Axel Meierjohann

Application of LC-MS/MS for Environmental Analysis

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“A man travels the world over in search of what he needs
and returns home to find it.”

George A. Moore

Preface

The work presented in this thesis was carried out at the Laboratory of Organic Chemistry at Åbo Akademi University between the years of 2010 and 2015. The work was financed by the Finnish Graduate School for Environmental Science and Technology, Maa- ja vesitekniikan tuki ry and the Advisory Board on Environmental and Sustainability Subjects in Research and Education at Åbo Akademi University.

I would like to sincerely thank my supervisor, Professor Leif Kronberg, for giving me the opportunity to work on this exciting subject and for all his support on both the scientific and technical questions I have had over these years. I would also like to thank Professor Reko Leino for welcoming me at the Department of Organic Chemistry and for his hard work in making this an enjoyable place to work at.

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A big thanks also goes to Thomas Lilley who, early on in my "career", gave me the challenge of creating an analysis method for TBT in anything from water to bat

fur. Our article on the transfer of TBT over the ecosystem boundary is still one that I feel particularly proud of having been a part of.

Also I would like to thank everybody at the lab who has helped me keep our instruments up-and-running, thank you for your help, Peter Holmlund, Roger Nordqvist and Karl-Johan Wikman.

My United Organic Tiger colleagues, thank you for your company in the lab, lively discussions in the coffee room as well as great parties and excursions. Especially I would like to thank Ida, Lucas, Jani, Heidi and Magnus for a friendship that extends beyond the doors of the lab.

A special thanks also goes to all my friends outside the department. Especially Thomas Sandberg, thank you for your company and all the interesting discussions during countless trainings.

Last but not least I would like to thank my family, my parents who have always supported all my decisions and encouraged me along the way, and my brother who has been great company on many trips around the world. Most of all I would like to thank Juhani. Thank you for putting up with me every day, supporting me, and giving me perspective whenever I started getting stressed. I would not be where I am without you.

Turku, July 2018

Axel Meierjohann

Abstract

With the advent of electrospray ionization (ESI), liquid chromatography coupled to mass spectrometry (LC-MS) became a powerful tool used in many scientific areas for the quantitative and qualitative analysis of organic compounds. In environmental sciences, this technology is now widely used for the analysis of water soluble compounds in the environment. This thesis covers work on three different areas of use of this technology.

The first area concerns the analysis of pharmaceuticals in the aquatic environment. For this, a new simplified analysis method for antibiotics in water was developed utilizing on-line SPE technology for sample preparation.

To determine fate scenarios of a number of commonly detected pharmaceuticals, the River Rakkolanjoki in eastern Finland, a river with high wastewater content from a single point source, was studied over four seasons during one year. Based on the seasonal distribution of the pharmaceutical concentrations and loads, likely removal pathways for the pharmaceuticals could be determined.

The second topic covered by the work presented in this thesis is the analysis of illicit drugs in municipal wastewater, for the comparison of spatial and temporal trends in illicit drug consumption. The wastewater of the cities of Turku and Helsinki were studied in 2011, 2012 and 2013, within the framework of a larger European collaboration. The studies showed that Finland has one of the highest per capita consumptions of amphetamines among the studied cities, while cocaine consumption in Turku and Helsinki was negligible.

The third topic of this thesis is the analysis of Tributyltin (TBT) in sediments of both shipping lanes and adjacent reed beds, as well as in chironomids (non-biting midges) that emerged from these reed beds. The study showed that TBT could pass the ecosystem boundary between the aquatic and the terrestrial ecosystem through chironomids.

Sammanfattning

Sedan uppfinningen av electrospray jonisering har vätskekromatografi kopplad till masspektrometri blivit en populär metod inom många vetenskapsområden för kvantitativ och kvalitativ analys av organiska föreningar. Inom miljökemin används metoden för analys av vattenlösliga föreningar i främst den akvatiska miljön. Denna avhandling behandlar tre användningsområden för denna analysmetod.

Första delen av avhandlingen handlar om analys av läkemedel i vattendrag. En förenklat metod för analys av antibiotika i vattenprov har utvecklats och testats för detta ändamål.

För att bestämma möjliga nedbrytningsvägar för ett antal olika läkemedel, undersöktes floden Rakkolanjoki i östra Finland. Floden innehåller en hög halt av renat avloppsvatten som härstammar från en enda källa. Koncentrationsmätningar vid olika årstider gav möjligheten att bestämma sannolika nedbrytningsvägar.

Andra delen av avhandlingen handlar om analys av narkotika i avloppsvatten. Analyserna utfördes på prov av vatten från Åbo och Helsingfors och arbetet utgjorde en del av ett större europeisk samarbetsprojekt. Resultaten visade att Finland hade bland den högsta konsumtionen av amfetaminer i Europa, medan kokain konsumtionen var bland de lägsta i Europa.

Det tredje projektet som beskrivs i avhandlingen behandlar triobutyltenn (TBT) och dess överföring från den akvatiska till den terrestriska miljön via myggor och dess larver. Larverna upptar TBT som ligger i bottensediment och överför sedan föreningen till den terrestriska miljön i vuxenstadiet som myggor. Myggorna utgör föda för vissa arter av fladdermöss.

List of Original Publications

I **Meierjohann, A.**; Brozinski, J-M.; Kronberg, L. Seasonal variation of pharmaceutical concentrations in a river/lake system in eastern Finland. *Env. Sci. Process. Impact*, **2016**, 18, 342-349

II **Meierjohann, A.**; Krzymyk, E.; Brozinski, J-M.; Kronberg, L. Development of an On-line SPE LC-MS/MS method for the analysis of antibiotics in waste- and surface water. *Eniviron Sci Pollut Res*, **2017**, 24, 8692-8699

III Thomas, K.; Bijlsma, L.; Castiglioni, S., Covaci, A.; Emke, E.; Grabic, R. Hernández, F.; Karolak, S.; Kasprzyk-Hordern, B.; Lindberg, R.; Lopez de Alda, M.; **Meierjohann, A.**; Ort, C.; Pico, Y.; Quintana, J.; Reid, M.; Rieckermann, J.; Terzic, S.; van Nuijs, A.; de Voogt, P. Comparing illicit drug use in 19 European cities through sewage analysis. *Sci Total Environ*, **2012**, 432, 432-439

IV Ort, C.; van Nuijs, A.; Berset, J-D.; Bijlsma, L.; Castiglioni, S.; Covaci, A.; de Voogt, P.; Emke, E.; Fatta-Kassinos, D.; Griffiths, P.; Hernández, F.; González-Mariño, I.; Grabic, R.; Kasprzyk-Hordern, B.; Mastroianni, N.; **Meierjohann, A.**; Nefau, T.; Östman, M.; Pico, Y.; Racamonde, I.; Reid, M.; Slobodnik, J.; Terzic, S.; Thomaidis, N.; Thomas, K. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. *Addiction*, **2014**, 109, 1338-1352

V Lilley, T.; **Meierjohann, A.**; Ruokolainen, L.; Peltonen, J.; Vesterinen, E.; Kronberg, L.; Nkinmaa, M. Reed beds may facilitate transfer of tributyltin from aquatic to terrestrial ecosystems through insect vectors in the Archipelago Sea, S-W Finland. *Environ Toxicol Chem*, **2012**, 31, 1781-178

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Hellström, P.; Hendolin, P.; Kaihovaara, P.; Kronberg, L.; Meierjohann, A. Millerhovf, A.; Paloheimo, L.; Sundelin, H.; Syrjänen, K.; Webb, D-L.; Salaspuro, M. Slow-release L-cysteine capsule prevents gastric Mucosa exposure to carcinogenic acetaldehyde: results of a randomized single-blinded, cross-over study of Heliobacter-associated atrophic gastritis. *Scand J Gastroenterol*, **2017**, 52, 230-237

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Turja, R.; Lehtonen, K.; Meierjohann, A.; Brozinski, J-M.; Vahtera, E.; Soirinsuo, A.; Sokolov, A.; Snoeijs, P.; Budzinski, H.; Devier, M-H; Peluhet, L.; Pääkkönen, J-P.; Viitasalo, M.; Kronberg, L. The mussel caging approach in assessing biological effects of wastewater treatment plant discharges in the Gulf of Finland (Baltic Sea). *Mar Pollut Bull*, **2015**, 97, 135-149

Kronlund, D.; Bergbreiter, A.; Meierjohann, A. Kronberg, L. Lindén, M. Grosso, D.; Smått, J-H. Hydrophobization of marble pore surfaces using a total immersion treatment method – Product selection and optimization of concentration and treatment time. *Prog Org Coat*, **2015**, 85, 159-167

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Sandler, N.; Määttänen, A.; Ihalainen, P.; Kronberg, L.; Meierjohann, A.; Viitala, T.; Peltonen, J. Inkjet printing of drug substrates and use of porous substrates-towards individualized dosing. J Pharm Sci, **2011**, 100, 3386-3395

Contribution of the author

Axel Meierjohann has contributed to the papers in this thesis as stated below:

I	Axel Meierjohann participated in the sampling, analyzed the samples, evaluated the data and was responsible for writing the paper.
II	Axel Meierjohann participated in the sampling, the method development and validation, and was responsible for writing the paper
III	Axel Meierjohann participated in the planning of the collaboration, participated in the sampling, developed the method, analyzed the samples and evaluated the data for Turku and Helsinki.
IV	Axel Meierjohann participated in the planning of the collaboration, organized the sampling, developed the method, analyzed the samples and evaluated the data for Turku and Helsinki.
V	Axel Meierjohann developed the extraction and analysis methods, and assisted in the sample analysis and data evaluation and participated in writing the paper.

List of abbreviations

2,8-DCDD	2,8-Dichlorodibenzo-p-dioxin
ACN	Acetonitrile
DBT	Dibutyltin
DMRM	Dynamic Multiple Reaction Monitoring
E1	Estrone
E2	17 β-estradiol
EE2	17 α-ethinylestradiol
EMCDDa	European Monitoring Centre for Drugs and Drug Addiction
ESI	Electrospray Ionization
FIMEA	Finnish Medicines Agency
GC	Gas Chromatography
HLB	Hydrophilic Lipophilic Balance Sorbent
HPH	High pH Column
IMO	International Maritime organization
LC	Liquid Chromatography
LD50	Dose lethal to 50% of test subjects
LOQ	Limit of Quantification
MALDI	Matrix Assisted Laser Desorption ionization
MBT	Monobutyltin

MCX	Mixed-mode Strong Cation-exchange
MDMA	Methylenedioximethamphetamine
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
NSAID	Nonsteroidal Anti Inflammatory Drugs
OTC	Organotin Compound
PE	Population Equivalents
PLRP-S	Polymeric Reverse Phase Column
POP	Persistent Organic Pollutant
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
TBT	Tributyltin
ToF	Time of Flight Mass Spectrometer
UV	Ultraviolet light
WWTP	Wastewater Treatment Plant

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1. Introduction

1.1. LC-MS/MS

Mass spectrometry (MS) has long been a powerful tool for both the structural elucidation as well as the quantification of organic molecules. However, the need to achieve isolated ions in vacuum for the mass spectrometric separation has previously limited this methodology to small volatile molecules. The development of ESI, MALDI and related ionization methods in the 1980's and 1990's has opened up this field to a large range of new compounds as well as the direct combination of liquid chromatographic (LC) separation with mass spectrometric detection[1]. This first chapter will briefly discuss the general concepts of mass spectrometry as well as the ionization techniques used to interface LC with MS.

1.1.1 Mass spectrometry

Mass spectrometry aims at determining the mass of isolated compounds, or the constituents of a mixture of compounds. This is achieved by first ionizing the compounds followed by a separation of the different mass-to-charge ratios (m/z), in an electric, magnetic or combined electric and magnetic field, depending on the type of mass analyzer used [2].

Mass spectrometry can be used either as a selective detector for the quantification of single compounds, often in complex matrixes[3-5], or for the structural elucidation of unknown molecules[6,7]. This has made mass spectrometry an important tool in many scientific fields including environmental and forensic sciences, pharmacology, drug development, quality control of drugs and food, clinical tests, as well as many biosciences such as proteomics[2].

There are many types of mass spectrometers available on the market today, depending on the type of sample to be analyzed and the type of information needed from the sample, but all mass spectrometers consist of the same three basic elements: An ion source where the compounds are ionized and separated from the matrix, resulting in isolated ions in vacuum, a mass analyzer, where the ions are separated by their m/z values, and a detector which registers the ions and creates an electronic signal that can be registered by a computer or similar output system[2].

1.1.2 Ion Sources

The ion source of a mass spectrometer is responsible for the ionization of the analyte. Depending on the ion source, a number of different types of ions will be created. Ions can be either positively or negatively charged; they can be created by the "simple" removal or addition of an electron, creating a molecular ion, by protonation or deprotonation, creating quasi-molecular ions, or through reactions with other ions or radicals, creating adduct ions [2].

Since the ions need to be isolated in vacuum for analysis in the mass analyzer, most early ion sources were kept under vacuum themselves. These ion sources, such as electron impact ionization, chemical ionization, field desorption and fast atom bombardment, had the disadvantage of being limited in the polarity, molecular size, volatility, and volume of sample that can be introduced without affecting the vacuum in the ion source, making it difficult or impossible to combine these sources with liquid chromatography[2].

In the late 1980's and 1990's, the first atmospheric pressure ionization sources were developed, of which electrospray ionization (ESI) quickly became the most common. The original idea and first experiments on ESI, were published by Malcom Dole in 1968[8]. In these experiments, Dole showed that it was possible to transfer the charge of a charged solvent onto macromolecules by evaporation of the solvent. This idea was adopted by John Fenn some years later, and had its breakthrough in 1984 when Fenn and Yamashita published the first ESI-MS measurements in their paper "Electrospray ion source. Another variation on the free-jet theme"[9].

The principle of ESI is that an electrically conducting solvent passes through a capillary held at a potential several kV higher or lower than a counter electrode. At the opening of this capillary, the fluid stream breaks up into small droplets each carrying a net charge, which is concentrated on the surface due to charge repulsion. As the solvent evaporates, assisted by an inert and heated dry gas, the repulsion between the charges on the surface rises, until it exceeds the surface tension of the droplet. At this point, the droplet undergoes a coulombic explosion, breaking up into a number of smaller droplets each carrying a net charge. This process repeats several times[1,2].

There are two theories on the formation of the final ion. The charge-residue model, explains the formation of ions by continued coulombic explosions until the final "droplet" consists of only one molecule, and its charge. The ion evaporation model is currently the more widely accepted model of ion formation. It explains the final ionization step as field ionization, where the electric field at the surface of very small droplets is sufficiently strong to expel single ions [1,2].

This entire ionization process takes place at atmospheric pressure. The formed ions subsequently pass through narrow orifices, skimmers and/or capillaries that separate the ion source from the high vacuum in the mass analyzer. This process makes it possible to interface mass spectrometry with liquid chromatography.

1.1.3 Mass analyzers

In the field of environmental analysis, two classes of mass analyzers are generally used. For target analysis of trace amounts of known compounds, triple quadrupoles are typically used [10,11], while high resolution instruments such as time of flight or Orbitrap mass spectrometers have the advantage of giving full high resolution spectra for non-target analysis [12,13].

