UMEÅ UNIVERSITY

Antimicrobials in sewage treatment plants

Occurrence, fate and resistance

Marcus Östman

Department of Chemistry
Umeå 2018

Dissertation for PhD
ISBN: 978-91-7601-938-2
Cover: Marcus Östman. Watercolour, 33x22 cm on Arches, 640g.
Photo on the back cover: Elin Näsström
Electronic version available at: http://umu.diva-portal.org/
Printed by: VMC-KBC, Umeå University
Umeå, Sweden 2018

Till min familj

"This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country"

-World Health Organization, 2014

Table of Contents

Abstract

The World Health Organization (WHO) has identified antibiotic resistance as a major threat to human health. The environment has been suggested to play an important role in the emergence of antibiotic resistant bacteria. The external environment can act as a source of resistance genes that could potentially be transferred into human pathogens. It is also an important route for the dissemination of antibiotic resistance genes and bacteria. Sewage treatment plants (STPs) are among the most important routes by which antibiotics and antibiotic resistance genes enter the environment. It has been suggested that STPs are hotspots for the development of antibiotic resistance because they contain relatively high concentrations of antibiotics as well as both human and environmental bacteria. Further complicating matters, there is evidence that other substances with antimicrobial properties, such as biocides and metals, can cause antibiotic resistance due to co- and cross-resistance.

This thesis contributes new knowledge on the concentrations, mass flows, and removal efficiencies of antimicrobials in STPs and their connections to the emergence of antibiotic resistance. Paper I presents data on the levels of 40 different antimicrobials in the incoming wastewater, treated effluent, and digested sludge of eleven different STPs. Although not previously detected in STPs, chlorhexidine is shown to be ubiquitous in such plants. In Paper II, mass flows and removal efficiencies are calculated for eleven antimicrobials over various treatment steps in three STPs, showing that polar antimicrobials were inefficiently removed from the wastewater. In Paper III, the minimum selective concentration (MSC) for the antibiotic tetracycline was determined in a complex bacterial aquatic biofilm using both phenotypic and genotypic endpoints. It was found that 10 µg/L selected for phenotypic resistance, and 1 µg/L selected for certain resistance genes. Paper VI used metagenomics to determine whether there is selection for antibiotic-resistant bacteria in STPs and whether the extent of this selection can be correlated to the concentrations of antimicrobial compounds. No clear evidence for selection was identified. Paper V evaluates advanced wastewater treatment techniques for removing antimicrobial compounds using ozonation and granular activated carbon (GAC). The identity of the GAC material was found to strongly affect removal efficiency, and GAC was more efficient than ozonation for most compounds at the tested concentrations.

Populärvetenskaplig sammanfattning

Antibiotika är viktiga läkemedel för behandling av infektionssjukdomar och de har utan tvivel har räddat livet på miljoner människor. Dessutom är de nödvändliga vid många operationer och cancerbehandlingar m.m. och spelar således en vital roll i den moderna sjukvården. Ett växande globalt problem är uppkomsten av antibiotikaresistens, som innebär att antibiotikan inte fungerar på de bakterier som orsakar sjukdomen man försöker behandla. I många fall finns det dessutom få eller inga andra antibiotika att sätta in för att bakterierna har blivit multiresistenta. WHO har uppgett antibiotikaresistens som ett av de största hoten mot den moderna sjukvården. En viktig orsak till uppkomsten av antibiotikaresistens är att bakterier kan plocka upp resistensgener från varandra. Det är troligt att många av de resistensgener vi hittar i sjukdomsframkallande bakterier idag har sitt ursprung i miljön där de till exempel kan ha varit en del av bakteriernas försvarsmekanism. Det finns troligtvis många fler oupptäckta resistensgener i den yttre miljön som möjligtvis skulle kunna överföras till bakterier som kan orsaka sjukdom hos människor. I dagsläget är kunskapen väldigt dålig om hur en sådan spridning skulle gå till och vilka mekanismer som styr. Det är troligt att miljöer där det finns höga halter av antibiotika kan selektera fram antibiotikaresistenta bakterier. En sådan miljö är avloppsreningsverk som innehåller en blandning av många olika antibiotika samt en mängd olika bakterier. För att komplicera saken ytterligare kan andra ämnen med antibakteriella egenskaper, till exempel så kallade biocider, och metaller kan ge uppkomst till antibiotikaresistenta bakterier.

Den här avhandlingen försöker besvara ett antal viktiga frågor kring reningsverkens bidrag till uppkomsten av antibiotikaresistens. I det första arbetet visar vi vilka halter av olika antibiotika, biocider och metaller som finns i slam och vatten från svenska avloppsreningsverk. Flera ämnen som inte tidigare har rapporteras hittas och vissa som klorhexidin, dessutom i nästan alla prover. I det andra arbetet så studerar vi hur effektiva reningsverken är att rena bort dessa ämnen för att förhindra att de släpps ut i miljön. Flera av de mer vattenlösliga ämnena släpps ut till stor del, även om mycket också bryts ned. I det tredje arbetet så visar vi att så låga halter som 1 µg/L av tetracyclin verkar kunna driva resistensutvecklingen i ett bakteriesamhälle. I det fjärde arbetet undersöker vi om förekomsten av resistens ökar i ett reningsverk genom att sekvensera allt DNA från bakterier och se om det har något samband med mängden antibakteriella ämnen som mätts upp. Ingen konsekvent ökning av resistensgener kunde ses. I det sista arbetet undersöker vi om det går att öka reningen av antibakteriella ämnen i avloppsvatten ytterligare genom att använda ozon och aktivt kol. Arbetet visar att båda metoderna fungerar men högre reningsgrad uppnås med aktivt kol.

Abbreviations

ARG	Antibiotic resistance gene
BAC-10	Benzyldimethyldecylammonium chloride
BAC-12	Benzyldimethyldodecylammonium chloride
BAC-14	Benzyldimethyltetradecylammonium chloride
BAC-16	Benzyldimethylhexadecylammonium chloride
BIT	Benzoisothiazolinone
BOD_7	Biological oxygen demand (7 days)
CFU	Colony forming unit
CPC	Hexadecylpyridinium chloride
CTAB	Hexadecyltrimethylammonium bromide
DC	Direct current
DCOIT	4,5-dichloro-2-octyl-3-isothiazolone
DDD	Defined daily dose
DDMAC	Didecyldimethylammonium chloride
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
ds	Dry substance
d.w.	Dry weight
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionization
EPA	Environmental protection agency
ESI	Electrospray ionization
EtOAc	Ethyl acetate
EU	European Union
FA	Formic acid
FDA	Food and drug administration
GAC	Granular activated carbon
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HESI	Heated electrospray ionization
HGT	Horizontal gene transfer
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
IS	Internal standard
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry

LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOEC	Lowest observed effect concentration
LOQ	Limit of quantification
MBBR	Moving bed biofilm reactor
MeOH	Methanol
MIC	Minimum inhibitory concentration
MIT	Methylisothiazolinone
MS	Mass spectrometry
MSC	Minimal selective concentration
MS/MS	Tandem mass spectrometer
MTBT	2-(methylthio)-benzothiazole
MWD	Microwave digestion
NOEC	No observed effect concentration
OIT	Octhilinone
PAC	Powdered activated carbon
PE	Population equivalents
PICT	Pollution induced community tolerance
PNEC	Predicted no effect concentration
QAC	Quaternary ammonium compound
Q_{dim}	Dimensioned flow
qPCR	Quantitative polymerase chain reaction
RF	Radio frequency
RG	Resistance gene
RNA	Ribonucleic acid
rpm	Rotations per minute
rRNA	Ribosomal ribonucleic acid
QqQ	Triple quadrupole
SPE	Solid phase extraction
STP	Sewage treatment plant
TBT	Tributyltin
TCMTB	2-(thiocyanomethylthio)-benzothiazole
TOC	Total organic carbon
UN	United Nations
US EPA	United States environmental protection agency
UV	Ultraviolet
WHO	World Health Organization

Definitions

Antibiotic – Pharmaceutical drugs used in human or veterinary medicine to treat bacterial infections. The term antibiotic originally referred to natural substances produced by living organisms to kill microorganisms (Waksman & Woodruff 1941, Pramer 1988), but has been broadened to include many semi-synthetic and synthetic compounds. Antibiotics are also used as growth promoters in livestock.

Antimicrobial – Any compounds with antimicrobial properties that are used for "destroying or inhibiting the growth of microorganisms" (Merriam-Webster Dictionary 2018).

Biocide – An antimicrobial compound not used as a pharmaceutical. The word biocide is a broader term referring to any chemical that inactivates microorganisms (McDonnell & Russell 1999); this thesis focuses primarily on antimicrobial biocides. These compounds can be used as disinfectants, antiseptics or preservatives.

Co-resistance – Resistance to both an antibiotic and a biocide/metal located on the same genetic element (i.e. plasmid or integron). May also refer to simultaneous resistance to different classes of antibiotics.

Cross-resistance – A single mechanism that confers resistance to both an antibiotic and a biocide/metal.

Horizontal gene transfer – Movement of genetic material between organisms other than the route from parent to offspring (Burmeister 2015).

Minimal selective concentration (MSC) – The lowest concentration of an antimicrobial that will give a resistant strain an advantage in growth rate compared to a susceptible strain (Andersson & Hughes 2014).

Resistance gene – A gene that will allow microorganisms to withstand increased antimicrobial concentrations (Martínez et al. 2015).

Selection pressure – "Any change in the environment that encourages particular mutations to succeed" (Medical Dictionary 2009).

List of publications

The thesis is based on the following papers that are henceforth referred to using the Roman numerals shown below.

I. **Marcus Östman**, Richard H Lindberg, Jerker Fick, Erik Björn, Mats Tysklind. Screening of biocides, metals and antibiotics in Swedish sewage sludge and wastewater.
Water Research (2017) 115, 318–328

II. **Marcus Östman**, Jerker Fick, Mats Tysklind. Detailed mass flows and removal efficiencies for biocides and antibiotics in Swedish sewage treatment plants.
Science of the Total Environment (2018) 640-641, 327-336

III. Sara Lundström, **Marcus Östman**, Johan Bengtsson-Palme, Carolin Rutgersson, Malin Thoudal, Triranta Sircar, Hans Blanck, K Martin Eriksson, Mats Tysklind, Carl-Fredrik Flach, DG Joakim Larsson. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms.
Science of the Total Environment (2016) 553, 587-595

IV. Johan Bengtsson-Palme, Richard Hammarén, Chandan Pal, **Marcus Östman**, Berndt Björlenius, Carl-Fredrik Flach, Jerker Fick, Erik Kristiansson, Mats Tysklind, DG Joakim Larsson. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics.
Science of the Total Environment (2016) 572, 697-712

V. **Marcus Östman**, Berndt Björlenius, Jerker Fick, Mats Tysklind. Effect of full-scale ozonation and granular activated carbon on the removal of biocides, antimycotics and antibiotics in a sewage treatment plant.
Science of the Total Environment (2019) 649, 1117-1123

See **https://www.researchgate.net/profile/Marcus_Oestman** for a full publication list of the author.

Author's contributions

Paper I

The author was involved in planning the study, performed part of the sampling campaign, developed the analytical methods, did all the experimental work, evaluated the data, and had the lead role in writing and revising the manuscript.

Paper II

The author was involved in planning the study, performed part of the sampling campaign, did all the experimental work, evaluated the data, and wrote and revised the manuscript.

Paper III

The author developed the analytical methods, did all the analytical experimental work, and wrote the sections of the manuscript relating to the analytical measurements.

Paper IV

The author developed some of the analytical methods, performed some of the analytical measurements, and wrote the sections of the manuscript relating to chemical analysis.

Paper V

The author was involved in planning the study, did all the analytical experimental work, evaluated the data, and wrote and revised most of the manuscript.

Introduction

Antibiotics are important medicines used to treat bacterial infections and are key components of modern healthcare systems. Unfortunately, the global use of antibiotics during the last 80 years has led to increasingly serious problems due to the emergence of antibiotic-resistant bacteria. These problems cause hundreds of thousands of deaths annually and are expected to become much more severe in the coming decades (O'Neill 2014). A lack of reliable antibiotics would greatly complicate many modern healthcare procedures including surgery, cancer treatment and neonatal care.

The main driving force of the global antibiotic resistance problem is likely to be the use and misuse of antibiotics in humans and animals. However, the role of the environment as a source of novel resistance genes and route for the transmission of both resistant bacteria and resistance genes is unclear (Martínez 2008, Ashbolt et al. 2013, Bengtsson-Palme, Boulund, et al. 2014). It is very likely that the environment contains many undiscovered antibiotic resistance genes (ARGs) that could become enriched and transferred to pathogens. This has probably happened in the past with many ARGs (Davies & Davies 2010). Many non-antibiotic compounds possess antimicrobial properties, such as various metals and biocides. Biocides are used in multiple applications in households, hospitals, and industries, and, like antibiotics, can enter the aquatic environment directly via discharge to water or through sewage treatment plants (STPs). Many studies have shown that exposure to biocides and heavy metals can increase antibiotic resistance over time due to co- or cross-resistance (Braoudaki & Hilton 2004, SCENIHR 2009, Andersson & Hughes 2012, Singer et al. 2016). Since human activities are polluting the environment with antimicrobials, there is a risk that these antibiotics, biocides and metals will impose selective pressure on environmental microorganisms, promoting the emergence of antibiotic-resistant bacteria.

However, there are many unanswered questions about this process. For example, at what concentrations do antibiotics become selective in the environment, and how is this selective pressure affected by the presence of co-selective agents such as biocides and metals? In addition, there is a clear need for reliable measurements of the mass flows and removal of antimicrobial compounds in sewage treatment plants.

Aim of this thesis

The overall aim of this thesis is to determine the identities and concentrations of the antimicrobial compounds in sewage treatment plants, their fate, and the scope for their removal. An additional goal is to determine whether these compounds impose selective pressure on the bacterial community favoring the emergence of antibiotic resistance.

Specific objectives are to (**Figure 1**):

- Quantify selected antimicrobial biocides, antibiotics and metals that are present in sewage treatment plants (**Paper I**)
- Investigate the fate of antimicrobials in Swedish STPs and what is the impact of different treatment processes (**Paper II**)
- Determine the minimal selective concentration (MSC) of an antibiotic (tetracycline) in a complex microbiological community (**Paper III**)
- Determine whether antibiotics impose direct selection pressure favoring resistant bacteria in STPs, and whether biocides, metals and antibiotics can co-select for resistance in STPs (**Paper IV**)
- Determine whether concentrations of antimicrobial compounds in STPs can be reduced by using ozonation or granular activated carbon as a tertiary treatment technology (**Paper V**)

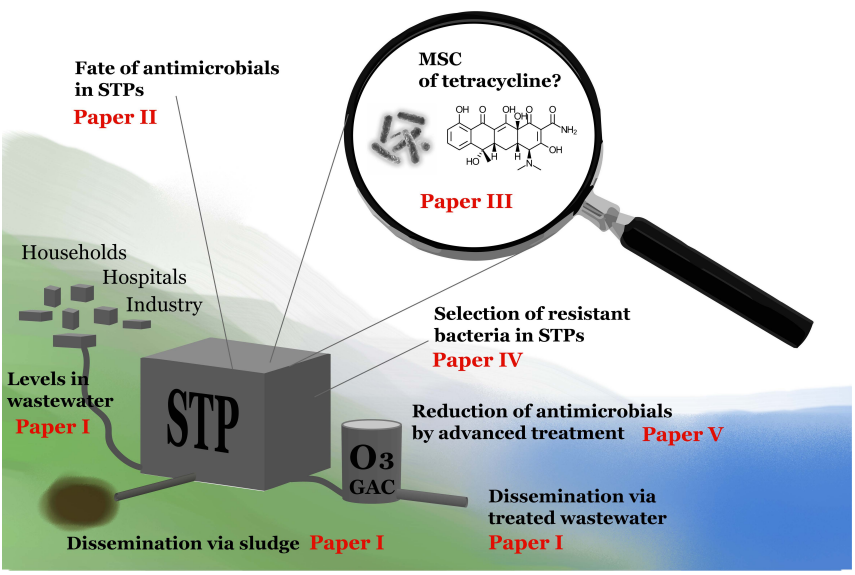


Figure 1. *Schematic overview, showing the objectives and papers of the thesis.*

Antibiotic resistance

It is estimated that we share this world with 1 trillion different microbial species (Locey & Lennon 2016), most of which are still unknown to us. Some are essential for our survival, but a small fraction can cause diseases and are commonly referred to as pathogens (Casadevall & Pirofski 2014). A bacterial infection can be a serious medical condition, and throughout history, humans have used various types of plants or different inorganic compounds to treat these diseases, with varying results (Frazer 1930, Tesch 2002). Despite these treatments, bacterial infections were often fatal and many people never reached adulthood in the pre-antibiotic era (Guyer et al. 2000). The microbiologist Paul Ehrlich, speculated that it should be possible to find a substance that selectively killed bacteria without being harmful to humans and he was eventually proved right (Tan & Grimes 2010). The discovery of penicillin in 1928 (Fleming 1929), changed the history of medicine. The sulphonamides, which emerged at around the same time, were the first antimicrobials produced on large scale (Domagk 1935, Aminov 2017). Penicillin, which was far more potent than previous antimicrobials, was introduced clinically in the 1940s (Chain et al. 1940), at which point mankind left the pre-antibiotic era behind.

Antibiotics really were wonder drugs and soon found many different applications. This had a dramatic effect: some infectious diseases almost disappeared and average human lifetimes increased significantly (Bérdy 2012). For a period of time, it seemed that antibiotics would solve the problems of bacterial infections for good (Cockburn 1964, Snowden 2008). As we now know, this was not the case. Although the first signs of bacterial resistance came very early, the situation seemed to be under control, with new antibiotics constantly reaching the market (Demain & Sanchez 2009). However, after the 1960s, changes in the pharmaceutical industry's priorities greatly reduced the rate at which new antibiotics were discovered (Bérdy 2012). Worse, most of the new antibiotics that did emerge were just variations of known substances; no new classes of antibiotics were introduced for over 40 years (Fischbach & Walsh 2009). Antibiotics have been extensively used in medicine since their introduction, both to treat bacterial infections and for prophylaxis in surgery and cancer treatment.

The extensive clinical use of antibiotics, together with their use in agriculture (Hoelzer et al. 2017) and aquaculture (Done et al. 2015), has imposed strong selective pressure on bacteria, resulting in the emergence of antibiotic-resistant strains. Resistance to antibiotics typically emerges quickly: an enzyme capable of inactivating penicillin was discovered even before the drug's clinical introduction (Abraham & Chain 1940), and resistant strains have emerged for every new antibiotic introduced to the market ever since (Davies & Davies 2010). Today

there are many strains of pathogens that exhibit multiresistance, i.e. resistance to several types of antibiotics; some infections with these strains are untreatable. The emerging problem of antibiotic resistance has been identified as a major threat to global health (World Health Organization 2014) because antibiotics are a cornerstone of modern healthcare, and their loss would be devastating.

The role of the environment

The main driving force of the global antibiotic resistance problem is likely to be the use and misuse of antibiotics in humans and animals. However, the environment is also likely to play a role and therefore warrants more study (Ashbolt et al. 2013). There are vastly more environmental bacteria than human pathogens, making it likely that the environment can act as a reservoir of antibiotic resistance genes that may be transferred into pathogens (D'Costa et al. 2006, Wright 2010, Finley et al. 2013, Pruden et al. 2013). Several resistance factors that are causing problems in healthcare today were probably transferred to pathogens from non-pathogenic bacteria that live around us (Bonomo & Szabo 2006). The presence of antibiotic resistance genes in bacteria is therefore not a modern phenomenon associated with clinical use of antibiotics, but a natural phenomenon based on genetic material that has existed in the environment for a very long time (Dcosta et al. 2011). Since many of the antibiotics in clinical use originate from natural sources such as soil bacteria, antibiotics are natural components of the ecosystem, making it likely that resistance genes emerged long before human use of antibiotics. It is very likely that the environment contain many undiscovered antibiotic resistance genes that could become enriched and transferred to pathogens (Bengtsson-Palme, Kristiansson, et al. 2018). This has almost certainly happened in the past with many ARGs (Davies & Davies 2010). The environment also probably plays a role in the spread of resistant bacteria between hosts. A schematic overview of the environment's role in the antibiotic resistance problem is presented in **Figure 2**. The environment can also act as a source of opportunistic pathogens (Berg et al. 2005). Examples where bacteria are disseminated include sewage treatment plants, pharmaceutical production facilities, and agricultural and aquaculture facilities (Larsson et al. 2018).

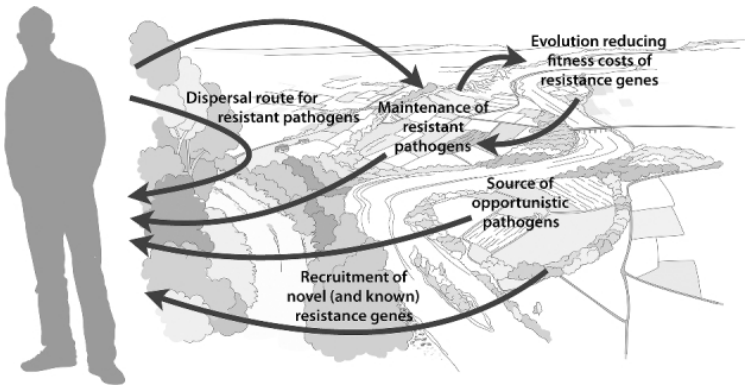


Figure 2. *The environment's role in the development and spread of antibiotic resistant bacteria. (Bengtsson-Palme, Kristiansson, et al. 2018) under the terms of Creative Commons Attribution Non-Commercial License.*

Selection and spread of antibiotic resistance genes and bacteria

Bacteria can acquire antibiotic resistance genes via mutation or by horizontal gene transfer (HGT). HGT allows ARGs to quickly spread within a bacterial population and adapt to selection pressure due to antimicrobials in the environment. HGT can occur by conjugation, transduction or transformation (von Wintersdorff et al. 2016). Conjugation means that the DNA is exchanged via a mobile genetic element, such as a plasmid, that is transferred between bacteria. Transduction is DNA exchange via viruses (bacteriophages), while transformation is the uptake of free DNA (von Wintersdorff et al. 2016). Given that the number of bacterial cells on Earth is estimated to exceed 10^{30} ("Microbiology by numbers" 2011, Kallmeyer et al. 2012) and that HGT can occur between species, it is likely that transfers of genes and mutations occur regularly.

Acquiring a resistance gene typically comes with a fitness cost such as reduced growth rate. If the fitness cost is high enough, and there is no selection pressure from antimicrobials, the mutation will probably not be fixated in the population (Andersson & Hughes 2010). Even so, compensatory mutations often arise (Handel et al. 2006); together with the existence of low-cost or cost-free ARGs, this will make it very difficult to reverse resistance even if the selection pressure is removed (Andersson & Hughes 2010). Furthermore, the genepools of different environments can easily be exchanged with genepools in more clinically relevant settings since there are no barriers to such exchanges (Aminov & Mackie 2007, Aminov 2011). This makes it important to adapt an interdisciplinary "One Health" approach to surveillance that treats humans, animals, and the environment as parts of the same system (Queenan et al. 2016).

Antibiotic resistance resulting from co-selection induced by biocides and heavy metals

To complicate things, many studies have shown that exposure to other antimicrobial compounds such as biocides and heavy metals can increase antibiotic resistance over time (Braoudaki & Hilton 2004, "SCENIHR" 2009, Andersson & Hughes 2012, Singer et al. 2016). A metal or biocide can thus co-select for antibiotic resistance. This can happen through three mechanisms, co-resistance, cross-resistance and co-expression/co-regulation, (**Figure 3**). Co-resistance occurs if resistance genes to an antibiotic and a biocide/metal are located on the same genetic element (for example a plasmid). Cross-resistance means that the same gene (or mechanism) confers resistance to several antibiotics/biocides/metals simultaneously, for example an efflux pump that can act on many different compounds. The third mechanism is co-expression/co-regulation, whereby one regulatory gene controls other resistance genes.

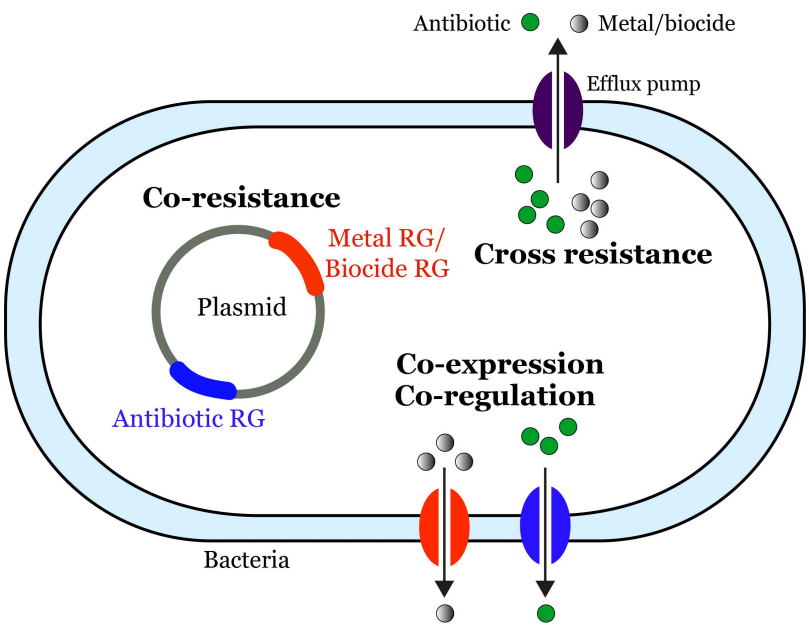


Figure 3. *The principles of co-selection. RG = resistance gene. Figure modified from (Pal et al. 2017)*

Many bacteria resistant to both an antibiotic and a biocide/metal are known (Pal et al. 2015). For example, there are bacteria resistant to mercury and several antibiotics (Drace et al. 2018), as well as copper/erythromycin (Amachawadi et al. 2011), and silver/fluoroquinolones (Fang et al. 2016). It was recently shown that the uptake of cadmium into bacterial cells can increase their minimum inhibitory concentration (MIC) values for several antibiotics (i.e. increased

antibiotic resistance) (Kaur et al. 2018). Co-selection makes it even more difficult to reverse antibiotic resistance since selection pressure may be maintained by other compounds even if the antibiotic is removed (Enne et al. 2004). This is especially worrying for metals since many of them are ubiquitous at present.

Sewage treatment plants as hotspots for antibiotic resistance

Sewage treatment plants (STPs) are of particular interest as environments that could promote antibiotic resistance in bacteria (Rizzo, Manaia, et al. 2013, Berendonk et al. 2015, Lood et al. 2017, Voolaid et al. 2017). They are unique because they contain a mixture of human faecal bacteria (including pathogens), a diverse set of environmental bacteria, and many chemicals, including antibiotics, biocides and metals (Rizzo, Manaia, et al. 2013). It has been reported that the concentrations of antibiotics in STPs are higher than in any environment other than those in the vicinity of antibiotic production facilities (Larsson et al. 2007, Fick et al. 2009). It is thus likely that environmental bacteria in STPs could donate new resistance elements to pathogens, and that resistant strains could be enriched in STPs due to selective pressure resulting from the high levels of antibiotics. Furthermore, STPs are probably the most important route for the transportation of antibiotics, resistance genes, and resistant bacteria into the aquatic environment (Ferreira da Silva et al. 2006, Kümmerer 2009a).

