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OCCURRENCE OF PHARMACEUTICALS IN MUNICIPAL WASTEWATER TREATMENT PLANTS AND RECEIVING SURFACE WATERS IN CENTRAL AND SOUTHERN FINLAND

BY

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Academic Dissertation for the Degree of
Doctor of Philosophy

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ABSTRACT

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Occurrence of pharmaceuticals in municipal wastewater treatment plants and receiving surface waters in central and southern Finland

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The presence of five selected pharmaceuticals, four anti-inflammatory drugs, diclofenac, ibuprofen, ketoprofen, naproxen, and an antiepileptic drug carbamazepine, was determined at four municipal wastewater treatment plants (WWTPs) and in the receiving waterway near the city of Jyväskylä, in central Finland and also in the River Vantaa. First, an analytical method was developed including a pretreatment and purification followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in the multiple reaction monitoring mode.

The studied pharmaceuticals were detected from influents and effluents of four municipal WWTPs and from surface water of eight locations along the water way and northern Lake Päijänne. In addition, samples of sedimented particles were collected among the water samples from five locations near the discharge point of the effluent. Furthermore, the studied compounds were sampled from the surface water of northern Lake Päijänne and the River Vantaa both by grab sampling and passive sampling. Passive sampling was performed by using the Chemcatcher® sampler with a SDB-RPS Empore disk as a receiving phase. In Lake Päijänne, the passive sampling was conducted at four locations near the discharge point of a wastewater treatment plant and at four locations along the River Vantaa.

The concentrations in the influents and effluents ranged from hundreds of nanograms per liter to micrograms per liter. In lake water, the concentrations ranged from tens of nanograms per liter in northern parts of the waterway to hundreds of nanograms per liter in northern Lake Päijänne near the city area. The concentrations of ketoprofen in sedimented particles ranged from tens to over one hundred micrograms per gram, while only trace amounts of other selected pharmaceuticals were detected. The results indicate that the concentrations of pharmaceuticals are affected by not only the biological and chemical reactions occurring in the wastewater treatment processes, but also by the UV light in the photic layer of Lake Päijänne.

Seasonal variation in the surface water of Lake Päijänne was studied by comparing the concentrations collected in the winter and in the summer. The concentrations were higher in the winter compared to summer time in surface

water due to decreased temperature and solar irradiation. On the other hand, higher concentrations of ibuprofen, ketoprofen, and naproxen were found in summer at the WWTPs, possibly due to seasonal variations in consumption.

It can be concluded that considerable amounts of selected pharmaceuticals are present in the influent and effluent of municipal WWTPs, in the water and sedimented particles of along the waterway and northern Lake Päijänne, and in the River Vantaa. Additionally, the results suggest that the Chemcatcher passive samplers are suitable for detecting pharmaceuticals both in lake and river waters.

Keywords: liquid chromatography, mass spectrometry, municipal wastewater treatment plant, passive sampling, pharmaceuticals, sedimented particles, solid phase extraction

PREFACE

This research was carried out in the Laboratory of Applied Chemistry at the Department of Chemistry, University of Jyväskylä in cooperation with the Finnish Environment Institute (SYKE), from April 2013 to May 2016.

I wish to express my deepest gratitude to my supervisors, Professor Juha Knuutinen and Research Professor Sirpa Herve, for their guidance and encouragement during this work. My sincere thanks go to Dr. Heidi Ahkola for her valuable comments and help with the laboratory work. I would also like to thank Research Manager Timo Huttula and Development Manager Taina Nystén at the Finnish Environment Institute as well as Professor Tuula Tuhkanen at the Department of Biological and Environmental Science, University of Jyväskylä. Finally, I would like to thank all my coworkers at the Department of Chemistry, especially at the Laboratory of Applied Chemistry, for guidance and creating a pleasant work atmosphere.

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My mother and my late father deserve the warmest thanks for all the support they have given me during my life and studies. Warm thanks go to all my friends, especially to Anne. I would also like to thank my late mother-in-law, Armi, for reminding me that dreams are meant to be pursued before it is too late. Finally, I express my deepest gratitude to my husband and best friend, Risto, for his love and support.

Jyväskylä, May 2016

Petra Lindholm-Lehto

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred in the text by Roman numerals (**I-V**).

I Lindholm, P., Knuutinen, J., Ahkola, H. & Herve, S., Analysis of trace pharmaceuticals and related compounds in municipal wastewaters by preconcentration, chromatography, derivatization, and separation methods, *BioResources*, 9(2014)3688-3732.

II II Lindholm-Lehto, P., Knuutinen, J., Ahkola, H. & Herve, S., Refractory organic pollutants and toxicity in pulp and paper mill wastewaters, *Environmental Science and Pollution Research*, 22(2015)6473-6499.

III Lindholm-Lehto, P., Ahkola, H., Knuutinen, J. & Herve, S., Occurrence of pharmaceuticals in municipal wastewater, in the recipient water, and sedimented particles of northern Lake Päijänne, *Environmental Science and Pollution Research*, 22(2015)17209-17223.

IV Lindholm-Lehto, P., Ahkola, H., Knuutinen, J. & Herve, S., Widespread occurrence and seasonal variation of pharmaceuticals in surface waters and municipal wastewater treatment plants in central Finland, *Environmental Science and Pollution Research*, 23(2016)7985-7997.

V Lindholm-Lehto, P., Ahkola, H., Knuutinen, J., Koistinen, J., Lahti, K., Vahtera, H. & Herve, S., Suitability of passive sampling for the monitoring of pharmaceuticals in Finnish surface waters, *Environmental Science and Pollution Research*, DOI: 10.1007/s11356-016-6778-y.

In the publications, the planning of the experimental work, laboratory analyses, interpreting the results and writing the manuscripts were carried out by the author. The manuscripts were completed with co-authors. In publication III and IV, experimental work was planned with Dr. Heidi Ahkola. In publication V, sample collection was carried out with Dr. Jaana Koistinen.

Other related publications by the author

Petra Lindholm-Lehto, Juha Knuutinen, Sirpa Herve, Heidi Ahkola, Lääkeaineet jäteveden puhdistamolla ja vesistössä, Ympäristö ja Terveys 46(2015)38–43.

Petra Lindholm-Lehto, Heidi Ahkola, Juha Knuutinen, Sirpa Herve, Study of pharmaceuticals in municipal wastewater, in the recipient water and sedimented particles of northern Lake Päijänne, 15th EuCheMS International Conference on Chemistry and the Environment-ICCE, Leipzig, Germany, September, (2015).

Martin A. Hubbe, Jeremy R. Metts, Daphne Hermosilla, M. Angeles Blanco, Laleh Yerushalmi, Fariborz Haghighat, Petra Lindholm-Lehto, Zahra Khodaparast, Mohammadreza Kamali, and Allan Elliott, Wastewater Treatment and Reclamation: A Review of Pulp and Paper Industry Practices and Opportunities, BioResources, in press.

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CONTENTS

ABBREVIATIONS

AA	Acetic acid
ACN	Acetonitrile
AcS	Activated sludge
ACT	Acetone
AF	Assessment factor
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionization
BOD	Biological oxygen demand
CA	Cellulose acetate
CAS	Chemical Abstracts Service
CBZ	Carbamazepine
CFDA-AM	5-Carboxyfluorescein diacetate, acetoxymethyl ester
COD	Chemical oxygen demand
CPE	Cloud point extraction
CZE	Capillary zone electrophoresis
DAD	Diode array detector
DCF	Diclofenac
DDD	Defined daily dose
DGT	Diffusive gradient in thin film
DHE	Dihexyl ether
DLLME	Dispersive liquid-liquid microextraction
DMLS	Diffusive multi-layer sampler
EC50	Effective concentration, 50 %
ESI	Electrospray ionization
ESI-	Negative ESI
ESI+	Positive ESI
GC	Gas chromatography
GPx	Glutathione peroxidase
HAc	Acetic acid
HDPE	High-density polyethylene
Hex	Hexane
HF-LPME	Hollow-fiber liquid phase microextraction
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time
IBP	Ibuprofen
ISO	International Organization for Standardization
K_d	Sorption distribution coefficient
KET	Ketoprofen
K_{ow}	Octanol-water partitioning coefficient
LC	Liquid chromatography
LC50	Lethal concentration, 50 %
LDPE	Low-density polyethylene
LLE	Liquid-liquid extraction

LOD	Level of detection
LOQ	Level of quantification
LPME	Liquid phase microextraction
LPO	Lipoperoxidation
MAE	Microwave-assisted extraction
MEC	Measured environmental concentration
MeOH	Methanol
MESCO	Membrane enclosed sorptive coating
MIP	Molecularly imprinted polymers
MISPE	Molecularly imprinted solid phase extraction
MRM	Multiple reaction monitoring
mRNA	Messenger RNA
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
m/z	Mass-to-charge ratio
N/DeN	Nitrification and denitrification
NH_4OH	Ammonium hydroxide
NIRA	Near infrared analysis
NOEC	No observed effect concentration
NOM	Natural organic matter
NPX	Naproxen
NSAID	Non-steroidal anti-inflammatory drug
o.d.	Oven dry
OECD	Organisation for Economic Co-operation and Development
PAH	Polyaromatic/ polycyclic aromatic hydrocarbon
PC	Polycarbonate
PCB	Polychlorinated biphenyls
PDBS	Passive diffusion bag sampler
PES	Polyethersulfone
PHWE	Pressurized hot water extraction
pK_a	Acid dissociation constant
PLE	Pressurized-liquid extraction
PNEC	Predicted no effect concentration
POCIS	Polar organic chemical integrative sampler
PRC	Performance reference compounds
PS	Polysulfone
PTFE	Polytetrafluoroethylene
QTRAP	Triple quadrupole/linear ion trap mass spectrometer
REACH	Registration, Evaluation, Authorization and Restriction of Chemical Substances
RQ	Risk quotient
R_s	Sampling rate
RSD	Relative standard deviation
SD	Standard deviation

S/N	Signal-to-noise ratio
SDB-RPS	Styrenedivinylbenzene-reversed phase sulfonated copolymer
SDB-XC	Styrenedivinylbenzene-exchange copolymer
SM-LLME	Stir membrane liquid-liquid microextraction
SOD	Superoxide dismutase
SPE	Solid phase extraction
SPMD	Semi-permeable membrane device
SPME	Solid phase microextraction
SRT	Solids retention time
TOC	Total organic carbon
TWA	Time-weighted average
UHQ	Ultra high quality
UPLC	Ultra-Performance Liquid Chromatography (Waters)
UV	Ultraviolet
UV-Vis	Ultraviolet-visible spectroscopy
WWTP	Wastewater treatment plant

1 INTRODUCTION

A wide variety of different pharmaceuticals are used in high quantities to prevent and treat diseases (Kümmerer 2009; Bottoni et al. 2010). Worldwide, the consumption of many categories of pharmaceuticals continues to increase, partly due to the increase of ageing-related and chronic diseases (OECD 2013). In Finland, the consumption of all pharmaceuticals reached 2.7 billion euros in 2014 of which anti-inflammatory drugs constitute 53 million euros per year (Finnish Medicines Agency Fimea 2014). In most countries, non-steroid anti-inflammatory drugs (NSAID) are among the top used pharmaceuticals (Gómez-Oliván et al. 2009). Worldwide, 30 million persons use NSAIDs on a daily basis (Salas et al. 2007). They are easily accessible and often sold over-the-counter (Bori Segura et al. 2009).

Thousands of tons of pharmaceuticals are used every year in human and veterinary medicine and released into the environment through metabolic excretion and improper treatment techniques (Bends et al. 2005; Castiglioni et al. 2006). Often, a great part of administered pharmaceutical is excreted unchanged or as an active metabolite entering wastewater treatment systems (Carlsson et al. 2006a). However, current wastewater treatment processes are unable to remove pharmaceuticals effectively (Gros et al. 2010; Ottmar et al. 2010). In addition to municipal wastewaters, hospitals are another main source of pharmaceuticals releasing into the environment. Hospital wastewaters have been estimated between 5 to 15 times more toxic than typical municipal wastewater effluents (Emmanuel et al. 2009). Other anthropogenic sources of pharmaceuticals are improper disposal of unused drugs, manufacture spill accidents, leakages from, *e.g.*, septic tanks, landfills, and via direct disposal into water bodies (Derksen et al. 2004; Carrara et al. 2008; Mompelat et al. 2009).

Pharmaceuticals are constantly released into the environment and surface waters via wastewater treatment systems (Loos et al. 2009; Ottmar et al. 2010). Even though most pharmaceuticals are not intrinsically persistent, they can be considered pseudo-persistent due to their constant release into the environment (Radke et al. 2010) and therefore considered potentially hazardous for aquatic organisms and the health of the ecosystem (Neto and Ferreira 2007; Al Aikido et al. 2012). Pharmaceuticals have been detected in ecosystems *e.g.*, in soil, surface water and groundwater (Santos et al. 2010) in a number of water bodies (Neto and Ferreira 2007; Al Aikido et al. 2012) and, in remote areas (Fent et al. 2006; Loos et al. 2008). The study of pharmaceuticals in the environment is a relatively recent issue and even more so is the detection and assessment of their metabolites (de García et al. 2013). However, metabolites should be included in the risk assessment studies to receive reliable information of their occurrence.

Even though pharmaceuticals are detected in the environment at concentrations from low nanograms per liter to micrograms per liter, they can have sub-lethal or chronic toxic effects to non-target organisms (Kraigher et al. 2008; Kümmerer

2009; WHO 2011; Kaplan 2013). For example, synthetic hormones and antibiotics have been found to cause adverse effects on aquatic organisms (Kristiansson et al. 2011; Lee et al. 2012). The potential threat to aquatic organisms originates from the ecotoxicity and bioaccumulation properties of pharmaceuticals (Fent et al. 2006; Kasprzyk-Hordern et al. 2008; Salem et al. 2012). Additionally, pharmaceuticals can accumulate and cause adverse effects in wildlife and humans (Daughton and Ternes 1999). Most pharmaceuticals occur in water due to their hydrophilic properties or interact with suspended organic material by the polar functional groups (carboxylic moieties, amines, aldehydes) and end up in sewage sludge (Ternes et al. 2004; Chen et al. 2013).

Pharmaceuticals and their metabolites are continuously released into aquatic environments as complex mixtures (Daughton and Ternes 1999; Carlsson et al. 2006a). Hundreds of pharmaceuticals and their metabolites have been detected in aquatic environments worldwide (*e.g.*, Ternes et al. 2001; Heberer 2002; Gros et al. 2010). Even though widely studied, the adverse effects of many pharmaceuticals and their metabolites still remain unknown. Moreover, synergistic effects of mixtures can be observed, which can pose a much more severe threat to the environment.

Pharmaceuticals have been classified as emerging pollutants because they are released into the environment in large amounts (Jones et al. 2005). Increasingly restrictive regulations in Europe, including the European Regulation for Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) consider the problem of chemicals being released into the environment (Dévier et al. 2011). However, there are approximately 16 million known compounds in Chemical Abstracts Service (CAS), and only a minor portion is regularly monitored compared to those potentially present in the environment (Brack et al. 2005; de García et al. 2013). Additionally, the debate about the presence of pharmaceuticals and their effects in the environment is relatively young. There is still a lack of good understanding and consensus on which substances should be regulated and at what concentrations. Even though pharmaceuticals have been considered as emerging pollutants for years, their discharge and environmental levels still remain legally uncontrolled, and no limit concentrations are being set (Daughton and Ternes 1999; Rivera-Utrilla et al. 2013).

In August 2013, diclofenac, 17-β-estradiol and 17-α-ethinylestradiol were added to the European Commission's watch list of priority substances (Vieno 2014). In 2014, another seven substances or groups of substances were proposed and added to the watch list, among them the antibiotics erythromycin and clarithromycin (Carvalho et al. 2014). Additionally, carbamazepine and ibuprofen have been suggested as additions to the chemical watch list (Vieno 2014). In Finland, the directive on priority substances (2013/39/EU) has been implemented into the national legislation with the government decree on substances dangerous and harmful to the aquatic environment (1022/2006 and 868/2010). In the future, wastewater treatment processes need to be modified to meet the requirements of the near future.

2 OBJECTIVES OF THE STUDY

The objective of this work was to study the occurrence of selected pharmaceuticals in Finnish municipal wastewater treatment plants (WWTP) and in surface waters in central and southern Finland. Lake Päijänne is one of the most important fresh water sources in Finland and is used for production of drinking water for the capital city area of Helsinki, comprising about 1 million inhabitants. In addition, the River Vantaa is used as a backup fresh water source for the capital area. Therefore, the monitoring of the water quality and its trace contaminants is of utmost importance. This objective included the following aspects.

- The analytical methods and trace contaminants both in municipal and industrial wastewaters were reviewed (Papers I and II). Based on these, the analytical method for the quantitative analysis was developed;
- Development of analytical methods for quantitative analysis of selected pharmaceuticals in wastewaters, surface waters and sedimented particles at low ng L^{-1} levels (Paper III);
- Development of an analytical method for determining the concentrations of selected pharmaceuticals by Chemcatcher® passive sampling (Paper V);
- Study of the occurrence of pharmaceuticals in influents and effluents at municipal WWTPs in central Finland, in Lake Päijänne at different depths, locations and along the waterway leading to Lake Päijänne (Papers III-IV);
- Study of the seasonal variation of pharmaceuticals at the selected WWTPs and in Lake Päijänne (Paper IV);
- Study the occurrence of pharmaceuticals in the River Vantaa leading to the Gulf of Bothnia and in Lake Päijänne by grab and passive sampling (Paper V).

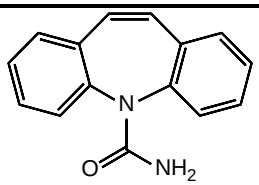
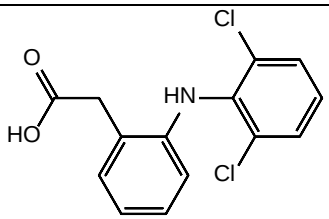
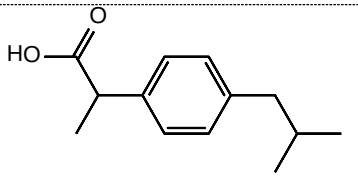
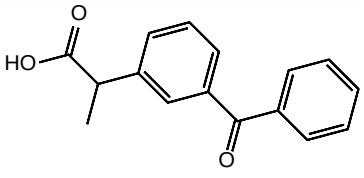
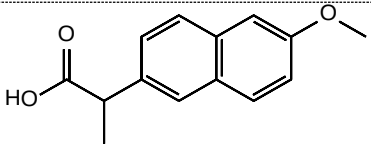
3 LITERATURE REVIEW

3.1 Properties of the selected pharmaceuticals

In Europe, NSAIDs are among the most frequently detected pharmaceuticals in aquatic environments and drinking water (*e.g.*, Heberer 2002; Santos et al. 2010; Grenni et al. 2013). NSAIDs are a heterogeneous group of pharmaceuticals, including diclofenac, ibuprofen, ketoprofen and naproxen, all of which are widely used due to their analgesic, antipyretic (lowering elevated body temperatures without impairing consciousness), and anti-inflammatory properties (Gómez-Oliván et al. 2009; Islas-Flores et al. 2013, Table 1).

Carbamazepine is one of the most commonly prescribed antiepileptic drugs. It has also anticonvulsant and mood stabilizing properties and can be used to treat epileptic seizures and bipolar disorders (Beutler et al. 2005; Lee et al. 2007).

TABLE 1 Names and structures of the selected pharmaceuticals

Therapeutic use	**Pharmaceutical**	**CAS-number**	**Chemical structure**	**MW (g mol^{-1})**	**IUPAC name**
anti-epileptic	Carbamazepine (CBZ)	298-46-4		236.26	Benzo[b][1] benzazepine-11-carbox amide
anti-inflammatory	Diclofenac (DCF)	15307-86-5		296.14	2-[2-(2,6-Dichloroanilino) phenyl] acetic acid
	Ibuprofen (IBP)	15687-27-1		206.28	2-[4-(2-methylpropyl)phenyl] propanoic acid
	Ketoprofen (KET)	22071-15-4		254.28	2-(3-benzoyl phenyl) propanoic acid
	Naproxen (NPX)	22204-53-1		230.26	2-(6-methoxy naphthalen-2-yl) propanoic acid

Most pharmaceuticals have a molecular mass <500 Da and are designed to be biologically active at low concentrations (Lipinski et al. 1997; Grenni et al. 2013). They are polar molecules with one or more ionizable group, and the degree of ionization and its properties depend on the pH of the medium. Additionally, they are typically soluble in water (Table 2). The polar nature of most pharmaceuticals makes them directly bioavailable to filter feeding organisms, such as bivalves (McEneff et al. 2014).

TABLE 2 Physical properties of the selected pharmaceuticals

Pharmaceutical	**Vapor pressure** (mm Hg)	**Solubility in water** (mg L^{-1})	**pKa** (20 °C)	**log K_{ow}**	**Henry's constant** (at m^3 m^{-1})
Carbamazepine	$1.8x10^{-7}$ [a]	17.7 [a, b]	14.0 [b]	2.45 [a, c]	$1.1 \cdot 10^{-10}$ [a]
Diclofenac	6.14×10^{-8} [b]	2.37 [b]	4.1–4.5 [b, d]	1.9–4.5 [b, e]	$4.7 \cdot 10^{-12}$ [e]
Ibuprofen	1.86×10^{-4} [b]	21 [b]	3.5–4.9 [d, f]	2.5–4.0 [b, g]	$1.5 \cdot 10^{-7}$ [e]
Ketoprofen	1.46×10^{-6} [f]	51 [h]	4.5 [i, j]	3.1 [i, j]	$2.1 \cdot 10^{-11}$ [e]
Naproxen	1.27×10^{-6} [f]	144 [h]	4.2–4.5 [f, i]	3.2–3.3 [d, i]	$3.4 \cdot 10^{-10}$ [e]

[a] Meylan and Howard (1991) [b] Scheytt et al. (2005) [c] Dal Pozzo et al. (1989) [d] Huber et al. (2005) [e] Tixier et al. (2003) [f] Lindqvist et al. 2005 [g] Cleuvers (2004) [h] US EPA (2009) [i] Vieno et al. 2007 [j] Kim and Tanaka (2009)

In Finland, ibuprofen is the most used anti-inflammatory drug (Finnish Medicines Agency Fimea 2014). A classification of the defined daily doses (DDD) and consumption of the selected pharmaceuticals in different countries is listed in Table 3. The DDDs is the unit in grams for the daily dose of a drug. However, the DDD value is only a technical unit and not necessarily equal to the real dose (Finnish Medicines Agency Fimea 2014). The consumption of pharmaceuticals is one of the most important factors explaining the concentration profile in wastewater.

TABLE 3 Consumption of selected pharmaceuticals in different countries

Pharmaceutical	**Consumption** [t year^{-1}]	**Country**	**References**
Carbamazepine DDD 1g	3.4	Finland[a] 2014	Finnish Medicines Agency Fimea 2014
Diclofenac DDD 0.1g	4.4	Australia[b] 1998	Tambosi et al. 2010
	26	England[c] 2000	Jones et al. 2002
	0.9	Finland[a] 2014	Finnish Medicines Agency Fimea 2014
	86	Germany[d] 2001	Nikolaou et al. 2007
	4.5	Switzerland[e] 2004	Fent et al. 2006
Ibuprofen DDD 1.2g	14.2	Australia[b] 1998	Tambosi et al. 2010
	162	England[c] 2000	Jones et al. 2002
	119	Finland[a] 2014	Finnish Medicines Agency Fimea 2014
	345	Germany[d] 2001	Nikolaou et al. 2007
	25	Switzerland 2004	Fent et al. 2006
Ketoprofen DDD 0.15g	0.38	Finland[a] 2014	Finnish Medicines Agency Fimea 2014
	0.25	Switzerland[e] 2004	Fent et al. 2006
Naproxen DDD 0.5g	22.8	Australia[b] 1998	Tambosi et al. 2010
	35	England[c] 2000	Jones et al. 2002
	6.1	Finland[a] 2014	Finnish Medicines Agency Fimea 2014

Population [a] 5,47 $\cdot 10^6$ in Finland, 2014; [b] 18.7 $\cdot 10^6$ in Australia 1998;
[c] 49.1 $\cdot 10^6$ in UK 2000; [d] 82.3 $\cdot 10^6$ in Germany 2001; [e] 7.3 $\cdot 10^6$ in Switzerland 2004
DDD defined daily dose

3.2 Metabolites and transformation products

In the human body, pharmaceuticals transform to one or several metabolites which are excreted as a mixture of parent compounds and metabolites (Pérez and Barceló 2007). The degree of metabolism depends on several parameters, such as age and gender of the patient and enzyme induction or inhibition caused by interactions between different drugs (Lemmer 1996). The pharmaceuticals and their metabolites then enter the environment. Studies regarding the occurrence of pharmaceuticals are often made by detecting the parent compounds. However, the absence of pharmaceutical does not necessarily imply their complete removal but rather their transformation to metabolites and other transformation products.

In the human body, the liver is the main site of metabolism (Pérez and Barceló 2007). Typically, this leads to the loss of pharmacological activity and an increase in hydrophilicity and elimination. The metabolism in humans is divided into two types of reactions. The first type includes hydrolytic cleavages, oxidations, reductions, alkylations, and dealkylations while the other type refers to conjugated reactions in which a polar group or molecule forms a conjugate with the parent drug or metabolite (Tables 4 and 5). Typically, this leads to sulfation, N-acetylation, or amino-acid conjugation, with glucuronidation being the most common. The conjugates are excreted via urine or bile in the form of more polar and hydrophilic derivatives, as a metabolite or as a mixture of multiple metabolites (Silverman and Hoffman 1984; Heberer 2002; Jones et al. 2005; Rivera-Utrilla et al. 2013).

TABLE 4 Percentage (%) of selected pharmaceuticals excreted as parent compound after metabolism in mammalian body

Pharmaceutical	Excreted unchanged (%)	References
Carbamazepine	1-2	Ternes 1998; Jones et al. 2002
Diclofenac	2-15	Ternes 1998; Khan and Ongerth 2004
Ibuprofen	1-10	Ternes 1998; Khan and Ongerth 2004
Ketoprofen	10	Khan and Ongerth 2004
Naproxen	1-10	Khan and Ongerth 2004

Even though the stable properties of pharmaceutical compounds are desirable in medicine, at WWTPs they lead to minimal degradation of these chemicals (Jakimska et al. 2014). The removal of pharmaceuticals during municipal wastewater treatment has been found to be incomplete (*e.g.*, Ternes 1998; Quintana and Reemtsma 2004), and residues of these pharmaceuticals have been found in surface waters in ng L^{-1} to µg L^{-1} concentrations (Quintana et al. 2005). Treatment technologies, such as ozonation, are investigated for the removal of pharmaceuticals and their residues (Ternes et al. 2003; Huber et al. 2003). The most important process for the removal of acidic pharmaceuticals during

wastewater treatment is microbial degradation (Quintana et al. 2005). However, the degradation pathways and products are still largely unidentified even though some metabolites have been recognized (Zwiener et al. 2002; Miao and Metcalfe 2003; Illés et al. 2013).

In aquatic environments, organic compounds undergo a series of transformation reactions (Jakimska et al. 2014). Some compounds can be less prone to degrade and, depending on their physicochemical properties (*i.e.*, hydrophobicity, lipophilicity) are able to bind to solid material, such as sludge. The sorption effect hinders the elimination of the compound during WWTP processes. Additionally, the compound can be transformed into more lipophilic derivatives than the primary compound. A stable and polar compound is not degraded in WWTP processes and ends up in aquatic environments (Halling-Sørensen et al. 1998).

TABLE 5 Human metabolites of selected pharmaceuticals detected in biological samples and in the environment

Pharmaceutical	Human metabolites	Human metabolites in the environment	References
Carbamazepine	•10,11-Dihydro-10,11-epoxycarbamazepine •10,11-Dihydro-10,11-dihydroxycarbamazepine •10,11-Dihydro-10-hydroxycarbamazepine •2-Hydroxycarbamazepine •3-Hydroxycarbamazepine •10-Hydroxy-carbamazepine and its conjugates	•10,11-Dihydro-10,11-epoxycarbamazepine •10,11-Dihydro-10,11-dihydroxycarbamazepine •2-Hydroxycarbamazepine •3-Hydroxycarbamazepine •10-Hydroxy-carbamazepine	Miao and Metcalfe 2003; Valentine et al. 1996; Lertratanangkoon and Horning 1982; Kitteringham et al. 1996
Diclofenac	•4´-Hydroxydiclofenac •5-Hydroxydiclofenac •3´-Hydroxydiclofenac •4´,5-Dihydroxydiclofenac •3´-Hydroxy-4´-methoxydiclofenac •Conjugates of diclofenac and its metabolites	Not reported	Wiesenberg-Boettcher et al. 1991
Ibuprofen	•Hydroxy-ibuprofen •Carboxy-ibuprofen •Carboxy-hydratropic acid •Conjugated ibuprofen	•Hydroxy-ibuprofen •Carboxy-ibuprofen	Kepp et al. 1997; von Bruchhausen et al. 1994; Mills et al. 1973; Ternes et al. 1998; Stumpf et al. 1998
Ketoprofen	•Glucuronide	Not reported	Foster et al. 1988
Naproxen	•*O*-Desmethyl-naproxen	Not reported	Sidelmann et al. 2001

In the environment, degradation can occur by hydrolysis, photodegradation or biodegradation depending on the environmental conditions and structures of compounds (Jakimska et al. 2014). The degraded products can exhibit pharmacological effects and can be more stable than their parent compounds (Halling-Sørensen et al. 2002). Pharmaceuticals, such as carbamazepine, diclofenac, naproxen, ibuprofen and ketoprofen, are susceptible to photodegradation in water, including surface and wastewater (Lin and Reinhardt 2005; Salgado et al. 2013). Phototransformation is an important process in the degradation of pharmaceuticals and includes both direct photolysis and indirect photolysis in which the reactions proceed via radicals, such as ·OH and ROO·, generated by photosensitizers (Andreozzi et al. 2003; Alapi and Dombi 2007). In constant UV irradiation, the degradation reactions are typically first order.

