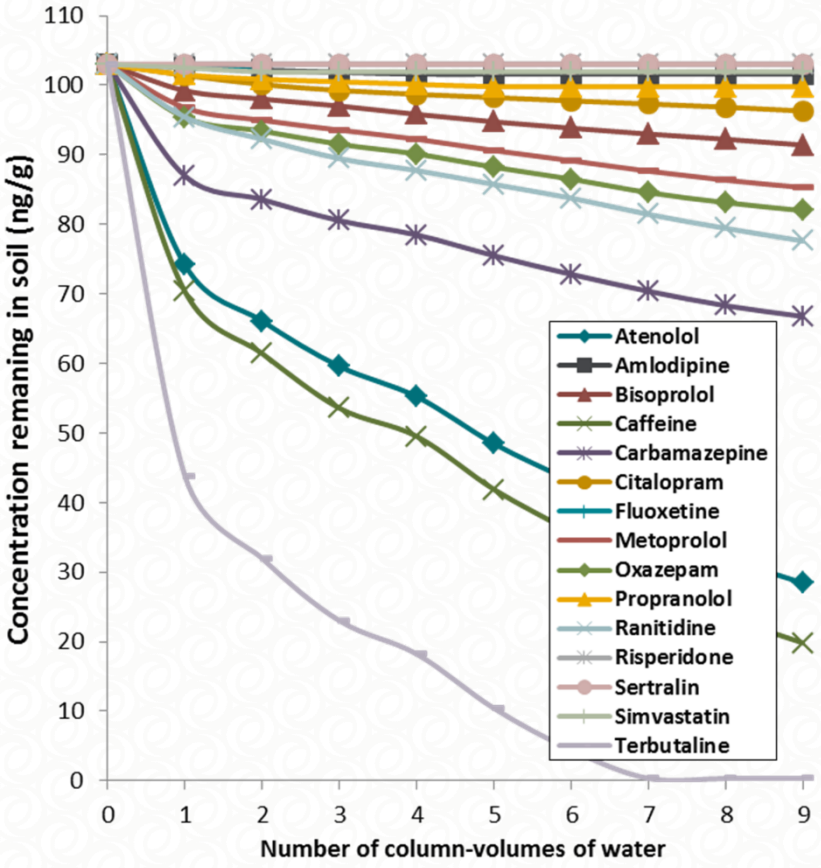

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Fate of pharmaceutical residues - in sewage treatment and on farmland fertilized with sludge

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Summary

Pharmaceutical products constitute a fundamental part of modern medicine and are in many cases crucial for health and wellbeing in our everyday life. However, the benefits from pharmaceutical come with drawbacks for the environment. These chemicals are designed to have a biological effect, which they unfortunately also might have on other living organisms than humans. They are moreover also considered to be semi-persistent due to the continuous discharge from our society. These characteristics make them problematic if they end up in the environment. For the last ten years pharmaceutical companies on the Swedish market can choose to publish environmental information about their products on the public web-based portal www.fass.se. Prior to publication the environmental information is reviewed by an external part (IVL Swedish Environmental Research Institute). Within the context as third party reviewer, IVL also performs research to increase the knowledge of pharmaceuticals in the environment to improve the reviewing process. This report describes the Fass research study conducted in 2014 and 2015. The focus of this study was to investigate the distribution and removal of a selection of pharmaceuticals within a sewage treatment plant (STP) and their final fate in the environment. For unclear reasons residues of pharmaceuticals can be represented in higher concentration in the effluent wastewater compared to the influent, which limits correct conclusions to be drawn regarding their removal during sewage treatment. Several studies on matrix effects and metabolism were performed to test different hypothesis that could explain the phenomena and to be able to estimate the "true" concentrations of pharmaceuticals within a STP. A mass balance was also performed to further study the pharmaceutical distribution. To assess the dispersion and fate of pharmaceuticals in the environment a farmland fertilized with sludge from the investigated STP were studied. Soil and sludge samples were analyzed as well as soil water collected by lysimeter techniques. In addition laboratory based soil sorption tests of the farmland soil exposed to pharmaceutical and sewage sludge were also performed.

The result of the study showed that analytical interferences (ion-suppression) due to competition with co-eluting matrix components during instrumental analysis was the main contributor to the observed increase in concentration of pharmaceuticals from influent to effluent wastewater, with an average ion-suppression of 49% in influent wastewater and 35% in effluent wastewater of the investigated pharmaceuticals.

The sludge from the STP used to fertilize the farmland contained 15 of the 24 investigated pharmaceuticals in concentrations between 1.9 to 1043 ng/g dry weights (dw). However, analysis of the lysimetric soil water revealed no detectable levels of the investigated pharmaceuticals. Analysis of soil samples exposed to sludge showed only traces of 4 out of 24 investigated pharmaceuticals in concentrations between 0.4 to 4.9 ng/g dw. A laboratory scale soil sorption test of unexposed soil from the field of Petersborg, spiked with a mixture of the 24 pharmaceuticals showed high soil retention of basic and neutral pharmaceuticals and a slightly higher diffusivity of acidic compounds. The four pharmaceuticals detected in the soil were among the compounds exhibiting high retention. The result of the study implies that the investigated pharmaceuticals are retained and eventually degraded at the surface of the soil. However, further investigations using representative leaching tests and additional degradation tests need to be performed in order to fully understand the fate of pharmaceutical residues in soil.

Sammanfattning

Läkemedel är en viktig del av den moderna medicinen och många gånger avgörande för människors hälsa och välbefinnande. Trots att fördelarna med läkemedel för behandling av sjukdomar är många kan rester av läkemedel orsaka skador i miljön. Läkemedel är kemikalier designade för att ha en biologisk effekt, vilket de även kan ha på andra levande organismer än enbart människor. Dessutom kan läkemedel vara semi-persistenta. Vilket gör dem problematiska om de hamnar i miljön. Under de senaste 10 åren har läkemedelsföretag etablerade på den svenska marknaden haft möjlighet att publicera miljöinformation om sina produkter på den publika webb-portalen www.fass.se. Innan miljöinformationen görs offentlig granskas den av en extern part (IVL Svenska Miljöinstitutet). Föreliggande studie har genomförts för att öka kunskapen kring läkemedel i miljön samt för att stärka granskningsprocessen.

Fokus för denna studie har varit att undersöka fördelningen och reningsgraden av ett urval av läkemedel i ett avloppsreningsverk samt läkemedlens slutliga öde i naturen. Vissa läkemedel har visat sig kunna förekomma i högre koncentration i utgående avloppsvatten än i inkommande, vilket begränsar slutsatser kring deras avskiljning i reningsverket. Studier av matriseffekter och metabolism har utförts i syfte att testa olika hypoteser som skulle kunna förklara fenomenet samt för att kunna uppskatta den ”sanna” koncentrationen av läkemedel i ett reningsverk. Även en massbalans studie har genomförts för att få kunskap om läkemedlens fördelning i reningsverket. För att bedöma spridningen samt läkemedlens öde i naturen studerades en jordbruksmark gödslad med slam från det undersökta reningsverket. Jord och slamprover analyserades liksom markvatten som samlats in med hjälp av lysimetrar. Dessutom utfördes även adsorptions tester av läkemedel till jord i laboratorieskala.

Resultaten av studien visade att jon-suppression på grund av konkurrens med sameluerande matriskomponenter under själva analysen var den största bidragande orsaken till den observerade ökningen i koncentration av läkemedelsrester från inkommande till utgående avloppsvatten, med en jon-suppression på 49 % i medel på inkommande avloppsvatten och 35% i medel på utgående avloppsvatten.

Analyserna av slammet som andvänts som gödning på åkermarken innehöll 15 av de 24 undersökta läkemedlen i koncentrationer från 1.9 till 1043 ng/g torrvikt. Däremot uppvisade ingen av markvattenproverna detekterabara halter av de undersökta läkemedlen. De jordarna som behandlats med slam uppvisade endast spår av 4 av de 24 studerade läkemedlen i koncentrationer från 0.4 till 4.9 ng/g torrvikt. Jordadsorptionsförsöket av oexponerad jord från Petersborgs gård, som spikats med en blandning av de 24 läkemedlen, uppvisade hög retention av basiska och neutrala läkemedel och en något sämre retention av sura läkemedel. De fyra läkemedel som detekterades i jordarna som behandlats med slam var bland de substanser som uppvisade hög adsorption till jord.

Resultatet av studien tyder på att de läkemedel som studerats fastläggs i jord för att med tiden brytas ned på plats. För att säkerställa resultatet av studien krävs ytterligare kompletterande tester i form av representativa lakningstester samt tester av nedbrytning av läkemedlen i jord.

1 Introduction

1.1 FASS the Swedish environmental classification system

Pharmaceutical products are essential for health and wellbeing in our everyday life. Medicines provide benefits, such as improvement in quality of life, and the demand will likely increase in the future due to a growing ageing population, chronic/lifestyle diseases, emerging-market expansion, and treatment and technology advances. Pharmaceuticals are chemicals designed to have a biological effect when administered to humans and animals. Many drugs are also designed to be stable in order to subsist when administered through the gastrointestinal tract. Furthermore, drugs need to be easily solubilized or to have the propensity to form polar soluble by-products when metabolized, in order to be successfully excreted from the body. These necessary characteristics in a pharmaceutical context can become problematic since excreted drugs and metabolites of drugs may end up in the aquatic environment and subsequently pose a risk to aquatic organisms, not initially considered when developing the pharmaceutical. Various studies have shown that the presence of some pharmaceuticals in the environment can result in the evolution and dissemination of antibiotic resistance genes (Gullberg et al., 2011) and other adverse effects, such as behavioral changes (Brodin et al., 2013) and skewed gender distribution of the aquatic wildlife (Hinfray et al., 2010; Sanchez et al., 2011; Tetreault et al., 2011).

In the year 2005, the Swedish Association of the Pharmaceutical Industry (LIF) took the initiative to develop and introduce a voluntary environmental classification guide for the pharmaceutical companies on the Swedish market. The initiative was a response to an increasing political and public demand for environmental information on pharmaceuticals. Each pharmaceutical company is responsible for the environmental information published on www.fass.se. An external part (IVL Swedish Environmental Research Institute) reviews the classifications to make sure they are based on a scientifically acceptable interpretation of the guidance. The work on the review of the environmental risk assessments on www.fass.se is conducted in close connection with related research studies, which form the basis for the development of the reviewing process. The study presented in this report is the result from the research project conducted in 2014 and 2015 as part of the ongoing work of improving the classification in www.fass.se.