Sewage treatment plants

Proper handling of human waste (including excreta) is critical to prevent diseases and protect the environment. For most of human history, this task has been poorly handled (Lofrano & Brown 2010). Wastewater systems in Sweden started to emerge in the late 1800s; the earliest systems released entirely untreated sewage into waterways (Naturvårdsverket 2014). This polluted lakes, causing high fish mortality and large-scale ecosystem change due to eutrophication, as well as epidemics in some cases. The first investment into STPs in Sweden took place in the 1940s but it was not until the 1960s that these investments were made on a large scale across the country. Today, essentially all households and industries are connected to an STP (Naturvårdsverket 2014). Most households are connected to municipal STPs, which treat the waste produced by 87% of Sweden's population, but people in rural areas are often connected to small on-site sewage treatment facilities (Blum et al. 2018).

Treatment technologies

Many different treatment technologies exist; this section briefly outlines those used most widely in Sweden. The key treatment steps are presented in **Figure 4**, which outlines the treatment process used at Rya STP. Each treatment performed in a conventional STP can roughly be classified as a mechanical, chemical, or biological treatment. Advanced tertiary treatment steps are discussed later in this thesis.

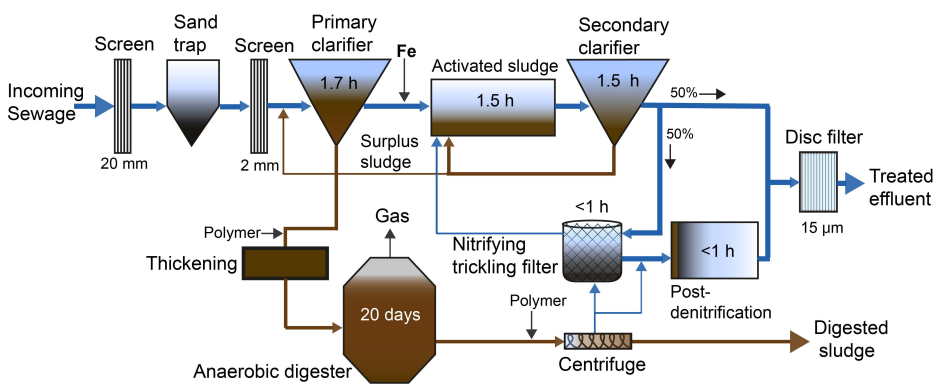


Figure 4. *Schematic overview of the wastewater treatment process at Rya STP, Göteborg showing the residence times for different treatment steps.*

Mechanical treatment

The first step in the treatment process used in all modern sewage treatment plants involves having the wastewater flow through a series of coarse and fine screens, as well as grit chambers, to remove larger objects that would otherwise interfere with subsequent treatment steps (*Operation of Water Resource Recovery Facilities* 2017). The screens remove objects such as plastics, paper, and wood, while the grit chambers remove particles that settle quicker than the organic matter, such as sand and gravel. The waste from these mechanical treatments is physically separated and typically incinerated.

Chemical treatment

After mechanical treatment, the wastewater is typically passed through sedimentation tanks at a low flow rate to allow organic material to settle to the bottom of the tank while material less dense than water is removed by floatation (Nelson 2017). The removed sludge from the bottom of the first clarifier is known as primary sludge and is usually thickened and digested further on in the process.

In many STPs the process of sedimentation is enhanced by adding various iron or aluminium salts that act as flocculation and coagulation agents (Samuelson 2009). This process is generally described as chemical treatment. For example, when ferric chloride is added to the wastewater, it forms insoluble $Fe(OH)_3$ that sweeps out suspended material when it settles to the bottom of the tank (Ebeling et al. 2003). Phosphorus is flocculated in the same way by the formation of $FePO_4$. The point in the wastewater treatment process at which these chemicals are applied differs between STPs.

Biological treatment

The biological treatment step uses microorganisms to decompose organic substances in the wastewater. The microorganisms may be grown as a biofilm on a fixed material, which is the approach used in trickling filters (Séguret et al. 2000) and moving bed bioreactors (MBBR) (Ødegaard 2006, Falås et al. 2012). Alternatively, they may be present in suspension, as is the case in activated sludge systems (Barnard 1976) and membrane bioreactors (Engelhardt et al. 1998, Krzeminski et al. 2017). Activated sludge systems are the most common type of biological treatment system in Sweden. Their operating principles were first demonstrated in 1914 (Ardern & Lockett 1914), and they continue to be widely used around the world.

Figure 5 presents a schematic depiction of a conventional activated sludge process. Pretreated wastewater enters the bioreactor where it is decomposed by microorganisms. Parts of the bioreactor are aerated to enhance microbial degradation and prevent organic material from sedimenting at the bottom of the

tank. The water is then allowed to settle in a secondary clarifier and much of the sludge is returned to the bioreactor. Excess sludge can be removed and used in another part of the process.

In principle, there are three ways that micropollutants, such as antimicrobials, can be removed during biological treatment: biodegradation, volatilization and removal by adsorption onto sludge flocs (Grandclément et al. 2017). Volatilization is usually not an important removal mechanism for pharmaceuticals and personal care products (Joss et al. 2006), but can be relevant for other compounds considered in this thesis.

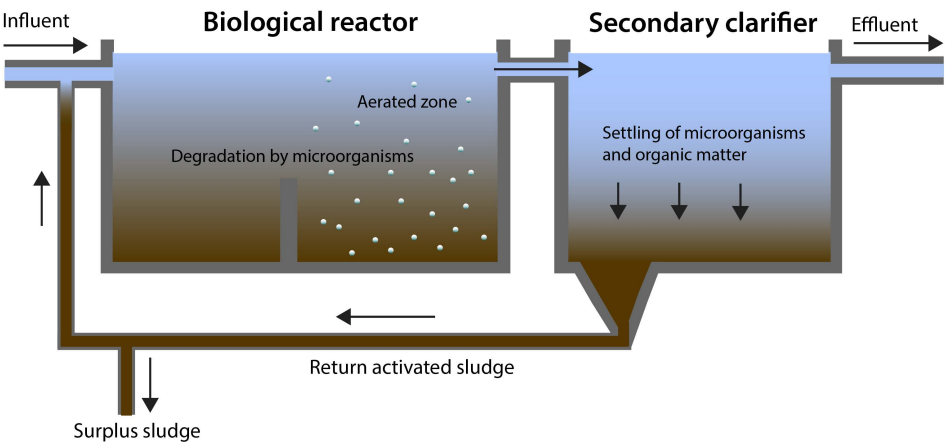


Figure 5. *Schematic depictions of an activated sludge system.*

Sewage sludge

Sludge is the solid residue that remains after wastewater treatment (Edwards et al. 2017). It consists of the various kinds of organic and inorganic matter present in the wastewater, including microorganisms, phosphorus, metals, and chemicals from the human technosphere. Inside the STP, different kinds of sludge are removed from the wastewater in different treatment steps depending on the technology used. The sludge in the STP is usually a slurry but its water content is typically reduced before it leaves the STP. Sludge is subjected to anaerobic digestion for stability in many STP (Nelson 2017). During digestion, organic compounds are converted into CO_2 and CH_4 by bacteria (Angelidaki & Sanders 2004). The handling of sludge from wastewater treatment presents many challenges (Zhang et al. 2017). It contains valuable nutrients such as phosphorus that ideally should be recycled (for applications in industries such as agriculture), but its high content of heavy metals and other micropollutants sometimes makes this difficult.

Sewage treatment plants studied in this thesis

This thesis is based on samples from 15 STPs in Sweden whose sizes and locations is presented in **Figure 6** and **Table 1**. The set of studied STPs span a wide range of sizes and includes the largest STP in Scandinavia, Rya STP, which treated 100 times more wastewater than the smallest plant included in the study (Knivsta STP).

Table 1. Information on the studied STPs. Data for 2014 – 2015 provided by each STP.

City	STP	PE	Raw wastewater (Mm^3 $year^{-1}$)	Sludge (tons d.w. $year^{-1}$)
Göteborg	Rya	806 575	147	14846
Stockholm	Henriksdal	890 000	107	15200
Lidingö	Käppala	440 000	61	8000
Stockholm	Bromma	208 000	54	5500
Uppsala	Kungsängsverket	153 800	18	3300
Norrköping	Slottshagen	140 900	16	2857
Eslöv	Ellinge	101 955	4.4	1214
Umeå	Ön	83 919	12	2048
Östersund	Göviken	66 114	7.1	912
Borås	Gässlösa	64 464	14	2301
Borlänge	Borlänge	37 145	5.7	968
Piteå	Sandholmen	24 623	3	1204
Alingsås	Nolhaga	21 268	3.9	724
Söderhamn	Granskär	12 500	2.2	770
Knivsta	Knivsta	10 430	1.4	191

PE = population equivalents (Based on 70 g of BOD7/day). STP = Sewage treatment plant, d.w. = dry weight, BOD = Biological oxygen demand.

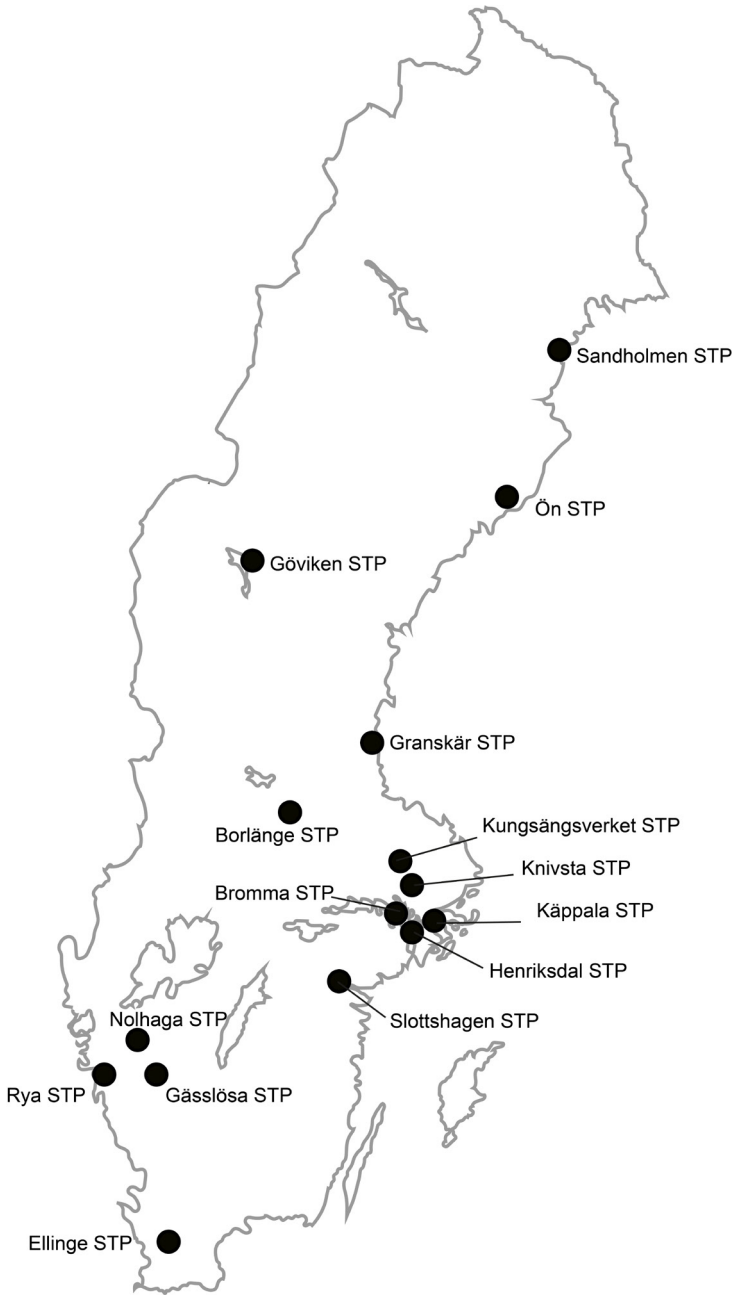


Figure 6*. Map of Sweden showing the locations of the sewage treatment plants studied in this thesis. STP = Sewage treatment plant.*

Full-scale ozonation at Knivsta STP

During 2015, Knivsta STP north of Stockholm was modified to reduce the levels of pharmaceuticals in the effluent by adding a final ozonation step. The full-scale ozonation system treated all of the wastewater with a dimensioned flow (Q_{dim}) of 200 m^3/h. The ozone was dosed at 7 g O_3/m^3 (0.55 g O_3/g TOC), and the treated wastewater was passed through a polishing pond before entering the recipient.

Mobile pilot plant at Knivsta STP

To study the impact of various treatment technologies, a mobile pilot plant was used to evaluate the ozonation and GAC treatments at Knivsta STP (Kårelid et al. 2017). The mobile pilot plant consisted of a 20-foot shipping container filled with treatment lines for GAC and ozonation, as well as equipment for process control and sampling. The tests presented in this thesis evaluated three different GAC materials along with ozonation at a dose of 5 g O_3/m^3 (0.39 g O_3/g TOC).

Quantitative analysis of antibiotics, biocides and metals

Detecting and quantifying micropollutants such as biocides and antibiotics in the environment is challenging due to their low concentrations and the presence of complex matrices that can cause interference during both extraction and analysis. To achieve reliable results, the entire analytical chain from sampling to data analysis must be considered.

Sample preparation

Preparation of samples is an essential part of the analytical chain and generally one of the most time consuming. Usually more than 80% of the analysis time is spent on collecting and preparing samples for analysis (Pawliszyn & Lord 2010). One of the most important factors in trace analysis of antimicrobials in environmental samples is the scope for pre-concentration of the analytes and removal of unwanted matrix-compounds.

All samples discussed in this thesis were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and inductively coupled plasma-mass spectrometry (ICP-MS). Three techniques were used for sample preparation: solid phase extraction (SPE), bead beating, and microwave digestion (MWD). A schematic overview of the sample preparation workflow for antimicrobial analysis used in **Papers I, II** and **V** can be seen in **Figure 7**. The sample preparation procedures are described at length in the Supplementary information of each paper, but are also outlined below for convenience.

Solid phase extraction for water samples

The technique of adsorbing analytes of interest on a solid material is well established (Braus et al. 1951, May et al. 1975) and has become a cornerstone of modern trace analysis methods for analytes in aqueous samples (Liška 2000). In SPE, the aqueous sample is passed through a cartridge filled with a solid adsorbent (typically a polymer) on which the analytes are adsorbed. Unwanted matrix components can be washed off before the analytes are eluted. The extract is then evaporated to a small volume to achieve a high pre-concentration factor.

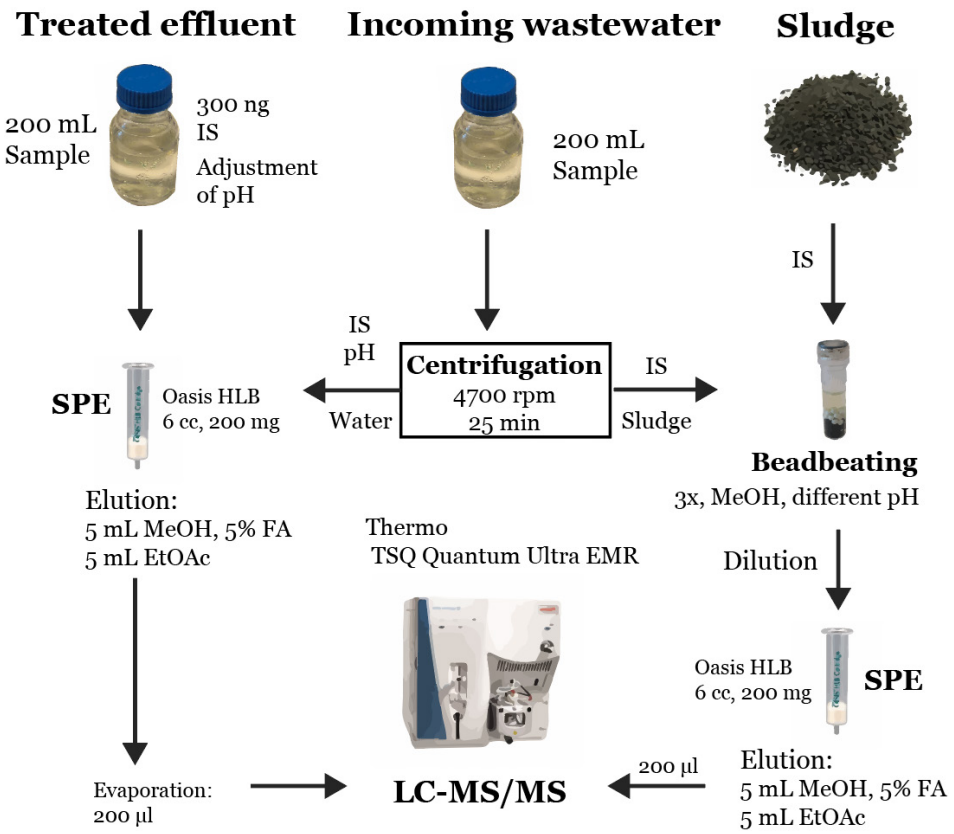


Figure 7. *Schematic overview of the analytical sample preparation workflow used to analyse biocides and antibiotics in* ***Papers I, II*** *and* ***V****. IS = Internal standard, MeOH = methanol, EtOAc = Ethyl acetate, FA = formic acid.*

Sample preparation of water samples in papers I, II, V

As shown in **Figure 7**, 200 mL of wastewater was used for extraction. Samples of treated effluent were extracted directly, while incoming wastewater samples were centrifuged before extraction to separate the particulate and water phases. The particulate phase was treated as sludge and extracted by bead beating as described below. The water samples were pH adjusted and spiked with 300 ng of isotopically labelled internal standards (IS) before extraction with SPE. Extraction was performed using Waters Oasis HLB cartridges because they are all-purpose SPE units capable of retaining compounds with a wide range of physicochemical properties. To achieve sufficient recovery of quaternary ammonium compounds (QACs), they were washed off the columns using 5% formic acid (FA) in methanol as the eluent. After evaporation, the resulting extracts were ready for LC-MS/MS analysis. The full method description is provided in the Supplementary information of **Paper I**.

Analysis of tetracycline in water for paper III

An LC-MS/MS method was developed to monitor tetracycline concentrations with the experimental setup used in **Paper III**. The analysis of tetracycline presents several challenges because tetracycline binds to proteins and divalent cations such as Ca^{2+} and Mg^{2+} (Neuvonen 1976), and undergoes different degrees of epimerization depending on the pH (McCormick et al. 1957). This epimerization is reversible and is most pronounced at pH values around 3 (Pena et al. 1998). Consequently, two peaks corresponding to tetracycline and 4-epitetracyline were consistently detected in this study.

A full description of the method is provided in the Supplementary information of **Paper III.** Briefly, a 10 ml sample was mixed with an extraction buffer containing acetic acid and EDTA. To obtain accurate results, it was essential to shake the mixed sample before removing aliquots for extraction; the average tetracycline concentrations measured using unshaken samples were less than 20% of those measured in shaken samples. Tetracyline-D6 was used as an internal standard; the use of this compound was crucial because none of the tested tetracycline surrogates yielded acceptable results. Samples were pre-concentrated and extracted by SPE using Oasis HLB cartridges before analysis by LC-MS/MS.

Analysis of antibiotics in water for paper IV

For the analysis of antibiotics in **Paper IV**, the samples were filtered through 0.45 µm syringe filters, acidified, and spiked with IS before being extracted by online SPE (Oasis HLB 2.1 x 20, 15 µm). The SPE system was coupled to the LC-MS/MS system before the analytical column. All details of the method are described in detail elsewhere (Lindberg et al. 2014).

Extraction of sludge samples with bead beating

In bead beating, a solid sample is shaken by a machine at high speed together with an extraction solvent and heavy beads. The technique is normally used for extraction and disruption of tissue samples (Grabicova et al. 2014), but has also been used for extraction of sludge samples (Zuloaga et al. 2012).

Bead beating using zirconium/silica beads was used to extract antibiotics and biocides from all sludge samples analyzed in **Papers I, II** and **IV**. In **Papers I** and **II**, the final extract was diluted and subjected to SPE-clean up using the same procedure as the water samples. No SPE was used for the sludge samples analyzed in **Paper IV**.

Microwave digestion

Microwave digestion (MWD) is a common technique used to extract metals and metalloids from samples rich in organic matter. The basic principle of the technique is that the sample is placed in a closed vessel with a strong acid and then heated by microwaves to digest the organic material and release the metals into solution (Araújo et al. 2002). The acid solution can then be diluted to a suitable concentration and analyzed using atomic spectroscopy techniques.

MWD was used in **Paper I** and **Paper IV** for the digestion of sewage sludge for metal analysis using Method 3051A from the US EPA (US EPA 2007). Sludge was placed in a sealed Teflon vessel with 4 mL of HNO_3 and 1 mL of HCl and the temperature was ramped in steps up to 175 °C. The final acid extract was diluted and filtered through 0.45 µm syringe filters. The incoming water samples analyzed in **Paper I** were also subjected to MWD extraction using US EPA method 3015H to remove organic matter and reduce non-spectral interference.

Chromatography and mass spectrometry

Put simply, mass spectrometry (MS) involves separating or isolating ions based on their mass-to-charge ratio (m/z). It is a technique for quantitative analysis of inorganic and organic compounds that has become a cornerstone of environmental analytical chemistry. The technique was invented over 100 years ago when J.J. Thomson built a Parabola Mass Spectrograph that deflected positive ions from a cathode ray using a magnetic field (Griffiths 2008). Following practical improvements and the discovery of isotopes (Aston 1921, Thomson 1921), the technique underwent a revolution during World War II when it was used in the petroleum industry and to separate uranium isotopes (Nier 1989). Gas chromatography (GC) was first coupled to MS at the end of the 1950's (Gohlke 1959), enhancing its applicability to complex samples and securing the mass spectrometer's position as an invaluable tool for environmental analysis.

GC-MS

In GC-MS, compounds in the sample are separated by volatility and their interactions with the column's stationary phase before entering the MS for identification (Sparkman et al. 2011). A capillary column is typically used to achieve good separation, and analytes can be positively identified by comparison to spectral libraries if electron impact (EI) ionization is used. A GC-MS method for triclosan, parabens and some phenolic compounds in sludge samples was developed for this thesis but not used for analysis in any papers.

LC-MS/MS

Coupling liquid chromatography (LC) to MS (LC-MS) was challenging until the invention of electrospray ionization (ESI) (Dole et al. 1968, Yamashita & Fenn 1984, Fenn et al. 1989). This opened up new possibilities in environmental analytical chemistry because it eliminated several drawbacks of GC-MS. Suddenly, it was possible to analyze non-volatile compounds in water without needing long extraction procedures and derivatization steps (McMaster 2005). The technique eventually became the method of choice for analyzing polar compounds such as antibiotics and other pharmaceuticals.

An LC-MS system has three major components. First is the sample introduction system, which consists of an LC unit and autosampler; second is an analytical column for separating compounds; and third is the MS system, which is used to ionize and detect analytes (de Hoffmann & Stroobant 2007). An overview of the LC-MS/MS system used in this thesis can be seen in **Figure 8**. A fixed volume of sample is injected into the system using an autosampler. The components of the sample are then separated based on their physicochemical properties, usually by partitioning between liquid and stationary phases inside a packed column (HPLC). Upon entering the MS system, the sample's components are ionized by ESI or some other ionization technique depending on their properties (Huang et al. 2010). The charged molecules then enter the high-vacuum part of the mass spectrometer, where they can be separated by the mass analyzer. A common type of mass analyzer used in LC-MS is the quadrupole (Paul & Steinwedel 1960) which consists of four parallel rods. RF and DC voltages are applied across these rods to allow only ions of a specific m/z to reach the detector. The quadrupole is a simple and robust design but unfortunately creates a high risk of isobaric interference due to low resolution. This can be solved by combining two quadrupoles in series with a collision cell in the middle to achieve a form of tandem mass spectrometry (MS/MS) known as triple quadrupole mass spectrometry (QqQ) (Yost & Enke 1978). This approach was used for all analyses of organic molecules presented in this thesis.

For all analyses of organic molecules presented in **Papers I-V**, a PAL autosampler was used to inject the sample into the injection valve followed by the loop. The sample was then directed to the analytical column for separation. Different kinds of reversed phase columns were used for all analyses (see respective paper for details). A heated electrospray ionization (HESI) source was used on the Thermo TSQ Quantum EMR triple quadrupole instrument.

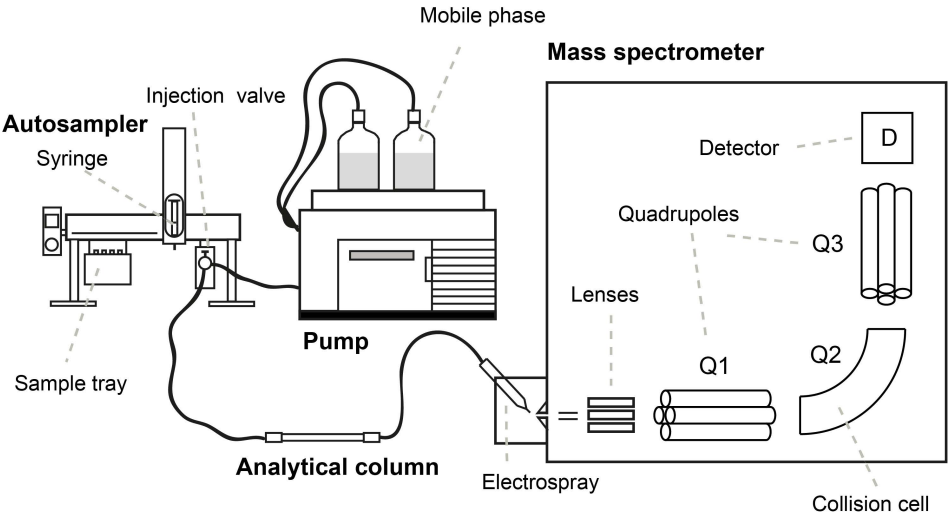


Figure 8. *Schematic overview of a LC-MS/MS system with a triple quadrupole mass analyzer.*

ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) (Houk 1986, Greenfield 2000) is the method of choice for most types of trace metal analysis due to its high sensitivity, robustness and relative insensitivity to spectral interference (Evans & Giglio 1993, Harris 2007). A typically configured ICP-MS instrument has three principal components: the sample introduction system, which converts the sample into an aerosol (or gas), a plasma source to atomize and ionize the sample, and a mass spectrometer for ion isolation and detection (see **Figure 9**). The sample is aspirated through a nebulizer that creates a fine mist. Larger droplets are removed in a spray chamber to allow efficient ionization in the plasma without unnecessary cooling. The plasma source (Houk et al. 1980) contains argon gas that is ionized inside a quartz tube using an induction coil to create a plasma with a temperature of 5000 – 10 000 K. This efficiently atomizes and ionizes the components of the sample that can be separated by their m/z ratio in the mass analyzer (in this case a quadrupole) and registered by the detector.

