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Fate of pharmaceuticals and perfluoroalkyl substances during source separated wastewater treatment

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Department of Aquatic Sciences and Assessment
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Abstract

In the past decade, water reuse and nutrient recycling of wastewater has gained more attention as sustainable water cycle management solutions, driven by the increasingly noticeable resource restrictions of the 21st century. One of these possible solutions is source separated treatment of latrine or blackwater for nutrient recovery. However, one major issue of wastewater recycling are micropollutants released into the environment, which can affect ecosystems and human health. This study investigated the fate and removal efficiency of two emerging groups of micropollutants, pharmaceutically active compounds (PhACs) and perfluoroalkyl substances (PFASs), in two source separating wastewater treatments. The first treatment investigated was laboratory-based anaerobic degradation of latrine under mesophilic (37 °C) and thermophilic (52 °C) conditions. The second was a full-scale blackwater treatment, including wet composting and sanitation with urea. Occurrences and concentrations in different steps of the treatments of 29 PhACs and 26 PFASs in the liquid and solid phase of latrine and blackwater were determined.

The results showed high environmental concentrations of PhACs in latrine and blackwater with values up to hundred $\mu g\ L^{-1}$ and $\mu g\ g^{-1}$ dry weight (d.w.) in the liquid and solid phase, respectively. The concentrations measured in latrine and blackwater were higher than those found in conventional wastewater effluents, due to lower dilution. The average removal rates of PhACs were 45 % under mesophilic and 31 % under thermophilic conditions of latrine and a slightly higher removal rate was determined in blackwater, 49 %. Some compounds showed close to complete removal, such as most antibiotics (up to 100 %, n=4). The majority of PFASs were not detected and the ones detected showed low environmental concentrations in the range of low ng L^{-1} and ng g^{-1} d.w. in the liquid and solid phase, respectively. In the removal analysis, increased concentrations have been found for PFASs in mesophilic treatment (in average 24 %), possibly due to degradation of PFAS precursors, and a low average removal rate in the thermophilic experiment (in average 4 %). No evaluation could be made about the fate of PFASs in blackwater, due to no significant concentrations measured. It is concluded that latrine and blackwater are no major sources of PFASs and therefore do not represent a major threat to the environment.

The removal efficiency of the two source separated treatments revealed moderate to low removal rates for PhACs and PFASs. But since the occurrence of PFASs in latrine and blackwater is low, their removal might not have to be considered in the source separated wastewater such as latrine and blackwater. Regarding the PhACs additional advance treatments might be necessary or efforts to find a better suitable treatment technique need to be made, as the treated end-product of blackwater is reused as fertilizer in agricultural fields.

Popular Science Abstract

Increased human population, intensified agriculture and industrial production associated with a noticeable resource restriction results in growing concerns about water management. Water reuse and nutrient recycling of wastewater can be adequate solutions for future water cycle management. One approach of nutrient recycling is source separated treatment of blackwater. In source separated systems, blackwater (urine, feces, toilet paper and flush water) is treated separately from greywater (bath and cleaning water) to recover nutrients. But a major problem of wastewater recycling is the presence of pollutants in the wastewater. If the nutrient-rich product from the wastewater is spread on agricultural fields it may contains pollutants and those can have negative affects on ecosystems and human health. This study investigated the fate of two groups of these pollutants, pharmaceutically active compounds (PhACs) and perfluoroalkyl substances (PFASs) during source separated treament. PhACs are pharmaceuticals or their degradation products and are designed to have curing effects on the human body, therefore they are bioactive and affect organisms. PFASs are fluorinated compounds that are used for instance as fire-fighting foams and water-resitant coating on textile products. They are toxic and very difficult to degrade, and therefore remain in the environment for a long time. Two source separating wastewater treatments were investigated. The first treatment was a laboratory-based anaerobic degradation (a biological treatment in absence of oxygen) of latrine under medium-warm (37 °C) and warm (52 °C) conditions. The second was a full-scale blackwater treatment, including wet composting (biological treatment with oxygen) and sanitation with urea. Urea is a nitrogous compound, which has sanitation properties towards pathogens (all organisms that cause diseases). The occurrences and removal efficiencies of 24 PhACs and 26 PFAS compounds were determined at different steps of the treatments. The results showed high concentrations of PhACs in latrine and blackwater with values up to hundred $\mu g\ L^{-1}$ in the water phase and $\mu g\ g^{-1}$ dry weight (d.w.) in the solid phase. The concentrations measured were higher than those found in conventional wastewater. That is because conventional wastewater is higher diluted since it contains also other lower concentrated wastewaters (e.g. laundry water). The average removal rates of PhACs were 45 % under medium-warm and 31 % under warm conditions of latrine and a slightly higher removal rate was determined in blackwater, 49 %. Some compounds showed close to complete removal, such as most antibiotics. In blackwater, the majority of PFASs were not detected and the ones detected showed low concentrations. The concentrations were significantly lower than in conventional wastewater. In the treatment of latrine, PFASs were not removed. Instead PFAS concentrations increased in the medium-warm treatment (on average 24 % increase), possibly due to degradation of PFAS precursors to their final degradation products. In the warm experiment the removal of PFASs was low, 4 %. No evaluation could be made about the removal of PFASs in blackwater, since concentrations were very low. It is concluded that latrine and blackwater are no major sources of PFASs and therefore do not represent a major threat to the environment. Regarding the removal efficiency of the two source separated treatments it was determind that they were not sufficient for PhACs and PFASs. But since the occurrence of PFASs in latrine and blackwater is low, their removal might not have to be considered in the source separated wastewater such as latrine and blackwater. Regarding the PhACs, additional advanced treatments might be necessary or efforts to find a better suitable treatment technique need to be made, as the treated end-product of blackwater is reused as fertilizer in agricultural fields.