Quadrupole mass analyzers are also known as mass filters, as they can selectively allow only a certain mass, or rather very narrow mass range, to pass through. Quadrupole mass filters were first developed by Wolfgang Paul in the 1950s [14]. They consist of four hyperbolical or rod shaped electrodes, with the opposite electrodes held at the same potential, while the other two electrodes are held at the opposite potential. By adding a frequency to the potential, ions entering along the z axis of the quadrupole, can be caused to oscillate in a stable trajectory through the quadrupole or collide with the rods depending on their m/z value and the frequency applied. This means that the mass filter can allow certain masses to pass through by adjusting the voltage[14]. To receive a full scan mass spectrum, the quadrupole scans through the chosen mass range, revealing all the ions present. Single quadrupoles are very compact and relatively low priced, and are therefore the mass analyzer of choice for most GC-MS applications where high selectivity is already achieved in the GC column. Due to the low selectivity of the single quadrupole mass spectrometers, these instruments are not very well suited for trace analyses in a complex matrix when coupled to LC. For this purpose, triple quadrupoles are the most common mass analyzers today. Triple quadrupoles consist of three consecutive quadrupole mass analyzers (denoted Q_1, Q_2, and Q_3), where the middle quadrupole functions as a collision cell, in which ions are collided with an inert gas to produce fragment ions. Triple quadrupoles can be run in different modes with the individual quadrupoles fulfilling different functions to achieve qualitative or quantitative information about the sample. Some of the most common methods are precursor ion scans, in which Q_1 scans through a given mass range, all ions are fragmented in the collision cell, and Q_3 is set to a specific mass, giving information about all compounds that create the same fragment. Product ion scans, in which Q_1 selects a specific ion, which is then fragmented in the collision cell and Q_3 scans through a given mass range, showing all fragments

coming from the chosen precursor ion. The most common experiment run on triple quadrupoles is multiple reaction monitoring where Q_1 monitors a selected parent ion which is subsequently fragmented in the collision cell and Q_3 monitors a selected fragment ion. This method gives both improved selectivity, and very high sensitivity making it the method of choice for target analysis of trace levels [2].

The second type of mass analyzers that are commonly used in environmental analyses, are high resolution mass spectrometers such as Time of Flight (ToF) or Orbitrap instruments. Despite being based on fundamentally different principles of mass separation and detection, both ToF and Orbitrap instruments have in common that they yield high mass resolution full spectra results for the analyzed samples. High resolution mass spectrometry in environmental analysis has seen a significant increase over the past ten years with the introduction of the Orbitrap and newer generation ToF instruments that are both more sensitive and affordable than previous generations of high resolution instruments. Although high resolution instruments are not as sensitive as a well set-up triple quadrupole, these instruments have the advantage of giving high resolution results, which can be used to determine possible elemental compositions of molecules, as well as giving full scan data for each sample making it possible to detect compounds that were not targeted [12]. The full scan data also means that it is possible to review old results for the presence of compounds that were not of interest at the time the samples were run.

1.2 Pharmaceuticals in wastewater and the environment

1.2.1 Presence of pharmaceuticals in the environment

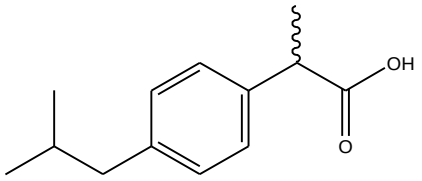
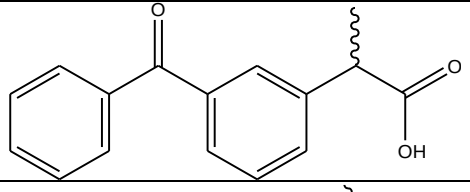
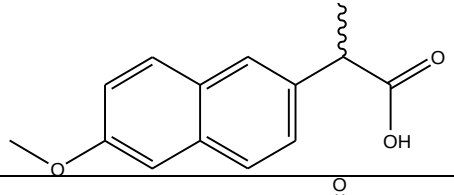
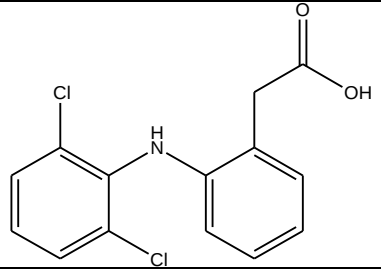
The term pharmaceutical describes a wide variety of chemically very diverse compounds that are used for the treatment of human and veterinary illnesses. The presence of pharmaceuticals in environmental waters was first reported in the 1970s by Tabak et al. [15] and Norpoth et al. [16] who both detected the active ingredients of contraceptives in waste and surface water. Since then, especially in the late 1990s and early 2000s when LC-MS instruments became more widely available, hundreds of different active pharmaceutical ingredients have been detected in waste and surface waters around the world [10,17-26].

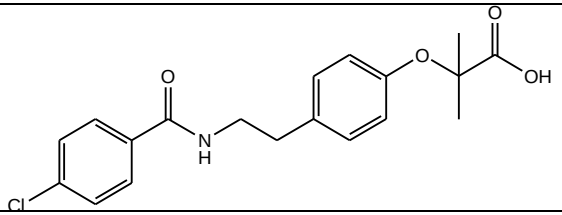
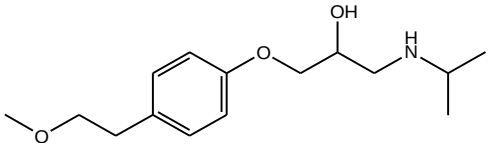
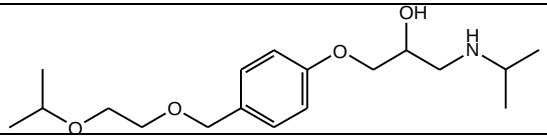
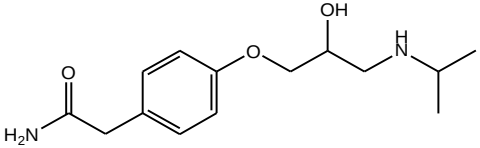
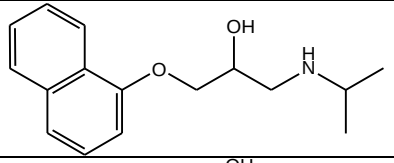
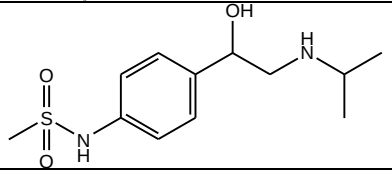
Pharmaceuticals enter the environment mainly through wastewater. After consumption pharmaceuticals are excreted, as metabolites, as the parent compounds or as both, through urine and feces. Removal of these pharmaceuticals during wastewater treatment varies greatly depending on the processes employed and the type of compound [27]. In many cases even the flow rate of wastewater at the WWTP has a strong effect on the removal rates [28]. Some compounds such as ibuprofen are readily degraded to over 90%, while compounds like carbamazepine can often be detected at higher concentrations in WWTP effluent than influent due to deconjugation of metabolites back to the parent compound [27].

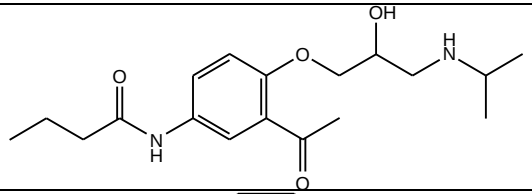
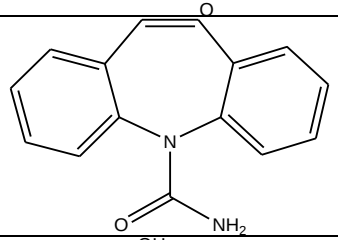
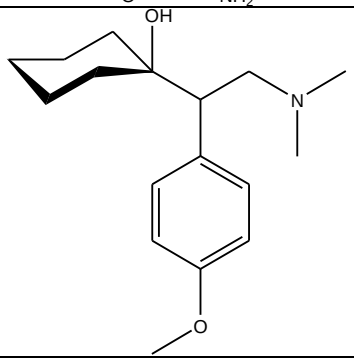
In environmental waters, pharmaceuticals are most often detected at concentrations ranging from low ng/l to low µg/l values. One exception to this being the very high concentrations of antibiotics (mg/l) detected in rivers receiving wastewater from pharmaceutical plants in India [29].

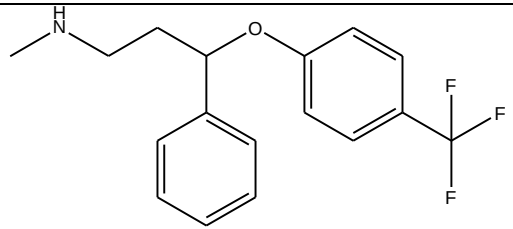
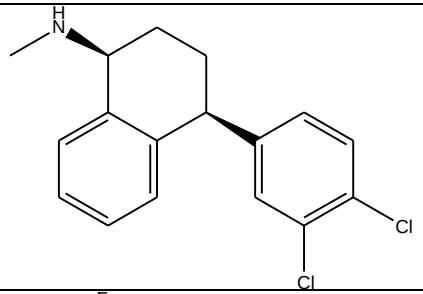
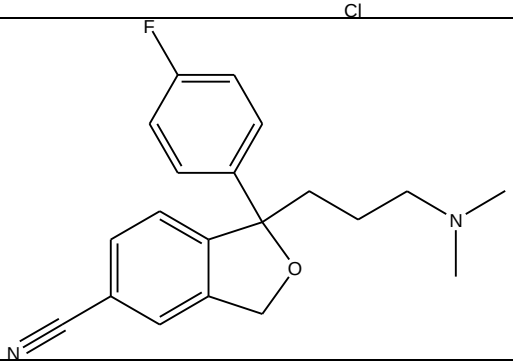
Although most pharmaceuticals are not stable in the environment and thus not persistent, the constant influx of these compounds leads to a permanent presence (pseudo persistence) of these compounds in affected waters [30]. A short list of some of the surface water concentrations reported in the literature for compounds discussed in this thesis can be found in Table 1.

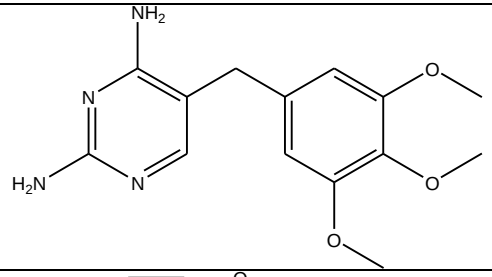
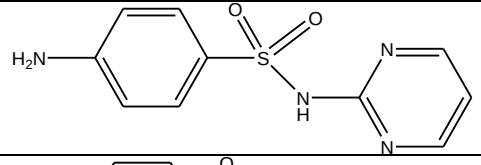
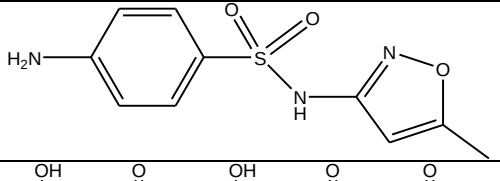
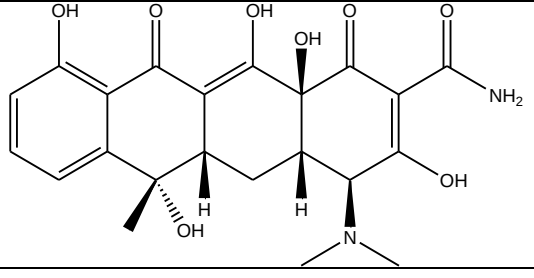
Table 1 Compound name, structure, compound class and reported surface water concentrations of the compounds discussed in this thesis.

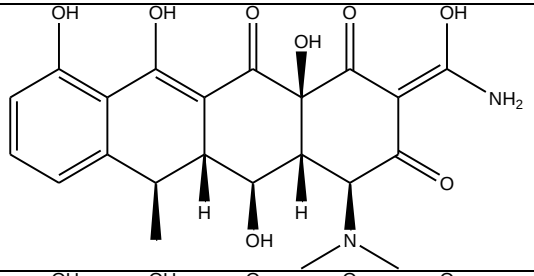
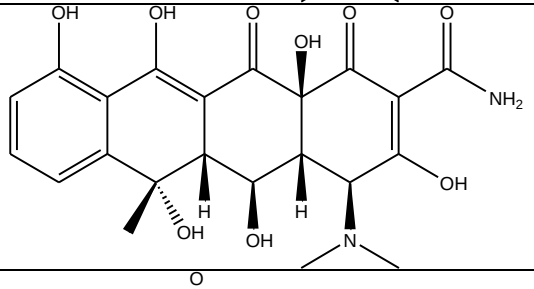
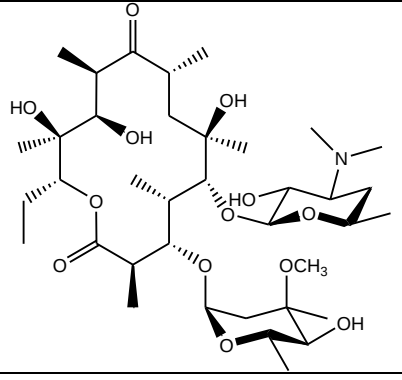
Compound	Structure	Class	Concentration ng l^{-1}	Reference
Ibuprofen		NSAID	2 – 64 5 – 90 1.5 – 7.8 64 – 141 144 – 2370 4.9 – 50.4	Lindqvist et al. Vieno et al. Buser et al. Metcalfe et al. Roberts et al. da Silva et al. [21,27,31-34]
Ketoprofen		NSAID	6.5 – 39 8 – 28 12 – 50 18 – 132	Lindqvist et al. Vieno et al. Metcalfe et al. da Silva et al.[27,31,32,34]
Naproxen		NSAID	17 – 313 5.5 – 57 13 – 32 94 – 207 29 – 87	Kosjek et al. Lindqvist et al. Vieno et al. Metcalfe et al. da Silva et al.[31,32,34-36]
Diclofenac		NSAID	9 – 282 2 – 40 10 – 55 26 – 194 4 – 148	Kosjek et al. Lindqvist et al. Vieno et al. Metcalfe et al. da Silva et al. [21,27,31,32]

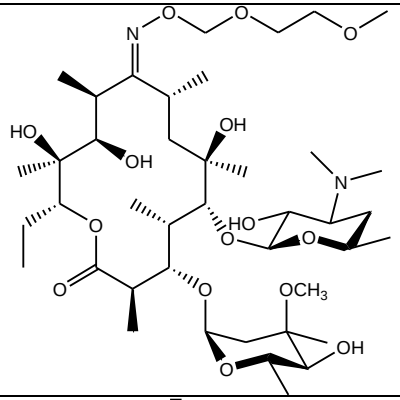
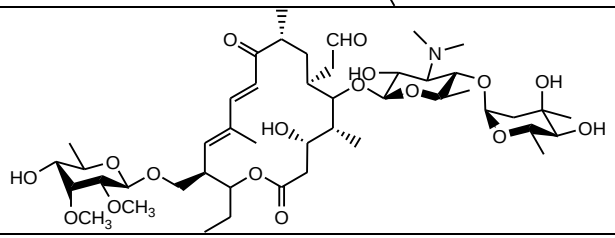
Bezafibrate		Lipid Regulator	6 – 64 52 3 – 24 3 – 20 0 – 10	Kasprzyk-Horden et al. Metcalfe et al. Lindqvist et al Vieno et al. Gros et al.[10,31,32,36,37]
Metoprolol		β-blocker	5 – 11 29 – 36 84 – 310 3 – 275 20 – 116	Kasprzyk-Horden et al. Alder et al. Kunkel et al. Daneschvar et al. Vieno et al. [4,18,37-39]
Bisoprolol		β-blocker	9.4 – 26	Gonçalves et al.[40]
Atenolol		β-blocker	4 – 488 0 – 250 58 – 83 3 – 271 2 – 25	Kasprzyk-Horden et al. Gros et al. Alder et al. Daneschvar et al. Vieno et al. 2[4,10,18,37,39]
Propranolol		β-blocker	6 – 40 7 – 8 1 – 3.5	Kasprzyk-Horden et al. Alder et al. Kunkel et al.[18,37,38]
Sotalol		β-blocker	0 – 70 45 – 52 61 – 160 2 – 169 15 – 52	Gros et al. Alder et al. Kunkel et al. Daneschvar et al. Vieno et al. 2[4,10,18,38,39]

