBUPROFEN | 105.1 | 10.1 | 9.6 | 79.2 | 13.3 | 16.8 | 120.5 | 7.0 | 5.8 |
| | CARBOXY-IBUPROFEN | >150 | 17.3 | 4.7 | >150 | 14.7 | 6.2 | 108.4 | 7.9 | 7.3 |
| **Psychiatric drugs** | **CARBAMAZEPINE** | 138.3 | 17.2 | 12.5 | >150 | 7.2 | 1.9 | 90.1 | 6.3 | 7.0 |
| | 10,11-EPOXY-CARBAMAZEPINE | 210.8 | 17.3 | 8.2 | 131.1 | 8.9 | 6.8 | 114.0 | 13.9 | 12.3 |
| | 2-HYDROXY-CARBAMAZEPINE | 150.0 | 2.8 | 1.9 | >150 | 19.3 | 8.1 | >150 | 11.0 | 6.4 |
| | **DIAZEPAM** | 138.7 | 11.6 | 8.4 | 141.7 | 1.6 | 1.1 | 137.3 | 10.1 | 7.4 |
| | NORDIAZEPAM | 139.2 | 0.5 | 0.4 | 128.9 | 35.4 | 27.5 | 108.1 | 6.8 | 6.3 |
| | **VENLAFAXINE** | 55.5 | 12.3 | 22.4 | >150 | 12.2 | 4.2 | 132.7 | 12.7 | 9.6 |
| | N-DESMETHYLVENLAFAXINE | 97.8 | 2.1 | 2.2 | 108.8 | 0.5 | 0.5 | 41.1 | 1.6 | 4 |
| | O-DESMETHYLVENLAFAXINE | 91.98 | 1.3 | 1.4 | 72.7 | 1.2 | 1.7 | - | - | - |
| **Antibiotics** | **SULFAMETHOXAZOLE** | >150 | 18.3 | 6.4 | 87.8 | 15.3 | 17.4 | 89.6 | 9.9 | 11.0 |
| | $N^4$-ACETYLSULFAMETHOXAZOLE | 131.3 | 9.2 | 6.7 | 72.1 | 8.5 | 11.8 | 98.8 | 11.1 | 11.2 |
| | **SULFAPYRIDINE** | 98.9 | 11.3 | 11.4 | 113.5 | 19.9 | 17.6 | 95.8 | 12.5 | 13.1 |
| | $N^4$-ACETYLSULFAPYRIDINE | 99.3 | 13.3 | 13.4 | 100.8 | 16.9 | 16.7 | 118.1 | 5.2 | 4.4 |

**Table SI 5.2.** Method limits of detection (LOD) and methods limits of quantification (LOQ) for river and WWTP water samples (ng L-1), and precision of the method expressed as relative standard deviation (n=5, %).

| | | Influent ($W_i$) | | | Effluent ($W_e$) | | | Surface Water ($I_u$ and $I_d$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Therapeutic Family** | **Compound** | **LOD** | **LOQ** | **RSD(%)** | **LOD** | **LOQ** | **RSD(%)** | **LOD** | **LOQ** | **RSD %** |
| **Analgesics/ anti-inflammatories** | **ACETAMINOPHEN** | 64.95 | 216.51 | 11.5 | 32.21 | 107.36 | 2.2 | 50.98 | 169.94 | 4.4 |
| | **DICLOFENAC** | 2.88 | 9.59 | 3.7 | 11.08 | 36.93 | 1.8 | 2.82 | 9.41 | 1.1 |
| | 4'-HYDROXY-DICLOFENAC | 0.98 | 3.27 | 3.6 | 0.29 | 0.97 | 6.8 | 1.84 | 6.14 | 1.9 |
| | **IBUPROFEN** | | | 2.2 | 43.14 | 143.79 | 7.9 | | | |
| | 1-HYDROXY-IBUPROFEN | 35.42 | 118.08 | 2.6 | 37.39 | 124.62 | 4.7 | 0.64 | 2.15 | 4.1 |
| | 2-HYDROXY-IBUPROFEN | 150.71 | 502.38 | 1.3 | 39.87 | 132.89 | 13.8 | 35.64 | 118.81 | 5.5 |
| | CARBOXY-IBUPROFEN | 635.97 | 2119.90 | 1.9 | 338.37 | 1127.89 | 3.4 | 15.20 | 50.67 | 8.3 |
| **Psychiatric drugs** | **CARBAMAZEPINE** | 0.53 | 1.77 | 0.54 | 0.01 | 0.03 | 2.2 | 1.59 | 5.30 | 5.4 |
| | 10.11-EPOXY-CARBAMAZEPINE | 4.30 | 14.32 | 2.3 | 3.25 | 10.82 | 2.9 | 16.67 | 55.56 | 9.2 |
| | 2-HYDROXY-CARBAMAZEPINE | 2.81 | 9.37 | 4.2 | 0.43 | 1.44 | 1.5 | 50.12 | 167.08 | 5 |
| | **DIAZEPAM** | 0.23 | 0.77 | 0.6 | 0.04 | 0.15 | 1.6 | 0.38 | 1.28 | 1.5 |
| | NORDIAZEPAM | 0.21 | 0.70 | 3 | 0.03 | 0.09 | 2.4 | 0.61 | 2.03 | 32.6 |
| | **VENLAFAXINE** | 1.95 | 6.51 | 0.9 | 0.37 | 1.24 | 9.6 | 7.86 | 26.20 | 5.8 |
| | N-DESMETHYLVENLAFAXINE | 2.73 | 9.11 | 0.9 | 0.28 | 0.93 | 2.9 | 32.23 | 107.43 | 4.5 |
| | O-DESMETHYLVENLAFAXINE | 3.28 | 10.93 | 1.7 | 0.19 | 0.65 | 3.7 | 0.05 | 0.18 | 8.6 |
| **Antibiotics** | **SULFAMETHOXAZOLE** | 43.39 | 144.65 | 1.2 | 3.36 | 11.19 | 3.4 | 0.81 | 2.70 | 13.9 |
| | $N^4$-ACETYLSULFAMETHOXAZOLE | 92.99 | 309.98 | 3.5 | 0.81 | 2.71 | 5.7 | 3.60 | 12.01 | 10.3 |
| | **SULFAPYRIDINE** | 9.66 | 32.19 | 1.2 | 0.86 | 2.88 | 1.3 | 2.17 | 7.24 | 14 |
| | $N^4$-ACETYLSULFAPYRIDINE | 8.49 | 28.30 | 3.5 | 0.46 | 1.52 | 2.7 | 0.66 | 2.21 | 3.6 |

**Table SI 5.3.** Sampling procedure, chemical analysis, and flow measurements uncertainties applied in the WWTP and river samples.

| **Sampling procedure and Chemical analysis uncertainty ($U_{sampling}$)** | | | | |
|---|---|---|---|---|
| **Pharmaceuticals** | $W_i$ (%) | $W_e$ (%) | $I_1$ (%) | $I_5$ (%) |
| **ACETAMINOPHEN** | 28.31 | - | - | - |
| **SULFAPYRIDINE** | 45.21 | 10.32 | 35.72 | 30.36 |
| $N^4$-ACETYLSULFAPYRIDINE | 49.24 | 23.97 | 20.21 | 47.04 |
| **SULFAMETHOXAZOLE** | 46.63 | 10.58 | 16.92 | 15.88 |
| $N^4$-ACETYLSULFAMETHOXAZOLE | 78.47 | - | - | - |
| **CARBAMAZEPINE** | 25.77 | 11.04 | 7.21 | 6.40 |
| 2-HYDROXY-CARBAMAZEPINE | 23.32 | 12.09 | 7.32 | 11.12 |
| 10.11-EPOXY-CARBAMAZEPINE | 27.99 | 13.58 | - | - |
| **VENLAFAXINE** | 28.48 | 13.49 | 7.72 | 5.19 |
| N-DESMETHYLVENLAFAXINE | NaN | 16.48 | 14.43 | 6.69 |
| O-DESMETHYLVENLAFAXINE | 23.19 | 12.00 | 12.08 | 4.45 |
| **IBUPROFEN** | 14.42 | 26.52 | 16.01 | 17.39 |
| CARBOXY-IBUPROFEN | 23.54 | 60.68 | 9.64 | 88.16 |
| 1-HYDROXY-IBUPROFEN | 59.67 | 29.65 | 6.46 | 122.31 |
| 2-HYDROXY-IBUPROFEN | 14.19 | 26.30 | 12.41 | 7.77 |
| **DICLOFENAC** | 27.30 | 24.14 | 8.55 | 18.56 |
| 4'-HYDROXY-DICLOFENAC | 20.25 | 14.98 | 13.30 | 43.09 |
| **DIAZEPAM** | 30.58 | 14.63 | 57.60 | - |
| NORDIAZEPAM | 20.89 | 12.60 | 12.17 | 19.17 |
| **Hydraulics uncertainty ($U_{Hydraulics}$)** | | | | |
| | WWTP (%) | | River (%) | |
| **Flow-rate** | 10 | | 13 | |
| **Travel times** | 10 | | 13 | |

**Table SI 5.4.** Load reduction (%) and half-life times (HLT, h) for the analyzed PhCs in the studied system, and elsewhere. IBU: Ibuprofen; DIC: Diclofenac; DIA: Diazepam; SUL: Sulfamethoxazole; VEN: Venlafaxine; CAR: Carbamazepine. Only conventional activated sludge systems are shown in the Table. NA: not applicable; NoA: no attenuation. A "/" between values is included to differentiate between different WWTPs in the same study. A "~" is used when the exact values were not reported in the references and hence were obtained from a graph. In the river variability is provided (±) when available in the original paper. * estimated from parameters given in the paper using first order equation.

| | ACM | SPY | SUL | CAR | VEN | IBU | DIC | DIA | Reference |
|---|---|---|---|---|---|---|---|---|---|
| **WWTP (Load reduction, %)** | | | | | | | | | |
| Puigcerdà, 2012 | 100 | 28 | 45 | 28 | 18 | 95 | 0.6 | 43 | This study |
| WWTPs with SRT < 6d | 84.6 -> 100 | 20 | 54 -> 71 | -5 -> 35% | - | 82 -> 99 | 7.1 -> 65% | 42 | Verlicchi et al., 2012 |
| WWTP B (Sept/Apr), 2009-2010 | - | - | - | 10/4.4 | 18/12 | - | - | - | Lajeunesse et al., 2012 |
| WWTP W, 2009-2010 | - | - | - | 3.6 | 39 | - | - | - | Lajeunesse et al., 2012 |
| Many WWTPs | - | - | - | - | - | - | 36 | - | Vieno & SillanäÄ, 2014 |
| WWTP Vuelta Ostrera (Spain) | 99.5 | NA | 58.9 | NA | - | 76.9 | NA | 15.2 | Urtiaga et al., 2013 |
| WWTP Athens/Mytilene (Greece), 2009 | - | - | - | - | - | 100/100 | 75/39 | - | Samaras et al., 2013 |
| WWTP Shore (Milwake, US), 2009-2010 | 100 | - | 100 | 100 | - | 100 | - | - | Blair et al., 2013 |
| Xiamen (China), 2012-2013 | ~100 | - | 52.4 | ~30 | - | 68.5/91 | ~-5->45 | - | Sun et al., 2014 |
| 2 WWTPs (Albany, US), 2013 | - | - | - | ~-60 | ~5 | - | - | ~40 | Subedi & Kanna, 2015 |
| 2 WWTPs (Q/MH), 2010–2012 | 99/99 | - | 0/47 | - | - | 71/99 | - | - | Guerra et al., 2014 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2 WWTPs (A/B), 2010-2011 | - | - | - | NoA | - | ~80/~97 | NoA | - | Fernández et al., 2014 |
| South-west China, 2012-2013 | | | 61 | -15 | - | <95 | 57 | - | Laurans et al., 2013 |
| Betzdorf | | | | ~-10 -> 0 | - | - | ~20 | - | Majewsky et al., 2013 |
| **River (Load reduction, %)** | | | | | | | | | |
| Segre, 2012 | ND | -6 | 16 | -8 | -1 | 58 | 12 | NA | |
| RoterMain (E2/E4/E5), 2007 | - | - | - | - | - | - | 4 ± 6/ -5 ± 7/ 5 ± 15 | - | Radke et al., 2010 |
| Gründlach, (Period 1/ Period 2), 2010 | - | - | 26/ 25 | - | - | NA/ NA | 69/ 41 | - | Kunkel&Radke, 2012 |
| Santa Ana, 2004 | - | - | - | - | - | 87 | - | - | Lin et al., 2006 |
| Santa Ana, 2002 | - | - | - | NA | - | 83 ± 25 | - | - | Gross et al., 2004 |
| River (HLT, h) | | | | | | | | | |
| Segre, 2012 | ND | NA | 16 | NA | NA | 3 | 21 | NA | |
| Segre, 2010 | 1.7 ± 1 | - | 5.8 ± 4.9 | 4.1 ± 2.4 | 2.7 ± 1.7 | 2 ± 1.1 | 1.6 ± 0.5 | 28.1 ± 45 | Acuña et al., 2014 |
| Boulder, 2011 | - | - | - | 21.0 ± 4.5 | NA | - | - | - | Writer et al., 2013 |
| Gründlach, (Period 1/ Period 2), 2010 | - | - | 41*/29* | NoA/NoA | - | - | 11*/16* | - | Kunkel&Radke, 2012 |
| SävaBrook, 2009 | - | - | - | - | - | 10 ± 1.3 | NoA | - | Kunkel&Radke, 2011 |
| Santa Ana, 2004 | - | - | - | - | - | 5.4 | - | - | Lin et al., 2006 |