In surface waters, UV light induced degradation reactions can precede only in the photic layer of a lake because the influence of the solar radiation rapidly decreases with increasing depth (Bartels and von Tümpling 2007). Based on the molecular structure of the compounds, photolysis leading to transformation reactions is connected to organic matter and NO_3^- ion because their absorption of light can provoke or diminish photolysis (Deviér et al. 2011; Zhang et al. 2011). For example, humic acids act as inner filters decreasing the decomposition reactions. Therefore, the content of humic substances and the depth of photic layer of different surface waters have a strong decreasing effect on the decomposition reactions (Huovinen et al. 2003). On the other hand, humic acids can react with oxygen forming reactive species of singlet oxygen or react directly with other organic substances promoting their phototransformation (Andreozzi et al. 2003). Typically, the concentrations are lower in the summer time with more intense UV irradiation compared to autumn and winter in boreal areas (Buser et al. 1998; Vieno et al. 2005).

For example, the photodegradation pathways of ibuprofen and ketoprofen include five degradation products of ibuprofen and seven of ketoprofen (Figs. 1-2). Some of them have been detected both in wastewater and surface water. Ketoprofen undergoes photodegradation reactions easier and at a higher reaction rate than ibuprofen. In the case of ketoprofen, the first degradation product, decarboxylated ketoprofen is formed in 10 s while the transformation products of ibuprofen are formed slower by hydrolysis or biodegradation (Borsarelli et al. 2000; Musa et al. 2007).

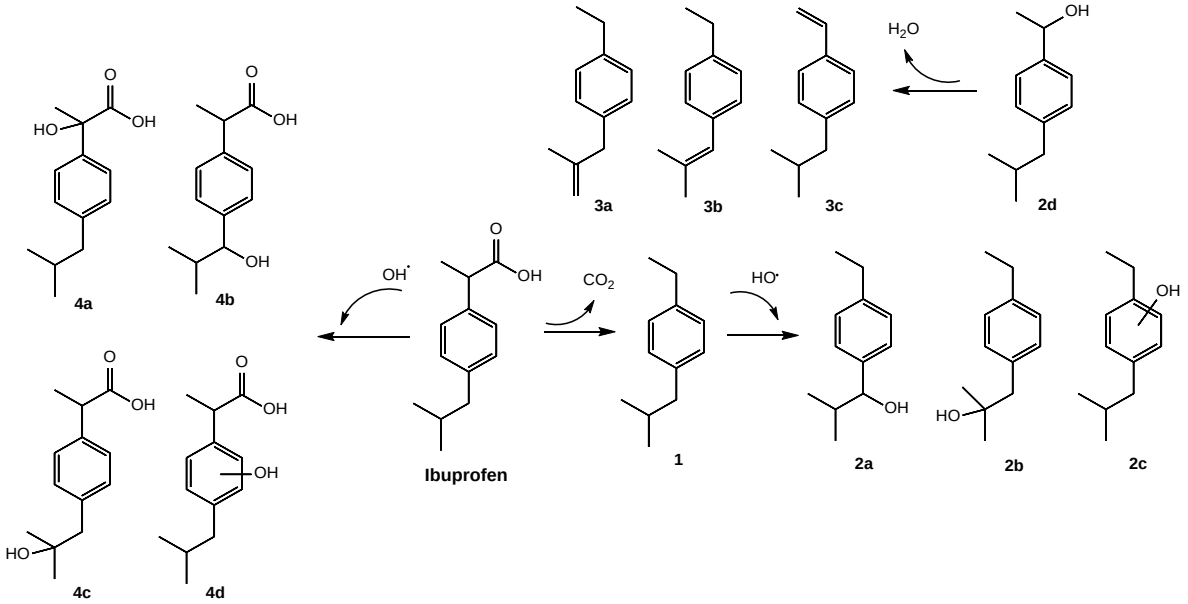


FIGURE 1 Suggested main phototransformation pathways of ibuprofen (Jakimska et al. 2014)

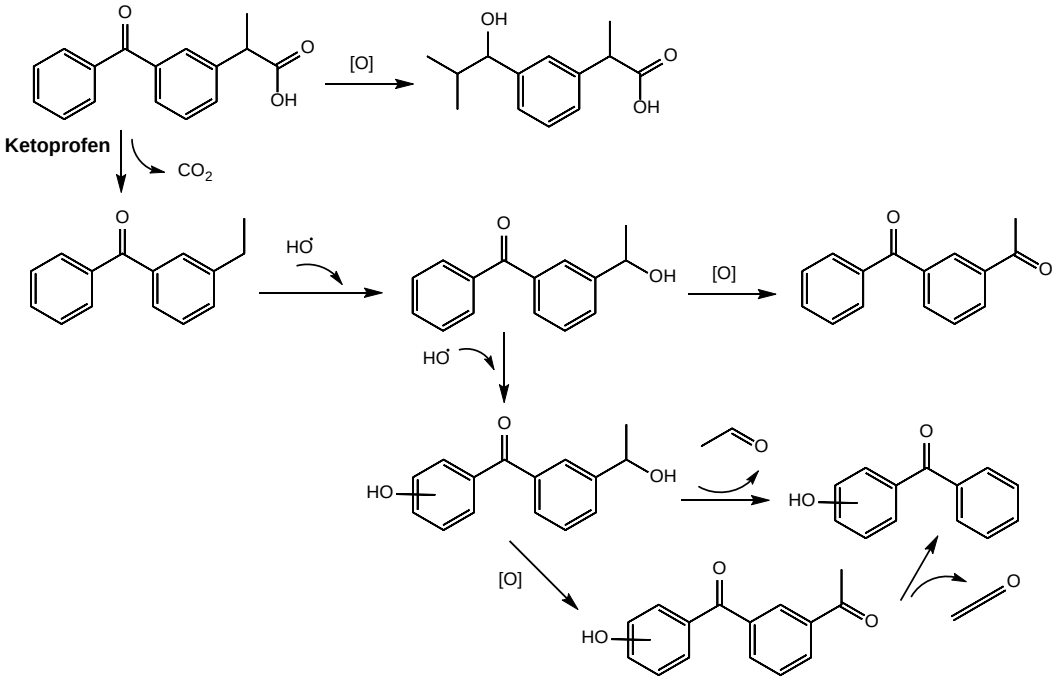


FIGURE 2 Suggested main phototransformation pathways of ketoprofen (Jakimska et al. 2014)

Naproxen is one of the most commonly used pharmaceuticals but only a few of its phototransformation products are known (DellaGreca et al. 2003; Aydin 2015). For example, the two main transformation products of naproxen (1) are benzyl succinic acid (2) and caffeic acid (3, Fig. 3). In surface water, reactions of naproxen are accelerated in the presence of nitrate and decreased in the pres-

ence of humic acids, including both naproxen degradation and transformation product formation (Aydin 2015).

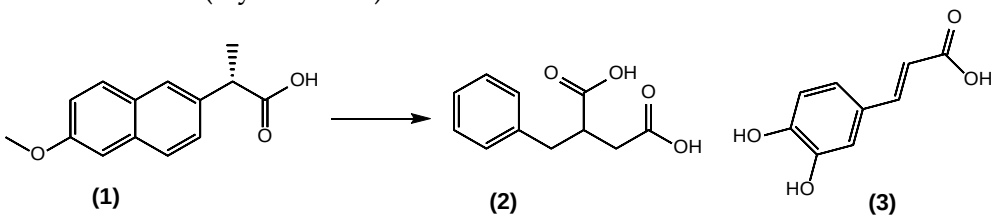


FIGURE 3 Naproxen (1) and its phototransformation products benzyl succinic acid (2) and caffeic acid (3) (Aydin 2015)

In wastewater treatment, naproxen degrades relatively slowly. For example, Quintana et al. (2005) reported a 60 % transformation within 28 days of biodegradation time (Fig. 4). They detected a metabolite *O*-desmethyl naproxen (2) after the cleavage of ether bond which is a known metabolite for mammalian metabolism and transformation by fungi (Sidelmann et al. 2001; He and Rosazza 2003). *O*-desmethyl naproxen is an unstable by-product and leads to mineralization after substantial reaction time. This suggests that the removal of naproxen in municipal wastewater treatment is a kinetic problem (Quintana et al. 2005).

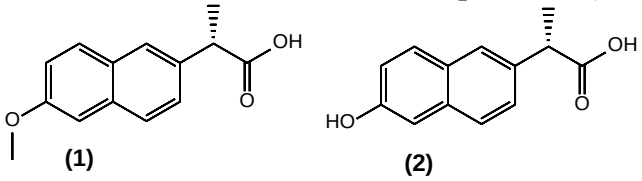


FIGURE 4 Naproxen (1) and its phototransformation product *O*-desmethyl naproxen (2) (Quintana et al. 2005)

Diclofenac is a polar compound that is relatively persistent in aquatic environments (Bartels and von Tümpling 2007). However, it shows only minimal adsorption on suspended matter and sediments. It has been shown to decompose under natural sunlight (*e.g.*, Buser et al. 1998; Packer et al. 2003). During photolysis of diclofenac, several photochemical transformation products are formed (Fig. 5). For example, hydration product 1-(2,6-dichlorophenyl) indolin-2-one (1), 2-(2-chloro-phenyl-amino)benzaldehyde (2), 2,6-dichloro-N-o-tolylbenzamine (3), 9H-carbazole-1-carbaldehyde (7), 2-(2,6-dichloro-phenylamino)-benzaldehyde (8), and 8-chloro-9H-carbazole-1-carbaldehyde (9) have been identified (Moore et al. 1990; Agüera et al. 2005; Bartels and von Tümpling 2007). Additionally, the trace photolysis products 2-chloroaniline (4), 2,6-dichloroaniline (5), and 2,6-dichlorophenol (6) have been detected (Bartels and von Tümpling 2007).

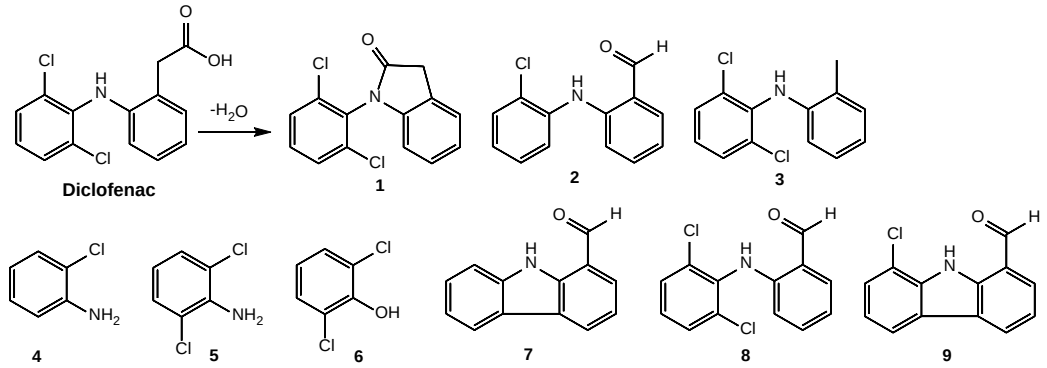


FIGURE 5 Diclofenac and some of its phototransformation products (Agüera et al. 2005; Bartels and von Tümpling 2007)

Carbamazepine is readily metabolized, and only 1-2 % of carbamazepine has been reported to be excreted unchanged (Ternes 1998; Jones et al. 2002). However, the parent compound has been found to be one of the most persistent pharmaceuticals in WWTPs and surface waters (Clara et al. 2004; Vieno et al. 2006a).

Carbamazepine has a tendency to form acridine products in environmental conditions (Fig. 6). Humic acids and nitrate, which are commonly present in aquatic environments, especially in the Nordic countries, act by enhancing decay of carbamazepine, most likely due to generation of reactive oxygen species (Hoigné 1998). The degradation of carbamazepine is believed to proceed via hydroxylation of carbamazepine at the 10 position giving a radical intermediate and later a 10,11-epoxycarbamazepine (1). The opening of the epoxide ring yields a labile species, leading to 9-acridine-9-carboxaldehyde (2) which can eventually decompose to acridine (3) (Furst and Uetrecht 1993). Alternatively, oxidation of carbamazepine by hydroxyl radicals can lead to an attack on the aromatic ring and hydroxylated derivatives (1- and 2-hydroxyacridine, 4) which are prone to breakdown via catechol (5), salicylic acid (6), and anthranilic acid (7) intermediates. Acridine belongs to azarenes, which are known pollutants in air and water with mutagenic and carcinogenic activity (Furst and Uetrecht 1993).

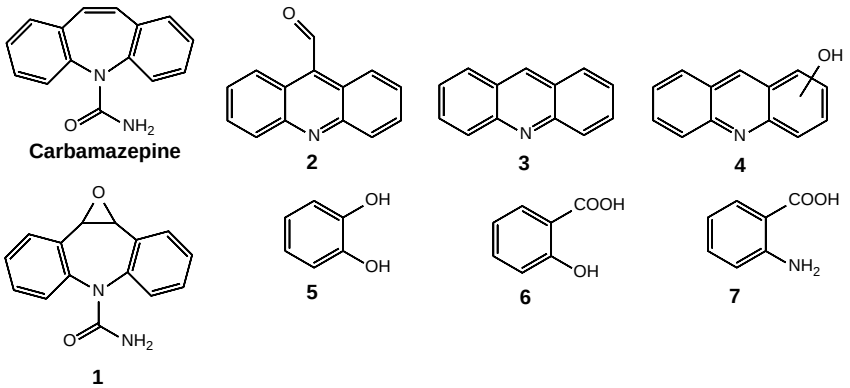


FIGURE 6 Carbamazepine and some of its UV light induced degradation products (Vogna et al. 2004)

3.3 Occurrence of pharmaceuticals

3.3.1 Wastewater influents and effluents

Typically, human and veterinary drugs are released into the environment via excreta (Petrović et al. 2005). Drugs consumed by humans enter the environment via effluents from WWTPs, while veterinary medicines, *e.g.*, via fish feed and agricultural soil leaching (Heberer 2002). Other anthropogenic sources of pharmaceuticals are manufacture spill accidents, improper disposal of unused drugs and leakages from, *e.g.*, landfills and septic tanks (Derksen et al. 2004; Carrara et al. 2008; Mompelat et al. 2009). Pharmaceuticals are excreted into the sewage system as a mixture of parent compounds and metabolites, such as transformation products and conjugated glucuronides (Heberer 2002).

The main purpose of WWTPs is to remove solids, dissolved organic matter and nutrients from wastewater (Vieno et al. 2007). Conventional wastewater systems with activated sludge process are still widely employed because they produce effluents that meet required quality standards at reasonable operating and maintenance costs (Jelić et al. 2011). However, they are not designed to remove pharmaceuticals and do not eliminate them efficiently (Carballa et al. 2004; Gros et al. 2010). Many pharmaceuticals are poorly eliminated in WWTP processes due to their hydrophilicity and persistent nature against biodegradation and their ability to be transformed into recalcitrant metabolites (Petrović et al. 2003; Dobor et al. 2012). However, ibuprofen is one of the most efficiently eliminated pharmaceuticals (60–100 %) in WWTP processes (Tauxe-Wuersch et al. 2005). Additionally, its conjugates are easily cleaved, releasing the parent compound into the wastewater and later into the environment (Ternes 1998; Jelić et al. 2011).

There is a wide variety of pharmaceuticals entering the WWTPs, including *e.g.*, antibiotics, anti-inflammatories, lipid regulators, and antiepileptic drugs (Santos et al. 2005). Numerous studies have reported the occurrence and levels of pharmaceuticals all over the world (McEneff et al. 2014). Over 80 pharmaceuticals and their metabolites have been detected in the range ng L^{-1} to low µg L^{-1} concentrations in municipal sewage effluent, surface water and groundwater worldwide (*e.g.*, Ternes 1998; Roberts and Thomas 2006; Fatta-Kassinos et al. 2011; Lapworth et al. 2012). Examples of detected concentrations of the selected pharmaceuticals in wastewater influents and effluents in different countries are listed in Table 6.

Elimination of pharmaceuticals at WWTPs is based on biodegradation by microbes in the biological treatment and adsorption to solid particles. Lipophilic substances are more likely adsorbed onto solid particles, while hydrophilic substances tend to remain in the aqueous phase (Ternes et al. 2003; Larsen et al. 2004). Many pharmaceuticals are relatively hydrophilic and their sorption to sludge is limited (Joss et al. 2006).

TABLE 6 Detected concentrations of selected pharmaceuticals in influents and effluents of municipal WWTP

Pharmaceutical	Location	Influent μg L^{-1}	Effluent μg L^{-1}	References
Carbamazepine	France	-	0.98–1.2	Andreozzi et al. 2003
	Germany	-	2.1–6.3	Ternes 1998
	Italy	-	0.3-0.5	Andreozzi et al. 2003
	South Korea	0.095-21.6	0.21–21.0	Sim et al. 2011
	Spain	0.046-0.095	0.01-0.16	Gros et al. 2012
	UK	0.71–2.93	0.64–4.60	Kasprzyk-Hordern et al. 2009
Diclofenac	Canada	0.14–1.01	0.14–0.75	Lishman et al. 2006
	South Korea	0.09-0.52	0.05-1.76	Sim et al. 2011
	Spain	0.40-0.44	0.03-0.38	Gros et al. 2012
	Sweden	0.19-0.54	0.22-0.23	Remberger et al. 2009
	Taiwan	0.15–0.19	0.10–0.13	Fang et al. 2012
	UK	0.03–0.26	0.03–0.14	Kasprzyk-Hordern et al. 2009
Ibuprofen	Canada	8.45–16.5	0.35–0.77	Lishman et al. 2006
		38.7–75.8	4.0–24.6	Metcalfe et al. 2003
	Japan	0.38–1.13	0.01–0.18	Nakada et al. 2006
	Spain	61.0–83.5	6.3–6.5	Santos et al. 2005
		3.81–9.48	nd	Gros et al. 2012
	Sweden	2.6–8.0	3.20–3.50	Remberger et al. 2009
	Taiwan	0.72–2.20	0.55–1.60	Fang et al. 2012
	UK	0.97–3.0	0.13–0.42	Kasprzyk-Hordern et al. 2009
Ketoprofen	Canada	0.14–0.29	0.11–0.21	Lishman et al. 2006
	Japan	0.11–0.37	0.07–0.22	Nakada et al. 2006
	Spain	nd-1.10	nd-0.98	Santos et al. 2005
		0.41–1.13	0.03-0.56	Gros et al. 2012
	Sweden	0.49–1.40	0.53–0.57	Remberger et al. 2009
	Taiwan	0.13–0.18	0.07–0.13	Fang et al. 2012
	UK	nd–0.12	nd–0.03	Kasprzyk-Hordern et al. 2009
Naproxen	Italy	-	0.29–5.22	Andreozzi et al. 2003
	South Korea	0.48–12.5	0.02-0.74	Sim et al. 2011
	Spain	4.7–6.0	1.5–2.6	Santos et al. 2005
		4.24–5.46	0.04-0.15	Gros et al. 2012
	Sweden	2.3–7.3	3.2–3.4	Remberger et al. 2009
	UK	0.40–1.46	0.23–0.70	Kasprzyk-Hordern et al. 2009

nd not detected
- not analyzed

Factors other than those of the compound itself affect the elimination rate of the pharmaceutical in wastewater treatment, such as the type of treatment process (Kanda et al. 2003; Joss et al. 2006), dilution by, *e.g.*, rain water (Ternes 1998; Tauxe-Wuersch et al. 2005; Joss et al. 2006), temperature (Vieno et al. 2005; Castiglioni et al. 2006), hydraulic retention time (HRT) (Tauxe-Wuersch et al. 2005), and solids retention time (SRT) (Clara et al. 2005). Therefore, elimination of pharmaceuticals can vary significantly from plant to plant. It has been suggested that high elimination of biodegradable pharmaceuticals occur in WWTPs with high levels of nitrogen removal and high SRT (Clara et al. 2005), but other studies do not support this (Vieno et al. 2007). Examples of removal rates of the selected pharmaceuticals in different countries and WWTPs with varying process solutions are listed in Table 7.

TABLE 7 Removal rates (%) of selected pharmaceuticals by WWTPs with different process solutions in different countries

Pharmaceutical	Removal (%)	Unit operations	Location	References
Carbamazepine	23–51	Conventional WWTP	Ulsan, South Korea	Behera et al. 2011
Diclofenac	75	Settling, AcS	Rio de Janeiro, Brazil	Stumpf et al. 1999
	23-60	AcS, P removal	Aura/ Tampere/ Harjavalta, Finland	Lindqvist et al. 2005
	9-25	AcS/N/DeN/ P removal	Helsinki/ Seinäjoki/ Turku, Finland	Lindqvist et al. 2005
	17	Conventional WWTP	Berlin, Germany	Heberer et al. 2002
Ibuprofen	78–100	AcS/P removal	Aura/ Tampere/ Harjavalta, Finland	Lindqvist et al. 2005
	92–99	1. settling, AcS, 2◦settling	Frankfurt, Germany	Ternes 1998
	60-70	1. settling, AsC, 2. settling	Galicia, Spain	Carballa et al. 2004
Ketoprofen	51-100	AcS/phosphate removal	Aura/ Tampere/ Harjavalta, Finland	Lindqvist et al. 2005
	15–72	AcS, ppt with FeCl3	Switzerland	Tauxe-Wuersch et al. 2005
	69	1◦settling, AcS	Rio de Janeiro, Brazil	Stumpf et al. 1999
Naproxen	55–98	AcS/phosphate removal	Aura/ Tampere/ Harjavalta, Finland	Lindqvist et al. 2005
	69–94	1. settling, AcS, 2. settling	Frankfurt, Germany	Ternes 1998
	50–80	AcS/N/DeN, sand filtration	Kloten/ Opfikon, Switzerland	Joss et al. 2005

AcS -activated sludge; N/DeN -nitrification and denitrification; P removal -phosphorous removal; Conventional WWTP -primary settling, physicochemical and activated sludge processes; ppt with $FeCl_3$ -precipitation with $FeCl_3$

3.3.2 Surface waters and sediments

Pharmaceuticals have been detected in all kinds of water bodies, including rivers (Kolpin et al. 2002; Valcárcel et al. 2013), lakes (Tixier et al. 2003; Loos et al. 2007; Daneshvar et al. 2010), marshlands (Vazquez-Roig et al. 2012), groundwater (Stuart et al. 2012), wetlands (Camacho-Muñoz et al. 2010; Vazquez-Roig et al. 2011) but also in drinking water (Heberer 2002; Valcárcel et al. 2011). Additionally, they have been detected both in industrialized and in remote areas (Fent et al. 2006; Loos et al. 2008).

Pharmaceuticals are mainly hydrophilic and present in the liquid phase, but some of them can undergo interactions with solid fraction and can be transferred to sediment (Moreno-González et al. 2015). Even though the presence of pharmaceuticals in water bodies is widely studied, less data is available regarding their presence in sediments because they are thought to have lower adsorption capacity in natural conditions (Shi et al. 2014; Lara-Martín et al. 2014). However, sediment analysis represents pollution of a longer time period which can give a broader overview for, *e.g.*, risk assessment purposes (Antonić and Heath 2007). Detected concentrations of selected pharmaceuticals in European surface waters and river sediments have been listed in Table 8.

The persistence of pharmaceuticals depends on their photostability, binding, adsorption capacity and the rate of degradation (Ternes et al. 2003). Hydrolysis and photolysis are the main mechanisms of abiotic transformations occurring in surface waters. Pharmaceuticals are generally resistant to hydrolysis, and the primary pathways for their transformations occur via direct and indirect photolysis (Andreozzi et al. 2003).

In addition to the amount released into the environment, concentrations of pharmaceuticals depend on their temporary evolution, synergic and antagonistic effects, geographical area, and climate conditions (Ternes et al. 2003; Larsen et al. 2004). Lipophilic substances are often adsorbed on solid particles and accumulate easily in soil or sediment (Ternes et al. 2003; Larsen et al. 2004). On the other hand, hydrophilic, highly mobile pharmaceuticals are often leached into the groundwater or transported to surface waters (Babić et al. 2006). For example, anti-inflammatory drugs are highly soluble in water and polar, making them able to penetrate the soil and enter groundwater (Reddersen et al. 2002; Ternes et al. 2002).

Even though the amounts of these trace pollutants are generally low, they are continuously present in the environment due to their continuous release, which may lead to adverse effects on aquatic organisms. One of the main concerns is the potential of pharmaceuticals to accumulate in biota. The polar nature of most pharmaceuticals makes them directly bioavailable to filter feeding organisms, such as bivalves (McEneff et al. 2014). However, very little is still known about the long-term effects and behavior of pharmaceutical residues in the aquatic environment (Schwarzenbach et al. 2006) and, especially, in groundwater (Massmann et al. 2008).

TABLE 8 Detected concentrations of selected pharmaceuticals in receiving surface waters and sediments in different countries

Pharmaceutical	Location	Surface water ng L^{-1}	Sediment µg kg^{-1}	References
Carbamazepine	EU[a]	248		Loos et al. 2009
	Spain	8-41		Gros et al. 2012
	UK	71–327		Kasprzyk-Hordern et al. 2009
Ibuprofen	EU[a]	395		Loos et al. 2009
	Hungary		4-31	Dobor et al. 2012
	Spain	nd-380		Gros et al. 2012
	Sweden	0.5-7.4		Remberger et al. 2009
	UK	12–62		Kasprzyk-Hordern et al. 2009
Ketoprofen	EU[a]	10		Loos et al. 2009
	Hungary		9-99	Dobor et al. 2012
	Slovenia	281	320	Antonić and Heath 2007
	Sweden	<0.5-1.2		Remberger et al. 2009
	UK	0–7		Kasprzyk-Hordern et al. 2009
Naproxen	EU[a]	38		Loos et al. 2009
	Hungary		7-57	Dobor et al. 2012
	Slovenia	308	60	Antonić and Heath 2007
	Spain	7-156		Gros et al. 2012
	Sweden	0.46-5.6		Remberger et al. 2009
	UK	17–146		Kasprzyk-Hordern et al. 2009
Diclofenac	EU[a]	17		Loos et al. 2009
	Hungary		11-144	Dobor et al. 2012
	Spain	16-52		Gros et al. 2012
	Sweden	<0.2		Remberger et al. 2009
	UK	9–40		Kasprzyk-Hordern et al. 2009

[a] 122 sampling sites in European rivers
nd -not detected

3.4 Toxicity of pharmaceuticals

Pharmaceuticals are designed to have pharmacological and physiological effects in humans, mammals and other vertebrates (Cleuvers 2003; Directive 2004/27/EC). Due to the biological activity of pharmaceuticals, they can induce undesired effects in the environment to non-target organisms (Daughton and Ternes 1999). Aquatic organisms are exposed to pharmaceuticals, *e.g.*, in rivers, lakes, and coastal waters, which receive discharge from WWTPs (Pal et al. 2010; Gelsleichter and Szabo 2013). Pharmaceuticals have relatively short environ-

mental half-lives but are continuously released into the environment and, therefore, are pseudo-persistent (Daughton and Ternes 1999). Pharmaceutical residues can also be transported through food chains, leading to harmful effects on non-target species (Caballo et al. 2015). The possible effects on aquatic organisms are hard to predict but also can remain undetected or accumulate slowly, leading to irreversible changes (Schnell et al. 2009).

According to EU-Directive 93/67/EEC (Commission of the European Communities 1996), substances are classified according to their median effective concentration (EC50) values to different classes: < 1 mg L^{-1} (very toxic to aquatic organisms), 1-10 mg L^{-1} (toxic to aquatic organisms), and 10-100 mg L^{-1} (harmful to aquatic organisms). Substances with EC50 values above 100 mg L^{-1} are not classified.

Typically, the risk quotient (RQ) of a compound is calculated by measuring the environmental concentration (MEC) divided by the predicted no-effect concentration (PNEC) (Eq. 1). According to REACH guidelines, a PNEC is estimated based on the toxicity data of short-term/acute toxicity data median effective or lethal concentration (EC50 or LC50) divided by an assessment factor (AF) of 1000. In the case that long-term/chronic NOEC values are available for one, two, or three trophic levels, AF values of 100, 50, or 10 can be used (ECHA 2008).

$$RQ = \frac{MEC}{PNEC} \tag{1}$$

Contamination of the environment by pharmaceuticals can alter important physiological processes, such as development, reproduction, and nervous system function of various aquatic organisms, such as primary producers, primary consumers, secondary consumers, and decomposers (Schweer 2002; Pal et al. 2010; Gelsleichter and Szabo 2013). For example, exposure to NSAIDs can cause disruption of the endocrine system by alternating aromatase activity and leading to imbalance of sex hormones (Kümmerer 2008; Ji et al. 2013). In addition, cytological and histological effects have been observed (Schwaiger et al. 2004; Mehinto et al. 2010).

3.4.1 Toxic effects in aquatic environment

Many pharmaceuticals, including anti-inflammatory drugs and antibiotics, are relatively hydrophobic enabling them to partition into the lipid portion of organisms and bioaccumulate (Li et al. 2012; Brozinski et al. 2013). For example, carbamazepine is potentially bioaccumulative, while diclofenac shows only low potential for bioaccumulation (Liu et al. 2015). In fish, pharmaceuticals bioaccumulate most eagerly in the liver, followed by the brain, gills, and muscles. However, the bioaccumulation of pharmaceuticals is still inadequately studied, and only a limited number of studies are available. Larsson et al. (1999) was the first to report bioaccumulation of 17α-ethinylestradiol in the bile of juvenile rainbow trout. Grabicova et al. (2014) reported that antidepressants, such as sertraline and citalopram, accumulate in the brain and liver of rainbow trout

(*Oncorhynchus mykiss*). Bioaccumulation of anti-inflammatory drugs, including diclofenac, naproxen, and ibuprofen, has also been detected in the tissues of wild fish and over 1000 times higher concentrations were found in the bile of fish than in water (Gao et al. 2012; Brozinski et al. 2013).