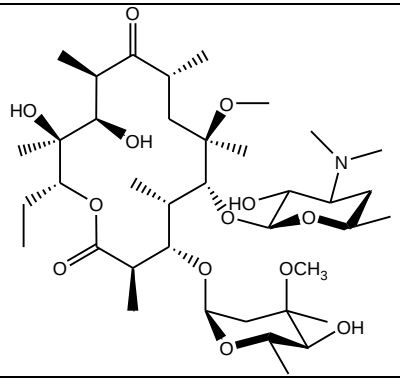
Acebutolol		β-blocker	0.5 – 2.2 2 – 8	Daneschvar et al. Vieno et al. 2[4,39]
Carbamazepine		Anti-Epileptic	5 – 356 20 – 185 0 – 110 20 – 66 20 – 1160 8 – 41	Kasprzyk-Horden et al. Metcalfe et al. Gros et al. Vieno et al. 2 Gonzales Alonso et al. Gros et al. 2[4,10,32,37,41,42]
Venlafaxine		Anti-depressant	35 – 71 8 – 44 13 – 45 100 – 1003	Baker et al. Gonzales Alonso et al. Gros et al. 2 Valcarcel et al.[3,41-43]

Fluoxetine		Anti-depressant	9 – 13.5 8 – 44	Baker et al. Gonzales Alonso et al.[3,41]
Sertraline		Anti-depressant	2.4 – 12.4	Conley et al.[44]
Citalopram		Anti-depressant	13 – 120 5 – 11 26 – 160	Gonzales Alonso et al. Gros et al 2. Valcarcel et al.[41-43]

Trimethoprim		Antibiotic	1 – 126 0 – 20 38 – 690 3 – 12	Kasprzyk-Horden et al. Gros et al. Valcarcel et al. Christian et al.[10,37,43,45]
Sulfadiazine		Antibiotic	270 0.5 – 72	Wei et al. Yan et al.[46,47]
Sulfamethoxazole		Antibiotic	1 – 2 32 – 952 100 4 – 52 1.5 – 57	Kasprzyk-Horden et al. Valcarcel et al. Wei et al. Christian et al. Yan et al.[37,43,45-47]
Tetracycline		Antibiotic	270 4 – 12 0.11	Wei et al. Yan et al. Lindsey et al. [46-48]

Doxycycline		Antibiotic	5.63	Yan et al.[47]
Oxytetracycline		Antibiotic	220 0.5 – 12 0.07 – 1.34	Wei et al. Yan et al. Lindsey et al. [46-48]
Erythromycin		Antibiotic	1 – 162* 0 – 30 90 – 3847 15.9 4 – 190 0.3 – 45	Kasprzyk-Horden et al. Gros et al. Valcarcel et al. Zuccato et al. Christian et al. Yan et al.[10,37,43,45,47,49]

Roxithromycin		Antibiotic	4 – 14 0.4 – 8	Christian et al. Yan et al.[45,47]
Tylosin		Antibiotic	0 – 2.2	Zuccato et al. 2[50]

Clarithromycin		Antibiotic	127 – 1727 20.3 1 – 37	Valcarcel et al. Zuccato et al. Christian et al.[43,45,49]

1.2.2 Fate of pharmaceuticals in the environment

In the years following the widespread observation of pharmaceuticals in the environment, a large part of the research in this field has focused on determining and understanding the fate of pharmaceuticals. Most pharmaceuticals are not persistent, meaning they are removed from the aquatic environment over time. There are four main pathways for the removal of pharmaceuticals: Sorption to particulate matter, biodegradation, photodegradation, and chemical hydrolysis. Many compounds not only undergo one of these processes, but a combination. For example, biodegradation is often preceded by sorption [51].

Sorption is driven by the partitioning of the pharmaceutical between solid particles and the liquid phase. For due to the diversity of group of chemicals that pharmaceuticals are, there are a number of processes involved in this partitioning. A value that is often used for the calculation of partitioning and equilibrium coefficients is the K_{ow} of pharmaceuticals. This value describes the partitioning of a compound between water and octanol, and is thus a measure of its polarity. The K_{ow} is well suited to estimate the partitioning between water and the organic carbon and lipids of microorganisms present in sediments, but due to the ionic nature of many pharmaceuticals their K_{ow} is strongly pH dependent, and other processes such as ionic interactions can have a strong impact on the partitioning. Table 2 gives an overview over the K_{ow} as well as the pK_a of all pharmaceuticals that are discussed in the experimental section of this thesis.

Table 2 Pharmaceuticals studied in this Thesis, Their $\log K_{OW}$ and pK_a

Compound	**$\log K_{ow}$**	**pK_a**
Ibuprofen	2.48(Scheytt)[52]	4.52(Rafols) [53]
Ketoprofen	2.81(Tadkaew)[54]	4.36(Rafols) [53]
Naproxen	3.00(Tadkaew)[54]	4.57(Rafols) [53]
Diclofenac	1.90(Scheytt)[52]	4.16(Rafols) [53]
Bezafibrate	4.25(Tiehm)[55]	3.61(Lindqvist) [31]
Metoprolol	1.80 (Narvaez)[61]	9.70(Jouyban)[56]
Bisoprolol	1.84(Detroyer)[57]	9.16(Detroyer)[57]
Atenolol	0.10(Tadkaew)[54]	13.88; 9.16 (Tadkaew)[54]
Propranolol	2.81(Narvaez)[61]	9.50(Jouyban)[56]
Sotalol	0.37(Detroyer)[57]	9.19 (Detroyer)[57]
Acebutolol	1.19(Detroyer)[57]	9.40(Jouyban)[56]
Carbamazepine	1.51(Scheytt)[52]	13.9; -0.49 (Tadkaew)[54]
Venlafaxine	2.8 (Minguez)[58]	9.6 (Singh)[59]
Fluoxetine	3.96(Narvaez)[61]	10.05 (Vasskog)[60]
Sertraline	5.1(Minguez)[58]	9.47 (Vasskog)[60]
Citalopram	3.5(Minguez)[58]	9.59 (Vasskog)[60]
Trimethoprim	0.79 (Tadkaew)[54]	7.20 (Tadkaew)[54]
Sulfadiazine	0.76 (Wehrhana)[62]	1.6; 6.5 (Wehrhana)[62]
Sulfamethoxazole	0.89 (Tadkaew)[54]	5.81; 1.39 (Tadkaew)[54]
Tetracycline	-1.3(Li)[63]	3.3; 7.7; 9.7 (Figuero)[64]
Doxycycline	-0.02 (Botelho)[65]	3.02; 7.97; 9.15 (Shariati)[66]
Oxytetracycline	-0.9(Li)[63]	3.57; 7.49; 9.88 (Figuero)[64]
Erythromycin	3.06(Botelho)[65]	8.88(Botelho)[65]
Roxithromycin	2.75(Botelho)[65,]	9.2 (Kees)[68]
Tylosin	1.63 (Yi)[67]	7.1 (Gingerich)[69]
Clarithromycin	0.41(Narvaez)[61]	9.2 (Goddard)[70]
Cloxacillin	2.53 (Chemspider)	2.7 (Newton)[71]
Cephalexin	-0.19(Narvaez)[61]	5.25; 7.1 (Newton)[71]
Ampicillin	1.35 (Hamscher)[72]	2.53; 7.24 (Newton)[71]
Benzylpenicillin	1.33(Narvaez)[61]	2.74 (Hamscher)[72]

In sorption studies tetracyclines and fluoroquinolone antibiotics showed the highest affinity to sediments and can be considered to bind irreversibly to most types of sediments. Due to the very low K_{ow} values of these compounds it can be concluded that these compounds bind via ionic interactions rather than lipophilic interactions[73].

Another group of compounds that has a strong tendency towards sorption to sediments are estrogens. These very lipophilic compounds follow hydrophobic partitioning mechanisms.

Antidepressants such as, fluoxetine, sertraline and venlafaxine can be found in sediments[74]. While citalopram was found to have a relatively low affinity to sediments in a study conducted in the USA, a Finnish study found citalopram to be the most common and abundant of 17 studied pharmaceuticals in suspended particulate matter near WWTPs[74,75] indicating a very high degree of sorption as citalopram has only been found at very low concentrations in Finnish WWTP effluent (**I**). One explanation for this discrepancy could be differences in the composition of these solid matrices.

The high affinity to sediments of the beta blockers salbutamol, timolol and nadolol compared to the very low affinity of sotalol, found by Ferreira da Silva et al.[34] in the Ebro river basin, shows that large differences can even be observed within one distinct compound group.

While sorption to sediments removes the parent compounds from the water phase, it is not a complete removal from the environment. Some compounds show very high stability in sediments and may still be bioavailable there over a longer period of time [76], such as estrogens. Nevertheless, sediments also play a very important role in the degradation of many pharmaceuticals. Biodegradation of some pharmaceuticals for example can only proceed within the sediments [51].

Unlike sorption, biodegradation is a true removal pathway, as it not only binds compounds, but chemically changes the parent compound. Biodegradation proceeds through various pathways; while biodegradation in higher organisms mainly produces phase I and phase II metabolites, bacterial degradation can lead to the complete mineralization of many organic compounds[77]. On the other hand bacterial biodegradation can also transform phase one metabolites back to the parent compound, as has been observed for e.g. carbamazepine [27].

As with sorption, biodegradation is a compound dependent process. While some compounds rarely undergo biodegradation, others are readily biodegraded only in sediments and pore water and again others undergo biodegradation even in the water column of rivers [51].

Compounds that are easily biodegraded are often already removed to a large extent during wastewater treatment. What is left of these compounds is usually removed very rapidly to levels below the limits of detection of most analytical methods once they enter the environment. Ibuprofen, a compound that easily undergoes biodegradation is, to some extent, an exception, due to the very high volumes that this nonsteroidal anti-inflammatory drug (NSAID) is used at. The removal rate of ibuprofen during wastewater treatment has been observed to be

significantly over 90 % in most studies[23]. Nearly all of this removal is due to biodegradation[77], making it the most easily biodegradable compound among the commonly studied pharmaceuticals.

Other compounds that easily undergo biodegradation are NSAIDs such as naproxen and ketoprofen [23]. Laboratory experiments by Kunkel et al. demonstrated that out of six tested pharmaceuticals (bezafibrate, clofibric acid, diclofenac, naproxen, gemfibrozil and ibuprofen), ibuprofen was the only one that was biodegraded in the water phase. None of the compounds were removed in abiotic experiments containing sediments, while bezafibrate, naproxen, diclofenac and ibuprofen all decreased in non-sterile tests with sediments indicating that sediments play a major role in the biodegradation of these pharmaceuticals[51].

Kunkel et al showed in a separate study, that while not undergoing biodegradation in the water phase, metoprolol, sulfamethoxazole and sotalol all were biodegraded in pore water. As none of these compounds showed significant sorption to the tested sediments, it can be concluded that much of the biodegradation linked to sediments takes place in the pore water rather than on the sediment particle surfaces[38].

Experiments by Löffler et al. and Quintana et al. indicate that biodegradation can often lead to full mineralization of pharmaceuticals making it a true removal pathway for many pharmaceuticals [77,78]. In contrast to this, phototransformation of pharmaceuticals has been linked to the formation of compounds that may be both more toxic and more persistent than the parent compound. Phototransformation can be divided into two different categories, direct photolysis and indirect photolysis. In direct photolysis the pharmaceutical itself contains functionalities that absorb UV light above a wavelength of 280 nm, the region where solar radiation begins. The absorbed energy can then lead to intra- or intermolecular reactions or dissociation of the parent compound.

Indirect photolysis describes a process, where not the parent compound itself absorbs the UV radiation, but other organic compounds present in environmental waters, such as humic acids, absorb the light, forming reactive species, mostly radicals, that then in turn react with the pharmaceutical compounds. As environmental waters are rarely free of these compounds, a combination of both pathways is most likely true for most compounds that undergo phototransformation[79].

The degree of phototransformation that can take place depends on a number of factors, such as turbidity, geographic location, and season. Most of the phototransformation takes place only in the very top layer of the water column. Nevertheless, it has been shown to play a significant role in the removal of a number of pharmaceuticals [38].

Several of the NSAIDs such as diclofenac, naproxen and ketoprofen have been shown to undergo direct photolysis. Figure 1 shows the main photoproducts of naproxen, of these, compounds a and b have been shown to be more toxic than naproxen itself[80,81].

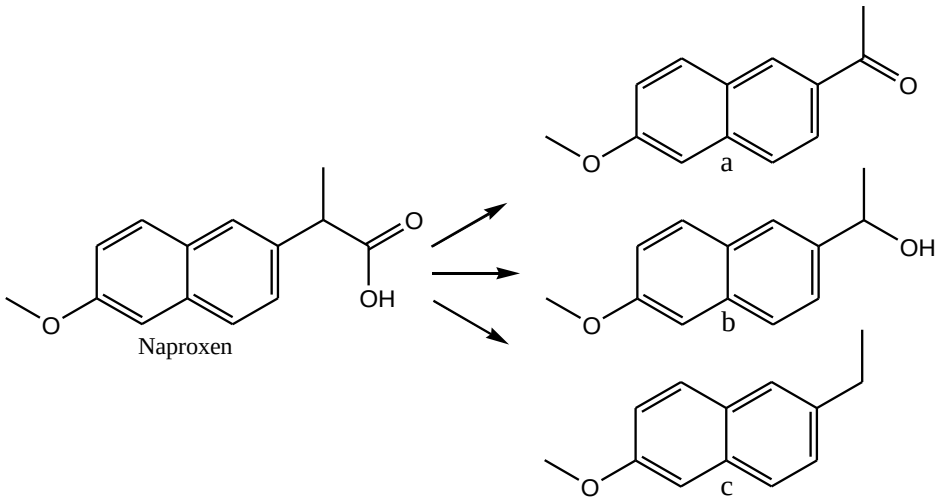


Figure 1. Phototransformation pathway of naproxen

On the other hand the photolysis end products of diclofenac (d,e,f) shown in Figure 2 are chemically unstable in water at environmental pH values [82].

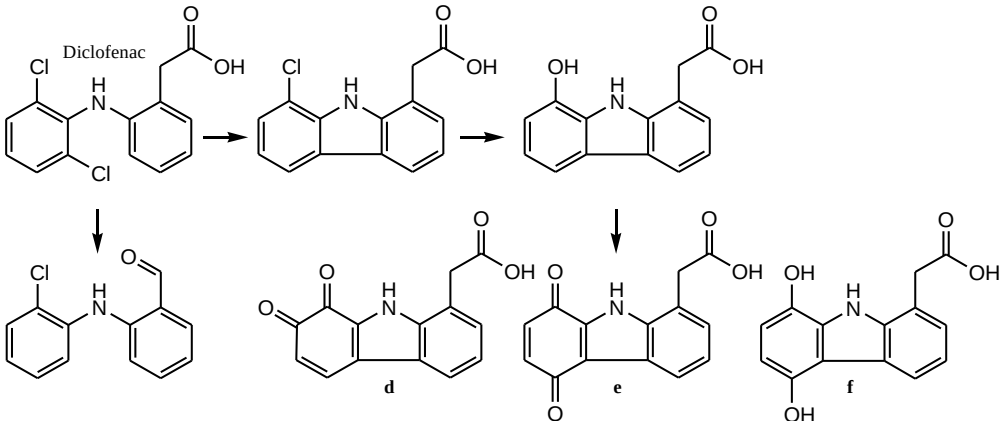


Figure 2. Phototransformation pathway of diclofenac

Ketoprofen is also readily phototransformed. Kosjek et al identified 22 phototransformation products, and concluded that phototransformation at 254 nm does not lead to complete mineralization of ketoprofen, and that a large

number of the products contained functional groups that have previously been associated with photo-allergic reactions as well as estrogenic activity[83]. Bezafibrate is another well studied compound that undergoes phototransformation under natural conditions.