1.2 Pharmaceuticals within the STPs

The chemical and physical properties of a pharmaceutical or group of pharmaceuticals determine their distribution within the Sewage Treatment Plants (STPs) and eventually in the environment. However, chemical analyses of pharmaceutical concentrations in wastewater are not straightforward and several studies have reported higher pharmaceutical concentrations in the effluent STP water than in the influent (Frick et al 2011, Paxéus 2004, Radjenovic et al 2007). The reason for this is not yet fully understood and different hypothesises exists:

1. **Matrix effects:** The presence of organic matter in the wastewater can interfere with the analyte of interest (in this case the pharmaceutical) during several steps of the analyses. This could lead to the detection of false concentration levels, an effect that could be larger for influent wastewater with higher amounts of organic matter. The organic matter may interfere with the analyte of interest both during sample preparation (leading to low recovery of the analyte) and during detection in the mass spectrometer (leading to either ion-suppression or ion-enhancement). In the mass spectrometer the excess of organic matter will compete with the analyte of interest for the electrospray ionisation beam. If the organic matter gives the same signal as the analyte, the result will be interpreted as a higher amount of the analyte than what is actually true (ion-enhancement). If the organic matter and the analyte have different signals the interpretation would instead be that less amount than reality is detected (ion-suppression). To account for recovery losses, ion-suppression and ion-enhancement it is possible to use isotopic labelled standards of the analytes of interest. However, the lack of isotopic labelled standards for all the pharmaceutical investigated makes it impossible to compensate for the losses during sample preparation and quantification.

   A common used method to determine the "true" concentration of an analyte in a complex matrix is by using standard addition, often referred to as "spiking" the sample. The idea is to add a range of known concentrations of the analyte (a standard curve) to several aliquots of the sample (i.e. to "spike" the sample) and analyse those together with the non-spiked sample with unknown background concentrations of the analyte. The change in instrument response between the unknown sample and the spiked samples is assumed to be due only to the change in analyte concentration. Linear regression is then used to calculate the concentration of the analyte in the unknown sample. By comparing differences in signal from pre-spiked samples (spiking before sample preparation) with post-spiked samples (spiking after sample preparation) analyte losses due to low recovery can be calculated. By comparing differences in signal from post-spiked samples with post-spiked blanks signal differences due to ion-suppression and ion-enhancement may be calculated. The approach of using standard addition to estimate the "true" concentration of an analyte is illustrated in Figure 1.

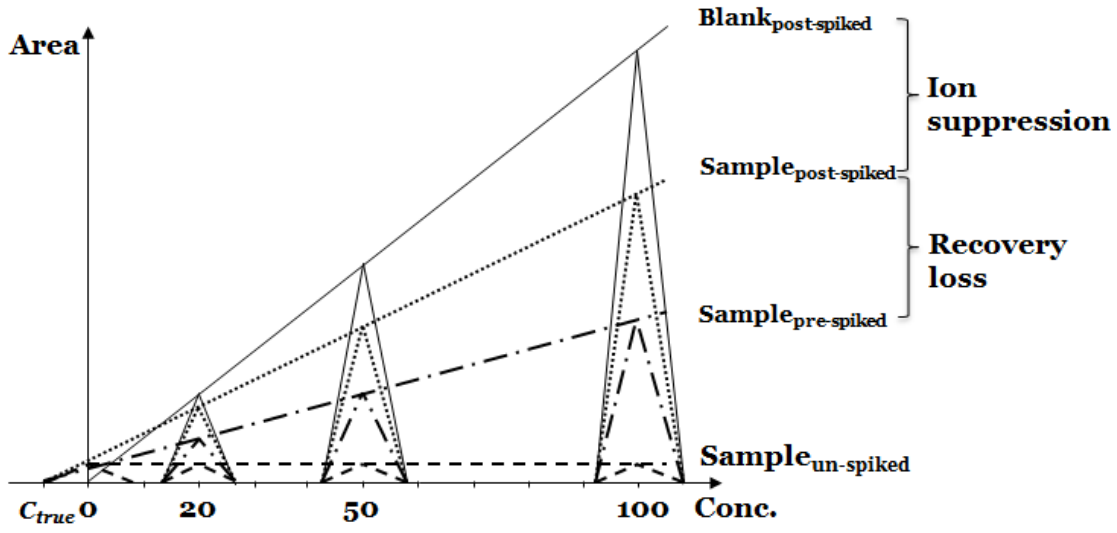

Figure 1. Relationship between ion-suppression and recovery loss utilizing standard addition to elucidate the "true" concentration in complex sample matrices.

2. **Metabolism:** Another hypothesis for detecting higher concentrations of different pharmaceuticals in the effluent wastewater compared to the influent water is referring to the metabolism of the pharmaceuticals in the human body before it reaches the STPs. In the human body xenobiotics, such as pharmaceuticals (denoted as "R" in Figure 2), are metabolised in a two-step process referred to as phase I and II to make the compound more water soluble and eliminated from the body through excretion via the urine (Figure 2). In phase I the pharmaceuticals are modified by either oxidation, hydrolysis (reduction) or cyclization. Some of these conditions may be reversible when the substance reaches different treatment steps in the STP, which include both reductive and oxidative environments. If the phase I metabolism is not enough to eliminate the pharmaceutical from the body the liver will try to attach water soluble groups through conjugation to the molecule in the second step of the metabolism, phase II (Figure 2). Thus, in many cases the pharmaceuticals entering the STP in the influent wastewater are not in the form of the parent substances, but chemically changed through the phase I and II metabolism reactions in the human body. In the STPs the conjugated metabolites from the phase II metabolism are de-conjugated by bacteria back to the initial parent substances through enzymatic cleavage. Since the chemical analyses of the influent and effluent wastewater only determine the amount of pharmaceuticals in the parent form, the result could be that higher concentrations are measured in the effluent wastewater than in the influent, if a compound is present as a transformed/metabolized entity in the influent water and is transformed back to the parent compound during the sewage treatment process.

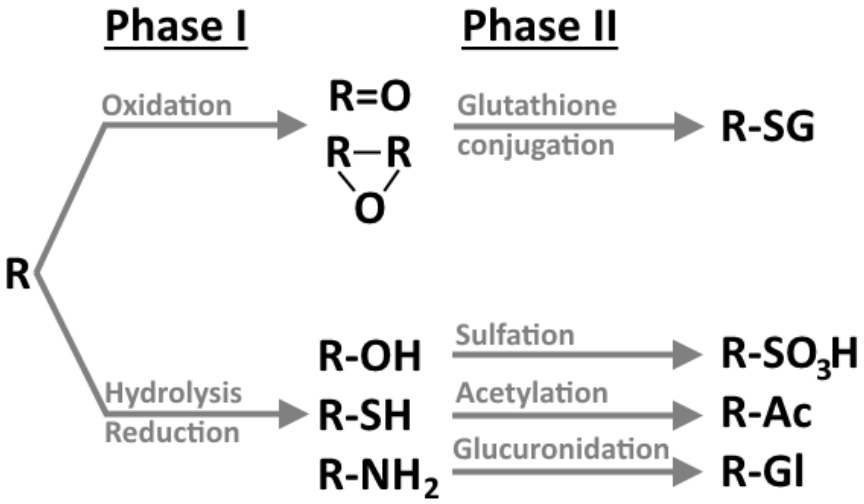

Figure 2. Phase I and II metabolism of xenobiotics (i.e. pharmaceuticals, denoted as "R" in the figure) in the liver.

1.3 Pharmaceuticals in the environment

Pharmaceuticals are as mentioned above a heterogenic group of substances with a large variety in physical and chemical properties which determines their distribution and fate in STP and in the environment. Pharmaceutical residues in the environment have become a prioritized area within environmental surveillance as well as within environmental risk assessment. It is a focus area in the EU Strategy for the Baltic Sea Region (European commission, 2015) and is being investigated in a number of national and international projects, such as Pharmas, MistraPharma, noPILLS, and within the Swedish screening program (Fick et al., 2011; Fick et al., 2015).

Many pharmaceuticals are not degraded in STP and may therefore be distributed via dispersion of sludge on farmland. In Sweden, approximately 25 % of the sewage sludge produced in the STPs is being used as fertilizers on farmlands (SCB, 2012). However, the use of sewage sludge as fertilizers is controversial due its contents of anthropogenic, organic compounds that may pose a risk to the environment, both through the dissemination of contaminants to the soil and groundwater as well as by its uptake in crops. Investigations have shown that the aerobic degradation of pharmaceuticals continue in soils that has been amended with sewage sludge (Kümmerer 2004; Gielen et al 2009). The uptake in crops occurs mainly through pore water and substances readily taken up in crops have an octanol-water distribution of Log K_{OW} ~1.8 (Wu et al., 2010a). In an experimental study where soil had been exposed to several different substances, including pharmaceuticals, less than 10% of the tolerable intake for humans (TDI) was found in the crops although the soil had a relatively high concentration of 1 mg/kg (Boxall et al., 2006). A study performed by Kenny et al., (2006) showed a low (<1) bioaccumulation factor (BAF) of the studied pharmaceuticals in earthworm. Even though many investigations have been performed regarding the degradation and dispersion of pharmaceuticals on farmland knowledge gaps still remains. Future technology advancements in wastewater treatment in the STP may result in higher concentrations of pharmaceuticals in the sludge, which further complicates the usage of sludge as fertilizer on farmland (Ek et al. 2014; Baresel et al 2015). Thus, more knowledge is needed about the actual degradation and the factors controlling the immobilization of the substances in the soil.

2 Aim and strategy

The overall aim of this study is to continue to develop and strengthen the Swedish environmental classification system of pharmaceuticals. The more specific aims of the two-year research project accomplished during 2014 and 2015 were to investigate the distribution and fate of pharmaceuticals within the STPs as well as in the environment. For this, a base set of 24 pharmaceuticals were selected as model substances for the analyses and several different approaches and matrices, specified below, were used in the studies.