Among anti-inflammatory and analgesic drugs, diclofenac has the highest acute toxicity (Paje et al. 2002; Fent et al. 2006). Compared to other NSAIDs, diclofenac shows lower EC50 and LC50 values and can be considered more toxic than other NSAIDs (Islas-Flores et al. 2013, Table 9). Even trace concentrations of diclofenac can induce toxic effects on aquatic organisms and affect their development and reproduction (Marques et al. 2004; Brun et al. 2006). Guiloski et al. (2015) showed that diclofenac can alter the levels of testosterone and affect the reproduction process, causing negative impacts in aquatic organisms. In addition, ingestion of diclofenac by birds leads to death shortly after exposure (Stülten et al. 2008).

TABLE 9 EC50 values for carbamazepine, diclofenac, ibuprofen, ketoprofen, and naproxen in green algae and rainbow trout, NOEC values in liver cells of rainbow trout with CFDA-AM assay and acute PNEC values by planktonic crustacean *Daphnia Magna* tests

Pharmaceutical	**EC50** (mg L^{-1}) Algae *D. subspicatus*	**EC50** (µg L^{-1}) Rainbow trout *Oncorhynchus mykiss*	**NOEC** (mg L^{-1}) Rainbow trout liver (CFDA-AM assay)	**PNEC** (ng L^{-1}) Crustacean *Daphnia Magna*
Carbamazepine, CBZ	85 [a]	nd	$2.5 \cdot 10^{-3}$ [e, f]	13800 [n]
Diclofenac, DCF	72 [b]	266.8 ± 39.8 [d]	$5 \cdot 10^{-5}$ [e, g, h]	22430 [e]
Ibuprofen, IBP	342 [b]	207.4 ± 27.3 [d]	$1 \cdot 10^{-5}$ [f, i, j]	9060 [o]
Ketoprofen, KTF	248 [c]	157.7 ± 18.8 [d]	$2 \cdot 10^{-3}$ [j, k]	nd
Naproxen, NPX	625 [b]	231.3 ± 51.7 [d]	$3.3 \cdot 10^{-4}$ [k, l, m]	nd

[a]Cleuvers (2002) [b]Cleuvers (2004) [c]Camacho-Muñoz et al. (2010) [d]Schnell et al. (2009) [e] Ferrari et al. (2003) [f] Yamamoto et al. (2007) [g] Quinn et al. (2011) [h] Hoeger et al. (2005) [i] Han et al. (2006) [j] Sanderson et al. (2003) [k]Harada et al. (2008) [l] Isidori et al. (2005) [m] Straub and Stewart (2007) [n]Ferrari et al. (2004) [o]Pounds et al. 2008)
nd - not defined

Diclofenac has a profound influence on the hematological, biochemical, and enzymological effect on the freshwater fish and the concentrations of 1 µg L^{-1} are reported to damage the liver and kidney cell functions in fish (Saravanan et al. 2011). Fish are often considered as sensitive bioindicators for exposure to aquatic pollutants (van der Oost et al. 2003). The common carp is often used as bioindicator species because cyprinids are quantitatively the most important group of teleost fishes cultured throughout the world for commercial purposes (Huang et al. 2007). For example, subchronic exposure of diclofenac during the early life stages on carp has an effect on mortality and oxidative stress (Ste-

panova et al. 2013). Diclofenac causes oxidative stress in liver of a carnivorous fish *Hoplias malabaricus*, which increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and lipoperoxidation (LPO) (Guiloski et al. 2015). Additionally, diclofenac has potentially adverse effects on various tissues of brown trout at concentrations typically found in the aquatic environment (Hoeger et al. 2005).

According to acute toxicity tests (Ferrari et al. 2004; Kim et al. 2007; Hampel et al. 2014), carbamazepine is unlikely to be lethal at environmental levels (ng L^{-1} to µg L^{-1}), with LC50 and EC50 values in mg L^{-1} ranges. In addition, carbamazepine does not have an effect on the major physiological processes, including growth and mobility. However, carbamazepine can interact with voltage gated Na^+, Ca^+ and K^+ channels and modulate release, uptake and receptor binding of neurotransmitters, and inhibit adenylate cyclase activity (Ambrosio et al. 2002; Beutler et al. 2005; Rao et al. 2007; Lee et al. 2007). Exposure to carbamazepine at concentrations commonly found in wastewater (ng L^{-1} to µg L^{-1} levels) can change brain pituitary hormone messenger RNA (mRNA) levels in Atlantic salmon parr *Salmo salar*, possibly leading to disruption with an ecological significance (Hampel et al. 2014). Additionally, carbamazepine has some effect on human embryonic cells, such as growth inhibition and morphological changes, even at environmental concentrations (Komtchou et al. 2015).

Chronic exposure of fish to ibuprofen can alter their reproduction and development (Flippin et al. 2007; David and Panchratna 2009). For example, exposure to ibuprofen inhibits the growth of the mollusk *Planorbis carinatus*, reduces the reproduction capacity of the crustacean *Daphnia magna*, and causes abnormal behavior of crustacean *Gammarus pulex* (Carlsson et al. 2006a, b). In addition, concentrations of ibuprofen higher that 100 mg L^{-1} are known to be fatal to the fish *Oryzias latipes* (Sanderson et al. 2003; Pounds et al. 2008). Consumed ibuprofen is rapidly excreted and forms various conjugates, such as hydroxy-and carboxy-ibuprofen (Buser et al. 1999; Méndez-Arriaga et al. 2008), which are acutely toxic and potentially have endocrine disrupting properties in human and wildlife (Buser et al. 1999).

3.4.2 Toxicity of mixtures

Pharmaceuticals are present in the environment in mixtures which are constituted by a variety of chemicals with diverse modes of action (Pomati et al. 2008). However, the various interactions among these substances and their effects on organisms still remain largely unknown (Cleuvers 2003). Typically, the evaluation of environmental toxicity strongly relies on the study of individual substances at a time, neglecting interactive effects of mixtures containing parent compounds, metabolites and transformation products (Backhaus and Faust 2012; Vasquez et al. 2014). This leads to a possibility of under- or overestimation of the actual impacts in the environment (Khetan and Collins 2007; Pomati et al. 2008; Backhaus and Karlsson 2014). In addition, individual and combined toxic effects at higher concentration levels are not able to predict individual or com-

binatorial effects at lower, more environmentally relevant doses. Therefore, the standard toxicity tests may not provide representative information.

Generally, there are two concepts in use for predicting the toxicity of mixtures; concentration addition and independent action. In general, the combination effect will be higher if the substances follow the concept of concentration addition. Concentration addition can be described mathematically for a mixture of substances by Eq. 2 (Berenbaum 1985).

$$\sum_{i=1}^{n} \frac{c_i}{ECx_i} = 1 \qquad (2)$$

In the equation, c_i are the concentrations of single substances present in the mixture with the total effect of x %. ECx_i are the concentrations of substances that alone cause the same effect as observed for the mixture. Concentration addition means that the substances with concentrations below their individual no observed effect concentration (NOEC) can contribute to the total effect of the mixture. The concept is based on similar action of chemicals, which means a specific interaction with a molecular target site in the observed organism (Pöch 1993). On the other hand, a similar action can be observed for all substances able to cause the same toxicological response, such as the death of test organisms.

Independent action is based on dissimilar action of compounds in a mixture and thus, different molecular target sites and modes of action (Bliss 1939; Barata et al. 2006). Therefore, the relative effect of one toxicant remains unchanged in the presence of another in a mixture. The combination effect can be calculated by Eq. 3, where $E(c_i)$ is the effect of a single substance and E_{mix} the total effect of the mixture. According to this equation, a substance with toxicity below its individual NOEC will not add to the total effect of the mixture. If the toxicity of all substances in a mixture remains below their NOEC, no mixture toxicity will be observed.

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_i)) \qquad (3)$$

Typically, acute toxicity tests have failed to detect effects caused by substances at environmental concentrations even though mixtures can have additive and synergistic properties (Daughton and Ternes 1999). For example, Pomati et al. (2006) studied a mixture of 13 pharmaceuticals which at low (ng L^{-1}) concentrations, inhibited cell proliferation of human embryonic cell affecting their physiology and morphology suggesting potential effectors on aquatic life. Cleuvers (2003) reported that exposure of ibuprofen causes higher mortality of *D. magna* in the presence of other NSAIDs than ibuprofen alone (Cleuvers 2003). In addition, the combined effects of ibuprofen and diclofenac in the *Daphnia* test were much stronger than their weak individual effects (Cleuvers 2003). On the other hand, Pomati et al. (2008) stated that in case of mixtures of certain antibiotics and anti-inflammatory drugs, antagonistic effects are more probable than synergistic effects when the chemicals have different modes of action. Therefore, as

the number of different compounds increases, the chance of antagonism also increases (Brack 2007).

Currently, the toxicological mechanisms of pharmaceuticals on non-target organisms are still rather unknown and require further investigation. Current data suggests that pharmaceuticals, with environmental concentrations below 1 μg L^{-1} in most cases, are unlikely to pose a risk of acute toxicity but mixture and chronic toxicity, especially in fish, still requires further research (Cleuvers 2003; Fent et al. 2006; Pomati et al. 2008).

3.5 Analysis of pharmaceuticals

3.5.1 Extraction

Many of the emerging pollutants, such as pharmaceuticals, which are present at trace levels (low ng L^{-1}) in the environment, are polar or semipolar. Their measurement in environmental matrices requires advanced analytical techniques (Harman et al. 2012). For example, pharmaceuticals often occur in low ng L^{-1} concentrations in surface waters (Budzinski and Togola 2006; Stackelberg et al. 2007). However, the level of quantification (LOQ) of typical tandem mass spectrometry (MS/MS) equipment (without preconcentration) is in the μg L^{-1} range. Therefore, the analysis of pharmaceuticals in environmental samples requires specific pre-treatment procedures, including extraction, minimizing their transformation and degradation (Olives et al. 2012).

Despite the fast development of analytical equipment and methods, most instruments are not able to handle complex sample matrices. In environmental samples, analytes need to be preconcentrated and cleaned to remove matrix constituents before analysis. An efficient cleanup procedure is crucial to avoid matrix interferences, the main cause of inaccuracy in the analysis of environmental samples (Sousa et al. 2011). Some compounds can react with target molecules during sampling and storage periods and affect the yield. Natural organic matter (NOM) can coelute with analytes leading to suppression or enhancement of analysis results (Van de Steene and Lambert 2008). Therefore, a pretreatment step is required to clean up and concentrate the sample compatibility for the chosen equipment (Olives et al. 2012).

Traditional liquid-liquid extraction (LLE) is still the one of the most commonly used extraction techniques for analysis of inorganic and organic pollutants (Tobiszewski et al. 2009; Pena-Pereira et al. 2010). LLE is based on transfer of analytes from an aqueous sample to a water-immiscible solvent. However, conventional LLE has some drawbacks, such as emulsion formation, use of large volumes of solvents, possible loss of analytes, and time-consuming steps. Nevertheless, the method is still widely used in analysis of pharmaceuticals in biological and environmental samples (Ojha et al. 2009; Radwan et al. 2010; Sakaguchi et al. 2011).

In recent decades, LLE has been widely replaced by solid phase extraction (SPE) in environmental analyses. Additionally, the aim has been to miniaturize the

traditional LLE principle by reducing the quantities of organic solvents. For example, in liquid phase micro extraction (LPME) only several μL of solvents are required (Sarafraz-Yazdi and Amiri 2010). Other pretreatment methods include *e.g.*, solid phase microextraction (SPME) (Yu et al. 2012), cloud point extraction (CPE) (Han et al. 2008), dispersive liquid-liquid microextraction (DLLME) (Cruz-Vera et al. 2009), pressurized-liquid extraction (PLE) (Pérez-Carrera et al. 2010), hollow-fiber liquid phase microextraction (HF-LPME) (Ramos Payán et al. 2010), pressurized hot water extraction (PHWE) (Saleh et al. 2011), and stir membrane liquid-liquid microextraction (SM-LLME) (Riaño et al. 2012).

Currently, SPE is the most commonly used pretreatment for extraction and cleanup of pharmaceuticals in environmental matrices (Farré et al. 2008; Olives et al. 2012). However, the method has drawbacks, such as a requirement of a large sample volume and time-consuming cartridge conditioning, washing, elution of analytes, and an extra step of concentration. Additionally, control of pH plays an important role in SPE because it has an effect on the dissociation on ionizable compounds, their hydrophobicity, and interaction with the sorbent. In case of traditional reverse phase sorbents, retention of an analyte increases at the pH of unionized form (Wu et al. 2008).

The isolation procedure comprises selective retention of analytes in a solid sorbent that can be eluted with organic solvent(s) (Olives et al. 2012). Analytes are selectively trapped on the sorbent mainly through hydrogen bonds, van der Waals forces, hydrophobic effects, π-electron interactions, and cation- or anion-exchange processes.

There are several SPE sorbents available from alkyl-modified silica material (*e.g.*, C8, C18) to new polymer sorbents with improved retention of polar compounds (Mahugo-Santana et al. 2011). Alkyl-modified silica materials retain the analytes via apolar interactions. Most pharmaceuticals, including NSAIDs, are lipophilic with log K_{ow} around 4, and good retention in C18 is achieved mainly via van der Waals forces between the flat areas of the molecules and the sorbent (Olives et al. 2012). On the other hand, polymer sorbents have different functional groups attached to a polymer and different kinds of interactions between analyte and sorbent are possible (hydrophilic, hydrophobic, π-π interactions). For example, polydivinylbenzene resin (Strata-X) with piperidone groups has been employed in the extraction of NSAIDs from wastewater, surface water, and ground water (Kosjek et al. 2005; Wu et al. 2008), and the hydrophobic-lipophilic copolymers of divinylbenzene and vinylpyrrolidone (Oasis HLB) for concentration of water and biological samples (Hashim and Khan 2011). The poly(divinylbenzeneco-N-vinylpyrrolidone) copolymer (Oasis MAX) combines non-polar retention with anion exchange functionalities (Rousseau et al. 2008).

Recently, new sorbent materials, including molecularly imprinted polymers (MIP), carbon nanotubes, metallic nanoparticles, hemimicelles, and admicelles, etc. have shown potential to improve the extraction efficiency of SPE (Rubio and Pérez-Bendito 2009; Wen et al. 2014). If the sample clean-up needs to be further optimized and the matrix suppression reduced, a two-step SPE can be

applied (Van De Steene et al. 2006). For example, NH_2- and florisil-cartridges have been employed to decrease the humic substances from the matrix (Westbom et al. 2004; Parera et al. 2004). Examples of pretreatment methods for environmental sample matrices are presented in Table 10.

TABLE 10 Different pretreatment and cleanup procedures of selected pharmaceuticals in variety of environmental samples

Sample	**Pharmaceutical**	**Pretreatment**	**Solvents**	**Obs**	**Ref**
River sediment	Diclofenac Ibuprofen Ketoprofen Naproxen	MAE / SPE	40 mL (MeOH/ACT) 1200 W, 170 °C, 40 min Condition: 3 mL Hex + 3 mL ACT + 2 x 3 mL MeOH + 2x 3 mL 1% MeOH/H_2O (pH < 3) Wash: 1% MeOH/ H_2O (pH < 3) Elution: 1.5 mL MeOH	5 g sediment (dried, crushed, sieved until 0.2 mm particles) Reconstitution: 3 mL MeOH, diluted to 500 mL H_2O (pH < 3) Strata X cartridge (60 mg, 3 mL), 0.5 mL toluene SPE prior to derivatization, GC-MS LOQ: 0.1- 0.25 μg g^{-1}	Antonić and Heath 2007
Ground water, surface water (stream, lake)	Diclofenac Ibuprofen Ketoprofen	SPE	Condition: 2 x 3 mL MeOH + 2 x 3 mL 1% EDTA/H_2O Wash: 2 mL 5 % MeOH Elution: 2 x 3 mL MeOH	250 mL sample (pH 5, H_2SO_4) Strata X (60 mg/3 mL) Evaporation until 200 μL, LC-MS-MS LOQ: 8.4-61 ng L^{-1} Recovery: 75 – 113 %	Wu et al. 2008
Tap water, sewage water, pond water	Diclofenac Ibuprofen Naproxen,	MISPE	Condition: ACN+ MeOH +10 mM ammonium formate buffer pH 3 Wash: 1 mL ACN / H_2O (4:6, v:v) + 1 mL toluene / heptane (4:6, v:v) Elution: 2x 0.9 mL acetic acid in ACT/MeOH (2:8, v:v)	Sample (pH 3) MISPE cartridge: Supel MIP NSAIDs (25 mg/ 3 mL) Reconstitution: 0.15 mL MeOH/H_2O (2:8, v:v), LC-MS/MS, LC-UV LOQ: 3.5-8 ng L^{-1} Recovery : 84-116 %	Zorita et al. 2008
Sewage water	Ketoprofen, ibuprofen, naproxen, diclofenac	HF-LPME	Adjusted to pH 1.5-1.9 Organic solvent: DHE Acceptor solvent: pH 9.5 $(NH_4)_2CO_3$ 0.1 M	Sample flow: 100 mL/min Polypropylen HF: Celgard (240 μm I.D., 30 μm wall thickness, 0.1 μm pore size) LC-DAD LOQ: 0.03- 0.17 μg L^{-1}	Larsson et al. 2009
Soil, sediments	Diclofenac, Ibuprofen (32 pharmaceuticals simultaneously)	PLE / SPE	NH_3 0.1 M/ MeOH (1:1 v:v) 1500 psi 5 cycles, 5 min, 80 °C Condition: 5 mL heptane + 5 mL ethyl acetate + 5 mL MeOH + 2x 5 mL H_2O (pH 7)	20 g sample PLE prior to SPE (MAX- HLB cartridges) Evaporation until 5 mL and redissolved: 30 mL buffer + 25 mL EDTA (pH 7)	Pérez-Carrera et al. 2010

TABLE 10 Different pretreatment and cleanup procedures of selected pharmaceuticals in variety of environmental samples (continued)

Soil, sediments	Diclofenac, Ibuprofen (32 pharmaceuticals simultaneously)	PLE / SPE	Wash: 5 mL heptane Elution: 1 mL ethyl acetate + 2 mL MeOH + 2 mL 2% acetic acid/MeOH + 2 mL MeOH, HLB: 3 mL ethyl acetate + 4 mL MeOH	Reconstitution with 0.2 mL mobile phase after SPE, LC-MS/MS LOQ: 0.3-7.1 ng g^{-1}	Pérez-Carrera et al. 2010
Wastewater, tap water, surface water	Ibuprofen, Ketoprofen Naproxen + enantiomers	SPE / SPE	Conditioning: 3 mL ACN + 3 mL MeOH + 3 mL H_2O (pH 2.5, 1M H_2SO_4) Wash: 2 x 10 mL H_2O Elution: 4 mL ACN Conditioning: 1 mL ethyl acetate + 1 mL MeOH + 1 mL H_2O (pH 9.5, NaOH 1M) Wash: 2x 1 mL H_2O (pH 9.5) Elution: 1 mL ethyl acetate	500 mL sample (filtration, centrifugation) pH 2.5 (H_2SO_4 1 M) Oasis HLB (60 mg, 3 mL) Evaporation until 300 µL, Derivatization + second SPE 3mL aqueous solution from derivatization step, Oasis HLB (10 mg, 1 mL) GC-MS/MS LOQ: 0.2-3.3 ng L^{-1}	Hashim and Khan 2011
Sewage sludge	Diclofenac Ibuprofen Ketoprofen Naproxen	PHWE/ HF-LPME	NaOH 0.01 M, 100 bar, 5 cycles, 5 min, 120 °C 90 mL adjusted to pH 1.5, diluted to 100 mL Organic solvent: DHE Acceptor solvent: pH 9.5 $(NH4)_2CO_3$ 0.1 M	0.5 g sludge PHWE prior to HF-LPME Polypropylen HF: Accurel Q3/2 (600 µm I.D., 200 µm wall thickness, 0.2 µm pore size, 25 µL) LC-ESI-MS LOQ: 1.5-12.2 ng g^{-1}	Saleh et al. 2011
Wastewater, tap water, surface water	Diclofenac Ibuprofen Naproxen	SPE	Conditioning: 12 mL of MeOH+10 mL UHQ Wash: 10 mL UHQ, elution: 15 mL MeOH Reconstitution: 1 mL ACN	C18 (1000 mg) Waters, LC-MS/MS LOQ: 0.02-0.03 µg L^{-1}	Eslami et al. 2015
River water	Carbamazepine Diclofenac	SPE	Conditioning: 3 mL MeOH, 3 mL of water (1.4 mM ETDA), pH 3 with formic acid. 45 min eluted with 3 mL MeOH and 3 mL MeOH with 0.5 M ammonium hydroxide Reconstitution: 200 mL of H_2O–MeOH (9:1,v/v)	Oasis MCX (60 mg, 3 cc) cartridges (Waters), LC-ESI-MS/MS LOQ: 0.16-3.5 ng L^{-1}	Brieudes et al. 2016

LC-ESI-MS/MS: liquid chromatography with electron ionization tandem mass spectrometry
GC-MS, GC-MS/MS: gas chromatography with (tandem) mass spectrometry
DAD: Diode array detector
EDTA: Ethylenediaminetetraacetic acid disodium salt
LOQ: level of quantification

3.5.2 Analysis methods

Several methods for the determination of pharmaceuticals have been reported (*e.g.*, Nödler et al. 2010; Huerta-Fontela et al. 2010; López-Serna et al. 2011; Gros et al. 2012; Gilart et al. 2013). The most widely used method includes separation by liquid chromatography (LC) because pharmaceuticals are usually polar and nonvolatile (Table 11). There are also methods based on gas chromatography (GC), but they require a derivatization step, which is time-consuming and unsuitable for thermolabile compounds (Togola and Budzinski 2008; Varga et al. 2010). LC is useful due to its versatility and lack of the need for the derivatization. In addition to mass spectrometry (MS), LC can be coupled to Ultraviolet-visible spectroscopy (UV-Vis), fluorescence, or a diode array detector (DAD). Currently, the most extensively used method for the analysis of pharmaceuticals is HPLC coupled with a triple quadrupole (QqQ) MS (Fig. 7).

LC-MS is a powerful technique for rapid screening of pharmaceuticals in aqueous matrices. Unknowns can be identified by full scan based on their masses (Herrera-Rivera et al. 2011). However, LC-MS/MS is preferred over LC-MS because of greater analytical sensitivity in case of complex sample matrices (Sim et al. 2011). Over the last years, tandem systems have become more popular. Additionally, LC-MS equipment do not meet the requirements of Commission Decision 96/23/EC for confirmatory purposes like LC-ESI-Ion trap-MS/MS (Igualada et al. 2007) and LC-ESI-MS/MS systems (Jedziniak et al. 2010). In some cases, even quantitation of less than 1 ng L^{-1} can be achieved (Jedziniak et al. 2010; Olives et al. 2012).

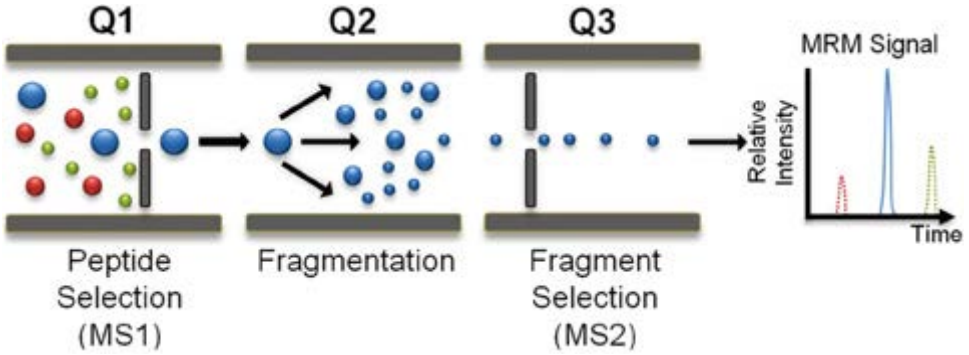


FIGURE 7 A schematic of a triple quadrupole mass spectrometer (QQQ-MS): Q1 and Q3 represent two mass filters for precursor and fragment ion selection, while Q2 (collision cell) creates fragment ions via collisionally-induced dissociation (CID) (Boja and Rodriguez 2011)

LC-MS/MS equipment with triple quadrupoles or time-of-flight is the best choice for analysis of pharmaceuticals in environmental matrices (Petrović et al. 2005). When developing an LC-MS/MS method, matrix effect needs to be evaluated. Co-eluting compounds originating from the matrix can enhance or suppress the signal. When compounds from the matrix and from analytes enter the ion source simultaneously, ionization efficiency of the analyte can be decreased (Taylor 2005). Therefore, matrix effect can have an effect on the reproducibility and accuracy of the method.

TABLE 11 Variety of separation and detection methods of selected pharmaceuticals in environmental samples.

Pharmaceutical	SPE	LC separation	Detection	LOQ	Ref
Diclofenac	Oasis MCX +Oasis HLB Plus, pH 2, elution with methyl-t-butyl ether/ MeOH/(30%) NH_4OH	HyPURITY AQUASTAR C18 column A:ammonium formate + formic acid, PH 4, B: MEOH	Micromass Quattro Ultima, ESI+	5 ng L^{-1}	Bartels and von Tümpling 2007
Diclofenac Ibuprofen Ketoprofen	OASIS HLB cartridges (6 m, 6 cc) pH 2, elution with 4 mL MeCN	LC (Acquity, Waters) Column: Acquity C18 ethylene bridged hybrid, 100 mm×2.1 mm 1.7 µm, eluents UHQ, ACN	ESI+, ESI- (Quattro Premier; Micromass) MS/MS	1 µg L^{-1}	Cimetiere et al. 2013
Carbamazepine Diclofenac	pH 4, Strata-X SPE cartridges (6 mL, 200 mg, Phenomenex) elution 50:50 (v:v) ethyl acetate: ACT	Column: Waters Sunfire 150 × 2.1 mm, 3.5 µm C18 A: 80% 13 mM ammonium acetate (aq.), 20% ACN B: ACN	Bruker Daltonics HCT ion trap MS with an API-ESI+, ESI-	CBZ 4-15, DIC 22-225 ng L^{-1}	McEneff et al. 2014
Carbamazepine Diclofenac Ibuprofen Ketoprofen Naproxen	SPE Oasis HLB 60 mg, 3 mL elution with MEOH	UPLC (Waters) PI: Acquity HHS T3, A: MEOH, B: 10 mM FA/ammonium formate, pH 3.2 NI: Acquity BEH C18, A: ACN, B: 5 mM ammonium acetate /NH_3, pH 8	ESI- (QqLIT) MS/MS 5500 Qtrap	0.1-30 ng L^{-1}	Moreno-González et al. 2014
Carbamazepine Diclofenac Ibuprofen Ketoprofen Naproxen	pH 2.5, Elution 2 x 3 mL of MeOH	UHLC Atlantis T3 C18 column (100 mm×2.1 mm, 3 µm, Waters) PI: 0.01 % (v/v) formic acid (A), MeOH (B), NI: 1 mM ammonium formate (A), MeOH (B)	Thermo Scientific QqQ, ESI+, ESI-	CBZ 64.2 DIC 63.6 IBU 47 KET 11.5 NPX 24.2 ng L^{-1}	Dasenaki and Thomaidis 2015
Carbamazepine Diclofenac Ibuprofen Ketoprofen Naproxen	OASIS HLB cartridges (3 mL /60mg), elution 3 x 4 mL MeOH	Agilent 1200 LC, Zorbax Eclipse XDB RP-C18 column, (Agilent 150 x 4.6 mm, 5 mm) A: UHQ/ACN (90:10 % v/v) + 0.01% formic acid (pH 3), B: ACN + 0.01% formic acid	4000 QTRAP tandem mass spectrometer AB SCIEX, ESI+, ESI-	0.2-0.5 µg L^{-1}	Al-Tarawneh et al. 2015

Liquid chromatography is the most used technique (70 %) for analysis of NSAIDs, while other techniques, such as GC, capillary zone electrophoresis (CZE), fluorimetry and voltammetry cover the remaining 30 % (Starek 2011; Olives et al. 2012). Partly, this is due to the possibility of coupling LC with mass spectrometry but in case of biological samples other detection methods (UV-Vis, DAD, and fluorescence) are useful (Barceló and Petrović 2007). In industrial applications, IR and near infrared analysis (NIRA) are useful for the quality control (Olives et al. 2012).

The most recent methods focus on the multiresidue analysis, which is able to determine multiple classes of drugs simultaneously in one procedure (Lavén et al. 2009; Boleda et al. 2013). Additionally, some methods focus on the determination of specific therapeutic classes (Ibáñez et al. 2009; Kim et al. 2013; Dorival-García et al. 2013).

3.5.3 Quantification

Individual peak area of a chromatogram needs to be determined related to an amount or concentration of the analyte, which is performed by calibration. There are three main calibration methods available: external and internal calibration and calibration by standard addition (Robards et al. 1997). In case of the external standard, standard solutions of pure analytes of varying concentrations are prepared and analyzed. Peak areas are determined from chromatograms and plotted as a function of concentration to form a calibration graph (Robards et al. 1997). The unknown analyte is analyzed, and its concentration is determined based on the measured peak area and the calibration graph. The method requires precise control of the analytical technique. Especially, injection of the sample needs to be reproducible. Typically, this can be achieved by using autoinjector, while syringe injection is relatively poor and can lead to error of 10-20 %.

In the standard addition method, a known quantity of analyte is added to the sample (Robards et al. 1997). From the increase in signal, the amount of analyte in the sample can be derived. The standard addition technique combines the features of both internal and external calibration. It is a useful technique when the detector response is known to be linear (Harris 2010). Standard addition is suitable if the sample contains a complex matrix that can change the analytic signal.

The internal standard method is based on a known amount of standard added to the sample (Harris 2010). The standard should resemble analyte closely in terms of physical and chemical properties (Robards et al. 1997). Standards can be analogues, homologues, isomers, enantiomers and isotopically labelled analogues of the analyte. However, the standard cannot be included in the sample or react with any component of the sample. If the sample contains multiple analytes, several internal standards can be used. Generally, the standard and the analyte should elute close together but can be distinguished, for example, in instances of isotopically labelled samples (Robards et al. 1997).