List of Abbreviations

ACN	acetonitrile
AOP	advanced oxidation processes
C	concentration in sample
d.w.	dry weight
GAC	granular activated carbon
HPLC-MS/MS	high-performance liquid chromatography coupled with tandem mass spectrometry
HRT	hydraulic retention time
IS	internal standard
JTI	Swedish Institute of Agricultural and Environmental Engineering
K_d	solid-water distribution coefficient
K_{oc}	organic carbon-water partition coefficient
K_{ow}	octanol-water distribution coefficient
log	logarithmic
n.a.	not available
n.d.	not detected
MDL	method detection limit
MQL	method quantification limit
MW	molecular weight
NF	nanofiltration
NH_3	ammonia
NH_4^+	ammonium ion
NI	negative electrospray ionization
PAC	activated carbon
PFAS	perfluoroalkyl substance
PhAC	pharmaceutically active compound
PI	positive electrospray ionization
pK_a	logarithmic acid dissociation constant
PNEC	predicted no-effect concentration
PP-tubes	polypropylene centrifuge tubes
RO	reverse osmosis
RQ	risk quotient
SD	standard deviation

SPE	solid-phase extraction
SRT	sludge retention time
UPLC/QTOF-MS	ultra-performance liquid chromatography system coupled with quadrupole-time-of-flight mass spectrometer
UV	ultraviolet
VS	volatile solids
WWTP	wastewater treatment plant

Perfluoroalkyl substances

PFCAs	**perfluoroalkyl carboxylates**
PFBA	perfluorobutanoate
PFPeA	perfluoropentanoate
PFHxA	perfluorohexanoate
PFHpA	perfluoroheptanoate
PFOA	perfluorooctanoate
PFNA	perfluorononanoate
PFDA	perfluorodecanoate
PFUnDA	perfluoroundecanoate
PFDoDA	perfluorododecanoate
PFTriDA	perfluorotridecanoate
PFTeDA	perfluorotetradecanoate
PFHxDA	perfluorohexadecanoate
PFOcDA	perfluorooctadecanoate
PFSAs	**perfluoroalkane (-alkyl) sulfonates**
PFBS	perfluorobutane sulfonate
PFHxS	perfluorohexane sulfonate
PFOS	perfluorooctane sulfonate
PFDS	perfluorodecane sulfonate
FOSAs	**perfluorooctanesulfonamides**
FOSA	perfluorooctanesulfonamide
N-MeFOSA	N-methylperfluorooctan- sulfonamide
N-EtFOSA	N-ethylperfluorooctane- sulfonamide
FOSEs	**perfluorooctane sulfonamidoethanols**
N-MeFOSE	N-methylperfluorooctane- sulfonamido-ethanol
N-EtFOSE	N-ethylperfluorooctane- sulfonamido-ethanol

FOSAAs	**perfluoroalkyl sulfonamidoacetic acids**
FOSAA	perfluorooctanesulfonamido- acetic acid
N-MeFOSAA	N-methylperfluorooctane- sulfonamidoacetic acid
N-EtFOSAA acid	N fluorotelomer sulfonates -ethylperfluorooctane- sulfonamidoacetic
FTSAs	**fluorotelomer sulfonates**
6:2 FTSA	6:2 fluorotelomer sulfonate

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1 Introduction

Increased human population, intensified agricultural and industrial activities resulted in growing concerns about water management and the pollution of water bodies by emerging environmental pollutants. Water reuse and nutrient recycling from domestic wastewater appear to be adequate solutions for future water cycle management.

Domestic wastewater contains high amounts of nutrients, such as phosphorus and nitrogen, which are eliminated in conventional treatment, to reduce nutrient loads in receiving waters. However, new approaches are to reuse these nutrients from domestic wastewater in order to obtain a treated end-product that can be used as fertilizer in agricultural fields. In some case sewage sludge from conventional wastewater treatment is already applied. Actually, the produced sewage sludge in Sweden contains about 5,800 tons phosphorus per year and 25 % (ca. 1340 tons) is used as fertilizer (Swedish EPA, 2013). By 2018, the Swedish government has established that 40 % of total phosphorous and 10 % of total nitrogen originating from sewage sludge should be applied in agricultural fields (Swedish EPA, 2013). Therefore in Sweden, wastewater treatments based on nutrient recovery have gained more importance and recognition in recent years, such as source separated treatments (Vinnerås, 2002).

Source separation distinguishes between blackwater (urine, feces, toilet paper and flush water) and separated from greywater (wastewater from bath, laundry and kitchen). Blackwater contains the majority of nutrients and is therefore used for nutrient recovery. Besides nutrient recovery, source separated systems offer the advantage to reduce pollution of organic contaminants entering the water cycle. Wastewater treatments used in source separated systems include anaerobic degradation for latrine and wet composting and sanitation with urea for blackwater (Kujawa-Roeleveld and Zeeman, 2006, Vidal Estévez, 2013, Larsen et al., 2009, Dumontet et al., 1999). Nevertheless, major issues that concern wastewater reuse are micropollutants that might be released into the terrestrial environment and groundwater bodies through its application as fertilizer. Some examples of micropollutants are pharmaceutically active compounds (PhAC) and perfluoroalkyl substances (PFAS), which are both considered as emerging contaminants.

PhACs and PFASs can pose negative impacts in ecosystems and pose health risks to humans if they occur in drinking water (Yuan et al., 2009, Sirés and Brillas, 2012, Svensk-Vatten, 2013, Halling-Sorensen et al., 1998, Harries et al., 1997, Rivera-Utrilla et al., 2013, Wu et al., 2010). Moreover, PhACs can exert biological effects in organisms (e.g. fish and invertebrates) (Halling-Sorensen et al., 1998, Harries et al., 1997, Rivera-Utrilla et al., 2013, Wu et al., 2010). Several monitoring studies have reported residues of multiple PhACs in significant concentration levels of ng L^{-1} to high μg L^{-1} in wastewaters (Carballa et al., 2004, Halling-Sorensen et al., 1998, Lindberg et al., 2014, Fick et al., 2011). Concentrations in sewage sludge were found in the range of μg kg^{-1} to mg kg^{-1} (Malmborg and Magnér, 2015).