Another example of phototransformation to more toxic and more persistent transformation products is that of the antimicrobial substance triclosan, which forms dioxins under UV radiation[84,85].

Figure 3 shows the transformation pathway determined by Latcher et al. including the compound 2,8-DCDD which belongs to the dioxin family. Dioxins are persistent organic pollutants (POPs) and have been shown to be persistent, bio-accumulated and toxic[86]. 2,8-DCDD originating from triclosan has been found in the aquatic environment[87].

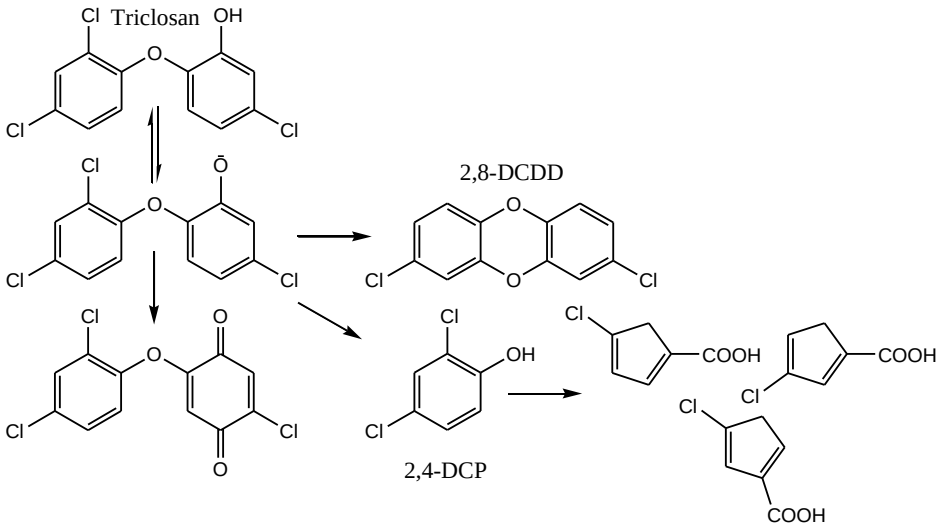


Figure 3. The Phototransformation pathway of triclosan.

1.2.3 Effects of pharmaceuticals in the environment

A number of effects attributed to the presence of pharmaceuticals in the environment have been observed. These effects can be divided into three categories of severity: a) effects that were observed in the environment and could later be attributed to the presence of pharmaceuticals; b) effects that were first detected in laboratory experiments, and later confirmed to also take place in the environment; c) effects that could be observed in laboratory experiments and may take place in the environment.

The first group is very short as it craves near catastrophic effects to the population of one or several species. A case that fits this category very well is that of diclofenac. Diclofenac is an anti-inflammatory drug that is commonly used in both human and veterinary treatment. Diclofenac has a very low acute toxicity to most mammals (LD_{50} = 240 mg/kg in Rat)[88], but is highly toxic to certain species of birds such as vultures (LD_{50} = 0.098 mg/kg in Oriental White-backed Vulture) where it causes renal failure[89]. The common use of this relatively inexpensive anti-inflammatory drug for the treatment of cattle in India caused the near extinction of several species of vulture in regions of India and Pakistan. Apart from the catastrophic effect on the vulture population which has declined by more than 95% (99.9% for the oriental white backed vulture)[90] for several species[91], this also had severe secondary effects on the areas environment and population. Starting from the economic losses due to the loss of income based around eco-tourism, and the additional costs related to the disposal of animal carcasses these problems extend further towards health concerns related to the large amount of animal carrion that was previously disposed of by vultures. Carcasses can be breeding grounds for a number of pathogens that can be very dangerous to humans, such as anthrax. Other problems with carcasses not being removed by vultures is the increase in the populations of other scavengers such as feral dogs[92]. India has the world's highest human rabies infection rate, 95% of which can be attributed to dog bites. It was long unclear what the cause of the decline in vulture population was until Oaks et al. could attribute the most common causes of death (visceral gout and renal failure) to residues of diclofenac[93].

The second case of pharmaceuticals identified as the cause of effects observed in the environment is the contraceptive 17 α-ethinylestradiol. In 1994, Purdom et al. observed the occurrence of hermaphrodite fish in lagoons of WWTP effluent, and experimentally proved that the effluent was estrogenic to fish[94]. In 1998, Desbrow et al. analyzed WWTP effluent and fractioned it to find the compounds responsible for the estrogenic effect. They found that the majority of the estrogenic effect in WWTP effluent came from the two natural estrogens estrone (E1) and 17

β-estradiol (E2) as well as the synthetic active ingredient in contraceptives 17 α-ethinylestradiol (EE2)[95,96]. Since then several studies have confirmed that WWTP effluent as well as environmentally relevant concentrations of the above mentioned estrogens cause physiological changes in male fish, most significantly vitellogenin (a protein used for the production of egg yolk in females) production and infertility [96].

Kidd et al. showed in an experiment in a natural lake that the entire population of certain fish species can collapse when exposed to worst-case-scenario concentrations of estrogens over a sustained period of time. Exposing a lake to 5 – 6 ngl^{-1} of EE2 caused reproductive failure of the fathead minnow population in this lake in the two consecutive years of its addition, leading to an almost complete collapse in the population. Other species were also affected by the addition of EE2, though not as dramatically as the fathead minnow[97,98].

Antibiotics resistance is an example of effects first observed in laboratory experiments, and then confirmed to take place in the environment. It has long been known that extended exposure to antibiotics gives rise to a selective pressure on bacterial communities, favoring resistant strains. These resistant bacteria then thrive significantly better in the presence of antibiotics, and can even pass on their resistance genes to other bacteria[99]. Based on this knowledge a number of studies have been performed with bacterial colonies in and near WWTPs as well as in and surrounding hotspots for veterinary use of antibiotics such as fish farms, manure pits and agricultural runoff ditches. Many of these studied areas had a significantly higher rate of antibiotics resistance than bacterial communities from pristine sites [100-103].

Antibiotics resistance can together with endocrine disruptors and the very specific case of diclofenac poisoning of vultures be considered one of the major causes of concern also for the human population. Antibiotics are a very important class of pharmaceuticals that can cure diseases that are potentially fatal without effective treatments.

Apart from these specific cases, a number of laboratory studies have shown potential physiological effects on aquatic organisms exposed to pharmaceuticals at environmental levels [104,105]. Behavioral changes attributed to psychoactive drugs have also been observed. These changes are difficult to confirm in the environment, but might be of interest as they are likely to start at exposure levels significantly below those needed to cause physiological changes[106].

1.3 Illicit drugs in wastewater

The consumption of illicit drugs is widespread, and causes various social, economic and health problems. The first critical step for the development of successful counter programs is the accurate assessment of the prevailing consumption trends and amounts. Traditionally this data has been collected using questionnaires, arrest reports, hospital records and confiscation data[107]. Arrest reports, hospital records and confiscation data only show a fraction of actual consumption, and questionnaire based data can be biased depending on who is asked, how truthfully these people feel they can reply, and whether or not the user is even fully aware of what substances they are consuming[107]. Another disadvantage with questionnaire based date is that it takes a long time to compile, and covers a large period of time, making it difficult to follow trends in a timely manner.

In recent years the discovery of illicit drug residues in untreated wastewater has coined the idea to follow illicit drug consumption by measuring the concentrations in WWTP influents and calculating consumption data from this value.

1.3.1 Methodology

The first step in the determination of illicit drug consumption by wastewater analysis is to define suitable target analytes. The analytes should be either the parent compound, or a metabolite of the illicit drug of interest, it should be specific for one particular illicit drug, and present in wastewater at detectable quantities.

The analytical methodology to determine the concentrations of these target molecules is almost exclusively based on solid phase extraction (on-line or off-line) followed by LC – MS analysis using either a high resolution or triple quadrupole instrument. The obtained concentrations are then converted into loads/population (e.g. mg/1000 inh/day). If only data on trends or comparisons between locations is required, results can be reported as such. For data on actual consumption these loads have to be converted into consumption, using a factor that takes into account the metabolism and excretion pattern of the consumed parent compound as well as possibly the stability in the wastewater system.

1.3.2 Results obtained in early studies

The presence of illicit drugs in wastewater was first observed in the late 90's and early 2000's [108] and Daughton suggested the possibility of monitoring illicit drug use through wastewater analysis already in 2001[109]. The first study that attempted to do this was carried out by Zuccato et al in Italy in 2005[110]. The only compound

studied in this first study was cocaine, targeting both the parent compound and its main metabolite benzoylecgonine. The study could identify the compounds in both the river Po as well as in wastewater, giving a first number on the consumption calculated from wastewater of 27 doses (100 mg) per 1000 young adults per day. This was a number that exceeded the official numbers from traditional data collection methods[110].

One year later the same group published results containing a significantly increased number of analytes such as amphetamine, methamphetamine, MDMA, as well as a number of cocaine and heroin metabolites[111]. Since then a number of studies both in Europe and the USA were conducted, including the two European comparison studies included in this thesis[112-116].

1.3.3 Comparison to conventional methods

To the best of the authors knowledge there has been only one study conducted that directly compares results obtained using wastewater analysis and two other methods, over the same time period and the same population. This study was carried out by Reid et al. in Norway[117], comparing wastewater analysis results to questionnaire based results and results obtained through saliva tests on drivers. Despite being voluntary, with about 90 % of the drivers participating, the saliva study gave a higher prevalence of cocaine use than the combined population surveys. Due to the relatively short period that cocaine is detectable in saliva, the possibility that drivers who had recently consumed cocaine were more likely to decline participating in the test and that socially marginalized heavy users are less likely to own a motor vehicle the recorded prevalence of 0.7 % of drivers can be considered to be a minimum value[117]. While underreporting in surveys on topics such as drug use can be expected, the total annual consumption calculated from wastewater loads and the survey did not differ significantly. This indicates that while underestimating the prevalence the amount consumed by each user might be overestimated, balancing out the total annual consumption. This can be attributed for example to purity of street cocaine or incorrect estimation of the own use by cocaine users[117].

Another possibility is that the wastewater study underestimated the total consumption due to factors explained in more detail in the following chapter.

1.3.4 Advantages and challenges

Wastewater epidemiology has been viewed with some criticism by many epidemiologists as it cannot provide the standard measure of drug use; the prevalence, neither can it differentiate between the consumption of different age- or other well defined population groups.

Even when seeing wastewater epidemiology as what it is, a strong objective additional tool to monitor trends and regional variations in real time, it is important to keep a number of challenges in mind.

The first challenge is the user. A small number of marginalized heavy users is responsible for a large fraction of the total consumption of drugs. Although in countries such as Finland, many of these heavy users still live in houses that are connected to the public sewer, this is not the case in all countries. This higher likelihood of heavy users not contributing to wastewater samples can cause a bias in the results.

The next challenge is the sewer system and the possible methods for sampling wastewater. Sewer systems generally cover large areas and populations, leading to big differences in residence time in the system for wastewater arriving at the WWTP from different parts of a city. Many compounds are continuously degraded while travelling through the sewer system and this can also cause a bias that is very difficult to quantify[118].

As the prevalence of drug use is relatively low the measured loads are due to a relatively low number of distinct toilet flushes, which makes it very important to have a sampling method that can give a representative sample of the entire population[119].

The final challenge is the back calculation. There are very little metabolic data on illicit drugs, and even less on how co-consumption of several drugs affects the metabolism. This makes it difficult to calculate consumed amounts from loads in wastewater. Not performing this back calculation step will give a definitive number for the load, but can be misleading, as the compounds found to have the highest loads are not necessarily the ones consumed the most. Performing the back calculation, will give consumption data that will correct for these effects, but should only be compared to data obtained using the same methods[118].

If all of these factors are kept in mind, wastewater epidemiology could be a very strong tool for continuous real time monitoring of drug consumption trends.

1.4 Tributyltin

The accumulation of fouling organisms on ships is a major problem in commercial as well as leisure shipping. Fouling organisms cause additional drag and weight, lowering the speed and fuel efficiency of ships and boats[120].

To inhibit the growth of fouling organisms on ships' hulls a number of technologies have been used. For over 30 years starting in the 1970s and ending with a final ban in 2008 tributyltin (TBT) containing polymer paints were the most popular anti-fouling systems, accounting at times for over 70 % of all anti-fouling systems used by the world's ocean-going fleet[121].

TBT has been claimed to be one of the most toxic compounds ever intentionally released into the environment causing a variety of disorders in marine organisms[122-124].

1.4.1 Organic tin compounds

Organotin compounds (OTCs) are a group of compounds with the basic structures R_nSnX_{4-n} where n is a number between one and four, R is an organic group covalently bound to the central tin atom and X is an anionic species.

The first organotin compounds were synthesized and studied in the mid-19th century [125] and the first application was as an additive in plastics. These compounds were soon found to have strong biocidal properties, leading to their extensive use as fungicides and anti-fouling agents.

1.4.2 Toxicity of organic tin compounds

The first systematic study of the toxicity of organic tin compounds was conducted by van der Kerk et al in 1954[125]. This study showed that tri organic tin compounds had the highest toxicity towards fungi, while mono organic tin compounds and inorganic tin showed very little toxicity. This is true for all side chains and all target species, although the effect of the side chain differs between target species, triethyltin is most toxic to mammals, trimethyltin is most toxic to insects and tributyltin is most toxic to fungi and aquatic organisms[125].

Also the acute toxicity of organic tin compounds depends strongly on the target species, and the developmental stage they are in. For aquatic invertebrates LC_{50} values for TBT between 0.2 and 32 µg l^{-1} have been published after 48h of exposure[122,126-128]. After eight days of exposure concentrations as low as 15 – 20 ng l^{-1} were shown to be lethal to *Acartia Tonsa* larvae[122].

Mammals are significantly less susceptible to TBT. Studies on Syrian hamsters and rats showed LD_{50} values between 146 and 277 mg kg^{-1} body weight[129]. The acute toxicity towards aquatic vertebrates lies in-between these regions[130].

Although the acute toxicity of TBT is high enough to be of concern even at environmental concentrations, especially near harbors, chronic toxicity which shows effects at far lower concentrations is of concern for significantly larger areas.

Soon after TBT had become a popular anti-fouling agent, significant effects were observed in the environment, which could later be linked to TBT exposure.

The first observations of the chronic toxicity were reported in the late 1970s in the Arcachon Bay in France. The bay only has a narrow access to the Atlantic and contains both large oyster beds and several marinas. The extensive use of TBT antifouling paints caused the oysters in the bay to form shell anomalies, slow or no growth of the oysters and finally a complete inhibition of reproduction[131,132].

Further studies revealed that oysters and clams experience severe cell damage and calcification anomalies of the shells at concentrations as low as 2 ng l^{-1} causing a high mortality rate as well as reproductive failure. These laboratory studies translate very well into the environment where clear connections between growth rates and the distance to known TBT hot-spots can be observed[133-135].

Oysters were the first organisms where the effect of environmental TBT concentrations was observed, as they are an important industry along the coastline and in estuaries, where the highest TBT concentrations are often observed.

The class of organisms with the highest sensitivity to chronic TBT exposure is the gastropods. Gastropods can experience imposex at concentrations below 0.5 ng l^{-1} [123]. Imposex is an endocrine disorder, which causes the female of a species to produce male genitalia. While early stages of imposex do not necessarily affect the fertility of a female, concentrations of more than 2 ng l^{-1} have proven to cause complete infertility to many species of gastropods[123].