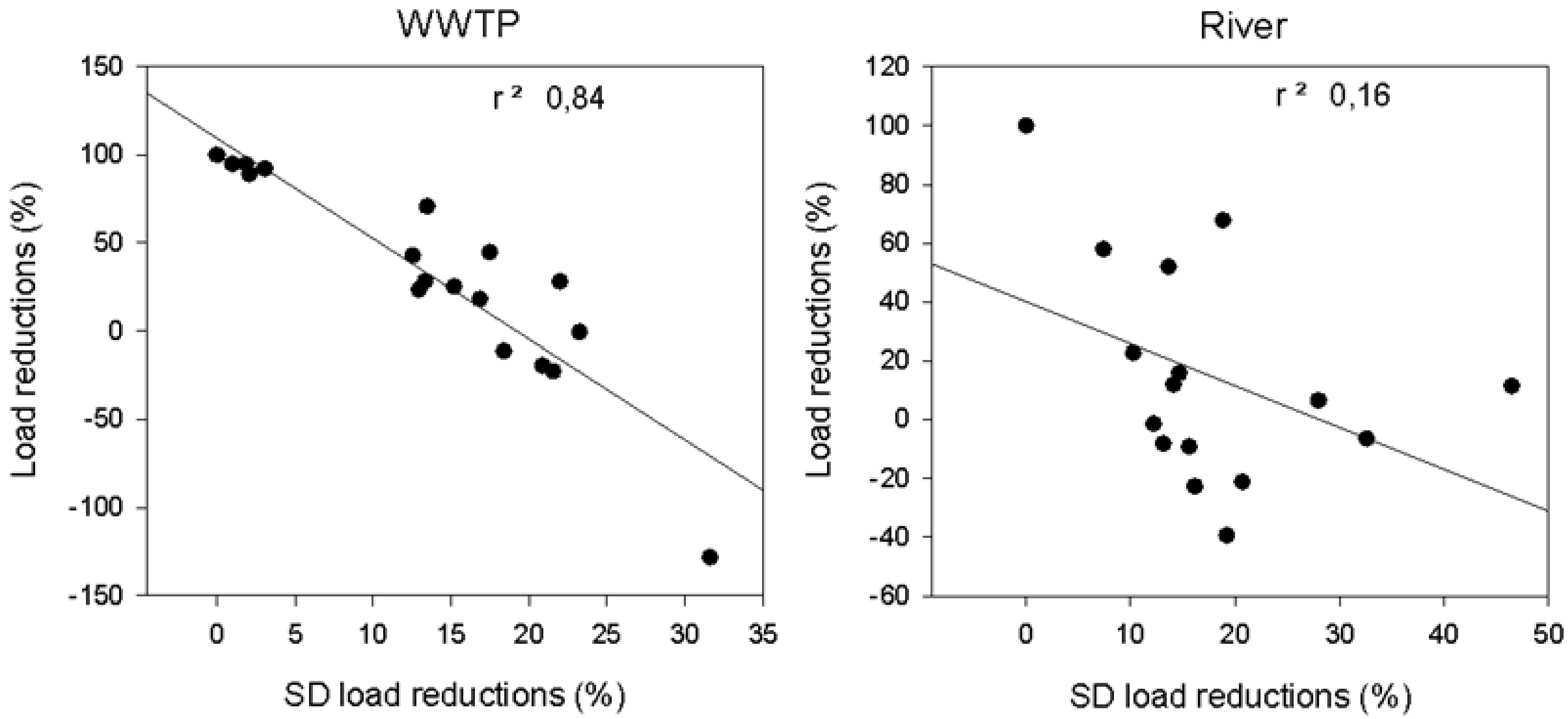


**Figure SI 5.1.** Linear regression between the load reductions and uncertainties of the analyzed compounds for the WWTP and river under study.

# Chapter 6

# Fate of organic micro-contaminants in wastewater and river systems: an uncertainty assessment in view of sampling strategy, compound consumption rate and degradability

The content of this chapter has been published as:

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## 6.1 Introduction

Many organic micro-contaminants enter freshwater ecosystems mainly via point-source discharges of wastewater treatment plants (WWTP) (Pal et al., 2010; Li, 2014). Although concentrations of these micro-contaminants are often below one microgram per litre, many micro-contaminants raise environmental and human health concerns and have become a key environmental problem (Acuña et al., 2015; Schwarzenbach et al., 2006). Unfortunately, the understanding of processes that control the occurrence and fate of these chemical compounds remains incomplete (Antweiler et al., 2014), mainly because of the predominance of lab-scale studies (Joss et al., 2006), the lack of integrated studies of both WWTP and rivers (Petrie et al., 2015), and the diversity of sampling strategies used to assess attenuation (Ort et al., 2010a, 2010b). Thus, many studies have been published reporting attenuation (net balance between removal and release) rates in either WWTP or rivers, but they often differ in the sampling strategy (i.e., sampling frequency and composite duration), thus limiting the comparability of the obtained results. In fact, there are few examples of studies estimating loads and attenuation in both WWTP and rivers in a comparable manner (Alder et al., 2010; Aymerich et al., 2016).

In the case of rivers, the most commonly used sampling strategies for the estimation of attenuation are "mass balances" and "Lagrangian". The mass balance strategy implies the calculation of micro-contaminant loads at different points along a stretch of river, normally using 24 h flow- or time-proportional composite samples (e.g., Alder et al., 2010; Kunkel and Radke, 2012). The Lagrangian strategy involves tracking and sampling a water parcel as it moves downstream, which it can be used either with natural river concentrations (e.g., Aymerich et al., 2016; Barber et al., 2013) or after artificially increasing river concentrations with a Dirac pulse (e.g., Kunkel and Radke, 2011; Writer et al., 2013). There are some recent studies on the effects of sampling strategy on the uncertainty of attenuation estimates (e.g., Antweiler et al., 2014), as well as some studies suggesting improved numerical methods to estimate attenuation (e.g., Riml et al., 2013; Aymerich et al., 2016).

In the case of WWTPs, the most commonly used sampling strategy is mass balance, calculating loads of micro-contaminants by taking one or multiple subsequent 24-hour composite samples from WWTP influent and effluent. Normally, the start of the effluent composite sample is delayed by a multiple of the hydraulic residence time. Such composite samples are composed of individual samples taken at a predefined frequency. This sampling strategy is equivalent to the previously described mass balance strategy for rivers, with the only difference being that in rivers the composite samples between the 2 sites along a river stretch are either not delayed or delayed one time for the hydraulic travel time. Additionally, similar to those proposed for rivers, recent studies have explored the effects of sampling strategy on the reliability of loads and attenuation rate estimates in WWTPs. For example, Ort et al. (2010a, 2010b) demonstrated that sampling mode (volume- or flow-proportional) and frequency matters in the estimation of loads at the influent of a WWTP. As for the estimation of attenuation, several authors have highlighted the importance of considering residence time distributions (Majewsky et al., 2011) applying time-shifted mass balancing approaches (Rodayan et al., 2014), or applying longer sampling durations (Ternes and Joss, 2006; Majewsky et al., 2013). Although (Ort et al., 2010a) proved that sampling strategy influences the load estimates at a WWTP influent, it is not yet clear how the sampling strategy influences the load estimates at the effluent of a WWTP, and most importantly, how it can influences in the calculation of attenuation rates. Overall, none of the studies assessing the effects of sampling strategies considered both engineered and natural systems (i.e., a WWTP and a river stretch).

Given this background, our goal was to assess how different sampling strategies can influence on the estimates of loads and attenuation rates in WWTPs and their receiving rivers. Specifically, we were interested in assessing the effect of different sampling frequencies and durations on estimated attenuation rates of organic micro-contaminants. Other investigated properties include compound characteristics (i.e., degradability) and served population (i.e., size and consumption rates). We expected higher uncertainty i) for small population size and/or consumption rates, ii) micro-contaminants with low degradability and iii) sampling strategies in the WWTP influent because of high temporal concentration/load variability at this point. The assessment was conducted by simulating a real case study of the integrated wastewater system of Puigcerdà (NE Iberian Peninsula).

## 6.2 Material and methods

Given our objectives, we built and calibrated a model for the Puigcerdà integrated system (catchment, sewer, WWTP and river). Then, we estimated attenuation rates in the WWTP and in the river for micro-contaminants with different degradability and consumption rates under several scenarios of population size and sampling strategies. To assess the effects of micro-contaminants consumption rates and population size, we generated realistic patterns of compounds in the study site. For the assessment of sampling strategies, we used different scenarios differing in frequency and duration of composite samples.

### 6.2.1 Catchment-Sewer model

The sewage pattern generator SPG (SPG, 2013) based on Ort et al. (2005) was used to model the Puigcerdà catchment to generate realistic flows and concentrations at the inlet of the WWTP ($W_i$). The model was calibrated against measured flows and ammonia concentrations (Aymerich et al. 2016) by adjusting parameters related to catchment characteristics, water consumption from households (110 L inhabitants$^{-1}$ d$^{-1}$), groundwater infiltration (1200 L s$^{-1}$), pulse mass (150 mg), and total number of pulses (e.g., toilet flushes containing the substance of interest) per person per day (assuming 5 toilet flushes per inhabitant per day). Industrial activity is negligible in the catchment. The number of expected pulses per day is an average and the effective number is generated with a non-homogenous Poisson process, following the diurnal pattern. The temporal resolution of simulated flows and load patterns was 2 minutes. Values for concentration and flow between the two-minute time steps are interpolated linearly.

### 6.2.2 WWTP-river coupled model

The model is the one developed in Aymerich et al. (2016), which is based on ordinary differential equations and includes a first-order attenuation kinetic on top of a hydraulic model for both the WWTP and the river. The WWTP hydraulics were modelled with a Combined Stirred Tank Reactor (CSTR) approach, with one biological reactor and one secondary clarifier. The hydraulics in the river were modelled following a Lagrangian approach with 5 river sections and including lateral flows. Both hydraulic models were constructed and calibrated using a tracer test (see **Chapter 4**). At the WWTP, inputs to the model were the dynamic flow and the influent concentrations of the pharmaceuticals at the $W_i$ (obtained from the catchment/sewer model). Simulations were run to obtain WWTP effluent concentration profiles at the $W_e$ and, in turn, concentrations at $R_u$ (equivalent to I1 in **Figure 3.1**) and $R_d$ (equivalent to I5 in **Figure 3.1**). This modelling approach was built in SIMBA® (version 6, IFAK, Germany).

### 6.2.3 Numerical methods to quantify attenuation rates

In total, we ran 12 simulations to cover all possible combinations of number of pulses per day at all households and target compound degradability (**Table 6.1**). All the simulations were run for 400 days at 2-min time resolutions. For each simulation, we applied the 8 combinations of sampling frequencies and composite durations using a flow-proportional sampling model. Composite durations corresponded to approximately 1 & 4 times the HRT of the WWTP and to 2 & 4 times the HRT of the river (**Table 6.1**). The total number of combinations evaluated was 96, as a result of 4 (number of pulses) x 4 (frequency) x 2 (composite duration) x 3 (degradability). Then, samples were composited using a script that is part of SPG (SPG, 2013).