Similarly, PFASs have been detected in wastewater with concentration levels of ng L^{-1} to μg L^{-1} (Arvaniti et al., 2014, Ahrens et al., 2009b). The uptake of PhACs and PFASs by plants has been already reported (Wu et al., 2010, Felizeter et al., 2012). Land application of sewage sludge from domestic wastewater can be a significant pathway of dissemination of these micropollutants in the environment and might contaminate soils and groundwater, due to leaching and surface water, due to runoff (Narumiya et al., 2013, Sepulvado et al., 2011, Blaine et al., 2014).

Therefore, studying the fate of PhACs and PFASs during source separated wastewater treatment is important to get an understanding if and how much of these pollutants are released into the environment and for further research, their potential risks and adverse effects on ecosystems and human health (Prevedouros et al., 2006, Ahrens, 2011, Martin et al., 2003, Giesy and Kannan, 2001, de Graaff et al., 2011, Suarez et al., 2010, Halling-Sorensen et al., 1998).

1.1 Aims

The overall aim of this study was to determine the fate and removal efficiency of 29 PhACs and 26 PFASs during two different source separated wastewater treatments: *i)* anaerobic degradation of latrine and *ii)* blackwater treatment. The overall aim is divided in the following three objectives:

1) To assess the removal efficiency of selected PhACs and PFASs in **latrine** during anaerobic degradation, under mesophilic and thermophilic conditions, in laboratory batch experiments.
2) To assess the removal efficiency of selected PhACs and PFASs during **blackwater** treatment using wet composting (aerobic degradation) combined with urea treatment in full-scale.
3) To compare the performance of the two treatments concerning the removal of PhACs and PFASs.

This study is part of the 'Läkemedel I kretsloppet' project (Läk, pharmaceuticals in the water cycle). Project partners are the Swedish Institute of Agricultural and Environmental Engineering (JTI), the pharmaceutical laboratory SPPD, the Department of Energy and Technology and the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences (SLU).

2 Background

2.1 Pharmaceuticals

Over the last 20 years, pharmaceuticals in the environment have received increased attention. Pharmaceuticals are developed with the intention of performing a biological effect and have specific physicochemical characteristics, e.g. being persistent so they will not be degraded before having a curing effect, they are lipophilic to be able to pass membranes and they can be highly metabolized before excretion (Halling-Sorensen et al., 1998). Many pharmaceuticals are bioaccumulative and can affect water quality, thus have adverse effects on human health and aquatic and terrestrial ecosystems (Yuan et al., 2009, Sirés and Brillas, 2012). For example, estrogenic affects have been found in male trouts in the UK several kilometers downstream of wastewater treatment plant (WWTP) inputs (Harries et al., 1997). Pharmaceuticals are also considered as pseudo-persistent pollutants, because they are continuously introduced into the environment, but long-term effects (chronic effects) are mostly unknown (Kolpin et al., 2002, Fent et al., 2006, Jjemba, 2006, Harries et al., 1997). New analytical methods have been developed (e.g. liquid chromatography coupled to mass spectrometry) which allow the detection of extremely low concentrations in solid (ng g^{-1} d.w.) and liquid samples (ng L^{-1}) (Petrović et al., 2005, Hernández et al., 2007).

In countries like Denmark, England and Sweden many pharmaceuticals are used in quantities of 1 to 30 tons annually (Halling-Sorensen et al., 1998). A British study in 1985 predicted concentrations of 0.1 μg L^{-1} or more in the River Lee for about 170 PhACs of which one ton of each were applied annually in North London (Richardson and Bowron, 1985). In Stockholm county in Sweden the following target pharmaceuticals investigated in this study have been the most prescripted to patients in the year 2014; the β-blocker metoprolol got prescript by far the most (~119,000 patients), followed by the antidepressants oxazepam (~53,000), citalopram (~48,000) and the antihypertensive valsartan (~39,000)(Socialstyrelsen, 2014).

2.1.1 Occurrence in Wastewater and Treatment Techniques

Pharmaceuticals are released into the environment either through direct disposal or through excretion via feces and urine (*Figure* 1). Studies reported that most pharmaceuticals are poorly removed during wastewater treatment and therefore effluents from a WWTP discharged into the receiving water are found to be the most important point source of pharmaceuticals (Castiglioni et al., 2006, Radjenovic et al., 2007). Most pharmaceuticals found in wastewater are analgesics, antibiotics, antiepileptics, β-blockers and lipid regulators, anti-inflammatories, antiepileptic's, tranquillizers, X ray contrast media and hormones (Carballa et al., 2004, Jones et al., 2001). PhACs can be excreted from the human body with no transformation or transformed by metabolic reactions in the liver as phase I or phase II metabolites (Halling-Sorensen et al., 1998). Phase I, in which oxidation, reduction, hydrolysis, and alkylation reactions happen, can produce products that are often more reactive and toxic than the parent drug (Halling-Sorensen et al., 1998, Silverman and Hoffman, 1984). In phase II, conjugates (glucuronide or sulfate) are formed, which are normally inactive compounds (Silverman and Hoffman, 1984, Heberer, 2002). Both metabolic reactions change the physicochemical character and behavior of the compounds, e.g. metabolites are always more water soluble than the parent compound (Halling-Sorensen et al., 1998). Phase II metabolites can be also reactivated into the parent compound or

into a phase I metabolite (Berger et al., 1986). Pharmaceuticals that produce bioactive metabolites are for example acetaminophen, carbamazepine and diclofenac.