1.4.3 Occurrence of TBT in the environment

While most of the OTC's are being used as stabilizers for PVC production, most of the TBT found in the aquatic environment today can be traced back to anti-fouling paints.

There were two types of TBT containing paints that have been used over the years. The first are so called Free Association paints, where the TBT is freely dispersed in the paint, and released uncontrolled into the surrounding water (Figure 4).

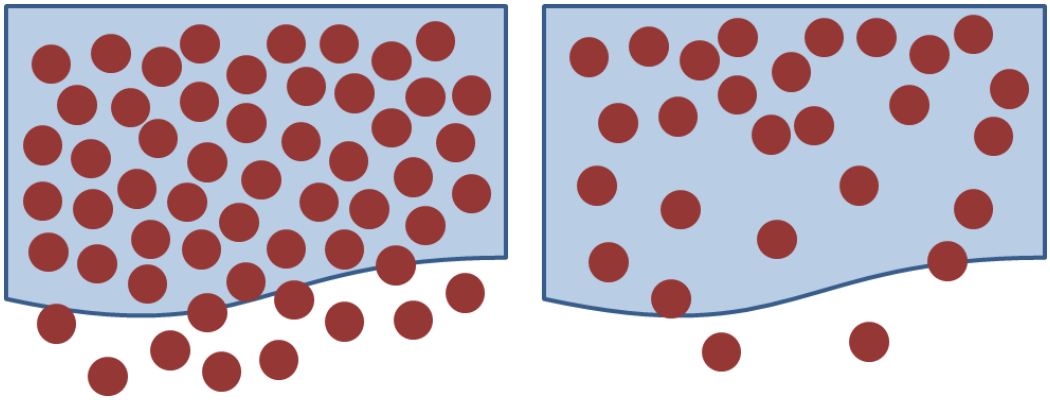


Figure 4 Schematic representation of a TBT free association paint, the TBT leached freely from the paint matrix.

The second type are the so called self-polishing polymer paints, where the TBT is covalently bound to the polymer paint, and released controlled layer by layer, (Figures 5 and 6) this creates a more durable paint, which can last for up to 5 years, and was the cheapest and most popular anti-fouling option[136].

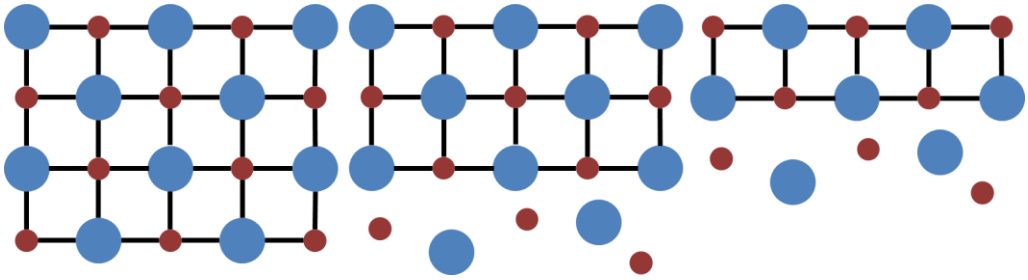


Figure 5. Schematic representation of a TBT self-polishing polymer paint. The polymer and biocide are released layer by layer

In both cases the anti-fouling effect is achieved by the high concentration of TBT in the water layer closest to the hull, which prevents fouling organisms from

attaching to the hull. The leaching rates of these paints lie between 0.1 and 5 μg/cm^2/day[136].

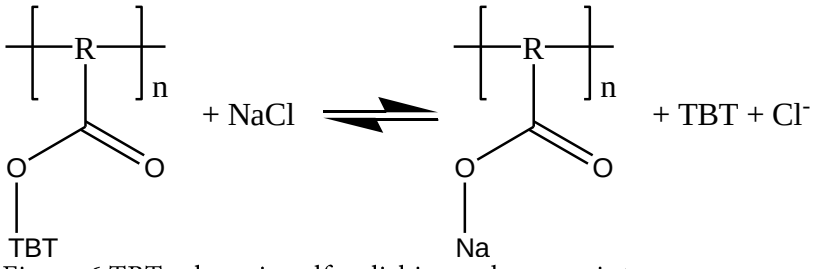


Figure 6 TBT release in self-polishing polymer paints.

Once in the aquatic environment TBT has a strong affinity to sediments. After 14 days, 72% of the TBT added to the water column of a model ecosystem can be detected in the sediments[137]. With further large amounts found in plants (6%) and snails (16%), and only 9% remaining in the water column[137]. This can also be observed in environmental samples with the highest concentrations of TBT found in the sediments close to harbors and shipping lanes, where concentrations in the μg kg^{-1} up to mg kg^{-1} ranges have been found[138-140].

TBT is readily bio accumulated, especially in fatty tissues, such as the liver, and has been found in the environment in among others mussels, fish, dolphins, sea otter and sea birds. The published concentrations range from 0.2 μg kg^{-1} wet weight in mussels in Greenland to 3020 μg kg^{-1} wet weight in the liver of a sea otter from the California coast[124,141-147].

Degradation in the environment, especially once bound to sediments in colder climates is very slow, and TBT is expected to be persistent for decades, which can be seen by the continued presence of TBT in sediments despite the general ban in 2008[148].

The main degradation pathway in the environment is believed to be biodegradation, which takes place through a stepwise de-alkylation of TBT via DBT and MBT to inorganic tin[149,150].

1.4.4 Legislation

France being the first country to detect the detrimental effect of TBT on the environment, and more importantly the oyster farming industry, were also the first to make regulations limiting the use of TBT as an anti-fouling agent. At this point (in 1982) the oyster industry in only the Arcachon Bay had already lost an estimated 147 million US dollars in revenue due to TBT contamination[131]. The first regulation only applied to leisure craft less than 25m in length and only to paints containing a high TBT concentration, but was already extended to all TBT paints later the same year. The ban had the desired effect, and the French oyster populations recovered by 1984[131]. The next countries to regulate the use of TBT were the UK (1986), the USA (1988) and Japan (1990)[151]. All these regulations only applied to smaller craft. Only 10 years later, in 2001, the IMO passed the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, which prohibits any application of TBT containing paints to ship hulls "twelve months after the date on which no less than twenty-five States, the combined merchant fleets of which constitute not less than twenty-five percent of the gross tonnage of the world's merchant shipping"[152] have signed the convention. The convention came into force in September 2008 and has since effectively banned any use of TBT in the marine environment.

2 Materials and methods

2.1 Instrumentation

Two different LC-MS/MS systems were used for the studies presented in this thesis. For papers (I), (III), (IV) and (V), a Micromass QuattroMicro triple quadrupole mass spectrometer equipped with an ESI source was used. The mass spectrometer was coupled to an Agilent 1100 series HPLC equipped with a binary pump, a vacuum degasser, and a thermostatted column oven.

In paper (II) an Agilent 6460 triple quadrupole mass spectrometer equipped with an Agilent jet stream ESI source was used. This mass spectrometer was coupled to an online SPE system consisting of an Agilent 1290 HPLC equipped with a binary pump (Pump 1), a vacuum degasser, and a thermostatted column oven for the analytical separation, as well as an Agilent 1100 series HPLC equipped with a binary pump (Pump 2) for loading the trapping column. An Agilent 1260 auto sampler with a 900 µl analytical head and a 900 µl needle seat capillary was used for high volume injections. Pumps one and two were coupled together via a two position six-port-valve (Figure 7).

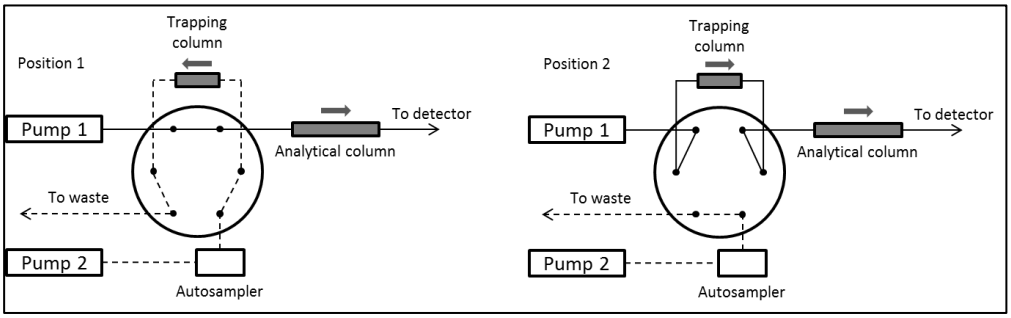


Figure 7 Schematic representation of the on-line SPE system used in II

2.2 Illicit drug analysis

2.2.1 Sampling

Samples were taken from the Kakola WWTP in Turku as well as the Viikinmäki WWTP in Helsinki as 24 h composite samples for seven consecutive days in the spring of 2011, 2012, and 2013.

2.2.2 Solid phase extraction

A modified version of the method described by Castegliони et al. was used for solid phase extraction[111]. In short, Waters Oasis MCX (3 cm^2, 60mg) cartridges were preconditioned with 6ml of methanol and 6ml of water at pH=2. The samples were then split into 300 ml triplicates, before adding the internal standards (100ng in 100 µl ACN) and filtration on Whatman GF-C filters. The cartridges were loaded with the samples, dried under nitrogen and subsequently eluted with 3 ml of MeOH and 3 ml of 2% NH_4 in MeOH. The eluates were dried under nitrogen and re-dissolved in 200 µl of water.

2.2.3 LC-MS/MS method

The concentrations of amphetamine, methamphetamine, MDMA (ecstasy) and cocaine, as well as the cocaine metabolite benzoylecgonine were monitored for this study. The analysis of illicit drugs was performed by two different methods. The first method was used for paper (III) while the second method was used for paper (IV).

2.2.3.1 Analysis method used in (III)

For the mass spectrometric analysis of illicit drugs the mass spectrometer was run in MRM mode. Two transitions were monitored for each compound, and individual stable isotope labeled internal standards were used (Table 3).

Table 3 Illicit drugs monitored as well as their MS conditions and internal standards

Analyte	Ion mode	Precursor ion (m/z)	Product ions (m/z)	Cone voltage (V)	Collision energy (eV)	Internal standard
Cocaine	ESI+	304.0	182.2	20	23	Cocaine-D_3
			150.1		23	
Benzoylecgonine	ESI+	290.0	168.3	27	21	Benzoylecgonine-D_3
			104.8		21	
MDMA	ESI+	194.0	163.1	30	12	MDMA-D_5
			135.1		18	
Methamphetamine	ESI+	150.0	119.3	20	14	Methamphetamine-D_5
			91.2		14	
Amphetamine	ESI+	136.0	119.1	10	10	Amphetamine-D_5
			90.9		14	

The chromatographic separation was performed on a Waters XBridge C_{18} column (2.1 x 50 mm; 3.5 µm) with water (A) and acetonitrile (B) as eluents. Both eluents contained 0.5% acetic acid as an additive. After one minute of isocratic elution at 3% (B) the percentage of organic modifier was increased in a liner gradient from 3% (B) to 65% (B) over 13 min. The conditions were held at 65% (B) for one minute before being returned to the initial conditions over the next minute. The system was given eight minutes to equilibrate between injections. The flowrate was 0.3 ml min^{-1} and the injection volume was 30 µl.

2.2.3.2 Analysis method used in (IV)

For the analysis of illicit drugs in (IV) the mass spectrometric conditions were kept as in (III) with changes made to the chromatographic conditions.

The chromatographic separation was performed on a Waters Atlantis C_{18} column (2.1 x 100 mm; 3.5 µm) with water (A) and acetonitrile (B) containing 0.5% acetic acid as eluent. After one minute of isocratic elution at 0% (B) the percentage of (B) was raised in a linear gradient to 55% (B) over 11 min. The eluent composition was kept at 55% (B) for one minute before raising the percentage of (B) to 95% over the next minute. The column was washed at 95% (B) for one minute before returning to the initial conditions over the following minute. The column was given eight minutes for equilibration between injections. The flowrate was 0.3 ml min^{-1} and the injection volume was 30 µl.

2.3 Rakkolanjoki

2.3.1 The River Rakkolanjoki

The River Rakkolanjoki (Figure 8) is situated in eastern Finland, and continues into Russia as the River Seleznevka before flowing into the Bay of Finland at Vyborg. The Finnish stretch of this river was chosen for this study as it gives the possibility to follow pharmaceuticals downstream from a single point-source for a long distance and residence time. The WWTP of Lappeenranta which serves approximately 60 000 inhabitants can be considered to be the source of the river.

The studied river stretch can be divided into three sections: the upper section is 20 km long, about two meters wide and half a meter deep, with a median residence time of 32 h. The middle section is the shallow eutrophic Lake Haapajärvi, with a surface area of 220 ha an average depth of 1.4 m and a median residence time of 159 days. In the third section, the river continues for another nine kilometers to the Russian border. Here the river is shallow and fast flowing with a median residence time of 5 hours. The average content of wastewater is 53% in the upper stretch of the river and 22% in the lower part of the river.

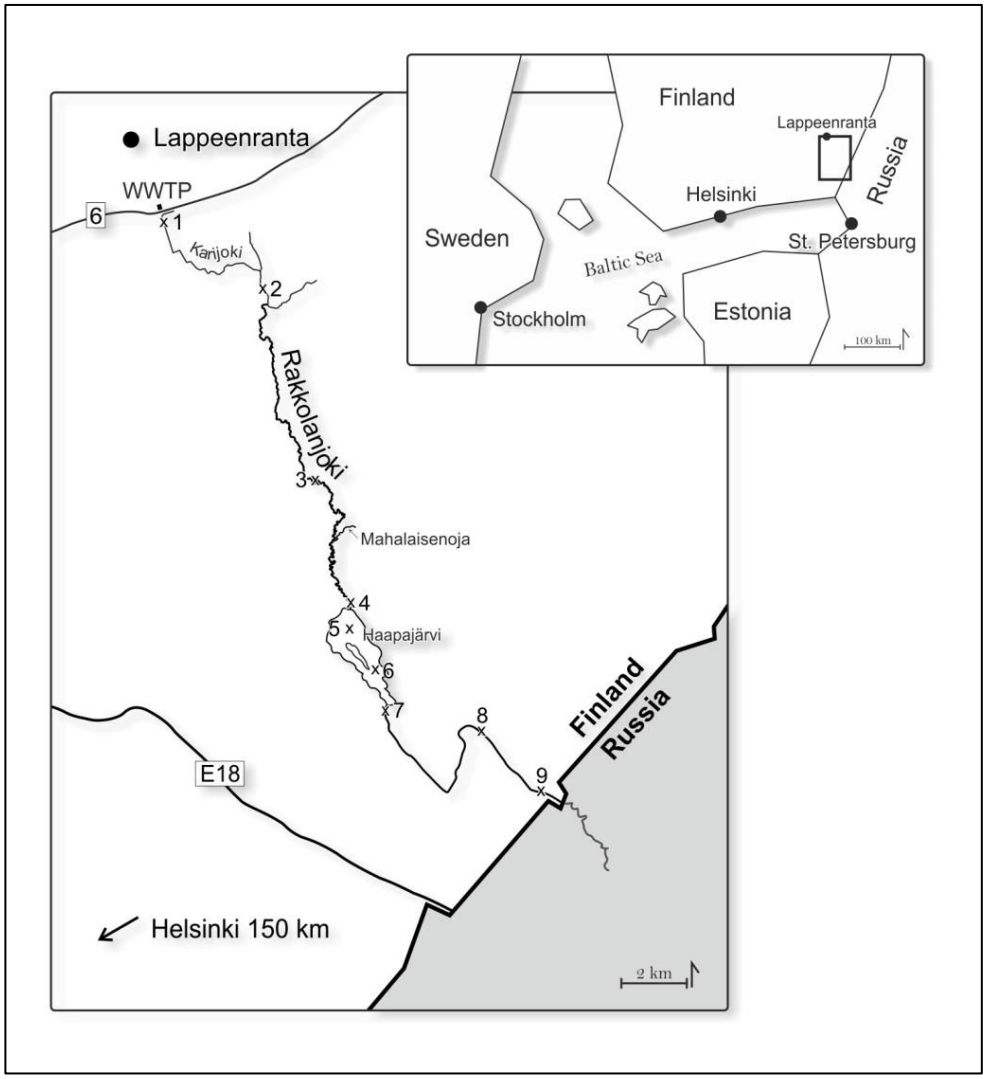


Figure 8 Map of the River Rakkolanjoki with sampling sites 1 to 9

2.3.2 Sampling

Samples were taken four times during 2010, in February, when the river is fully covered with ice and snow, in May when there is plenty of sunlight and rising temperatures, in June with plenty of sunlight and high temperatures as well as November with little sunlight and sinking temperatures.