The amount of internal standard is selected by estimating the amount of analyte in the sample aiming at the internal standard peak, and analyte peaks are roughly the same size (Robards et al. 1997; Harris 2010). The peak area of the analyte is compared with the standard peak and the concentration of the analyte is calculated based on it. Internal standards are useful when the quantity of analytes varies only slightly from run to run. However, given complex samples with multiple analytes, it is difficult to find a suitable substance for internal standard (Robards et al. 1997). Internal standards are desirable when sample loss is expected during sample preparation prior to analysis.

The best option to deal with the matrix effect is to use isotopically labelled internal standards (Benijts et al. 2004; Petrović et al. 2005). Structural analogues are the second best option. However, in environmental application it can be difficult to find analogues, such as drugs from the same class that are definitely not present in the sample. In addition, the matrix effect can vary when studying different kinds of matrices, such as wastewater influent and effluent, lake water and river water (Petrović et al. 2005; Taylor 2005). Pigini et al. (2006) compared the use of internal and external standards in the LC-MS/MS analysis and concluded that the results are consistent by both methods. However, with external standards, complex matrix can lead to under- or overestimation.

3.6 Passive sampling

Since the 1970s, passive sampling devices have been used to measure time-weighted average or equilibrium concentrations of pollutants in various environmental matrices, including soils, sediments, and water (Kozdroń-Zabiegała et al. 1995; Kot et al. 2000; Górecki and Namieśnik 2002; Mills et al. 2011). In 1974, the first passive sampling device was applied to monitor inorganic compounds in surface water (Benes and Steinnes 1974). Since then, several passive sampling devices have been developed and applied for monitoring trace compounds in environmental matrices. Passive sampling can cover the drawbacks of grab sampling where events of pollution can be missed due to low concentrations or the variation of concentration over time (Alvarez et al. 2005; Mazzella et al. 2008; Martínez Bueno et al. 2009).

Passive samplers can be divided into equilibrium and kinetic or integrative samplers (Bopp 2004; Vrana et al. 2005). Contaminants can diffuse into the receiving phase until the equilibrium between the collecting phase and its surroundings is reached. After that, further enrichment cannot take place. In case of equilibrium samplers, the equilibrium is reached rapidly but the analytes can diffuse back into the surrounding media. Equilibrium samplers include the passive diffusion bag sampler (PDBS), the diffusive multi-layer sampler (DMLS), and polydimethylsiloxane, better known as silicon rubber sheets (Ronen et al. 1987; Booij et al. 2002; Bopp 2004).

Integrative samplers have a high collecting capacity of contaminants and do not reach equilibrium with the surrounding media (Bopp 2004). Therefore, they are continuously enriched throughout the sampling period and much less likely to

diffuse back out of the sampler. The average concentration determined by integrative samplers during the entire sampling period is also referred to as time-weighted average (TWA) concentration (Grathwohl and Schiedek 1997). Most sampler types are employed as TWA samplers (Bopp 2004; Vrana et al. 2005). However, in cases with multiple analytes, all the analytes may not have reached the equilibrium at the end of the deployment time, leading to unclear distinction between equilibrium and integrative sampling.

The passive sampling enables estimates of the average concentration over the sampling period, detects trace amounts of contaminants, permits monitoring the effects of episodic events, and provides monitoring of contaminants over extended periods of time (Alvarez et al. 2004; Petty et al. 2004). Passive sampling eliminates the need of multiple sampling operations, and the samplers are often easier to handle, store, and transport than water samples of multiple liters. The main advantage of passive samplers is that only one device is needed for the whole sampling period. Therefore, the amount of samples can be reduced. Furthermore, this reduces the number of samples analyzed and eventually the sampling costs.

Passive sampling is suitable for sampling of both organic and inorganic contaminants. There are several passive sampler designs for organic pollutants: the polar organic chemical integrative sampler (POCIS), the semi-permeable membrane device (SPMD), the ceramic dosimeter, the membrane enclosed sorptive coating (MESCO), and Chemcatcher, to name but a few (Kingston et al. 2000; Alvarez et al. 2004; Stuer-Lauridsen 2005; Paschke et al. 2006; Seethapathy et al. 2008), while DGT (Diffusive Gradient in Thin film) or Chemcatcher® are suitable for inorganic analytes (Huckins et al. 1993; Kingston et al. 2000; Vrana et al. 2001).

Over the last few decades, robust passive samplers have been developed for the measuring of TWA concentrations of non-polar pollutants, such as PAHs and PCBs (Vrana et al. 2006). Passive sampling devices for monitoring organic pollutants in water include SPMD and MESCO for non-polar compounds and POCIS and the polar version of Chemcatcher for polar compounds (Huckins et al. 1993; Vrana et al. 2001; Alvarez et al. 2004; MacLeod et al. 2007). Most of the published research is about POCIS and less about Chemcatcher (Schäfer et al. 2008; Mills et al. 2014). For example, over 300 compounds have been shown to accumulate in POCIS, including pesticides (>100), pharmaceuticals (>90), and industrial chemicals (>30) (Mazzella et al. 2007; Togola and Budzinski 2007; Harman et al. 2012). However, most passive sampling devices for organic compounds in water are developed for hydrophobic substances (Vrana et al. 2006).

The passive samplers are placed at the sampling site for a known time during which the sampler collects freely dissolved part of analytes (Kingston et al. 2000; Aguilar-Martínez et al. 2009). The sampling period can vary, depending on the type of sampler and the aim of the study, from days to months. The concentration of an analyte is integrated over the exposure time making the meth-

od immune to accidental and occasional variations of concentration and allowing a long-term overview of pollutant levels.

3.6.1 Theory

The uptake of pollutants by passive samplers is based on permeation of analyte through the surface of the phase material (Mayer et al. 2003; Vrana et al. 2005). The permeation proceeds by Fickian diffusion due to the high affinity of the receiving phase for the analyte. The rate of permeation depends on physical properties of the pollutant, such as molecular size (Kingston et al. 2000). Diffusion and separation mechanisms depend on the chemical potentials of collected and still remaining analytes (Kozdroń-Zabiegała et al. 1995). All in all, the mass transfer of compounds from water to the sampler includes diffusion and transport across barriers and depends on the receiving phase, the aqueous boundary layer, diffusion limiting membrane (if used) and, in the case of field studies, the biofouling layer (Huckins et al. 1999; Kot-Wasik et al. 2007). The overall mass transfer resistance is the sum of barrier resistances to mass transfer.

Chemicals diffuse and partition until the aqueous concentrations of substances reach the equilibrium between uptake and elimination into and from the sampling device over time or until the end of the deployment time (Vermeirssen et al. 2009). Under constant aqueous concentration, the concentration in the sampler increases nearly linearly with time (Vermeirssen et al. 2008). In case of linear uptake, the amount of substance in the receiving phase can be calculated as expressed in Eq. 4 (Kingston et al. 2000; Shaw et al. 2009).

$$M_s(t) = C_w R_s t \qquad (4)$$

where $M_s(t)$ is the amount of pollutant (ng) measured in the receiving phase after the deployment time t (days), C_w is the average concentration (ng L^{-1}) in water and the sampling rate (R_s), which is the extracted water volume per unit of time (L day^{-1}) (Vrana et al. 2006).

R_s is established for each device and compound by performing lab or field experiments (MacLeod et al. 2007). The amount of chemical in a sampler can be divided by the R_s and the deployment time to calculate the TWA concentration. However, it is not straightforward to calculate TWA concentrations from passive sampling data because the diffusion and partitioning are influenced by, *e.g.*, temperature, turbulence around the sampler, salinity and biofouling, and by the type of sorbent, sampling period, and physical-chemical parameters, such as pH, conductivity, and content of dissolved organic compounds (Vrana et al. 2006; Booij et al. 2006; Togola and Budzinski 2007; Vermeirssen et al. 2008; Li et al. 2010). For example, R_s has been reported to increase with the temperature and flow and to decrease due to diffusion limitation in the membrane (Kingston et al. 2000; Schäfer et al. 2008; Li et al. 2010).

R_s needs to be determined for each substance, which can be time and resource-consuming if a large number of substances is analyzed (Harman et al. 2012).

However, adding chemical standards called performance reference compounds (PRCs) to the receiving phase prior to exposure of the passive sampler has been suggested as a means to calibrate the exchange rates *in situ* (Booij et al. 1998; Huckins et al. 2002; Harman et al. 2012; Mills et al. 2014). The PRCs are typically deuterated or C^{13} labelled chemical standards. The use of isotopically labeled PRCs in an *in situ* calibration method was first introduced for SPMDs but has later been applied to other sampling devices (Huckins et al. 2002; Bartkow et al. 2006; Vrana et al. 2006; Shaw et al. 2009).

Even though passive sampling devices have been available for several years, they are still poorly characterized in terms of modeling the uptake rates and the effects of environmental factors (Harman et al. 2012). Additionally, there are no theoretical models available to predict the uptake of a chemical into POCIS or Chemcatcher based on the physicochemical properties of a compound. Therefore, an extensive laboratory experiment is required to estimate the uptake rate of each compound. An ISO standard has been published (ISO 2011) to guide the application of passive samplers for monitoring of metals and organic pollutants in surface waters (Mills et al. 2011).

3.6.2 Chemcatcher®

The passive sampler Chemcatcher is designed for both hydrophobic and hydrophilic substances and can be adapted to organic, organometallic, and inorganic contaminants depending on the receiving phase and diffusion membrane. The first application of Chemcatcher was reported for organic and later for inorganic contaminants (Kingston et al. 2000; Björklund Persson et al. 2001). Chemcatcher passive samplers have been used for *e.g.*, polar herbicides (Stephens et al. 2005; Tran et al. 2007) and pesticides (Gunold et al. 2008) but rarely to determine the concentrations of pharmaceuticals in aquatic environments (Vermeirssen et al. 2009). For example, metals including mercury, organotin compounds, PAHs, and nonylphenol ethoxylates have been studied by using the Chemcatcher passive sampler (Vrana et al. 2006; Allan et al. 2007; Aguilar-Martínez et al. 2009; Ahkola et al. 2014).

The Chemcatcher is composed of a receiving phase (Empore disk) and a membrane with three different housing geometries (Charriau et al. 2016). The device is composed of two parts made of PTFE or PC, which are screwed or clipped together to seal the disk and the membrane (Kingston et al. 2000; Vrana et al. 2005). Additionally, a copper mesh can be added to protect the disk from mechanical damage and from biofilm development, even though nowadays it is only rarely used. In all designs, a lid is used to protect the disk during transport prior to the deployment.

Four types of Empore disks have been used as receiving phases: C18, SDB-RPS, SDB-XC, and chelating disks (Charriau et al. 2016). A C18 silica sorbent bonded with octadecylgroups is suitable for low polarity or nonpolar compounds, while SDB-RPS (styrenedivinylbenzene-reverse phase sulfonated) copolymer is modified with sulfonic acid groups to make it hydrophilic. The SDB-XC

(styrenedivinylbenzene-exchange) copolymer is used as a reversed phase sorbent. SDB-RPS and SDB-XC contain spherical, porous, and cross-linked copolymer particles suitable for both polar and low-polarity compounds. Chelating disks are made of a polystyrene-divinylbenzene copolymer modified with iminodiacetic acid groups and are suitable for metals. The receiving phases can be overlaid with different types of membranes or applied without (Stephens et al. 2009). Membranes are often made of cellulose acetate (CA), low-density polyethylene (LDPE), polysulfone (PS) or polyethersulfone (PES) (Kingston et al. 2000).

For Chemcatcher, the membrane is optional but can be used to extend the linear uptake phase (Moschet et al. 2015). A membrane can be used to protect the disk, bring selectivity of the accumulated compounds, and control the uptake of analytes (Kingston et al. 2000; Schäfer et al. 2008; Harman et al. 2009). Empore disks collect a wider range of chemicals with higher values of R_s when used without a membrane (Vermeirssen et al. 2012). High values of R_s are suitable with the sampling time of a few days and for monitoring of selected peak events (Camilleri et al. 2012; Vermeirssen et al. 2013). However, a diffusion-limiting membrane extends the time until the receiving phase reaches equilibrium with its surroundings (Kingston et al. 2000).

Depending on the polarity of organic compounds, Chemcatcher (for SDB-XC or SDB-RPS with PES membrane) can be compared to POCIS (Oasis HLB sorbent) and SPMD (for the C18 with LDPE membrane). Only a few studies (de la Cal et al. 2008; Allan et al. 2009; Vermeirssen et al. 2012; Jacquet et al. 2014) have been conducted by comparing the performances of the passive samplers and investigating the various uptake kinetics and R_s (Vermeirssen et al. 2012; Jacquet et al. 2014). R_s values differ because of the intrinsic characteristics of each device: material for the receiving phase and membrane, diffusion and accumulation mechanisms, and sampling area. Chemcatchers are shown to have slightly higher values of R_s compared to POCIS when differences in sample surface areas have been taken into account (Vermeirssen et al. 2012). However, in both sampler types, a similar number of analytes is likely to accumulate in SDB disks (Moschet et al. 2015).

4 EXPERIMENTAL

4.1 Sampling

4.1.1 Wastewater influents and effluents

Samples were collected from four municipal wastewater treatment plants (in Saarijärvi, Äänekoski and Jyväskylä) in central Finland (Fig. 8, publications **III-V**). The samples were taken as pooled 24-h composite samples from influents and effluents. Information about the WWTPs, including treatment processes, inhabitants served, and efficiencies are presented in Table 12.

TABLE 12 Properties of WWTPs selected for sampling

WWTPs:	**Saarijärvi (Pylkönmäki)**	**Saarijärvi (Saarilampi)**	**Äänekoski (Teräväniemi)**	**Jyväskylä (Nenäinniemi)**
WW load	250 m^3 d^{-1}	440000 m^3 a^{-1}, 1200 m^3 d^{-1}	5700 m^3 d^{-1}	$13 \cdot 10^6$ m^3 a^{-1}, 35600 m^3 d^{-1}
Inhabitants served	400	5600	15000	150000
Process	Aerobic biological with chemical coagulation	Aerobic biological with chemical coagulation	Aerobic biological with chemical coagulation	Aerobic biological with chemical coagulation
Chemicals	$FeSO_4$ for phosphorous removal	$FeSO_4$ (213 g m^{-3}) for coagulation	$FeSO_4$ for phosphorous removal, polyelectrolyte Zetag 8140 for flocculation	$FeSO_4$ (120 g m^{-3}) for phosphorous removal, polyaluminum chloride and polymer for coagulation
Efficiency	BOD_7 ≥ 93 % P ≥ 93 % Total solids 97 %	BOD_7 ≥ 95 % P ≥ 95 % Total solids ≥ 90 %	BOD_7 97 % P 96 % Total solids ≥ 90 %	BOD_7 98 % P 96 % Total solids 96 %
Effluent discharged	Lake Karankajärvi and River Leuhunjoki	Lake Karankajärvi and River Leuhunjoki	Lake Kuhnamo	Northern Lake Päijänne

BOD_7: biological oxygen demand in seven days
P: removal of phosphorous
Zetag 8140: Solid grade cationic polyelectrolyte (Copolymer of acrylamide and quaternized cationic monomer)

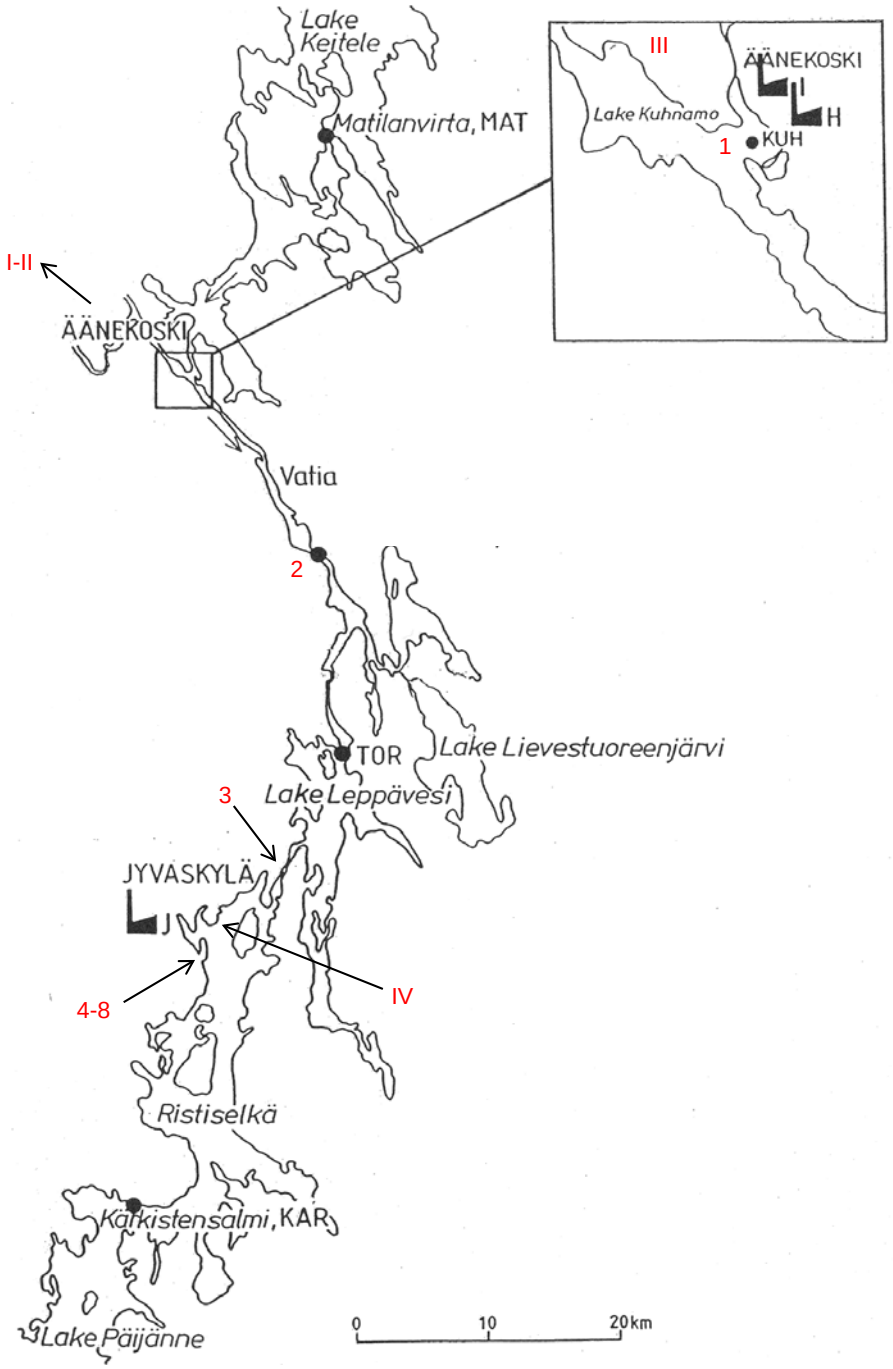


FIGURE 8 Sampling sites (I-IV) indicate WWTPs Pylkönmäki (I), Saarilampi (II), Äänekoski (III), Jyväskylä (IV) and water sampling sites in Lake Kuhnamo (1), Kapeenkoski Rapids (2), Haapakoski Rapids (3) and northern Lake Päijänne (4-8), Herve (1991)

4.1.2 Surface water sampling

Water samples were collected with a Ruttner water sampler from Lake Kuhnamo, Kapeenkoski Rapids, Haapakoski Rapids (Fig. 8), northern Lake Päijänne (surface area 1080 km^2, average depth 16.2 m, Fig. 9), and the River Vantaa (Fig. 10; Table 16). From northern Lake Päijänne, water samples were collected from five locations (sites 4-8, Fig. 9) and from two or three depths (1 m, 5 m/10 m and 14 m/20 m), depending on the depth of water every two weeks (**III**). Passive samplers were deployed in Lake Päijänne (sites 4, 6-8, Fig. 9), and duplicate samplers were exposed at a depth of 1 m for two weeks at a time (**V**). Water samples were collected before and after the deployment time of passive samplers. Later, water samples were collected from Lake Päijänne, Lake Kuhnamo, Kapeenkoski Rapids, and Haapakoski Rapids (sites 1-8, Figs. 8 and 9) in the winter when the lakes were covered with ice (**IV**). All water samples were collected to glass bottles but stored as frozen in HDPE bottles at −18 °C before analysis. Environmental conditions in Lake Päijänne over the time of sampling are collected in Table 13.

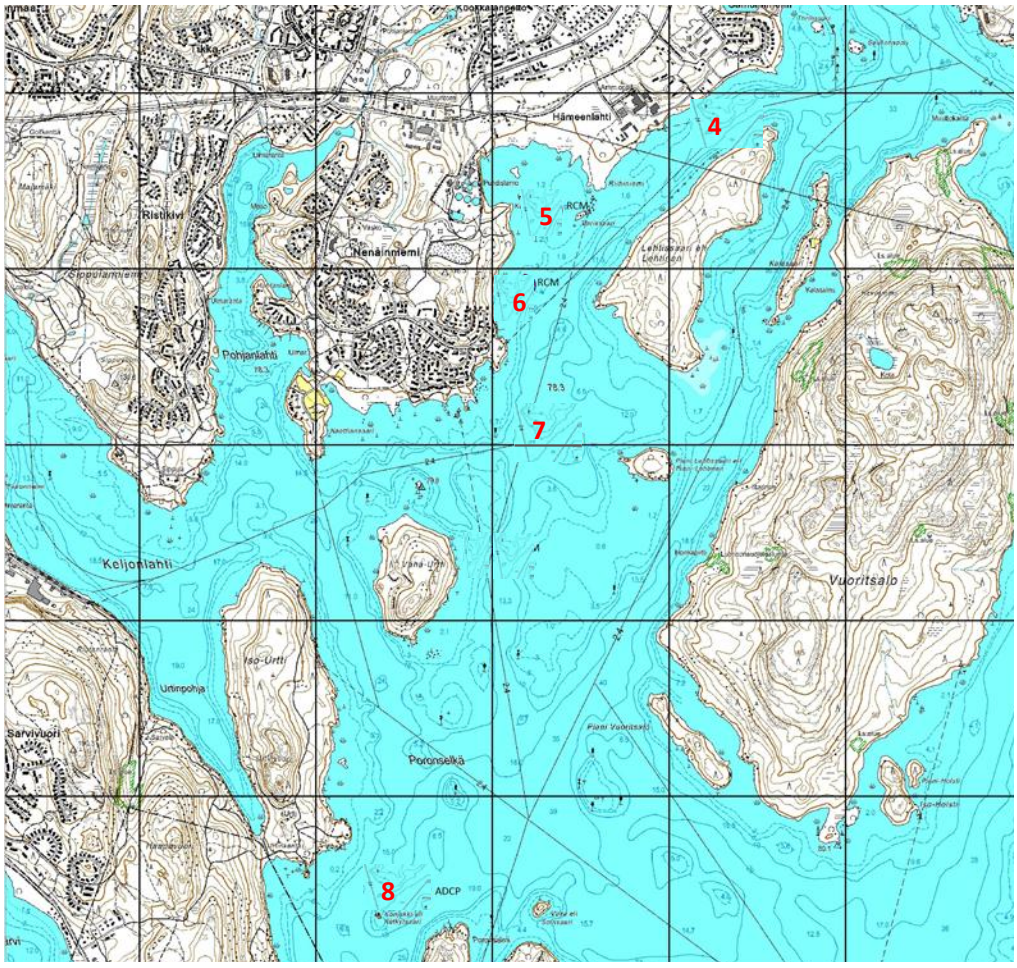


FIGURE 9 Sampling sites (sites 4-8) in northern Lake Päijänne

Sedimented particle samples were collected from sampling sites 4, 6-8 (Fig. 9) by deploying sedimentation tubes. Sedimentation tubes were 50 cm long (Ø 9.3

cm, 67.9 cm^2) in which the particle matter in water settles. Every 2 weeks, the tubes were emptied, particles collected, and the tubes were redeployed. Samples were collected to plastic HDPE bottles and stored at −18 °C.

TABLE 13 Conditions of Lake Päijänne and Jyväskylä region, summer 2013 and winter 2015[a]

Month	June 2013	July 2013	August 2013	March 2015
Temperature[b]	16.8	15.8	15.0	-0.4
Rain/snowfall (mm)[b,c]	95	76	89	46
Solar radiation (kWh m^{-2})	5.4	5.2	3.6	1.9
Water temperature, 1 m (°C)[d]	20.2	18.0	20.0	0.4-0.5
Secchi depth (m)[d]	1.6-1.8	1.6-1.7	1.8-2.0	2.6-2.8
Water color (mg Pt L^{-1})	nd	nd	50-70	60-70
Turbidity (FNU)	nd	nd	1.5-1.7	0.52-0.53

[a] HERTTA (2015)
[b] Average temperature, Jyväskylä Airport, 2013, 2015
[c] Cumulative
[d] Values measured from the deepest sampling site at Lake Päijänne (41.5 m)
nd - not defined

From the River Vantaa, grab water samples (500 mL) were collected with a Ruttner water sampler from four locations (sites V1-V4), six from each location, from a depth of 1 m (Fig. 10, **V**). Additionally, long-term concentrations were monitored by using passive sampling with the deployment time of two weeks. The samples were taken in August 2013 and again in April-May 2015. Average flow velocities along the River Vantaa are listed in Table 14. Duplicate passive samplers were deployed at four sampling sites (V1-V4) in the River Vantaa at a depth of 1 m for two weeks (Fig. 10). Water samples were taken in glass bottles before and after each deployment time period of the passive samplers. All samples were frozen in HDPE bottles at -18 °C in the laboratory before analysis.

TABLE 14 Average flow velocities (min…max) of the River Vantaa (m^3 s^{-1}) in August 2013, April and May 2015 (OIVA 2015)

Distance from the sea shore (km)	August 2013	April 2015	May 2015
64	1.3 (0.9-2.3)	7.8 (3.0-14.2)	6.8 (2.4-12.2)
39	1.3 (0.9-2.3)	7.8 (3.0-14.2)	6.8 (2.4-12.2)
15	3.2 (2.0-6.8)	19.6 (6.4-52)	14.4 (4.8-41)
1	4.8 (3.0-10.1)	28 (8.4-75)	19.6 (6.7-55)

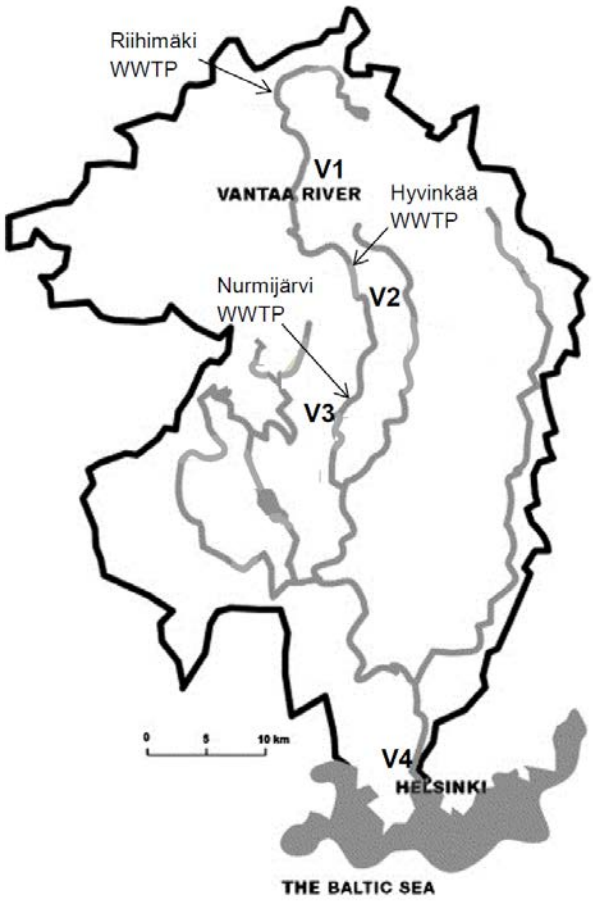


FIGURE 10 Sampling sites (V1-V4) along the River Vantaa and the municipal WWTPs of Riihimäki, Hyvinkää and Nurmijärvi

There are several municipal wastewater treatment plants along the River Vantaa. One of them, Riihimäki WWTP (Fig. 10, Table 15) treats the municipal wastewater of 28 000 residents (12 600 m^3 d^{-1}) and industrial wastewater from a dairy product producer. Its nitrogen removal is based on a biological denitrification-nitrification process with a tertiary treatment stage based on phosphorous precipitation mainly by ferrosulphate.

TABLE 15 Average discharge of effluent (m^3 d^{-1}) from the WWTPs along the River Vantaa in August 2013, April and May 2015 (OIVA 2015) and the share (%) of effluent compared to the total water flow at the point of discharge

WWTP	August 2013	April 2015	May 2015
Riihimäki	11300	16200	14500
Hyvinkää	9100	13100	12100
Nurmijärvi	1600	2700	3000
% of river flow	19.6	4.7	5.1

Hyvinkää WWTP (Fig. 10, Table 15) serves 32 900 residents and treats 10 300 m^3 d^{-1} of municipal wastewater (Kalteva WWTP 2011). The treatment process is based on chemical and biological nitrification-denitrification with chemical precipitation of phosphorous by ferrosulphate. Nurmijärvi WWTP (Fig. 10, Table 15) treats municipal wastewater (2 700 m^3 d^{-1}) of 5 800 residents (Nurmijärvi WWTP 2011). The treatment process includes biological nitrogen removal combined with chemical precipitation by ferrosulphate.

Altogether, samplings from different sampling sites, times, and by varying methods are listed in Table 16.

TABLE 16 Samplings of wastewaters, surface waters, and sedimented particles collected during the study

Type of sample	Sampling site	Sampling time	Procedure	Publication
Municipal wastewater influent and effluent	Jyväskylä (Nenäinniemi)	May 2013 - Sep 2013	24-h composite sampling	III,IV
		Mar 2015	24-h composite sampling	IV
	Äänekoski	Mar 2015	24-h composite sampling	IV
	Saarijärvi (Saarilampi)	Mar 2015	24-h composite sampling	IV
	Saarijärvi (Pylkönmäki)	Mar 2015	24-h composite sampling	IV
Surface water	Lake Päijänne	May 2013 - Sep 2013	Grab sampling, Passive sampling	III V
		May 2015	Grab sampling	IV
	Lake Kuhnamo	May 2015	Grab sampling	IV
	Kapeenkoski Rapids	May 2015	Grab sampling	IV
	Haapakoski Rapids	May 2015	Grab sampling	IV
	River Vantaa	Aug 2013, Apr-May 2015	Grab sampling, Passive sampling	V
Sedimented particles	Lake Päijänne	May 2013 - Sep 2013	Composite sampling (2 weeks)	III

4.2 Analytical methods

4.2.1 Sample preparation

The methods of pretreatment and chromatographic separation of wastewater, surface water, sedimented particle samples and passive samplers are listed in Tables 17-20 (**III-V**). All chemicals used in this study are listed in Appendix I.