**Table 6.1.** Summary of the combinations of number of pulses, degradability, sampling frequencies and composite durations evaluated in this study.

| | WWTP | River |
|---|---|---|
| **Number of pulses (pulses · day$^{-1}$)** | 50, 100, 1000, 10000 | |
| **Degradability (%)** | 0, 50, 90 | |
| **Sampling frequencies (min)** | 5, 15, 30, 60 | 15, 60, 120, 240 |
| **Composite durations (h)** | 24, 96 | 12, 24 |

#### 6.2.3.1 Calculation of sampling errors at the different sampling points

For each of the composite samples generated at the different sampling points ($W_i$, $W_e$, $R_u$, and $R_d$) we calculated the associated sampling error between the "true" average concentration and the "estimated" average concentration for the sampling strategy evaluated. For a given sampling strategy (i.e. sampling frequency and composite duration, the "estimated" average concentration was calculated following **Eq 6.1**, according to Ort et al. (2010a). The "true" concentration was calculated using the same approach as before but with the highest possible frequency allowed by the simulation (i.e., 2 minutes). This "true" concentration would be equivalent to the case of using a continuous sampling strategy.

$$\bar{C}_x = \frac{\sum_i c_{x_i} \cdot v_{x_i}}{\sum_i v_{x_i}}, \quad V_x = \sum_i v_{x_i}$$
$$i = t, t + t_{int}, t + 2t_{int}+, t + 3t_{int}, \dots, +t_{cdur} \qquad \textbf{(Eq 6.1)}$$

Wherex is the sampling location ($W_i$, $W_e$, $R_u$ and $R_d$), *i* is the time step, $c_{xi}$ is the instantaneous concentration at the sampling interval applied, $v_{xi}$ is the volume of a flow-proportional discrete sample, $V_x$ is the total wastewater volume of the composite sample, $t_{int}$ is the sampling interval and $t_{cdur}$ is the composite duration applied. Then, the relative sampling error for the load at each site was calculated with **Eq 6.2**:

$$\text{relative sampling error}_x = \frac{\bar{C}_{\text{true}_x} - \bar{C}_{\text{flow-prop}_x}}{\bar{C}_{\text{true}_x}} \qquad \textbf{(Eq 6.2)}$$

#### 6.2.3.2 Attenuation error calculation

The approach to calculate the attenuation error associated with each sampling strategy consisted of taking one sample pair at the WWTP ($W_i$ and $W_e$) and one sample pair at the river ($R_u$ and $R_d$). The starting times of samples taken at $W_e$ and $R_d$ were delayed by 1 time for the HRT of the evaluated subsystems (time-shifted mass balancing approach, as defined in Rodayan et al., 2014). The attenuation for each sample pair was calculated as the proportion of load removed between the two sampling points ($W_i$ and $W_e$, $R_u$ and $R_d$), where loads ($L$) were calculated as the product of the average concentration ($C_x$) and the wastewater volume ($V_x$) obtained for each of the composite samples. Then, loads calculated for each sample pair were used to calculate the "true" attenuation ($A_{true}$) (**Eq 6.3**), "estimated" attenuation ($A_{flow\text{-}prop}$) (**Eq 6.4**) and the relative attenuation error between the true and the estimated through flow-proportional sampling (**Eq 6.5**):

$$\mathrm{A_{true}} = \frac{\mathrm{L_{true_{in}}} - \mathrm{L_{true_{out}}}}{\mathrm{L_{true_{in}}}} \qquad \textbf{(Eq 6.3)}$$

$$\mathrm{A_{flow-prop}} = \frac{\mathrm{L_{flow-prop_{in}}} - \mathrm{L_{flow-prop_{out}}}}{\mathrm{L_{flow-prop_{in}}}} \qquad \textbf{(Eq 6.4)}$$

$$\text{relative attenuation error } = \frac{\mathrm{A_{true}} - \mathrm{A_{flow-prop}}}{\mathrm{A_{true}}} \qquad \textbf{(Eq 6.5)}$$

In order to obtain a distribution in sampling and attenuation errors, the procedure explained in the previous paragraph was repeated 100 times; hence 100 sample pairs taken randomly at the WWTP ($W_i$ and $W_e$) and 100 pairs at the river ($R_u$ and $R_d$). The result is a distribution of the load and attenuation errors for both the WWTP and river. To characterize the load and attenuation error, we used two measures: bias and uncertainty. Bias is calculated as the difference between the true value and the central value of our estimates (expressed as the median of the error distribution). Uncertainty is the dispersion of the error distribution of our estimates (expressed as the 90-interquartile range of the error distribution, calculated from the difference between the 95$^{th}$and 5$^{th}$ percentiles).

## 6.3 Results

### 6.3.1 Temporal and spatial concentration patterns

The 24-hour temporal and spatial concentration patterns are shown in **Figure 6.1** (shown as relative to the maximum value of each time-series), including the influent of the WWTP ($W_i$), the effluent of the WWTP ($W_e$) and the river ($R_d$). For the studied system, if only a few such wastewater pulses are expected over the course of a day (e.g., 50 pulses day$^{-1}$), the observed pattern at $W_i$ is intermittent with large short-term fluctuations. Thus, in these cases, it is evident that a very high sampling frequency would be necessary to capture these pulses. When increasing the number of wastewater pulses (e.g., 1000 pulses day$^{-1}$), a smoother pattern with more systematic diurnal variation is observed, which is very similar to the typical ammonia pattern observed at the influent of a WWTP (e.g., a compound that would be present in the majority of toilet flushes). At $W_e$, we can see that the mixing processes occurring in the WWTP smooth the patterns observed at the influent of the WWTP between the different wastewater pulses (e.g., 50 and 1000 pulses day$^{-1}$), and thus in such cases a similar sampling frequency would be valid for both compounds. At the river ($R_u$), the difference in the concentrations between the different wastewater pulses also becomes negligible and similarly as observed in $W_e$, where relative concentration variations are slightly increased due to the flow variations occurring in the WWTP. The dynamics at $R_d$ (results not shown), are the same as for $R_u$ because of the plug-flow behaviour of the river, but with a time-shift of 5.3 hours.

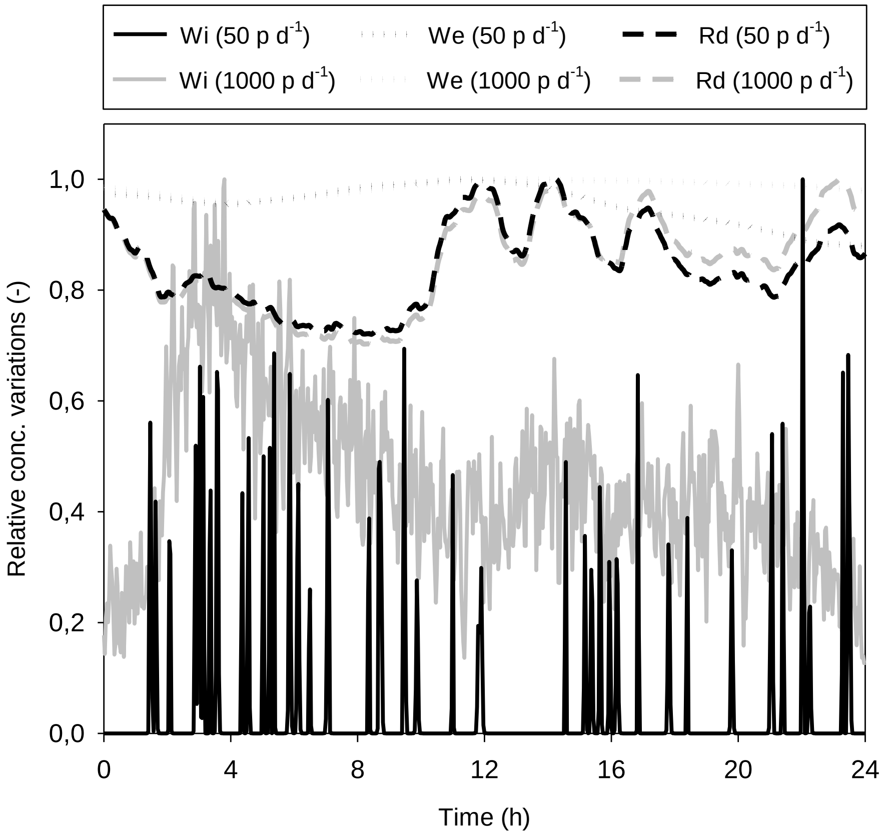


**Figure 6.1.** Illustrative example of the variations of concentrations (scaled on the maximum value) at different sampling locations for the system under study, for 50 and 1000 pulses d-1.Wi = influent of the WWTP; We = effluent of the WWTP; Rd = Downstream river 4500 m after the WWTP discharge.

### 6.3.2 Effects of sampling strategies on the estimation of loads and attenuation rates in the WWTP

The effects of sampling strategies on the load and attenuation estimations at the WWTP for a conservative compound (i.e., 0% degradability) are shown in **Figure 6.2**, which shows the effects of different sampling frequencies (5 to 60 min) and duration of composite samples (24 to 96 h) for different wastewater pulses (from 50 to 10,000 pulses $d^{-1}$). The results for all the compounds are all the combinations evaluated are summarized in **Table SI 6.1-12** (Supporting Information). The results show that the sampling frequency is especially critical at Wi in cases with a small number of toilet flushes of a compound (≤100 pulses day-1), where sampling errors (90-interquartile range of the obtained error distribution) can range from 171% (50 pulses day-1 and 60-min sampling frequency) to 14% (100 pulses day-1 and 5-min sampling frequency) (**Figure 6.2A**). Errors at We are far lower (<1%) than those at $W_i$, with almost no influence from the sampling frequency and number of pulses (**Figure 6.2B**), owing to the mixing effects within the WWTP.

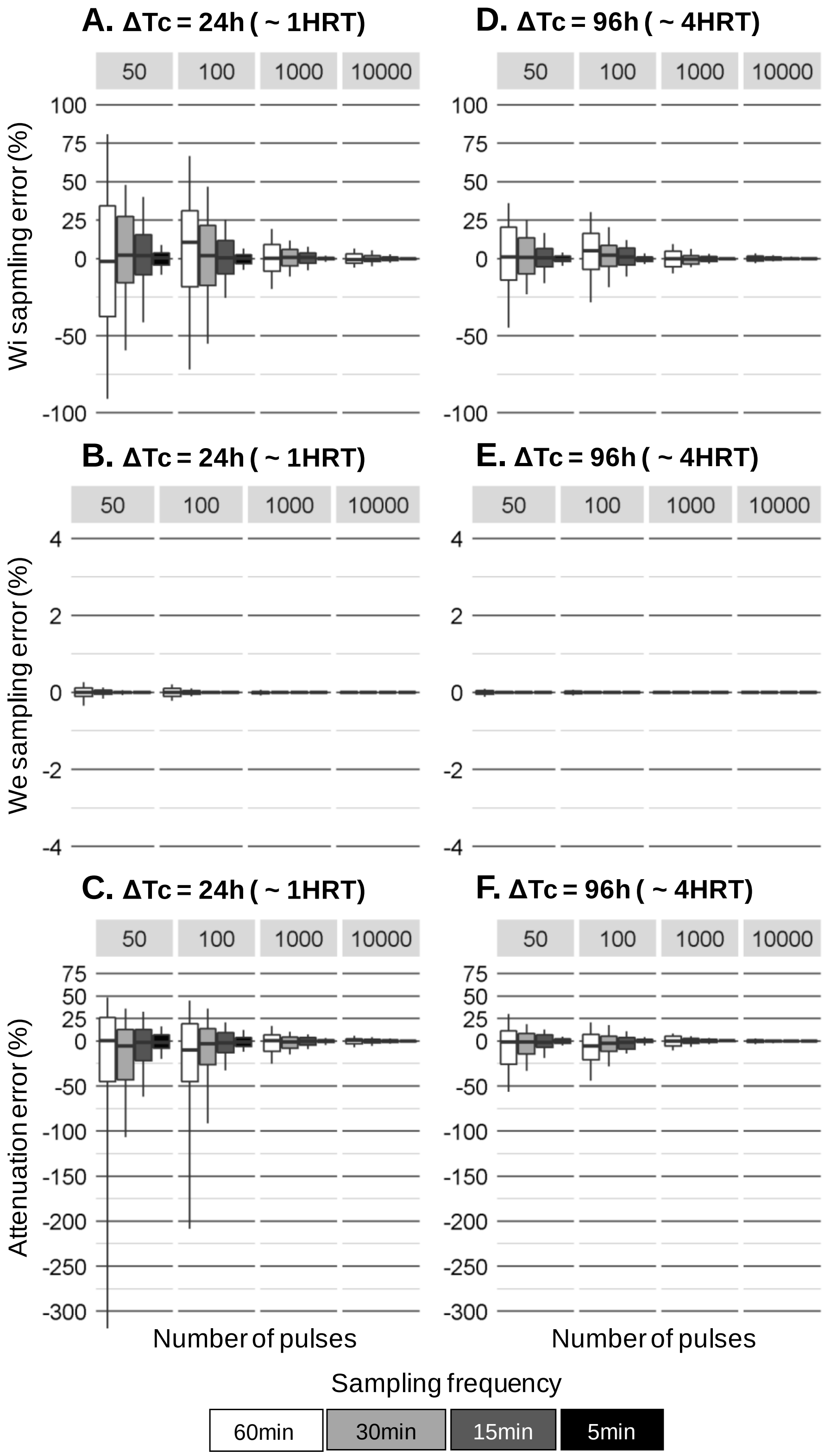


**Figure 6.2.** Loads and attenuation errors at the WWTP for a flow-based sampling mode and different wastewater pulses (from 50 to 10 000 pulses d-1) and sampling frequencies (from 5 to 60 min). A-C: composite duration of 24-h. D-F: composite duration of 96-h. The results presented as boxplots stands for the 5-percentile, 25-percentile, 50-percentile, and 95-percentile.