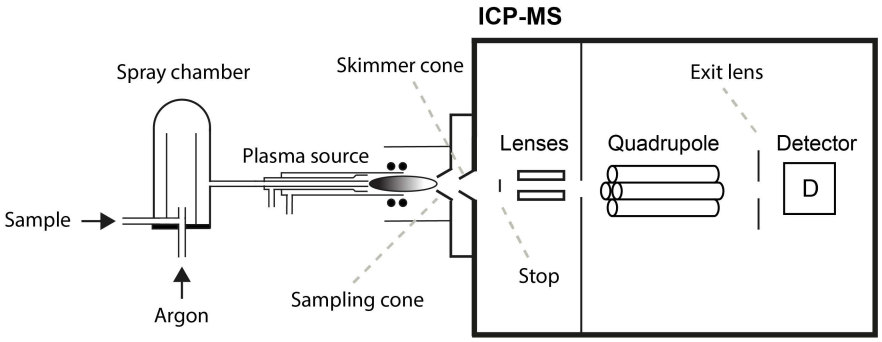


Figure 9. *Schematic overview of an ICP-MS instrument with a standard pneumatic nebulization-spray chamber sample introduction system and quadrupole mass filter.*

An ICP-MS instrument was used for the analyses of metals and metalloids in **Papers I** and **IV**. Details of the instrument's settings and the analysis are presented in each paper. Briefly, sludge and incoming wastewater samples were extracted using MWD as described above. The treated effluent samples were then filtered through 0.45 µm filters and diluted 2x with HNO_3/HCl (4:1, v/v) to a final acid concentration of 2%. The sample introduction system consisted of a MicroFlow PFA-ST nebulizer and cyclone spray chamber. The ICP-MS used was a Perkin Elmer/Sciex Elan DRC-e instrument, measuring two isotopes per element where possible.

Occurrence and fate of antimicrobials in sewage treatment plants

It is well established that many of the chemicals we use will end up in sewage treatment plants (Kümmerer 2001, Verlicchi et al. 2012, Loos et al. 2013, Olofsson et al. 2013, Luo et al. 2014, Östman et al. 2014, Blum et al. 2017). As discussed above, it has been suggested that antimicrobial compounds present in STPs can impose selective pressure on bacteria and thereby enrich antibiotic resistance genes (Rizzo, Manaia, et al. 2013). To understand these processes, it is essential to know which antimicrobial compounds are present in sewage treatment plants and at what levels. It is also important to know the concentrations at which antimicrobials are disseminated to receiving waters from the STP via the release of treated effluent and in digested sludge that may be used for agricultural fertilization. Finally, to reduce the levels at which antimicrobials are disseminated from STPs, it is important to understand the fate of these compounds within the STP and the processes responsible for their removal.

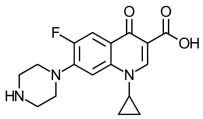
Antimicrobial compounds

A very large number of compounds possess antimicrobial properties, of which antibiotics and biocides are two major classes. Biocides and antibiotics have been defined in several different ways; the definitions used in this thesis are given on page vii.

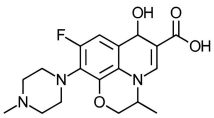
Antibiotics

Antibiotics are a group of pharmaceuticals used to treat bacterial infections. Since the discovery of the first natural antibiotics, many new compounds with antibiotic activity have been identified or synthesised. The antibiotics studied in this thesis are shown in **Figure 10**. Antibiotics can be divided into different classes based on their structures and their targets in the bacteria. Antibiotics usually target structures or functions in bacteria that are not present in human cells, resulting in selective toxicity (Walsh 2003). Notable targets of antibiotics include enzymes involved in cell wall synthesis and folate synthesis, DNA gyrases, and RNA polymerases (Walsh 2003). Antibiotics are not only used in human and veterinary medicine, but also as growth promotors in livestock; at present, the quantities of antibiotics given to animals greatly exceed those consumed by humans (Marshall & Levy 2011). The use of antibiotics as growth promotors is banned in the EU (Castanon 2007).

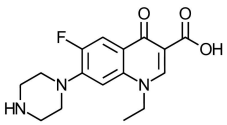
Flouroquinolone antibiotics



Ciprofloxacin

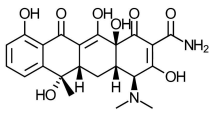


Ofloxacin

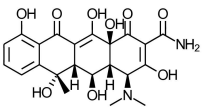


Norfloxacin

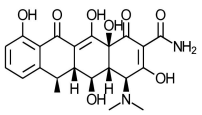
Tetracycline antibiotics



Tetracycline

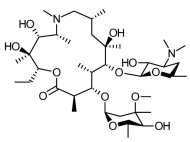


Oxytetracycline

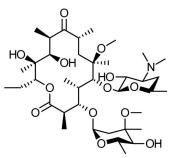


Doxycycline

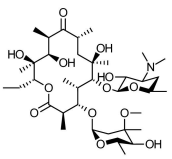
Macrolide antibiotics



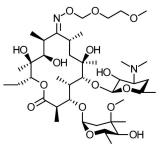
Azithromycin



Clarithromycin

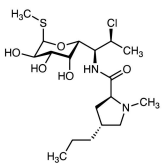


Erythromycin

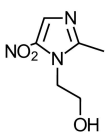


Roxithromycin

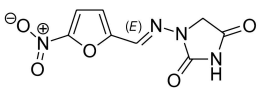
Other antibiotics



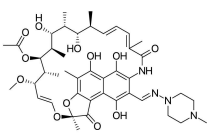
Clindamycin



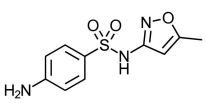
Metronidazole



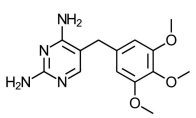
Nitrofurantoin



Rifampicin



Sulfamethoxazole



Trimethoprim

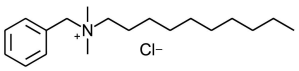
Figure 10. *Molecular structures of the antibiotics studied in this thesis.*

Biocides

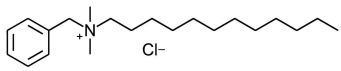
Biocides are used to prevent microbial growth in unwanted places. Some of them have been used for centuries, since long before the discovery of bacteria (Maillard 2018). The word biocide comes from “bio”, meaning life, and the suffix “cide”, meaning to kill (McDonnell & Russell 1999). A biocide is simply a substance used to kill organisms such as plants, rodents and insects. The number of applications for these chemicals is immense; among other things, they are used in food hygiene (Meyer 2006) and as disinfectants/antiseptics in healthcare settings (Murtough et al. 2002, Rutala & Weber 2004). They are also found in many consumer products (Hahn et al. 2010, Wieck et al. 2016), including as preservatives in cosmetics and personal care products (Vita et al. 2018). Finally, they are used to prevent biofouling in building materials (Burkhardt et al. 2012), on ship hulls (Callow 1990), and in various industrial installations (Klahre & Flemming 2000) – for example, biocides are used as slimicides in ballast water tanks (Ahn et al. 2013), fuel storage chambers (Bautista et al. 2016), and in hydraulic fracturing fluids (Kahrilas et al. 2014). The biocides considered in the remainder of this thesis are biocides used to control microorganisms, i.e. antimicrobial biocides, because they are the class of biocides most relevant in the context of antibiotic resistance.

The use of biocidal products in Sweden and other EU countries is regulated by the law of the European Union (EU regulation 528/2012 2012). However, a limitation of this legislation is that it is based on the intended use of the product rather than the substances themselves. A biocide that is used in a biocidal product is regulated by the biocidal products act, but the same substance used in a non-biocidal application may be subject to different laws and might not be regulated. The complexity of the legislation on biocides may lead to underestimation of the risks they pose to the environment because of incomplete risk assessments (Wieck et al. 2016). A product inventory in Germany showed that only 36% of the observed uses of biocides were covered by the EU Biocidal Products Regulation (Wieck et al. 2016). There are many different groups of biocides; those discussed in this thesis are shown in **Figure 11** and **Figure 12**.

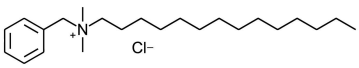
QACs



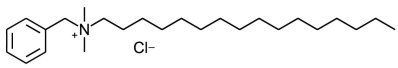
10-BAC



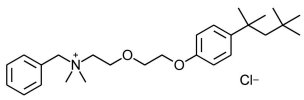
12-BAC



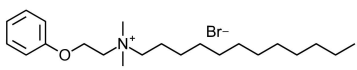
14-BAC



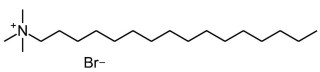
16-BAC



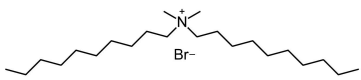
Benzethonium chloride



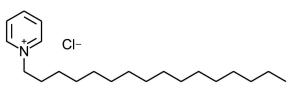
Domiphen bromide



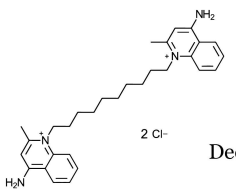
CTAB



DDMAB



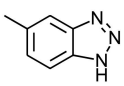
CPC



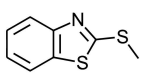
Dequalinium chloride

Benzotriazoles and benzothiazoles

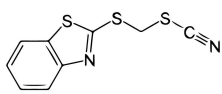
Benzotriazole



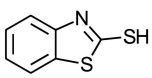
Methyl benzotriazole



MTBT

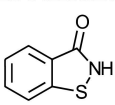


TCMTB

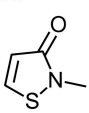


Mercaptobenzothiazole

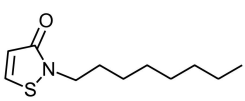
Isothiazolones



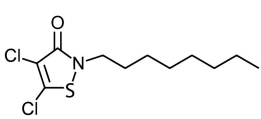
BIT



MIT



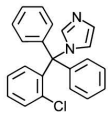
OIT



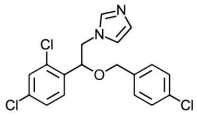
DCOIT

Figure 11. *Quaternary ammonium compounds (QACs), benzotriazoles, benzothiazoles and isothiazolones discussed in this thesis. MTBT = 2- (methylthio)-benzothiazole, TCMTB = 2-(thiocyanomethylthio)benzothiazole BIT = Benzoisothiazolinone. MIT = Methylisothiazolinone. OIT = Octhilinone. DCOIT =4,5-dichloro-2-octyl-3-isothiazolone. Abbreviations of QACs are written in Figure 13.*

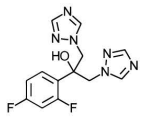
Antimycotics



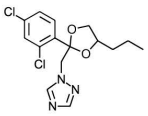
Clotrimazole



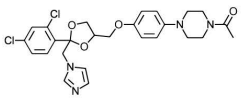
Econazole



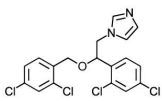
Fluconazole



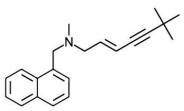
Propiconazole



Ketoconazole

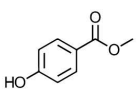


Miconazole

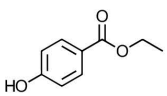


Terbinafine

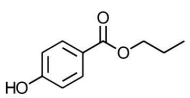
Parabens



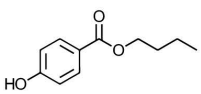
Methyl paraben



Ethyl paraben

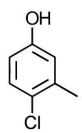


Propyl paraben

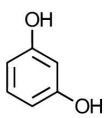


Butyl paraben

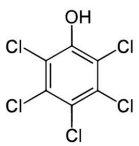
Phenols



4-chloro-3-methylphenol

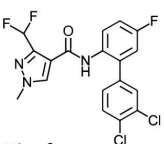


Resorcinol

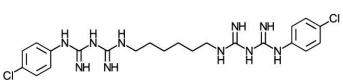


Pentachlorophenol

Others

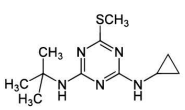


Bixafen

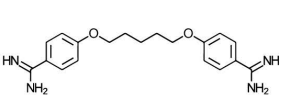


Chlorhexidine

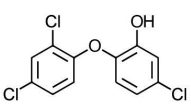
Formaldehyde



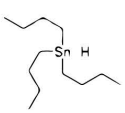
Irgarol



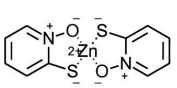
Pentamidine



Triclosan



TBT



Zn pyrithone

Figure 12. *Antimycotics, parabens, phenols and other biocide compounds discussed in this thesis. TBT = tributyltin.*

Metals and metalloids

Heavy metals are toxic to bacteria and show broad spectrum antimicrobial activity (Pal et al. 2017). Metals are naturally present and anthropogenic activities has released additional amounts into the environment for thousands of years (Nriagu 1996). Among the many uses of metals in society are several applications as antimicrobials. Various forms of zinc, copper, silver, gold, arsenic, tellurium, and antimony are typically used for this purpose (Hobman & Crossman 2015). It has been shown that antifouling boat paint that contains copper and zinc has the potential to co-select for antibiotic resistant bacteria due to cross-resistance (Flach et al. 2017). Unlike antibiotics and biocides, which are organic molecules, metals will not degrade over time. Furthermore, metals can exist as a number of different species, depending on the redox conditions and the available ligands, which can significantly affect their behavior and toxicity (Achterberg et al. 2018). Metals can also be bound to organic groups, as in the organotin biocides. The metals and metalloids studied in this thesis are listed in **Paper I**.

Selection of target compounds

There are many chemicals with antimicrobial properties, so it was necessary to select a representative subset of compounds to study. The EU list of approved biocides includes 750 substances (ECHA 2018), but only antimicrobial biocides were considered. Selection was based on usage statistics ("Swedish Chemicals Agency" 2018), previous environmental analyses (e.g. (Kümmerer et al. 1997, Kümmerer 2009a, 2009b, Tjus 2014)), stability, and analytical considerations. Compounds of interest in the context of resistance were also investigated using the BacMet database (Pal et al. 2014). More information on the selection of target compounds included in this thesis can be found in **Paper I**.

Levels of antimicrobials and metals in Swedish STPs

To determine which antimicrobial compounds are present in Swedish STPs and to quantify their concentrations, a screening campaign was conducted. Samples of incoming wastewater, treated effluent, and digested sludge were collected from 11 Swedish STPs and analysed for the presence of 36 organic antimicrobials and 11 metals (**Paper I**). **Figure 13** - **Figure 15** show all the concentration measurements reported in **Papers I, II** and **IV** and all the concentrations of antimicrobial biocides measured during screening campaigns conducted by the Swedish EPA between 2000 and 2013 (Tjus 2014) to provide an overview of the antimicrobial compounds found in Swedish STPs. The risk of resistance development for these substances is discussed in the chapter on "Antibiotic resistance in sewage treatment plants" at page 40.

Antibiotics

The antibiotic detected at the highest concentrations in the incoming wastewater was tetracycline, followed by ciprofloxacin and sulfamethoxazole. Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic that is classified as being critically important in human medicine (WHO 2016). It is a common antibiotic with over 2 million defined daily doses (DDD) prescribed in Sweden during 2015 ("Statistikdatabas för läkemedel" 2018). Both tetracycline and ciprofloxacin were occasionally detected at levels >1 µg/L in incoming wastewater. The levels of ciprofloxacin in treated effluent were lower (well below 100 ng/L). Ciprofloxacin tends to partly adsorb to sludge particles and was the antibiotic detected at the highest concentration in sludge. Several antibiotics were detected in the treated effluent, including clindamycin, erythromycin, trimethoprim and oxytetracycline.

QACs

Quaternary ammonium compounds (QACs) are widely used as disinfectants, antiseptics in various cosmetics, cleaning agents, and other household products such as fabric softeners (McDonnell & Russell 1999, Lara-Martín et al. 2010). They also have industrial applications as surfactants; among other things, they are used as phase transfer agents and antistatics (Zhang et al. 2015). Because of this wide usage, they have become high production volume chemicals (Tezel & Pavlostathis 2015), with over 100 tons sold in Sweden annually (Swedish Chemicals Agency 2018). In addition to their household and industrial sources, QACs also enter wastewater from hospitals (Kümmerer et al. 1997), laundry facilities (Kreuzinger et al. 2007), and runoff from roofs (Van de Voorde et al. 2012). Some QACs such as benzyldimethyldodecylammonium chloride (BAC-12) and hexadecyltrimethylammonium bromide (CTAB) were detected at concentrations of >10 µg/L in incoming wastewater in **Papers I**, **II** and **IV**. The levels of QACs in treated effluent were generally <100 ng/L, but those in sludge were much higher, exceeding 100 mg/kg in several cases.

Benzotriazoles

Benzotriazoles are primarily used as corrosion inhibitors, especially in dishwashers (Janna et al. 2011). However they do have other industrial applications (Weiss et al. 2006), including as antimicrobials (Jamkhandi & Disouza 2012). Benzotriazoles were detected in almost all water samples analysed during this thesis. The highest concentration measured for any compound of this class in incoming water was 24.6 µg/L, which was observed for benzotriazole itself. Benzotriazoles were also detected at high concentrations in treated effluent, with levels exceeding 1000 ng/L for both benzotriazoles in many samples. These high concentrations are consistent with EU data; benzotriazole concentrations of up to 221 µg/L have been detected in treated effluent (Loos et al. 2013).

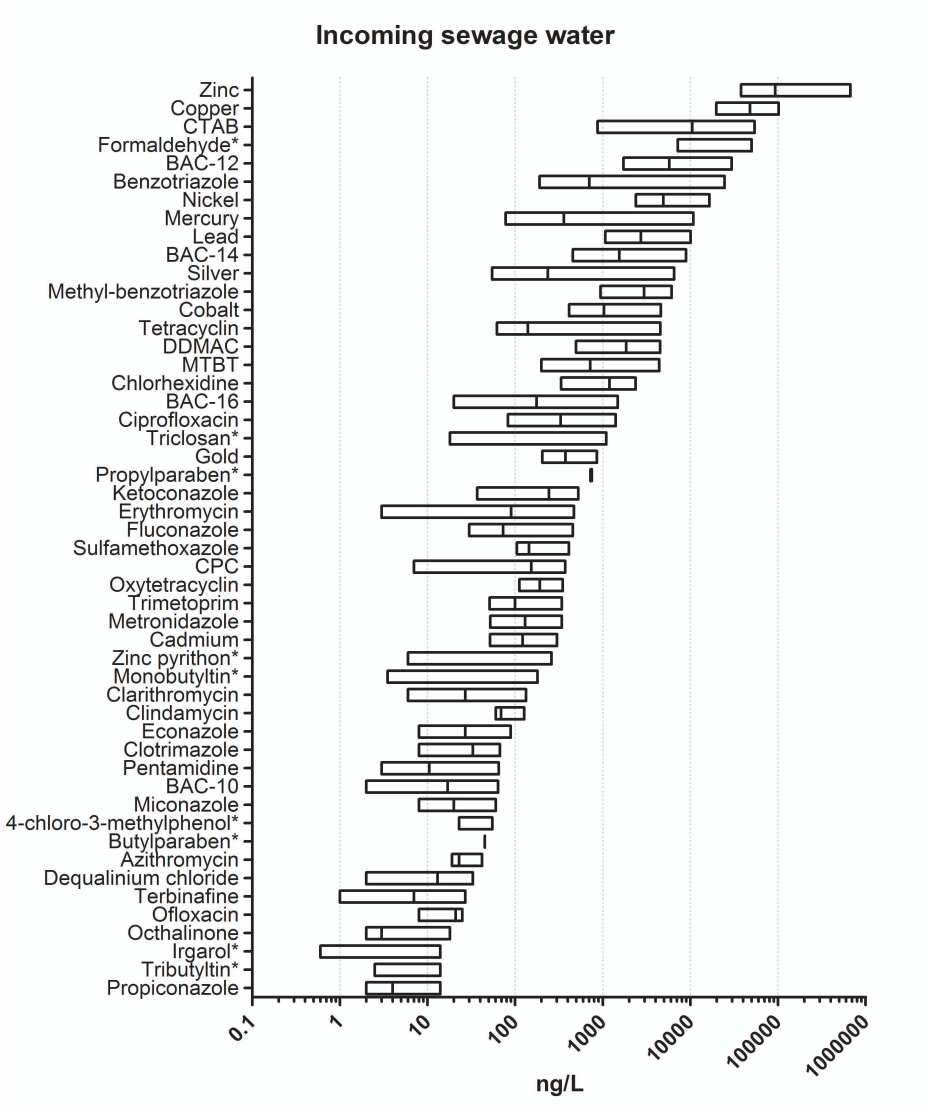


Figure 13. *Levels of antimicrobial compounds in incoming wastewater from Swedish STPs. The bars indicate the minimum and maximum measured values; the central lines represent median values. The data are derived from* ***Papers I***, ***II*** *and* ***IV****. *Data derived from screening campaigns conducted by the Swedish EPA (Tjus 2014). CTAB = hexadecyltrimethylammonium bromide, BAC-12 = benzyldimethyldodecylammonium chloride, BAC-14 = benzyldimethyltetradecylammonium chloride, DDMAC = didecyldimethylammonium chloride, MTBT = 2-(methylthio)-benzothiazole, BAC-16 = benzyldimethylhexadecylammonium chloride, CPC = hexadecylpyridinium chloride, BAC-10 = Benzyldimethyldecylammonium chloride.*

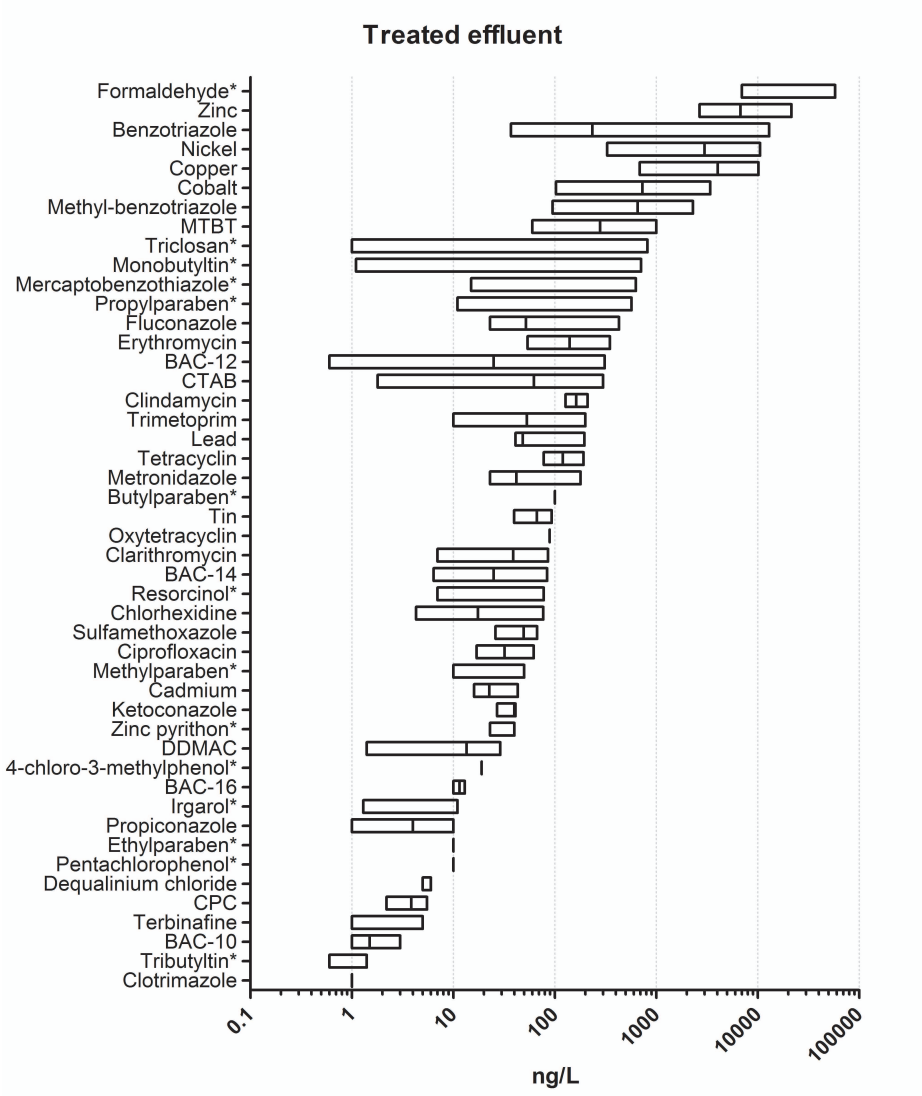


Figure 14. *Levels of antimicrobial compounds in treated wastewater effluents from Swedish STPs. The bars indicate the minimum and maximum measured values; the central lines represent median values. The data are derived from* ***Papers I, II*** *and* ***IV****. *Data derived from screening campaigns conducted by the Swedish EPA (Tjus 2014). CTAB = hexadecyltrimethylammonium bromide, BAC-12 = benzyldimethyldodecyl ammonium chloride, BAC-14 = benzyldimethyltetradecylammonium chloride, DDMAC = didecyldimethylammonium chloride, MTBT = 2-(methylthio)-benzothiazole, BAC-16 = benzyldimethylhexadecylammonium chloride, CPC = hexadecylpyridinium chloride, BAC-10 = Benzyldimethyldecylammonium chloride.*

Benzothiazoles

Benzothiazole compounds have diverse applications such as slimicides, fungicides, preservatives, corrosion inhibitors, de-icing fluids and additives in rubber manufacturing (Asimakopoulos et al. 2013). Two common compounds of this class are mercaptobenzothiazole and 2-(thiocyanomethylthio)-benzothiazole (TCMTB). TCMTB is unstable in water and has only been detected rarely in Swedish wastewater (Tjus 2014); it is rapidly degraded into 2-(methylthio)-benzothiazole (MTBT) (De Wever & Verachtert 1997). MTBT was found in almost all water samples analysed for this thesis. As noted in **Paper I**, its average concentrations in incoming wastewater and treated effluent were 1200 ng/L and 330 ng/L, respectively.

Isothiazolones

Isothiazolones (or isothiazolinones) are biocides with applications as preservatives in cosmetics and paint, and as slimicides in various industrial applications (Jayjock et al. 1996, Russell 2003, Johansson & Somasundaran 2007). Washing and cleaning agents are important sources of isothiazolones in wastewater (Wieck et al. 2018a). They are also found in facade coatings, so their levels in STPs vary with the rain if the STP is connected to stormwater drains or stormwater leaks into the STP's pipes (Bollmann et al. 2014). The isothiazolone octhilinone (OIT) was detected in 36% of the incoming wastewater samples analysed in **Paper I** at an average concentration of 6 ng/L. Various isothiazolones have been reported in STPs in northern Europe (Rafoth et al. 2007, Bollmann et al. 2014, El-taliawy et al. 2017, Wieck et al. 2018a). Earlier screening campaigns measuring methylisothiazolinone (MIT) in Sweden did not detect it. (Tjus 2014).