1. **Distribution and fate of pharmaceuticals within the STP**
    a. *The role of matrix effects for the pharmaceutical concentrations in the wastewater:* influent and effluent STP wastewater were analysed in order to investigate the possible influence matrix effects, such as ion-suppression and ion-enhancement, can have on establishing the "true" removal efficiency of pharmaceuticals during sewage treatment.

    b. *The role of matrix effects for the pharmaceutical concentrations in the sludge:* STP sludge were analysed in order to determine the possible influence matrix effects can have on estimating the final concentration of pharmaceutical residues in sludge.

    c. *The role of pharmaceutical metabolism in the human body for the concentrations in the wastewater*: influent and effluent STP wastewater were analysed to determine the possible influence metabolism, such as phase I and phase II transformations, can have on establishing the "true" removal efficiency of pharmaceuticals during sewage treatment.

    d. *Mass balance calculations on the pharmaceutical concentrations:* by utilizing the results from step a, b, and c a mass balance were performed to estimate the distribution of pharmaceuticals between the different matrices within the STPs and to assess the main route of distribution of different classes of pharmaceuticals to the environment.

2. **Distribution and fate of pharmaceuticals in the environment**
    e. *Pharmaceutical concentration in sewage sludge, soil and soil water:* soil and soil water from a farmland treated with STP sludge as fertilizer, were sampled and analysed in order to investigate the retention of pharmaceuticals in soil and the potential dispersion to the groundwater.

    f. *Soil sorption tests with soil exposed to sludge from the farmland:* soil sorption tests were performed, with soils from the sampling site exposed to sludge or a mixture of pharmaceuticals, in order to further investigate the soils capacity to retain pharmaceuticals of different chemical and physical subclasses.

3 Methods

3.1 Pharmaceuticals

A subset of 24 pharmaceuticals was selected for the analyses (Table 1). The selected substances represent a commonly studied base set of pharmaceuticals frequently detected in sludge and water samples from STPs. With their wide range of chemical properties they represent different classes of pharmaceutical substances. This facilitates the drawing of general conclusions on the fate of different classes of pharmaceuticals, since it is not possible to perform studies on all of the thousands of pharmaceuticals that exist on the market today.

Table 1. The 24 investigated pharmaceuticals including mode of action and chemical properties such as the partition coefficients logKow[1)] and logD[2)] and if the pharmaceutical molecule is an acid, a base or neutral.

		Chemical properties		
Substance	**Mode of action**	Acid/Base	Log K_{ow} [1)]	Log $D_{pH7.4}$ [2)]
Diclofenac	*Anti-inflammatories*	Acid	4.06	1.37
Furosemide	*Diuretics*	Acid	3.10	-0.78
Hydrochlorothiazide	*Antihypertensives*	Acid	-0.07	-0.01
Ibuprofen	*Anti-inflammatories*	Acid	3.72	0.45
Naproxen	*Anti-inflammatories*	Acid	3.00	0.45
Ramipril	*Antihypertensives*	Acid	3.41	-0.13
Warfarin	*Anticoagulants*	Acid	3.42	0.30
Atenolol	*Antihypertensives*	Base	0.10	-1.85
Amlodipine	*Antihypertensives*	Base	4.16	1.91
Bisoprolol	*Antihypertensives*	Base	2.14	0.12
Caffeine	*Stimulant*	Neutral	-0.13	0.28
Carbamazepine	*Sedatives*	Neutral	2.67	2.28
Citalopram	*Antidepressants*	Base	2.51	1.27
Fluoxetine	*Antidepressants*	Base	4.09	1.75
Ketoprofen	*Anti-inflammatories*	Acid	2.81	0.06
Metoprolol	*Antihypertensives*	Base	1.79	-0.25
Oxazepam	*Sedatives*	Neutral	2.31	2.06
Paracetamol	*Anti-inflammatories*	Acid	1.08	0.74
Propranolol	*Antihypertensives*	Base	3.10	1.15
Ranitidine	*Antiulcers*	Base	1.23	-0.63
Risperidone	*Antipsychotic*	Base	2.88	1.81
Sertralin	*Antidepressants*	Base	4.81	3.14
Simvastatin	*Lipid-regulating*	Neutral	4.41	4.60
Terbutaline	*Asthma medication*	Base	0.48	-1.61

(1)) **LogKow:** the logarithm of Kow (the octanol/water partition coefficient), where Kow = a chemicals concentration in octanol phase / a chemicals concentration in aqueous phase. The logKow-value describes the chemicals water solubility and its tendency to partition to an organic phase (e.g. biota and soil) or an aqueous phase. Chemicals with high logKow-values (e.g. ≥4) are considered hydrophobic, whereas chemicals with low logKow-values (e.g. <4) are considered relatively hydrophilic and thus have high water solubility (LIF 2012). LogKow describes partitioning of the neutral (uncharged) form of the molecule.

(2) **$LogD_{pH7.4}$:** the logarithm of the distribution-coefficient at pH 7.4 (the physiological pH of blood serum). The majority of the pharmaceuticals contains ionisable groups and is therefore likely to be charged at environmental relevant pH. Since LogKow describes the partition coefficient of uncharged molecules, the logD-value is a better indicator to estimate the lipophilicity of ionisable compounds like pharmaceuticals. The logD-value is calculated from the logKow and the acid dissociation constant (pKa) or the base dissociation constant (pKb).

3.2 Sampling sites

3.2.1 Sjölunda STP

Sjölunda STP is located in Malmö and is one of the largest STPs in Sweden. The plant was commissioned in 1963 and today it collects wastewater from approximately 300 000 persons in

nearby municipalities. Influent and effluent wastewaters were collected from Sjölunda STP for studies on matrix effects. Sludge from Sjölunda was used in the soil sorption tests and data on concentrations and flows of the wastewater in Sjölunda STP were used for the mass balance calculations.

3.2.2 Hendriksdals STP

Henriksdals STP is the largest STP in Sweden and was dedicated in 1941. It is located in the southern part of Stockholm and collects wastewaters from approximately 780 000 persons in the municipalities nearby. Due to the limited access to influent and effluent composite sewage water samples from Sjölunda STP sewage water from Henriksdal STP was used instead to evaluate the influence of metabolism on the removal efficiency of the investigated pharmaceuticals. This implied the following assumption that metabolites ratio of pharmaceuticals from humans excretion are equal between populations and that the two STPs use similar wastewater treatment techniques.

3.2.3 Petersborg

To study the long term effects of sludge amendment on storage and dispersion of pharmaceuticals in arable soil, sampling of soil and soil water was performed in an experimental arable field (Petersborg) in Skåne. The arable field at Petersborg has a long history of sewage sludge amendment. Long term field studies of the effects of sludge amendment on arable land have been conducted since year 1981 (Andersson, 2012). The total area of the field is 36 * 120 m (4 320 m2). The field is cultivated according to a field specific crop rotation. In the year 2014 and 2015 the crops grown were winter wheat and sugar beet respectively. The topsoil (A-horizon, i.e. plough layer) at Petersborg has a pH of 6,8 and contains 2-3 percent organic matter and 15-25 percent clay (Andersson, 2012). The climate is temperate with a mean annual temperature of 7-8 °C and a mean annual precipitation of around 600 mm for the period 1960-1990. The latest application of sludge was in August year 2013 (SLU, 2013). The sludge applied at Petersborg was derived from the Sjölunda STP in Malmö.

3.3 Distribution and fate of pharmaceuticals within the STP

3.3.1 Collection of samples

Influent and effluent wastewaters from Sjölunda STP were collected as composite samples between 12-18th of October 2015. The corresponding sewage sludge was collected as a composite sample between 2-6th of November 2015. The collected samples were stored at -20°C before sample preparation and analysis.

3.3.2 Chemical analyses

The wastewater and sewage sludge from Sjölunda STP collected during autumn 2015, as well as the sludge that was used as a fertilizer on the Petersborgs farm during autumn 2013 (also from Sjölunda STP) were analyzed regarding their concentration of the selected 24 pharmaceuticals.

Different sample treatment techniques such as matrix effects, chemical reduction and oxidation as well as enzymatic cleavage were utilized to elucidate the "true" concentration of the targeted analytes in the samples. Table 2 shows a summary of the applied analytical techniques on each sample.

Table 2. Analytical methods applied to the wastewater and sewage sludge from Sjölunda STP.

Matrix	Number of Samples	Untreated	Matrix effects	Chemical reduction	Chemical oxidation	Enzymatic cleavage
Influent wastewater	1	Yes	Yes	Yes	Yes	Yes
Effluent wastewater	1	Yes	Yes	Yes	Yes	Yes
Sludge in STP	1	Yes	Yes	Yes	Yes	Yes
Sludge amended on farmland	1	Yes	No	No	No	No
Sum:	4					

Authentic reference standards were used in the chemical analyses as well as spiked and non-spiked procedural blanks. Blank subtraction was performed in appropriate studies, for additional information on quality control; see the description of each study.

3.3.2.1 Preparation of water samples

The solid phase extraction (SPE) procedure of water was modified based on a method previously described by Gros et al., 2006. Thawed composite samples were spiked with 50 µl of the respective isotopic labeled standards diclofenac-$^{13}C_6$, hydrochlorotiazide-$^{13}C_6$, carbamazepine-$^{13}C^{15}N$ and ibuprofen- d_3 with a concentration of 2 µg/ml. The samples were shaken at 125 rpm (rotations per minute) with an addition of 50 mg ethylenediaminetetraacetic acid (EDTA) for 30 minutes before the samples were pre-concentrated on the SPE cartridges (Oasis HLB, 6cc, Waters). The SPE cartridges were conditioned with methanol followed by MQ water. Thereafter, the samples were applied to the columns at a flow rate of two drops per second (<10 ml/minute). The analytes were eluted from the SPE cartridges using 6 ml methanol followed by 6 ml acetone. The eluates were evaporated to dryness under nitrogen at 40° C. The samples were reconstituted in 1.0 ml methanol:water (1:1) and centrifuged at 10 000 rpm in 10 minutes. The supernatants were transferred to vials for final determination on a high performance liquid chromatography-triple quadrupole mass spectrometry (HPLC-MS/MS).