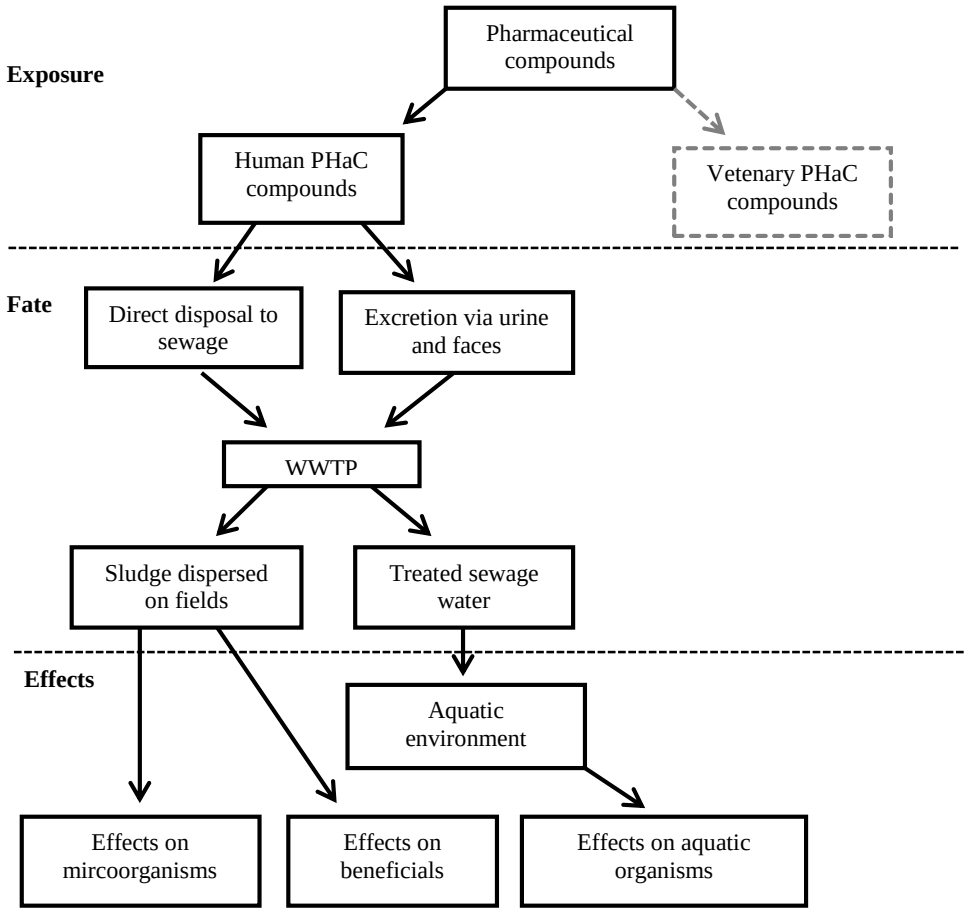

Figure 1. The exposure, fate and effects of human pharmaceutical compounds (after Halling-Sorensen et al., 1998).

Concentration of PhACs in wastewater influent and effluents range from a few ng L^{-1} to a 100 μg L^{-1} in liquid phases and up to 100 ng g^{-1} in the solid phases (Jelic et al., 2011, Malmborg and Magnér, 2015). During wastewater treatment, Halling-Sorensen et al. (1998) determined three principal possible fates of pharmaceuticals: a) the compound is completely mineralized to carbon dioxide and water, e.g. aspirin is usually mineralized (Richardson and Bowron, 1985); b) sorption onto sewage sludge; c) the compound is fully or partially biodegraded and the remaining concentrations is in the receiving water. The removal during wastewater treatment depends on several parameters, e.g. sludge retention time (SRT), hydraulic retention time (HRT), temperature, pH, biomass concentration, compound's polarity, biodegradability and cation-exchange properties (Radjenovic et al., 2009).

Additionally, the distribution of PhACs between the solid and liquid phase depends on their physicochemical properties, the octanol-water distribution coefficient (K_{ow}), the logarithmic acid dissociation constant (pK_a), the organic carbon-water partition coefficient K_{oc}, and most importantly the solid-water distribution coefficient (K_d) (*Table 1, Table 8, Table E1,*). K_{ow} expresses the ratio of a compound's concentration between the octanol (organic phase) and aqueous phase at equilibrium. The pK_a states how strong a compound acts as an acid, the larger

the value of pK_a the weaker the acid. K_{oc} is the ratio of concentrations in the organic carbon (mg kg^{-1}) and the water phase (mg L^{-1}), which displays the sorption of hydrophobic organic substances to organic carbon (Seth et al., 1999). K_d can be used to predict whether sorption is a major removal process of target compounds or degradation (Ternes et al., 2004). A study conducted by Ternes et al. (2004) about the occurrence of pharmaceuticals in sewage sludge from several WWTPs found low K_d values for most substances in the range of < 1 to 500 L kg^{-1}, indicating negligible sorption. Therefore biodegradation was the major removal process. Some compounds, however, had different K_d values in different treatment steps, for example diclofenac had a K_d value of 459 L kg^{-1} in primary sludge at pH 6.6 and 16 L kg^{-1} in secondary sludge at pH 7.5. Lower pH increases the tendency of a compound to sorb onto solids, indicating the importance of sludge composition and pH (Ternes et al., 2004). The K_d coefficient is based on the measured concentration of PhACs and can be calculated as followed:

$$K_d = \frac{C_{solid}}{C_{liquid}} \times 10^3$$

K_d is expressed as L kg^{-1}, C_{solid} is the concentration of PhAC measured in the solid phase (ng g^{-1} d.w.) and C_{liquid} is the one measured in the liquid phase (ng L^{-1}). In general, when predicting the fate of PhACs in treatment (e.g blackwater sanitation with urea) changes in the distribution between liquid and solid phase should be considered (Narumiya et al., 2013).