Antimycotics

Antimycotics are mainly pharmaceuticals used to treat fungal infections (Casado et al. 2014). Some antimycotics such as propiconazole are also used as preservatives, mainly for wood (Fernández-Calviño et al. 2017). Fluconazole was the most commonly detected antimycotic in treated effluent and the one detected at the highest levels; its measured concentrations exceeded 200 ng/L. The other antimycotics were predominantly associated with sludge and were less commonly detected in treated effluent. However, they were common in digested sludge, with levels exceeding 1 mg/kg d.w. in some cases.

Parabens

Parabens are compounds used for their antimicrobial properties (Haman et al. 2015). They are especially common in cosmetics, being used in over 20 000 products (Andersen 2008). Several parabens have recently become more strictly

regulated in the EU (EU 358/2014 2014, EU 1004/2014 2014). They have been detected in wastewater for over 20 years (Paxéus 1996); their concentration ranges in Swedish wastewater and sludge can be seen in **Figure 13** – **Figure 15.** There are many different parabens but the most common are the methyl– butyl paraben homologues. Propyl paraben was the most commonly detected paraben in treated Swedish effluent; its measured concentrations range from 11 to 570 ng/L (Tjus 2014).

Phenols

Several phenolic compounds are used as biocides, notably as disinfectants in healthcare settings (Russell 2002, Tluczkiewicz et al. 2010). They can also be used as preservatives, as exemplified by pentachlorophenol (McLellan et al. 2007). No phenols were analysed in this work, but earlier screening campaigns in Sweden showed that resorcinol, 4-chloro-3-methylphenol, and pentachlorophenol were detected in STPs (**Figure 13** – **Figure 15**). Resorcinol is widely used in various industrial applications, and was detected at levels of 7 – 78 ng/L (Tjus 2014).

Metals

Zinc and copper dominated the incoming wastewater, with average concentrations exceeding 50 µg/L; the measured concentrations of Zn were as high as 670 µg/L. Zn and Cu are used as biocides in products such as antifouling boat paint (Yebra et al. 2004, Thomas & Brooks 2010) but these are unlikely to be their main sources in wastewater. Industrial point sources have historically been the dominant sources of metal ions in wastewater (Lester et al. 1979), but sources today are more varied and include households, traffic, and atmospheric deposition, among others (Sörme & Lagerkvist 2002, Karvelas et al. 2003). Another important source of copper is domestic plumbing (Houhou et al. 2009). Other common metals were nickel, lead, cobalt, mercury and silver. Levels of silver in Swedish sewage sludge have fallen almost ten-fold over the last 20 years ("Silver i rötslam - Stockholms miljöbarometer" 2018) because silver was used to develop photographic films but traditional film cameras have been almost completely displaced by digital cameras (Eckelman & Graedel 2007). However, silver has broad use as an antimicrobial (often in the form of nanosilver) in healthcare settings and household products such as clothes, washing machines, and cosmetics (Diener & Palme 2012). These metals therefore remain among the most highly concentrated target analytes in the treated effluent (**Figure 14**). Most metals tend to adsorb to particulate material and are therefore found at high concentrations in the digested sludge (**Figure 15**). Notably, Cu has been detected at levels exceeding 1 g/kg d.w. of sludge and other elements such as aluminium have been detected at concentrations of >50 g/kg d.w. sludge (Suanon et al. 2018).

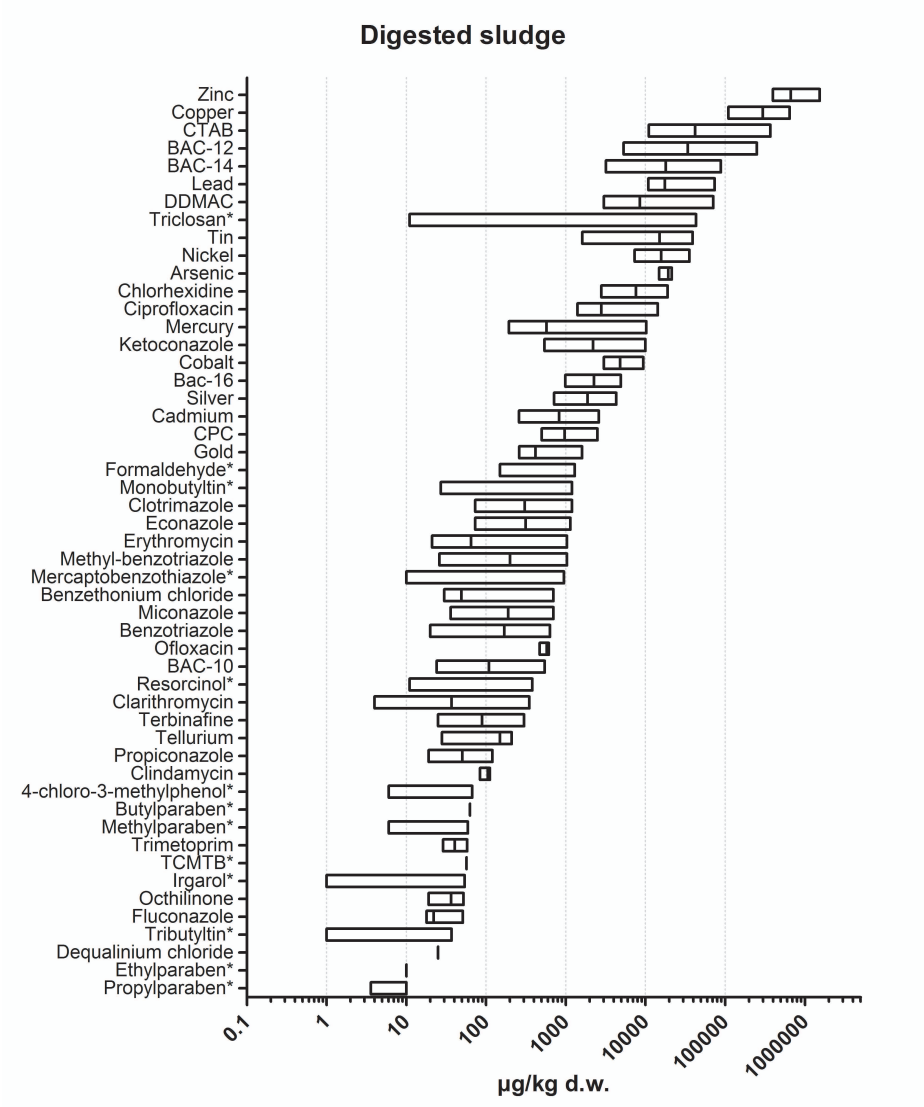


Figure 15 *Levels of antimicrobial compounds in digested sludge from Swedish STPs. The bars indicate the minimum and maximum measured values; the central lines represent median values. The data are derived from* ***Papers I, II*** *and* ***IV****. *Data derived from screening campaigns conducted by the Swedish EPA (Tjus 2014). CTAB = hexadecyltrimethylammonium bromide, BAC-12 = benzyldimethyldodecylammonium chloride, BAC-14 = benzyldimethyltetradecylammonium chloride, DDMAC = didecyldimethylammonium chloride, TCMTB = 2-(thiocyanomethylthio)-benzothiazole, BAC-16 = benzyldimethylhexadecylammonium chloride, CPC = hexadecylpyridinium chloride, BAC-10 = Benzyldimethyldecylammonium chloride.*

Other compounds

Among the organic antimicrobials listed in **Figure 13**, formaldehyde (which is not analysed further here) was detected at the highest concentrations. Formaldehyde is a naturally occurring compound and is the most common aldehyde in the environment (Wilbourn et al. 1995) but is also used for disinfection and preservation as well as in manufacturing plastics, glues, paint, etc. (Cheney & Collins 1995, McDonnell & Russell 1999, Tjus 2014).

Chlorhexidine is a very common antiseptic used in both households and hospitals (Russell & Path 1986). It was detected in all sludge and incoming water samples, and in 12 % of the treated wastewater samples. Its concentrations were high, averaging 1305 ng/L in incoming wastewater from 11 STPs. Before the studies presented in this thesis, chlorhexidine had only been detected in hospital effluent in 1984 (Matsushima & Sakurai 1984) and an earlier ambitious Swedish screening study failed to detect it (Törneman 2011).

Triclosan is arguably one of the more well-known antibacterial agents. It is found in many personal care products such as toothpaste and mouth washes as well as in functional clothes and plastic materials (Engelhaupt et al. 2007). It was not quantified in any samples analysed in this work, but previous screening campaigns in Sweden have frequently detected it in the incoming wastewater of STPs at levels below 1000 ng/L (**Figure 13**). The use of triclosan has decreased in parts of the world due to increasingly strict regulation by authorities such as the EU (EU 2016/110 2016) and the FDA (Food and Drug Administration 2017). This decreased usage is reflected in gradually decreasing concentrations in Swedish sludge (Olofsson et al. 2012).

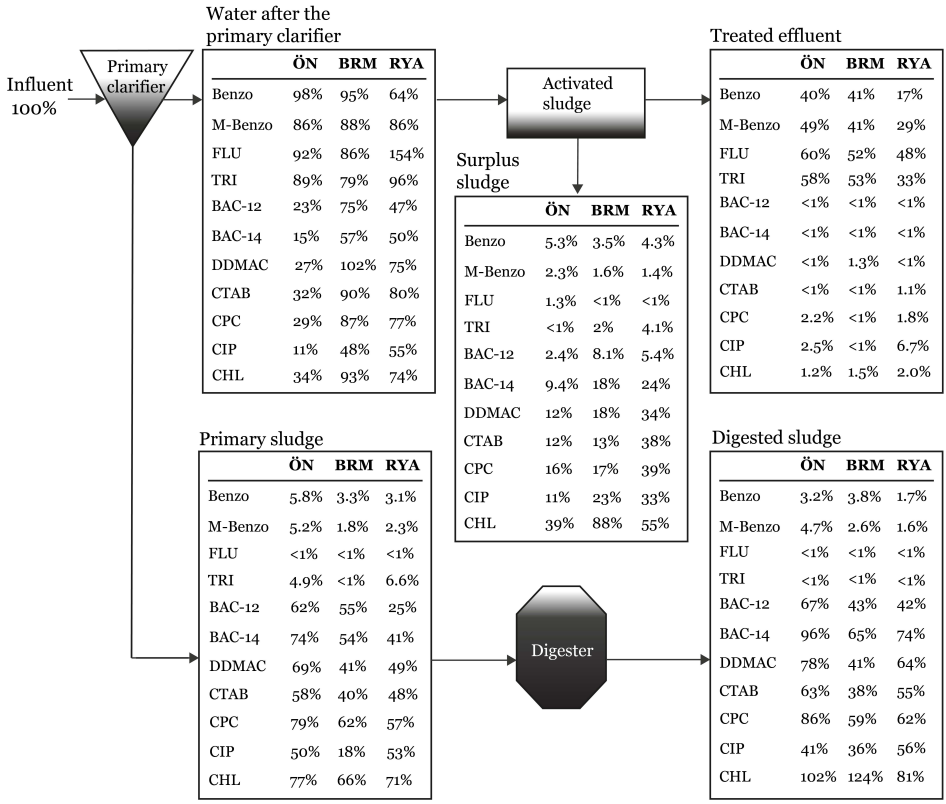
Removal efficiency of antimicrobials in STPs

As shown in **Figure 14**, many antimicrobials are found in treated effluent that enters the recipient aquatic environment. It is well known that many compounds are inefficiently removed in STPs (Loos et al. 2013). Removal efficiencies depend on both the properties of the micropollutants and the type of treatment as well as parameters of the treatment process, such as the temperature (Castiglioni et al. 2006), hydraulic retention time (Tauxe-Wuersch et al. 2005, Guerra et al. 2014), solid retention time (Clara et al. 2005), nitrification (Tran et al. 2009) and dilution by storm water (Ternes 1998).

The data from **Paper I** provided clear indications of overall removal efficiency for many studied compounds, albeit with the limitation that only three sample types were studied over three days. To better characterize the fate of the studied compounds in STPs, a larger sampling campaign was performed for **Paper II,** including eight sample types sampled over nine days in three different STPs. This gave more detailed information on the compounds' distribution and fate in the STPs, including their fate during different treatment steps.

The removal efficiencies for antimicrobials during the different treatment steps at the Bromma, Rya and Ön STPs are listed in **Figure 16**. The figure shows the average mass flow for each compound as a percentage of that in the incoming wastewater at various sampling points. The benzotriazoles, fluconazole and trimethoprim were all inefficiently removed from the wastewater, with removal efficiencies of 40 – 83% (i.e. 17 – 60 % of the mass flow in the incoming wastewater remained in the treated effluent). The QACs, ciprofloxacin and chlorhexidine were removed more efficiently, with removal efficiencies of 93 - >99%. The opposite was observed in the digested sludge: less than 5% of the benzotriazoles, fluconazole, and trimethoprim ended up there. The mass flows of QACs, ciprofloxacin and chlorhexidine in the digested sludge were in the range of 36 – 124% compared to the incoming wastewater.

Two mechanisms can explain the removal of the studied compounds from wastewater: sorption to sludge particles, and biodegradation/transformation (Khan & Ongerth 2002). Evaporation to air is also possible but not expected to be relevant here because the Henry's law constants (Sander 2015) of the studied compounds are below 10^{-7} atm-m^3/mole (Olsen & Davis 1990).



Water after the primary clarifier	ÖN	BRM	RYA
Benzo	98%	95%	64%
M-Benzo	86%	88%	86%
FLU	92%	86%	154%
TRI	89%	79%	96%
BAC-12	23%	75%	47%
BAC-14	15%	57%	50%
DDMAC	27%	102%	75%
CTAB	32%	90%	80%
CPC	29%	87%	77%
CIP	11%	48%	55%
CHL	34%	93%	74%

Surplus sludge	ÖN	BRM	RYA
Benzo	5.3%	3.5%	4.3%
M-Benzo	2.3%	1.6%	1.4%
FLU	1.3%	<1%	<1%
TRI	<1%	2%	4.1%
BAC-12	2.4%	8.1%	5.4%
BAC-14	9.4%	18%	24%
DDMAC	12%	18%	34%
CTAB	12%	13%	38%
CPC	16%	17%	39%
CIP	11%	23%	33%
CHL	39%	88%	55%

Treated effluent	ÖN	BRM	RYA
Benzo	40%	41%	17%
M-Benzo	49%	41%	29%
FLU	60%	52%	48%
TRI	58%	53%	33%
BAC-12	<1%	<1%	<1%
BAC-14	<1%	<1%	<1%
DDMAC	<1%	1.3%	<1%
CTAB	<1%	<1%	1.1%
CPC	2.2%	<1%	1.8%
CIP	2.5%	<1%	6.7%
CHL	1.2%	1.5%	2.0%

Primary sludge	ÖN	BRM	RYA
Benzo	5.8%	3.3%	3.1%
M-Benzo	5.2%	1.8%	2.3%
FLU	<1%	<1%	<1%
TRI	4.9%	<1%	6.6%
BAC-12	62%	55%	25%
BAC-14	74%	54%	41%
DDMAC	69%	41%	49%
CTAB	58%	40%	48%
CPC	79%	62%	57%
CIP	50%	18%	53%
CHL	77%	66%	71%

Digested sludge	ÖN	BRM	RYA
Benzo	3.2%	3.8%	1.7%
M-Benzo	4.7%	2.6%	1.6%
FLU	<1%	<1%	<1%
TRI	<1%	<1%	<1%
BAC-12	67%	43%	42%
BAC-14	96%	65%	74%
DDMAC	78%	41%	64%
CTAB	63%	38%	55%
CPC	86%	59%	62%
CIP	41%	36%	56%
CHL	102%	124%	81%

Figure 16. *Average mass flows expressed as percentages of those in the incoming wastewater (based on samples collected over 9 days) for selected biocides and antibiotics in three Swedish STPs. ÖN = Öns STP, Umeå. BRM = Bromma STP, Stockholm. RYA = Rya STP, Göteborg. Benzo = 1H-benzotriazole, M-Benzo = 5-methyl-benzotriazole + 4-methyl-benzotriazole, FLU = fluconazole, TRI = triemethoprim, BAC-12 = benzalkonium chloride-12, BAC-14 = benzalkonium chloride-14, CTAB = cetrimonium bromide, DDMAC = didecyldimethylmethyl ammonium chloride, CPC = hexadecylpyridinium chloride, CIP = ciprofloxacin, CHL= chlorhexidine.*

The benzotriazoles, trimethoprim and fluconazole were only removed to sludge to a small extent; instead, they followed the water (**Figure 17**). This was expected looking at the phase distribution between water and particles (**Figure 18**), showing that the amount of these compounds were >90% detected in the water phase in the incoming wastewater.

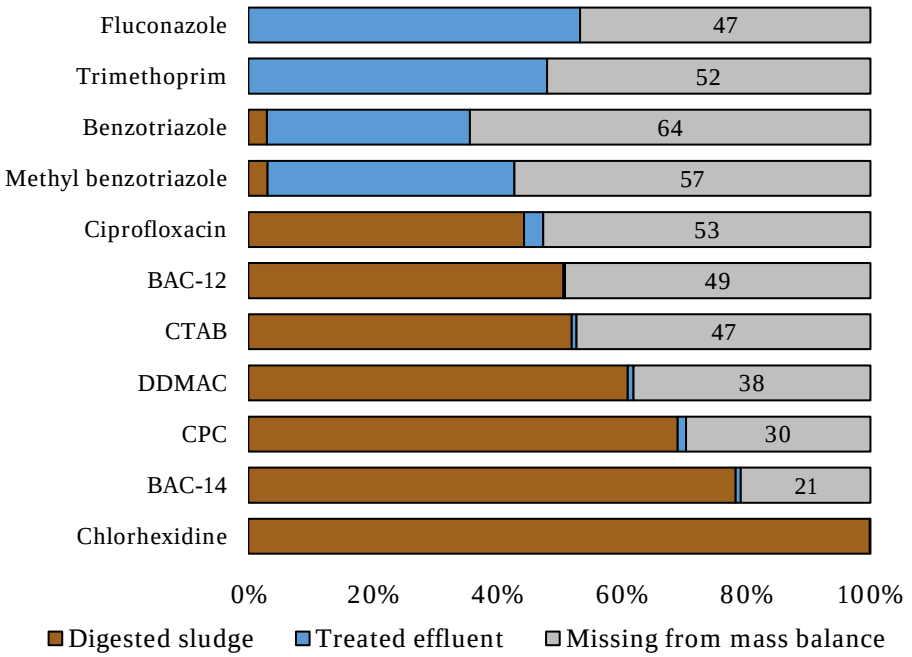


Figure 17. *Average mass flows for eleven antimicrobials, expressed as percentages of the values for incoming water, in digested sludge and treated effluent in three Swedish STPs (**Paper II**) and the percent missing from the mass balance (compared to incoming wastewater). Material missing from the mass balance was assumed to have undergone biodegradation. Chlorhexidine has been set to 100% in sludge in the figure for illustrative purposes since the calculated value exceeds 100% (true value = 102%).*

The compounds that were removed from the wastewater primarily via the primary and surplus sludge, such as QACs and chlorhexidine, were predominantly (>90%) detected in the particle phase in the incoming wastewater. As shown in **Figure 18**, compounds predominantly detected in water generally have lower Log K_{OW} values than those predominantly detected in sludge. The correlation is very weak, however, so models based on hydrophobicity alone are too simplistic. Other factors such as the compound's ionic state are probably important (Pan et al. 2009, Rybacka & Andersson 2016). The sorption of several compounds has been shown to vary with the pH of the sludge (Hörsing et al. 2011), and a compound's chemical structure is known to affect its removal by biological treatment systems (Tadkaew et al. 2011).

On average, 0 – 64% of the mass flow in incoming wastewater was found to be missing from the mass balance in **Paper II**. This was tentatively attributed to biodegradation. As shown in **Figure 17**, all compounds except chlorhexidine were apparently degraded to some extent. The main treatment step responsible

for this degradation in the STPs was the biological treatment step with activated sludge. The removal efficiency varied between the STPs, as reported previously (Chen et al. 2015). The QACs that entered the biological treatment step were not just biotransformed, they were also partly removed through the surplus sludge to a greater extent than compounds less associated with sludge such as benzotriazoles and fluconazole. The aerobic environment of the activated sludge process was much more favourable for degradation than the anaerobic environment of the digester, which only affected trimethoprim.

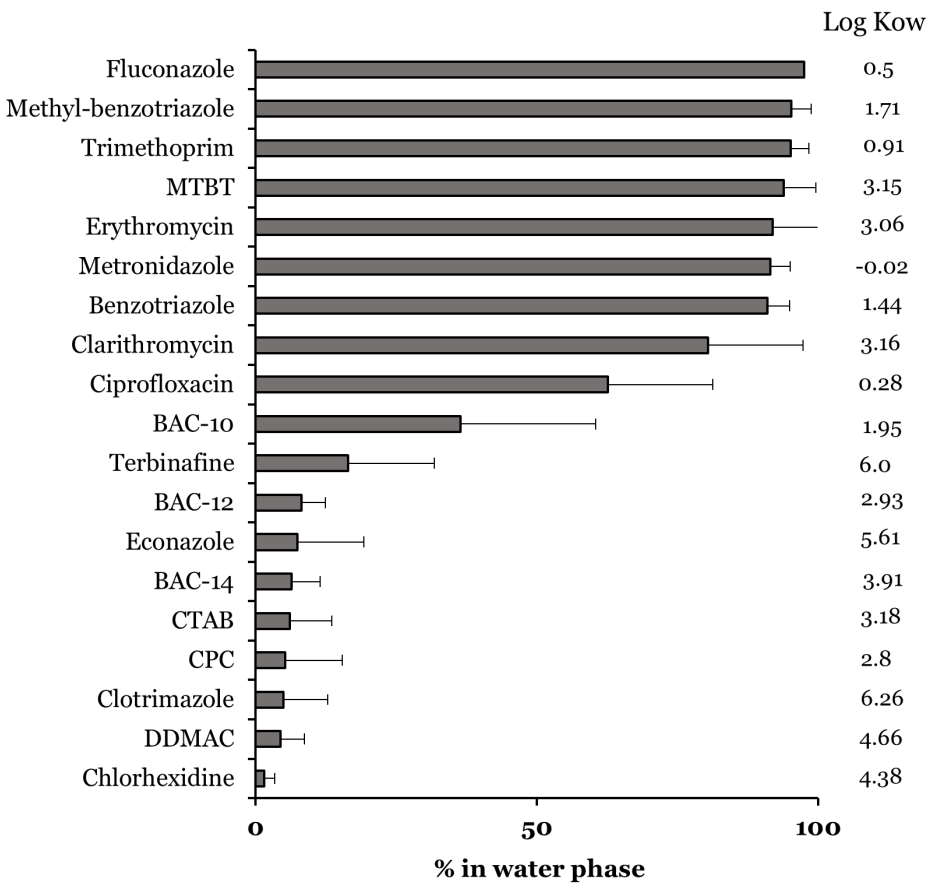


Figure 18. *Phase distributions of antimicrobials in incoming wastewater as reported in* ***papers I-II****. Levels detected in the water phase are expressed as percentages of the total detected amount (water + particle phase). All values below LOQ were replaced with LOQ/2 and only compounds detected at >80% in at least one phase were included. MTBT and metronidazole were only detected in the water phase. Log* K_{OW} *values were calculated using EPI suite (US EPA 2015).*

Dissemination of antimicrobials via sludge and treated wastewater

As shown by the preceding discussion, biocides and antibiotics are present in treated effluent and digested sludge. Accurate environmental risk assessments require reliable estimates of the total load of these compounds emitted to the environment from Swedish STPs. **Table 2**, presents estimated total emissions for selected antimicrobials from all Swedish STPs larger than 10 000 PE. The STPs considered in **Papers I** and **II** were classified into four different categories based on their size. The mean emissions of each antimicrobial for each STP size category were then multiplied by the number of Swedish STPs belonging to that category to estimate total yearly emissions. The resulting numbers are only rough estimates because some of the size categories are very broad. For example, Ryaverket is much larger than the other STPs in its class, which affects the mean. Additionally, the 237 STPs with PE values between 2000 – 10 000 were not included because no STPs in that size range were studied. The numbers in **Table 2** are therefore likely to be underestimated.

The combined estimated emissions to effluent and sludge for all antimicrobials included in **Table 2**, were 42 000 kg per year, of which 4 000 kg was emitted to the aquatic environment via treated effluent and 38 000 kg ended up in the digested sludge. In Sweden, sludge can be used as an agricultural fertilizer if its contents of heavy metals and selected organic compounds are below specific threshold values (European Commission 2001). At present, about 25% of all sludge produced in Swedish STPs is used in agriculture (Hörsing 2018). The handling of sludge has been debated extensively, and the Swedish government is considering banning its agricultural use (Regeringskansliet 2018).

The antimicrobials with the highest emissions to treated effluent were the benzotriazoles (~2500 kg/year), followed by MTBT (686 kg/year). Although the QACs were predominantly emitted to sludge, over 350 kg/year were emitted in treated effluent. Additionally, emissions of the antibiotics ciprofloxacin and trimethoprim to effluent exceeded 200 kg/year.

The antimicrobials with the highest emissions to sludge were the QACs; the combined emissions of benzalkonium chlorides, DDMAC, and CTAB via sludge exceed 34 000 kg/year. The substance with the highest emissions via sludge was CTAB, which was emitted at a rate of 15 383 kg/year. Emissions of chlorhexidine to sludge were also high, at almost 2500 kg per year. The antibiotic with the highest emissions via sludge was ciprofloxacin, whose total emissions exceed 1000 kg/year.