The effects of sampling strategies on attenuation estimates are shown in **Figure 6.2C**, where we can see that the uncertainty is of similar magnitude to the uncertainty of loads at $W_i$ and $W_e$ (**Figure 6.2A-B**). For example, for 5-min sampling frequency and 50 pulses d-1, sampling errors in the load estimations in $W_i$ range from -11% to 9% ($5^{th}$and $95^{th}$percentile), which are similar to corresponding attenuation estimation errors ranging from -20% to 16%. With decreasing sampling frequencies, we can see that the under estimation exceeds -200% in some cases. In particular, we see that the distribution of errors in the attenuation estimations is not as symmetric as those that occur at the effluent and influent of the WWTP; instead, the distribution is skewed towards negative values, which means that we would tend to underestimate attenuation. Negative skewness in attenuation error distributions originates due to mathematical issues when differences in the magnitudes and distributions of sampling errors at the different sampling location are obtained. For example, for an overestimation of $W_i$ sampling error of 75% or 50% (and $W_e$ sampling error of practically 0%), an overestimation of attenuation error of 43% and 33% would be obtained; while for an underestimation of the $W_i$ sampling error of 50% and 75%, an underestimation of attenuation error of -100% and -300% is obtained. Finally, results show that the effects of duration of composite samples are is lower than those of frequency, as the gains of increasing the frequency far exceed those of increasing the duration (**Figure 6.2D-F**). However, increasing the composite durations under low wastewater pulses can be used as a strategy to reduce errors in those cases when the sampling frequency cannot be further reduced, such as in the case of 50 pulses $day^{-1}$ and 15-min sampling frequency, where the uncertainty can be decreased from 94% to 32%. However, in that case, the uncertainty would still be too high (32%), and a higher sampling frequency (e.g., continuous sampling or sampling frequency lower than 5-min) or a longer composite duration would thus be required.

### 6.3.3 Effects of sampling strategies on the estimation of loads and attenuation rates in the river

The effects of sampling strategies on the estimation of sampling and attenuation rates in the river are shown in **Figure 6.3** and summarized in **Table SI 6.7** to **Table SI 6.12**. For the sampling frequencies evaluated (15 to 240 min), uncertainty associated with the sampling and attenuation estimations always remains lower than 10%. However, when decreasing the sampling frequency from 15 to 240 min, uncertainty increases (from 1 to 10%) but to a lower extent than that obtained at the influent of the WWTP (Figure 3). Overall, the distribution width of the errors depends on both sampling frequency and the number of wastewater pulses, although similar errors were observed for Ru and Rd sampling locations due to the plug-flow behaviour of the river hydraulics. Similar to observations at the WWTP (**Figure 6.2**), when estimating attenuation, precision decreases but the bias disappears. The increase of composite duration also shows some benefits for reducing errors but only in cases when low sampling frequencies are applied (i.e., 240 min), especially for shorter composite durations (i.e., 12 h). When increasing the sampling duration to 24-h (approximately 4 times the hydraulic residence time of the studied river section), the uncertainty in all cases remains lower than 2%.

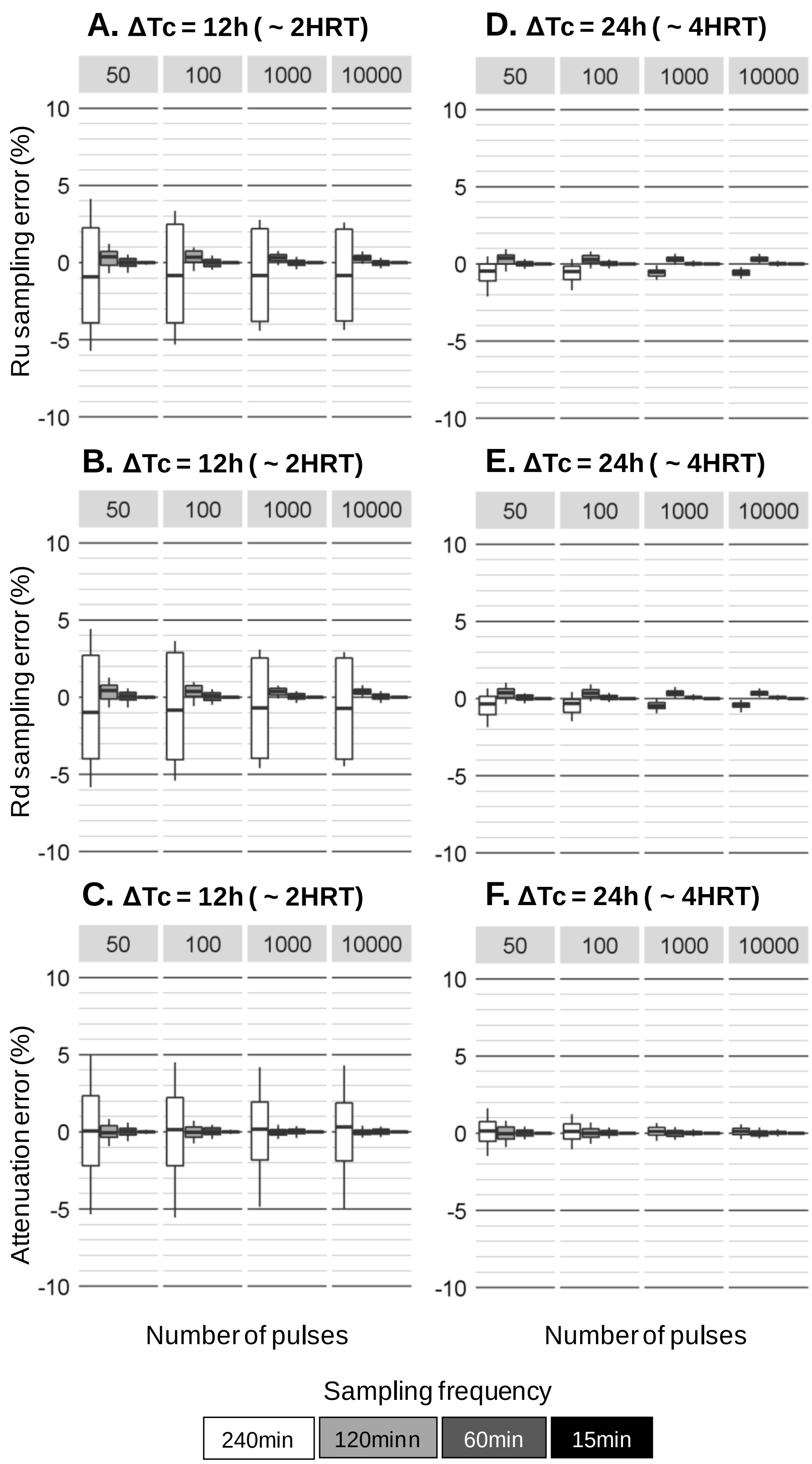


**Figure 6.3.** Loads and attenuation errors at the river for a flow-based sampling mode and different wastewater pulses (from 50 to 10 000 p d-1) and sampling frequencies (from 15 to 240 min). A-C: composite duration of 12-h. D-F: composite duration of 24-h. The results presented as boxplots stands for the 5-percentile, 25-percentile, 50-percentile, and 95-percentile.

### 6.3.4 Effects of compound degradability on the estimation of loads and attenuation rates

The effects of different degradability levels (0%, 50%, and 90% attenuation from $W_i$ to $W_e$ and $R_u$ to $R_d$) for a given sampling strategy are shown in **Figure 6.4**. At the influent of the WWTP (**Figure 6.4A**), we observe the same sampling errors for the different degradability levels. At the effluent of the WWTP (**Figure 6.4B**), sampling error increases when degradability increases, but always remain lower than 0.5%. When propagating those errors to the attenuation calculations (**Figure 6.4C**), uncertainty decreases when increasing degradability. This is a consequence of higher signal to noise ratios at higher degradability, which in fact result in higher differences which are not so affected by the errors in the estimates of $W_e$ or $W_i$. Again, we also observe attenuation error distributions skewed to negative values for the different degradability cases. In particular, for the same sampling strategy but different degradability rates, for the most critical case (50 pulses $d^{-1}$), uncertainty in attenuation estimations can change from -62% to 32% for a compound which attenuates 0% and from -6% to 3% for a compound which attenuates 90%. At the river (**Figure 6.4D-E**), we observe trends in sampling error distributions similar to those observed at the effluent of the WWTP (**Figure 6.3B**), but slightly increased due to the different sampling frequencies (15-min at the WWTP and 240-min at the River) and increased concentration variations due to the flow variations occurring in the WWTP (**Figure 6.1**). Again, when propagating those errors in attenuation calculations, we observe that the lowest uncertainties occur with high degradability levels, even the highest sampling errors are applied in the calculations for the latter. With regards to attenuation error distributions, we see that symmetric distributions are obtained due to similar magnitude and distributions in sampling errors obtained at the different sampling locations ($R_u$ and $R_d$).

To better understand the effect of degradability on attenuation uncertainty **Figure 6.5** shows the relationship between sampling uncertainty and attenuation uncertainty for different degradability levels (0%, 50%, 90%), and for numbers of wastewater pulses (from 50 to 10,000 pulses $d^{-1}$). In both the WWTP (**Figure 6.5A**) and the river (**Figure 6.5B**), we observe that sampling errors in the load estimations and attenuation estimations show a linear relationship. In particular, the results show that 0% degradability is the most critical case, as any error in the estimate of $W_i$ is directly translated in errors in attenuation (e.g., an error of 40% in Wi equals 40% in attenuation); except for low number of wastewater pulses (< 100 pulses $d^{-1}$) and inappropriate sampling frequencies where attenuation errors can increase up to a factor of 1.8 to 2.5 (respect to sampling errors). Instead, higher degradability implies that errors in the estimate of $W_i$ are minimized when estimating attenuation (e.g., an error of 40% in $W_i$ equals 5%). In the river, similarly to what described for the WWTP, increases in degradability also imply that the transmission of errors from load estimates to attenuation is minimized. However, this minimization is not as pronounced as for the WWTP, as the slope between load and attenuation errors for a degradability of 90%, is 0.1 at the WWTP and 0.25 at the river.