The concentrations of PhACs found in effluents from WWTPs proof that many of them are not sufficiently removed by conventional treatments, since the WWTPs are not designed to remove micropollutants. In general, they have primary treatment (physicochemical) and secondary treatment (biological treatment). The insufficient removal is suspected to be due their low concentrations in wastewater and their complex molecular structure (Ternes et al., 2002, Jones et al., 2005). Thus more effective treatments are required to reduce environmental impacts of micropollutants (Rivera-Utrilla et al., 2013). According to literature, adsorption on activated carbon and advanced oxidation/reduction processes are demonstrated as good removal techniques, but none are applied yet on an industrial scale (Rivera-Utrilla et al., 2013). The advantage of activated carbon is that it has a high capacity to adsorb PhACs and it does not generate toxic or pharmacologically active products (Dutta et al., 1999, Adams et al., 2002). Also advanced oxidation processes (AOPs) can remove up to 100 % of PhACs when H_2O_2 or activated carbon are present during treatment (Rivera-Utrilla et al., 2013). Additionally membrane filtration (nanofiltration and reverse osmosis) proved to be an efficient technique for PhACs removal (Summers et al., 1989, Newcombe et al., 1997). PhACs can also be photo degraded, because many of the compounds contain aromatic rings, heteroatoms and other functional groups that allow absorption of solar radiation or promote photo degradation reactions (Rivera-Utrilla et al., 2013). UV radiation alone is not very sufficient, but combined with H_2O_2, TiO_2, or TiO_2/activated carbon removal up to 100 % can be reached (Rivera-Utrilla et al., 2013).

Besides advanced wastewater treatment, anaerobic degradation treatment to reduce PhAC loads can be used. The removal efficiency of PhACs during anaerobic degradation of conventional wastewater has been investigated in several studies (Suarez et al., 2010, Narumiya et al., 2013, Bergersen et al., 2012, Samaras et al., 2014, de Graaff et al., 2011). All studies reported that the majority of PhACs investigated had a high removal efficiency (80-100 %) for example fluoxetine, sulfamethoxazole and trimethoprim, but some compounds were only moderately

removed (around 30-50 %) or even persistent to treatment, e.g. diazepam, carbamazepine and diclofenac (Suarez et al., 2010, Narumiya et al., 2013).

Alternative to treatment of conventional wastewater is the treatment of source separated wastewater, high concentrated blackwater and less concentrated greywater. Therefore blackwater contains higher concentrations of PhACs (μg L^{-1} to mg L^{-1}) than the more diluted conventional wastewater, thus separated treatment has the potential to minimize their release into surface waters (de Graaff et al., 2011). Graaff et al. (2011) investigated the fate of several PhACs in anaerobic treatment of vacuum collected blackwater. Determined concentrations ranged from 1.1 μg L^{-1} for carbamazepine to > 1,000 μg L^{-1} for paracetamol. Only paracetamol showed high removal rates (> 90 %). Metoprolol showed moderate removal (67 %) and diclofenac, carbamazepine and cetirizine were persistent. The study concluded that the persistence of PhACs during treatment makes the application of advanced physical and chemical treatment, in addition to anaerobic treatment, unavoidable.

To our knowledge, no studies have been conducted on PhACs removal in source separated systems of blackwater with wet composting and urea sanitation.

2.1.2 Impacts on the Environment

Many PhACs have been detected in the environment (Jones et al., 2001), e.g. Verlicchi et al. (2012) reviewed up to 118 PhACs found in wastewater effluents. Studies found pharmaceutical residues in groundwater, rivers, sediments, soils and oceans (Halling-Sorensen et al., 1998). Even though the concentrations detected in the environment are low (ng L^{-1} to μg L^{-1}), many compounds have been shown to have effects on aquatic life (e.g. oestrogens) (Larsson et al., 1999). Certain PhACs that are designed for modulation of human endocrine and immune systems are potential endocrine disrupters (Ayscough et al., 2000). An increasingly serious threat are antibiotics found in the environment developing increased antimicrobial resistance, a major issue concerning public health (Jones et al., 2001, WHO, 2014). The World Health Organization (WHO, 2014) stated that resistance of antimicrobial resistance has reached alarming levels in many parts of the world, which is a threat to the achievements of modern medicine.

Verlicchi et al. (2012) determined environmental risks posed by PhACs in secondary effluent by calculating a risk quotient (RQ) based on the ratio between the average PhAC concentrations measured in the effluent and its corresponding predicted no-effect concentrations (PNECs, *Figure* 2). Species assayed were mainly daphnia, fish, algae and invertebrates. If the predicted RQ is higher than one, it is expected that the compound has a high risk. Out of the 67 compounds investigated by Verlicchi et al. (2012), 12 are target compounds included in this study (*Table 1*). Five of these 12 PhACs pose potentially high risk to aquatic organisms (RQ > 1), three antibiotics (azithromycin, clarithromycin and sulfamethoxazole) and two antidepressants (diazepam and fluoxetine). Four compounds were determined with a medium risk; two β-blocker (atenolol and propranolol), one antibiotic (trimethoprim) and one analgesic (codeine).

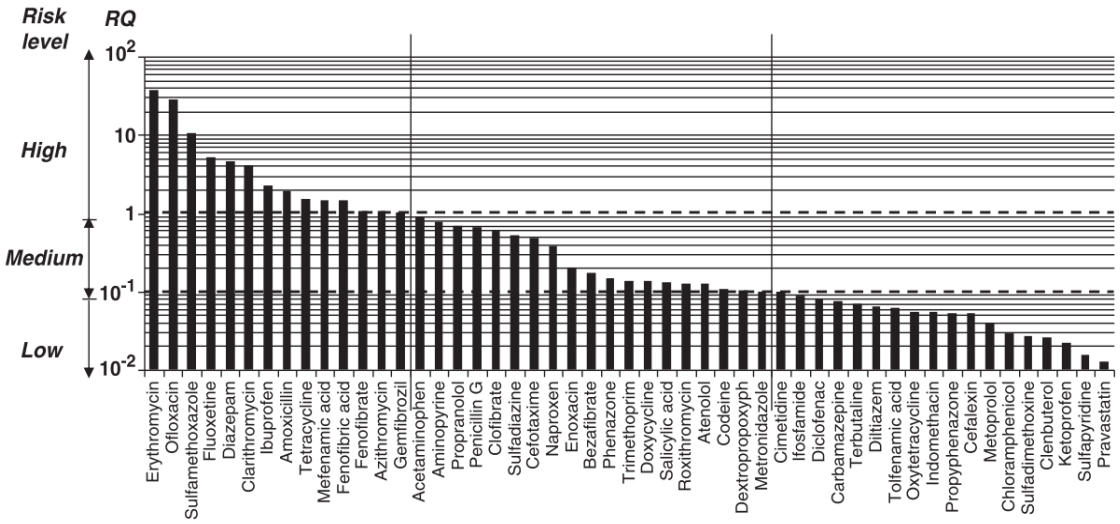

Figure 2. Risk quotient (RQ) of the investigated compounds. RQ < 0.1 low risk to aquatic organisms, 0.1≤RQ≤1 medium risk and RQ ≥ 1 poses high risk (Verlicchi et al., 2012).