Table 2. *Calculated total yearly emissions of antimicrobials from Swedish STPs of different sizes*

Compound		Emissions in kg/year				
	PE	>100k	50 – 100k	20 – 50k	10 – 20k	Sum:
Benzotriazole	Effluent	360	139	409	40	948
	D. sludge	27	8.7	11	11	58
M-benzotriazole	Effluent	681	341	381	103	1506
	D. sludge	34	12	3.9	4,9	55
Clotrimazole	Effluent	64	42.5	19.2	4.4	130
	D. sludge	77	15	13	5.4	110
Econazole	Effluent	x	x	x	x	x
	D. sludge	89	15	15	4.6	123
Miconazole	Effluent	x	x	x	x	x
	D. sludge	76	8.8	15	4.7	105
Fluconazole	Effluent	64	43	19	4.4	130
	D. sludge	x	x	x	x	x
Ciprofloxacin	Effluent	61	9.5	13	x	84
	D. sludge	418	215	260	199	1092
Trimethoprim	Effluent	70	33	19	9.3	131
	D. sludge	x	x	x	x	x
MTBT	Effluent	389	194	86	17	686
	D. sludge	x	x	x	x	x
Chlorhexidine	Effluent	30	6.1	x	x	36
	D. sludge	1443	479	393	183	2498
BAC-10	Effluent	x	x	x	x	x
	D. sludge	13	7.5	6.1	2.3	29
BAC-12	Effluent	32	8.7	46	x	88
	D. sludge	4733	2265	1640	730	9368
BAC-14	Effluent	31	7,9	12	1.9	53
	D. sludge	2909	1360	878	517	5664
DDMAC	Effluent	35	3.4	4.7	0.7	44
	D. sludge	2433	564	615	187	3798
CTAB	Effluent	101	21	48	1.9	172
	D. sludge	7941	4197	2529	716	15 383

The STPs from **papers I** and **II** were divided into four groups according to size and the average mass flow for each group was multiplied by the total number of Swedish STPs belonging to that group according to the SCB (SCB 2016). 10 – 20k PE: 68 STPs, 20 – 50k PE: 62 STPs, 50 – 100k PE: 28 STP, and >100k PE: 21 STPs. D. sludge = Digested sludge, PE = Population equivalents. x = No value available because most or all measurements were below LOQ.

Antibiotic resistance in sewage treatment plants

Since the concentrations of many antimicrobial compounds in sewage treatment plants were established in **Papers I** and **II**, and in earlier studies, the next step was to investigate the connections between these concentrations and the development of resistance.

Studying environmental antibiotic resistance

There are several ways to obtain information about phenotypic and genotypic resistance. The sections below briefly outline the techniques used in **Papers III** and **IV**.

Culturing

Culturing of microorganisms in the lab has been an important tool for the last 150 years (Overmann et al. 2017). Microorganisms are grown in a culture medium, which makes it possible to isolate certain strains for study. Modifying the medium, for example agar, by adding an antibiotic prevents the growth of non-resistant strains (Madigan et al. 2000). The main disadvantage of culturing is that most bacteria can't be cultivated using standard methods and thus cannot be studied using this approach (Amann et al. 1995).

qPCR

Quantitative real-time PCR (qPCR) is used to measure the abundance of certain genes (Heid et al. 1996). It has been widely used to quantify resistance genes in various environmental compartments, including sewage treatment plants (Gao et al. 2012). qPCR is a culture-independent and highly sensitive method that makes it possible to detect and quantify resistance genes from bacterial species that cannot be studied using traditional culturing methods. The drawback is that there is a limited set of resistance genes that can be studied at the same time, and the genes of interest must be known in advance.

Metagenomics

Metagenomics was developed partly in response to the inability of qPCR to study unknown genes (Handelsman et al. 1998). The idea of metagenomics is that many different genomes from an environmental sample can be studied at once without worrying about which species they originate from.

The original version of metagenomics was termed functional metagenomics (Handelsman et al. 1998). Briefly, it involves extracting DNA from the entire

community of an environmental sample and cutting it into shorter fragments. These fragments can then be inserted into host bacteria that can be cultivated. To study antibiotic resistance, the strains are then grown on antibiotic-containing media. Resistant strains that survive on the antibiotic plates can then have their DNA fragment sequenced and studied. This has revealed many resistance genes that have not yet found their way into pathogens (Sommer et al. 2010, Moore et al. 2011).

Functional metagenomics has several disadvantages. First, genes must be intact (or almost intact) when inserted in to the recipient bacteria to remain functional. Given that the genes can come from a huge number of bacterial species, one cannot assume that all of them can be expressed (and remain functional) inside the host bacterial species (typically *Escherichia coli*). Additionally, it is a very time-consuming method and it can be difficult to reliably quantify the resistant genes.

A method that overcomes many drawbacks of functional metagenomics is commonly referred to as shotgun metagenomics (Wooley et al. 2010). In shotgun metagenomics, the DNA of an entire community is extracted, fragmented randomly and sequenced at high speed. The fragments can be assembled or compared to databases for similarity. The drawback is of course that to get a positive hit, the gene (or a closely related one) must be known in advance. Metagenomics is less sensitive than qPCR but is good for measuring many resistance genes at once or detecting changes in taxonomic composition (Bengtsson-Palme 2016). Shotgun metagenomics was used in **Papers III** and **IV**.

PICT

Pollution-induced community tolerance (PICT) (Blanck et al. 1988) is an eco-toxicological tool for measuring a community's response to an introduced pollutant. The community (for example, bacteria or algae) will change due to the selection pressure of the pollutant by replacing the sensitive species with more tolerant ones, selecting for better genotypes, or physiological (phenotypic) adaptation. PICT was used in **Paper III**.

Minimal selective concentrations in the environment

An important question to ask is at what levels do antibiotics and substances such as biocides and metals select for resistance in bacteria? For a long time, it was believed that selection of resistant bacteria could only occur at concentrations above the minimum inhibitory concentration (MIC) of a susceptible strain. At these concentrations (shown in orange in **Figure 19**), only resistant bacterial strains can grow. However, there is also a sub-MIC selective window in which both sensitive and resistant strains can grow, but at different rates. This MSC concept was proposed by Gullberg et al. (Gullberg et al. 2011) and tested by a competition experiment where two strains that differ only in their resistance factor were grown in antibiotic concentrations below the MIC. This showed that the sub-MIC selective window can be much larger than the traditional selective window; some MSC values were found to be as low as 1/230 of the corresponding MIC. More recent studies have also reported large sub-MIC selective windows (Murray et al. 2018). Interestingly, selection in the sub-MIC region can give rise to different types of resistance mutations than the traditional selective window (Wistrand-Yuen et al. 2018).

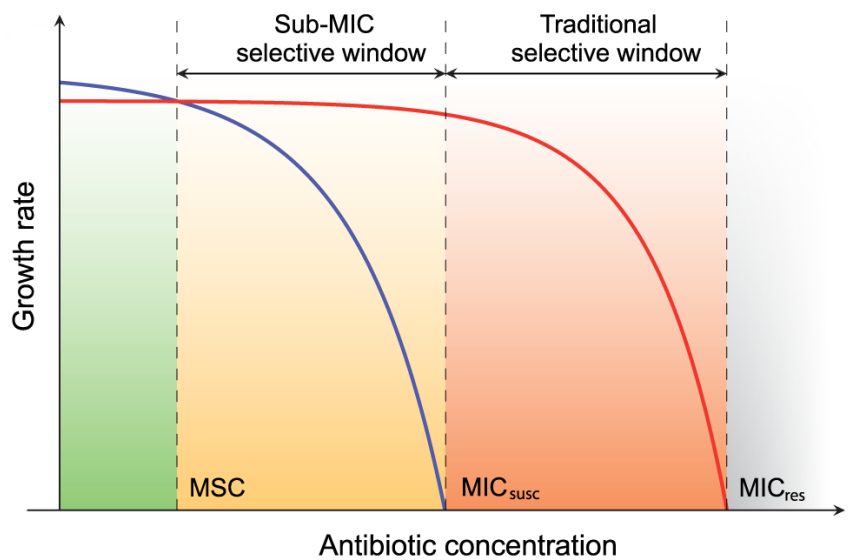


Figure 19. *Bacterial growth rates as functions of the antibiotic concentration. In the green window (concentration range), the susceptible strain (blue line) will outcompete the resistant strain (red line). In the yellow and orange windows (concentration ranges), the resistant strain will outcompete the susceptible strain. MSC = minimal selective concentration, MIC_{susc} = minimum inhibitory concentration of the susceptible strain, MIC_{res} = minimum inhibitory concentration of the resistant strain. Reprinted from (Gullberg et al. 2011) under the terms of the Creative Commons Attribution License.*

The experiments using two competing strains (Gullberg et al. 2011, Liu et al. 2011) to determine MSCs are very important but it is unclear how transferable their results are to real world situations, given that they were performed under simplified laboratory conditions. In the environment, the situation is more complex: microbial communities are diverse rather than consisting of just two strains of one species. The different species will likely vary in susceptibility to antibiotics, both in comparison to the lab strains and to each other. Under selective pressure due to antibiotics, it is possible that many different bacteria with higher tolerances will replace more sensitive ones as their ecological niches become available. Another important factor is that the selective pressure of antibiotics does not exist in isolation in the environment; many factors, such as nutrient scarcity, will also affect selection (Bengtsson-Palme, Alm Rosenblad, et al. 2014). These effects are not addressed in studies that include only two strains in a nutrient-rich lab environment. It is therefore important to determine MSC values in more complex environments resembling those of interest so as to establish safe levels of antibiotics that can be used in mitigation.

Minimal selective concentrations of tetracycline

The objective of **Paper III** was to determine MSC values for an antibiotic in a complex community (a bacterial biofilm) to maximize the ecological relevance of the results obtained. Tetracycline was chosen as the antibiotic for study because it belongs to an important class of broad-spectrum antibiotics used in large quantities in both animals and humans (Kools et al. 2008, Coenen et al. 2011). Furthermore it is excreted mostly unchanged from the body (Agwuh & MacGowan 2006), meaning that large amount will enter the STPs. In **Paper I**, tetracycline was detected in incoming wastewater at concentrations of up to 510 ng/L. It has previously been detected in Sweden at levels exceeding 1 µg/L in incoming wastewater and >6 µg/L in hospital effluent (Lindberg et al. 2014).

MSC values can have different meanings depending on the studied endpoint. In **Paper III**, several endpoints were considered to achieve broad coverage of both the genotypic and phenotypic effects of tetracycline exposure. Notable endpoints considered included changes in the abundance of resistance genes (measured with both qPCR and metagenomics), PICT, changes in taxonomic diversity and community composition, and the CFU count on R2A plates. The MSC values determined in this way are thus community MSC values. For these experiments, bacterial biofilms were grown in aquaria containing different tetracycline concentrations for nine days. The biofilms were then harvested and subjected to different conditions. Among the tested endpoints, the change in *tet* gene (*tet* A and *tet* G) abundance was the most sensitive, producing an MSC of 1 µg/L (**Figure 20**).

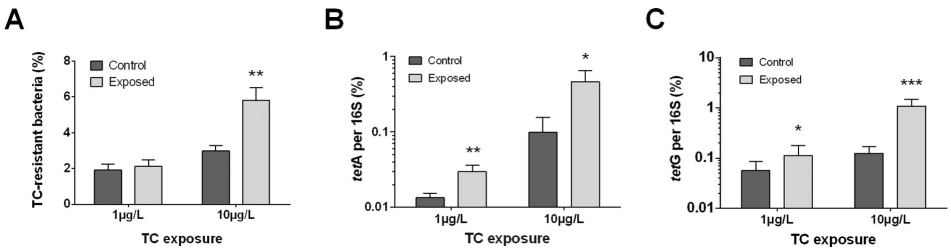


Figure 20. *Measurements of MSC based on phenotypic (A) and genotypic (B,C) tetracycline resistance in a complex bacterial community. A: Number of tetracycline-resistant bacteria (%) in biofilms exposed to 1 and 10 µg/L tetracycline, vs unexposed controls, as determined by CFU counts on R2A plates (with or without TC at 20 µg/L). B: changes in tetA per 16S determined by qPCR. C: changes in tetG per 16S determined by qPCR. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ using one-tailed student's t-test. Figure derived from* ***Paper III****.*

Comparing MSC to environmental concentrations

When an MSC value for an antimicrobial has been established, it is interesting to compare it to the levels of that antimicrobial measured in environmental samples. Whether or not the derived MSC value actually represents a safe level will depend on many factors. First, different endpoints give different MSC values, as shown in **Paper III**. Second, even if the MSC is determined for a complex community it will still be specific to that community, and a different MSC value would likely be obtained under different conditions. Third, only a fraction of the quantity of antimicrobial nominally present in the system will actually be accessible to the bacteria in most cases (Menz et al. 2018). The bioavailable fraction will probably vary between systems, making it difficult to meaningfully compare measured concentrations. Finally, the microorganisms in a given environment, for example a STP, will never be exposed to one antimicrobial at a time; there will always be a cocktail of chemicals with various (mostly unknown) mixture effects (Backhaus et al. 2011, Backhaus 2014, Menz et al. 2017). Similar compounds that share a mode of action are likely to have additive effects (Backhaus et al. 2011), suggesting that it might be best (for example) to look at all tetracycline antibiotics together rather than focusing on tetracycline in isolation.

Despite the difficulties described above, it is still interesting to compare MSC values to environmental concentrations. Unfortunately, experimentally determined MSC values for antimicrobials are rare; a collection of literature values is presented in **Table 3**. In cases where different studies have yielded different values, the lowest reported value is given. The table also contains estimated predicted no effect concentration (PNEC) values (Bengtsson-Palme & Larsson 2016).

The community MSC of 1 µg/L for tetracycline reported in **Paper III**, has been exceeded in many measurements of wastewater (Karthikeyan & Meyer 2006, Batt et al. 2007, Lindberg et al. 2014). Other antibiotics whose concentrations exceeded one or more of the calculated PNEC or MSC values in incoming wastewater in **Paper I, II** or **IV** are ciprofloxacin, metronidazole and fluconazole.

Table 3. *MSC values and predicted no effect concentrations from the literature and* ***Paper III*** *compared to measured concentrations in incoming wastewater from* ***Papers I, II*** *and* ***IV****.*

Compound	MSC (strain specific) (µg/L)	Community MSC (µg/L)	PNEC[a] (µg/L)	Conc. incoming wastewater (µg/L)
Ciprofloxacin	0.1-2.5[b]	<1[c,d,e]	0.064	0.1 - 1.4
Norfloxacin			0.5	0.11 - 0.14
Ofloxacin			0.5	0.01 - 0.03
Tetracycline	15[b]	<1[e,f]	1	0.06 - 4.6
Oxytetracycline		20 µg/kg[g,h]	0.5	0.11 - 0.35
Trimethoprim	<2[i]		0.5	0.05 - 0.34
Clarithromycin			0.25	0.01 - 0.13
Clindamycin			1	0.06 - 0.13
Erythromycin	<200[b]		1	0.01 - 0.47
Sulfamethoxazole			16	0.11 - 0.41
Metronidazole			0.125	0.05 - 0.34
Fluconazole			0.25	0.03 - 0.46
$CuSO_4$	90[i]			20 - 102

a: Bengtsson-Palme & Larsson 2016
b: Gullberg et al. 2011
c: Kraupner et al. 2018
d: NOEC = 0.1 µg/L, LOEC = 1 µg/L
e: aquatic biofilm
f: **Paper III**
g: Shentu et al. 2015
h: soil
i: Gullberg et al. 2014

It is known that ciprofloxacin will start to select for resistance in *E. coli* at 5-10 µg/L in complex biofilms, but even concentrations as low as 1 µg/L induced taxonomic shifts and an increase in the abundance of mobile quinolone resistance genes (Kraupner et al. 2018). Consequently, Kraupner et al. recommended 0.1 µg/L as a maximum safe level for this antibiotic. The data presented in **Papers I** and **II** clearly show that this level was exceeded at the studied STPs. However, as

noted above, factors such as the unknown size of the bioavailable fraction make it difficult to directly compare the concentrations measured in STPs to those used in lab experiments (Boxall et al. 2012). It has also been shown that complexation with metals alters both the fate and antimicrobial properties of fluoroquinolones (Cuprys et al. 2018).

MSC values for metals and biocides are less well studied compared to antibiotics. A strain-specific MSC of 90 µg/L was determined for $CuSO_4$ (Gullberg et al. 2014), which is lower than the maximum concentration of 102 µg/L determined in this work. Another study reported that Cu induced HGT at concentrations between 5 – 50 µg/L (Zhang et al. 2018). The same study also investigated Ag, Cr, and Zn but the concentrations reported to increase HGT of ARGs were above the highest concentrations measured in this work. Previously reported estimates of the levels at which various heavy metals induce co-selection (Seiler & Berendonk 2012) suggest that Cu and Zn could possibly be co-selective at the concentrations observed in this work. Other compounds such as triclosan and chlorhexidine have also been reported to induce HGT at sub-MIC concentrations (Jutkina et al. 2018). It should be noted that increased HGT rates do not necessarily mean that the newly transferred resistance genes will become fixed in the bacterial population. Comparing various MSC values for metals to measured concentrations is particularly complex because metals can exist as different species at different conditions, which can strongly affect their behaviour and toxicity to bacteria.

Promotion of resistance in sewage treatment plants

As discussed above, some of the antimicrobials were detected at concentrations high enough to potentially promote resistance development in STPs. To determine whether there is selection for resistance genes in STPs, a shotgun metagenomics study was performed (**Paper IV**). Samples were collected during different parts of the treatment process at three different STPs, and were subjected to whole genome sequencing as well as chemical analysis to quantify their contents of antibiotics, biocides, and metals. There was no evidence of enrichment of resistance genes for antibiotics, biocides, or metals in the STPs. As shown in **Figure 21** (A and B), the abundance of resistance genes decreased during the treatment process. In the treated effluent, resistance genes for QACs and trimethoprim were more abundant than in the incoming water, but the changes were not significant (**Figure 21** C). Resistance genes for sulphonamides were enriched in the digested sludge (**Figure 21** D).

Paper IV also investigated the potential for other antimicrobials to induce co-selection of resistance genes by determining the frequency at which biocide and metal resistance genes were found on the same DNA fragment as an antibiotic resistance gene. However, this was rare and no clear evidence for co-selection was found.

Taxonomic information about bacterial communities (i.e. species composition) is also important because factors other than selection may favour bacterial species that carry certain resistance genes. There was a readily apparent taxonomic shift in the bacterial community present in the wastewater as it moved through the treatment process. Even in the incoming wastewater, many obligate anaerobes (can only grow in the absence of oxygen) found in human faeces had been replaced by facultative anaerobes (can grow both with and without oxygen). It is therefore likely that changes in antibiotic resistance gene abundance are more heavily influenced by factors affecting taxonomic composition (such as oxygen levels) rather than antibiotic-induced selection pressure.

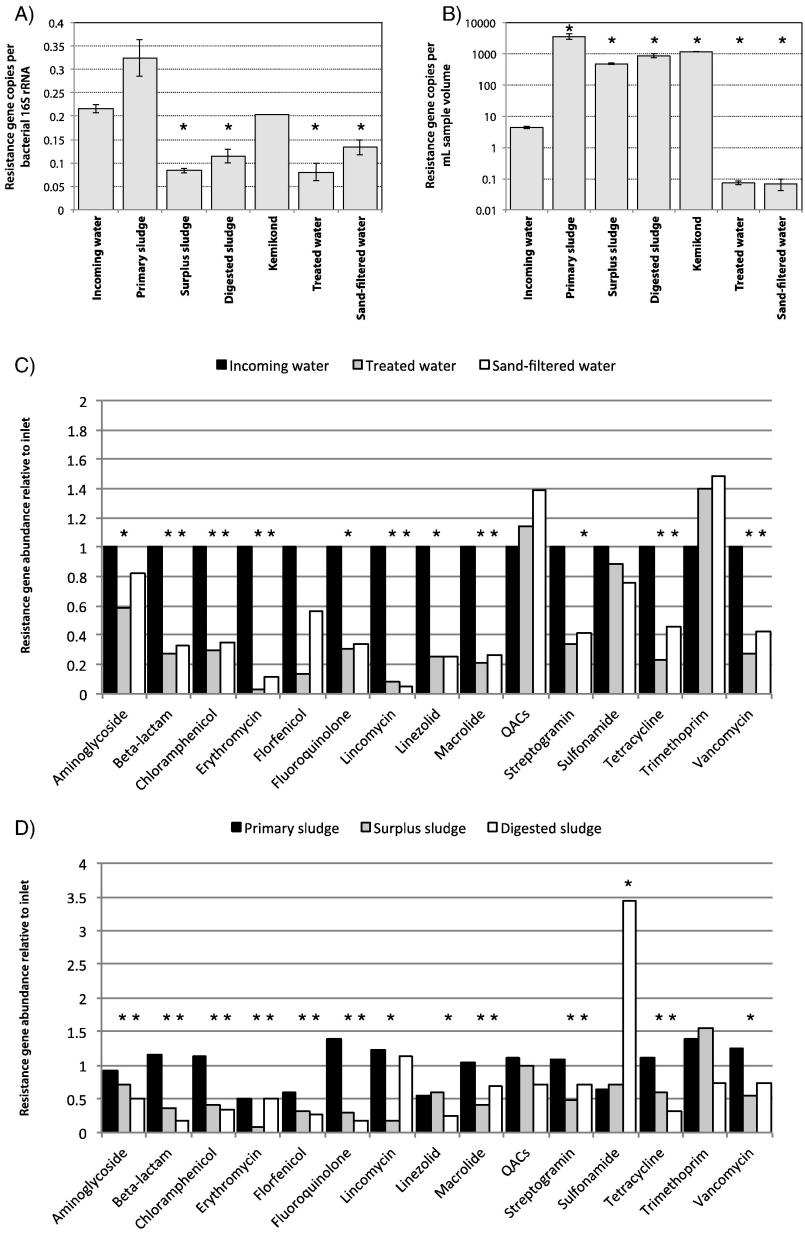


Figure 21*. Abundance of resistance genes during different treatment steps at three STPs (Henriksdal, Käppala and Kungsängsverket), A: Total abundance of resistance genes per 16S rRNA, B: Total abundance of resistance genes per mL sample, C: Total abundance of resistance genes per 16S rRNA in treated effluent (before and after sand filtering) relative to incoming water, D: Total abundance of resistance genes in sludge per 16S rRNA relative to incoming water, * Changes from incoming water with a false discovery rate of <0.05. Figure derived from* ***Paper IV*** *(Bengtsson-Palme et al. 2016).*

Dissemination of bacteria and resistance genes from STPs

In addition to potentially inducing selection for antibiotic resistance, STPs are important routes for the dissemination of both bacteria and antibiotic resistance genes from humans to the environment (Pruden 2014, Berendonk et al. 2015).

Paper IV shows that STPs reduced the abundance of resistance genes in the effluent by a factor of more than 50 when sample volume is accounted for. The quantity of bacteria in the effluent was also lower than in the influent. Other studies have also noted that STPs can greatly reduce the abundance of bacteria and resistance genes in the effluent (Yang et al. 2014, Karkman et al. 2016). Despite the pronounced reduction of ARG abundance in many cases, STPs are very important routes for the dissemination of ARGs into the aquatic environment due to the large volumes of effluent. The fate of ARGs in the aquatic environment is poorly understood. It was recently shown that many observed increases in ARG abundance in the environment are attributable to faecal pollution rather than onsite selection pressure (Karkman et al. 2018).

Removal of antimicrobials by tertiary treatment technologies

Conventional STPs are not designed to remove polar chemicals, such as pharmaceuticals, so the value of adding extra treatment steps specifically designed to remove or degrade these compounds was investigated. Since both **Papers I – II** and earlier studies (Verlicchi et al. 2012, Loos et al. 2013) showed that several antimicrobials are poorly removed during conventional treatment, their response to advanced targeted treatments was investigated in Knivsta STP. This STP is the first full-scale ozonation treatment plant in Sweden, and was also equipped with a mobile pilot STP capable of both ozonation and GAC filtration (**Paper V**).

Ozonation

When ozone is applied to water, it is partially decomposed to form OH radicals (Staehelin & Hoigne 1985). This gives rise to two different oxidation processes, a more selective one involving ozone and an unselective reaction pathway involving OH radicals (von Gunten 2003a). Ozone has long been used for disinfection and to reduce levels of natural organic matter in drinking water (Papageorgiou et al. 2017), and attracted wider interest after being shown to reduce levels of pharmaceuticals in water (Zwiener & Frimmel 2000) and wastewater (Ternes et al. 2003).

Ozonation has been successfully used to reduce micropollutant levels in STPs in Switzerland and Germany for over a decade (Hollender et al. 2009, Margot et al. 2013, Eggen et al. 2014), and more countries are adopting this approach (Bourgin et al. 2018). In Sweden, there have been several pilot-scale projects investigating the effects of ozonation (Baresel et al. 2016, Beijer et al. 2017, El-taliawy et al. 2017, Nilsson et al. 2017). To investigate the effects of full-scale ozonation, Knivsta STP, north of Stockholm, was upgraded with an ozonation facility. The first permanent full-scale ozonation treatment plant in Sweden was recently set up in Linköping (Tekniska verken 2018).

As reported in **Paper II**, several compounds such as benzotriazoles, fluconazole, and trimethoprim were inefficiently removed in conventional STPs. The same was observed at Knivsta STP, where the conventional treatment reduced the levels of these compounds by <60% (**Figure 22**). Ozonation of the treated effluent improved the results observed for these and other antimicrobial compounds, reducing the levels of the studied antibiotics by >90%. The compounds that were most resistant to ozone were fluconazole and the benzotriazoles. The ozone dose required to achieve a given degree of elimination

varies between compounds (Lee et al. 2013) and depends on the water's DOC content (El-taliawy et al. 2017). It was clear that the specific ozone dose of 0.55 g O_3/g TOC, used in our experiment was too low for high removal of these compounds. A dose of 7 g O_3/m^3 is commonly used in studies on wastewater ozonation (Gomes et al. 2017) but the specific dose in **Paper V** was lower than intended due to high TOC level. A dose of 0.55 g O_3/g DOC has been recommended to achieve >80% elimination of many micropollutants (Bourgin et al. 2018).

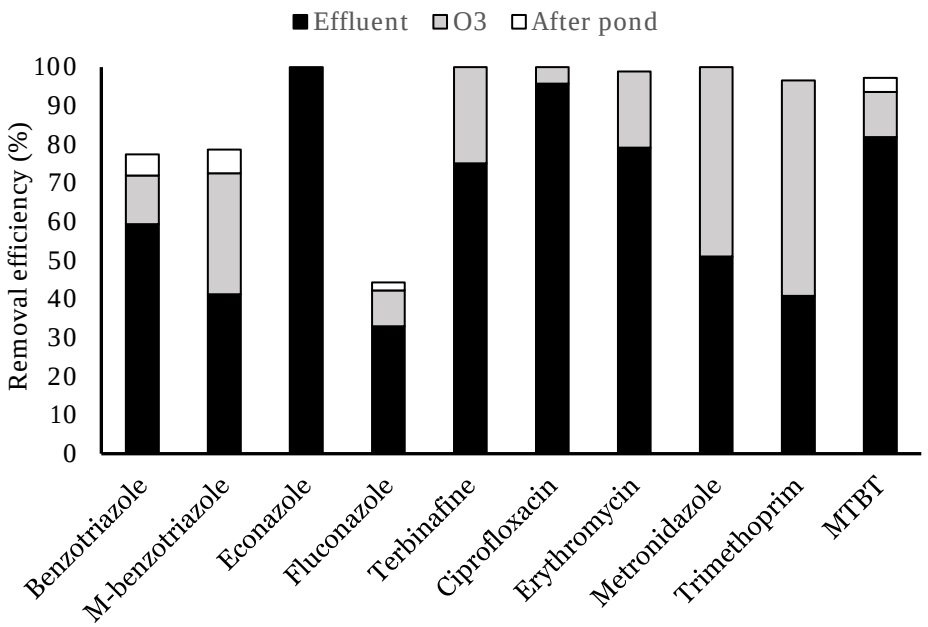


Figure 22. *Removal efficiencies for some benzotriazoles, benzothiazoles, antimycotics, and antibiotics at Knivsta STP, which has been upgraded with a full-scale ozonation treatment step and a polishing pond. Specific ozone dose: 0.55 g O_3/g TOC. M-benzotriazole = Methyl-benzotriazole. MTBT = 2-(methylthio)-benzothiazole.*

A drawback of ozonation is the formation of transformation products, since micropollutants are typically not mineralized at the applied ozone doses (Hübner et al. 2015, Schollée et al. 2018). Since the original compounds are transformed, they lose their original properties, such as antimicrobial activity (Suarez et al. 2007, Dodd et al. 2010). However, they may be converted into new unknown degradation products (Hollender et al. 2009, Hübner et al. 2015). Ozonation has been shown to both decrease (Margot et al. 2013) and increase (Magdeburg et al. 2014) the toxicity of wastewater in different cases. This is likely due to the

formation of toxic by-products whose abundance will vary with the composition of the wastewater. The drawback of toxic by-products can be overcome by combining ozonation with an additional treatment step such as passage through activated carbon.