The standard solutions (100 µg mL^{-1}) of analytical standards carbamazepine, diclofenac, ibuprofen, ketoprofen, and naproxen were prepared by diluting an accurate amount of pure standard in HPLC grade methanol and filtered with membrane filter 0.2 µm pore size, ME 24.

Water and wastewater samples
The samples were concentrated with SPE cartridges, which were conditioned with 3 mL methanol followed by 3 mL UHQ water before loading the samples. The analytes were eluted with 3 mL of acetone and the extracts evaporated to dryness under a stream of nitrogen. Subsequently, they were redissolved in 300 µL of methanol and water (1:1, v/v), filtered through syringe cartridges and analyzed immediately with LC-MS/MS (Table 17).

Sedimented particles
Sedimented particles were extracted in an ultrasonic bath with 20 mL of solvent. The slurry of sedimented particles and solvent were first thoroughly hand shaken and then ultrasonicated for 45 min. The slurries were centrifuged for 15 min at 3000 rad min^{-1}. The procedure was repeated three times by using UHQ water, hexane, and acetone as solvents. The supernatant of solvents were collected, combined, and evaporated to a small volume by a rotary evaporator. The samples were then filtrated through syringe cartridges and evaporated to dryness under a stream of nitrogen. Finally, they were redissolved in 300 µL of methanol and water (1:1, v/v) and analyzed with LC-MS/MS (Table 18).

Passive sampling
In passive sampling, a SDB-RPS Empore disk (diameter 47 mm, surface area 15.9 cm^2) was used as a receiving phase. It was conditioned before use by immersing in methanol followed by immersing in UHQ water (Vermeirssen et al. 2009). The conditioned disk was fitted in a polycarbonate Chemcatcher sampler housing, which was kept in methanol overnight and rinsed with UHQ water, closed and stored in zip-lock bags at 4 °C until exposure. After the deployment time, the SDB-RPS disk was carefully removed from the sampler into Kimax tube and stored at -18 °C before analysis.

For analysis, the Empore disks were eluted with 10 ml of acetone in an ultrasonic bath for 10 minutes. The solvent was collected and the procedure repeated by using 10 mL of methanol. The solvents were combined and evaporated to a small volume by a rotary evaporator. The samples were then filtrated through syringe cartridges and evaporated to dryness under a stream of nitrogen. Finally, they were redissolved in 300 µL of methanol and water (1:1, v/v) and analyzed with LC-MS/MS (Table 19).

TABLE 17 Methods used for analysis of wastewater and surface water samples

	Method 1	**Method 2**
Pharmaceuticals	Diclofenac, Ibuprofen, Ketoprofen, Naproxen	Carbamazepine
Influent/Effluent volume	300 mL	300 mL
Surface water volume	300 mL	300 mL
SPE sorbent	C 18 LO (500 mg)[a,c]/ C 18 (500mg)[b,d]	C 18 LO (500 mg)[a,c]/ C 18 (500mg)[b,d]
Sorbent preconditioning	3 mL MeOH, 3 mL H_2O	3 mL MeOH, 3 mL H_2O
Elution	3 mL ACT	3 mL ACT
Evaporation	Under stream of N_2	Under stream of N_2
Sample filtration	0.45µm PTFE[e]	0.45µm PTFE[e]
Sample	300 µL (MeOH:H_2O, 1:1)	300 µL (MeOH:H_2O, 1:1)
HPLC column	Zorbax Eclipse Plus C18 (1.8 µm, 2.1×50 mm)[d]	Zorbax Eclipse Plus C18 (1.8 µm, 2.1×50 mm)[d]
HPLC Guard column	Zorbax Eclipse Plus C18 (5 µm 2.1×12.5 mm)[d]	Zorbax Eclipse Plus C18 (5 µm 2.1×12.5 mm)[d]
Injection volume	15 µL	15 µL
Flow rate	0.2 mL/min.	0.2 mL/min.
Eluents	A: 10 mM NH_4OH (aq) B: 9 ACN : 1 10 mM NH_4OH (aq)	A: 0.5 % AA (aq) B: MeOH
Elution	7 % B→ 3 min. 10 % B →14 min. 60 % B → 15 min. 60 % B → 16 min. 7 % B	0 → 1 min. 3 % B, 97 % A → 12 min. 80 % B → 15 min. 80 % B → 16 min. 3 % B

[a] Paper III
[b] Paper IV, V
[c] Varian
[d] Agilent Technologies
[e] Cronus

AA acetic acid
ACN acetonitrile
ACT acetone
MeOH methanol
NH_4OH ammonium hydroxide

TABLE 18 Methods used for analysis of sedimented particle samples

	Method 1	**Method 2**
Pharmaceuticals	Diclofenac, Ibuprofen, Ketoprofen, Naproxen	Carbamazepine
Sedimented particle sample	1 g, o.d.	1g, o.d.
Eluents	20 mL H_2O, 20 mL Hex, 20 mL ACT	20 mL H_2O, 20 mL Hex, 20 mL ACT
Extraction	3× ultrasonicated (45 min.), centrifuged (15 min. 3000 rad min^{-1})	3× ultrasonicated (45 min.), centrifuged (15 min. 3000 rad min^{-1})
Sample filtration	1.2 µm GF[a]	1.2 µm GF[a]
Evaporation	First to small volume with rotary evaporator, until dry with N_2 gas	First to small volume with rotary evaporator, until dry with N_2 gas
Sample	300 µL (MeOH:H_2O, 1:1)	300 µL (MeOH:H_2O, 1:1)
HPLC column	Zorbax Eclipse Plus C18 (1.8 µm, 2.1×50 mm)[b]	Zorbax Eclipse Plus C18 (1.8 µm, 2.1×50 mm)[b]
HPLC Guard column	Zorbax Eclipse Plus C18 (5 µm 2.1×12.5 mm)[b]	Zorbax Eclipse Plus C18 (5 µm 2.1×12.5 mm)[b]
Injection volume	15 µL	15 µL
Flow rate	0.2 mL/min.	0.2 mL/min.
Eluents	A: 10 mM NH_4OH (aq) B: 9 ACN : 1 10 mM NH_4OH (aq)	A: 0.5 % AA (aq) B: MeOH
Elution	7 % B → 3 min. 10 % B →14 min. 60 % B → 15 min. 60 % B → 16 min. 7 % B	0 → 1 min. 3 % B, 97 % A → 12 min. 80 % B → 15 min. 80 % B → 16 min. 3 % B

[a] Cronus
[b] Agilent Technologies
o.d. oven dry

AA acetic acid
ACN acetonitrile
ACT acetone
Hex hexane
MeOH methanol
NH_4OH ammonium hydroxide

TABLE 19 Methods used for analysis of passive samplers

	Method 1	Method 2
Pharmaceuticals	Diclofenac, Ibuprofen, Ketoprofen, Naproxen	Carbamazepine
Receiving phase	SDB-RPS Empore disk	SDB-RPS Empore disk
Chemcatcher body	polycarbonate	polycarbonate
Eluents	10 mL ACT, 10 mL MeOH	10 mL ACT, 10 mL MeOH
Extraction	2× ultrasonicated (10 min.)	2× ultrasonicated (10 min.)
Sample filtration	0.45 µm PVDF[a]	0.45 µm PVDF[a]
Evaporation	until dry with N_2 gas	until dry with N_2 gas
Sample	300 µL (MeOH:H_2O, 1:1)	300 µL (MeOH:H_2O, 1:1)
HPLC column	Zorbax Eclipse Plus C18 (1.8 µm, 2.1×50 mm)[b]	Zorbax Eclipse Plus C18 (1.8 µm, 2.1×50 mm)[b]
HPLC Guard column	Zorbax Eclipse Plus C18 (5 µm 2.1×12.5 mm)[b]	Zorbax Eclipse Plus C18 (5 µm 2.1×12.5 mm)[b]
Injection volume	15 µL	15 µL
Flow rate	0.2 mL/min.	0.2 mL/min.
Eluents	A: 10 mM NH_4OH (aq) B: 9 ACN : 1 10 mM NH_4OH (aq)	A: 0.5 % AA (aq) B: MeOH
Elution	7 % B → 3 min. 10 % B →14 min. 60 % B → 15 min. 60 % B → 16 min. 7 % B	0 → 1 min. 3 % B, 97 % A → 12 min. 80 % B → 15 min. 80 % B → 16 min. 3 % B

[a] Cronus
[b] Agilent Technologies

AA acetic acid
ACN acetonitrile
ACT acetone
Hex hexane
MeOH methanol
NH_4OH ammonium hydroxide

4.2.2 Instrumental analysis

The chromatographic separation was performed with an Agilent 1290 Infinity series liquid chromatograph connected to a 6460 Triple Quad triple-quadrupole mass analyzer (MS/MS) equipped with an electrospray ionization source (Agilent Technologies). Argon was used as the collision gas and nitrogen as a desolvation gas with a temperature of 300 °C and a flow rate of 300 L h^{-1}. The capillary voltage was set to 4000 V for acids and 3500 V for basics. The mass spectrometer was operated in the MRM mode and the mass transitions, cone voltages, and collision energies were optimized for each analyte (Table 20). External standard method was used for the quantification of the studied pharmaceuticals.

TABLE 20 Tandem mass spectrometric parameters

	Ionization mode	Cone voltage (V)	Collision Energy (eV)	Precursor ion(s), (m/z)	Product ion(s), (m/z)
Carbamazepine	ESI (+)			237.1; 238.2	194.1, 193.1, 192.1, 179.1
Diclofenac	ESI (-)	5	0	294.0; 296.0	250.0, 252.0
Ibuprofen	ESI (-)	3	0	205.2	161.2
Ketoprofen	ESI (-)	3	0	253.1	209.1
Naproxen	ESI (-)	5	0, 8, 20	229.1	185.1, 170.1, 169.1

4.3 Calibration experiment

A calibration experiment was performed in order to determine the R_s of each studied pharmaceutical. Ten passive samplers were exposed in a constant concentration system under controlled conditions of temperature, water turbulence, and analyte concentration. The experiment was performed in a dark room at 18 °C. The system consisted of a 31 L cylindrical glass tank filled with 19 L of UHQ water spiked with a 100 µL of selected pharmaceutical standards in methanol. The nominal concentration of 500 ng L^{-1} was maintained throughout the experiment.

The spiked UHQ water was renewed every 3 or 4 days. Grab samples of 500 mL were collected from the exposure tank and the concentrations of pharmaceuticals were measured before and after each renewal. Ten samplers were tied to an overhead stirrer with cable ties, two in each tie. The holder was interconnected to an overhead stirrer and it was rotated at a constant stirring speed of 90 rpm (33 cm s^{-1}). The exposure took place for 14 days expecting a linear uptake. Duplicate samplers were removed after 3, 6, 8, 10 and 14 days, and the concentrations of accumulated chemicals were determined. The values of R_s were calculated according to Eq. 4.

5 RESULTS AND DISCUSSION

The more detailed results are available in papers **III-V**. In this section, the results are briefly summarized.

5.1 Quantification

The purpose of the method development was to find a suitable procedure for the quantification of the selected anti-inflammatory and antiepileptic drugs in different media. Additionally, methods for the extraction of water samples, sedimented particles, and passive samplers were developed.

In the method development (**III**), the methods were evaluated for precision (*i.e.*, intra-day, interday), repeatability, linearity, level of detection (LOD), and level of quantification (LOQ). The analysis method, including the extraction phase, was validated by performing experiments with water samples spiked with standards (recoveries listed in table 21).

TABLE 21 Absolute recoveries (%) of spiked pharmaceuticals in effluent, surface water and passive samplers

Pharmaceutical	Recovery of effluent (%)	Recovery of surface water (%)	Recovery of passive samplers (%)
Carbamazepine	75	90	93
Diclofenac	81	88	91
Ibuprofen	87	91	91
Ketoprofen	77	86	87
Naproxen	81	89	89

The linearity of the method was evaluated by plotting the correlation between peak areas and concentrations of the standard solutions from 10 ng mL^{-1} to 100 µg mL^{-1} on a line for each pharmaceutical. Each standard curve included five points and was measured on three different days. Linear regression analysis was performed for each standard curve, which led to the regression parameters listed in Table 22. Errors in the slope and intercept of the regression lines were calculated at a confidence interval of 95 %.

The results of regression coefficients were close to 1, which indicates a good fit of the actual data points with the calculated standard curves. Because internal standards were not available, the slopes of linearity analysis were later used for the calculations of the sample concentrations (**III-V**).

The LODs and LOQs were determined in the MRM mode (**III, V**). They were based on a signal to noise (S/N) ratio of 3 and 10 for LOD and LOQ, respectively. In the case of water samples, the LOD and LOQ were further calculated for 300 mL, which was the volume of each water sample. The LOD and LOQ of

passive samples were calculated by converting the instrumental value to ng per sampler and further to ng L^{-1} by using the sampling rate and the deployment time of 14 days (Eq. 4). The results for LOD and LOQ of each pharmaceutical in water samples and in passive samplers are presented in Table 22 and in good agreement with those reported earlier (*e.g.*, Hernando et al. 2006; Wille et al. 2010).

TABLE 22 Regression coefficients, LODs, and LOQs (ng L^{-1}) of water samples and passive samples

Pharmaceutical	**Linearity**[a] (regression coefficient r)	**LOD**		**LOQ**	
		Water samples	Passive samples	Water samples	Passive samples
Carbamazepine	0.998	0.1	0.1	0.1	0.1
Diclofenac	0.973	7.5	38	25	130
Ibuprofen	0.993	3.7	9.7	12	32
Ketoprofen	0.998	1.5	6.6	5.1	22
Naproxen	0.996	2.8	7.0	9.2	23

[a] Experimental data fitted in a linear mode, concentration range from 10 ng mL^{-1} to 100 µg mL^{-1}

The precision of the method and possible daily variations were evaluated at low and high concentration levels by making repeated analyses on different days. A single factor analysis (ANOVA) was used to evaluate the intraday and interday precisions (**III**). A standard mixture of selected pharmaceuticals was analyzed six times (n=6) during 3 days. The results were used to calculate the intraday and interday precisions (Table 23). The intraday (Eq. 5) and interday (Eq. 6) precisions were expressed as relative standard deviation (RSD), where σ_r is the residual error, σ_A error due to day factor and $\bar{x}$ the average (Destandau et al. 2005; Käkölä and Alén 2006).

$$RSD_{\text{int}\,raday}(\%) = \frac{\sigma_r}{\bar{x}} 100\ \% \quad (5)$$

$$RSD_{\text{int}\,erday}(\%) = \frac{\sigma_A}{\bar{x}} 100\ \% \quad (6)$$

TABLE 23 Interday (n=3) and intraday (n=6) precision

Pharmaceutical	Low concentration level		High concentration level	
	RSD (%) intraday	**RSD (%) interday**	**RSD (%) intraday**	**RSD (%) interday**
Carbamazepine	3.2	10.3	5.1	11
Diclofenac	1.9	7.1	7.7	14
Ibuprofen	2.2	4.5	5.3	8.9
Ketoprofen	6.9	6.1	6.1	22
Naproxen	7.9	4.0	9.4	6.8

Overall, the results were satisfactory, indicating good method precision. The linearity of the methods was also evaluated giving values of regression coefficients (r) between 0.97-0.99, respectively.

5.2 Matrix effects

Nowadays, triple quadrupole mass spectrometry with ESI is the most commonly used method for the detection of pharmaceuticals in environmental matrices (Table 11). It allows selective and sensitive quantification of pharmaceuticals. However, analyte signals can be suppressed or, in some cases, enhanced by matrix constituents and insufficient analyte purification (Petrović et al. 2005; Hernando et al. 2004; Wille et al. 2010).

In this study, signal suppression was determined for the pharmaceuticals by using the standard addition method. The SPE extracts of surface water, influent and effluent of municipal wastewater were spiked with pharmaceutical standards with concentration of 50 µg L^{-1} and compared with the signals of unspiked and standard solutions. The signal suppression was calculated according to Eq. 7 (Vieno et al. 2006b; Garcia-Ac et al. 2009).

$$Matrix\ effect\ (\%) = \frac{A_s - (A_{SP} - A_{USP})}{A_s} 100\% \quad (7)$$

A_S is the peak area of the spiked standard solution; A_{SP} denotes the peak area of the spiked surface water or wastewater sample, and A_{USP} the peak area of the un-spiked sample. Values less than hundred indicate signal suppression and those over hundred, signal enhancement (Garcia-Ac et al. 2009). Values of matrix effects and retention times of each studied pharmaceuticals are listed in Table 24.

TABLE 24 Retention times of each pharmaceutical (min) in LC-MS/MS analysis and matrix effects (%) of surface water, influent and effluent samples

Pharmaceutical	Retention time (min)	Influent (%)	Effluent (%)	Surface water (%)
Carbamazepine	10.2	85	86	89
Diclofenac	8.9	88	88	97
Ibuprofen	8.0	92	93	99
Ketoprofen	6.6	90	90	96
Naproxen	6.5	89	90	98

Minor signal suppression was detected in influent and effluent samples and even smaller in surface waters (Table 24). The highest signal suppression was detected for carbamazepine. For example, Vieno (2007) reported signal suppression of a variety of pharmaceuticals, particularly carbamazepine. Carbamazepine is eluted at the longest retention time, consistent with the previous findings, which indicate that late eluting compounds are more prone to matrix

interferences (Antignac et al. 2005). However, in case of acidic drugs, decreasing signal suppression with increasing retention times has been reported (Quintana and Reemtsma 2004). On the other hand, Wille et al. (2010) found negligible matrix effects of carbamazepine, diclofenac, and ketoprofen. In this case, the observed matrix effects of all selected pharmaceuticals were relatively small, and the results can be considered reliable.

5.3 Pharmaceuticals at municipal WWTPs

The selected pharmaceuticals (carbamazepine, diclofenac, ibuprofen, ketoprofen, and naproxen) were detected in the influents and effluents of the WWTPs I-IV (Appendices II, IV). The mean concentrations in the influents and effluents both in summer and in winter seasons are depicted in Figs. 11-14.

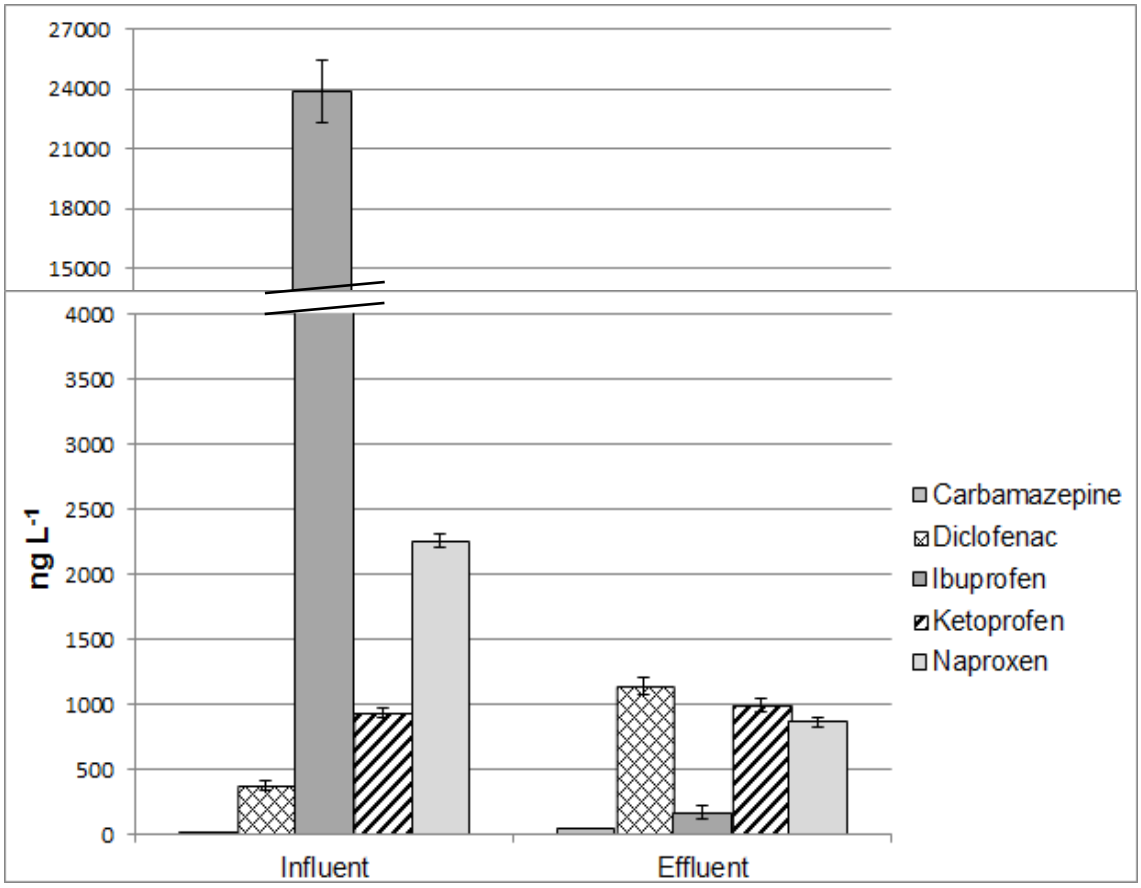


FIGURE 11 Mean concentrations of selected pharmaceuticals in influents and effluents of the WWTP of Jyväskylä (IV), summer 2013.

Ibuprofen was the major pharmaceutical in the influent, but only a small proportion was detected in the effluent (Figs. 11 and 12). Ibuprofen forms carboxyhydratropic acid, hydroxy- and carboxy-ibuprofen in the human body, which can be found among the parent compound from the untreated sewage (Buser et al. 1999; Heberer 2002). A significant proportion of ibuprofen and especially of carboxy ibuprofen is removed during the sewage treatment process, and even above 98 % removal rates have been reported (Stumpf et al. 1998; Quintana et al.

2005). However, concentrations of hydroxy-ibuprofen in the effluent are close to those of influents. The results suggest that the major part of ibuprofen in the influent is converted and altered to other conjugates of ibuprofen during the sewage treatment, mostly via microbial processes.

As shown in Figures 11 and 13, the concentrations of ibuprofen and naproxen decreased during the treatment processes, while those of diclofenac and carbamazepine were higher in effluent than in influent (Figs. 13 and 14). Most likely, this was due to enzymatic cleavage of the glucuronic conjugates. Pharmaceuticals are mainly excreted as biologically active metabolites, which originate from biochemical oxidation and reduction reactions or form conjugated metabolites (Holčapek et al. 2008). The conjugates are degraded and transformed during the wastewater treatment processes, typically during biological treatment back to their parent compounds. When the conjugates are degraded and, *e.g.*, glucuronic moiety is cleaved, higher concentrations can be detected. Typically, diclofenac and carbamazepine are excreted as conjugates, which deconjugate back to parent compounds during wastewater treatment explaining their higher concentrations in effluents (Ferrer and Thurman 2010; Fig. 14). Similar results have previously been reported by *e.g.*, Ternes (1998); Vieno et al. (2007) and Behera et al. (2011). The cleavage of adduct occurs during the wastewater treatment showing higher amounts of parent compounds in the effluent.

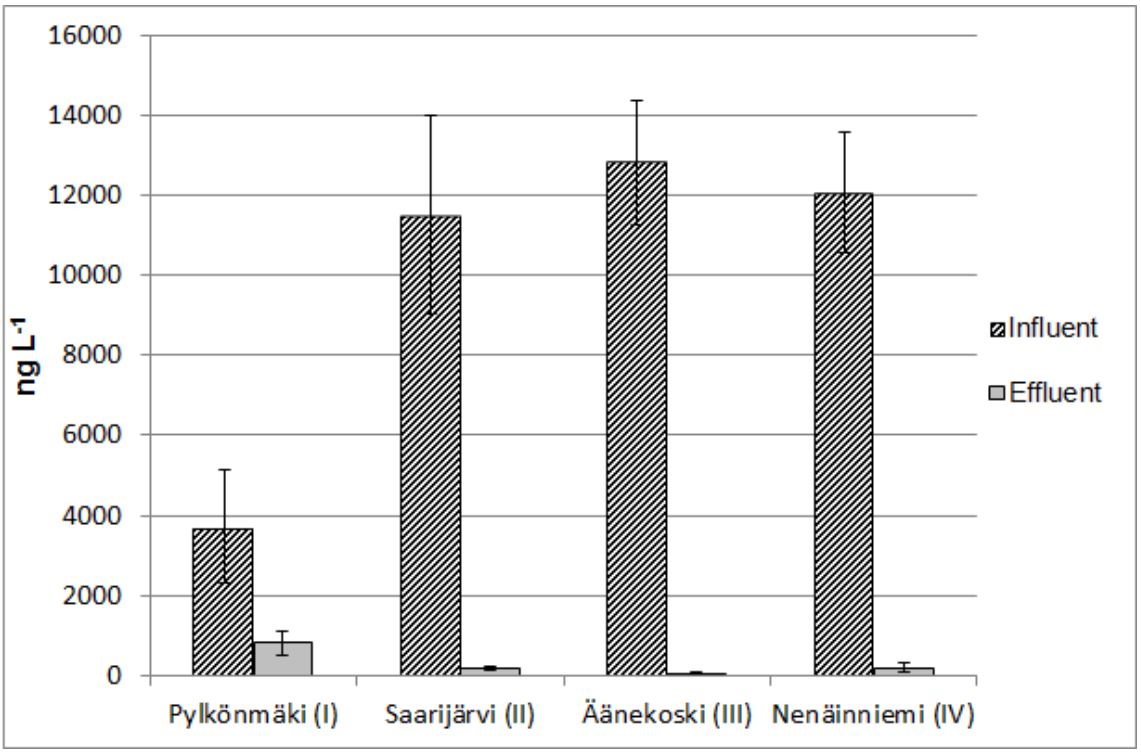


FIGURE 12 Mean concentrations of ibuprofen in influents and effluents at WWTPs I-IV, winter 2015

In addition, carbamazepine is fairly resistant toward treatment processes, leading to low or moderate removal of carbamazepine (Ternes 1998; Quintana et al. 2005). For example, 23-26 % removal of carbamazepine has been reported

(Quintana et al. 2005; Behera et al. 2011). The low removal efficiency of carbamazepine is caused by its persistent properties, water solubility, and its high persistency to biodegradation (Andreozzi et al. 2003; Quintana et al. 2005; Behera et al. 2011).

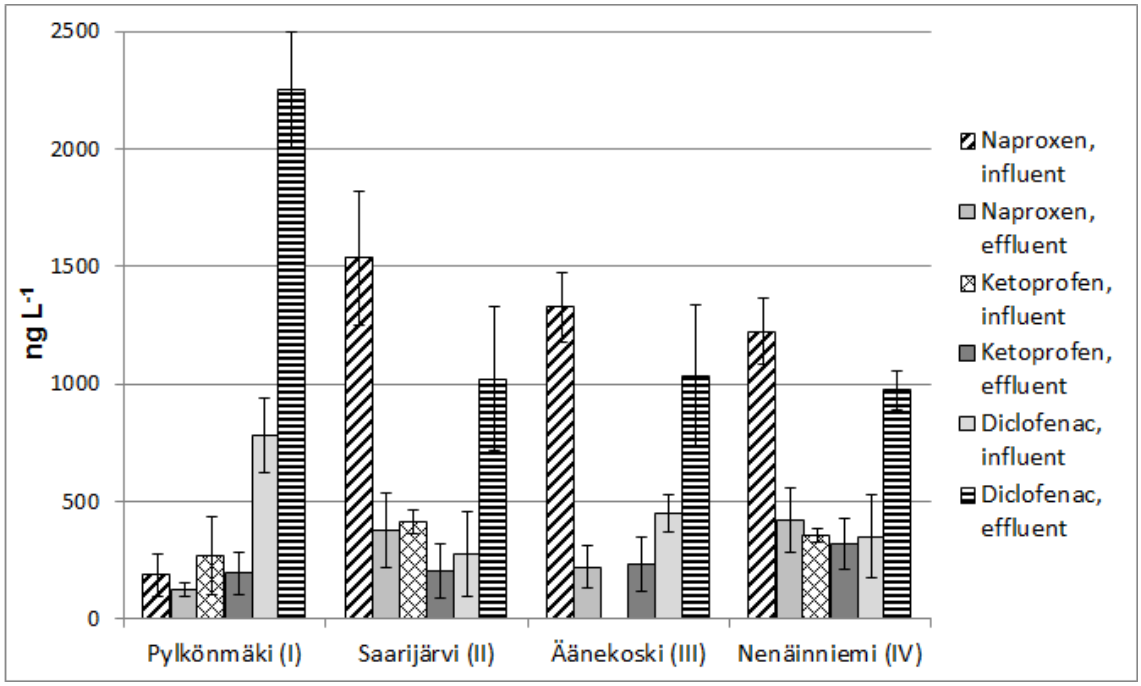


FIGURE 13 Mean concentrations of naproxen, ketoprofen, and diclofenac in influents and effluents of municipal WWTPs I-IV in central Finland, winter 2015

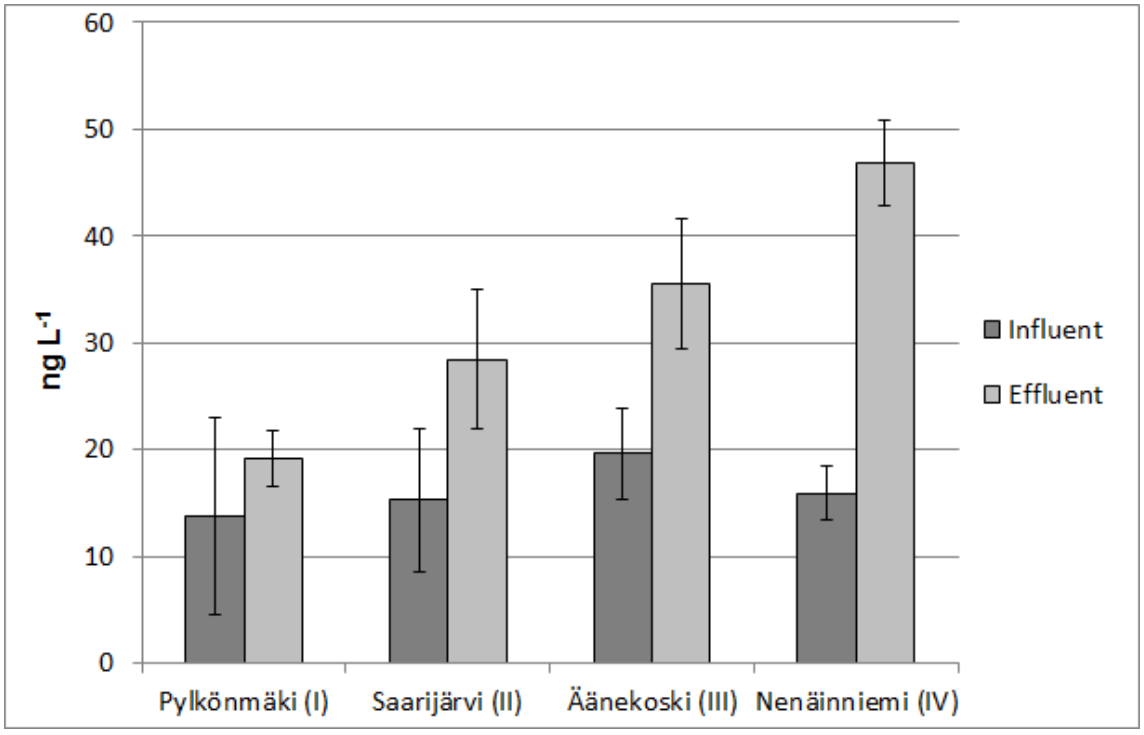


FIGURE 14 Mean concentrations of carbamazepine in influents and effluents at WWTPs I-IV, winter 2015

The concentrations of pharmaceuticals in influents and effluents at WWTPs II–IV are in the similar range (Figs. 12-14). In the case of WWTP I, the concentrations of the selected pharmaceuticals show different patterns, especially those of anti-inflammatory drugs. At WWTP I, wastewaters of only 400 residents are treated, while the other facilities serve larger communities. In addition, the WWTP I is the least efficient in terms of typical variables measuring treatment efficiency (BOD_7 and phosphorous removal, Table 12) and in terms of the removal efficiencies of the selected pharmaceuticals (Table 25). The microbial degradation is the most important process in biological wastewater treatment for removing polar pharmaceuticals (Quintana et al. 2005; Behera et al. 2011). Therefore, the less efficient removal of BOD_7 at WWTP I refers to less efficient microbial degradation and, thus, lower removal rates of selected pharmaceuticals.