2.2 Perfluoroalkyl Substances

PFASs are a group of chemicals with a completely fluorinated carbon chain. Their stable structure makes them useful in a broad range of applications, e.g. surface treatment (oil-, grease-, and water-resistant coatings on paper and textile products) and performance chemicals (fire-fighting foams, industrial surfactants, acid mist suppression, insecticides, etc.) (USEPA, 2002, Hekster et al., 2003). Their production increased rapidly during the last decades and therefore also their release into the environment (Saez et al., 2008). The global historical emissions of PFASs is estimated to be about 3,200 – 7,300 tonnes, both directly and indirectly discharged (Prevedouros et al., 2006). PFASs pose a potential risk and have received increased attention, because they are persistent, bioaccumulative and toxic (Martin et al., 2003, Giesy and Kannan, 2001, Ahrens, 2011, Prevedouros et al., 2006). They have been associated with adverse effects for humans for example on implications in birth weight, fertility disorders, phenomena of early menopause in women, carcinogenesis and thyroid malfunction (Rahman et al., 2014). Furthermore, they are able to be spread through long-range transport in the atmosphere and water (Yamashita et al., 2005, Shoeib et al., 2006). Their bioaccumulation potential and their behavior in the environment depends on the physicochemical properties, such as chain length, branched and linear chain and functional group (Inoue et al., 2012, Ahrens and Bundschuh, 2014). Since 2002, the production of perfluorooctane sulfonate (PFOS) is globally restricted (Ahrens and Bundschuh, 2014).

2.2.1 Occurrence in Wastewater and Treatment Techniques

PFASs have been found in surface and drinking water, sediments, soils, air and biota in large parts of the world including the Arctic (Saez et al., 2008). PFASs can be released during their whole life cycle, e.g. during production, transport, use and disposal (Ahrens and Bundschuh, 2014). Avaniti and Stasinakis's review (2015) mentioned that many studies have presented monitoring data on the occurrence of PFASs in wastewater before, during and after treatments. Little is known about the distribution of PFASs during wastewater treatment, between the liquid and solid phase (Arvaniti et al., 2012, Stasinakis et al., 2013). In general compounds with a long

carbon chain (> C_6) tend to partition onto the solid phase and compounds with a short chain occur most likely in the liquid phase (Ahrens and Bundschuh, 2014). The review Arvaniti and Stasinakis (2015) reported that PFDoA, PFTeDA, PFHpS, PFDS and PFOSA were detected in solid phases, whereas mainly PFHpA, PFOA, PFNA and PFHxS were found in the liquid phase. That shows that the analysis of both phases is of importance in order to avoid underestimations. A study determined K_d values of four selected PFASs in sludge and concluded the lower the pH the higher the compounds sorb onto the sludge (Arvaniti et al., 2014). Several studies detected PFASs at concentration up to some hundreds ng L^{-1} and some thousands in ng g^{-1} d.w., in untreated wastewater e.g. concentrations of 470 ng L^{-1}, 640 ng L^{-1} and 61205 ng L^{-1} for PFOS, PFOA and PFOSA, respectively were detected (Arvaniti and Stasinakis, 2015). In sewage sludge, PFOS has been determined with up to 7300 ng g^{-1} d.w., which makes it the most dominant compound (Arvaniti and Stasinakis, 2015).

During conventional wastewater treatment most monitoring studies reported that during secondary treatment (biological) the removal of PFASs seems not consistent (Arvaniti et al., 2012, Stasinakis et al., 2013, Schultz et al., 2006). Other studies found that specific PFAS concentrations in treated wastewater are higher compared to concentration measured in wastewater influent (Loganathan et al., 2007, Arvaniti et al., 2012, Stasinakis et al., 2013), indicating that they were transformed via biodegradation of precursor compounds. Biodegradation and sorption can be important mechanisms concerning PFASs removal during wastewater treatment (Wang et al., 2011, Wang et al., 2005, Sinclair and Kannan, 2006).

Since the removal of PFASs was insufficient during biological wastewater treatment, various advanced physicochemical treatment methods have been tested, such as adsorption, use of membranes (filtration), oxidation and reduction processes (Arvaniti and Stasinakis, 2015). It should be stressed that all advanced treatments have been conducted using water (natural or ultrapure). Several studies investigated adsorption processes with various sorbents; activated carbon (PAC), granular activated carbon (GAC), resin, zeolite, mineral materials (alumina, silica, goethite), cross linked chitosan beads, carbon nanotubes and molecularly imprinted polymer (Arvaniti and Stasinakis, 2015). It should be noted that mainly the removal of PFOS and PFOA was investigated. Arvaniti and Stasinakis (2015) drew the conclusion that the most effective adsorbents for PFOS and PFOA removal from groundwater were GAC and anion exchange resin, with removal rates more than 98 %. Filtration techniques like sand filtration are not successful in removal of PFAS, but advanced techniques such as nanofiltration (NF) and reverse osmosis (RO) achieve good removals up to 99 % (Eschauzier et al., 2012, Tang et al., 2007). Only a few studies have been investing reduction processes. Zero-Valent Iron (ZVI) is a process which reduces some compounds, e.g. PFOA was degraded up to 73.1 % in aqueous solution (Lee et al., 2010).