Grab samples were taken at seven points along the river and two points in the lake, with additional samples taken from the main tributaries in November as well as the entry points of these tributaries into the river.

2.3.3 Extraction and analysis

Table 4 shows the studied compounds as well as the monitored MRM transitions and their respective internal standards.

For sample preparation a modified version of the extraction and analysis method published by Vieno et al. and Daneshvar et al. was used[4,39,153]. In short, the samples were divided into two subsamples, one for the analysis of acidic compounds, and one for the analysis of basic and neutral compounds. The samples were filtered, before adjusting the pH to 2 and 10 for acidic and basic compounds respectively. The internal standards were added (200 ng) before loading the samples onto Water Oasis HLB SPE cartridges that had been preconditioned with 5 ml of MeOH and 5 ml of water at the pH of the respective sample. After loading, the cartridges were dried under nitrogen, and subsequently eluted with 4 ml of acetonitrile. The eluate was evaporated to dryness, and re-dissolved in 500 µl 4% acetonitrile in a 0.5% aqueous solution of acetic acid for the analysis of basic compounds and 10% acetonitrile in 10 mM NH_4OH for the analysis of acidic compounds.

Table 4 Monitored analytes, their internal standards, precursor and product ion m/z as well as the mass spectrometric conditions used.

Analyte	Ion mode	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)	Internal standard
Bezafibrate	ESI-	360	274	23	18	Fenoprop
Diclofenac	ESI-	294	250	15	12	Fenoprop
Ketoprofen	ESI-	253	209	14	8	Fenoprop
Naproxen	ESI-	229	170	11	16	Naproxen-D3
Ibuprofen	ESI-	205	161	15	8	Fenoprop
Fenoprop	ESI-	267	195	15	13	IS
Naproxen-D3	ESI-	232	188	11	16	IS
Sotalol	ESI+	273	255	19	12	Alprenolol
Atenolol	ESI+	267	145	33	25	Alprenolol
Acebutolol	ESI+	337	116	37	29	Alprenolol
Metoprolol	ESI+	268	133	32	24	Alprenolol
Bisoprolol	ESI+	326	116	33	21	Alprenolol
Citalopram	ESI+	325	109	20	23	Alprenolol
Venlafaxine	ESI+	278	121	27	20	Alprenolol
Carbamazepine	ESI+	237	194	30	19	Dihydrocarbamazepine
Fluoxetine	ESI+	310	148	13	10	Sertraline-D3
Sertraline	ESI+	306	275	23	13	Sertraline-D3
Dihydrocarbamazepine	ESI+	239	194	36	23	IS
Sertraline-D3	ESI+	309	275	23	13	IS

For the quantitative analysis of pharmaceuticals the mass spectrometer (see 2.1) was run in MRM mode. The monitored MRM transitions can be found in Table 4. Acidic and basic compounds were run in separate methods with negative or positive electrospray ionization respectively.

The chromatographic separation was performed on a Waters XBridge C_{18} column (2.1 x 50 mm; 3.5 µm) for both the acidic and the basic compounds. Acetonitrile (B) and 10mM aqueous NH_4OH (A) were used as eluents for acidic compounds. The column was eluted isocratically with 3% B for one minute followed by a linear gradient to 65% B over the course of 9 min. The percentage of acetonitrile was held at this value for 1 min and was then returned to the initial value over the next min. The system was given eight minutes to equilibrate before the next injection. The flow rate was 0.3 ml min^{-1} and the injection volume was 30 µl.

For basic compounds elution was carried out with acetonitrile and water, both containing 0.5% acetic acid. The eluent initially consisted of 3% acetonitrile and the percentage of acetonitrile was raised to 65% in a linear gradient over 14 min. The percentage of acetonitrile was kept at 65% for one minute before returning to the initial conditions over the next minute. The system was then given eight minutes to equilibrate before the next injection. The flow rate was 0.3 ml min^{-1} and the injection volume was 30 µl.

2.4 Antibiotics

2.4.1 Studied compounds and samples

The 17 antibiotics monitored in this study were selected from annual human prescription data (FIMEA 2013), as well as some of the most used veterinary antibiotics in Finland. A full list of all compounds can be found in Table 5.

The method was tested on grab samples taken from the River Vantaajoki. Samples were taken at three points; the discharge point of the WWTPs of Hyvinkää and Riihimäki (67 000 PE combined) 45 km downstream, close to the point of entry into the Gulf of Finland, as well as from the pristine River Kervajoki.

2.4.2 Online-SPE method

For analysis of antibiotics the on-line SPE LC – MS/MS system described in chapter 2.1 was used. An Agilent Poroshell HPH C_{18} column (2.1 x 50 mm, 2.7 µm) was used for the analytical separation; while an Agilent BE online PLRP-S (2.1 x 12.5 mm) column was used as a trapping column. Acetonitrile (B) and water (A), each containing 0.1% formic acid, were used as eluent for both pump 1 and 2. Prior to analyses 1 ng of the internal standards was added to 10 ml of the samples. The samples were then centrifuged and 6 ml were transferred to 6 ml vials for analysis. After injection of 1.8 µl, the sample was loaded onto the trapping column for 4 min with pump 2 using 100 % eluent (A). After 4 min the six-port-valve was switched to position 2 (Figure 7) and pump 1 was run in a linear gradient from 4 % (B) to 90 % (B) over 6 minutes. The eluent composition was held at 90 % (B) for one minute before being returned to the initial conditions over the next 0.1 minutes and given three minutes for equilibration. The flowrate of pump 1 was 0.45 ml min^{-1} while pump 2 was run at 1 ml min^{-1}.

Table 5 Antibiotics monitored as well as MS conditions

Target compound	Prec Ion MS1	Prod Ions	Frag (V)	CE (V)	Ret Time (min)	Ret Window (min)
Ampicillin	350.1	159.9 106.2	110	12 20	5.39	0.94
Benzylpenicillin	335.1	176.1 160	100	9 9	6.7	0.76
Carbamazepine	237.3	194 179	130	17 37	6.77	0.84
Cephalexin	348.1	174.1 158	95	9 5	5.43	1.23
Clarithromycin	748.5	158.1 83.1	165	29 60	6.83	1.04
Cloxacillin	436.1	277.1 160.1	100	9 9	7.31	0.92
Doxycycline	445.2	428.1 98.1	125	17 53	6.12	0.92
Erythromycin enol ether	716.5	558.4 158.1	165	13 29	6.87	1.08
Erythromycin	734.5	576.2 158	160	16 32	6.46	1.04
N-acetyl sulfadiazine	293.1	134.1 65.1	110	21 49	5.47	1.57
N-acetyl sulfamethoxazole	296.1	134.1 65.1	110	25 49	6.22	0.92
Oxytetracycline	461.2	443.2 426.1	120	9 17	5.52	1.04
Roxithromycin	837.5	679.4 158.1	190	17 37	6.88	0.92
Sulfadiazine	251.1	156 92.1	105	13 25	5.34	0.79
Sulfamethoxazole	254.1	156 92.1	105	13 25	6.12	1.29
Tetracycline	445.2	427 410	110	8 16	5.63	1
Trimethoprim	291.2	230.1 123.1	130	21 25	5.42	1.57
Tylosin	916.5	174 101.1	215	44 56	6.6	1.21
4-epi-tetracycline-D_6	451.2	416.2	135	17	5.49	0.55
Benzylpenicillin-D_7	342.1	183.1	90	9	6.69	0.76
Sulfadiazine-D_4	255	96.1	110	25	5.34	0.92
Trimethoprim-D_3	294	123.1	145	25	5.41	1.44
Cephalexin-D_5	353	158	95	5	5.43	0.93
Carbamazepine-D_8	245	202.1	105	20	6.73	1.27

2.4.3 Mass spectrometry

The mass spectrometer was run in MRM mode. The parent and daughter ions, as well as the optimal fragmentor voltage and collision energy, were found through injection of individual standards using the Mass Hunter Optimizer software, while other mass spectrometric conditions were optimized manually for the entire method.

Two MRM transitions were monitored for each compound. Individual isotope-labeled internal standards were used when available, for other compounds an internal standard was selected by compound group, similarity in retention time, and matrix effect. Tables 5 and 6 (page 62) show all the compounds, the monitored MRM transitions as well as the mass spectrometric conditions and the internal standards used.

The method was tested for linearity, repeatability as well as matrix suppression and relative matrix suppression. The LOQ for each compound was defined as the concentration giving a signal-to-noise value of 10 or an area 5 times higher than that of blank samples.

2.5 Tributyltin

2.5.1 Sampling area

All samples were taken from the Airisto Canal in the Archipelago Sea. The area is characterized by a high inflow of river water, leading to low salinity and an accumulation of organic and clay rich sediments, leading to regular dredging and marine dumping.

There are two large harbors (in Turku and Naantali) as well as a dry dock in the study area.

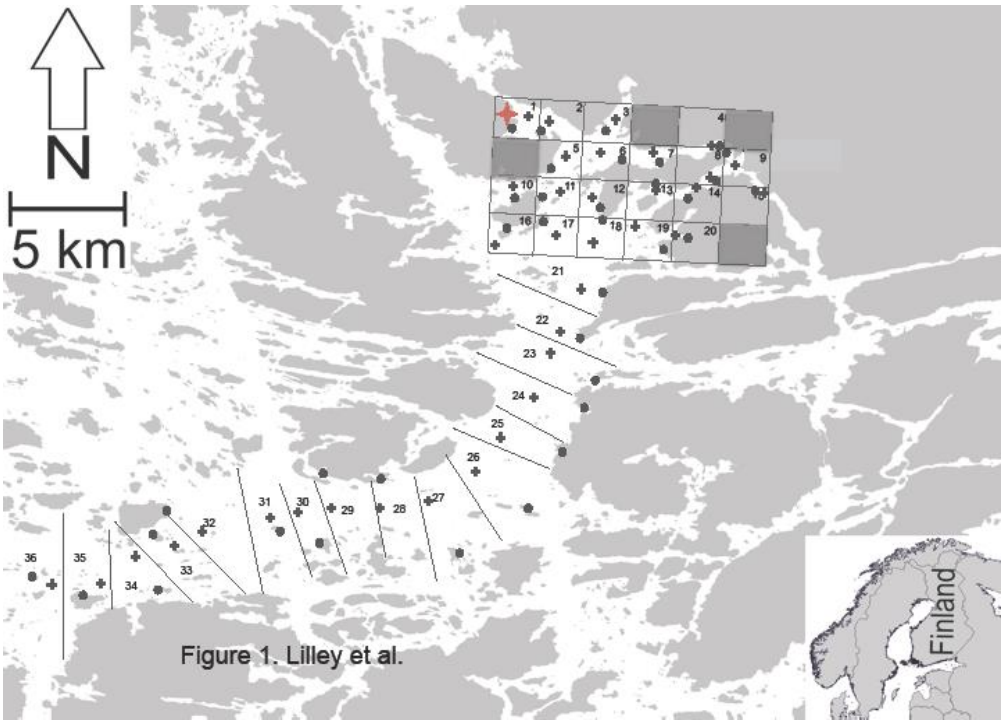


Figure 8 A Map of the Archipelago Sea with the sampling points and the dry dock (red star)

The northern archipelago sea, with the harbors and dry dock was divided into a 2 x 2 km grid and samples were taken in the shipping lane and one reed bed in each segment. The remaining samples were taken along the Airisto Canal in two km intervals. Samples were taken from the deepest point in the shipping lane as well as from reed beds along the coast (Figure 8). The sediment samples were taken in triplicate from the top 3 cm.

2.5.2 Chironomid trapping

Chironomids were caught using floating emergence traps that direct chironomids emerging from the water surface into a collection bottle filled with 70% ethanol, but do not allow entrance from above the water surface. The traps were placed at collection sites 1, 2, 12, 19, 22, 25, and 27 for three weeks in August 2011.

2.5.3 Sediment extraction

For analyses of the sediment samples, the sediments were freeze-dried and 1 µg of the internal standard (D_{27}-TBT) was added to 1 g of each sample. The sediments were extracted with 25 ml of glacial acetic acid in an ultrasonic bath. The slurry was then filtered before adding 500ml of distilled water and loading the sample on to a solid phase extraction cartridge (Waters Oasis HLB, 60 mg, 3cc) that had been preconditioned with 5 ml of methanol and 5 ml of water at pH 2. The cartridges were dried under nitrogen, and eluted with 4 ml of acetonitrile. The eluate was subsequently evaporated to dryness the residue was re-dissolved in 500 µl of acetonitrile and 500 µl of purified water with 0.1% trifluroacetic acid, and transferred into vials for analysis.

2.5.4 Chironomid extraction

The mixture of chironomids in ethanol was filtered, and the filtrate was transferred to a round-bottom flask. The Chironomids were then freeze-dried, crushed under a mortar and extracted by sonication with 3 x 4 ml of MeOH after addition of the internal standard. The methanol and ethanol of each sample were pooled and evaporated to dryness under vacuum. The residue was taken up in 4 ml of MeOH, transferred to a test tube, and evaporated to dryness under a stream of nitrogen. The residue was now taken up in 500 µl of acetonitrile and 500 µl of purified water with 0.1% trifluroacetic acid, and transferred into vials for analysis.

2.5.5 LC-MS/MS method

The LC-MS/MS analysis was performed on the Micromass Quattro Micro system described in 2.1 equipped with a Waters X-Bridge C_{18} column (2.1 x50 mm, 3.5 µm) and a guard column of the same material. The eluents used in this study were 0.5% formic acid in water (A) and 2-propanol, acetonitrile 1:1(B). Both eluents contained 0.01% tropolone. The eluent composition was initially held at 5% (B) and the system was run isocratic for the first minute. Over the next 11 min the percentage of (B) was raised to 85% in a liner gradient held there for one minute before being returned to the initial conditions over the next minute. The

system was given 8 min to equilibrate between injections. The flow rate was 350 µl min^{-1} and the injection volume was 30 µl.

2.5.6 Method validation

The limits of detection and quantification, set to signal-to-noise values of 3 and 10 were determined from injections of standard solutions. The extraction recoveries were calculated using spiked artificial sediment and the method was tested for repeatability and matrix suppression.

3 Results and discussion

In this section, the results of the studies are summarized. A more detailed presentation of the results can be found in papers **I** to **V**

3.1 Illicit Drugs

3.1.1 Occurrence of illicit drugs in the wastewater of Turku and Helsinki

For the analysis of illicit drugs in wastewater, 24 h composite samples were taken in Helsinki and Turku during one week in the spring of 2011, 2012 and 2013. All samples taken in Helsinki and Turku for the scope of this study contained at least one of the studied illicit drugs. In 2011, all of the studied illicit drugs could be detected in both Turku and Helsinki, although amphetamine could not be quantified due to strong matrix disturbance. In 2013 no data could be obtained for methamphetamine due to analytical problems.