3.3.2.2 Preparation of soil and sludge samples

The extraction of pharmaceutical residues from sludge and soil was based on a method previously described by Malmborg and Magnér (2015). Freeze-dried sludge or soil samples of 0.2 to 0.5 mg were introduced to 12 mL polypropylene (PP) tubes and spiked with 50 µl of the respective surrogate standards diclofenac-$^{13}C_6$, hydrochlorotiazide-$^{13}C_6$, carbamazepine-$^{13}C^{15}N$ and ibuprofen-d_3 with a concentration of 2 µg/ml. 0.5 mL of 2 M magnesium nitrate ($Mg(NO_3)_2$) and 10 mL of acetonitrile:dichloromethane (1:1) were added to the samples. Thereafter the samples were extracted by shaking with a vortex mixer for 30 seconds, followed by agitation on a horizontal shaking table at 1400 rpm for 30 minutes. After extraction the samples were centrifuged at 3000 rpm for 5 minutes. The supernatants were transferred to new 12 mL PP tubes and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The samples were reconstituted in 1.0 mL methanol:water (1:1) containing 0.1% (w/w) EDTA on an ultrasonic bath for 5 minutes, followed by centrifugation at 10 000 rpm for 10 minutes. The supernatants were transferred to vials for final determination on a HPLC-MS/MS.

3.3.2.3 Instrumental

The determination of pharmaceutical residues in the samples was performed on a binary liquid chromatography (UFLC) system equipped with an autoinjector (Shimadzu, Kyotu, Japan) coupled to an API 4000 triple quadrupole (MS/MS) (Applied Biosystems, Foster City, USA) with an electrospray ionization interface (ESI) performed in both positive and negative mode. The chromatographic separation was carried out using gradient elution on a Xbridge (Waters Corporation, Milford, USA) C_{18} reversed phase column (50 x 3 mm, 5-micron particle size) at 35 °C and a flow rate of 0.3 mL/minute. The mobile phase consisted of 10 mM acetic acid in water (mobile phase A) and methanol (mobile phase B). The gradient was initiated with 100% of mobile phase A and 0% of mobile phase B. The percentage of mobile phase B was increased linearly to 95% in 11 minutes and maintained at 95% for 5 minutes. Thereafter the mobile phase composition was returned to the initial composition in 1 minute and maintained for 4 minutes before the next injection. The total sample run-time was 21 minutes.

3.3.3 Matrix effects

Standard addition was used to evaluate the influence from matrix effects on the sample preparation and mass spectrometer analysis of the 24 investigated pharmaceuticals. For each set of matrix (sewage sludge, influent and effluent wastewater) triplicates were made and pre-spiked with 50, 100 and 200 ng of a standard mixture with the 24 investigated pharmaceuticals (Sample pre-spiked), i.e. the standard mixture was added to the matrix before sample preparation. An additional set of three samples were post-spiked (Sample post-spiked), i.e. where the standard mixture was added to the sample after sample preparation. The results of the analysis of the pre- and post-spiked samples were compared to triplicates of non-spiked samples of respective matrix (Sample un-spiked) and with a set of spiked tap water samples (Blank post-spiked) that haven't been subjected to sample preparation. The results of the standard additions were used to elucidate losses due to matrix effects, such as recovery losses during sample preparation and ion-suppression or ion-enhancement, during mass spectrometer analysis.

3.3.4 Metabolism

Influent and effluent wastewater (each 250 mL) from Henriksdal STP were sampled and extracted as previously described. The final extracts were evaporated to dryness under nitrogen at 40° C. The influent and effluent extracts were reconstituted in acetone (5.5 and 2.75 mL, respectively). Five aliquots (0.5 mL) of the re-dissolved extracts were transferred to new test tubes and evaporated to dryness using nitrogen gas and mild heat. The subsamples were subjected to different treatments procedures – utilizing the same protocol for the corresponding influent and effluent samples. The five treatments consisted of; i) reduction with sodiumborohydrate dissolved in acetonitrile (0.1 M $NaBH_4$, 1 mL), ii) reduction with sodiumborohydrate dissolved in methanol/water (0.1 M $NaBH_4$, 1:1, 1 mL), iii) oxidation with hydrogenperoxide (0.1 M, 1 mL), iv) incubation with β-Glucuronidas from *Helix pomatia* (from Sigma-Aldrich, this enzyme also commonly exhibits sulfatase activity) for 3 h at 55°C in a sodium acetate buffer (0,01 M, pH = 5, 2 mL), v) control sample, no treatment performed. The reductive and oxidative treatments, i.e. i-iii), were selected to reverse, if possible, the effects of phase I metabolism (see Figure 2). For instance an oxidized transformation product could be back-transformed to the mother compound if exposed to a reducing agent (sodium borohydride in the experiment). The enzymatic treatment, i.e. iv), was performed to investigate if any phase II metabolites were present in the water samples (see Figure 2). It should be noted that the enzyme extract from *Helix pomatia* is only known to express activity towards glucuronide and sulfate conjugated metabolites, in other words metabolites conjugated with different substrates

will not be cowered by this study. The results of the oxidation, reduction and de-conjugation (enzymatic) experiments were used to determine the possible influence metabolism, such as phase I and phase II transformations can have on establishing the "true" removal efficiency of pharmaceuticals during sewage treatment.

3.3.5 Mass balance

In a mass balance the mass flow in equals the mass flow out taken into account any production or reduction in the system. For the influent, effluent and sludge samples from Sjölunda STP a mass balance were set up based on concentrations and flows. The influent, effluent and sludge flows were given from the process data from Sjölunda STP. Two mass balances were calculated, one with the measured concentrations in each sample and one with the "true" concentrations, where the measured concentrations had been adjusted based on data from the matrix effect and metabolism experiments.

3.4 Distribution and fate of pharmaceuticals in the environment

3.4.1 Soil and soil water sampling

The field at Petersborg is equally divided in 36 sections. Every section is 6 x 20 m (120 m^2). In each section a defined amount of sludge: 0 (control), 4 or 12 tons dw/ha, has been applied repeatedly every fourth year since the experiment started. A schematic picture of the experimental design of the field trial at Petersborg is shown in **Error! Reference source not found.**3. In the present study sampling was performed in eight sections in the Petersborg field. The sections sampled represent two sludge application levels, i.e. no sludge (A2) and 4 tons sludge dw/ha. In both sections A2 and B2 sludge has been applied in combination with NPK (nitrogen, phosphorous and potassium) fertilizer (normal dose to meet crop demand). A sludge application rate of 4 tons dw/ha is considered to be representative to common Swedish agricultural practice.

1 A0	7 B0	13 C0	19 B0	25 C0	31 A0
2 A1	8 B1	14 C1	20 B1	26 C1	32 A1
3 A2	**9 B2**	15 C2	**21 B2**	27 C2	33 A2
4 A1	10 B1	16 C1	22 B1	28 C1	34 A1
5 A0	11 B0	17 C0	23 B0	29 C0	35 A0
6 A2	**12 B2**	18 C2	**24 B2**	30 C2	36 A2

Figure 3. Schematic design of the study field at Petersborg in Skåne, southern part of Sweden. Each numbered square represents a field section of 6x20 m, which is fertilized with the combination of sewage sludge and mineral NPK (nitrogen, phosphorus and potassium) fertilizer. The squares highlighted in green marks the sections sampled in the present study. These represent sections receiving 4 tons sludge dw/ha (text in bold) and sections where no sewage sludge have been applied.

The sampling of soil and soil water was performed by staff at Hushållningssällskapet in Malmöhus County. Originally it was intended that the sampling period would extend over two years (2014-2015). However, based on the initial results from chemical analysis of soil and soil water it was decided to end the field sampling after one year and instead continue with laboratory based soil sorption tests.

Sampling of soil was performed at on two occasions, in spring (April) and in late autumn (November) 2014. In spring 2014, soil samples were taken from the 0-0.3 m depth (plough layer) in untreated sections (no sludge applied) and from both 0-0.3 m and 0.3-0.6 m depth in sections receiving 4 tons sludge dw/ha. At the second sampling occasion in late autumn 2014, soil samples were solely taken from 0-0.3 m depth. Soil was sampled using a handheld soil drill made of steel. Ten soil samples from each section were pooled to form one composite sample per section and depth.

Soil water from beneath the plough layer (0.5 m depth) was sampled by Teflon suction lysimeters (Prenart Super Quartz: www.prenart.dk) in the sections that had been subjected to sewage sludge amendment (i.e. 9B2, 12B2, 21B2 and 24B2 in Figure 3). The installations of the lysimeters were performed by Prenart, staff at Hushållningssällskapet and IVL. In each section, five lysimeters were evenly distributed along a row, located at one meter distance from the edge of the long side. A total of 20 lysimeters were thus installed in the four sections. The lysimeters were installed several months prior to the first sampling occasions and soil water were discarded on several occasions before sampling to allow the lysimeters to equilibrate with the soil water. Collection of soil water was performed twice, in August 2015 (after harvest) and in November 2015 (before ploughing). Soil water was collected during approximately 1-2 weeks at each sampling occasion. Replicate soil water collected from the five lysimeters at each section was bulked in the field and the volume in each collecting bottle noted.

The lysimeter system consists of a porous body (i.e. a suction cup) which is connected via tubing to a collection vessel and a vacuum pump (**Error! Reference source not found.**4). Pictures taken during the lysimeter installation at Petersborg in March 2014 are shown in **Error! Reference source not found.**5.

At the laboratory, soil and soil water samples were stored in a freezer (-20° C) until analyses.

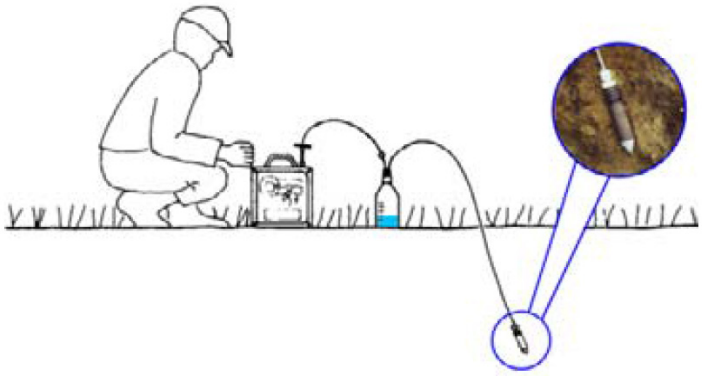

Figure 4. Illustration of the collection of soil water with a suction lysimeter system (picture from www.prenart.dk).

For the installation of a suction cup a hole was drilled with an auger. The hole was made at an angle (45°) to the ground surface.

A special tool was used to place the lysimeter at the bottom of the installation hole, together with a silica flour slurry.