No studies have investigated the removal of PFAS in source separated treatments, such as anaerobic degradation and urea sanitation.

2.2.2 Impacts on the Environment

PFASs are widely distributed in the environment (Ahrens and Bundschuh, 2014). In biota they distribute through tissue due to their affinity to bind to specific proteins (Martin et al., 2003, Ahrens et al., 2009b, Shi et al., 2012). The bioaccumulation potential is different for species or even individual organisms and depends on their physicochemical properties, e.g. branched PFAS isomers are easier to be eliminated (Benskin et al., 2009). Several studies reported that PFASs

biomagnify along food chains. PFOS has a high bioaccumulation potential (Giesy and Kannan, 2001, Gebbink et al., 2011, Loi et al., 2011, Tomy et al., 2004), whereas PFOA shows a low bioaccumulation potential, which might be explained by the different functional group (carboxylate) and shorter perfluorocarbon chain length (C_7) (Ahrens and Bundschuh, 2014, Martin et al., 2003). PFOS concentrations are generally decreasing in biota due to its global restriction (Gebbink et al., 2011). The concentrations of other PFASs show no clear trend (Ahrens and Bundschuh, 2014). Giesy and Kannan (2001) stated that PFOS concentrations found in wildlife are lower than the concentration levels required to cause a harmful effect in laboratory animals.

2.3 Source Separated Treatment

Nowadays source separation gets more acknowledged in sustainable and decentralized wastewater treatment concepts, driven by the increasingly noticeable resource restrictions of the 21st century (Kujawa-Roeleveld and Zeeman, 2006, Larsen et al., 2013). Source separation refers to separation of domestic wastewater at source. In a household different wastewater is produced; blackwater (feces, urine, toilet paper and flush water), grey water (originating from shower, bath, laundry and kitchen) and kitchen waste (*Figure* 3). Commonly all wastewaters are combined (often also with street runoff) and treated in centralized WWTPs (Kujawa-Roeleveld and Zeeman, 2006). However, in source separated systems, blackwater or urine is separated from greywater, and both wastes are managed separately and decentralized. Other source separation systems are vacuum collected blackwater, dry toilets and urine separation. The term latrine is used for blackwater collected from pit latrines (outhouses). Blackwater is up to 25 times higher concentrated than wastewater influents of WWTPs, which are highly diluted (de Graaff et al., 2011). It contains half the load of organic material in domestic wastewater, high amounts of the nutrients nitrogen and phosphorus (82 % and 68 % of the total domestic wastewater) and low concentrations of heavy metals (Kujawa-Roeleveld and Zeeman, 2006, de Graaff et al., 2011). Feces contain high quantities of organic matter and macronutrients such as phosphorus and potassium, whereas urine is rich in plant-available nitrogen (Jönsson et al., 2004). However, they also contain most of the pathogens and micropollutants (e.g. PhACs) (Kujawa-Roeleveld and Zeeman, 2006).

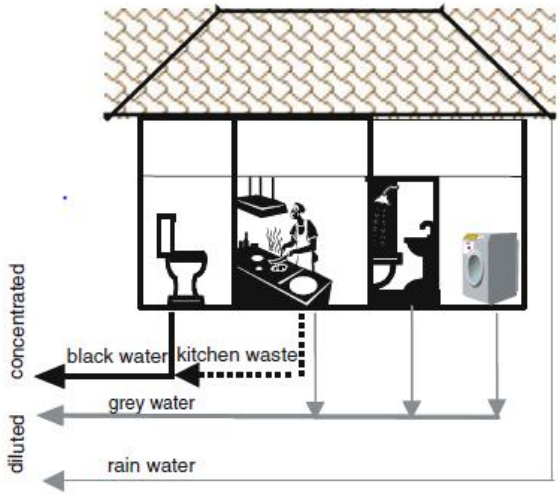

Figure 3. General types of wastewater streams in a household (Kujawa-Roeleveld and Zeeman, 2006)

Source separated treatments do not only provide nutrient recovery and reduced nutrient inputs into receiving waters (pollution control), but also energy recovery, cost benefits and potentially enhance the control of micropollutants (Larsen et al., 2013). The treatments offer a treated wastewater with high nutrient content, which can be re-used as fertilizer in agriculture. Suitable treatment techniques for blackwater or latrine are anaerobic treatment and wet composting combined with urea sanitation, which will be discussed in chapter 3.3 (Kujawa-Roeleveld and Zeeman, 2006, Häfner, 2014, Vidal Estévez, 2013). In Sweden, several source separated systems already exist, such as composting toilets and latrine pits commonly used in national parks and roadside facilities. In these toilets human excreta are collected and only little to no water is used. The municipality of Värmdö near Norrtälje in Sweden collects between 6 to 8 tonnes of latrine waste from subscribers every year and treats it with wet composting (Öberg and Elfström, 2013). Likewise in Salmunge waste facility latrine is collected (source of samples investigated in this study) and transported to Karby, a pilot plant were latrine is treated also with wet composting (Eveborn et al., 2007). Another pilot project is the full-scale treatment plant at Hölö, Södertälje municipality, treating collected blackwater from 600 households (source of samples investigated in this study), using wet composting combined with urea sanitation. Several other projects with blackwater systems have recently been developed and implemented in Sweden. They include different treatments that combine urea application and heat supply, e.g. in the municipalities of Uddevalla, Södertalje, Örebro, Strängnas and Västervik (Vidal Estévez, 2013)

High concentration of nutrients and pollutants in blackwater allows better control, nutrient recovery and treatment, and it offers the possibility to reduce the pollution of the aquatic environment and possible risks.

3 Method

3.1 Target Compounds

The removal of 29 PhACs and 26 PFASs has been investigated in latrine and blackwater samples following different treatment steps (*Table 1* and *Table* 2). The target PhACs were chosen due to high consumptions and applications whereas PFASs were chosen due to applications and occurrences in the environment.