The concentrations of ketoprofen increased after the treatment processes in the summer (Fig. 11) but decreased in the winter (Fig. 13). Ketoprofen is known to have the ability to accumulate in the environment during winter but in the summer it has more labile properties (Daneshvar et al. 2010; Vystavna et al. 2013). Therefore, it may accumulate to the particulate matter at the WWTP in the winter explaining the reduced concentrations.

The daily loads and capacities between WWTPs I-IV vary greatly but the main process solutions are relatively similar (Table 12). The removal efficiencies of the pharmaceuticals at the WWTPs are listed in Table 25. The results are in agreement with previously reported results (*e.g.*, Lindqvist et al. 2005; Santos et al. 2009; Behera et al. 2011). Only the removal rates of ketoprofen were unusually low.

TABLE 25 Mean removal efficiencies (n=8) of selected pharmaceuticals after wastewater treatment processes of WWTPs I-IV, winter 2015 and WWTP IV, summer 2013 (n=19)

Pharmaceutical	**WWTP I**	**WWTP II**	**WWTP III**	**WWTP IV, summer**	**WWTP IV, winter**
Carbamazepine	-	-	-	-	-
Diclofenac	-	-	-	-	-
Ibuprofen	78	99	99	99	98
Ketoprofen	27	50	-	-	10
Naproxen	33	75	83	63	66

5.4 Pharmaceuticals in surface waters

All selected pharmaceuticals were found in lake water in concentrations from a few ng L^{-1} to hundreds of ng L^{-1} (Appendix III). The main source of the detected pharmaceuticals in lake water is most likely the discharges from the municipal WWTPs. This is in agreement with previous results, suggesting that the direct discharges of wastewater are one of the main reasons for the increased levels of pharmaceuticals in surface water (Buser et al. 1999). When pharmaceuticals are discharged into the water body, dilution takes place. Northern Lake Päijänne is a deep (deepest point 41.5 m) water body with a large water mass leading to decreased concentrations. Additionally, conjugates formed in the WWTP can be degraded in aquatic environments back to their parent compounds.

Generally, the highest concentrations are found from sampling site 6, closest to the WWTP IV (Fig. 9; Appendix III). Somewhat lower concentrations are found from the other sampling sites, even in those upstream from the WWTP IV. At sampling site 6, ibuprofen is detected in northern Lake Päijänne on average in concentrations of 180 ng L^{-1} (Figs. 15 and 16). The results are in a similar range with those reported in the literature. For example, Ternes (1998) and Stumpf et al. (1999) reported median concentrations in the range of <10 to 400 ng L^{-1} for acidic pharmaceuticals, including diclofenac, ibuprofen, ketoprofen, and naproxen, in surface waters.

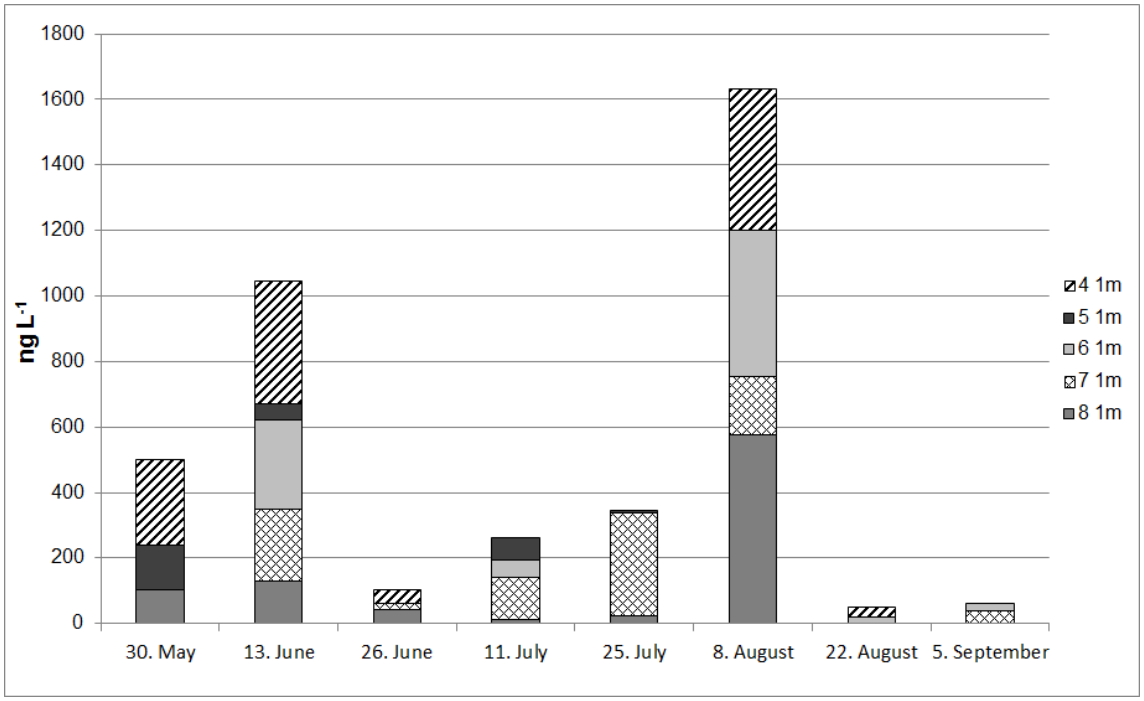


FIGURE 15 Mean concentrations of ibuprofen in northern Lake Päijänne at sampling sites 4-8 (1 m), May-September 2013

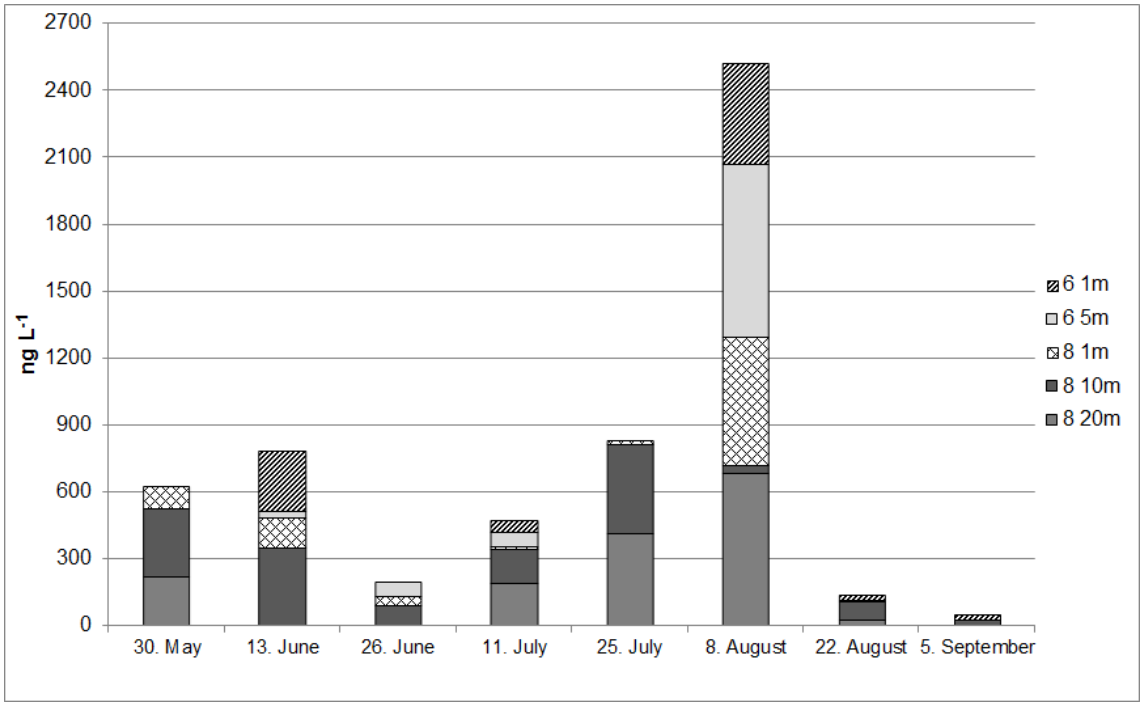


FIGURE 16 Mean concentrations of ibuprofen at sampling site 6 (1 m, 5 m), close to the point of effluent discharge and site 8 (1 m, 10 m, 20 m), the most remote of the selected sampling sites, May–September 2013

Based on current knowledge of water currents in northern Lake Päijänne, sampling site 4 is countercurrent to the WWTP IV (Krogerus et al. 2013). Additionally, the concentrations (mean 170 ng L^{-1}) in the effluent were lower than those detected at site 4 suggesting that the detected ibuprofen may not totally originate from the WWTP IV but from the smaller WWTPs upstream further north or another unknown source.

Generally, the highest concentrations of selected pharmaceuticals were detected in deeper water layers and the lowest in 1 m depths both near the WWTP and at the most distant sampling site (Figs. 15-18). Northern Lake Päijänne is a mesotrophic lake with low visibility and humic-containing properties (Table 13). Humic acids filter UV light, reducing solar radiation penetration into deeper water and preventing UV radiation-mediated degradation of compounds (Andreozzi et al. 2003). Secchi depth ranges from 1.6 to 2.0 m in northern Lake Päijänne, which represents the thickness of the photic layer and conditions for UV light-induced reactions (Table 13). Especially, diclofenac and carbamazepine showed higher concentrations in deeper water layers (Figs. 17 and 18). Ibuprofen is rapidly biodegraded in natural waters, but it cannot undergo direct UV-light induced reactions in environmental conditions (Buser et al. 1999; Lin and Reinhard 2005).

Carbamazepine transforms readily under UV radiation in aqueous environments and forms, *e.g.*, acridine and acridine intermediates (Vogna et al. 2004). This is consistent with results showing higher concentrations in deeper water

layers (Fig. 17). The concentrations are smaller near the water surface, suggesting that solar radiation degrades and transforms the parent compound.

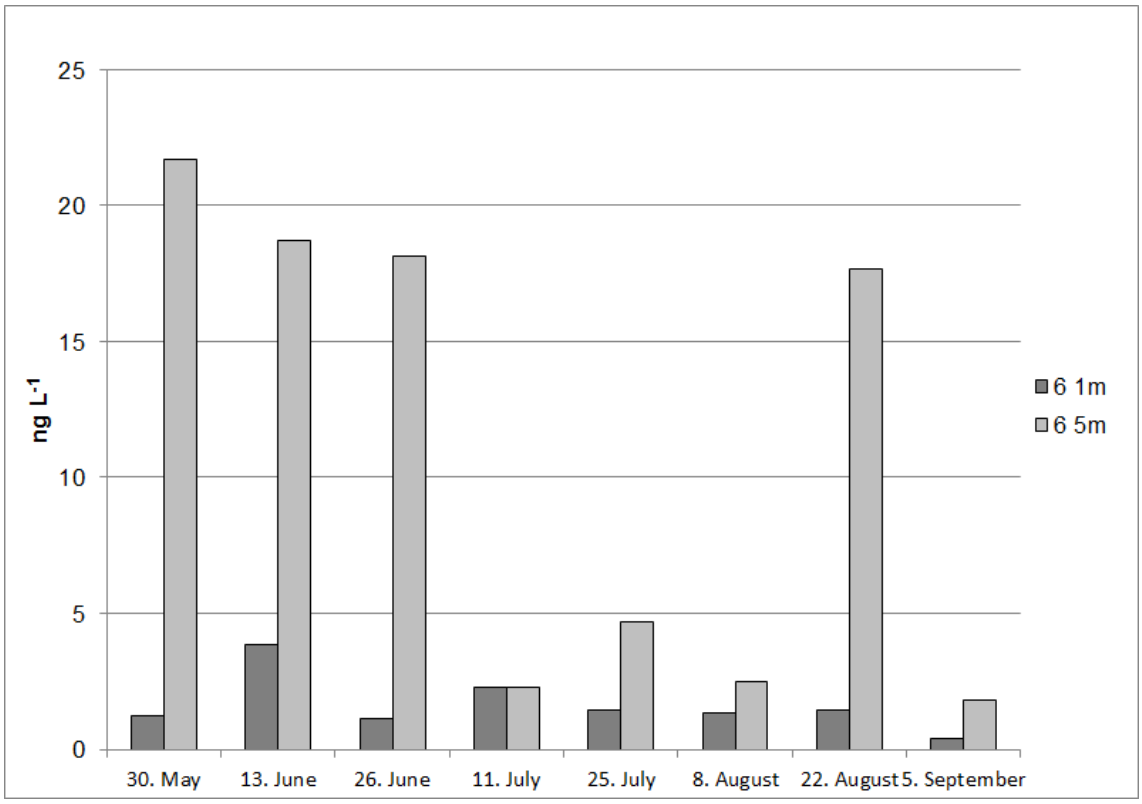


FIGURE 17 Mean concentrations of carbamazepine in northern Lake Päijänne, sampling site 6 (1 m, 5 m), May-September 2013

Diclofenac shows minimal degradation via chemical and biological reactions in the environment but is readily photodegraded by sunlight (Bartels and von Tümpling 2007; Buser et al. 1998). The half-life of diclofenac is less than a day in the summer time when exposed to direct solar radiation in aqueous environments (Andreozzi et al. 2003). The results (Fig. 18) are in good agreement with the previous conclusions regarding the influence of water depth on photolysis reactions (Bartels and von Tümpling 2007). Even at a 1 m water depth, the photodegradation of diclofenac is reduced to one third. However, intensive sunlight can lead to meta-UV stable products with slow photochemical degradation properties (Bartels and von Tümpling 2007).

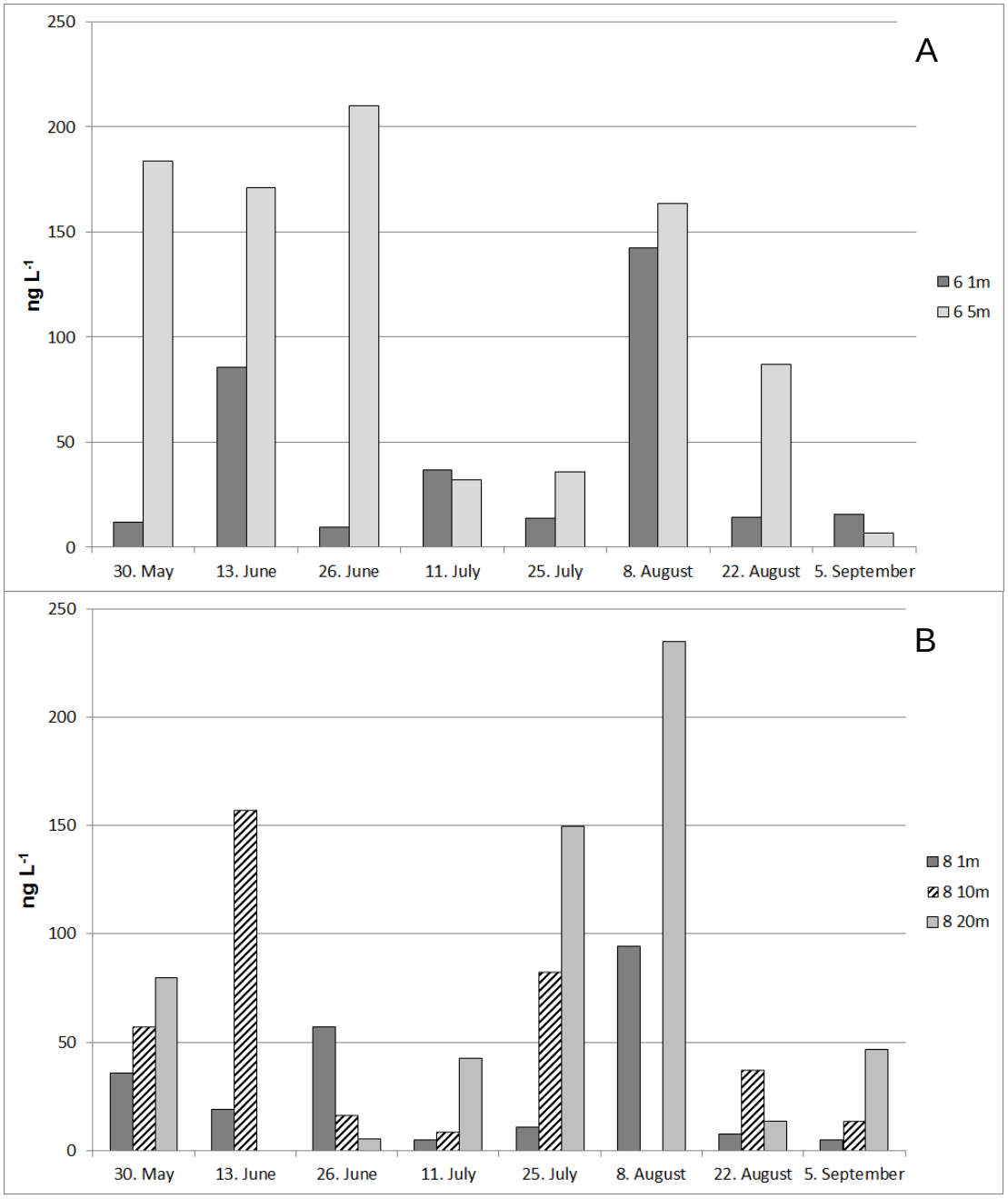


FIGURE 18 Concentrations of diclofenac detected in northern Lake Päijänne, (A) sampling site 6 (1 m, 5 m), (B) sampling site 8 (1 m, 10 m, 20 m), May-September 2013. High concentrations are detected in deep water where UV light-induced degradation reactions are limited.

In the winter, the concentrations of selected pharmaceuticals at each sampling site are depicted in Fig. 19. The concentrations in upstream areas (sites 1-3) are lower than those in Lake Päijänne (sites 4, 6, and 8). Sampling site 6 is located close to the effluent discharge pipe from the WWTP of Jyväskylä (IV). Therefore, the discharge from the WWTP IV most likely is responsible for the detected concentrations at sites 6 and 8.

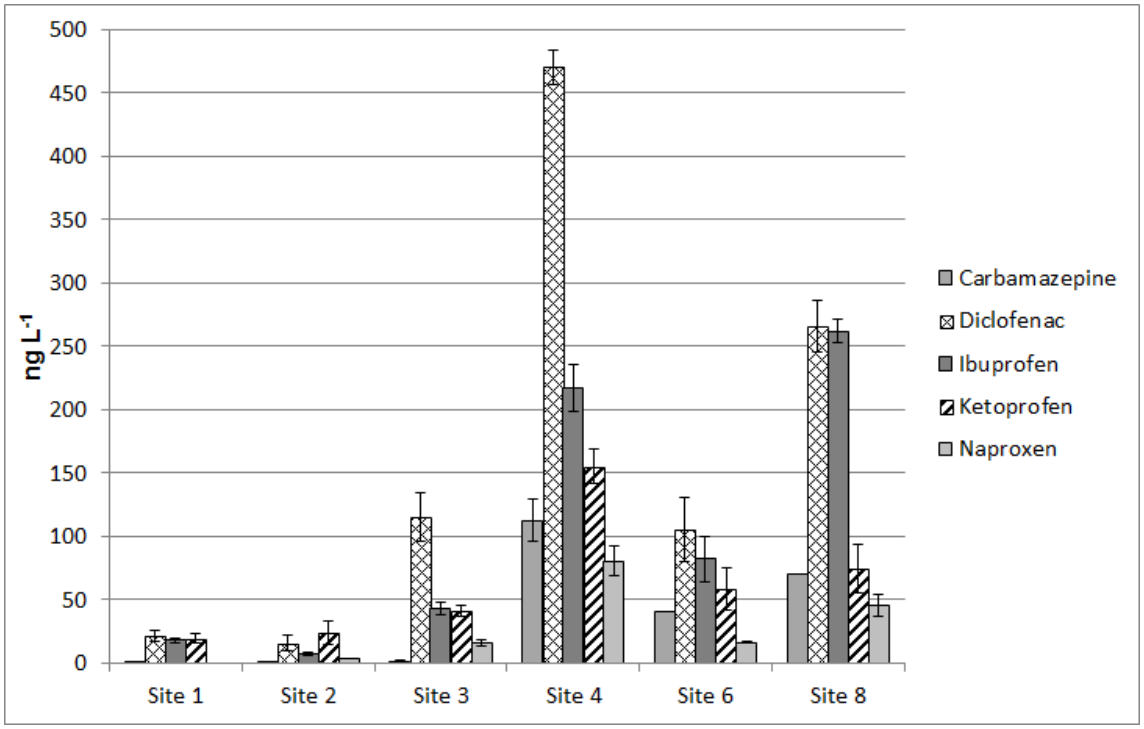


FIGURE 19 Mean concentrations of selected pharmaceuticals in Lake Kuhnamo (1), Kapeenkoski Rapids (2), Haapakoski Rapids (3), and Lake Päijänne (4, 6, 8), winter 2015

The detected concentrations at sampling sites 1-3 are much lower than those in the effluents of WWTPs I–III (Figs. 12-14, 19). First, the WWTPs I and II are located relatively far away from the first sampling site along the waterway, including the relatively large Lake Kuhnamo (622 ha). Therefore, the discharged pharmaceuticals are highly diluted, showing only small concentrations (Fig. 19). On the other hand, high concentrations are detected at sampling site 4, which is upstream from WWTP IV and, in the case of carbamazepine, even higher than those released from WWTPs I-III. The current transfers water to the opposite direction (Krogerus et al. 2013). However, Andreozzi et al. (2003) showed that the half-life of carbamazepine can be 100 days in northern latitudes during winter. Therefore, the concentrations can build up during winter due to their slow rate of degradation. It is likely that the concentrations detected at site 4 may not entirely originate from the effluents released by the northern WWTPs but also from other sources.

Generally, in addition to wastewater effluents, pharmaceuticals can be released from other sources, *e.g.*, unintentional discharges from hospitals, pharmaceutical industry, and runoffs from landfills or agriculture. However, none of these events are known to occur in the area. In addition to the waterway, Lake Päijänne receives water from Lake Jyväsjärvi, which is located on the northern side of Lake Päijänne. It is a 3.3 km^3 large lake by which the city of Jyväskylä is located and receives water mostly via River Tourujoki (2 km, 3.4 m^3 s^{-1}). Even though wastewaters of the city area are led to the municipal WWTP (IV), some discharges can be transferred to Lake Päijänne via this route.

In the previous decades, Lake Jyväsjärvi has been widely polluted by industrial activities and municipal load and was highly polluted in the 1960s before the municipal WWTP was built in 1973. The area by the lake was used as a landfill during years 1950-1970. Even though wastewaters as well as solid waste are now properly treated in Jyväskylä, contaminants may have remained in the lake sediment from which they can be released back into the water. For example, relatively large amounts of the selected pharmaceuticals have been found from the lake sediment of Lake Jyväsjärvi (unpublished data, Lindholm et al. 2014). However, in this study, no samples were taken from Lake Jyväsjärvi, and the initial source of the high concentrations at site 4 remains unclear, leaving Lake Jyväsjärvi an interesting area for further research.

5.5 Pharmaceuticals in sedimented particles

Sedimented particle samples are less sensitive to seasonal variations, rainfall, and other environmental changes compared to water samples (Antonić and Heath 2007). They provide long-term information about the concentrations of selected pharmaceuticals.

Excluding naproxen, the selected pharmaceuticals were consistently found in sampling site 6 near WWTP IV while only occasionally in others (Table 26). Even though the highest values of single samples were found at sites 7 and 8, the local municipal WWTP proved to be the main source of pharmaceuticals. However, minor concentrations were also found at site 4 upstream.

Ketoprofen was found the most abundantly in majority of the samples with the mean content of 15.5 µg g^{-1} and the maximum content of 135 µg g^{-1} (Table 26). The high concentrations of ketoprofen in the water samples and the tendency of ketoprofen attaching to particles are the most probable reasons for the highest detected concentrations in the sedimented particles.

TABLE 26 Concentrations of selected pharmaceuticals (ng g^{-1}; µg g^{-1} for ketoprofen, o.d.) in sedimented particles collected from sampling sites 4, 6–8 in northern Lake Päijänne, June–September 2013

Pharmaceutical	n	Site 4	n	Site 6	n	Site 7	n	Site 8
Carbamazepine	6	0.02–0.1	8	0.04–1.7	4	0.05–0.2	5	0.08–0.2
Diclofenac	1	1.6	8	3.2–3.6	1	11	5	nd
Ibuprofen	1	14	8	6.2–8.4	1	140	5	nd
Ketoprofen	6	0.08-66	8	0.1–13	4	2.9–39	5	6.3–135
Naproxen	6	nd	8	nd	1	77	5	nd

nd -not detected

Diclofenac, ibuprofen, and naproxen were less frequently detected from the sedimented particles (Table 26). In the case of diclofenac, the results follow those of previous studies (Buser et al. 1998; Antonić and Heath 2007). Diclofenac shows negligible adsorption onto sedimented particles, which can explain

its absence in the samples. Additionally, only small concentrations of carbamazepine (average 0.27 ng g^{-1}) were detected (Table 26; Fig. 20). This is most likely caused by the fact that carbamazepine forms a range of degradation intermediates in soil, which further reduces the amount of parent compound (Li et al. 2013).

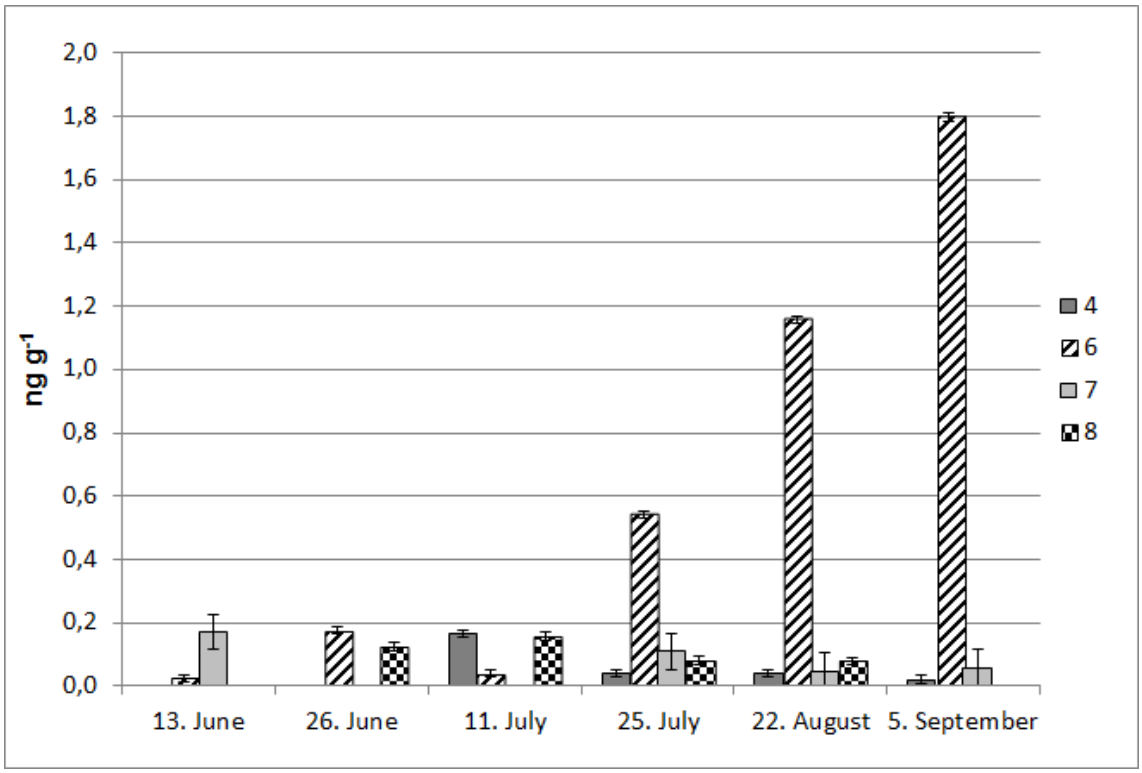


FIGURE 20 Mean concentrations of carbamazepine in sedimented particles in Lake Päijänne at sampling sites 4, 6-8.