All sampling collectors were placed in soil pits in sealed opaque containers to protect the samples from heat and sunlight.

The suction cup is connected via tubing to a collection vessel and a vacuum pump. During collection of soil water the lysimeters are operated at continuous vacuum.

Figure 5. Picture illustrating the installation of suction lysimeters at Petersborg in March 2014.

3.4.2 Soil sorption tests

To investigate the capacity of the soil to retain/immobilize the pharmaceuticals a set of soil sorption tests were performed. Soil from level 0-25 cm in square 3, lane A2 at the investigation area in Petersborg was packed in 6 ml polypropylene cartridges. Two different cartridges were prepared. One of the two cartridges was spiked with 0.5 g of freeze dried sludge (from the same sludge used as fertilizer on the field). The second cartridge was spiked with a solution containing 200 ng of each one of the 24 investigated pharmaceuticals. The cartridges were eluted with maximum 9 times the soil-column volumes of tap water (9 x 1.5 ml). The method is illustrated in Figure 6. Each volume was collected separately in vials and spiked with 50 µl of the respective surrogate standards diclofenac-$^{13}C_6$, hydrochlorotiazide-$^{13}C_6$, carbamazepine-$^{13}C^{15}N$ and ibuprofen- d_3 with a concentration of 2 µg/ml. Thereafter the eluates were sent for final determination on a HPLC-MS/MS.

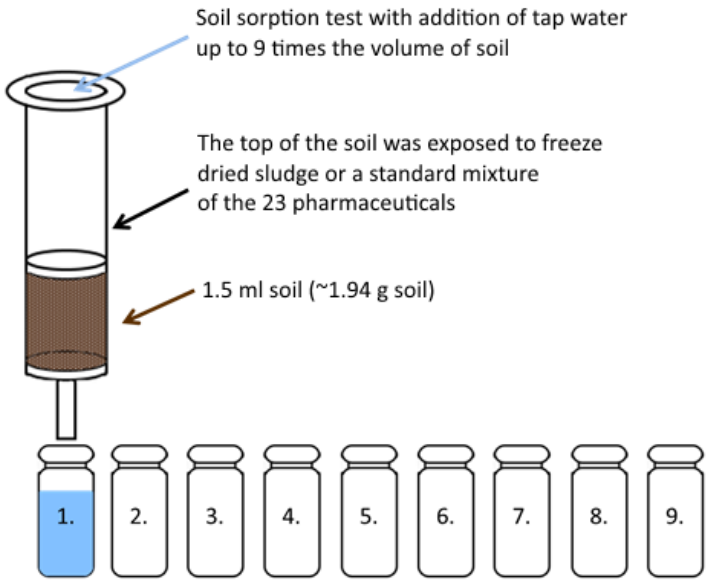

Figure 6. Illustration of the soil sorption test.

3.4.3 Chemical analyses and instrumental

Same methods were applied for the extraction and the mass spectrometer analyses of the soil, sewage sludge and soil water as previously described (see section 3.2.2.1, 3.2.2.2 and 3.2.2.3 above).

4 Results and discussion

4.1 Distribution and fate of pharmaceuticals within the STP

4.1.1 Matrix effects

Table 3, 4 and 5 shows the impact of matrix effects on the estimated true concentration of the 24 investigated pharmaceuticals in influent and effluent wastewater and sludge samples. For each substance, the Tables give information on Total recovery (%), Loss to ion-suppression (%), and Loss in recovery (%).

"Total recovery" gives the estimated abundance in percentage of a pharmaceutical in the sample, relative to the control, if standard addition is not used to compensate for the losses to ion-suppression and losses in recovery.

"Loss to ion-suppression" expresses suppression of the quantitative signal of a substance during instrumental analysis due to competition with co-eluting matrix component present in the sample for the ionisation energy in the ion-source of the mass-spectrometric detector. Negative values shows that the quantitative signal for a pharmaceutical is enhanced by the presence of co-eluting background matrix components, so called ion-enhancement.

"Loss in recovery" refers to losses during sample pre-treatment, such as sample pre-cleaning and sample pre-concentration. The recovery losses can be due to inefficient recovery of the pharmaceutical during sample preparation or due to competition with matrix components for the adsorptive sites of the SPE filter column used to extract the pharmaceuticals from the sample.

Negative values for "Loss in recovery" represent the residual or uncertainty between ion-suppression and loss in recovery when using standard addition to estimate the impact of matrix effects on the results.

The results which are summarized in Figure 7 show that 16 of the investigated pharmaceuticals have a lower total recovery in influent than effluent sewage water. Ion-suppression during analysis in the mass spectrometer showed to be the main contributor to the observed increase in concentration of pharmaceuticals from influent to effluent wastewater. The average ion-suppression of the investigated pharmaceuticals in the influent wastewater was 49% (median: 49%) and 35% (median: 34%) in the effluent wastewater (see Table 3 & 4). Ion-suppression also dominates the losses in sludge (Table 5). However, the loss to ion- suppression is less pronounced in sludge compared to wastewater, while the total recovery of the pharmaceuticals in general is lower in sludge compared to the wastewater (Figure 7). The reason why the loss in recovery is more substantial in sludge may be due the sample pre-concentration of pharmaceuticals from sludge using liquid-liquid extraction (LLE), which has shown to be a less effective extracting technique than solid phase extraction (SPE) utilized to pre-concentrate pharmaceuticals from wastewater (Filippov et al., 2003). Nevertheless, ion-suppression dominates the observed overall losses in wastewater and sludge (Table 3, 4, 5 & Figure 7). Caffeine showed inconsistent values of recovery and ion-suppression in the experiment and was therefore excluded from Table 3 and 4

and from Figure 7. The unrealistic values of caffeine could be an artefact due to the high abundance of caffeine in wastewater, which made the relative small addition of standard negligible. Since caffeine is less prone to partitioning to more lipophilic matrices, the observed concentration of caffeine in sludge was well within the concentration range of the standard addition and the result was therefore illustrated in Table 5.

Table 3. Matrix effect on the total recovery during analysis (loss to ion-suppression) and sample preparation (loss in recovery) for the investigated pharmaceuticals in influent wastewater.

Substance	Total recovery %	Loss to ion-suppression %	Loss in recovery %
Diclofenac	56	50	-6.1
Furosemide	29	70	1.4
Hydrochlorothiazide	63	36	1.1
Ibuprofen	84	70	-54
Naproxen	51	60	-11
Ramipril	68	73	-41
Warfarin	83	32	-15
Atenolol	93	3.6	3.8
Amlodipine	31	73	-3.8
Bisoprolol	58	41	0.2
Carbamazepine	57	49	-5.3
Citalopram	50	48	2.7
Fluoxetine	33	67	0.3
Ketoprofen	31	73	-4.3
Metoprolol	59	42	-0.4
Oxazepam	49	73	-22
Paracetamol	88	3.3	8.3
Propranolol	54	48	-1.5
Ranitidine	39	64	-3.1
Risperidone	39	47	14
Sertralin	27	73	-0.1
Simvastatin	56	37	7.5
Terbutaline	92	2.3	6.1
Average:	*56*	*49*	*-5.3*
Median:	*56*	*49*	*-0.4*

Table 4. Matrix effect on the total recovery during analysis (loss to ion-suppression) and sample preparation (loss in recovery) for the investigated pharmaceuticals in effluent wastewater.

Substance	Total recovery %	Loss to ion-suppression %	Loss in recovery %
Diclofenac	68	37	-5.3
Furosemide	34	65	1.3
Hydrochlorothiazide	69	30	1.1
Ibuprofen	38	62	0.6
Naproxen	50	52	-2.2
Ramipril	79	29	-7.7
Warfarin	71	32	-2.9
Atenolol	89	8.8	1.8
Amlodipine	28	69	3.3
Bisoprolol	75	26	-0.8
Carbamazepine	66	35	-0.9
Citalopram	65	34	0.8
Fluoxetine	45	52	3.1
Ketoprofen	37	65	-1.8
Metoprolol	73	27	0.0
Oxazepam	63	41	-4.3
Paracetamol	74	12	15
Propranolol	69	31	-0.8
Ranitidine	47	58	-5.1
Risperidone	55	29	16
Sertralin	39	51	10
Simvastatin	62	-53	91
Terbutaline	77	4.4	18
Average:	***60***	***35***	***5.7***
Median:	***65***	***34***	***0.6***

Table 5. Matrix effect on the total recovery during analysis (loss to ion-suppression) and sample preparation (loss in recovery) for the investigated pharmaceuticals in sludge.

Substance	Total recovery %	Loss to ion-suppression %	Loss in recovery %
Diclofenac	12	82	6.3
Furosemide	8.3	62	29
Hydrochlorothiazide	60	9.8	30
Ibuprofen	9.7	85	5.4
Naproxen	15	75	9.7
Ramipril	41	6.6	52
Warfarin	66	15	20
Atenolol	31	12	57
Amlodipine	20	65	15
Bisoprolol	65	2.4	33
Caffeine	48	29	24
Carbamazepine	25	59	16
Citalopram	78	-46	68
Fluoxetine	19	62	19
Ketoprofen	11	80	8.6
Metoprolol	77	-19	42
Oxazepam	13	74	13
Paracetamol	41	11	48
Propranolol	56	11	33
Ranitidine	0.7	99	0.8
Risperidone	49	26	25
Sertralin	25	25	50
Simvastatin	7.2	90	3.0
Terbutaline	44	4.5	52
Average:	***34***	***38***	***27***
Median:	***28***	***27***	***24***

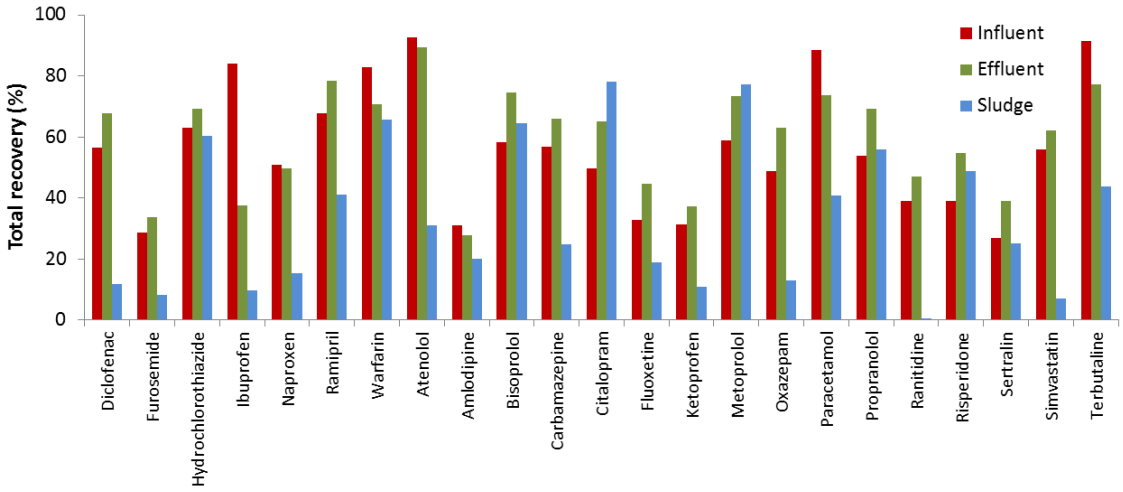

Figure 7. Total recovery of the investigated pharmaceuticals in influent wastewater, effluent wastewater and sludge.