Activated carbon

Activated carbon refers to a wide range of carbonaceous materials that can be used as adsorbents (Simate et al. 2016). They all have a highly developed internal pore structure, which is important for their characteristics. Activated carbon in the form of charcoal has been used for thousands of years for various purposes, including purifying drinking water (Çeçen & Aktaş 2011). It can be produced from various carbon-containing materials (Teng & Lin 1998, Simate et al. 2016); the properties of the resulting activated carbon such as its porosity, surface area, and adsorption capacity depend on the starting material and treatment (Yeganeh et al. 2006, Okhovat et al. 2012).

Since activated carbon has large adsorption capacity for organic molecules, it can be used to treat wastewater. Activated carbon is most often used in two forms: granular activated carbon (GAC) or powdered activated carbon (PAC). PAC has a much smaller particle size than GAC, which typically leads to better adsorption kinetics and therefore a higher removal efficiency (Nowotny et al. 2007). It can also be dosed directly into the biological treatment step, making it easy to use and to modify the dose depending on the STP's load (Boehler et al. 2012). The PAC material is removed from the wastewater together with the sludge, which can cause problems if it is intended for any use other than incineration. In Sweden however, the goal has been to recycle sludge in applications such as land restoration and as fertilizer in agriculture as long as its content of contaminants is low (Finnson 2013, Hörsing 2018). This goal is incompatible with the use of PAC in wastewater treatment. However, GAC is not added to the wastewater; instead, it is placed in columns that the water flows through after undergoing conventional treatment. This is more compatible with the requirements of Sweden's sludge strategy. We therefore decided to study the removal of various antimicrobial compounds on GAC. GAC has the advantage over PAC that it can be regenerated to some extent using various methods and reused (McQuillan et al. 2018). A downside is that it is difficult or impossible to compensate for differences in load over time in the STP. An alternative to using activated carbon derived from coal is to use biochar as an adsorbent in wastewater treatment. Biochar is produced by pyrolysis of biomass, which can have a lower environmental impact than conventional activated carbon production methods (Lehmann 2007). It is even possible to produce biochar from sewage sludge (Liu et al. 2018).

As described above, ozonation did not achieve an adequate removal efficiency for several compounds. To investigate the removal efficiency using activated carbon, pilot-scale GAC filtration columns were used to treat effluent from the conventional treatment process at Knivsta STP, as described in **Paper V**.

Pilot-scale ozonation was also performed so its performance could be compared directly to that of GAC filtration. As shown in **Figure 23**, the two tested GAC materials performed differently; the material with the smallest particle size (GAC 1) performed better for all tested compounds. Grinding activated carbon to reduce its particle size often improves its adsorption kinetics by improving mass transfer (Matsui et al. 2015). The GAC 1 material reduced the levels of all tested compounds by >95%, with the levels of benzotriazoles, trimethoprim and MTBT being below the LOQ.

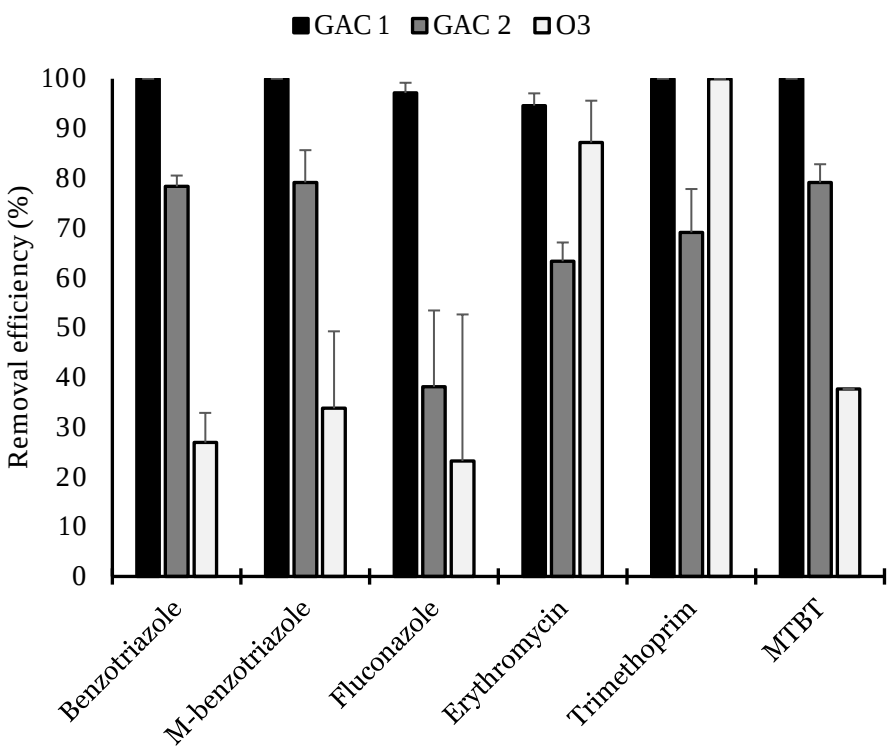


Figure 23. *Removal efficiencies for selected micropollutants in a pilot-scale plant at Knivsta STP. GAC 1 = granular activated carbon (Chemviron Fitrasorb 300, bituminous coal, 0.8 – 1.0 mm, 950 m^2/g), GAC 2 = granular activated carbon (Miljø-Teknologi AS MT-ACARB SIL 40, anthracite, 4 mm, 1100 m^2/g), O3 = pilot scale ozonation (5 g O_3/m^3, specific ozone dose: 0.39 g O_3/g TOC). MTBT = 2-(methylthio)-benzothiazole.*

The GAC 2 material still outperformed ozonation at the tested dose for all compounds other than erythromycin and trimethoprim. The ozone doses applied at both the full- and pilot-scales were lower than intended due to increases in the wastewater's TOC content, which were not compensated for by increasing the ozone dosage. The results presented in **Paper V** indicate that the choice of GAC material strongly affects the efficiency of antimicrobial removal. Similarly large differences in performance between different GAC materials have been reported previously (Kårelid et al. 2017). GAC filtration has been applied in full-scale facilities (Benstoem et al. 2017) and is a promising technique. While GAC outperformed ozonation in our study, it is likely that a higher ozone dose would have resulted in a higher removal efficiency. The choice of technique should be based on both economic and technical considerations in addition to removal efficiency when making decisions about future investments.

One advantage of using adsorbents such as activated carbon in preference to ozone is that they can remove metals and metalloids from the water. Several studies have demonstrated the removal of heavy metals such as Hg, Cd, Pb and Cr using coal-based materials (Namasivayam & Yamuna 1995, Arpa et al. 2000, Anwar et al. 2009).

Antibiotic resistance genes

As mentioned previously in this thesis, and shown in **Paper IV**, STPs release both bacteria and antibiotic resistance genes into the aquatic environment. Although not tested in this thesis, several studies have demonstrated the potential to reduce levels of bacteria and ARG in wastewater using advanced treatment technologies such as various oxidation processes (Lüddeke et al. 2015, Sharma et al. 2016, Sousa et al. 2017) and UV-radiation (Rizzo, Fiorentino, et al. 2013). Ozonation also has the advantage of being able to inactivate microorganisms including pathogens such as *E. coli* (von Gunten 2003b). Wastewater ozonation will therefore reduce the dissemination of both antibiotic resistance bacteria and antibiotic resistance genes to the aquatic environment. The effects of advanced treatment processes on antibiotic resistant genes and bacteria needs further study, however. It has been shown that some techniques can induce SOS responses (Beaber et al. 2004) in bacteria that can increase the mutation rate (Karkman et al. 2017). Some techniques may thus have undesired effects on antibiotic resistance, which needs further evaluation.

Concluding remarks and future perspectives

The problem of antibiotic resistance is both very important and very difficult to solve. As described in this thesis, environmental considerations and the roles of other selective agents (i.e. biocides and metals) require further study.

This thesis shows that many compounds with antimicrobial properties are frequently detected at high levels in STPs. **Paper I** quantifies the levels of 40 compounds in wastewater and sludge from 11 STPs, and this thesis provides a comprehensive overview of antimicrobial levels in Swedish STPs. The list of quantified compounds is likely to be extended further by future measurement campaigns that will use both targeted and non-targeted approaches.

Paper II presents detailed mass flow data for 11 different antibiotics and biocides during different treatment steps in three STPs. Evaluations of common wastewater technologies and mass flow measurements are important for estimating emissions of antimicrobials from STPs and because they increase understanding of the removal process, which can be used to minimize releases to the environment.

A key goal is to define safe concentrations of antimicrobials in the environment. To this end, MSC values were determined for the important antibiotic tetracycline in a complex bacterial community derived from treated sewage effluent (**Paper III**). It was found that concentrations as low as 1 µg/L can select for different *tet* genes. The method used to determine MSC values was appropriate for the task, but future studies of this kind should include additional experiments to account for intra-species selection (Kraupner et al. 2018). One factor that makes it difficult to compare MSC values is the impact of mixture toxicity (Backhaus et al. 2011). Bacteria in STPs are exposed to thousands of chemicals and the effect of this combined exposure is currently unclear, although research is ongoing (Menz et al. 2017). Furthermore, it is unlikely that measured antimicrobial concentrations accurately reflect the bioavailability of the measured compounds to bacteria; further research is needed to clarify this issue.

To determine whether there is selection for antibiotic-resistant bacteria during the treatment process at STPs, a metagenomic study was performed and its results were compared to measured antimicrobial concentrations (**Paper IV**). There was no detectable evidence for any increase in resistance gene abundance during the treatment process, nor any high risk of co-selection of biocides or metals. The evidence for co-selection of antibiotic resistance at sub-lethal

concentrations is still inconclusive and warrants further study (Pal 2017). It is possible that metagenomic techniques alone will be unable to detect changes in resistance genes abundance due to large taxonomic changes induced by other factors in STPs. Culture-based approaches targeting relevant species will therefore probably be useful complements to metagenomic analyses in the future (Flach et al. 2018).

Since conventional wastewater treatment processes cannot remove several antimicrobials, we evaluated additional treatment steps involving ozonation and GAC (**Paper V**). Both techniques reduced the levels of antimicrobials in the effluent, although GAC had a higher removal efficiency than ozonation at the tested doses. However, these evaluations were not comprehensive; there are many other treatment technologies and combinations of technologies to evaluate, and future evaluations should also include a wider range of antimicrobial compounds.

The cost of upgrading STPs with advanced treatment technologies is significant and is likely to increase the cost of Sweden's water and sewage operations by up to 10% per year if all STPs >2000 pe are upgraded (Baresel et al. 2017, Swedish EPA 2017). Ozonation is the cheapest technique, and can cost as little as 5 € per household and year, depending on the size of the STP (Baresel et al. 2017), although earlier estimates suggested higher costs (Wahlberg et al. 2010). Advanced treatment techniques will also reduce levels of other known and unknown micropollutants in the effluent. Micropollutants in the aquatic environment cause many direct and indirect effects in the ecosystem (Saaristo et al. 2018), including behavioural changes (Brodin et al. 2013) and sexual dimorphism in fish (Jobling et al. 1998). We currently use over 21 000 different chemicals within the EU (ECHA 2018), and compounds typically not used for antimicrobial purposes can still affect microorganisms. For example, PAHs were shown to increase ARG abundance in environmental bacteria (Wang et al. 2017). Furthermore the use of pharmaceuticals is increasing globally (Aitiken 2015), which will put more pressure on the aquatic environment if removal efficiencies in STPs are not increased. Care must also be taken to avoid potential negative effects due to toxic treatment by-products (Andrä et al. 2018). From a global perspective, about 80% of the world's sewage is still untreated (Wilson et al. 2015); as such, increasing the number of STPs would make a large difference, even if they lacked advanced treatment facilities. Thus, improving the water quality worldwide, in line with the UN sustainable development goal 6 – "Clean water and sanitation" is challenging (UN 2018).

Another important strategy for minimizing the load of antimicrobials and other micropollutants in the environment is to minimize their unnecessary use (Kümmerer et al. 2018). Antibiotics should be used prudently in healthcare, and

the use of broad-spectrum antibiotics in particular should be minimized. Antibiotic use in animals must also be greatly reduced, and antibiotics that are important in human medicine should be avoided completely (Tang et al. 2017, World Health Organization 2017).

Many biocides have very important functions (such as disinfection in health care settings), but some of their uses are of questionable importance (for example, the use of silver for odour control in training clothes). Consequently, there should be efforts to minimize their use in cases where the positive effect is unclear. It is likely to be difficult to limit household use of biocides by public information campaigns alone, so other measures such as new regulations will probably be needed (Wieck et al. 2018b).

The problem of antibiotic resistance is predicted to become increasingly severe. While this thesis focuses on environmental considerations, a holistic approach to the problem involving diverse social competences will be needed to overcome the problem. The development of new antibiotics will be crucial, although it will only buy us time since resistance mechanisms will continue to emerge if they are biochemically possible. It is possible that we in the future will use alternative strategies such as vaccines that target bacteria instead of viruses (Mishra et al. 2012) or innovative technologies such as CRISPR-Cas to erase bacterial populations carrying antibiotic resistance genes (Bikard et al. 2014, Gomaa et al. 2014, Pursey et al. 2018). It is possible that these technologies will make it possible to turn back the clock and reverse resistance after all. If so, the future looks a lot brighter.

Acknowledgement

There are many people to thank that have contributed to this thesis, one way or another. Först och främst vill jag tacka min huvudhandledare **Mats Tysklind** för att jag fick chansen att genomföra det här projektet, alla vetenskapliga diskussioner, stöd och uppmuntran under åren samt att jag har fått fria tyglar under hela projekttiden. Min biträdande handledare, **Jerker Fick** för all entusiasm, stöd och idéer. Tack för alla roliga diskussioner på och utanför jobbet, konferensresor m.m. Min andra biträdande handledare **Erik Björn** för hjälp med ICP-MS samt all korrekturläsning av avhandlingen. Tack också för värdefull kunskap från tiden som exjobbare för länge sedan. Ett särskilt tack också till **Richard Lindberg** för alla råd om LC-MS, reningsverk etc., och för all tid på och utanför jobbet, IKSU, Bishops etc.

Stort tack till alla inom INTERACT. Det har verkligen varit både roligt, spännande och meningsfullt att vara en del av projektet, och jag har lärt mig massor: **Joakim Larsson**, för att jag har fått vara en del av flera av era projekt. **Johan Bengtsson-Palme** för gott samarbete, **Sara Lundström** för alla tetracyline- och CTAC prover. De var både utmanande och roliga att analysera. **Chandan**, **Jekaterina**, **Carl-Fredrik**, **Dan**, **Lisa**, **Erik**, **Hans**, **Martin, Triranta, Åsa** och **Jessica**. It has been a highlight to meet all of you once a year to discuss the progress of the various parts of the project.

Berndt Björlenius för att jag fick möjlighet att provta både från Knivsta och piloten samt allt jobb och noggrann korrekturläsning av sista artikeln till denna avhandling.

Ett stort tack till all personal på alla reningsverk som skickat prover, utan er hade det inte blivit mycket till avhandling. Särskilt till **Stefan Remberger** och **Nicklas Paxéus** för hjälp med provtagningsstrategi samt **Lucica Enache** för provtagning. **Pia Trygg** för utmärkt provtagning i Knivsta, trots att jag själv inte visste vad jag ville ha för prover och till **Anna Sundelin** som körde upp dem till Umeå. Ett stort tack till Vakins personal i Umeå (**Erik Nilsson**, **Tomas Johansson**, **Ulrika Lindberg** mfl.) som lät mig vandra runt och provta själv i verket under några veckor.

Former and present co-workers at Environmental Chemistry, among others: **Lisa**, **Patrik**, **Pierre, Jana**, **Peter**, **Anna**, **Malin**, **Chistine**, **Kristin**, **Terry**, **Mirva**, **Darya**, **Ziye**, **Kavitha**, **Alexandra**, **Kenneth**, **Eva, Sara, Sture, Sandra, Hanna**.

Majid Mustafa and **Ivan Kosyatnyk** for collaborations on other ozonation related projects. **Per Liljelind** för hjälp med GC-MS och Mass Lynx. **Maria Hjelt** för diverse labbhjälp med frystork m.m. **Van-Liem Nguyen** for solving the ICP-MS troubles. **Cathrin Veenaas** for voluntarily proofreading the whole thesis! **Tomas Brodin** för SETAC-konferenser och road trip i Florida.

Referensgruppen på de årliga uppföljningsmötena: **Tomas Hedlund** (även för hjälp med SolGasWater)**, Staffan Lundstedt** och sedermera **Stina Jansson**.

För användbara kunskaper i hur man reparerar/underhåller diverse analysinstrument står jag i tacksamhetsskuld till **Lars Lundmark** för grundkunskaperna, **Roman Grabic** for LC-MS/MS skills samt diverse serviceingenjörer från Thermo och Waters som har delat med sig av sina kunskaper och låtit mig hjälpa till. **Solomon Tesfalidet** för inspirerande förläsningar i analytisk kemi under min grundutbildning som på sätt och vis utgör startpunkten på den här resan.

Jan Roslin och **Erik Klinga** för traditionsenlig söndagsskådning som har gett värdefulla avbrott och nya fåglar.

Lena och **Sigurd** som kunnat rycka in med barnpassning när det behövts och en hel massa andra saker.

Tack till **Mamma**, **Pappa** och mina bröder **Simon** och **Alexander** för allt.

Elin, tack för all hjälp med avhandlingsrelaterade saker som Excel, Simca, Graphpad, statistik, korrekturläsning m.m. Tack för att du står vid min sida och delar livet. Slutligen tack till vår dotter **Matilda** som förgyller tillvaron med alla upptåg.

"It has all been very interesting"

-Lady Mary Wortley Montagu

References

Abraham, E.P. and Chain, E., (1940) An Enzyme from Bacteria able to Destroy Penicillin. Nature **146**, 837.

Achterberg, E.P., Browning, T.J., Gledhill, M. and Schlosser, C., (2018) Transition Metals and Heavy Metal Speciation☆, in: Reference Module in Earth Systems and Environmental Sciences. Elsevier.

Agwuh, K.N. and MacGowan, A., (2006) Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J. Antimicrob. Chemother. **58**, 256–265.

Ahn, S., Peterson, T.D., Righter, J., Miles, D.M. and Tratnyek, P.G., (2013) Disinfection of Ballast Water with Iron Activated Persulfate. Environ. Sci. Technol. **47**, 11717–11725.

Aitiken, M., (2015) Global Medicines Use in 2020. IMS Institute for healthcare informatics, USA.

Amachawadi, R.G., Shelton, N.W., Shi, X., Vinasco, J., Dritz, S.S., Tokach, M.D., Nelssen, J.L., Scott, H.M. and Nagaraja, T.G., (2011) Selection of Fecal Enterococci Exhibiting tcrB-Mediated Copper Resistance in Pigs Fed Diets Supplemented with Copper. Appl Env. Microbiol **77**, 5597–5603.

Amann, R.I., Ludwig, W. and Schleifer, K.H., (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59**, 143–169.

Aminov, R., (2017) History of antimicrobial drug discovery: Major classes and health impact. Biochem. Pharmacol., Antibiotics - Meeting the Challenges of 21st Century Health Care: Part I **133**, 4–19.

Aminov, R.I., (2011) Horizontal Gene Exchange in Environmental Microbiota. Front. Microbiol. **2**.

Aminov, R.I. and Mackie, R.I., (2007) Evolution and ecology of antibiotic resistance genes. FEMS Microbiol. Lett. **271**, 147–161.

Andersen, F.A., (2008) Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products. Int. J. Toxicol. **27**, 1–82.

Andersson, D.I. and Hughes, D., (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat. Rev. Microbiol. **8**, 260–271.

Andersson, D.I. and Hughes, D., (2012) Evolution of antibiotic resistance at non-lethal drug concentrations. Drug Resist. Updat. **15**, 162–172.

Andersson, D.I. and Hughes, D., (2014) Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. **12**, 465–478.

Andrä, J., Beyer, F., Cornelissen, G., Einfeldt, J., Heseding, J., Kümmerer, K., Oelkers, K. and Floeter, C., (2018) PharmCycle: a holistic approach to reduce the contamination of the aquatic environment with antibiotics by developing sustainable antibiotics, improving the environmental risk assessment of antibiotics, and reducing the discharges of antibiotics in the wastewater outlet. Environ. Sci. Eur. **30**, 24.

Angelidaki, I. and Sanders, W., (2004) Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotechnol. **3**, 117–129.

Anwar, J., Shafique, U., Salman, M., Waheed-uz-Zaman, Anwar, S. and Anzano, J.M., (2009) Removal of chromium (III) by using coal as adsorbent. J. Hazard. Mater. **171**, 797–801.

Araújo, G.C.L., Gonzalez, M.H., Ferreira, A.G., Nogueira, A.R.A. and Nóbrega, J.A., (2002) Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials. Spectrochim. Acta Part B At. Spectrosc., 7th Rio Symposium on Atomic Spectroscopy **57**, 2121–2132.

Ardern, E. and Lockett, W.T., (1914) Experiments on the oxidation of sewage without the aid of filters. J. Soc. Chem. Ind. **33**, 523–539.

Arpa, Ç., Başyilmaz, E., Bektaş, S., Genç, Ö. and Yürüm, Y., (2000) Cation exchange properties of low rank Turkish coals: removal of Hg, Cd and Pb from waste water. Fuel Process. Technol. **68**, 111–120.

Ashbolt, N.J., Amézquita, A., Backhaus, T., Borriello, P., Brandt, K.K., Collignon, P., Coors, A., Finley, R., Gaze, W.H., Heberer, T., Lawrence, J.R., Larsson, D.G.J., McEwen, S.A., Ryan, J.J., Schönfeld, J., Silley, P., Snape, J.R., Van den Eede, C. and Topp, E., (2013) Human Health Risk Assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ. Health Perspect. **121**, 993–1001.

Asimakopoulos, A.G., Ajibola, A., Kannan, K. and Thomaidis, N.S., (2013) Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece. Sci. Total Environ. **452**, 163–171.

Aston, F.W., (1921) Isotopes and Atomic Weights. Nature 334–338.

Backhaus, T., (2014) Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. Phil Trans R Soc B **369**, 20130585.

Backhaus, T., Porsbring, T., Arrhenius, Å., Brosche, S., Johansson, P. and Blanck, H., (2011) Single-substance and mixture toxicity of five pharmaceuticals and personal care products to marine periphyton communities. Environ. Toxicol. Chem. **30**, 2030–2040.

Baresel, C., Magnér, J., Magnusson, K. and Olshammar, M., (2017) Tekniska lösningar för avancerad rening av avloppsvatten (No. C 235).

Baresel, C., Malmborg, J., Ek, M. and Sehlén, R., (2016) Removal of pharmaceutical residues using ozonation as intermediate process step at Linköping WWTP, Sweden. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. **73**, 2017–2024.

Barnard, J.L., (1976) A Review of Biological Phosphorus Removal in the Activated Sludge Process. Water SA **2**.

Batt, A.L., Kim, S. and Aga, D.S., (2007) Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. Chemosphere **68**, 428–435.

Bautista, L.F., Vargas, C., González, N., Molina, M.C., Simarro, R., Salmerón, A. and Murillo, Y., (2016) Assessment of biocides and ultrasound treatment to avoid bacterial growth in diesel fuel. Fuel Process. Technol. **152**, 56–63.

Beaber, J.W., Hochhut, B. and Waldor, M.K., (2004) SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature **427**, 72–74.

Beijer, K., Björlenius, B., Shaik, S., Lindberg, R.H., Brunström, B. and Brandt, I., (2017) Removal of pharmaceuticals and unspecified contaminants in sewage treatment effluents by activated carbon filtration and ozonation: Evaluation using biomarker responses and chemical analysis. Chemosphere **176**, 342–351.

Bengtsson-Palme, J., (2016) Antibiotic resistance in the environment: a contribution from metagenomic studies, PhD thesis. University of Gothenburg. Sahlgrenska Academy, Gothenburg.

Bengtsson-Palme, J., Alm Rosenblad, M., Molin, M. and Blomberg, A., (2014) Metagenomics reveals that detoxification systems are underrepresented in marine bacterial communities. BMC Genomics **15**.

Bengtsson-Palme, J., Boulund, F., Fick, J., Kristiansson, E. and Larsson, D.G.J., (2014) Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front. Microbiol. **5**.

Bengtsson-Palme, J., Hammarén, R., Pal, C., Östman, M., Björlenius, B., Flach, C.-F., Fick, J., Kristiansson, E., Tysklind, M. and Larsson, D.G.J., (2016) Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Sci. Total Environ. **572**, 697–712. **Paper IV**

Bengtsson-Palme, J., Kristiansson, E. and Larsson, D.G.J., (2018) Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol. Rev. **42**.

Bengtsson-Palme, J. and Larsson, D.G.J., (2016) Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environ. Int. **86**, 140–149.

Benstoem, F., Nahrstedt, A., Boehler, M., Knopp, G., Montag, D., Siegrist, H. and Pinnekamp, J., (2017) Performance of granular activated carbon to remove micropollutants from municipal wastewater—A meta-analysis of pilot- and large-scale studies. Chemosphere **185**, 105–118.

Bérdy, J., (2012) Thoughts and facts about antibiotics: Where we are now and where we are heading. J. Antibiot. (Tokyo) **65**, 385–395.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.-N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F. and Martinez, J.L., (2015) Tackling antibiotic resistance: the environmental framework. Nat. Rev. Microbiol. **13**, 310–317.

Berg, G., Eberl, L. and Hartmann, A., (2005) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environ. Microbiol. **7**, 1673–1685.

Bikard, D., Euler, C.W., Jiang, W., Nussenzweig, P.M., Goldberg, G.W., Duportet, X., Fischetti, V.A. and Marraffini, L.A., (2014) Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat. Biotechnol. **32**, 1146–1150.

Blanck, H., Wängberg, S.-Å. and Molander, S., (1988) Pollution-Induced Community Tolerance—A New Ecotoxicological Tool. Funct. Test. Aquat. Biota Estim. Hazards Chem.