The highest concentrations were always observed for the amphetamines (31 to 1420 ng l^{-1}) (amphetamine and methamphetamine) which were detected in all samples that included measurements for these analytes. This is also in good agreement with data from conventional studies that indicate a high consumption of amphetamines in Finland (EMCDDA). Benzoylecgonine and cocaine were found in all samples taken in Helsinki, but only in samples taken in 2011 in Turku, when the flow rate and thus the dilution factor was low. In Turku the concentrations were very close to the LOQ (0 – 34 ng l^{-1}) while they were slightly higher in Helsinki (11 to 19 ng l^{-1}). These results also agree well with conventional studies that show only a marginal use of cocaine in Finland and a tendency to higher use of illicit drugs in larger cities[154].

MDMA, when detected, was found at higher average concentrations than cocaine in both Helsinki and Turku (8 – 232 ng l^{-1}). It was not detected in Turku in 2012 and only in 5 of 7 samples in 2013.

3.1.2 Spatial distribution in Finland

The combined loads of all studied substances per 1000 inhabitants were higher in Helsinki than Turku in all study years. This is a tendency that was also observed in other countries where higher amounts of drugs per 1000 inhabitants were observed in larger cities compared to smaller towns. This finding can also be confirmed by conventional epidemiology studies[154]. The difference was particularly marked for the drugs less common in Finland, such as cocaine and MDMA, while the tendency was not so obvious for the more common drugs such as

amphetamine and methamphetamine. The latter was even found in higher per capita amounts in Turku than Helsinki at some instances.

3.1.3 Short term temporal variation

When considering short term temporal variation, two different trends could be observed; drugs such as MDMA and cocaine had a significantly higher load during the weekend than on weekdays, while the other amphetamines had no significant weekend effect. The best example for a drug showing a considerable weekend effect is MDMA (Figure 9). In Helsinki the MDMA loads rose tenfold in the samples taken on Saturday and Sunday, which correspond to drugs consumed during Friday and Saturday, compared to samples taken during the week. Drugs like amphetamine and methamphetamine that are mainly used by heavy users in Finland do not show this trend to the same degree.

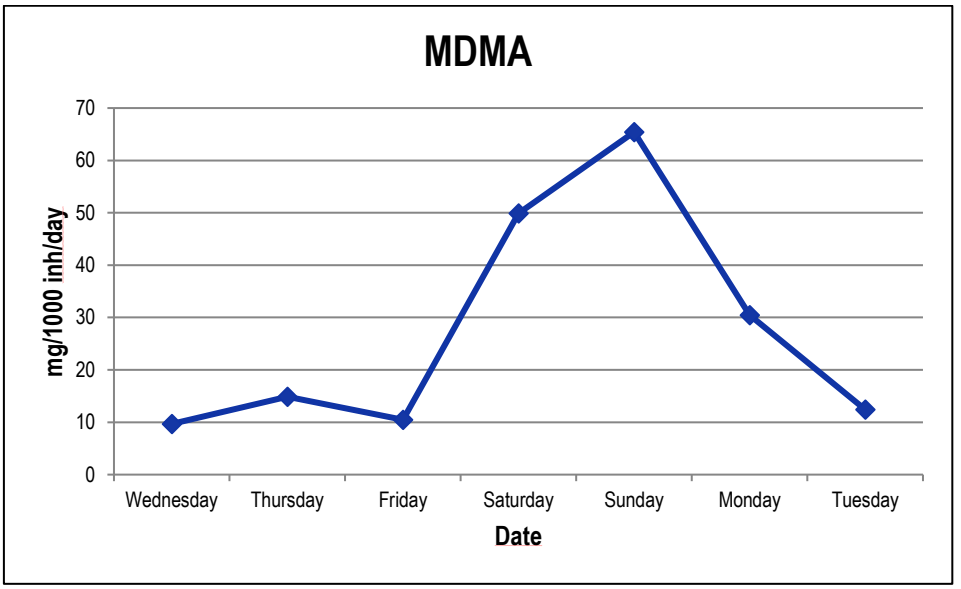


Figure 9. MDMA loads in Helsinki wastewater during one week starting on Wednesday the 06.03.2011

A slight weekend effect could be seen for cocaine as well, but due to the very low concentrations of cocaine in Finnish wastewater it is difficult to quantify this effect.

The clear weekend effect found for certain drugs, demonstrates the strength of this approach in generating real-time data of trends and changes in consumption patterns.

3.1.4 Long term temporal variation

When looking at the long term temporal variation data from this study it has to be remembered that the data only gives snapshots for the situation during three one-week periods in consecutive years. For more accurate long term data samples would have to be collected daily or weekly for the duration of the study.

Cocaine and MDMA, did not show any significant change over the three year period, indicating that amphetamines are still the main drugs of abuse in Finland. There was however a fluctuation in the concentrations of methamphetamine and amphetamine (Figure 10). For both compounds data could only be collected for two years, so it is not clear whether there is a true trend or only fluctuation. It is not uncommon for there to be large fluctuations in the loads of these drugs found in wastewater as they are interchangeable for consumers and the drug more easily and cheaply available will be used[155].

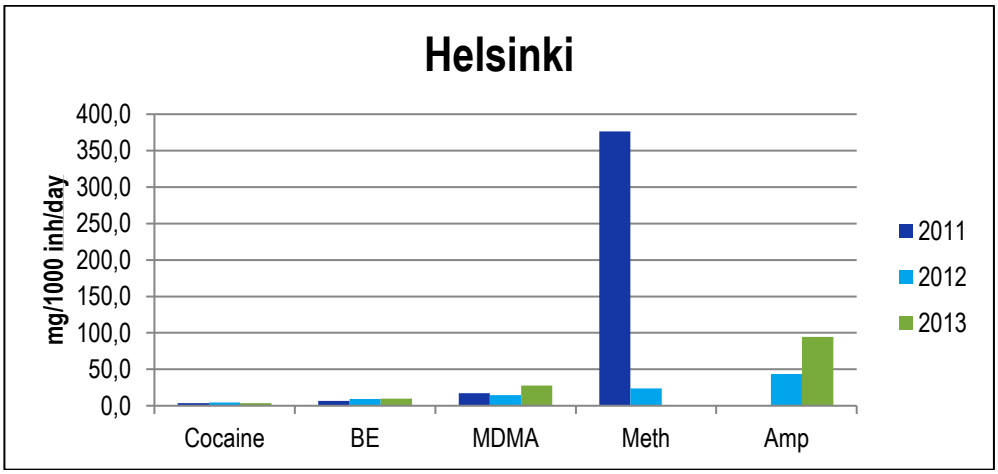


Figure 10. Average loads of the studied drugs found in Helsinki during the three sampling weeks in 2011, 2012 and 2013.

3.1.5 European comparison

With data from a total of 21 European countries included in articles III and IV, it is possible to find distribution and consumption patterns of the studied illicit drugs throughout most of Europe.

A clear spatial distribution in cocaine consumption could be observed in Europe with the highest loads found in western and southern Europe (Antwerp, London, Zürich, Amsterdam and Barcelona) and lowest concentrations in northern and eastern Europe (Finland, Slovakia, Romania and Czech Republic).

A similar although opposite geographical distribution could be observed for methamphetamine, with the highest loads found in northern and eastern Europe (Finland, Norway and Czech Republic), while only trace amounts could be detected in most of western and southern Europe.

Amphetamine use was highest in the Benelux region and the Nordic region, as well as parts of Germany. MDMA was detected at the highest loads in the Benelux region, but was also above average in large cities such as London, Paris and Barcelona.

3.2 Seasonal variation of pharmaceutical concentrations in the river Rakkolanjoki

3.2.1 Occurrence of pharmaceuticals in the river Rakkolanjoki

Fourteen out of the 15 studied pharmaceuticals could be detected at the first sampling point (Figure 8), the WWTP discharge point, during all seasons. Only fluoxetine was not detected in any samples. All of the compounds detected at the first sampling point were also found at the last sampling point in February. In May and July only 8 and 9 out of the studied compounds could be detected at the last sampling point, while 12 compounds could be detected at the last sampling point in November. The same seasonal pattern can be found when looking at all compounds over all sampling points. In February the total incidence of detection over all compounds and sampling points was highest at 94% followed by November with 84%, July with 74% and May with 73%.

Apart from fluoxetine, which was never detected, the lowest incidence of detection was observed for sertraline. Bezafibrate, diclofenac, naproxen, sotalol, atenolol, metoprolol, bisoprolol and carbamazepine were detected in all samples. No pharmaceuticals could be detected in the analyzed tributaries. This indicates that the Lappeenranta WWTP is the only source of pharmaceuticals in this watercourse.

3.2.2 Concentrations of pharmaceuticals

At the discharge point, the concentrations of the detected pharmaceuticals varied from low µg/l for metoprolol, carbamazepine, naproxen and ibuprofen, to low ng/l for the antidepressants.

The incoming concentrations of most pharmaceuticals were constant over the different sampling periods. The main exceptions were the nonsteroidal anti-inflammatory drugs (NSAIDs) ibuprofen and naproxen that occurred at elevated concentrations in May and the antidepressants citalopram and venlafaxine, which occurred at higher concentrations in November. While the higher concentration of the antidepressants in November can only be explained by an apparent higher consumption, the high concentrations of ibuprofen and naproxen in May are most likely due to the high flow rate of the effluent and river at this time. Ibuprofen and naproxen are known to be well removed in WWTPs[27] and the higher flow rate can cause short retention times in the WWTP and thus the treatment efficiency declines significantly[28].

Along the water course there are two points where significant concentration decreases were observed. The first one is located between the first and second sampling point, and the second one is located at the entry point of the river into the lake (Figure 11).

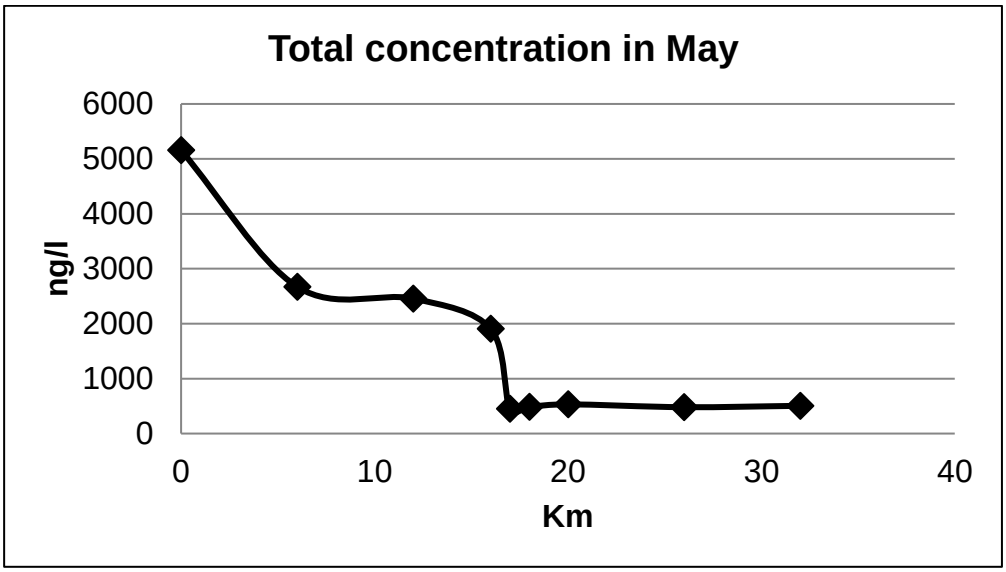


Figure 11 Total concentration of the monitored pharmaceuticals over the course of the river.

The first decrease is mainly due to dilution as several streams converge in this area. The second drop is due to dilution as well as removal during the long residence time in the lake. This drop accounts for most of the seasonal variation in concentrations at the last sampling point.

3.2.3 Loads and removal

The total load of the studied pharmaceuticals entering the watercourse at sampling point one was very constant at 75 g/day during February, July and November, but doubled to 142 g/day in May. This rise was mainly due to the loads of naproxen and ibuprofen and can be explained by the high flow rate during this sampling period possibly affecting the efficiency of the WWTP.

While the total load did not change significantly due to this, the loads of the antidepressants citalopram and venlafaxine increased significantly in November.

At the last sampling point there was a distinct seasonal variation in the total load of the studied pharmaceuticals observed. While 70 g/day were measured in February only 52 g/day were measured in May, 21 g/day in July and 28 g/day in November. The comparatively high loads of pharmaceuticals in May might be due to the higher flow rate during that time, leading to a shorter residence time in the river/lake system.

The individual compounds can be divided into four categories according to their seasonal removal patterns (Figures 12a-d).

The first group contains compounds that do not undergo any significant removal during any season. Carbamazepine is the only representative of this group. No significant degradation was observed for carbamazepine during any season. This is in good agreement with earlier studies that have shown carbamazepine to be very stable in the environment[156].

The second category contains compounds that are degraded to a certain degree all year round, with little to no seasonal variation. The studied compounds that fit into this category are the beta blockers acebutolol and bisoprolol, as well as the lipid modifier bezafibrate. Of these compounds bezafibrate was found to be the most stable in the water column, with more than half the load reaching the last sampling point. A little less than half of the initial load of acebutolol reached the last sampling point, and over 75% of bisoprolol was lost along the watercourse. As these compounds do not show any signs of a seasonal dependence of the removal, sorption to suspended particles and sediment is the suggested pathway of removal. Bezafibrate is not known to have a very strong affinity to sediments, but it is not known to undergo any other biotic or abiotic degradation in water either[34,157,158]. Thus the low degree of removal from the water column can most likely be attributed to sorption during the long residence time in the river lake system. Acebutolol and bisoprolol on the other hand are well known to bind to sediments, and not undergo any other biotic or abiotic degradation in the water column[157,159,160].

The third category contains the NSAIDs. These compounds show a significant difference in removal between summer and winter time, with a low rate of removal during the winter and near complete or complete removal during the summer months. This pattern is characteristic for compounds that undergo biodegradation and photodegradation. While ibuprofen is known to almost exclusively undergo biodegradation and diclofenac is known to readily photodegrade, naproxen and ketoprofen are known to be prone to both pathways[21,77,78,161-163]. This group of compounds is also the most difficult for comparing loads at the discharge point to those at the last sampling point, as they are consumed sporadically for short time periods, leading to higher fluctuations in incoming loads than were observed for other compounds. However, the very large difference in loads at the final sampling point between summer and winter can only be attributed to a significantly higher removal in summer than winter.