4.1.2 Metabolism

Figure 8 and Figure 9 show the results from the investigation of the impact metabolism/transformation before and after sewage treatment process of the 24 investigated pharmaceuticals residues in wastewater. Relative concentrations of the treated compared to the non-treated effluent water samples are given in percent (%). Concentrations below the limit of detection (LOD) and below the limit of quantification (<LOQ) were substituted with LOD/2 and LOQ/2, respectively. When all samples, treated and/or non-treated, were below LOD or LOQ, the concentrations were set to zero, but the analyte was included in the figure for comparative reasons (e.g. ibuprofen, amlodipine and simvastatin). The height of the bars in Figure 8 and 9 corresponds to the estimated concentration of a pharmaceutical in the sample after treatment and normalised against the concentration in the non-treated control sample expressed in percent. The blue and red bars represent reductive treatment to regain pharmaceutical residues that may exist in an oxidated state in the non-treated original sample. The green bars represent oxidative treatment to regain pharmaceutical residues that may exist in a reductive state in the original sample. The purple bars represent enzymatic de-conjugative treatment to regain pharmaceutical residues that may exist in a conjugated state in the non-treated sample.

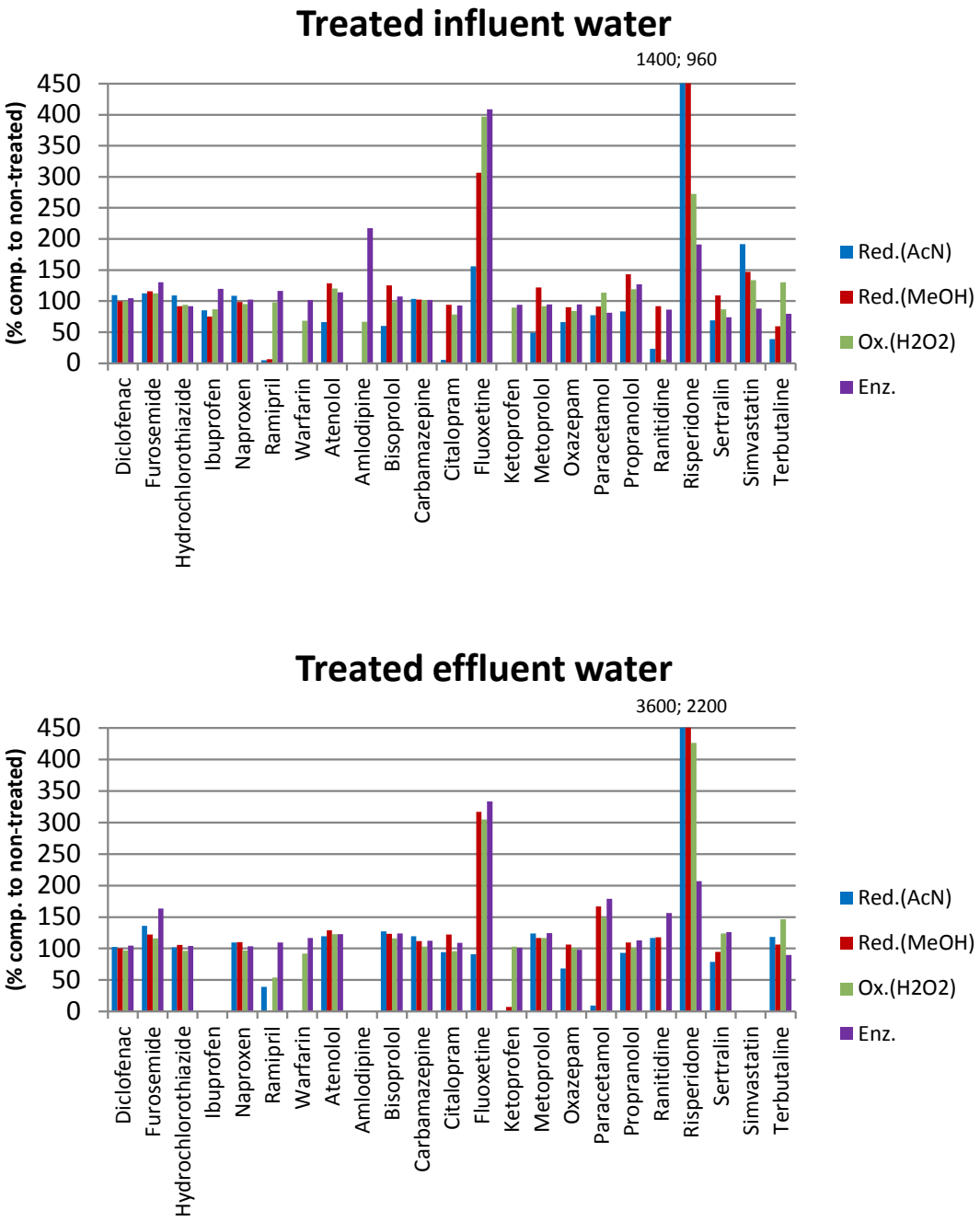

Figure 8 and Figure 9. The presence of transformation products of investigated pharmaceuticals in influent and effluent wastewater, respectively. The height of the bars corresponds to the estimated abundance in percentage of a pharmaceutical in the sample after the different treatments and relative to the control. Legend entries; Red.(AcN) = reductive treatment using sodium borohydride in acetonitrile, Red.(MeOH) = reductive treatment using sodiumborohydrate dissolved in methanol/water, Ox. (H_2O_2) = oxidative treatment using hydrogenperoxide and Enz. = incubation with enzymes.

In comparison to the non-treated samples the influent and effluent display similar patterns (Figure 8 & 9), e.g. Fluoxetine and Risperidone showed a significant increase in relative concentrations when the samples were subjected to a treatment prior to analysis. For Fluoxetine it seems to be less favorable with a reductive treatment in acetonitrile compared to the other protocols. It should be

noted that there was some co-elution with matrix residues in the chromatogram, possibly obstructing an accurate concentration determination. However, most distinguished is the change in concentration of Risperidone in the treated compared to the non-treated samples. In this case as well, there are indications of co-elution but these are most prominent in the untreated influent water samples and hence, should not lead to an overestimation of the pharmaceutical in the treated samples – rather the opposite. A high increase in the concentration of Amlodipine in the enzymatically treated, i.e. most likely de-conjugated, influent sample was also observed.

Compared to ion-suppression, the influence from metabolism seems to be of less importance for the observed increase in concentration of pharmaceuticals from influent to effluent wastewater. In comparison to the non-treated samples the results for influent and effluent display similar patterns (Figure 8 & 9). For example, both Fluoxetine and Risperidone showed a significant increase when the samples were subjected to a treatment prior to analysis. It seems that treatment of the extract with the reducing agent sodium borohydride dissolved in methanol showed better results, compared to sodium borohydride dissolved in acetonitrile, i.e. higher relative concentrations were in general observed for samples treated by the methanol protocol. Whether this is due to solubility of sodium borohydride and/or the analytes or some other unknown factor is not clear. In most cases (17 out of 24 pharmaceuticals in both influent and effluent), the concentration is close to or higher than the untreated sample after treatment with sodium borohydride dissolved in methanol. Oxidative treatment with hydrogen peroxide also shows some improvement for numerous pharmaceuticals, 20 and 18 out of 24 in influent and effluent, respectively, and only a decrease for a few compounds. This also applies to enzymatic treatment, for all investigated pharmaceuticals, with somewhat smaller increase compared with sodium borohydride dissolved in methanol (Figure 8 & 9). None the less, enzymatic treatment of the samples seem to have one distinct advantage over the other investigated treatment types, the concentration is slightly lower or elevated, but never clearly lower than non-treated control, compare to the other treatment types of for instance Ramipril in effluent water (Figure 9). In other words, the advantage of using enzymatic treatment is due to its high specificity and that it only cleaves conjugations compared to reductive and oxidative treatments, which may destroy or transform non-transformed/metabolized pharmaceutical residues in a sample. The case of Risperidone however, does show much smaller increase in concentration of the analyte compared to the other treatment types. This could be due to that a transformation product of Risperidone is present in STP but in such a moiety that the enzymes could not restore the mother compound via de-conjugation of a glucorinide or sulfate metabolite, but chemical reduction or oxidation could. It is important to stress that it is outside the scope of this study to investigate if the different treatment types actually changes (reduces, oxidizes or de-conjugates) any transformed pharmaceuticals, i.e. metabolites or other transformation products, on a molecular level – or if the changes observed within this study is due to additional clean-up associated with the additional treatment step. Furthermore, the benefit seems in general to be somewhat more profound in the effluent sample than the influent sample, perhaps due to less interference from the greater amount of matrix in influent water samples – irrespectively of treatment type applied.

4.1.3 Mass balance

A mass balance was performed to estimate the distribution of pharmaceuticals between matrices in STPs and to assess the main route of distribution of groups of pharmaceuticals to the environment. Data on flowrate and concentrations of the wastewater from Sjölunda STP were used in the calculations. In Table 6 the mass flows are presented for the corrected "true" concentrations, where the measured concentrations had been adjusted based on data from the matrix effect and metabolism experiments. The flowrate in and out was calculated as a mean flow over the sampling

period and were 96 545 m³/day. The sludge flow, calculated as dried sludge, was during the period at average 6.7 m³/day. This calculation is not considering any internal recirculation streams of flow and sludge.