Blum, K.M., Andersson, P.L., Renman, G., Ahrens, L., Gros, M., Wiberg, K. and Haglund, P., (2017) Non-target screening and prioritization of potentially persistent, bioaccumulating and toxic domestic wastewater contaminants and their removal in on-site and large-scale sewage treatment plants. Sci. Total Environ. **575**, 265–275.

Blum, K.M., Haglund, P., Gao, Q., Ahrens, L., Gros, M., Wiberg, K. and Andersson, P.L., (2018) Mass fluxes per capita of organic contaminants from on-site sewage treatment facilities. Chemosphere **201**, 864–873.

Boehler, M., Zwickenpflug, B., Hollender, J., Ternes, T., Joss, A. and Siegrist, H., (2012) Removal of micropollutants in municipal wastewater treatment plants by powder-activated carbon. Water Sci. Technol. **66**, 2115.

Bollmann, U.E., Tang, C., Eriksson, E., Jönsson, K., Vollertsen, J. and Bester, K., (2014) Biocides in urban wastewater treatment plant influent at dry and wet weather: Concentrations, mass flows and possible sources. Water Res. **60**, 64–74.

Bonomo, R.A. and Szabo, D., (2006) Mechanisms of Multidrug Resistance in Acinetobacter Species and Pseudomonas aeruginosa. Clin. Infect. Dis. **43**, S49–S56.

Bourgin, M., Beck, B., Boehler, M., Borowska, E., Fleiner, J., Salhi, E., Teichler, R., von Gunten, U., Siegrist, H. and McArdell, C.S., (2018) Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: Abatement of micropollutants, formation of transformation products and oxidation by-products. Water Res. **129**, 486–498.

Boxall, A.B.A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Beazley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., DeLeo, P.C., Dyer, S.D., Ericson, J.F., Gagné, F., Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M.V., Larsson, D.G.J., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray-Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.R.,

Trudeau, V.L. and Van Der Kraak, G., (2012) Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions? Environ. Health Perspect. **120**, 1221–1229.
Braoudaki, M. and Hilton, A.C., (2004) Adaptive Resistance to Biocides in Salmonella enterica and Escherichia coli O157 and Cross-Resistance to Antimicrobial Agents. J. Clin. Microbiol. **42**, 73–78.
Braus, H., Middleton, F. and Walton, G., (1951) Orangic Chemical Compounds in Raw and Filtered Surface Waters. Anal. Chem. **23**, 1160–1164.
Brodin, T., Fick, J., Jonsson, M. and Klaminder, J., (2013) Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science **339**, 814–815.
Burkhardt, M., Zuleeg, S., Vonbank, R., Bester, K., Carmeliet, J., Boller, M. and Wangler, T., (2012) Leaching of Biocides from Façades under Natural Weather Conditions. Environ. Sci. Technol. **46**, 5497–5503.
Burmeister, A.R., (2015) Horizontal Gene Transfer. Evol. Med. Public Health **2015**, 193.
Callow, M., (1990) Ship fouling: problems and solutions. Chem. Ind.
Casadevall, A. and Pirofski, L., (2014) Microbiology: Ditch the term pathogen. Nat. News **516**, 165.
Casado, J., Rodríguez, I., Ramil, M. and Cela, R., (2014) Selective determination of antimycotic drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography quadrupole time-of-flight mass spectrometry. J. Chromatogr. A **1339**, 42–49.
Castanon, J.I.R., (2007) History of the Use of Antibiotic as Growth Promoters in European Poultry Feeds. Poult. Sci. **86**, 2466–2471.
Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D. and Zuccato, E., (2006) Removal of Pharmaceuticals in Sewage Treatment Plants in Italy. Environ. Sci. Technol. **40**, 357–363.
Çeçen, F. and Aktaş, Ö., (2011) Activated Carbon for Water and Wastewater Treatment: Integration of Adsorption and Biological Treatment, 2nd ed. John Wiley & Sons.
Chain, E., Florey, H.W., Gardner, A.D., Heatley, N.G., Jennings, M.A., Orr-Ewing, J. and Sanders, A.G., (1940) Penicillin as a Chemotherapeutic Agent. The Lancet, Originally published as Volume 2, Issue 6104 **236**, 226–228.
Chen, X., Vollertsen, J., Nielsen, J.L., Gieraltowska Dall, A. and Bester, K., (2015) Degradation of PPCPs in activated sludge from different WWTPs in Denmark. Ecotoxicology **24**, 2073–2080.
Cheney, J.E. and Collins, C.H., (1995) Formaldehyde disinfection in laboratories: limitations and hazards. Br. J. Biomed. Sci. **52**, 195–201.
Clara, M., Kreuzinger, N., Strenn, B., Gans, O. and Kroiss, H., (2005) The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res. **39**, 97–106.
Cockburn, T.A., (1964) The Evolution and Eradication of Infectious Diseases. Perspect. Biol. Med. **7**, 498–499.
Coenen, S., Adriaenssens, N., Versporten, A., Muller, A., Minalu, G., Faes, C., Vankerckhoven, V., Aerts, M., Hens, N., Molenberghs, G. and Goossens, H., (2011) European Surveillance of Antimicrobial Consumption (ESAC): outpatient use of tetracyclines, sulphonamides and trimethoprim, and other antibacterials in Europe (1997–2009). J. Antimicrob. Chemother. **66**, vi57–vi70.
Cuprys, A., Pulicharla, R., Brar, S.K., Drogui, P., Verma, M. and Surampalli, R.Y., (2018) Fluoroquinolones metal complexation and its environmental impacts. Coord. Chem. Rev. **376**, 46–61.
Davies, J. and Davies, D., (2010) Origins and Evolution of Antibiotic Resistance. Microbiol. Mol. Biol. Rev. MMBR **74**, 417–433.

Dcosta, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W.L., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G.B., Poinar, H.N. and Wright, G.D., (2011) Antibiotic resistance is ancient. Nature **477**, 457–461.

D'Costa, V.M., McGrann, K.M., Hughes, D.W. and Wright, G.D., (2006) Sampling the Antibiotic Resistome. Science **311**, 374–377.

De Wever, H. and Verachtert, H., (1997) Biodegradation and toxicity of benzothiazoles. Water Res. **31**, 2673–2684.

Demain, A.L. and Sanchez, S., (2009) Microbial drug discovery: 80 years of progress. J. Antibiot. (Tokyo) **62**, 5–16.

Diener, D.L. and Palme, U., (2012) Silver i produktkedjan: Från konsumentvaror till avloppsslam med aktörsperspektiv (No. 2012–18). Stockholm.

Dodd, M.C., Rentsch, D., Singer, H.P., Kohler, H.-P.E. and von Gunten, U., (2010) Transformation of β-Lactam Antibacterial Agents during Aqueous Ozonation: Reaction Pathways and Quantitative Bioassay of Biologically-Active Oxidation Products. Environ. Sci. Technol. **44**, 5940–5948.

Dole, M., Mack, L.L., Hines, R.L., Mobley, R.C., Ferguson, L.D. and Alice, M.B., (1968) Molecular Beams of Macroions. J. Chem. Phys. **49**, 2240–2249.

Domagk, G., (1935) Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. DMW - Dtsch. Med. Wochenschr. **61**, 250–253.

Done, H.Y., Venkatesan, A.K. and Halden, R.U., (2015) Does the Recent Growth of Aquaculture Create Antibiotic Resistance Threats Different from those Associated with Land Animal Production in Agriculture? AAPS J. **17**, 513–524.

Drace, K., Giangiuli, S., LeSar, C. and Kiefer, A.M., (2018) Draft Genome Sequence of Mercury-Resistant Pseudomonas putida Strain DRA525. Genome Announc. **6**.

Ebeling, J.M., Sibrell, P.L., Ogden, S.R. and Summerfelt, S.T., (2003) Evaluation of chemical coagulation–flocculation aids for the removal of suspended solids and phosphorus from intensive recirculating aquaculture effluent discharge. Aquac. Eng. **29**, 23–42.

ECHA, (2018) Registered substances - European Chemials Agency [WWW Document]. URL https://echa.europa.eu/sv/information-on-chemicals/registered-substances

Eckelman, M.J. and Graedel, T.E., (2007) Silver Emissions and their Environmental Impacts: A Multilevel Assessment. Environ. Sci. Technol. **41**, 6283–6289.

Edwards, J., Othman, M., Crossin, E. and Burn, S., (2017) Anaerobic co-digestion of municipal food waste and sewage sludge: A comparative life cycle assessment in the context of a waste service provision. Bioresour. Technol. **223**, 237–249.

Eggen, R.I.L., Hollender, J., Joss, A., Schärer, M. and Stamm, C., (2014) Reducing the Discharge of Micropollutants in the Aquatic Environment: The Benefits of Upgrading Wastewater Treatment Plants. Environ. Sci. Technol. **48**, 7683–7689.

El-taliawy, H., Ekblad, M., Nilsson, F., Hagman, M., Paxeus, N., Jönsson, K., Cimbritz, M., la Cour Jansen, J. and Bester, K., (2017) Ozonation efficiency in removing organic micro pollutants from wastewater with respect to hydraulic loading rates and different wastewaters. Chem. Eng. J. **325**, 310–321.

Engelhardt, N., Firk, W. and Warnken, W., (1998) Integration of membrane filtration into the activated sludge process in municipal wastewater treatment. Water Sci. Technol., Water Quality International '98 **38**, 429–436.

Engelhaupt, E., Betts, K., Renner, R., Christen, K., Lubick, N. and Booth, B., (2007) More triclosan trouble. Environ. Sci. Technol. **41**, 2072–2077.

Enne, V.I., Bennett, P.M., Livermore, D.M. and Hall, L.M.C., (2004) Enhancement of host fitness by the sul2-coding plasmid p9123 in the absence of selective pressure. J. Antimicrob. Chemother. **53**, 958–963.

EU 358/2014, (2014) Commission Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products 5.

EU 1004/2014, (2014) Commission Regulation (EU) No 1004/2014 of 18 September 2014 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products 4.

EU 2016/110, (2016) Commission Implementing Decision (EU) 2016/ 110 - of 27 January 2016 - not approving triclosan as an existing active substance for use in biocidal products for product-type 1 2.

EU regulation 528/2012, (2012) Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.

European Commission, (2001) Disposal and recycling routes for sewage sludge - Part 2 - Regulatory report. Luxembourg.

Evans, E.H. and Giglio, J.J., (1993) Interferences in inductively coupled plasma mass spectrometry. A review. J. Anal. At. Spectrom. **8**, 1–18.

Falås, P., Baillon-Dhumez, A., Andersen, H.R., Ledin, A. and la Cour Jansen, J., (2012) Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals. Water Res. **46**, 1167–1175.

Fang, L., Li, X., Li, L., Li, S., Liao, X., Sun, J. and Liu, Y., (2016) Co-spread of metal and antibiotic resistance within ST3-IncHI2 plasmids from E. coli isolates of food-producing animals. Sci. Rep. **6**, 25312.

Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M., (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science **246**, 64–71.

Fernández-Calviño, D., Rousk, J., Bååth, E., Bollmann, U.E., Bester, K. and Brandt, K.K., (2017) Ecotoxicological assessment of propiconazole using soil bacterial and fungal growth assays. Appl. Soil Ecol. **115**, 27–30.

Ferreira da Silva, M., Tiago, I., Veríssimo, A., Boaventura, R.A.R., Nunes, O.C. and Manaia, C.M., (2006) Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. FEMS Microbiol. Ecol. **55**, 322–329.

Fick, J., Söderström, H., Lindberg, R.H., Phan, C., Tysklind, M. and Larsson, D.G.J., (2009) Contamination of surface, ground, and drinking water from pharmaceutical production. Environ. Toxicol. Chem. **28**, 2522–2527.

Finley, R.L., Collignon, P., Larsson, D.G.J., Mcewen, S.A., Li, X.-Z., Gaze, W.H., Reid-Smith, R., Timinouni, M., Graham, D.W. and Topp, E., (2013) The scourge of antibiotic resistance: The important role of the environment. Clin. Infect. Dis. **57**, 704–710.

Finnson, A., (2013) Slamanvändning och strategier för slamanvändning (No. M137). Svenskt Vatten, Stockholm.

Fischbach, M.A. and Walsh, C.T., (2009) Antibiotics for Emerging Pathogens. Science **325**, 1089–1093.

Flach, C.-F., Genheden, M., Fick, J. and Joakim Larsson, D.G., (2018) A Comprehensive Screening of Escherichia coli Isolates from Scandinavia's Largest Sewage Treatment Plant Indicates No Selection for Antibiotic Resistance. Environ. Sci. Technol. (ahead of print)

Flach, C.-F., Pal, C., Svensson, C.J., Kristiansson, E., Östman, M., Bengtsson-Palme, J., Tysklind, M. and Larsson, D.G.J., (2017) Does antifouling paint select for antibiotic resistance? Sci. Total Environ. **590–591**, 461–468.

Fleming, A., (1929) On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. Br. J. Exp. Pathol. **10**, 226–236.

Food and Drug Administration, (2017) Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use.

Frazer, A.D., (1930) Tellurium in the treatment of Syphilis. The Lancet, Originally published as Volume 2, Issue 5577 **216**, 133–134.

Gao, P., Munir, M. and Xagoraraki, I., (2012) Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a

conventional municipal wastewater treatment plant. Sci. Total Environ., Special Section: Reviews of Trace Metal Pollution in China **421**–**422**, 173–183.
Gohlke, R.S., (1959) Time-of-Flight Mass Spectrometry and Gas-Liquid Partition Chromatography. Anal. Chem. **31**, 535–541.
Gomaa, A.A., Klumpe, H.E., Luo, M.L., Selle, K., Barrangou, R. and Beisel, C.L., (2014) Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. mBio **5**, e00928-00913.
Gomes, J., Costa, R., Quinta-Ferreira, R.M. and Martins, R.C., (2017) Application of ozonation for pharmaceuticals and personal care products removal from water. Sci. Total Environ. **586**, 265–283.
Grabicova, K., Lindberg, R.H., Östman, M., Grabic, R., Randak, T., Joakim Larsson, D.G. and Fick, J., (2014) Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Sci. Total Environ. **488**–**489**, 46–50.
Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N. and Doumenq, P., (2017) From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: A review. Water Res. **111**, 297–317.
Greenfield, S., (2000) Invention of the Annular Inductively Coupled Plasma as a Spectroscopic Source. J. Chem. Educ. **77**, 584.
Griffiths, J., (2008) A Brief History of Mass Spectrometry. Anal. Chem. **80**, 5678–5683.
Guerra, P., Kim, M., Shah, A., Alaee, M. and Smyth, S.A., (2014) Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci. Total Environ. **473**–**474**, 235–243.
Gullberg, E., Albrecht, L.M., Karlsson, C., Sandegren, L. and Andersson, D.I., (2014) Selection of a Multidrug Resistance Plasmid by Sublethal Levels of Antibiotics and Heavy Metals. mBio **5**, e01918-14.
Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D. and Andersson, D.I., (2011) Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. PLOS Pathog. **7**, e1002158.
von Gunten, U., (2003a) Ozonation of drinking water: Part I. Oxidation kinetics and product formation. Water Res. **37**, 1443–1467.
von Gunten, U., (2003b) Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Res. **37**, 1469–1487.
Guyer, B., Freedman, M.A., Strobino, D.M. and Sondik, E.J., (2000) Annual summary of vital statistics: trends in the health of Americans during the 20th century. Pediatrics **106**, 1307–1317.
Hahn, S., Schneider, K., Gartiser, S., Heger, W. and Mangelsdorf, I., (2010) Consumer exposure to biocides - identification of relevant sources and evaluation of possible health effects. Environ. Health **9**, 7.
Haman, C., Dauchy, X., Rosin, C. and Munoz, J.-F., (2015) Occurrence, fate and behavior of parabens in aquatic environments: A review. Water Res. **68**, 1–11.
Handel, A., Regoes, R.R. and Antia, R., (2006) The Role of Compensatory Mutations in the Emergence of Drug Resistance. PLOS Comput. Biol. **2**, e137.
Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J. and Goodman, R.M., (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem. Biol. **5**, R245–R249.
Harris, D.C., (2007) Quantitative Chemical Analysis, Seventh Edition. ed. W. H. Freeman and Company.
Heid, C.A., Stevens, J., Livak, K.J. and Williams, P.M., (1996) Real time quantitative PCR. Genome Res. **6**, 986–994.
Hobman, J.L. and Crossman, L.C., (2015) Bacterial antimicrobial metal ion resistance. J. Med. Microbiol. **64**, 471–497.

Hoelzer, K., Wong, N., Thomas, J., Talkington, K., Jungman, E. and Coukell, A., (2017) Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence? BMC Vet. Res. **13**.

de Hoffmann, E. and Stroobant, V., (2007) Mass Spectrometry - Principles and Applications, Third Edition. ed. John Wiley & Sons.

Hollender, J., Zimmermann, S.G., Koepke, S., Krauss, M., McArdell, C.S., Ort, C., Singer, H., von Gunten, U. and Siegrist, H., (2009) Elimination of Organic Micropollutants in a Municipal Wastewater Treatment Plant Upgraded with a Full-Scale Post-Ozonation Followed by Sand Filtration. Environ. Sci. Technol. **43**, 7862–7869.

Hörsing, M., (2018) Avloppsslam på åkermark (No. 2018–4). Svenskt Vatten Utveckling, Stockholm.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., Jansen, J. la C. and Andersen, H.R., (2011) Determination of sorption of seventy-five pharmaceuticals in sewage sludge. Water Res. **45**, 4470–4482.

Houhou, J., Lartiges, B.S., Montarges-Pelletier, E., Sieliechi, J., Ghanbaja, J. and Kohler, A., (2009) Sources, nature, and fate of heavy metal-bearing particles in the sewer system. Sci. Total Environ. **407**, 6052–6062.

Houk, R.S., (1986) Mass spectrometry of inductively coupled plasmas. Anal. Chem. **58**, 97A-105A.

Houk, R.S., Fassel, V.A., Flesch, G.D., Svec, H.J., Gray, A.L. and Taylor, C.E., (1980) Inductively coupled argon plasma as an ion source for mass spectrometric determination of trace elements. Anal. Chem. **52**, 2283–2289.

Huang, M.-Z., Yuan, C.-H., Cheng, S.-C., Cho, Y.-T. and Shiea, J., (2010) Ambient Ionization Mass Spectrometry. Annu. Rev. Anal. Chem. **3**, 43–65.

Hübner, U., von Gunten, U. and Jekel, M., (2015) Evaluation of the persistence of transformation products from ozonation of trace organic compounds – A critical review. Water Res. **68**, 150–170.

Jamkhandi, C.M. and Disouza, J.I., (2012) Benzotriazole Derivatives as Antimicrobial Agents. Asian J Biochem Pharm Res **2**, 123–130.

Janna, H., Scrimshaw, M.D., Williams, R.J., Churchley, J. and Sumpter, J.P., (2011) From Dishwasher to Tap? Xenobiotic Substances Benzotriazole and Tolyltriazole in the Environment. Environ. Sci. Technol. **45**, 3858–3864.

Jayjock, M.A., Hazelton, G.A., Lewls, P.G. and Wooder, M.F., (1996) Formulation effect on the dermal bioavailability of isothiazolone biocide. Food Chem. Toxicol. **34**, 277–282.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G. and Sumpter, J.P., (1998) Widespread Sexual Disruption in Wild Fish. Environ. Sci. Technol. **32**, 2498–2506.

Johansson, I. and Somasundaran, P., (2007) Handbook for Cleaning/Decontamination of Surfaces. Elsevier Science.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A. and Siegrist, H., (2006) Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. **40**, 1686–1696.

Jutkina, J., Marathe, N.P., Flach, C.-F. and Larsson, D.G.J., (2018) Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. Sci. Total Environ. **616–617**, 172–178.

Kahrilas, G.A., Blotevogel, J., Stewart, P.S. and Borch, T., (2014) Biocides in Hydraulic Fracturing Fluids: A Critical Review of Their Usage, Mobility, Degradation, and Toxicity. Environ. Sci. Technol. **49**, 16–32.

Kallmeyer, J., Pockalny, R., Adhikari, R.R., Smith, D.C. and D'Hondt, S., (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. Proc. Natl. Acad. Sci. **109**, 16213–16216.

Kårelid, V., Larsson, G. and Björlenius, B., (2017) Pilot-scale removal of pharmaceuticals in municipal wastewater: Comparison of granular and powdered activated carbon treatment at three wastewater treatment plants. J. Environ. Manage. **193**, 491–502.

Karkman, A., Do, T.T., Walsh, F. and Virta, M.P.J., (2017) Antibiotic-Resistance Genes in Waste Water. Trends Microbiol.

Karkman, A., Johnson, T.A., Lyra, C., Stedtfeld, R.D., Tamminen, M., Tiedje, J.M. and Virta, M., (2016) High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant. FEMS Microbiol. Ecol. **92**.

Karkman, A., Pärnänen, K. and Larsson, D.G.J., (2018) Fecal pollution explains antibiotic resistance gene abundances in anthropogenically impacted environments. bioRxiv 341487.

Karthikeyan, K.G. and Meyer, M.T., (2006) Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Sci. Total Environ. **361**, 196–207.

Karvelas, M., Katsoyiannis, A. and Samara, C., (2003) Occurrence and fate of heavy metals in the wastewater treatment process. Chemosphere **53**, 1201–1210.

Kaur, U.J., Preet, S. and Rishi, P., (2018) Augmented antibiotic resistance associated with cadmium induced alterations in Salmonella enterica serovar Typhi. Sci. Rep. **8**, 12818.

Khan, S.J. and Ongerth, J.E., (2002) Estimation of pharmaceutical residues in primary and secondary sewage sludge based on quantities of use and fugacity modelling. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. **46**, 105–113.

Klahre, J. and Flemming, H.-C., (2000) Monitoring of biofouling in papermill process waters. Water Res. **34**, 3657–3665.

Kools, S.A.E., Moltmann, J.F. and Knacker, T., (2008) Estimating the use of veterinary medicines in the European union. Regul. Toxicol. Pharmacol. **50**, 59–65.

Kraupner, N., Ebmeyer, S., Bengtsson-Palme, J., Fick, J., Kristiansson, E., Flach, C.-F. and Larsson, D.G.J., (2018) Selective concentration for ciprofloxacin resistance in Escherichia coli grown in complex aquatic bacterial biofilms. Environ. Int. **116**, 255–268.

Kreuzinger, N., Fuerhacker, M., Scharf, S., Uhl, M., Gans, O. and Grillitsch, B., (2007) Methodological approach towards the environmental significance of uncharacterized substances — quaternary ammonium compounds as an example. Desalination **215**, 209–222.

Krzeminski, P., Leverette, L., Malamis, S. and Katsou, E., (2017) Membrane bioreactors – A review on recent developments in energy reduction, fouling control, novel configurations, LCA and market prospects. J. Membr. Sci. **527**, 207–227.

Kümmerer, K., (2001) Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere **45**, 957–969.

Kümmerer, K., (2009a) Antibiotics in the aquatic environment – A review – Part II. Chemosphere **75**, 435–441.

Kümmerer, K., (2009b) Antibiotics in the aquatic environment – A review – Part I. Chemosphere **75**, 417–434.

Kümmerer, K., Dionysiou, D.D., Olsson, O. and Fatta-Kassinos, D., (2018) A path to clean water. Science **361**, 222–224.

Kümmerer, K., Eitel, A., Braun, U., Hubner, P., Daschner, F., Mascart, G., Milandri, M., Reinthaler, F. and Verhoef, J., (1997) Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and high-performance liquid chromatography with post-column ion-pairing and fluorescence detection. J. Chromatogr. A **774**, 281–286.

Lara-Martín, P.A., Li, X., Bopp, R.F. and Brownawell, B.J., (2010) Occurrence of Alkyltrimethylammonium Compounds in Urban Estuarine Sediments:

Behentrimonium As a New Emerging Contaminant. Environ. Sci. Technol. **44**, 7569–7575.

Larsson, D.G.J., Andremont, A., Bengtsson-Palme, J., Brandt, K.K., de Roda Husman, A.M., Fagerstedt, P., Fick, J., Flach, C.-F., Gaze, W.H., Kuroda, M., Kvint, K., Laxminarayan, R., Manaia, C.M., Nielsen, K.M., Plant, L., Ploy, M.-C., Segovia, C., Simonet, P., Smalla, K., Snape, J., Topp, E., van Hengel, A.J., Verner-Jeffreys, D.W., Virta, M.P.J., Wellington, E.M. and Wernersson, A.-S., (2018) Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. Environ. Int. **117**, 132–138.

Larsson, D.G.J., de Pedro, C. and Paxeus, N., (2007) Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J. Hazard. Mater. **148**, 751–755.

Lee, Y., Gerrity, D., Lee, M., Bogeat, A.E., Salhi, E., Gamage, S., Trenholm, R.A., Wert, E.C., Snyder, S.A. and von Gunten, U., (2013) Prediction of Micropollutant Elimination during Ozonation of Municipal Wastewater Effluents: Use of Kinetic and Water Specific Information. Environ. Sci. Technol. **47**, 5872–5881.

Lehmann, J., (2007) Bio-energy in the black. Front. Ecol. Environ. **5**, 381–387.

Lester, J.N., Harrison, R.M. and Perry, R., (1979) The balance of heavy metals through a sewage treatment works I. Lead, cadmium and copper. Sci. Total Environ. **12**, 13–23.

Lindberg, R.H., Östman, M., Olofsson, U., Grabic, R. and Fick, J., (2014) Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. **58**, 221–229.

Liška, I., (2000) Fifty years of solid-phase extraction in water analysis – historical development and overview. J. Chromatogr. A **885**, 3–16.

Liu, A., Fong, A., Becket, E., Yuan, J., Tamae, C., Medrano, L., Maiz, M., Wahba, C., Lee, C., Lee, K., Tran, K.P., Yang, H., Hoffman, R.M., Salih, A. and Miller, J.H., (2011) Selective Advantage of Resistant Strains at Trace Levels of Antibiotics: a Simple and Ultrasensitive Color Test for Detection of Antibiotics and Genotoxic Agents. Antimicrob. Agents Chemother. **55**, 1204–1210.

Liu, Z., Singer, S., Tong, Y., Kimbell, L., Anderson, E., Hughes, M., Zitomer, D. and McNamara, P., (2018) Characteristics and applications of biochars derived from wastewater solids. Renew. Sustain. Energy Rev. **90**, 650–664.

Locey, K.J. and Lennon, J.T., (2016) Scaling laws predict global microbial diversity. Proc. Natl. Acad. Sci. 201521291.

Lofrano, G. and Brown, J., (2010) Wastewater management through the ages: A history of mankind. Sci. Total Environ. **408**, 5254–5264.

Lood, R., Ertürk, G. and Mattiasson, B., (2017) Revisiting Antibiotic Resistance Spreading in Wastewater Treatment Plants – Bacteriophages as a Much Neglected Potential Transmission Vehicle. Front. Microbiol. **8**.

Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D. and Gawlik, B.M., (2013) EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. **47**, 6475–6487.

Lüddeke, F., Heß, S., Gallert, C., Winter, J., Güde, H. and Löffler, H., (2015) Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques. Water Res. **69**, 243–251.

Lundström, S.V., Östman, M., Bengtsson-Palme, J., Rutgersson, C., Thoudal, M., Sircar, T., Blanck, H., Eriksson, K.M., Tysklind, M., Flach, C.-F. and Larsson, D.G.J., (2016) Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Sci. Total Environ. **553**, 587–595. **Paper III**

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S. and Wang, X.C., (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. **473–474**, 619–641.

Madigan, M., Martinko, J. and Parker, J., (2000) Brock Biology of Microorganisms, 9th ed. ed. Prentice-Hall, Inc., New Jersey.

Magdeburg, A., Stalter, D., Schlüsener, M., Ternes, T. and Oehlmann, J., (2014) Evaluating the efficiency of advanced wastewater treatment: Target analysis of organic contaminants and (geno-)toxicity assessment tell a different story. Water Res. **50**, 35–47.

Maillard, J.-Y., (2018) Resistance of Bacteria to Biocides. Microbiol. Spectr. **6**.

Margot, J., Kienle, C., Magnet, A., Weil, M., Rossi, L., de Alencastro, L.F., Abegglen, C., Thonney, D., Chèvre, N., Schärer, M. and Barry, D.A., (2013) Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? Sci. Total Environ. **461–462**, 480–498.

Marshall, B.M. and Levy, S.B., (2011) Food Animals and Antimicrobials: Impacts on Human Health. Clin. Microbiol. Rev. **24**, 718–733.

Martínez, J.L., (2008) Antibiotics and Antibiotic Resistance Genes in Natural Environments. Science **321**, 365–367.

Martínez, J.L., Coque, T.M. and Baquero, F., (2015) What is a resistance gene? Ranking risk in resistomes. Nat. Rev. Microbiol. **13**, 116–123.

Matsui, Y., Nakao, S., Sakamoto, A., Taniguchi, T., Pan, L., Matsushita, T. and Shirasaki, N., (2015) Adsorption capacities of activated carbons for geosmin and 2-methylisoborneol vary with activated carbon particle size: Effects of adsorbent and adsorbate characteristics. Water Res. **85**, 95–102.

Matsushima, H. and Sakurai, N., (1984) A selected ion monitoring assay for chlorhexidine in medical waste water. Biol. Mass Spectrom. **11**, 203–206.

May, W.E., Chesler, S.N., Cram, S.P., Gump, B.H., Hertz, H.S., Enagonio, D.P. and Dyszel, S.M., (1975) Chromatographic Analysis of Hydrocarbons in Marine Sediments and Sea water. J. Chromatogr. Sci. **13**, 535–540.

McCormick, J.R.D., Fox, S.M., Smith, L.L., Bitler, B.A., Reichenthal, J., Origoni, V.E., Muller, W.H., Winterbottom, R. and Doerschuk, A.P., (1957) Studies of the Reversible Epimerization Occurring in the Tetracycline Family. The Preparation, Properties and Proof of Structure of Some 4-epi-Tetracyclines. J. Am. Chem. Soc. **79**, 2849–2858.

McDonnell, G. and Russell, A.D., (1999) Antiseptics and Disinfectants: Activity, Action, and Resistance. Clin. Microbiol. Rev. **12**, 147–179.

McLellan, I., Carvalho, M., Pereira, C.S., Hursthouse, A., Morrison, C., Tatner, P., Martins, I., Romão, M.V.S. and Leitão, M., (2007) The environmental behaviour of polychlorinated phenols and its relevance to cork forest ecosystems: a review. J. Environ. Monit. **9**, 1055–1063.

McMaster, M., (2005) LC/MS - A practical user's guide. John Wiley & Sons.

McQuillan, R.V., Stevens, G.W. and Mumford, K.A., (2018) The electrochemical regeneration of granular activated carbons: A review. J. Hazard. Mater. **355**, 34–49.

Medical Dictionary, (2009) Selection pressure [WWW Document]. Med. Dict. URL https://medical-dictionary.thefreedictionary.com/selection+pressure

Menz, J., Baginska, E., Arrhenius, Å., Haiß, A., Backhaus, T. and Kümmerer, K., (2017) Antimicrobial activity of pharmaceutical cocktails in sewage treatment plant effluent – An experimental and predictive approach to mixture risk assessment. Environ. Pollut. **231**, 1507–1517.

Menz, J., Müller, J., Olsson, O. and Kümmerer, K., (2018) Bioavailability of Antibiotics at Soil–Water Interfaces: A Comparison of Measured Activities and Equilibrium Partitioning Estimates. Environ. Sci. Technol. **52**, 6555–6564.

Merriam-Webster Dictionary, (2018) Merriam-Webster Dictionary [WWW Document]. URL https://www.merriam-webster.com/dictionary/antimicrobial

Meyer, B., (2006) Does microbial resistance to biocides create a hazard to food hygiene? Int. J. Food Microbiol. **112**, 275–279.

Microbiology by numbers, (2011) . Nat. Rev. Microbiol. **9**, 628.

Mishra, R.P., Oviedo-Orta, E., Prachi, P., Rappuoli, R. and Bagnoli, F., (2012) Vaccines and antibiotic resistance. Curr. Opin. Microbiol., Antimicrobials • Genomics **15**, 596–602.

Moore, A.M., Munck, C., Sommer, M.O.A. and Dantas, G., (2011) Functional Metagenomic Investigations of the Human Intestinal Microbiota. Front. Microbiol. **2**.

Murray, A.K., Zhang, L., Yin, X., Zhang, T., Buckling, A., Snape, J. and Gaze, W.H., (2018) Novel Insights into Selection for Antibiotic Resistance in Complex Microbial Communities. mBio **9**, e00969-18.

Murtough, S.M., Hiom, S.J., Palmer, M. and Russell, A.D., (2002) A survey of rotational use of biocides in hospital pharmacy aseptic units. J. Hosp. Infect. **50**, 228–231.

Namasivayam, C. and Yamuna, R.T., (1995) Adsorption of chromium (VI) by a low-cost adsorbent: Biogas residual slurry. Chemosphere **30**, 561–578.

Naturvårdsverket, (2014) Wastewater treatment in Sweden.

Nelson, M.D. (chair), (2017) Operation of Water Resource Recovery Facilities, Seventh. ed, Manual of practice No. 11. McGraw-Hill Education, New York.

Neuvonen, P.J., (1976) Interactions with the absorption of tetracyclines. Drugs **11**, 45–54.

Nier, A.O., (1989) Some reminiscences of mass spectrometry and the Manhattan Project. J. Chem. Educ. **66**, 385.

Nilsson, F., Ekblad, M., la Cour Jansen, J. and Jönsson, K., (2017) Removal of pharmaceuticals with ozone at 10 Swedish wastewater treatment plants. Water Pract. Technol. **12**, 871–881.

Nowotny, N., Epp, B., von Sonntag, C. and Fahlenkamp, H., (2007) Quantification and Modeling of the Elimination Behavior of Ecologically Problematic Wastewater Micropollutants by Adsorption on Powdered and Granulated Activated Carbon. Environ. Sci. Technol. **41**, 2050–2055.

Nriagu, J.O., (1996) A History of Global Metal Pollution. Science **272**, 223–223.

Ødegaard, H., (2006) Innovations in wastewater treatment: –the moving bed biofilm process. Water Sci. Technol. **53**, 17–33.

Okhovat, A., Ahmadpour, A., Ahmadpour, F. and Khaki Yadegar, Z., (2012) Pore Size Distribution Analysis of Coal-Based Activated Carbons: Investigating the Effects of Activating Agent and Chemical Ratio [WWW Document]. Int. Sch. Res. Not. URL https://www.hindawi.com/journals/isrn/2012/352574/

Olofsson, U., Bignert, A. and Haglund, P., (2012) Time-trends of metals and organic contaminants in sewage sludge. Water Res. **46**, 4841–4851.

Olofsson, U., Brorström-Lundén, E., Kylin, H. and Haglund, P., (2013) Comprehensive mass flow analysis of Swedish sludge contaminants. Chemosphere **90**, 28–35.

Olsen, R.L. and Davis, A., (1990) Predicting the fate and transport of organic compounds in groundwater: part 1. Hazard. Mater. Control **3**, 40–64.

O'Neill, J., (2014) AMR Review Paper - Tackling a crisis for the health and wealth of nations.

Östman, M., Björlenius, B., Fick, J. and Tysklind, M., (2019) Effect of full-scale ozonation and pilot-scale granular activated carbon on the removal of biocides, antimycotics and antibiotics in a sewage treatment plant. Sci. Total Environ. **649**, 1117–1123. **Paper V**

Östman, M., Fick, J., Näsström, E. and Lindberg, R.H., (2014) A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci. Total Environ. **472**, 862–871.

Östman, M., Fick, J. and Tysklind, M., (2018) Detailed mass flows and removal efficiencies for biocides and antibiotics in Swedish sewage treatment plants. Sci. Total Environ. **640**–**641**, 327–336. **Paper II**

Östman, M., Lindberg, R.H., Fick, J., Björn, E. and Tysklind, M., (2017) Screening of biocides, metals and antibiotics in Swedish sewage sludge and wastewater. Water Res. **115**, 318–328. **Paper I**

Overmann, J., Abt, B. and Sikorski, J., (2017) Present and Future of Culturing Bacteria. Annu. Rev. Microbiol. **71**, 711–730.

Pal, C., (2017) Effects of biocides and metals on antibiotic resistance: a genomic and metagenomic perspective, PhD thesis. University of Gothenburg, Göteborg.

Pal, C., Asiani, K., Arya, S., Rensing, C., Stekel, D.J., Larsson, D.G.J. and Hobman, J.L., (2017) Chapter Seven - Metal Resistance and Its Association With Antibiotic Resistance, in: Poole, R.K. (Ed.), Advances in Microbial Physiology, Microbiology of Metal Ions. Academic Press, pp. 261–313.

Pal, C., Bengtsson-Palme, J., Kristiansson, E. and Larsson, D.G.J., (2015) Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics **16**, 964.

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E. and Larsson, D.G.J., (2014) BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Res. **42**, D737–D743.

Pan, B., Ning, P. and Xing, B., (2009) Part V - Sorption of pharmaceuticals and personal care products. Environ. Sci. Pollut. Res. **16**, 106–116.

Papageorgiou, A., Stylianou, S.K., Kaffes, P., Zouboulis, A.I. and Voutsa, D., (2017) Effects of ozonation pretreatment on natural organic matter and wastewater derived organic matter – Possible implications on the formation of ozonation by-products. Chemosphere **170**, 33–40.

Paul, W. and Steinwedel, H., (1960) Apparatus for separating charged particles of different specific charges.

Pawliszyn, J. and Lord, H., (2010) Handbook of sample preparation. Wiley, New Jersey.

Paxéus, N., (1996) Organic pollutants in the effluents of large wastewater treatment plants in Sweden. Water Res. **30**, 1115–1122.

Pena, A., Carmona, A., Barbosa, A., Lino, C., Silveira, I. and Castillo, B., (1998) Determination of tetracycline and its major degradation products by liquid chromatography with fluorescence detection. J. Pharm. Biomed. Anal. **18**, 839–845.

Pramer, D., (1988) Some Choice Words From Waksman [WWW Document]. Sci. Mag. URL https://www.the-scientist.com/news/some-choice-words-from-waksman-63029

Pruden, A., (2014) Balancing Water Sustainability and Public Health Goals in the Face of Growing Concerns about Antibiotic Resistance. Environ. Sci. Technol. **48**, 5–14.

Pruden, A., Larsson, D.G.J., Amézquita, A., Collignon, P., Brandt, K.K., Graham, D.W., Lazorchak, J.M., Suzuki, S., Silley, P., Snape, J.R., Topp, E., Zhang, T. and Zhu, Y.-G., (2013) Management Options for Reducing the Release of Antibiotics and Antibiotic Resistance Genes to the Environment. Environ. Health Perspect. **121**, 878–885.

Pursey, E., Sünderhauf, D., Gaze, W.H., Westra, E.R. and Houte, S. van, (2018) CRISPR-Cas antimicrobials: Challenges and future prospects. PLOS Pathog. **14**, e1006990.

Queenan, K., Häsler, B. and Rushton, J., (2016) A One Health approach to antimicrobial resistance surveillance: is there a business case for it? Int. J. Antimicrob. Agents **48**, 422–427.

Rafoth, A., Gabriel, S., Sacher, F. and Brauch, H.-J., (2007) Analysis of isothiazolinones in environmental waters by gas chromatography–mass spectrometry. J. Chromatogr. A **1164**, 74–81.

Regeringskansliet, R. och, (2018) Utredning ska föreslå förbud mot spridning av avloppsslam på åkrar och krav på återvinning av fosfor [WWW Document]. Regeringskansliet. URL https://www.regeringen.se/pressmeddelanden/2018/07/utredning-ska-foresla-forbud-mot-spridning-av-avloppsslam-pa-akrar-och-krav-pa-atervinning-av-fosfor/

Rizzo, L., Fiorentino, A. and Anselmo, A., (2013) Advanced treatment of urban wastewater by UV radiation: Effect on antibiotics and antibiotic-resistant E. coli strains. Chemosphere **92**, 171–176.

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I. and Fatta-Kassinos, D., (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Sci. Total Environ. **447**, 345–360.

Russell, A., (2003) Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect. Dis. **3**, 794–803.

Russell, A.D., (2002) Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. J. Appl. Microbiol. **92 Suppl**, 121S–35S.

Russell, A.D. and Path, F.R.C., (1986) Chlorhexidine: Antibacterial action and bacterial resistance. Infection **14**, 212–215.

Rutala, W.A. and Weber, D.J., (2004) Disinfection and Sterilization in Health Care Facilities: What Clinicians Need to Know. Clin. Infect. Dis. **39**, 702–709.

Rybacka, A. and Andersson, P.L., (2016) Considering ionic state in modeling sorption of pharmaceuticals to sewage sludge. Chemosphere **165**, 284–293.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M. and Arnold, K.E., (2018) Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. Proc R Soc B **285**, 20181297.

Samuelson, J.P., (2009) Industrial Waste: Environmental Impact, Disposal and Treatment. Nova Science Publishers, Incorporated, Hauppauge, United States.

Sander, R., (2015) Compilation of Henry's law constants (version 4.0) for water as solvent. Atmospheric Chem. Phys. **15**, 4399–4981.

SCB, (2016) Reningsverk efter region, storleksklass och reningsmetoder. Vartannat år 2014 - 2016 [WWW Document]. Statistikdatabasen. URL http://www.statistikdatabasen.scb.se/pxweb/sv/ssd/START__MI__MI0106/MI0106T04/

SCENIHR, (2009) Assessment of the antibiotic resistance effects of biocides.

Schollée, J.E., Bourgin, M., von Gunten, U., McArdell, C.S. and Hollender, J., (2018) Non-target screening to trace ozonation transformation products in a wastewater treatment train including different post-treatments. Water Res. **142**, 267–278.

Séguret, F., Racault, Y. and Sardin, M., (2000) Hydrodynamic behaviour of full scale trickling filters. Water Res. **34**, 1551–1558.

Seiler, C. and Berendonk, T.U., (2012) Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front. Microbiol. **3**, 399.

Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J. and Kim, H., (2016) A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere **150**, 702–714.

Shentu, J.-L., Zhang, K., Shen, D.-S., Wang, M.-Z. and Feng, H.-J., (2015) Effect from low-level exposure of oxytetracycline on abundance of tetracycline resistance genes in arable soils. Environ. Sci. Pollut. Res. Int. **22**, 13102–13110.

Silver i rötslam - Stockholms miljöbarometer [WWW Document], (2018) . URL http://miljobarometern.stockholm.se/miljogifter/miljogifter-i-rotslam/silver-i-rotslam/

Simate, G.S., Maledi, N., Ochieng, A., Ndlovu, S., Zhang, J. and Walubita, L.F., (2016) Coal-based adsorbents for water and wastewater treatment. J. Environ. Chem. Eng. **4**, 2291–2312.

Singer, A.C., Shaw, H., Rhodes, V. and Hart, A., (2016) Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. Front. Microbiol. **7**.

Snowden, F.M., (2008) Emerging and reemerging diseases: a historical perspective. Immunol. Rev. **225**, 9–26.

Sommer, M.O.A., Church, G.M. and Dantas, G., (2010) The human microbiome harbors a diverse reservoir of antibiotic resistance genes. Virulence **1**, 299–303.

Sörme, L. and Lagerkvist, R., (2002) Sources of heavy metals in urban wastewater in Stockholm. Sci. Total Environ. **298**, 131–145.

Sousa, J.M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M.F.R., Silva, A.M.T., Nunes, O.C. and Manaia, C.M., (2017) Ozonation and UV254nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. J. Hazard. Mater., Special Issue on Emerging Contaminants in engineered and natural environment **323**, 434–441.

Sparkman, D.O., Penton, Z.E. and Kitson, F.G., (2011) Gas Chromatography and Mass Spectrometry - A practical guide, Second Edition. ed. Academic Press.

Staehelin, J. and Hoigne, J., (1985) Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environ. Sci. Technol. **19**, 1206–1213.

Statistikdatabas för läkemedel [WWW Document], (2018) . URL http://www.socialstyrelsen.se/statistik/statistikdatabas/lakemedel

Suanon, F., Chi, Q., Yang, X., Wang, H., Rashid, A., Asefi, B., Mama, D., Yu, C.-P. and Sun, Q., (2018) Diagnosis and ecotoxicological risk assessment of 49 elements in sludge from wastewater treatment plants of Chongqing and Xiamen cities, China. Environ. Sci. Pollut. Res.

Suarez, S., Dodd, M.C., Omil, F. and von, G., (2007) Kinetics of triclosan oxidation by aqueous ozone and consequent loss of antibacterial activity: Relevance to municipal wastewater ozonation. Water Res. **41**, 2481–2490.

Swedish Chemicals Agency, (2018) Kemikalieinspektionen [WWW Document]. URL http://www.kemi.se/hitta-direkt/databaser

Swedish EPA, (2017) Avancerad rening av avloppsvatten för avskiljning av läkemedelsrester och andra oönskade ämnen (No. 6766). Naturvårdsverket, Bromma.

Tadkaew, N., Hai, F.I., McDonald, J.A., Khan, S.J. and Nghiem, L.D., (2011) Removal of trace organics by MBR treatment: The role of molecular properties. Water Res. **45**, 2439–2451.

Tan, S.Y. and Grimes, S., (2010) Paul Ehrlich (1854-1915): man with the magic bullet. Singapore Med. J. **51**, 842–843.

Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., Polachek, A.J., Ganshorn, H., Sharma, N., Kellner, J.D. and Ghali, W.A., (2017) Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet. Health **1**, e316–e327.

Tauxe-Wuersch, A., De Alencastro, L.F., Grandjean, D. and Tarradellas, J., (2005) Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. Water Res. **39**, 1761–1772.

Tekniska verken, L., (2018) Rening av läkemedelsrester [WWW Document]. URL https://www.tekniskaverken.se/om-oss/innovation/innovativa-projekt/rening-av-lakemedelsrester/

Teng, H. and Lin, H., (1998) Activated carbon production from low ash subbituminous coal with CO2 activation. AIChE J. **44**, 1170–1177.

Ternes, T.A., (1998) Occurrence of drugs in German sewage treatment plants and rivers1Dedicated to Professor Dr. Klaus Haberer on the occasion of his 70th birthday.1. Water Res. **32**, 3245–3260.
Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M. and Teiser, B., (2003) Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. **37**, 1976–1982.
Tesch, B.J., (2002) Herbs commonly used by women:an evidence-based review. Dis. Mon. **48**, 671–696.
Tezel, U. and Pavlostathis, S.G., (2015) Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. Curr. Opin. Biotechnol. **33**, 296–304.
Thomas, K.V. and Brooks, S., (2010) The environmental fate and effects of antifouling paint biocides. Biofouling **26**, 73–88.
Thomson, J.J., (1921) Discussion on isotopes. Proc R Soc Lond A **99**, 87–104.
Tjus, S., (2014) Biocider spridning i miljön och deras hälso- och miljörisker : screening år 2000-2013. En kunskapsöversikt (In swedish).
Tluczkiewicz, I., Bitsch, A., Hahn, S. and Hahn, T., (2010) Emission of biocides from hospitals: comparing current survey results with European Union default values. Integr. Environ. Assess. Manag. **6**, 273–280.
Törneman, N., (2011) Chlorhexidine and p-chloroaniline. Sweco Environment Screening Report for Swedish Environmental Protection Agency (No. 1270481000). Sweco Environment AB, Malmö.
Tran, N.H., Urase, T. and Kusakabe, O., (2009) The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds. J. Hazard. Mater. **171**, 1051–1057.
UN, (2018) Water and Sanitation [WWW Document]. U. N. Sustain. Dev. URL https://www.un.org/sustainabledevelopment/water-and-sanitation/
US EPA, (2007) Method 3051A, Microwave assisted acid digestion of sediments, sludges, soils, and oils.
US EPA, O., (2015) EPI Suite™-Estimation Program Interface [WWW Document]. US EPA. URL https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
Van de Voorde, A., Lorgeoux, C., Gromaire, M.-C. and Chebbo, G., (2012) Analysis of quaternary ammonium compounds in urban stormwater samples. Environ. Pollut. **164**, 150–157.
Verlicchi, P., Al, A. and Zambello, E., (2012) Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. Sci. Total Environ. **429**, 123–155.
Vita, N.A., Brohem, C.A., Canavez, A.D.P.M., Oliveira, C.F.S., Kruger, O., Lorencini, M. and Carvalho, C.M., (2018) Parameters for assessing the aquatic environmental impact of cosmetic products. Toxicol. Lett. **287**, 70–82.
Voolaid, V., Donner, E., Vasileiadis, S. and Berendonk, T.U., (2017) Bacterial Diversity and Antibiotic Resistance Genes in Wastewater Treatment Plant Influents and Effluents, in: Antimicrobial Resistance in Wastewater Treatment Processes. Wiley-Blackwell, pp. 157–178.
Wahlberg, C., Björlenius, B. and Paxéus, N., (2010) Läkemedeslrester i Stockholms vattenmiljö- Förekomst, förebyggande åtgärder och rening av avloppsvatten. Stockholm Vatten, Stockholm.
Waksman, S.A. and Woodruff, H.B., (1941) Actinomyces antibioticus, a New Soil Organism Antagonistic to Pathogenic and Non-pathogenic Bacteria. J. Bacteriol. **42**, 231–249.
Walsh, C.T., (2003) Antibiotics: actions, origins, resistance. ASM press, Washington.
Wang, J., Wang, J., Zhao, Z., Chen, J., Lu, H., Liu, G., Zhou, J. and Guan, X., (2017) PAHs accelerate the propagation of antibiotic resistance genes in coastal water microbial community. Environ. Pollut. **231**, 1145–1152.

Weiss, S., Jakobs, J. and Reemtsma, T., (2006) Discharge of Three Benzotriazole Corrosion Inhibitors with Municipal Wastewater and Improvements by Membrane Bioreactor Treatment and Ozonation. Environ. Sci. Technol. **40**, 7193–7199.

WHO, (2016) Critically Important Antimicrobials for Human Medicine.

Wieck, S., Olsson, O. and Kümmerer, K., (2016) Possible underestimations of risks for the environment due to unregulated emissions of biocides from households to wastewater. Environ. Int. **94**, 695–705.

Wieck, S., Olsson, O. and Kümmerer, K., (2018a) Not only biocidal products: Washing and cleaning agents and personal care products can act as further sources of biocidal active substances in wastewater. Environ. Int. **115**, 247–256.

Wieck, S., Olsson, O. and Kümmerer, K., (2018b) Consumers' perceptions of biocidal products in households. Int. J. Hyg. Environ. Health **221**, 260–268.

Wilbourn, J., Heseltine, E. and Møller, H., (1995) IARC evaluates wood dust and formaldehyde. International Agency for Research on Cancer. Scand. J. Work. Environ. Health **21**, 229–232.

Wilson, D.C., Rodic, L., Modak, P., Soos, R., Carpintero, A., Velis, K., Iyer, M. and Simonett, O., (2015) Global Waste Management Outlook (Monograph).

von Wintersdorff, C.J.H., Penders, J., van Niekerk, J.M., Mills, N.D., Majumder, S., van Alphen, L.B., Savelkoul, P.H.M. and Wolffs, P.F.G., (2016) Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. Front. Microbiol. **7**.

Wistrand-Yuen, E., Knopp, M., Hjort, K., Koskiniemi, S., Berg, O.G. and Andersson, D.I., (2018) Evolution of high-level resistance during low-level antibiotic exposure. Nat. Commun. **9**, 1599.

Wooley, J.C., Godzik, A. and Friedberg, I., (2010) A Primer on Metagenomics. PLOS Comput. Biol. **6**, e1000667.

World Health Organization (Ed.), (2014) Antimicrobial resistance: global report on surveillance. World Health Organization, Geneva, Switzerland.

World Health Organization, (2017) WHO guidelines on use of medically important antimicrobials in food-producing animals.

Wright, G.D., (2010) Antibiotic resistance in the environment: a link to the clinic? Curr. Opin. Microbiol., Antimicrobials/Genomics **13**, 589–594.

Yamashita, M. and Fenn, J.B., (1984) Electrospray ion source. Another variation on the free-jet theme. J. Phys. Chem. **88**, 4451–4459.

Yang, Y., Li, B., Zou, S., Fang, H.H.P. and Zhang, T., (2014) Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. Water Res. **62**, 97–106.

Yebra, D.M., Kiil, S. and Dam-Johansen, K., (2004) Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. Prog. Org. Coat. **50**, 75–104.

Yeganeh, M.M., Kaghazchi, T. and Soleimani, M., (2006) Effect of Raw Materials on Properties of Activated Carbons. Chem. Eng. Technol. **29**, 1247–1251.

Yost, R.A. and Enke, C.G., (1978) Selected ion fragmentation with a tandem quadrupole mass spectrometer. J. Am. Chem. Soc. **100**, 2274–2275.

Zhang, C., Cui, F., Zeng, G., Jiang, M., Yang, Z., Yu, Z., Zhu, M. and Shen, L., (2015) Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. Sci. Total Environ. **518–519**, 352–362.

Zhang, Q., Hu, J., Lee, D.-J., Chang, Y. and Lee, Y.-J., (2017) Sludge treatment: Current research trends. Bioresour. Technol. **243**, 1159–1172.

Zhang, Y., Gu, A.Z., Cen, T., Li, X., He, M., Li, D. and Chen, J., (2018) Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. Environ. Pollut. **237**, 74–82.

Zuloaga, O., Navarro, P., Bizkarguenaga, E., Iparraguirre, A., Vallejo, A., Olivares, M. and Prieto, A., (2012) Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review. Anal. Chim. Acta **736**, 7–29.

Zwiener, C. and Frimmel, F.H., (2000) Oxidative treatment of pharmaceuticals in water. Water Res. **34**, 1881–1885.