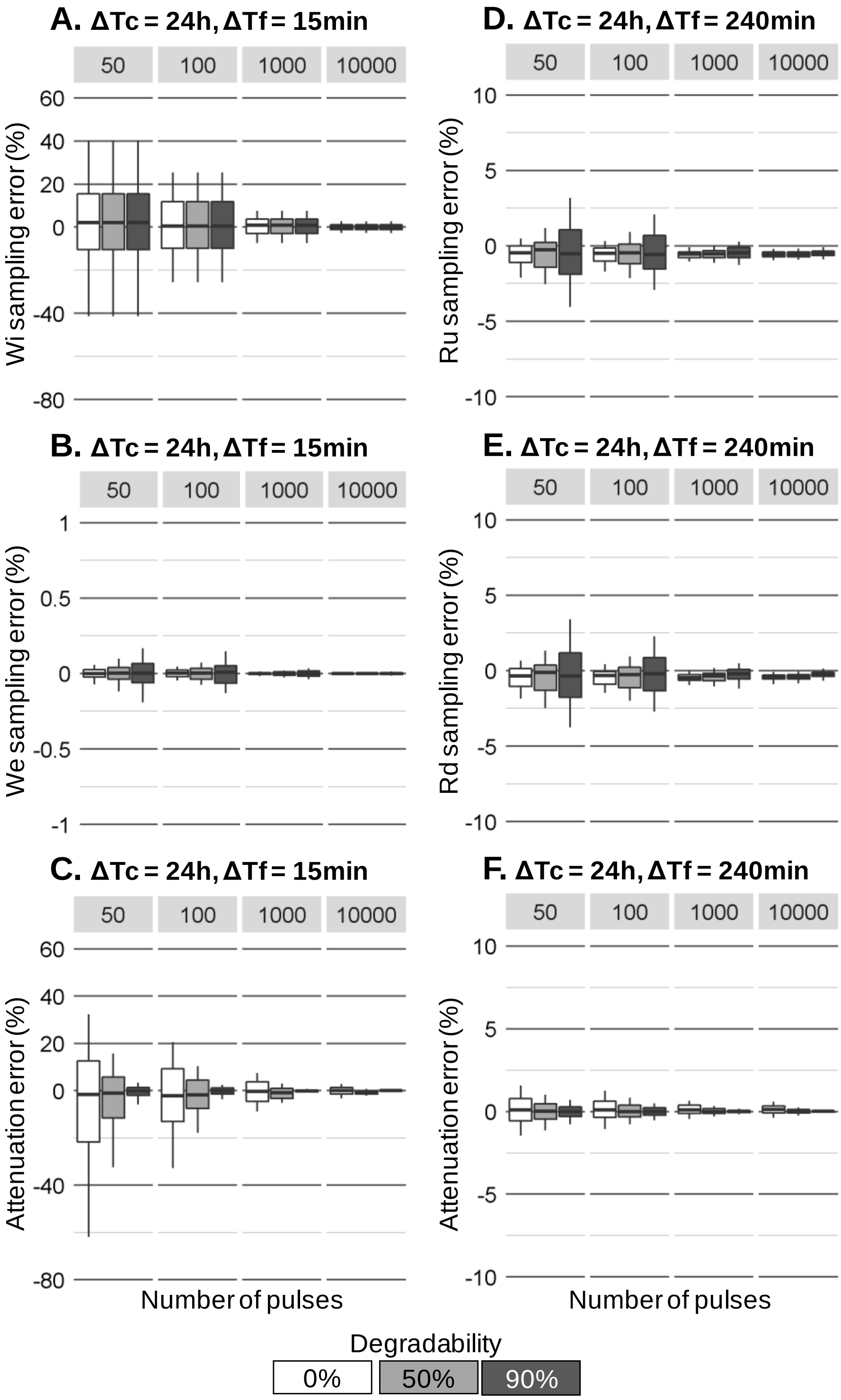


**Figure 6.4.** Load and attenuation errors for compounds at degradability rates of 0%, 50% and 90%. At the WWTP for a composite duration of 24-h and a sampling frequency of 15-min (A-C) and at the river for a composite duration of 24-h and a sampling frequency of 240-min (D-F).

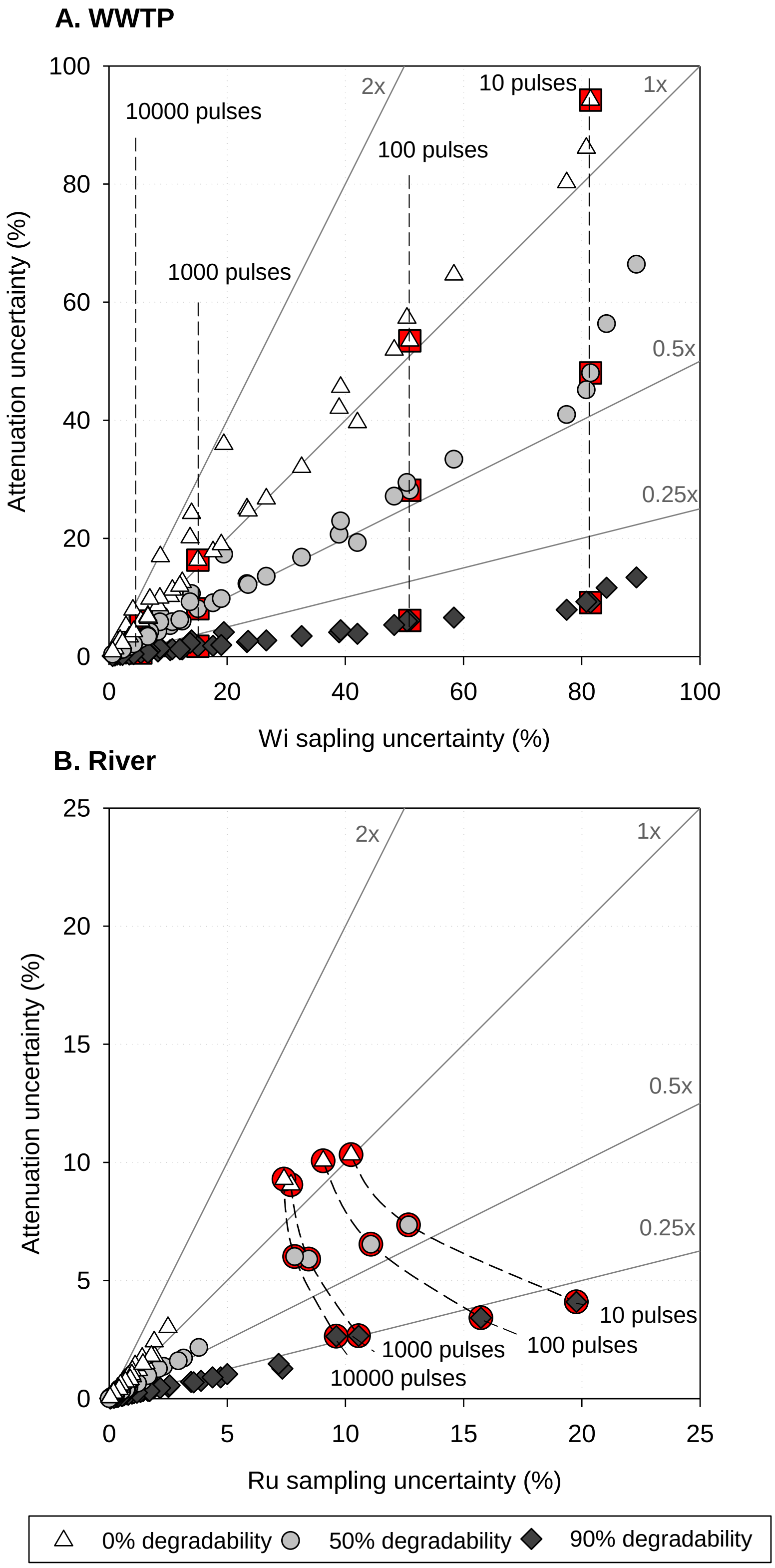


**Figure 6.5.** Relationships between $W_i$ sampling and attenuation errors in the WWTP (A) and Ru sampling and attenuation errors in the river (B) at different degradability levels. All number of pulses, composite frequencies and durations evaluated in the study are plotted. Red symbols show the sampling strategy presented in Figure 4. Uncertainty is expressed as the 90-interquartile range of the error distributions obtained. Note that only load and attenuation errors lower than 100% are presented.

### 6.3.5 Discussion

Based on the results from this study, we can conclude that sampling strategy matters, as errors in loads and attenuation change considerably as a function of sampling strategy. Regarding bias and uncertainty, the most important factor is the number of pulses (combination of served population and compound consumption rate), followed by the frequency, then the composite duration, and finally the compound degradability.

**Number of pulses (served population and consumption rate).** We confirmed our initial hypothesis that the sampling strategy has a significant influence on the estimation of organic micro-contaminant attenuation in WWTPs and rivers when the number of pulses is low (< 100 pulses day$^{-1}$). In particular, the effect of the number of pulses is especially critical at the influent of the WWTP (where the highest short-term variations occur), and to a lower extent at the effluent of the WWTP and at the river when short-term variations are reduced due to mixing processes in the WWTP. Hence, we must increase our sampling effort when working in i) small municipalities, ii) medium-large municipalities with compounds with a very low or low consumption rate, or iii) large catchments or catchments where pumping activity in the sewer system generates patterns that dominate the fluctuation in the influent of a WWTP (rather small number of pump events than number of toilet flushes). Hence, the calculation of the number of pulses would be the first step when designing a sampling campaign. Specifically, a similar effort should be devoted to rivers with a low dilution capacity, where other factors should also be accounted for, such as the impact of WWTP flow variations (Antweiler et al., 2014).

**Sampling frequency.** We confirmed the hypothesis that it is important to sample at sufficiently high frequencies at the influent of the WWTP to obtain attenuation estimates with low uncertainty. Proper guidance on sampling at the influent of WWTPs is provided in Ort et al. (2010a, 2010b), but more information is needed when sampling for integrated studies (Petrie et al., 2015). In this study, the results showed that the frequency of sampling in the effluent of the WWTP (and in the river) can be reduced (as compared to the influent of the WWTP), as uncertainty is much lower due to the mixing effects occurring in the WWTP. However, for inappropriate sampling frequencies (≥ 30-min) and low number of pulses (≤ 100 pulses day$^{-1}$), attenuation uncertainty can be greater than the obtained in the loads estimation, with attenuation error distributions skewed to negative values. Thus, when designing a sampling strategy, we should be aware that sampling strategies that appear to be sufficiently accurate for loads representation are not always appropriate for attenuation estimations. In the river, we recommend following the mass balance approach (same approach applied for WWTPs) in integrated studies (WWTP and river) and when evaluating the influence of the discharge of the WWTP on the river. In the river, the frequency of the sampling should be sufficiently high to capture concentrations variability governed due to the flow variations at the WWTP effluent (Antweiler et al., 2014). In fact, the importance of sampling frequency has been underestimated in river studies on the attenuation of micro-contaminants, as it has been rarely reported in studies on micro-contaminant attenuation (e.g., Alder et al., 2010).

**Composite and sampling duration.** We evaluated the influence of composite duration and concluded that longer composite durations decrease uncertainty. In the case of WWTPs, given equivalent costs in modifying frequency and duration of composite samples, it is preferable to increase frequency, as errors in magnitude and dispersion decrease to a major extent when frequency is increased. The common practice in WWTPs and in rivers (for the mass balancing approach) is to set composite duration to at least 1 times the HRT of the system and adjust the sampling frequency. However, in this study, we see that 1 times the HRT sometimes is not enough to ensure low bias and uncertainty in attenuation estimations (e.g., very low number of pulses); thus, for the estimation of attenuation, it is further recommended to sample along consecutive days. This can be accomplished by increasing the duration of the composite samples or by compositing daily samples over several consecutive days, as suggested for WWTPs in Majewsky et al. (2011; 2013) and in Ternes and Joss (2006). Note that a 96-h composite sample as presented in this study would mean taking 4 consecutive 24-h composite samples; one sample of 96-h would be subject to degradation inside the sampling bottle. In the case of rivers, a composite duration of at least 24-h is advisable, as the mechanisms behind attenuation experience 24 hour changes due to temperature and irradiation cycles (Schwientek et al., 2016). In our study, the attenuation uncertainty of a 12-h composite sample doubled that of a 24-h cycle, thus stressing the need to cover at least one full diel cycle.

**Compound degradability.** We have demonstrated that the lowest uncertainties in attenuation estimates are observed for compounds with high degradability levels and the highest uncertainties for compounds with low degradability. The propagation of uncertainty in the calculation of attenuation using influent-effluent (or upstream and downstream) loads is dependent on the degradability of the compound. For compounds with high degradability (e.g., 90%), the uncertainty in influent or upstream loads is much higher than the uncertainty in attenuation estimations. For compounds with low degradability (e.g., 0%), the uncertainty in the estimation of attenuation of similar magnitude than the uncertainty in the influent or upstream loads. In fact, the same relationship between degradability and uncertainty can be observed in the review from Luo et al. (2014); in there, compounds that degrade more than 90% show a deviation between 0 and 8%, and compounds that degrade less than 50% show a larger deviation between 13 and 34% (see Figure SI2). In agreement with the results obtained in this study, one of the explanations of this deviation could be the compounds' degradability and the use of different (and in some cases not optimal) sampling strategies in the reviewed studies, as well as combined with the fact of not addressing sampling uncertainty (Ort et al., 2010b). Hence, when designing a sampling campaign, one should consider the compounds' consumption and theoretical degradability as well as the desired level of accuracy in attenuation estimations, as compounds with lower degradability require higher sampling frequencies. As it is expected that studies will not only focus on one compound, the sampling strategy should be designed for the compound with the most important combination of number of pulses and non-degradability case.