Table 6. Mass flux at Sjöunda STP

Substance	In, g	Out, g	Sludge, g	Mass balance
Diclofenac	78	100	0.29	-23
Furosemide	270	190	0.12	77
Hydrochlorothiazide	120	1400	0.02	-16
Ibuprofen	41	19	0.79	21
Naproxen	220	69	0.00	150
Ramipril	0.09	2.7	0.00	-2.7
Warfarin	81	130	0.26	-48
Atenolol	130	38	0.14	89
Amlodipine	7.9	0.35	1.0	6.6
Bisoprolol	11	8.5	0.15	2.1
Carbamazepine	33	47	0.31	-14
Citalopram	44	14	3.8	26
Fluoxetine	5.3	0.49	0.87	4.0
Ketoprofen	72	70	0.01	1.8
Metoprolol	170	160	2.2	11
Oxazepam	23	57	0.20	-34
Paracetamol	15	4.1	0.05	11
Propranolol	8.2	4.5	0.83	2.9
Ranitidine	22	7.1	0.01	15
Risperidone	15	2.2	0.07	12
Sertralin	35	1.2	5.1	29
Simvastatin	12	0.36	0.01	12
Terbutaline	1.4	0.76	0.01	0.60

For six of the investigated pharmaceuticals, the sum calculated is negative which indicates that for those specific substances the analytic method needs further development, but it could also be an effect of how the sampling were performed. A mass balance were also calculated with the uncorrected analyses (not shown) and compered with the result in Table 6. The comparison showed that when using the uncorrected concentrations the negative sums were increased by a factor 1.5.

4.2 Distribution and fate of pharmaceuticals in the environment

4.2.1 Pharmaceutical concentrations in sewage sludge, soil and soil water

The results from the analyses of the 24 investigated pharmaceuticals in the Sjölunda STP sewage sludge used as fertilizer on the farmland Petersborg, in Skåne, are presented in Figure 10. The

graph shows that 15 of the 24 investigated pharmaceuticals were detected in concentrations between 1.9 to 1000 ng/g dry weight (dw) and that citalopram occurred in the highest concentration (Figure 10). In addition, the graph shows that, the pharmaceuticals with neutral and basic chemical properties (atenolol to terbutaline) have a higher abundance in sludge than pharmaceuticals with acidic chemical properties (diclofenac to warfarin) (Figure 10).

Soil samples from the farmland taken in spring 2014 and soil water collected in August 2014 were analyzed for the pharmaceuticals. This includes 8 composite soil samples from 0-0.3 m depth (from all sections sampled), 4 composite soil samples from 0.3-0.6 m depth (from sections receiving sewage sludge) and 4 composite soil water samples from 0.5 m depth (from sections receiving sewage sludge). Analysis of the lysimetric soil water reviled no detectable levels of the investigated pharmaceuticals, other than 69 ng/l of Caffeine in sample 24 B2 collected in August 2o14 (data not showed). The results from the determination of pharmaceuticals in soil, both treated and non-treated with sewage sludge, from the fields of Petersborg are presented in Figure 11. The soil samples not treated with sludge reviled no detectable levels of the investigated pharmaceuticals (Figure 11). However, all the 4 composite soil samples from 0-0.3 m depth showed detectable levels of up to 4 of the investigated pharmaceuticals in the range 0.4-4.9 ng/g dw, while composite samples collected at larger depth (0.3-0.6 m) revealed no detectable levels of pharmaceuticals (Figure 11).

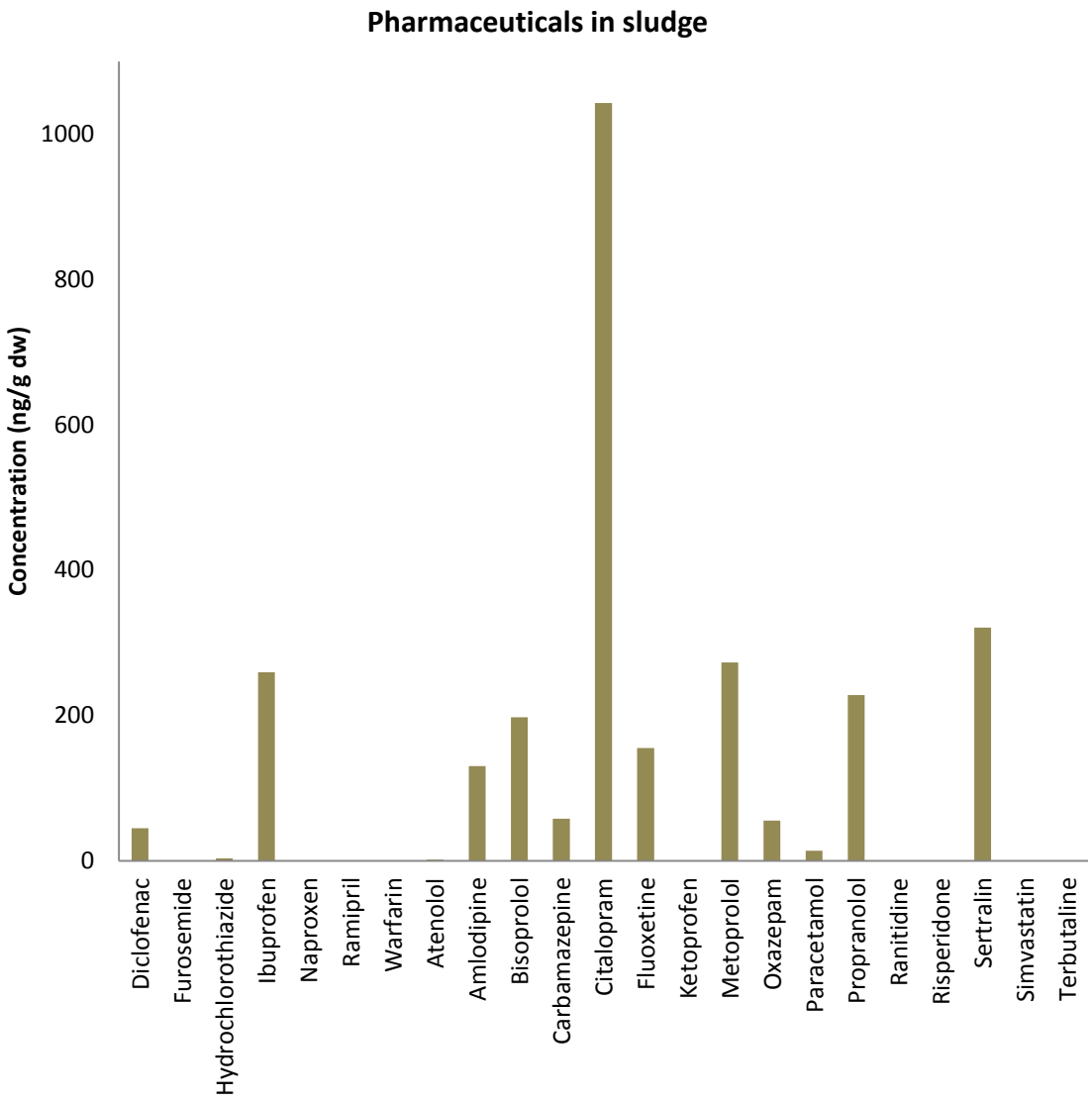

Figure 10. Concentrations of pharmaceuticals in sewage sludge (ng/g dw) deposited autumn 2013 as fertilizer on the farmland at Petersborg in Skåne, southern part of Sweden.

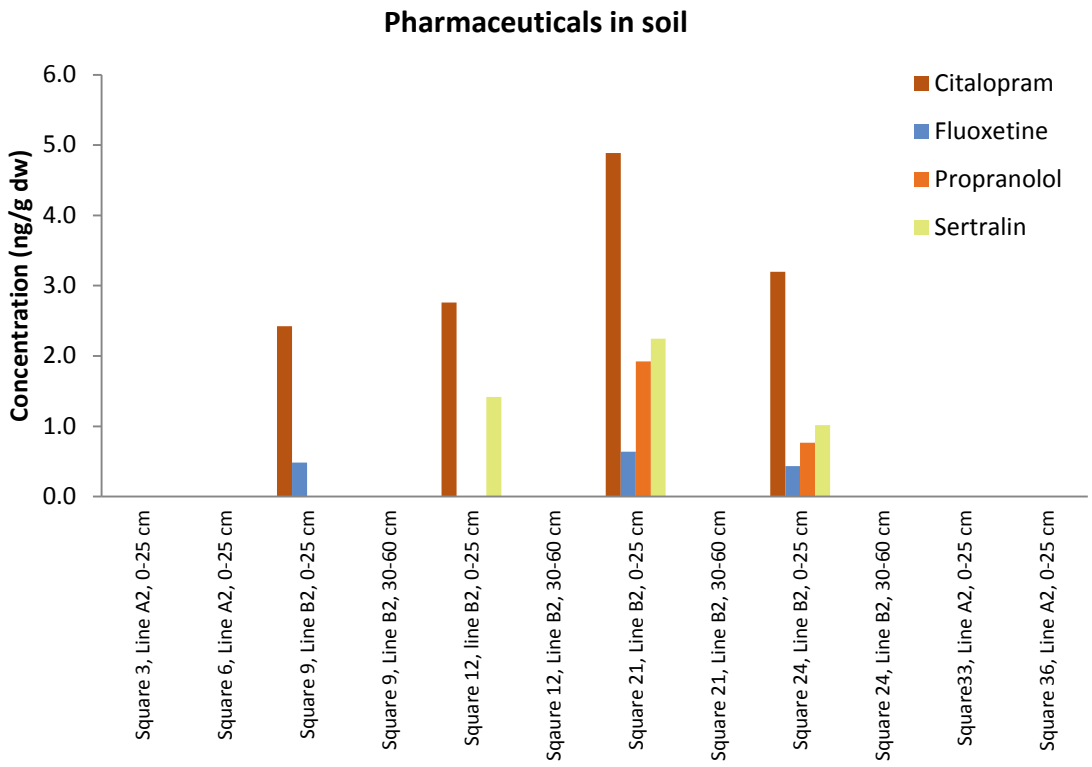

Figure 11. Concentrations of pharmaceutical residues in soil (ng/g dw) after treatment with sewage sludge as fertilizer.