Generally, the selected pharmaceuticals can form a variety of conjugates when bound to sedimented particles and therefore were not detected in this study. However, little information is still available about the behavior and sedimentation of the selected pharmaceuticals in aquatic environments, and a detailed assessment requires further research.

5.6 Seasonal variation

According to current knowledge, the main elimination processes of pharmaceuticals in the wastewater treatment are biodegradation and sorption, both of which are temperature dependent (Urase and Kikuta 2005; Vieno et al. 2005). Therefore, the treatment efficiency at WWTPs often decreases during winter, which leads to decreased degradation and transformation reactions and lower treatment efficiency.

In the case of diclofenac, the concentrations in influents were in the same range both in summer and winter, but in effluents, they seem to be higher in the summer (Fig. 21). Diclofenac is the effective ingredient in pharmaceutical products to treat symptoms, such as osteoarthritis and rheumatoid arthritis. These

conditions are not season related, such as *e.g.*, flu, which occurs more frequently during winter months. On the other hand, deconjugation reactions decrease in winter due to lower temperature and lower efficiency at WWTPs. Therefore, a lower amount of parent compound can be released in the winter (Fig. 21).

In the case of ketoprofen, higher concentrations in influents and effluents are detected at the WWTP IV in summer than in winter (Fig. 21). Possibly, the consumption of ketoprofen increases during spring and summer months as reported by Santos et al. (2009). The reported removal efficiency of ketoprofen during wastewater treatment has a great variability from none to almost complete removal (*e.g.*, Santos et al. 2009; Behera et al. 2011). In this study, the rate of removal remains minimal (Table 25). There were no drastic changes (7 % decrease compared to the year earlier) in the consumption of ketoprofen (Finnish Medicines Agency Fimea 2014) nor large changes in the number of residents in the Jyväskylä area. However, there are a number of festivals and events organized annually in the Jyväskylä area in the summer time (*e.g.*, Neste Oil Rally, 270,000 visitors; Suomi pop festival, 19,000 visitors), which attract a lot of visitors and possibly increase the use of anti-inflammatory drugs, such as ketoprofen. Similarly, the concentrations of common pain killers, ibuprofen, and naproxen also increased during summer months (Figs. 21 and 22). Daneshvar et al. (2012) reported increased concentrations of pain killers after festivals with thousands of visitors. In addition, both showed a lower degree of treatment efficiency during winter, most likely due to a lower temperature at the WWTP (Figs. 21 and 22).

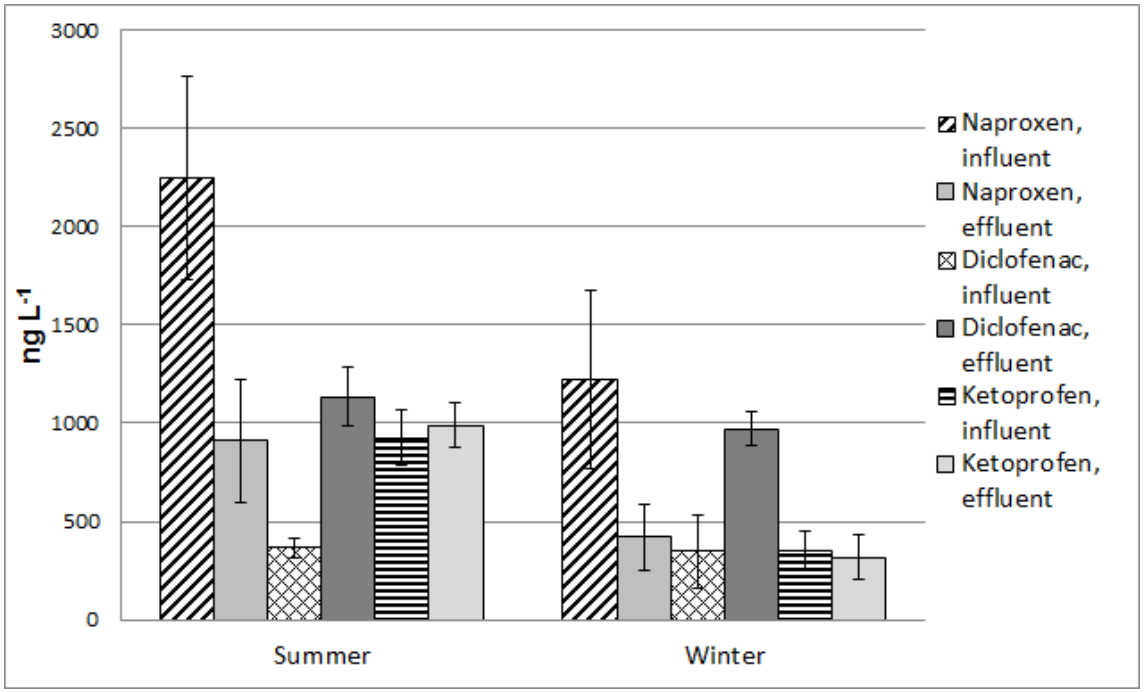


FIGURE 21 Mean concentrations of naproxen, diclofenac, and ketoprofen in influent and effluent at WWTP IV, summer 2013 and winter 2015

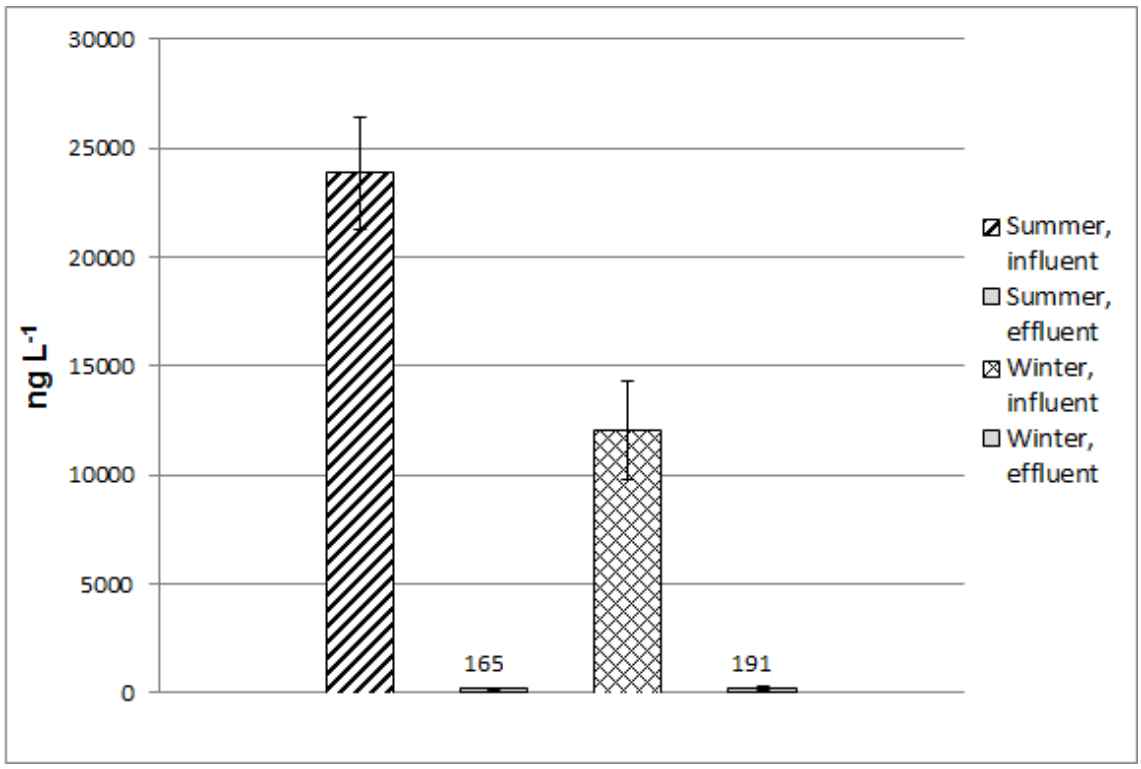


FIGURE 22 Mean concentrations of ibuprofen in influents and effluents at WWTP IV, summer 2013 and winter 2015

Antibiotic consumption is known to be seasonal, increasing during winter (García del Pozo et al. 2004). Therefore, a part of the observed differences can be due to variation of drug consumption. However, according to our knowledge, no data about seasonal variation of pharmaceutical consumption in Finland is available. In the case of carbamazepine, the concentrations in influents are higher during winter season compared to summer (Fig. 23). This can be due to an increased use of antidepressants during winter. It is known that the use of psycho active pharmaceuticals, such as carbamazepine, peaks in autumn and spring, including March (García del Pozo et al. 2004).

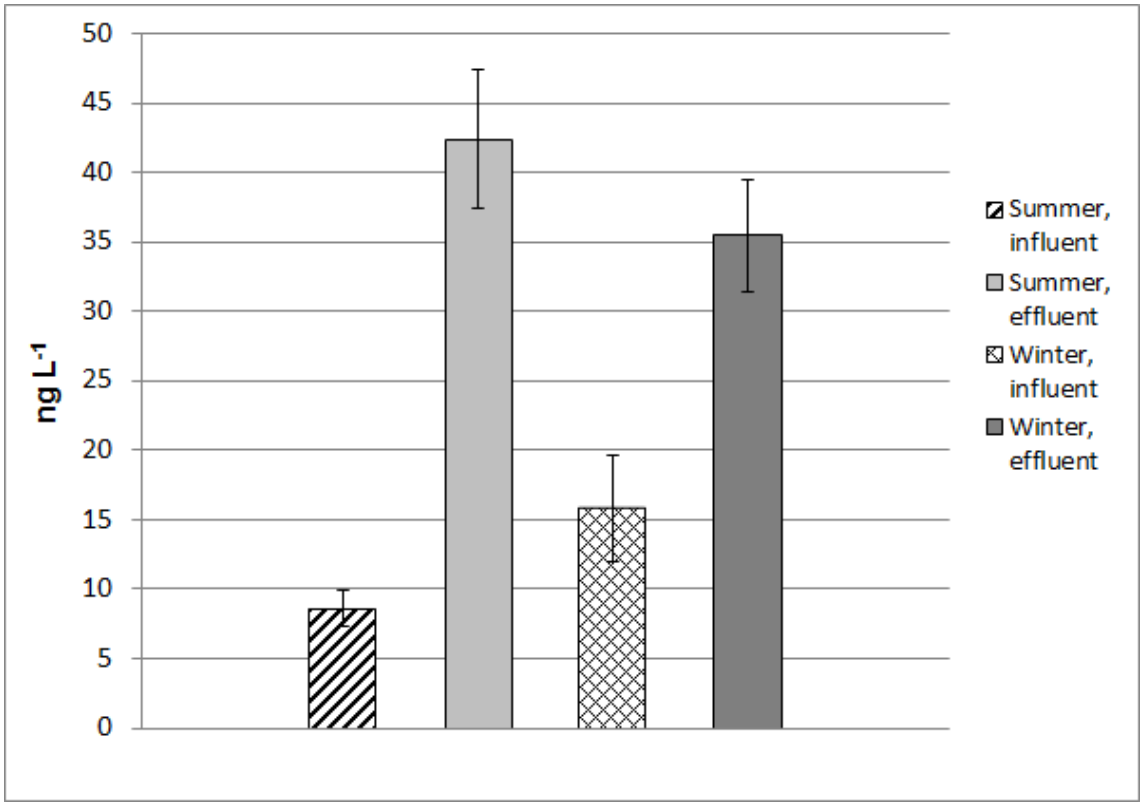


FIGURE 23 Mean concentrations of carbamazepine in influents and effluents at WWTP IV, summer 2013 and winter 2015

Generally, the concentrations of pharmaceuticals detected in Lake Päijänne in winter are higher than in summer (Fig. 24). Similarly, studies of different geographical areas have shown decreased concentrations of selected compounds in summer compared to winter time (Vieno et al. 2005; Daneshvar et al. 2010). For example, diclofenac and ketoprofen, which are known to undergo rapid photolysis reactions under UV light, show higher concentrations in the winter when UV light is limited (Bartels and von Tümpling 2007; Kotnik et al. 2014). Diclofenac undergoes fast degradation reactions with a half-life of less than a day during spring and summer and up to 5 days during winter (Andreozzi et al. 2003). Additionally, ketoprofen transforms into benzophenone derivatives, 3-ethylbenzophenone and 3-acetylbenzophenone when exposed to UV radiation (Kosjek et al. 2011). In this case, the lake was covered with ice and snow, leading to nonexistent UV light-induced reactions in the winter and increased concentrations.

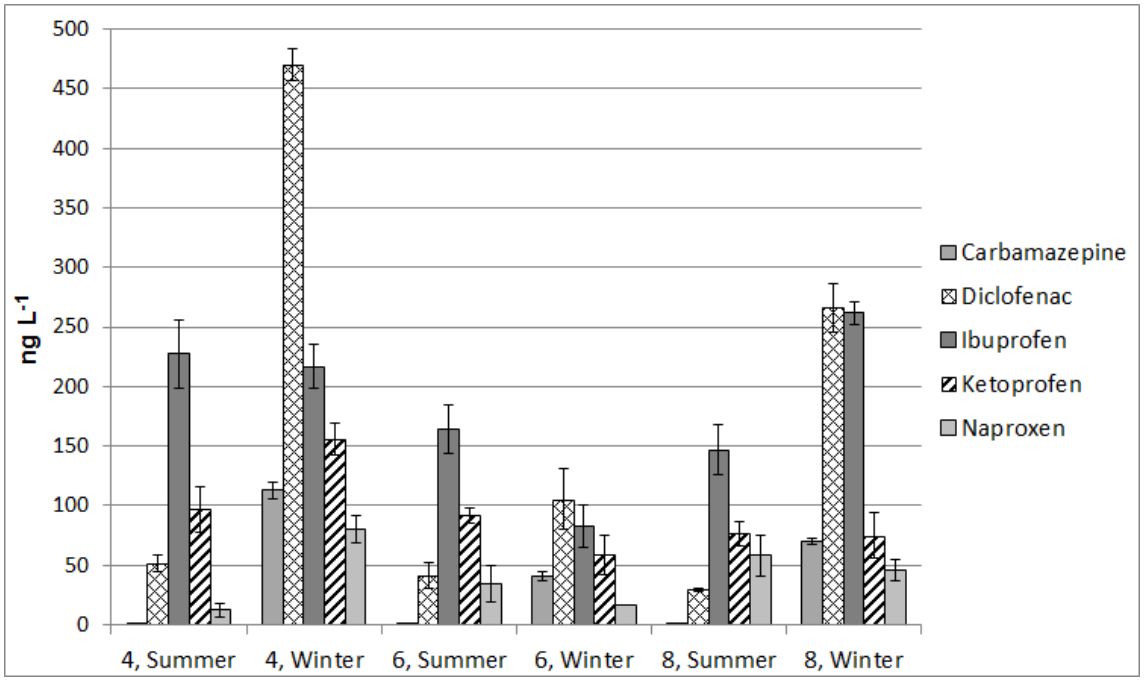


FIGURE 24 Mean concentrations of selected pharmaceuticals in Lake Päijänne at sampling sites 4, 6 and 8, summer 2013 and winter 2015

Concentrations of carbamazepine are much higher in all selected sampling sites during winter compared to summer time (Fig. 24). Carbamazepine has limited ability to be eliminated by sedimentation and by natural processes, such as adsorption (Ternes 1998; Andreozzi et al. 2003), but it transforms readily under UV radiation and forms, *e.g.*, acridine and acridine intermediates (Vogna et al. 2004). However, photodegradation of carbamazepine is found to be low with a half-life of over 100 days in the wintertime and increasing latitude (Andreozzi et al. 2003). In this case, higher concentrations of carbamazepine were found during winter due to lower UV radiation and ice cover preventing phototransformation reactions compared to summertime (Table 13). The results of seasonal variation are consistent with previous results (*e.g.*, Daneshvar et al. 2010).

Ibuprofen, on the other hand, undergoes biodegradation instead of UV light-induced reactions leading to fairly similar concentrations in the summer and winter (Packer et al. 2003; Illés et al. 2013). However, there are some differences between seasons because biodegradation reactions are temperature-dependent and proceed slower in colder conditions (Tables 13 and 25).

5.7 Passive sampling of pharmaceuticals

5.7.1 Calibration experiment

The values of R_s were determined by the laboratory calibration (Table 27). The highest R_s value was determined for carbamazepine and the lowest for diclofenac, while the R_s of ibuprofen, ketoprofen, and naproxen were essentially at similar levels and similar to those reported in the literature. For example, DiCarro et al. (2014) and Tanwar et al. (2015) studied pharmaceuticals with POCIS passive samplers and reported somewhat higher R_s values of ibuprofen and ketoprofen than those of diclofenac (IBP 0.075, KET 0.066, DCF 0.058). Additionally, MacLeod et al. (2007) and Li et al. (2010) reported higher R_s values of carbamazepine than ibuprofen, ketoprofen, and naproxen (CBZ 0.348-0.397, IBP 0.254, KET 0.135, NPX 0.116-0.298).

TABLE 27 The sampling rate, R_s (L day^{-1}) and standard deviation (SD) determined by the sampler calibration experiment for the selected pharmaceuticals

Pharmaceutical	R_s SD
Carbamazepine	0.158 ± 0.047
Diclofenac	0.048 ± 0.022
Ibuprofen	0.091 ± 0.030
Ketoprofen	0.099 ± 0.033
Naproxen	0.094 ± 0.033

5.7.2 Lake Päijänne

Generally, the highest concentrations of all selected pharmaceuticals were detected at sampling site 6, which is located at the point of effluent discharge from the WWTP in case of both passive and grab sampling (Table 28, Fig. 25). The concentrations decreased with increasing distance from WWTP IV. Furthermore, all analytes were detected at site 4 even though it is located upstream from the WWTP (Krogerus et al. 2013), suggesting another source of contaminants. In grab samples, ibuprofen dominated at all sites, with the highest mean concentration at site 4. In passive samples at site 6, the mean concentrations of diclofenac, ibuprofen, ketoprofen and naproxen were at a similar level, while at other sites, ketoprofen showed the highest concentrations (Table 28, Fig. 25).

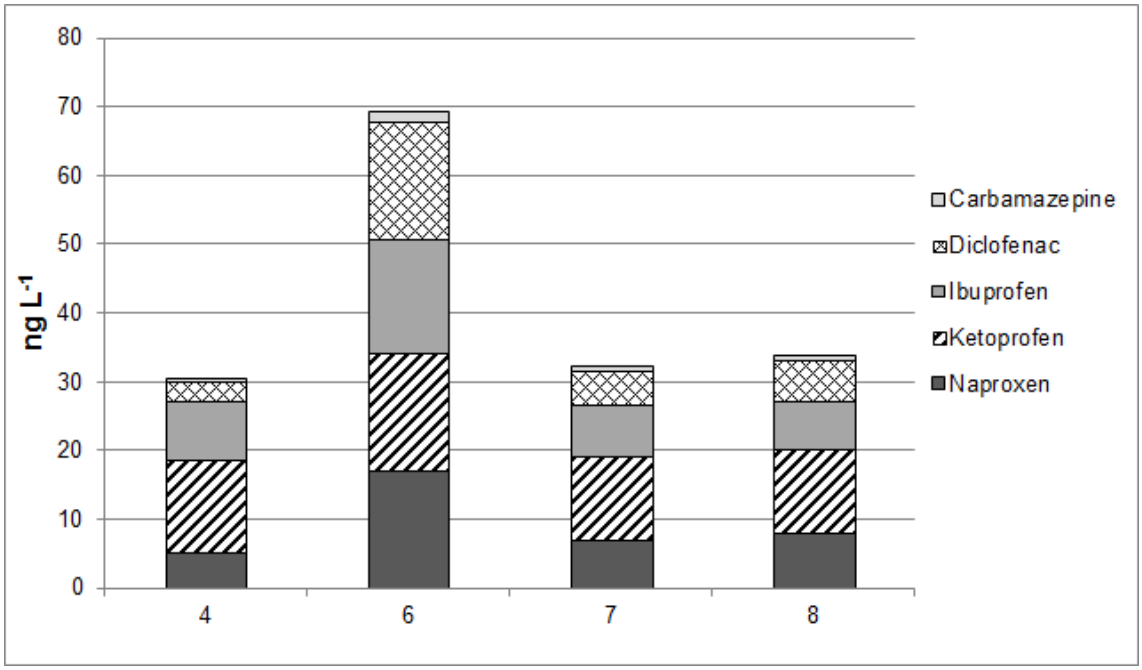


FIGURE 25 Mean concentrations of selected pharmaceuticals in Lake Päijänne at sampling sites 4, 6-8 by passive sampling, June-September 2013

The concentrations of selected pharmaceuticals are in a similar range, tens of nanograms per liter and in the similar range with previous results of grab and passive sampling of lake and river waters (Lindqvist et al. 2005; Vieno 2007; Moschet et al. 2015). Even though occasional fluctuations and high concentrations were detected by grab sampling, the results are generally in the same range (Table 28). Additionally, others have reported higher concentrations by grab sampling compared to passive sampling. For example, Moschet et al. (2015) reported concentrations of *e.g.*, naproxen and diclofenac ten times higher by grab sampling compared to passive sampling. However, in the case of carbamazepine, the concentrations are similar both by passive and grab sampling (Table 28, Fig. 25).

TABLE 28 The mean concentrations and standard deviations (SD), ng L^{-1}, of the studied pharmaceuticals in Lake Päijänne by grab (n=8) and passive (n=14) sampling, June–September 2013

Compound	Grab 4	Passive 4	Grab 6	Passive 6	Grab 7	Passive 7	Grab 8	Passive 8
Carbamazepine	1.0 (± 0.6)	0.4 (± 0.1)	1.7 (± 0.6)	1.5 (± 0.6)	2.1 (± 2.1)	0.8 (± 0.2)	1.1 (± 0.6)	0.8 (± 0.1)
Diclofenac	52 (± 53)	2.8 (± 0.9)	41 (± 48)	17 (± 11)	40 (± 42)	4.8 (± 2.1)	29 (± 32)	6.1 (± 1.9)
Ibuprofen	227 (± 186)	8.6 (± 1.0)	164 (± 191)	17 (± 14)	149 (± 112)	7.7 (± 4.4)	61 (± 214)	6.9 (± 2.8)
Ketoprofen	97 (± 70)	14 (± 4.7)	92 (± 39)	17 (± 7.0)	59 (± 33)	12 (± 4.1)	76 (± 28)	12 (± 3.7)
Naproxen	12 (± 6.1)	5.1 (± 4.2)	34 (± 15)	17 (± 9.7)	27 (± 7.3)	6.8 (± 2.8)	58 (± 67)	8.0 (± 4.5)

The concentrations of carbamazepine detected by passive sampling follow those of grab sampling (Fig. 26). The values are fairly constant over the sampling period, but in case of samples collected by passive sampling, they increase slightly towards fall. The solar radiation is the most intense in central Finland during summer months, especially in June and July, decreasing towards fall (Table 13). The grab samples and the passive samples were taken from a depth of 1 m, which is clearly included in the photic layer of the lake, enabling the UV light induced reactions (Table 13). Carbamazepine is known to be prone to UV light induced reactions in aquatic environments (Andreozzi et al. 2003), which can explain the slight increase in concentrations in late summer (Fig. 26).

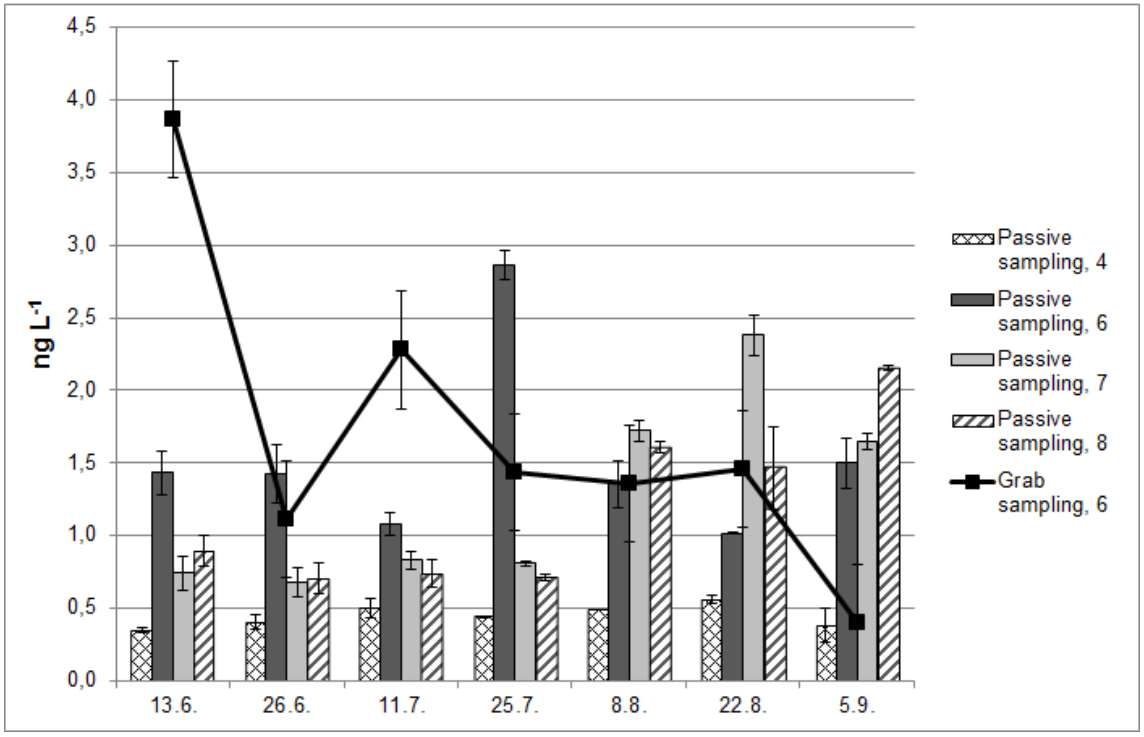


FIGURE 26 Mean concentrations of carbamazepine in Lake Päijänne at sampling sites 4, 6-8 by passive sampling and at site 6 by grab sampling, June-September 2013

5.7.3 River Vantaa

The concentrations of the studied pharmaceuticals ranged from few nanograms per liter of passive samples to hundreds of nanograms per liter of grab samples (Tables 29 and 30, Figs. 27-28). Despite the variation, they are in a similar range as in other studies. For example, similar levels of carbamazepine and diclofenac were reported by Äystö et al. (2014) in the River Vantaa. Moschet et al. (2015) detected 6-110 ng L^{-1} of carbamazepine, 1.4-320 ng L^{-1} diclofenac, and 26-87 ng L^{-1} naproxen in Swiss rivers by Chemcatcher passive samplers.

Overall, the concentrations were roughly ten times higher in August 2013 than in May 2015 (Tables 29 and 30; Figs. 27-28), excluding diclofenac. Most likely, the lower levels in 2015 are caused by the higher water flow in the spring time and dilution of the trace substances in the river water (Tables 14 and 15, OIVA 2015). Year 2013 was unusually dry and warm (79 % of average rainfall and 1.2-

1.8 °C warmer; OIVA 2015). The water flow has an effect on the accumulation factor of selected pharmaceuticals, which can affect the results of passive sampling (Ahkola et al. 2014). Furthermore, the WWTP in Riihimäki was rebuilt in 2014, which might have improved the removal efficiency of pharmaceuticals.

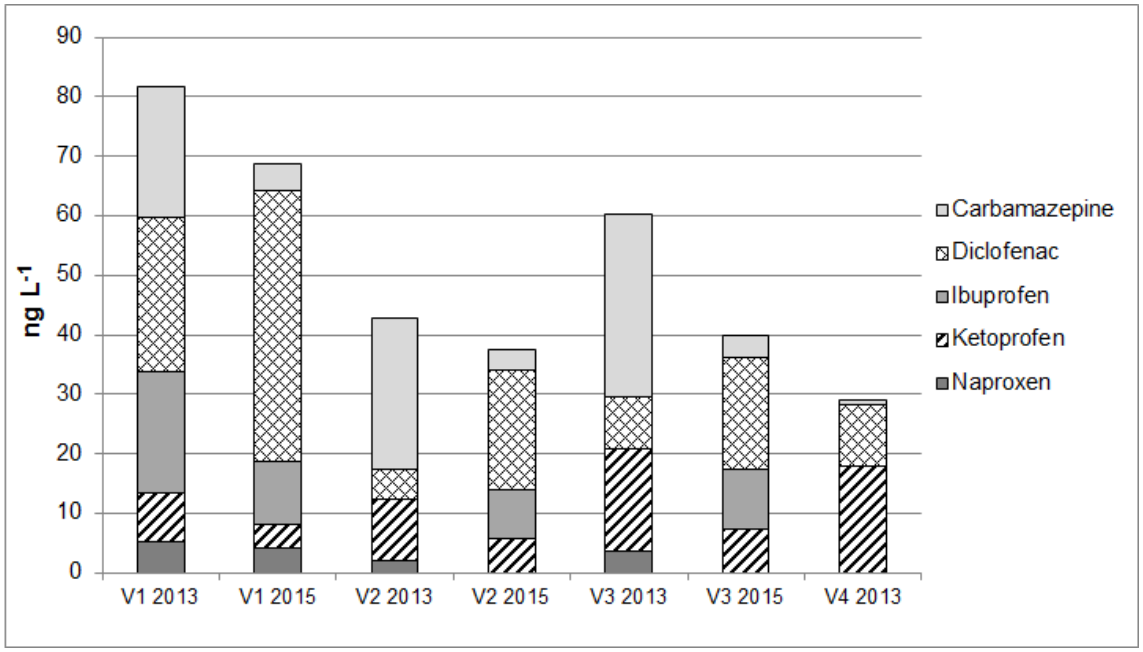


FIGURE 27 Mean concentrations of selected pharmaceuticals in the River Vantaa by passive sampling, at sampling sites V1-V4 in August 2013 and V1-V3 in April-May 2015

In 2013, the lowest levels of pharmaceuticals were measured at site V4 near the sea shore, excluding ketoprofen (Table 23; Figs. 27-28). The average flow of the River Vantaa increases towards the sea shore, suggesting increased dilution of trace substances (Table 14). However, the similar levels of pharmaceuticals indicate that there are several point sources of pharmaceuticals between sites V2 and V3. There are several municipal WWTPs along the River Vantaa releasing their effluents to the river, and the points of discharge from the WWTPs are located near the selected sampling sites (Fig. 10). In addition to the Riihimäki WWTP close to site V1, there is the point of effluent discharge from the Hyvinkää WWTP (10 300 m^3 d^{-1}) close to the site V2 and, near the site V3 the point of effluent discharge from the Nurmijärvi WWTP (2700 m^3 d^{-1}) (Fig. 10; Table 15).