## 6.4 Supporting Information

**Table SI 6.1.** *Wi* load errors information for a flow-based sampling mode and 24h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 60.00 | -1.61 | 71.72 | 171.60 | -90.87 | 80.73 |
| **50.00** | 30.00 | 2.23 | 42.98 | 107.57 | -59.51 | 48.05 |
| **50.00** | 15.00 | 2.03 | 25.88 | 81.50 | -41.37 | 40.14 |
| **50.00** | 5.00 | 0.43 | 7.89 | 19.45 | -10.53 | 8.93 |
| **100.00** | 60.00 | 10.61 | 49.34 | 138.59 | -72.00 | 66.59 |
| **100.00** | 30.00 | 1.92 | 39.07 | 101.90 | -55.16 | 46.74 |
| **100.00** | 15.00 | 0.49 | 21.66 | 50.91 | -25.54 | 25.36 |
| **100.00** | 5.00 | 0.13 | 5.72 | 13.99 | -7.33 | 6.66 |
| **1000.00** | 60.00 | 0.22 | 17.28 | 38.94 | -19.60 | 19.34 |
| **1000.00** | 30.00 | 0.94 | 10.77 | 23.36 | -11.45 | 11.91 |
| **1000.00** | 15.00 | 0.87 | 6.66 | 15.04 | -7.39 | 7.64 |
| **1000.00** | 5.00 | 0.27 | 1.45 | 4.05 | -2.05 | 2.00 |
| **10000.00** | 60.00 | -0.54 | 6.22 | 12.41 | -5.82 | 6.60 |
| **10000.00** | 30.00 | -0.17 | 3.84 | 10.38 | -4.95 | 5.43 |
| **10000.00** | 15.00 | -0.15 | 2.12 | 5.40 | -2.65 | 2.75 |
| **10000.00** | 5.00 | 0.08 | 0.55 | 1.38 | -0.61 | 0.77 |

**Table SI 6.2.** *Wi* load errors information for a flow-based sampling mode and 96h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 60.00 | 1.24 | 34.25 | 80.78 | -44.82 | 35.97 |
| **50.00** | 30.00 | 0.74 | 23.55 | 48.27 | -23.08 | 25.19 |
| **50.00** | 15.00 | 0.42 | 11.90 | 32.59 | -15.89 | 16.70 |
| **50.00** | 5.00 | -0.46 | 3.68 | 8.62 | -4.64 | 3.98 |
| **100.00** | 60.00 | 5.26 | 23.32 | 58.39 | -28.22 | 30.16 |
| **100.00** | 30.00 | 2.38 | 13.48 | 39.21 | -18.59 | 20.62 |
| **100.00** | 15.00 | 1.15 | 10.84 | 23.56 | -11.54 | 12.02 |
| **100.00** | 5.00 | -0.43 | 2.77 | 6.92 | -3.39 | 3.53 |
| **1000.00** | 60.00 | -0.06 | 10.18 | 19.00 | -9.42 | 9.58 |
| **1000.00** | 30.00 | -0.34 | 5.42 | 12.01 | -5.62 | 6.39 |
| **1000.00** | 15.00 | 0.02 | 3.12 | 6.58 | -3.28 | 3.29 |
| **1000.00** | 5.00 | 0.01 | 0.81 | 1.90 | -0.97 | 0.92 |
| **10000.00** | 60.00 | 0.16 | 3.23 | 6.60 | -3.03 | 3.57 |
| **10000.00** | 30.00 | 0.15 | 1.94 | 4.19 | -1.89 | 2.29 |
| **10000.00** | 15.00 | 0.04 | 1.11 | 2.31 | -0.99 | 1.32 |
| **10000.00** | 5.00 | 0.03 | 0.31 | 0.62 | -0.29 | 0.33 |

**Table SI 6.3.** *We* load errors information for a flow-based sampling mode and 24h composite duration.

| **Nº Pulses (pulses $d^{-1}$)** | **Sampling freq. (min)** | **Median** | **50-IQR** | **90-IQR** | **P5** | **P95** |
|---|---|---|---|---|---|---|
| **50.00** | 60.00 | 0.00 | 0.22 | 0.61 | -0.35 | 0.26 |
| **50.00** | 30.00 | 0.00 | 0.10 | 0.28 | -0.16 | 0.12 |
| **50.00** | 15.00 | 0.00 | 0.05 | 0.13 | -0.07 | 0.06 |
| **50.00** | 5.00 | 0.00 | 0.01 | 0.03 | -0.02 | 0.01 |
| **100.00** | 60.00 | 0.00 | 0.20 | 0.43 | -0.22 | 0.21 |
| **100.00** | 30.00 | 0.00 | 0.09 | 0.21 | -0.10 | 0.10 |
| **100.00** | 15.00 | 0.00 | 0.04 | 0.09 | -0.05 | 0.05 |
| **100.00** | 5.00 | 0.00 | 0.01 | 0.02 | -0.01 | 0.01 |
| **1000.00** | 60.00 | 0.00 | 0.06 | 0.14 | -0.08 | 0.06 |
| **1000.00** | 30.00 | 0.00 | 0.03 | 0.07 | -0.04 | 0.03 |
| **1000.00** | 15.00 | 0.00 | 0.01 | 0.03 | -0.02 | 0.01 |
| **1000.00** | 5.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| **10000.00** | 60.00 | 0.00 | 0.02 | 0.05 | -0.03 | 0.02 |
| **10000.00** | 30.00 | 0.00 | 0.01 | 0.02 | -0.01 | 0.01 |
| **10000.00** | 15.00 | 0.00 | 0.00 | 0.01 | -0.01 | 0.01 |
| **10000.00** | 5.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table SI 6.4.** *We* load errors information for a flow-based sampling mode and 96h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 60.00 | 0.00 | 0.09 | 0.21 | -0.12 | 0.10 |
| **50.00** | 30.00 | 0.00 | 0.04 | 0.10 | -0.05 | 0.04 |
| **50.00** | 15.00 | 0.00 | 0.02 | 0.05 | -0.02 | 0.02 |
| **50.00** | 5.00 | 0.00 | 0.00 | 0.01 | -0.01 | 0.00 |
| **100.00** | 60.00 | 0.00 | 0.07 | 0.15 | -0.08 | 0.07 |
| **100.00** | 30.00 | 0.00 | 0.03 | 0.07 | -0.04 | 0.03 |
| **100.00** | 15.00 | 0.00 | 0.02 | 0.03 | -0.02 | 0.01 |
| **100.00** | 5.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| **1000.00** | 60.00 | 0.00 | 0.02 | 0.05 | -0.02 | 0.02 |
| **1000.00** | 30.00 | 0.00 | 0.01 | 0.02 | -0.01 | 0.01 |
| **1000.00** | 15.00 | 0.00 | 0.00 | 0.01 | -0.01 | 0.01 |
| **1000.00** | 5.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **10000.00** | 60.00 | 0.00 | 0.01 | 0.02 | -0.01 | 0.00 |
| **10000.00** | 30.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| **10000.00** | 15.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **10000.00** | 5.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table SI 6.5.** WWTP attenuation errors information for a flow-based sampling mode and 24h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 60.00 | 0.41 | 71.23 | 441.61 | -393.47 | 48.14 |
| **50.00** | 30.00 | -5.33 | 56.00 | 142.71 | -106.81 | 35.90 |
| **50.00** | 15.00 | -1.64 | 34.36 | 94.23 | -61.81 | 32.43 |
| **50.00** | 5.00 | -1.42 | 14.58 | 35.94 | -19.80 | 16.14 |
| **100.00** | 60.00 | -9.98 | 64.23 | 253.61 | -208.72 | 44.89 |
| **100.00** | 30.00 | -3.24 | 39.97 | 127.81 | -91.60 | 36.21 |
| **100.00** | 15.00 | -2.22 | 22.34 | 53.45 | -32.83 | 20.62 |
| **100.00** | 5.00 | -1.02 | 10.09 | 24.25 | -12.06 | 12.19 |
| **1000.00** | 60.00 | 0.31 | 18.22 | 42.06 | -25.19 | 16.87 |
| **1000.00** | 30.00 | -0.95 | 12.28 | 25.02 | -14.77 | 10.25 |
| **1000.00** | 15.00 | -0.28 | 8.19 | 16.33 | -8.81 | 7.52 |
| **1000.00** | 5.00 | -0.23 | 3.18 | 7.89 | -4.37 | 3.52 |
| **10000.00** | 60.00 | 0.86 | 5.88 | 12.56 | -6.94 | 5.62 |
| **10000.00** | 30.00 | 0.06 | 4.09 | 10.21 | -5.68 | 4.54 |
| **10000.00** | 15.00 | -0.01 | 2.68 | 6.00 | -3.21 | 2.78 |
| **10000.00** | 5.00 | -0.15 | 1.00 | 2.51 | -1.31 | 1.20 |

**Table SI 6.6.** WWTP attenuation errors information for a flow-based sampling mode and 96h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 60.00 | -0.83 | 37.04 | 86.10 | -56.15 | 29.95 |
| **50.00** | 30.00 | -1.06 | 22.75 | 51.91 | -33.40 | 18.51 |
| **50.00** | 15.00 | -1.59 | 13.79 | 32.03 | -19.06 | 12.97 |
| **50.00** | 5.00 | -0.24 | 5.12 | 9.93 | -4.85 | 5.08 |
| **100.00** | 60.00 | -5.74 | 28.07 | 64.62 | -44.00 | 20.62 |
| **100.00** | 30.00 | -2.80 | 16.64 | 45.61 | -28.14 | 17.47 |
| **100.00** | 15.00 | -1.55 | 12.72 | 24.67 | -13.86 | 10.81 |
| **100.00** | 5.00 | -0.11 | 3.05 | 9.75 | -5.17 | 4.58 |
| **1000.00** | 60.00 | -0.31 | 10.73 | 18.95 | -10.40 | 8.55 |
| **1000.00** | 30.00 | 0.50 | 5.20 | 11.92 | -6.53 | 5.39 |
| **1000.00** | 15.00 | 0.10 | 3.44 | 6.87 | -3.63 | 3.23 |
| **1000.00** | 5.00 | 0.18 | 0.93 | 2.77 | -1.30 | 1.47 |
| **10000.00** | 60.00 | -0.10 | 3.21 | 6.69 | -3.69 | 3.01 |
| **10000.00** | 30.00 | -0.04 | 2.02 | 4.24 | -2.33 | 1.91 |
| **10000.00** | 15.00 | -0.11 | 1.17 | 2.21 | -1.27 | 0.94 |
| **10000.00** | 5.00 | -0.03 | 0.44 | 0.83 | -0.44 | 0.39 |

**Table SI 6.7.** *Ru* load errors information for a flow-based sampling mode and 12h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 240.00 | -0.93 | 6.16 | 9.85 | -5.72 | 4.13 |
| **50.00** | 120.00 | 0.37 | 0.89 | 1.90 | -0.70 | 1.20 |
| **50.00** | 60.00 | 0.00 | 0.49 | 1.19 | -0.68 | 0.51 |
| **50.00** | 15.00 | 0.00 | 0.09 | 0.23 | -0.14 | 0.10 |
| **100.00** | 240.00 | -0.85 | 6.37 | 8.69 | -5.33 | 3.36 |
| **100.00** | 120.00 | 0.33 | 0.71 | 1.53 | -0.55 | 0.98 |
| **100.00** | 60.00 | -0.01 | 0.47 | 0.93 | -0.47 | 0.45 |
| **100.00** | 15.00 | 0.00 | 0.09 | 0.17 | -0.09 | 0.08 |
| **1000.00** | 240.00 | -0.83 | 6.02 | 7.20 | -4.42 | 2.78 |
| **1000.00** | 120.00 | 0.31 | 0.41 | 0.92 | -0.16 | 0.76 |
| **1000.00** | 60.00 | -0.02 | 0.33 | 0.81 | -0.44 | 0.37 |
| **1000.00** | 15.00 | 0.00 | 0.06 | 0.12 | -0.06 | 0.05 |
| **10000.00** | 240.00 | -0.83 | 5.93 | 6.97 | -4.36 | 2.60 |
| **10000.00** | 120.00 | 0.30 | 0.34 | 0.80 | -0.09 | 0.71 |
| **10000.00** | 60.00 | 0.00 | 0.28 | 0.71 | -0.39 | 0.32 |
| **10000.00** | 15.00 | 0.00 | 0.06 | 0.11 | -0.06 | 0.05 |