Although the sludge from Sjölunda STP, used to fertilize the farmland on Petersborg during autumn 2013, contained 15 of the 24 investigated pharmaceuticals in a relative high abundance (between 1.9 to 1000 ng/g dw) only caffeine was detected (69 ng/L) in one lysimetric soil water sample (24 B2) collected in August 2014. Analysis of soil samples exposed to sludge showed only traces (between 0.4 to 4.9 ng/g dw) of 4 out of 24 investigated pharmaceuticals. An explanation to the near-absence of detectable levels of pharmaceutical residues in soil exposed to sludge could be that the sludge was amended to the fields of Petersborg in late autumn 2013 and the first soil samples were collected in spring 2014, which means that the pharmaceuticals could have followed the melting water down into the ground in late winter and early spring 2014. The lysimeters were also first mounted in the field after the melting water in mid spring 2014, and in accordance with the soil samples, could have missed the actual dispersion of the pharmaceutical residues in the soil column.

4.2.2 Soil sorption tests

The near-absence of detectable levels of the investigated pharmaceuticals, in the soil and soil water samples from the farmland at Petersborg, made it difficult to draw conclusions of the mobility of the pharmaceutical residues in the soil. As a complement soil sorption tests (at laboratory scale) were therefore performed to further investigate the soils capacity to retain pharmaceuticals of different subclasses, see 3.3.3. Figure 12 shows the results from the soil sorption test exposed to a standard mixture of the 24 pharmaceuticals which is presented in two graphs divided into acidic pharmaceuticals and basic or neutral pharmaceuticals. Figure 13 presents the results from the soil sorption tests exposed to freeze-dried sewage sludge. The sewage sludge was an aliquot of the sludge that was applied to the fields of Petersborg during autumn 2013 and the soil used in the experiment was soil unexposed to sludge also from the fields of Petersborg. Both the soil exposed

to a standard mixture of 24 pharmaceuticals and the soil exposed to the original sludge showed a general higher mobility of pharmaceuticals with acidic chemical properties compared to pharmaceuticals with basic or neutral chemical properties (Figure 12 & 13). The four pharmaceuticals detected in the soil exposed to sludge on the fields of Petersborg (Figure 11) were among the compounds exhibiting low or no mobility in the soil sorption tests (Figure 12).

There are still scares of reports in the scientific literature comparing the mobility of pharmaceuticals in soil with regards to differences in chemical and physical properties of the substances. However, a study by Wu et al., (2010b) showed low mobility of five pharmaceuticals with neutral and basic chemical properties. Carbamazepine and fluoxetine were among the pharmaceuticals studied showing low mobility in soil, which agree with the results from this study (Figure 12). Lin & Gan (2011) showed medium to high mobility for pharmaceuticals with acidic chemical properties, such as non-steroidal anti-inflammatory drugs (NSAIDs), which also are reflected in this study (Figure 12). The finding that pharmaceuticals with neutral and basic chemical properties are more retained by the soil than pharmaceuticals with acidic chemical properties could be explained in two ways. Pharmaceuticals with neutral chemical properties are generally more hydrophobic than charged spices (Table 1) and partition to higher extent to the organic content in soil (Schwarzenbach et al., 2003). Pharmaceuticals with basic chemical properties are cationic with a positive charge that is retained to the surface of soil particles which are mostly negatively charged (Haderlein & Schwarzenbach 1993; Magnér et al., 2009). That means that pharmaceuticals with acidic chemical properties are anionic with a negative charge and is repelled by the soil particles.

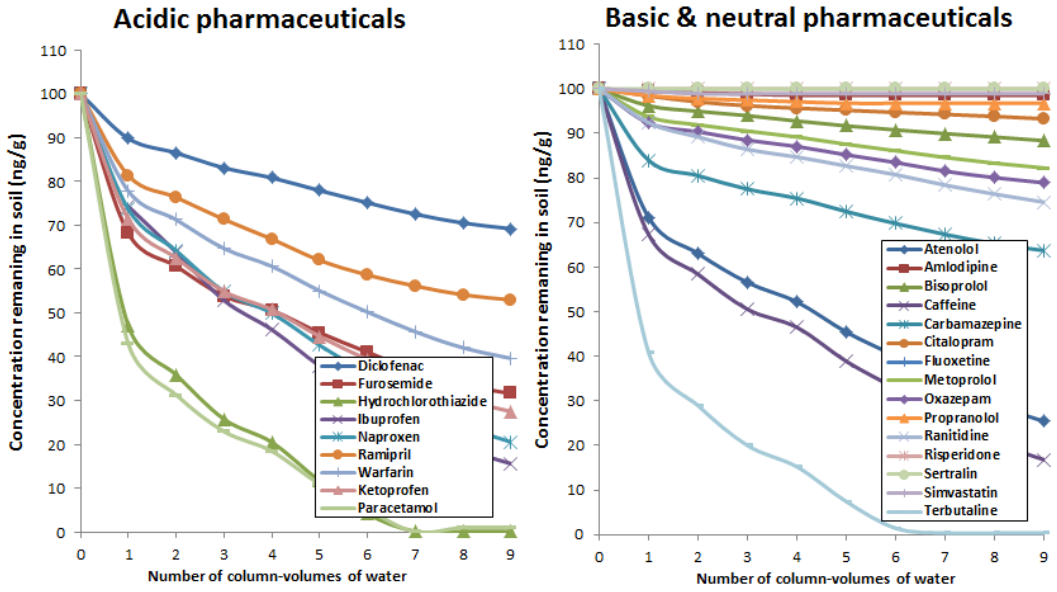

Figure 12. Results from the soil sorption tests with soil from the sampling site exposed to a mixture of the 24 pharmaceuticals.

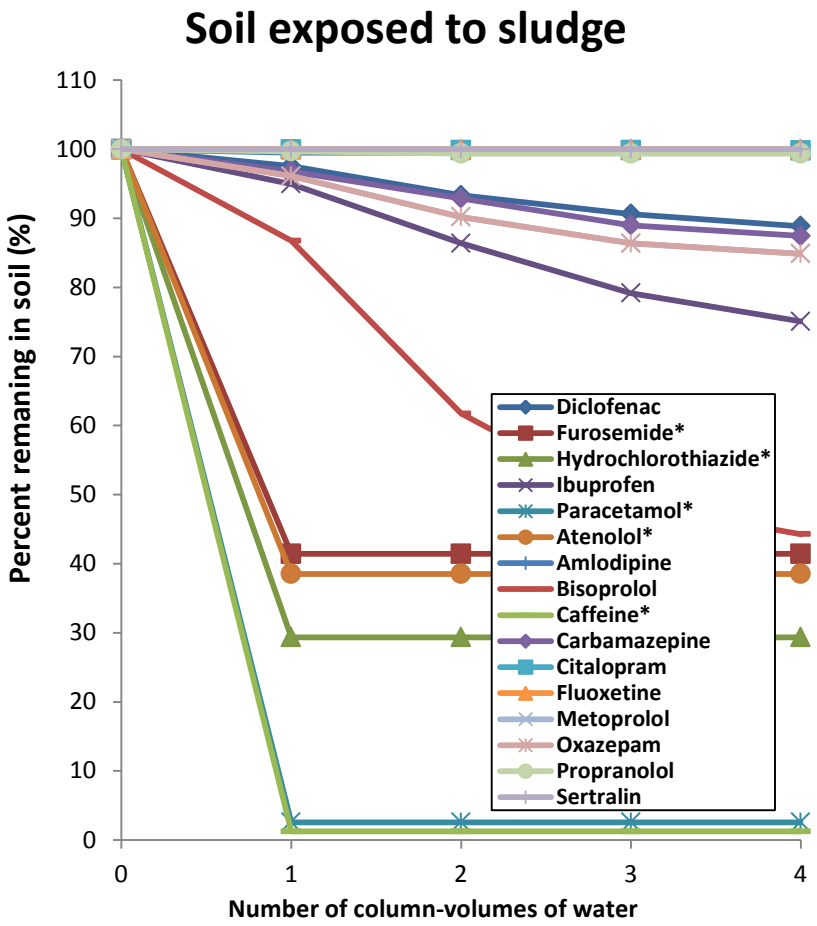

Figure 13. Results from the soil sorption tests with soil from Petersborg farmland in Skåne, southern part of Sweden. The soil in the sorption test was exposed to sludge that was applied to the fields in autumn 2013. Substances marked with asterix (*) end up below the limit of detection (LOD).

The poor recovery of pharmaceutical residues in the soil exposed to sludge in combination with the observed relatively low mobility of pharmaceuticals in the soil sorption experiment implies that the investigated pharmaceuticals are retained and degraded at the surface of the soil. However, due to the lack of measurements directly after that the sludge was applied to the fields of Petersborg and during the snow melting it is hard to exclude that the pharmaceutical residues have followed the water further down into the ground or degraded at the surface of the soil. To assess the "true" fate of pharmaceutical residues on farmland more frequent lysimetric soil water samples need to be taken already from the start, when the sludge is amended to the field. Furthermore, investigations using representative leaching tests and additional degradation tests need to be performed to fully establish the fate of pharmaceuticals in soil.

5 Conclusion

The result of the study showed that ion-suppression due to competition with co-eluting matrix component during instrumental analysis was the main contributor to the observed increase in concentration of pharmaceuticals from influent to effluent wastewater, with an average ion-suppression of 49% in influent wastewater and 35% in effluent wastewater of the investigated pharmaceuticals.

The sludge from the STP used to fertilize the farmland contained 15 of the 24 investigated pharmaceuticals in concentrations between 1.9 to 1000 ng/g dry weights (dw). However, the lysimetric soil water revealed no detectable levels of the investigated pharmaceuticals. Soil samples exposed to sludge showed only traces of 4 out of 24 investigated pharmaceuticals in concentrations between 0.4 to 4.9 ng/g dw. A laboratory scale soil sorption test of unexposed soil from the field of Petersborg, spiked with a mixture of the 24 pharmaceuticals showed high soil retention of basic and neutral pharmaceuticals and a slightly higher diffusivity of acidic compounds. The four pharmaceuticals detected in the soil were among the compounds exhibiting high retention. The result of the study implies that the investigated pharmaceuticals are retained and eventually degraded at the surface of the soil. However, further investigation using representative leaching test and additional degradation test need to be performed in order to fully establish the fate of pharmaceutical residues in soil.

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