Generally, the concentrations of carbamazepine and diclofenac decreased towards the sea shore (Fig. 27). The concentrations are tens of nanograms per liter when detected by passive samplers (Tables 29 and 30) and similar to those reported by Äystö et al. (2014). Additionally, Äystö et al. (2014) reported decreased levels of carbamazepine on the sea shore. Carbamazepine in river water most likely originated from the multiple municipal WWTPs along the River Vantaa. Carbamazepine is known to undergo transformation and dissociation reactions under sun light (Andreozzi et al. 2003). However, the reactions of car-

bamazepine proceed relatively slowly with half-life times of hundreds of days, and it is constantly released by the effluents. In addition, the water of the River Vantaa is turbid and contains humic substances, which are known to decrease or even reverse UV light-induced transformation reactions (Andreozzi et al. 2003). The highest amounts of humic substances are observed in the upstream of the River Vantaa due to forests and soil properties (OIVA 2015).

TABLE 29 Mean concentrations and standard deviations (SD), ng L^{-1}, of studied pharmaceuticals in the River Vantaa by passive (n=8) and grab sampling (n=12) at sites V1-V4 in August 2013

Compound	Grab V1	Passive V1	Grab V2	Passive V2	Grab V3	Passive V3	Grab V4	Passive V4
Carbamazepine	57 (± 43)	22 (± 8.0)	50 (± 28)	25 (± 7.9)	65 (± 28)	31 (± 13)	15 (± 7.8)	0.8 (± 0.5)
Diclofenac	187 (± 80)	26 (± 27)	96 (± 57)	5.1 (± 3.8)	99 (± 49)	8.7 (± 10)	11 (± 2.7)	11 (± 8.5)
Ibuprofen	89 (± 28)	20 (± 18)	67 (± 19)	nd	50 (± 1.0)	nd	9.7 (± 1.0)	nd
Ketoprofen	230 (± 2.9)	8.2 (± 2.5)	100 (± 4.3)	10 (± 3.0)	140 (± 6.0)	17 (± 2.8)	120 (± 4.6)	18 (± 2.7)
Naproxen	12 (± 1.0)	5.3 (± 1.6)	20 (± 9.3)	2.2 (± 1.0)	40 (± 38)	3.5 (± 1.0)	nd	nd

nd - not detected

TABLE 30 Mean concentrations and standard deviations (SD), ng L^{-1}, of studied pharmaceuticals in the River Vantaa by passive (n=6) and grab sampling (n=12) at sites V1-V4 in April-May 2015. In 2015, passive samples from site V4 were not collected due to technical reasons

Compound	Grab V1	Passive V1	Grab V2	Passive V2	Grab V3	Passive V3	Grab V4
Carbamazepine	14 (± 6.7)	4.6 (± 0.2)	8.3 (± 3.6)	3.4 (± 0.1)	7.6 (± 3.2)	3.9 (± 1.0)	4.7 (± 0.5)
Diclofenac	34 (± 18)	46 (± 28)	23 (± 9)	20 (± 5.4)	15 (± 12)	19 (± 21)	19 (± 11)
Ibuprofen	8.6 (± 4.1)	11 (± 6.4)	9.7 (± 1.8)	8.2 (± 8.9)	5.2 (± 1.2)	9.9 (± 11)	10 (± 1.0)
Ketoprofen	19 (± 8.6)	3.9 (± 1.0)	25 (± 4.0)	5.6 (± 1.0)	35 (± 3.4)	7.4 (± 2.7)	29 (± 5.2)
Naproxen	5.6 (± 1.7)	3.1 (± 1)	nd	nd	nd	nd	nd

nd - not detected

Ketoprofen showed the highest concentrations collected by grab sampling (Table 29; Fig. 28) while Äystö et al. (2014) detected no ketoprofen in fall 2013 in the River Vantaa. Carbamazepine showed the highest concentrations collected by passive samplers in 2013, while diclofenac dominated in passive samplers in 2015 (Table 30), excluding site V4.

In addition to point sources, other factors can contribute to the greater amount of ketoprofen towards the sea shore (Fig. 28), especially when studied by passive samplers. Ketoprofen has the ability to accumulate in the environment dur-

ing winter, but in the summer, it is more labile (Daneshvar et al. 2010; Vystavna et al. 2013). Apart from human consumption released via WWTPs, ketoprofen is used as a veterinary drug, which might end up in the fields after consumption by farm animals and later back to the river (Vystavna et al. 2013). Along the River Vantaa, there are several animal farms with horses, sheep and cattle which may contribute to the load of ketoprofen. Therefore, it is possible that the concentration of ketoprofen builds up in the river water towards the sea shore (Fig. 28).

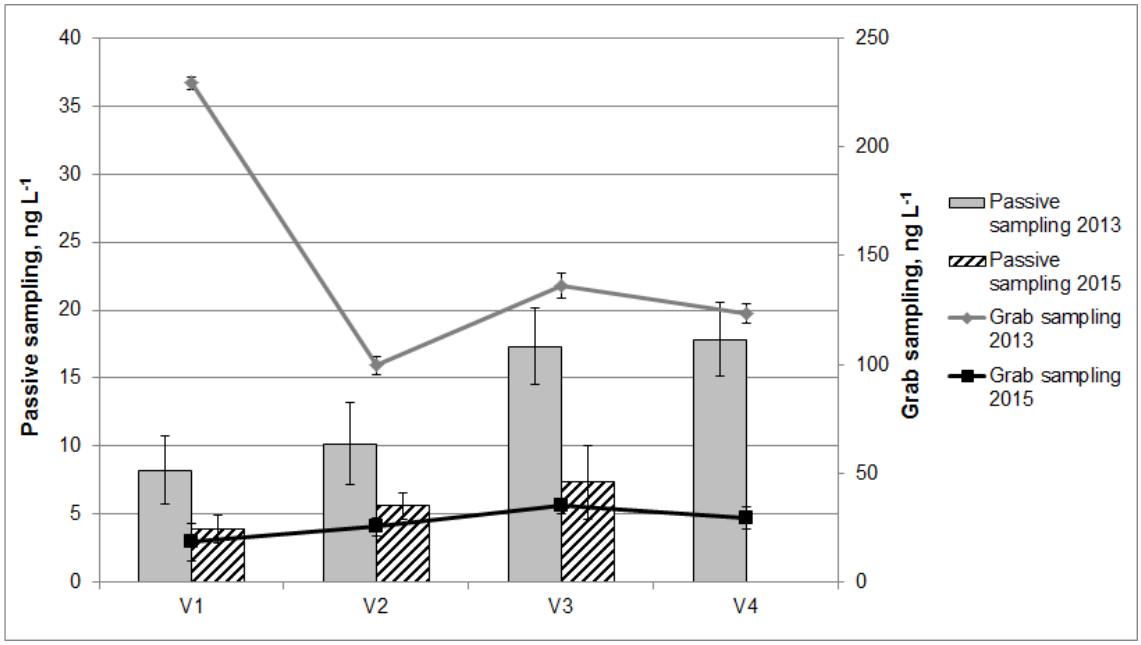


FIGURE 28 Mean concentrations of ketoprofen in the River Vantaa, collected by grab and passive sampling, August 2013 and April-May 2015

6 CONCLUDING REMARKS

6.1 Conclusions

This study shows that pharmaceuticals are present both in influents and effluents of the WWTPs and in the receiving water bodies. The concentrations at the WWTP IV in Jyväskylä are 10 to 100 times higher compared to those detected in the lake water. Ibuprofen was the major component in the influent due to its high consumption. However, only ibuprofen is almost completely transformed to its transformation products, while the others show more recalcitrant properties during treatment processes. Pharmaceuticals were found also from the sedimented particles, ketoprofen being the dominating compound.

All selected pharmaceuticals were detected in along the waterway and northern Lake Päijänne as well as in the River Vantaa. Concentrations varied from a few to hundreds of nanograms per liter. Higher concentrations were detected in northern Lake Päijänne than upstream, even at the sampling site upstream from WWTP IV. This suggests another source of discharge, possibly via Lake Jyväsjärvi which is located by the city area and connected to Lake Päijänne. Additionally, based on the results, UV light-induced degradation of pharmaceuticals occurs in the surface water leading to increased levels in deeper water layers.

The concentrations in lake water were higher in winter than in summer due to the decreased transformation and degradation reactions. In the winter, lower temperature and lower or nonexistent UV radiation, in the case of ice and snow cover, are the main reasons for decreased reactions in the environment. However, in some cases, the concentrations of selected pharmaceuticals were higher at the WWTPs in the summer. In the case of typical pain killer drugs (ibuprofen, ketoprofen, and naproxen), their consumption can increase in summer time. On the other hand, consumption of carbamazepine, which is also used as an antidepressant, is known to increase during the darker period of the year partly explaining the higher concentrations in the winter. In the case of diclofenac, concentrations in influents on both seasons are rather similar, but the concentrations in effluent decrease in the winter, most likely due to decreased, temperature-dependent efficiency at the WWTP.

Generally, the concentrations detected by passive samplers follow those of grab samples. Therefore, the results can be considered reliable and the Chemcatcher passive samplers suitable for the monitoring of pharmaceuticals in lake and river waters. Passive sampling gives results of a long-term situation, while grab sampling shows, if regularly taken, occasional fluctuations of concentrations. The highest concentrations were detected by passive sampling in Lake Päijänne at the point of effluent discharge from the WWTP. Decreasing concentrations with greater distance from the WWTP confirm that the WWTP is the main source of pharmaceuticals. The occurrence of pharmaceuticals upstream from the WWTP IV at a similar level but with a different profile suggests another source, calling for further research. Although high concentrations of selected

pharmaceuticals are only occasionally detected, the cocktail of different substances requires further studies, especially related to long-term toxicity to aquatic organisms.

Pharmaceuticals, such as carbamazepine and diclofenac, occurred at greater concentrations in the River Vantaa than in Lake Päijänne. The difference in the dominating pharmaceuticals between the River Vantaa and Lake Päijänne is most likely explained by the different sources of pharmaceuticals. There are several different types of point sources along the River Vantaa unlike in Lake Päijänne. Additionally, the environmental conditions and the dilution of the pharmaceuticals vary due to different loads and different types of water bodies. Sampling time and environmental factors most likely are the reason for the differences in river water in different years.

In conclusion, the Chemcatcher passive sampler with an SDB-RPS disk is suitable for detecting pharmaceuticals in river and lake waters. It can be expected that there will be the need for more extensive monitoring of pharmaceuticals in the near future due to restricting environmental legislation. Passive sampling gives a good alternative for long-term monitoring of environmental pollutants compared to traditional grab sampling and gives an inexpensive and less labor-intensive option also for screening of pollutants in obligatory environmental monitoring.

6.2 Suggestions for further research

Pharmaceuticals can accumulate and concentrate in aquatic organisms, including fish with magnitudes higher concentrations. Therefore, information is required regarding, not only concentration levels in a water body, but the toxicity and their effects on aquatic organisms. Furthermore, very little information is available about the effects of pollutant mixtures in the environment. This study shows that there is a mixture of pharmaceuticals present in Lake Päijänne and the River Vantaa. Their long-term effects on aquatic organisms, toxicity, and their antagonistic or synergic effects as a mixture remain subjects for further research.

In this study, parent compounds of the selected pharmaceuticals were detected and quantified. The degradation and transformation products both at the WWTPs and in surface water are an interesting and important subject of further studies when aiming at understanding their behavior in the environment. Furthermore, ways to degrade pharmaceuticals already at WWTPs leads to decreased environmental load and decreased effects on aquatic organisms. Methods for the degradation of pharmaceuticals at WWTPs remain an important subject for further studies.

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APPENDICES

APPENDIX I: Chemicals and matrials applied in study

APPENDIX II: Concentrations of selected pharmaceuticals (ng L^{-1}) in influent and effluent of WWTP IV, May-August 2013

APPENDIX III: Concentrations of selected pharmaceuticals (ng L^{-1}) in different depths and sampling sites of Lake Päijänne, May-August 2013

APPENDIX IV: Mean concentrations, number of samples (n), and standard deviations (SD) of selected pharmaceuticals (ng L^{-1}) in influents and effluents of WWTPs I-IV, March 2015

APPENDIX V: Mean concentrations and standard deviations (SD) of selected pharmaceuticals (ng L^{-1}) in water (depth 1 m) of Lake Kuhnamo (1), Kapeenkoski Rapids (2), Haapakoski Rapids (3) and northern Lake Päijänne (4, 6, 8), March 2015

APPENDIX I

Chemicals and materials applied in study

Chemical	Purity (%)	Supplier	Paper
Acetic acid	99.8 %	VWR	III-V
Acetonitrile	HPLC grade	J.T. Baker	III-V
Acetone	≥ 99.5 %	Sigma-Aldrich	III-V
NH_4OH	≥ 99.9 %	Sigma-Aldrich	III-V
Carbamazepine	98 %	Alfa Aesar	III-V
Diclofenac sodium salt	98 %	Alfa Aesar	III-V
Hexane	HPLC grade	Rathburn	III
Ibuprofen	99 %	Alfa Aesar	III-V
Ketoprofen	98 %	Alfa Aesar	III-V
Membrane filter 0.2 µm, ME 24		Cronus	III-V
Membrane filter 0.45 µm, PTFE		Cronus	III-V
Membrane filter 0.45 µm, PVDF		Cronus	V
Membrane filter 1.2 µm, GF		Cronus	III
Methanol	HPLC grade	J.T. Baker	III-V
Naproxen sodium	98 %	Alfa Aesar	III-V
SPE cartridge Bond Elut C18 LO, 500 mg/3 mL		Varian	III
SPE cartridge Bond Elut C18, 500 mg/3 mL		Agilent Technologies	IV-V

APPENDIX II 1/2

Concentrations of selected pharmaceuticals (ng L^{-1}) in influent of WWTP IV, May-August 2013

Date of sampling	Carbamazepine	Diclofenac	Ibuprofen	Ketoprofen	Naproxen
8.5.	6.3	368.7	14162.9	1092.7	1545.4
21.5.	5.4	348.0	20670.0	983.4	1966.9
29.5.	6.4	145.8	9785.5	445.2	799.3
12.6.	8.7	202.6	21226.5	759.7	1452.9
19.6.	8.7	248.9	16456.5	544.6	1481.0
26.6.	12.4	933.1	33701.2	1682.0	5161.2
3.7.	13.4	255.0	17909.4	567.7	1426.2
10.7.	6.4	319.4	32515.3	784.0	3501.1
16.7.	9.4	338.3	27879.6	492.2	3372.9
17.7.	7.4	271.1	29806.4	606.1	2129.8
18.7.	17.4	331.8	25701.2	782.5	2350.4
19.7.	12.0	121.0	14716.3	516.3	1148.3
24.7.	5.0	237.1	19057.3	372.1	1394.5
25.7.	4.4	248.1	14554.9	438.2	970.7
26.7.	19.7	369.1	20522.8	673.0	1983.8
31.7.	7.0	233.8	23322.9	605.6	2391.2
14.8.	12.1	691.9	42375.7	4292.9	4828.9
15.8.	1.0	631.9	32662.8	1279.1	3893.1
16.8.	5.5	478.8	23267.4	761.8	1238.8
19.8.	7.2	499.3	34829.2	726.0	1767.9
20.8.	4.4	456.3	25969.7	1084.9	2457.1
Mean	**8.6**	**368.1**	**23861.6**	**928.1**	**2250.5**

2/2

Concentrations of selected pharmaceuticals (ng L^{-1}) in effluent of WWTP IV, May-August 2013

Date of sampling	Carbamazepine	Diclofenac	Ibuprofen	Ketoprofen	Naproxen
8.5.	22.4	675.3	33.7	573.1	38.8
21.5.	31.7	614.7	53.3	404.4	242.8
29.5.	33.0	1305.1	86.0	864.1	1384.6
12.6.	91.9	nd	nd	nd	nd
19.6.	35.8	462.8	135.9	671.3	1269.8
26.6.	86.0	nd	nd	nd	nd
3.7.	36.9	972.9	58.4	697.7	672.9
10.7.	49.2	1577.4	239.5	1159.4	3070.0
16.7.	88.0	2755.1	208.4	1201.7	1753.6
17.7.	9.1	1105.7	126.7	1053.6	759.2
18.7.	47.3	644.4	419.0	1885.1	365.7
19.7.	54.1	1758.0	104.7	986.2	1703.8
24.7.	45.0	838.9	134.6	543.4	986.8
25.7.	54.3	1328.8	136.8	563.6	490.2
26.7.	31.2	1337.4	203.1	646.2	679.7
31.7.	42.2	1199.2	636.7	1109.1	995.9
14.8.	37.1	1072.9	76.8	3265.2	370.9
15.8.	42.8	1588.6	84.0	1259.0	552.4
16.8.	25.2	810.7	110.8	1004.0	664.3
19.8.	15.3	815.6	123.8	479.2	120.4
20.8.	12.1	687.7	164.7	427.3	287.2
Mean	**42.4**	**1134.3**	**165.1**	**989.1**	**863.6**

nd -not detected

APPENDIX III 1/5

Concentrations of carbamazepine (ng L^{-1}) in water of Lake Päijänne, May-August 2013 (Sampling site 4: 1, 5, 14m; 5: 1, 5m; 6: 1, 5m; 7: 1, 10, 24m; 8: 1, 10, 20m)

Date	Depth (m)	Site 4	Site 5	Site 6	Site 7	Site 8
30.5.	1	0.7	3.6	1.3	nd	1.0
	5/10	1.0	0.2	21.7	0.8	0.8
	14/20/24	0.2	na	na	1.4	1.1
13.6.	1	1.2	0.7	3.9	3.1	2.4
	5/10	0.4	1.0	18.7	1.9	3.9
	14/20/24	0.7	na	na	0.7	1.1
26.6.	1	0.6	0.6	1.1	0.5	0.9
	5/10	1.1	1.1	18.2	1.1	1.4
	14/20/24	1.5	na	na	na	0.7
11.7.	1	0.6	1.5	2.3	2.3	1.0
	5/10	0.9	3.6	1.4	0.9	0.7
	14/20/24	0.9	na	na	0.9	1.0
25.7.	1	1.0	1.4	1.4	6.3	0.6
	5/10	0.6	1.0	4.7	0.9	1.4
	14/20/24	0.8	na	na	1.3	2.8
8.8.	1	1.8	na	1.4	1.2	1.6
	5/10	0.8	na	2.5	0.9	1.5
	14/20/24	0.9	na	na	2.9	1.6
22.8.	1	1.9	1.4	1.5	0.5	0.6
	5/10	0.3	1.5	17.7	1.4	2.8
	14/20/24	0.4	na	na	1.2	1.5
5.9.	1	0.3	1.4	0.4	0.8	0.5
	5/10	0.3	2.4	1.8	3.9	2.3
	14/20/24	3.3	na	na	1.7	0.6

nd -not detected
na -not analyzed

APPENDIX III 2/5

Concentrations of diclofenac (ng L^{-1}) in water of Lake Päijänne, May-August 2013 (Sampling site 4: 1, 5, 14m; 5: 1, 5m; 6: 1, 5m; 7: 1, 10, 24m; 8: 1, 10, 20m)

Date	Depth (m)	Site 4	Site 5	Site 6	Site 7	Site 8
30.5.	1	58.6	83.3	11.8	5.6	35.7
	5/10	38.7	12.6	183.5	6.5	56.9
	14/20/24	nd	na	na	166.5	80.0
13.6.	1	129.7	29.2	85.6	64.1	18.9
	5/10	19.8	11.2	170.8	35.6	157.1
	14/20/24	4.5	na	na	6.7	nd
26.6.	1	9.4	4.2	9.4	8.3	57.0
	5/10	21.5	10.5	209.8	6.2	15.8
	14/20/24	31.0	na	na	6.4	5.3
11.7.	1	nd	20.2	36.9	46.5	5.2
	5/10	35.6	79.9	32.2	25.0	8.3
	14/20/24	nd	na	na	30.5	42.8
25.7.	1	22.6	9.6	14.0	130.7	11.1
	5/10	8.5	32.9	36.0	nd	82.0
	14/20/24	11.6	na	na	12.9	149.7
8.8.	1	119.9	na	142.5	37.3	94.4
	5/10	76.8	na	163.5	5.3	na
	14/20/24	115.7	na	na	59.5	234.8
22.8.	1	10.4	48.7	14.4	14.9	7.8
	5/10	1.3	19.1	86.8	8.8	36.6
	14/20/24	9.5	na	na	3.7	13.5
5.9.	1	9.9	62.0	15.5	13.2	5.2
	5/10	45.6	41.0	6.6	17.7	13.1
	14/20/24	47.3	na	na	60.6	46.5

nd -not detected
na -not analyzed

APPENDIX III 3/5

Concentrations of ibuprofen (ng L^{-1}) in water of Lake Päijänne, May-August 2013 (Sampling site 4: 1, 5, 14m; 5: 1, 5m; 6: 1, 5m; 7: 1, 10, 24m; 8: 1, 10, 20m)

Date	Depth (m)	Site 4	Site 5	Site 6	Site 7	Site 8
30.5.	1	263.1	136.9	nd	nd	100.9
	5/10	112.8	nd	nd	18.6	307.5
	14/20/24	nd	na	na	836.8	218.3
13.6.	1	374.7	47.8	273.8	216.9	130.5
	5/10	nd	nd	30.5	108.5	350.6
	14/20/24	nd	na	na	nd	nd
26.6.	1	39.2	nd	nd	19.7	42.3
	5/10	60.8	21.5	65.6	nd	90.2
	14/20/24	56.6	na	na	na	nd
11.7.	1	nd	70.1	52.5	127.2	12.2
	5/10	159.4	191.3	65.8	39.9	149.3
	14/20/24	149.4	na	na	77.1	191.3
25.7.	1	nd	6.7	nd	315.9	21.0
	5/10	nd	15.8	nd	nd	394.6
	14/20/24	nd	na	na	340.0	415.6
8.8.	1	429.9	na	449.8	177.5	574.4
	5/10	202.3	na	778.9	26.9	33.0
	14/20/24	239.8	na	na	176.0	685.3
22.8.	1	29.3	nd	20.0	nd	nd
	5/10	nd	nd	5.8	124.7	82.8
	14/20/24	nd	na	na	13.5	27.7
5.9.	1	nd	nd	24.1	37.9	nd
	5/10	nd	101.1	nd	nd	22.4
	14/20/24	nd	na	na	197.4	nd

nd -not detected
na -not analyzed

APPENDIX III 4/5

Concentrations of ketoprofen (ng L^{-1}) in water of Lake Päijänne, May-August 2013 (Sampling site 4: 1, 5, 14m; 5: 1, 5m; 6: 1, 5m; 7: 1, 10, 24m; 8: 1, 10, 20m)

Date	Depth (m)	Site 4	Site 5	Site 6	Site 7	Site 8
30.5.	1	84.3	nd	146.6	97.5	87.1
	5/10	nd	nd	nd	132.2	nd
	14/20/24	nd	na	na	282.8	nd
13.6.	1	117.9	79.3	119.3	26.6	89.8
	5/10	192.4	nd	441.7	nd	68.2
	14/20/24	64.9	na	na	nd	nd
26.6.	1	nd	nd	63.8	33.0	952.6
	5/10	70.8	nd	95.4	53.9	nd
	14/20/24	nd	na	na	nd	nd
11.7.	1	218.7	63.1	70.8	nd	nd
	5/10	nd	nd	nd	45.3	191.0
	14/20/24	148.0	na	na	65.0	nd
25.7.	1	nd	37.3	nd	96.2	118.9
	5/10	36.6	147.5	nd	nd	51.5
	14/20/24	nd		na	476.0	80.2
8.8.	1	84.3	na	58.4	23.3	64.8
	5/10	45.7	na	59.2	nd	26.3
	14/20/24	77.7	na	na	133.5	60.6
22.8.	1	9.4	182.4	nd	85.6	51.6
	5/10		167.1	106.9	nd	106.7
	14/20/24	60.3	na	na	nd	27.3
5.9.	1	65.6	62.4	nd	50.6	45.6
	5/10	102.3	nd	77.1	nd	19.2
	14/20/24	nd	na	na	nd	100.1

nd -not detected
na -not analyzed

APPENDIX III 5/5

Concentrations of naproxen (ng L^{-1}) in water of Lake Päijänne, May-August 2013 (Sampling site 4: 1, 5, 14m; 5: 1, 5m; 6: 1, 5m; 7: 1, 10, 24m; 8: 1, 10, 20m)

Date	Depth (m)	Site 4	Site 5	Site 6	Site 7	Site 8
30.5.	1	nd	nd	nd	na	nd
	5/10	nd	nd	nd	nd	nd
	14/20/24	nd	na	na	nd	nd
13.6.	1	16.6	nd	13.6	na	nd
	5/10	nd	nd	28.3	nd	20.1
	14/20/24	nd	na	na	nd	nd
26.6.	1	nd	nd	nd	31.8	105.9
	5/10	nd	nd	129.4	nd	nd
	14/20/24	nd	na	na	na	nd
11.7.	1	nd	55.2	nd	nd	nd
	5/10	nd	nd	nd	nd	nd
	14/20/24	nd	na	na	12.0	nd
25.7.	1	nd	2.8	nd	21.5	nd
	5/10	9.3	19.5	nd	nd	16.1
	14/20/24	nd	na	na	nd	27.1
8.8.	1	7.9	na	23.2	nd	10.9
	5/10	3.9	na	2.0	nd	nd
	14/20/24	nd	na	na	23.2	31.6
22.8.	1	nd	nd	nd	nd	nd
	5/10	nd	nd	23.1	nd	nd
	14/20/24	nd	na	na	nd	nd
5.9.	1	nd	nd	nd	nd	nd
	5/10	nd	nd	nd	nd	nd
	14/20/24	nd	na	na	nd	nd

nd -not detected
na -not analyzed

APPENDIX IV

Mean concentrations, number of samples (n), and standard deviations (SD) of selected pharmaceuticals (ng L^{-1}) in influents and effluents of WWTPs I-IV, March 2015

WWTP		Carbamazepine	Diclofenac	Ibuprofen	Ketoprofen	Naproxen
WWTP I: Influent	n	7	7	7	5	6
	mean	**13.8**	**779.4**	**3713.2**	**267.0**	**187.6**
	SD	9.3	159.3	1420.8	140.7	64.5
Effluent	n	9	9	9	8	8
	mean	**19.1**	**2247.8**	**821.3**	**195.5**	**124.8**
	SD	2.6	246.0	300.4	91.2	28.2
WWTP II: Influent	n	6	6	6	6	6
	mean	**15.3**	**277.8**	**11499.5**	**412.0**	**1534.8**
	SD	6.7	179.6	2483.0	49.9	286.0
Effluent	n	8	8	8	3	5
	mean	**28.4**	**1020.9**	**163.0**	**206.5**	**379.4**
	SD	6.5	306.5	43.3	115.1	157.0
WWTP III:	n	5	5	5	5	5
Influent	**mean**	**19.6**	**449.8**	**12817.5**	nd	**1325.4**
	SD	4.3	77.5	1558.1	nde	148.8
Effluent	n	4	4	2	4	4
	mean	**46.8**	**1033.9**	**59.1**	**234.7**	**222.4**
	SD	6.1	304.9	42.4	116.4	86.9
WWTP IV:	n	12	12	12	10	12
Influent	**mean**	**15.9**	**350.4**	**12056.2**	**353.9**	**1223.7**
	SD	2.5	177.6	1513.2	29.9	141.7
Effluent	n	16	15	13	9	13
	mean	**35.5**	**972.6**	**191.0**	**317.4**	**420.7**
	SD	3.9	83.3	113.2	107.6	138.6

nd -not detected
nde -not determined

APPENDIX V

Mean concentrations and standard deviations (SD) of selected pharmaceuticals (ng L^{-1}) in water (depth 1 m) of Lake Kuhnamo (1), Kapeenkoski Rapids (2), Haapakoski Rapids (3) and northern Lake Päijänne (4, 6, 8), March 2015

Date	Site 1	Site 2	Site 3	Site 4	Site 6	Site 8
Carbamazepine	**0.7**	**0.3**	**1.6**	**112.9**	**40.7**	**70.2**
SD	0.3	0.1	0.6	16.9	0.2	0.3
Diclofenac	**21.4**	**16.1**	**115.0**	**470.5**	**105.4**	**266.2**
SD	4.2	6.1	18.9	13.8	25.4	20.4
Ibuprofen	**18.4**	**6.9**	**43.1**	**217.0**	**82.6**	**261.9**
	1.9	1.2	4.6	18.4	17.9	9.5
Ketoprofen	**19.6**	**23.8**	**41.1**	**155.5**	**59.1**	**74.8**
	3.4	9.2	4.0	13.2	16.6	19.3
Naproxen	**nd**	**nd**	**15.6**	**80.4**	**16.5**	**46.0**
	nde	nde	2.6	11.8	0.2	8.5

nd -not detected
nde -not determined

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