**Table SI 6.8.** *Ru* load errors information for a flow-based sampling mode and 24h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 240.00 | -0.47 | 1.12 | 2.60 | -2.11 | 0.48 |
| **50.00** | 120.00 | 0.37 | 0.59 | 1.44 | -0.48 | 0.96 |
| **50.00** | 60.00 | 0.02 | 0.28 | 0.65 | -0.33 | 0.32 |
| **50.00** | 15.00 | 0.00 | 0.06 | 0.16 | -0.09 | 0.07 |
| **100.00** | 240.00 | -0.50 | 0.86 | 2.00 | -1.69 | 0.30 |
| **100.00** | 120.00 | 0.29 | 0.46 | 1.08 | -0.28 | 0.79 |
| **100.00** | 60.00 | 0.02 | 0.23 | 0.58 | -0.29 | 0.29 |
| **100.00** | 15.00 | 0.00 | 0.04 | 0.12 | -0.07 | 0.05 |
| **1000.00** | 240.00 | -0.55 | 0.38 | 0.96 | -1.04 | -0.08 |
| **1000.00** | 120.00 | 0.29 | 0.25 | 0.70 | -0.05 | 0.65 |
| **1000.00** | 60.00 | 0.01 | 0.13 | 0.41 | -0.18 | 0.23 |
| **1000.00** | 15.00 | 0.00 | 0.03 | 0.07 | -0.04 | 0.03 |
| **10000.00** | 240.00 | -0.55 | 0.31 | 0.75 | -0.95 | -0.20 |
| **10000.00** | 120.00 | 0.27 | 0.26 | 0.63 | 0.03 | 0.66 |
| **10000.00** | 60.00 | 0.02 | 0.15 | 0.36 | -0.17 | 0.19 |
| **10000.00** | 15.00 | 0.00 | 0.02 | 0.06 | -0.03 | 0.03 |

**Table SI 6.9.** *Rd* load errors information for a flow-based sampling mode and 12h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 240.00 | -0.98 | 6.72 | 10.24 | -5.82 | 4.42 |
| **50.00** | 120.00 | 0.43 | 0.88 | 1.93 | -0.67 | 1.26 |
| **50.00** | 60.00 | 0.06 | 0.49 | 1.24 | -0.67 | 0.57 |
| **50.00** | 15.00 | 0.00 | 0.10 | 0.25 | -0.14 | 0.11 |
| **100.00** | 240.00 | -0.83 | 6.95 | 9.06 | -5.41 | 3.65 |
| **100.00** | 120.00 | 0.36 | 0.68 | 1.56 | -0.58 | 0.98 |
| **100.00** | 60.00 | 0.06 | 0.50 | 0.99 | -0.48 | 0.51 |
| **100.00** | 15.00 | 0.00 | 0.09 | 0.19 | -0.10 | 0.09 |
| **1000.00** | 240.00 | -0.70 | 6.50 | 7.69 | -4.60 | 3.09 |
| **1000.00** | 120.00 | 0.36 | 0.45 | 0.84 | -0.09 | 0.75 |
| **1000.00** | 60.00 | 0.02 | 0.35 | 0.80 | -0.39 | 0.41 |
| **1000.00** | 15.00 | 0.00 | 0.07 | 0.15 | -0.08 | 0.07 |
| **10000.00** | 240.00 | -0.72 | 6.57 | 7.40 | -4.48 | 2.92 |
| **10000.00** | 120.00 | 0.35 | 0.32 | 0.86 | -0.07 | 0.79 |
| **10000.00** | 60.00 | 0.03 | 0.33 | 0.79 | -0.38 | 0.40 |
| **10000.00** | 15.00 | 0.00 | 0.07 | 0.13 | -0.07 | 0.06 |

**Table SI 6.10.** *Rd* load errors information for a flow-based sampling mode and 24h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 240.00 | -0.34 | 1.18 | 2.49 | -1.84 | 0.65 |
| **50.00** | 120.00 | 0.37 | 0.57 | 1.40 | -0.36 | 1.04 |
| **50.00** | 60.00 | 0.09 | 0.32 | 0.66 | -0.32 | 0.34 |
| **50.00** | 15.00 | 0.00 | 0.05 | 0.16 | -0.08 | 0.08 |
| **100.00** | 240.00 | -0.33 | 0.85 | 1.91 | -1.48 | 0.43 |
| **100.00** | 120.00 | 0.34 | 0.44 | 1.10 | -0.18 | 0.92 |
| **100.00** | 60.00 | 0.06 | 0.25 | 0.59 | -0.22 | 0.37 |
| **100.00** | 15.00 | 0.00 | 0.04 | 0.11 | -0.05 | 0.05 |
| **1000.00** | 240.00 | -0.45 | 0.40 | 0.98 | -0.95 | 0.02 |
| **1000.00** | 120.00 | 0.34 | 0.29 | 0.74 | 0.00 | 0.74 |
| **1000.00** | 60.00 | 0.06 | 0.14 | 0.40 | -0.13 | 0.27 |
| **1000.00** | 15.00 | 0.00 | 0.03 | 0.06 | -0.03 | 0.03 |
| **10000.00** | 240.00 | -0.45 | 0.26 | 0.81 | -0.89 | -0.08 |
| **10000.00** | 120.00 | 0.35 | 0.23 | 0.57 | 0.10 | 0.66 |
| **10000.00** | 60.00 | 0.07 | 0.14 | 0.36 | -0.12 | 0.24 |
| **10000.00** | 15.00 | 0.00 | 0.02 | 0.06 | -0.03 | 0.03 |

**Table SI 6.11.** River attenuation errors information for a flow-based sampling mode and 12h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 240.00 | 0.06 | 4.54 | 10.32 | -5.35 | 4.97 |
| **50.00** | 120.00 | -0.05 | 0.73 | 1.78 | -0.93 | 0.85 |
| **50.00** | 60.00 | 0.02 | 0.43 | 1.20 | -0.60 | 0.60 |
| **50.00** | 15.00 | -0.01 | 0.11 | 0.31 | -0.16 | 0.15 |
| **100.00** | 240.00 | 0.16 | 4.41 | 10.06 | -5.55 | 4.51 |
| **100.00** | 120.00 | -0.03 | 0.68 | 1.48 | -0.76 | 0.71 |
| **100.00** | 60.00 | -0.01 | 0.45 | 0.93 | -0.47 | 0.46 |
| **100.00** | 15.00 | 0.00 | 0.13 | 0.27 | -0.14 | 0.13 |
| **1000.00** | 240.00 | 0.17 | 3.76 | 9.05 | -4.86 | 4.19 |
| **1000.00** | 120.00 | -0.03 | 0.35 | 0.93 | -0.46 | 0.47 |
| **1000.00** | 60.00 | 0.04 | 0.29 | 0.79 | -0.41 | 0.38 |
| **1000.00** | 15.00 | -0.01 | 0.12 | 0.22 | -0.11 | 0.11 |
| **10000.00** | 240.00 | 0.30 | 3.74 | 9.29 | -4.99 | 4.30 |
| **10000.00** | 120.00 | -0.02 | 0.28 | 0.79 | -0.39 | 0.40 |
| **10000.00** | 60.00 | 0.03 | 0.31 | 0.71 | -0.35 | 0.36 |
| **10000.00** | 15.00 | -0.01 | 0.13 | 0.20 | -0.11 | 0.10v |

**Table SI 6.12.** River attenuation errors information for a flow-based sampling mode and 24h composite duration.

| Nº Pulses (pulses $d^{-1}$) | Sampling freq. (min) | Median | 50-IQR | 90-IQR | P5 | P95 |
|---|---|---|---|---|---|---|
| **50.00** | 240.00 | 0.13 | 1.31 | 3.02 | -1.46 | 1.57 |
| **50.00** | 120.00 | -0.02 | 0.73 | 1.72 | -0.85 | 0.87 |
| **50.00** | 60.00 | 0.02 | 0.36 | 0.81 | -0.40 | 0.41 |
| **50.00** | 15.00 | 0.00 | 0.08 | 0.19 | -0.10 | 0.09 |
| **100.00** | 240.00 | 0.14 | 0.95 | 2.41 | -1.13 | 1.28 |
| **100.00** | 120.00 | -0.01 | 0.57 | 1.40 | -0.73 | 0.67 |
| **100.00** | 60.00 | 0.02 | 0.31 | 0.73 | -0.36 | 0.38 |
| **100.00** | 15.00 | 0.00 | 0.06 | 0.14 | -0.07 | 0.07 |
| **1000.00** | 240.00 | 0.12 | 0.48 | 1.18 | -0.46 | 0.72 |
| **1000.00** | 120.00 | 0.00 | 0.36 | 0.87 | -0.45 | 0.43 |
| **1000.00** | 60.00 | 0.03 | 0.20 | 0.46 | -0.19 | 0.26 |
| **1000.00** | 15.00 | 0.00 | 0.04 | 0.09 | -0.04 | 0.05 |
| **10000.00** | 240.00 | 0.12 | 0.44 | 0.94 | -0.39 | 0.56 |
| **10000.00** | 120.00 | -0.01 | 0.33 | 0.64 | -0.31 | 0.33 |
| **10000.00** | 60.00 | 0.02 | 0.18 | 0.44 | -0.19 | 0.25 |
| **10000.00** | 15.00 | 0.00 | 0.03 | 0.09 | -0.04 | 0.04 |

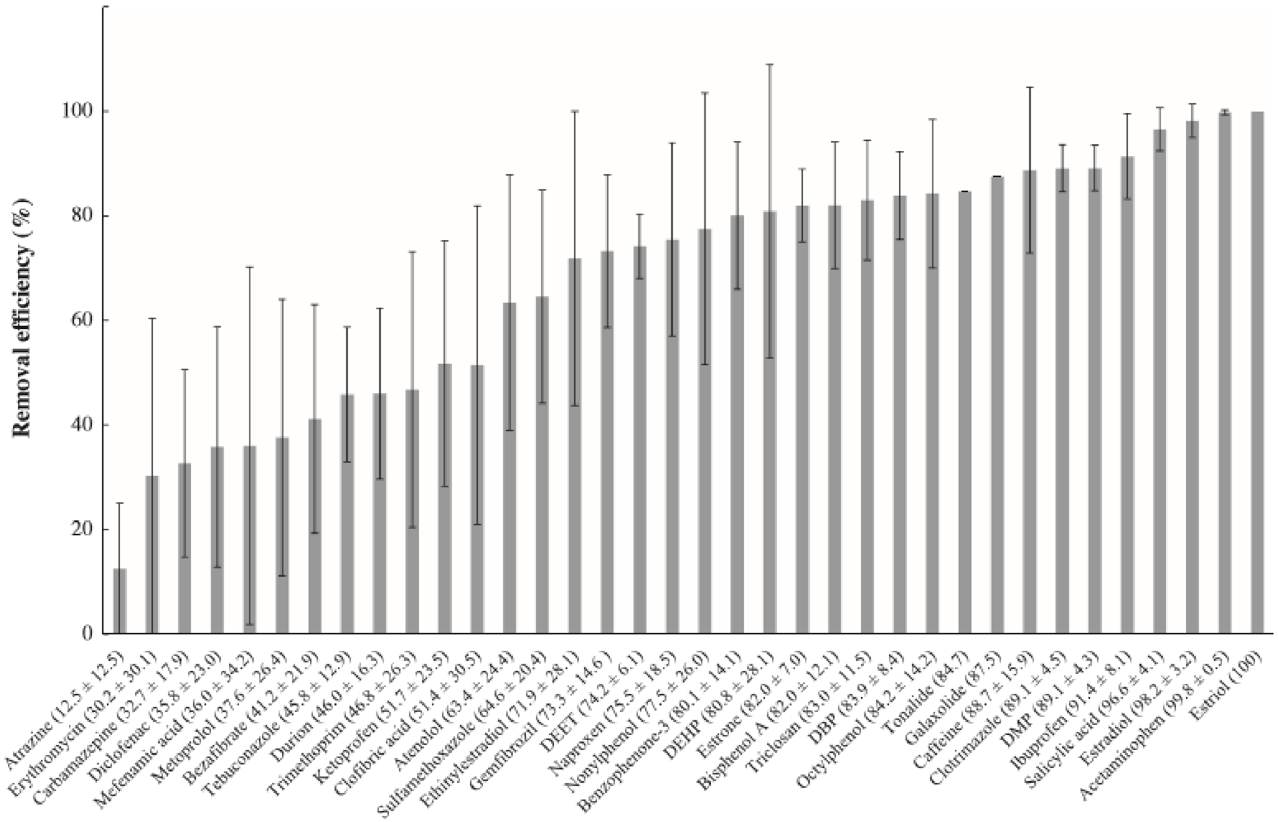


**Figure SI 6.1.** Removals of reported micro-pollutants in WWTP studies in the review from Luo et al. (2014). Note that negative removals were not included. X – axis displays the selected compounds and their mean concentrations and standard deviations(in the brackets).Error bars represent the standard deviations of the data.