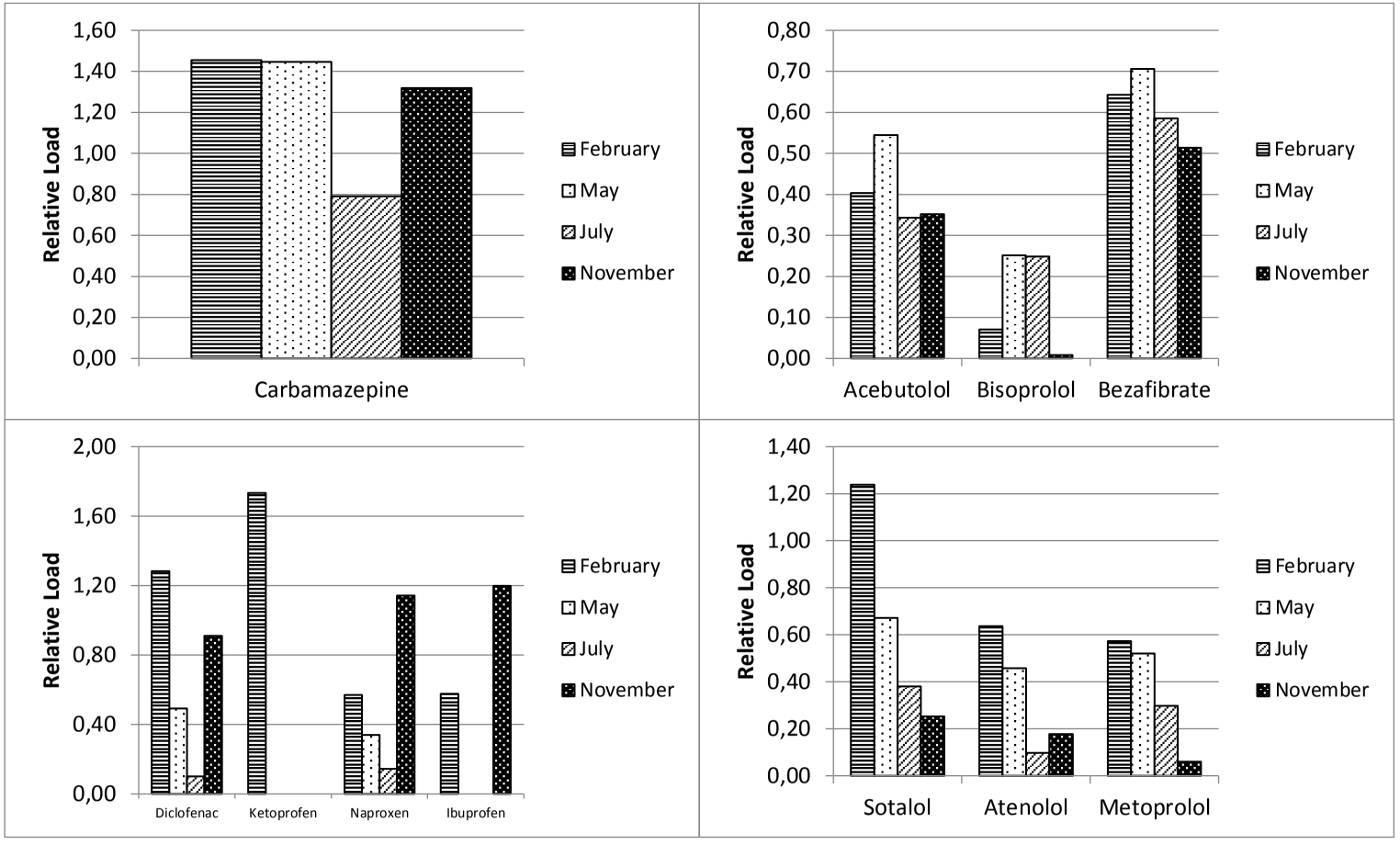


Figure 12 a-d relative loads of individual pharmaceuticals at the last sampling point

The fourth category of the studied compounds is represented by the beta blockers sotalol, atenolol and metoprolol. For these compounds the lowest degree of removal was observed in February followed by a steady increase in removal during the subsequent sampling instances. Atenolol and metoprolol were removed to a certain extent all year round indicating that sorption to particles and sediments contributes partially to the degradation while biodegradation, by microbial communities that are still active in the autumn, most likely accounts for the rest. No removal could be observed for sotalol in February, indicating that biodegradation is the main removal pathway of the drug. The low loads found at the last sampling point in November are likely due to the long residence time (over three months) in the river lake system at this time of year.

3.3 Antibiotics

3.3.1 Method development and validation

The MS/MS optimization of the antibiotics was carried out using flow injection of individual standard solutions. All compounds could be analyzed in positive ESI mode and gave at least two transitions. The two strongest daughter ions were monitored as the quantifier (Q) and qualifier (q) ions. The q/Q ratios for each compound were calculated over the entire range and showed no dependence on concentration. A dynamic MRM (DMRM) method with a cycle time of 700 ms was chosen, giving dwell times ranging from 14.92 ms to 346.5 ms depending on the number of MRM transitions monitored concurrently. This gave at least ten data points per peak for each compound.

Formic acid at various concentrations as well as ammonium formate were tested as possible eluent additives with 0.1% formic acid giving higher sensitivity.

Multiple columns were tested, and an Agilent HPH C_{18} (50 mm x 2mm, 2,6µm) Poroshell column gave the best peak shapes while staying under the 400 bar pressure limit of the trapping column of the online SPE system.

Injection volumes of 0.9 ml and 1.8 ml were tested with loading times of two and four minutes respectively. The larger injection volume gave better sensitivity while no negative effect on the peak shapes could be observed, thus the injection volume of 1.8 ml was chosen.

The method was linear ($r^2 > 0.99$) for all compounds over the calibration range, and gave very good detection limits for most compounds (1 – 10 ng l^{-1}) (Table 6). The repeatability was measured at 5 ng l^{-1} and 100 ng l^{-1} and the RSD of ten injections was below 20% for all compounds at the high concentration, and below 20 % for all compounds, with the exception of tylosin, at the low concentration (Table 6). The low concentration was below the LOQ for six compounds, so no RSD could be determined.

The matrix suppression ranged from 0% for sulfadiazine to 90% for cephalexin. Although compounds with short retention times appeared to experience less matrix suppression than compounds with long retention times the degree of matrix suppression depended more on the individual compound than only the retention time.

The accuracy in matrix, against a calibration curve spiked in milliQ water was tested, and a total of ten compounds (sulfadiazine, ampicillin, cephalexin, trimethoprim, oxytetracycline, doxycycline, benzylpenicillin, carbamazepine, tylosin, and cloxacillin) showed very good accuracies between 80 and 120 % of the nominal concentration in matrix, while a further six compounds (N-acetyl sulfadiazine, tetracycline, erythromycin, erythromycin enol ether, clarithromycin

and roxithromycin) gave accuracies between 50 and 150 %. Sulfamethoxazole and its metabolite N-acetyl sulfamethoxazole only had accuracies of 29%. Matrix-matched calibration or individual stable isotope labeled internal standards should be used for the quantification of these compounds.

3.3.2 Water Samples

In the tested water samples from the River Vantaajoki carbamazepine as well as seven antibiotics could be detected. The concentration of carbamazepine at the discharge point of the two WWTPs (Hyvinkää and Riihimäki) was 72 ng l^{-1} and 37 ng l^{-1} at the sampling point 45 km downstream of the discharge point. Carbamazepine is used as a marker for wastewater contamination, as it is ubiquitous in wastewater, very stable, and easily analyzed. No carbamazepine was detected in the River Kervajoki, confirming that the river is not contaminated by municipal wastewater. The antibiotics trimethoprim, sulfadiazine, cephalexin, sulfamethoxazole, clarithromycin, roxithromycin and erythromycin were detected at the discharge point. The measured concentrations ranged from 5 ng l^{-1} to 81 ng l^{-1}. Apart from sulfamethoxazole and roxithromycin all of these compounds could also be detected at the second sampling point. The measured concentrations at this point ranged between 5 ng l^{-1} to 38 ng l^{-1}. All compounds and concentrations can be found in Table 7. Despite the absence of carbamazepine, indicating that the River Kervajoki does not receive any input of municipal wastewater, cloxacillin was detected at a concentration of 45 ng l^{-1}. Cloxacillin is a commonly used veterinary antibiotic, and the presence of this compound in the otherwise pristine River Kervajoki is most likely due to agricultural activity in the surroundings of the river. The compounds and concentrations found in the River Vantaajoki are similar to those previously published in the literature.[10,37,43,45,46,47,49]

Table 6. Antibiotics studied with their validation results and internal standards

Compound class	Target compound	LOQ (ng l^{-1})	Matrix accuracy (%)	Repeatability (RSD, %)		Linearity (r^2)	q/Q ratio	IS
				5 ng l^{-1}	100 ng l^{-1}			
Tetracyclines	Tetracycline	20	54	N/A	5	0.993	0.5	4-epi-tetracycline-D_6
	Doxycycline	10	88	N/A	19	0.994	0.37	
	Oxytetracycline	5	113	16	17	0.989	0.39	
ß-lactams	Benzylpenicillin	1	92	15	3	0.996	0.9	Benzylpenicillin-D_7
	Cloxacillin	1	99	7	10	0.992	0.98	
	Ampicillin	1	102	11	6	0.992	0.55	Cephalexin-D_5
	Cephalexin	1	92	18	7	0.992	0.52	
Sulfonamides	Sulfadiazine	10	88	N/A	2	0.993	0.87	Sulfadiazine-D_4
	N-acetyl sulfadiazine	5	150	13	4	0.996	0.67	
	Trimethoprim	5	95	3	12	0.991	0.75	Trimethoprim-D_3
	Sulfamethoxazole	1	29	11 (n=9)	10	0.996	0.92	Sulfadiazine-D_4
	N-acetyl sulfamethoxazole	10	36	N/A	9	0.997	0.69	
Macrolides	Erythromycin	1	135	7	15	0.999	0.52	Trimethoprim-D_3
	Erythromycin enol ether	10	63	N/A	9	0.998	0.96	
	Roxithromycin	50	142	N/A	9	0.991	0.65	4-epi-tetracycline-D_6
	Clarithromycin	5	77	5	11	0.991	0.32	
	Tylosin	1	90	39	11	0.991	0.52	
Anticonvulsant	Carbamazepine	1	80	12	3	0.994	0.52	Carbamazepine-D_8

Table 7 Antibiotics concentrations in the studied rivers.

Compound class	Target compound	Mean (ng l^{-1}) and RSD (%) at the sampling points		
		River Kerava (upstream)	River Vantaa (downstream)	River Vantaa (WWTP discharge)
Tetracyclines	Oxytetracycline	<LOQ	<LOQ	<LOQ
	Doxytetracycline	<LOQ	<LOQ	<LOQ
	Tetracycline	<LOQ	<LOQ	<LOQ
ß-lactams	Cloxacillin	45 ±2.1	<LOQ	<LOQ
	Cephalexin	<LOQ	13 ±4.2	35 ±3.7
	Ampicillin	<LOQ	<LOQ	<LOQ
	Benzylpenicillin	<LOQ	<LOQ	<LOQ
Sulfonamides	Sulfadiazine	<LOQ	18 ±2.2	66 ±2.6
	N-acetyl sulfadiazine	<LOQ	<LOQ	<LOQ
	Trimethoprim	<LOQ	21 ±4.8	81 ±1.7
	N-acetyl sulfamethoxazole	<LOQ	<LOQ	<LOQ
	Sulfamethoxazole	<LOQ	<LOQ	5 ±3.7
Macrolides	Erythromycin	<LOQ	5 ±1.3	14 ±3.0
	Clarithromycin	<LOQ	8 ±2.0	26 ±0.7
	Roxithromycin	<LOQ	<LOQ	10 ±1.6
	Tylosin	<LOQ	<LOQ	<LOQ
	Erythromycin enol ether	<LOQ	<LOQ	<LOQ
Anti-epileptic	Carbamazepine	<LOQ	38 ±1.3	72 ±1.0

3.4 Tributyltin

The analysis of sediment samples in the Airisto Canal showed that there were considerable amounts of TBT in the sediments. Along the shipping lane, TBT was detected at all sampling sites, while TBT could not be detected at five of the reed bed sites. Over the sampling area there was a clear correlation between the sediment TBT concentrations and the distance to the dry dock which was the main source of TBT in the area at the time of sampling. There was no correlation between the concentrations of TBT found in the shipping lane and the adjacent reed beds. While the highest concentration (527 μg kg^{-1}) was found in reed beds, the concentrations in the shipping lane were on average higher than those found in the adjacent reed beds (Table 8).

Table 8 TBT concentrations in the study area

Sample Site	Mean TBT reed bed (µg kg^{-1})	Mean TBT shipping lane (µg kg^{-1})	Depth shipping lane (m)
1	165	93	24
2	527	76	18
3	2	39	13
4	30	34	3
5	2	79	35
6	41	34	10
7	30	39	9
8	14	90	10
9	3	27	11
10	8	30	12
11	3	31	50
12	40	11	5
13	40	12	10
14	46	114	10
15	30	22	2
16	<LOQ	16	47
17	7	12	13
18	38	14	9
19	34	6	5
20	10	16	2
21	120	19	15
22	13	14	20
23	<LOQ	41	44
24	<LOQ	4	58
25	2	5	65
26	<LOQ	2	45
27	3	15	50
28	1	73	46
29	2	18	25
30	<LOQ	6	61
31	4	20	42
32	1	8	55
33	3	6	22
34	2	6	16
35	2	2	39
36	1	1	35

Chironomid samples taken from the three most contaminated sites contained detectable amounts of TBT. The concentrations ranged from 88 μg kg^{-1} to 1487 μg kg^{-1}dry weight, and were slightly higher than the sediment concentrations in the reed bed (Table 9). There was a clear correlation between the reed bed TBT concentration and the chironomid TBT concentration. The discovery of TBT in chironomids indicates the possibility of TBT transfer from the aquatic to the terrestrial ecosystem through ecosystem boundaries such as reed beds.

Table 9 Comparison of TBT concentrations in the reed bed sediments and Chironomids

Sample	Mean TBT μg kg^{-1}	
site	Chironomids (*n*=2)	Reed bed sediment (*n*=3)
1	260 (±9)	165 (±37.2)
2	1487 (±90)	527.5 (±46)
12	88 (±3)	40.5 (±12)
19	<LOQ	33.7 (±24.7)
22	<LOQ	<LOQ
25	<LOQ	2 (±1.4)
27	<LOQ	3 (±1.4)

4 Conclusions and future perspectives

This thesis describes three different projects with the use of LC-MS/MS technology for the quantitative analysis of trace amounts of chemicals in common.

Two studies are concerned with the presence and fate of pharmaceuticals in surface waters. These compounds have been widely studied in the environment since the late 1990's. Two groups of compounds in particular have emerged as compounds of concern; endocrine disruptors, and antibiotics. However also the large number of unknown transformation products, as well as the combined effect of the cocktail of pharmaceuticals, transformation products and other xenobiotic compounds may pose a threat to the aquatic environment. In this work, the likely transformation pathways of 15 pharmaceuticals were studied under environmental conditions in an effluent dominated river. Due to the potential importance of these transformation products, further determination of transformation products in the environment as well as their toxicity or other biological effects is needed.

In the case of antibiotics in the environment, there has been very little research on their presence apart from a small number of selected compounds. The analysis method presented here has the capability to determine the presence and concentrations of 18 different antibiotics using a fast and cost-efficient method. This method can be used to learn more about the presence and distribution of antibiotics in the different environmental compartments. In the future, these studies can be combined with microbiological tests determining the presence of antibiotic resistant bacteria in water and sediments, to determine the risk posed by antibiotics in our wastewater.

The second part of this thesis deals with the presence of illicit drugs in wastewater and the possibility of back calculating community drug use from these measurements. The two papers presented here are part of a proof of concept study to show the applicability of the approach for the comparison of illicit drug use over time, or between different communities.

Although wastewater epidemiology has shown to be a very strong comparative tool for this purpose, there are still a number of issues that make the exact back calculation difficult. More data on the exact excretion patterns and behavior in the wastewater system of the compounds in question are needed for more precise back calculations.

The next step would be to start regular monitoring programs that would be a strong addition to the drug data received from questionnaires and other sources.

Another opportunity in this field would be the development of strong non-target analysis methods that may be able to identify new popular synthetic drugs (legal highs) long before they are observed using conventional methods.

The third part of this thesis describes the method development and application of an LC-MS/MS method for the analysis of TBT in sediments and chironomids. The method was successfully applied to show TBT crossing the ecosystem boundary between the aquatic to the terrestrial environment. This might have implications on a number of species that feed on the chironomids emerging from TBT polluted sediments.

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