# SECTION III

# DISCUSSION AND CONCLUSIONS

# Chapter 7

## General Discussion

Knowledge on the spatial and temporal transformations of organic matter, nutrients and micro-contaminants in WWTPs and their receiving freshwater ecosystems is rather fragmented. Within this thesis we have contributed to better understand such transformations through integrated monitoring and integrated modelling of a system consisting of a WWTP and its receiving freshwater ecosystem.

The goals established for the thesis were accomplished and these are the main contributions:

1) Better understanding of the performance of the integrated system with regards to organic matter, nitrogen and phosphorus transformations, through proper mass balancing and integrated modelling and calibration approaches (**Chapter 4**). Key messages are:

    - The observed attenuation patterns in the river reaches were shaped by the discharge of the WWTP, as the temporal and spatial dynamics of the C, N, and P compounds resulted from the patterns at the effluent of the WWTP. The river functioning downstream the WWTP was coupled to the WWTP. We observed that whereas the WWTP could not remove nitrogen, the river had the capacity to nitrify 80% of the ammonia load coming from the catchment with just five kilometers.
    - An integrated model was developed for a WWTP and its receiving river system. The most relevant aspect of this model is that there was a harmonization not only in terms of subsystems model complexity (using ASM3BioP and RWQM1 water quality models), but also in terms of data availability. We ran a targeted campaign to develop and calibrate such integrated model, which collected simultaneously data from the different subsystems. A relevant aspect is the execution of an integrated tracer test which allowed reducing any uncertainties related to hydraulics. To the best of our knowledge there is no integrated study which has addressed data availability in a similar manner.

2) Better understanding of the influence of global change to the performance of integrated systems, through a combined model-based approach and the incorporation of immission-based criteria for the evaluation of river chemical status (**Chapter 4**). Key messages are:

    - The integrated model developed and calibrated for the WWTP-river system was extended with a calibrated influent generator and included immission-based criteria to evaluate the performance of the system. This allowed the generation of different global change scenarios.
    - The model-based approach allowed us to conclude that under future foreseen population growth and decrease in the river flow (leading to increased loads from the catchment discharged to the river and decreased dilution capacity) the chemical status of the system will turn into bad conditions due to organic, nitrogen and phosphorus compounds concentrations.
    - This study calls for the soon definition of a water management plan in the studied catchment, where improving nitrogen removal in the WWTP, the upgrade of the system due to its limit capacity and the reduction of pollutants concentrations in the river are some of the concerns that should be addressed.

3) A proposal of a method to estimate the attenuation of pharmaceuticals and their transformation products in a comparable manner for both a WWTP and a receiving freshwater ecosystem (**Chapter 5**). Key messages are:

    - Only 5 out of the 19 pharmaceuticals were reduced by more than 90% at the WWTP, while the rest were partially or non-attenuated and discharged into the receiving river. Higher attenuation efficiencies were obtained in the river compared to the WWTP, while higher load reductions were obtained in the WWTP.
    - Routing between some pharmaceuticals and their transformation products was investigated and analyzed for the first time along the WWTP and the receiving river system, where linkages in the routing of some pharmaceuticals (venlafaxine, carbamazepine and ibuprofen) and their corresponding transformation products were identified.
    - A model-based approach was presented to model attenuation in the coupled WWTP and river system by the combination of experimental and modeling approaches, and by the consideration and propagation of different uncertainty sources. The results showed that dynamic attenuation could be predicted with simple first order attenuation kinetics in both systems.

4) Better understanding of the influence of sampling strategies to the estimation of attenuation of micro-contaminants in a WWTP and a receiving freshwater ecosystem (**Chapter 6**). Key messages are:

    - Sampling matters when investigating micro-contaminants in WWTPs and rivers, as errors in loads and attenuation calculations can change considerably as a function of several factors. In other words, uncertainty in the loads and attenuation estimations is not the same, and this should be considered in the design of sampling campaigns.
    - The influence of sampling frequencies and composite durations was evaluated under different number of pulses and degradability rates of a compound, showing that short sampling intervals and longer sampling durations are needed when estimating attenuation, especially for low served populations and/or low consumptions rates (e.g. low number of pulses).
    - The WWTP influent is especially critical when designing sampling strategies, and thus higher sampling frequencies and composite durations are needed at the influent of the WWTP to obtain estimates with low uncertainty. This is especially important for compounds with low degradability rates and low-intermittent presence at the influent of the WWTP.
    - Degradability and desired attenuation accuracy should be defined upfront. This study demonstrates that lowest uncertainties are observed for high degradability levels and the highest uncertainties for compounds with low degradability. This has been also observed in the reported values in the literature. This demonstrates that better sampling methods as well as proper propagation and quantification of uncertainty in our estimates should be conducted when representing data.

From the results obtained in this thesis it is clear that an integrated perspective is needed for UWWS decision-making. This is especially important in urban water systems that the contribution of the WWTP in the river is important, e.g. in Mediterranean catchments. We have learnt that the discharge of the WWTP influences the performance of the receiving freshwater ecosystem. We have been able to track compounds transformations along the entire system, and have been able to compare the performance of processes in the engineered and in the natural system. We could demonstrate that global change and population growth will lead to changes in the different sub-systems. Thus, it is therefore logical that the assessment of urban wastewater systems cannot take as system boundary the effluent of the WWTP, but include as well the receiving freshwater ecosystem.

Even though some of these statements have been published before, there are only few examples of complete integrated studies including monitoring and modelling approaches. We fully support initiatives such as Working Group on Modelling of Integrated Urban Water Systems (MIUWS, Chair: Peter Bach), with an interest on the integration of models of all components of the water cycle in urban areas, including the waste, nutrients and energy cycles for the various operational processes as they relate to the water cycle. Their main focus lies on model and software integration issues, e.g. on how to solve integration problems and how to exploit integration opportunities. This thesis is aligned with these goals and hope to contribute to the discussion.

Research on integrated systems should come alone with upgrades in legislation. The importance of integrated management has been stated by international directives (e.g. WFD). However, the process of incorporating the outcomes of research into practice is slow. In addition, there is need for an integration of freshwater environmental policies and WWTP management. For instance, Corominas et al. (2013) showed that there is a gap in EU environmental policies (between EU Directive 91/271/EEC and EU Water Framework Directive (2000/60/EEC)) leading to non-integrated management, which may result on adverse environmental and economical consequences. We believe that these policies should be updated and tuned to account for an integrated perspective, allowing a more efficient and sustainable management of wastewater treatment plants, maximizing the ecological, economical and social benefits of the system as a whole.

Finally, I would like to stress the importance of including uncertainty in research activities, and more specifically, in integrated studies and management. A limited number of studies in literature have approached monitoring, mass balancing, and modelling from an integrated perspective and with the same level of detail for each of the subsystems. Such approaches require an effort of integration of knowledge (and practices) from different disciplines (from water engineering and from natural sciences). Overall, we came up with an approach that is relevant for decision-makers to design new UWWS or upgrade existing ones. For the specific case of organic matter and nutrients we demonstrated the usefulness of the proposed approach for decision-making. Still, the approach can be further expanded to include other scenarios (e.g. wet weather conditions) or criteria. Reporting on uncertainties is extremely important to facilitate the exchange of knowledge between researchers and to communicate with managers. We have incorporated uncertainty in different sections of the thesis and hence we hope to inspire future studies to continue this practice.

# Chapter 8

## Future Perspectives

## 7.1 Current management

I imagine a world where integrated management of sanitation systems and rivers is common practice. Research has been made since the 1990-2000s in this topic, but there is still lack of acceptance of integrated approaches. Even though the Water Framework offers a new paradigm and great opportunities, actions taken are rather fragmented. I imagine a world where water utilities have integrated models of their WWTPs and freshwater ecosystems where they discharge, and where these models are used for decision-making. In the wastewater treatment field, and more intensely in North-America, consultancies are running projects to optimize WWTP operation which are based on models. I envision an expansion of consultancy services, expanding the WWTP system boundaries when water authorities request an integrated assessment when planning new developments in municipalities. This comes alone with clear definition of legislation, which is still ambiguous. Better definition of immission-based criteria is needed. A good example is UK & Wales, which have integrated immission-based criteria in new legislations. This should be conducted not only for conventional contaminants but also for emerging contaminants. Global changes and the impact in urban wastewater systems and their receiving water bodies require and integrated management.

## 7.2 Research needed to define global change scenarios

Besides the research conducted to build proper integrated models and to reduce the uncertainties associated to these models research is needed to define accurately global change scenarios. Proper methods are needed to transfer the global change projections of temperature and rainfall into the UWWS integrated models. Hence, global change research should be aligned to integrated modelling research and tools to properly execute this interfacing are needed. An example of such a tool can be found in the influent generator from Talebizadeh et al. (2016) which accounts for explicit variability and uncertainty into the generated influent time series. On the other hand, the use of integrated models for predicting the chemical status in freshwater ecosystems is in good progress. Research is needed to develop models to predict the ecological status. In that sense, research has been conducted at university of Ghent (Holguin et al., 2014). However, the applicability of these models to Mediterranean catchments has not been demonstrated, and thus more intensive monitoring/modelling/calibration efforts would be needed. Within this context, proper interaction between water authorities which are collecting ecological status information and research institutes is needed to make best use of these data.

## 7.3 Micro-contaminants

The fate and removal of micro-contaminants is a hot topic. The upgrade of WWTP with tertiary treatment is a reality in some countries (e.g. Switzerland). The precautionary principles for protecting the environment are applied in these countries even though the transformation processes along the entire sanitation system (from the human body, to the sewer system and to their receiving water bodies) are not well known. We currently care about compounds for which we have analytical capacity ready that allows their quantification. However, there are many other compounds which might be more toxic as compared to the ones we can currently measuring, as we could see in this PhD thesis. Research is needed to expand current analytical capacity and the linkage between pharmaceuticals and their transformation products,

# Chapter 8

# Conclusions

The fate and removal of contaminants along the urban wastewater system cannot be fully understood without including the WWTP and the receiving river ecosystem within the same system boundaries. The conclusions achieved in the different chapters of the thesis are:

In **Chapter 4** an assessment of attenuation of organic matter, nutrients in a WWTP and its receiving river ecosystem was conducted through the combination of integrated sampling and modelling approaches. The analysis of organic and nutrients showed that whereas the WWTP could not remove nitrogen, the river had the capacity to nitrify 80% of the ammonia load coming from the catchment with just five kilometers. At the present the chemical status as defined by the Catalan Water Agency is in bad conditions in the first two km after the discharge, which improves in the next segments to good thanks to dilution and removal processes in the river. Under future foreseen population growth and decrease the river flow the chemical status of the system will turn into bad conditions as well for the last three kilometers studied.

In **Chapter 5** the attenuation of pharmaceuticals and some of their transformation products in an integrated system composed by a WWTP and its receiving river ecosystem was investigated. The study showed that pharmaceuticals load reductions was much higher at the WWTP, but attenuation efficiencies were higher at the river. Only 5 out of 19 pharmaceuticals were reduced by more than 90% at the WWTP, while the rest were only partially or non-attenuated (or even released) and discharged into the receiving river. At the river only ibuprofen was reduced by more than 50% out of the 6 parent compounds, while partial or non-attenuation were observed for some of their transformation products. Moreover, the integrated approach allowed the identification of close causal relationships between the parental compounds and their transformation products, such as venlafaxine, carbamazepine, ibuprofen and diclofenac. Finally, the followed model-based approach showed that dynamic attenuation in the WWTP and river could be predicted with simple first order kinetics for most modelled compounds after considering uncertainty.

In **Chapter 6** an assessment of the effects of the number of pulses, sampling frequency, composite duration and compound degradability on loads and attenuation estimations of micro-contaminants in WWTP and rivers was conducted. We could conclude that sampling strategy matters, as errors in loads and attenuation rates change considerably as a function of sampling strategy. Regarding bias and uncertainty, the most important factor is the number of pulses, followed by the sampling frequency, composite duration and compound degradability. Overall, the results showed that we must do our sampling effort when dealing with low number of pulses, that is, in small municipalities or with compounds with a low consumption rate, especially critical for those compounds with low degradability rates where uncertainty is increased. We also seen that we must focus our sampling efforts at the WWTP influent, as the errors in the load estimations are higher than at the effluent or at the river. Increasing sampling frequency or sampling periods are recommended strategies to reduce the uncertainty associated in our load and attenuation estimations. Finally, the study shows that when designing the sampling strategy, we must not only consider population size and compound characteristics and use, but also the maximum acceptable error in the attenuation rates as well as the compound degradability. As it is expected that studies will not only focus on one compound, the sampling strategy should be designed for the compound with the most critical combination of number of pulses and degradability rate.

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