3.1.1 Target PhACs

The 29 PhACs can be classified into nine therapeutic groups; analgesics (painkiller), β-blockers, antibiotics, antidepressants, antihypertensives (to treat high blood pressure), diuretics (promotes the production of urine), lipid regulator (reduces cholesterol), anti-ulcer agent (used as part of the treatment for ulcers) and local anesthetic (nerve block). The anaerobic degradation experiment was also performed with added ('spiked') PhACs. The eight PhACs spiked were atenolol, propranolol, metoprolol, sulfamethoxazole, trimethoprim, ciprofloxacin, carbamazepine and furosemide.

Table 1. Target list of PhACs. PhACs (n=29) analyzed and their therapeutic group (marked bold), the corresponding internal standard (IS) used for quantification and their physicochemical properties: molecular weight (MW), logarithmic dissociation constant (pk_a), logarithmic octanol-water distribution coefficient (K_{ow}) and the organic carbon-water partition coefficient K_{oc}.

Compound	Chemical Formular	MW (g mol^{-1})	pK_a	Log K_{OW}	Log K_{oc}	Corresponding IS
Analgesics						
Codeine	$C_{18}H_{21}NO_3$	299.37[a]	8.21[a]	1.19[a]	3.12[b]	IS-Codeine-d_3
β-blockers						
Atenolol	$C_{14}H_{22}N_2O_3$	266.34[a]	9.60[d]	0.16[a]	2.17[b]	IS-Atenolol-d_7
Sotalol	$C_{12}H_{20}N_2O_3S$	272.37[a]	8.20[h]	0.24[a]	1.58[b]	IS-Bisoprolol-d_5
Metoprolol	$C_{15}H_{25}NO_3$	267.37[a]	9.60[d]	1.88[a]	1.79[b]	IS-Bisoprolol-d_5
Propranolol	$C_{16}H_{21}NO_2$	259.35[a]	9.40[a]	3.48[a]	3.09[b]	IS-Atenolol-d_7
Antibiotics						
Azithromycin	$C_{38}H_{72}N_2O_{12}$	748.98[a]	8.70[a]	4.02[a]	n.a.	IS-Azithromycin-d_3
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.95[a]	8.90a	3.16[a]	n.a.	IS-Trimethoprim-d_9
Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.33[a]	6.10/8.75[d]	0.46[d]	1.97[b]	IS-Ofloxacin-d_3
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.34[a]	6.16/8.63[a]	0.28[a]	1.55[b]	IS-Ciprofloxacin-d_8
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28[a]	5.70[d]	0.89[a]	3.19[b]	IS-Sulfamethoxazole-d_4
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.32[a]	7.12[d]	0.91[a]	2.96[b]	IS-Trimethoprim-d_9
Antidepressants						
Carbamazepine	$C_{15}H_{12}N_2O$	236.27[a]	7.00[d]	2.45[a]	3.59[b]	IS-Carbamazepine-d_{10}
Citalopram	$C_{20}H_{21}FN_2O$	324.39[a]	9.59e	3.74[a]	4.40[b]	IS-Venlafaxine-d_6
Diazepam	$C_{16}H_{13}ClN_2O$	284.74[a]	3.40[a]	2.82[a]	4.05[b]	IS-Diazepam-d_5
Lamotrigine	$C_9H_7Cl_2N_5$	256.09[a]	5.70[c]	0.99[c]	3.13[b]	IS-Trimethoprim-d_9
Oxazepam	$C_{15}H_{11}ClN_2O_2$	286.70[a]	10.90[d]	2.24[a]	3.08[b]	IS-Diazepam-d_5
Venlafaxine	$C_{17}H_{27}NO_2$	277.40[a]	3.28[a]	3.28[a]	3.17[b]	IS-Venlafaxine-d_6
Fluoxetine	$C_{17}H_{18}F_3NO$	309.30[a]	10.05[e]	4.05[a]	5.32[b]	IS-Fluoxetine-d_5
Amitryptiline	$C_{20}H_{23}N$	277.40[a]	9.40[a]	4.92[c]	5.70[b]	IS-Carbamazepine-d_{10}

Antihypertensives						
Losartan	$C_{22}H_{23}ClN_6O$	422.90[a]	5.50[a]	4.01[a]	5.96[b]	IS-Irbesartan-d_7
Valsartan	$C_{24}H_{29}N_5O_3$	435.52[b]	3.60[c]	4.00[c]	6.01[b]	IS-Irbesartan-d_7
Irbesartan	$C_{25}H_{28}N_6O$	428.53c	4.08/4.29[c]	5.31[c]	7.94[b]	IS-Irbesartan-d_7
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.52[a]	8.18/12.86[f]	2.79[a]	3.98[b]	IS-Diltiazem-d_4 HCL
Diuretics						
Furosemide	$C_{12}H_{11}ClN_2O_5S$	330.70[a]	3.80/7.50[c]	2.03[a]	2.28[b]	IS-Furosemide-d_5
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	297.70[a]	7.90[a]	-0.07[a]	1.90[b]	IS-Hydrochlorothiazide-$^{13}C,d_2$
Lipid regulator						
Atorvastatin	$C_{33}H_{35}FN_2O_5$	558.60[a]	-2.70/4.33[f]	5.7[c]	n.a.	IS-Atorvastatin-d_5
Bezafibrate	$C_{19}H_{20}ClNO_4$	361.82[a]	-0.84/3.83[f]	4.25[a]	3.17[b]	IS-Bezafibrate-d_4
Anti-ulcer agent						
Ranitidine	$C_{13}H_{22}N_4O_3S$	314.41[a]	8.08[f]	0.27[a]	4.44[b]	IS-Ranitidine-d_6 HCL
Local anesthetic						
Lidocaine	$C_{14}H_{22}N_2OClH$	234.34[a]	8.01[a]	2.44[a]	2.96[b]	IS-Lidocaine-d_{10}

[a](ChemIDplus Advanced, 2015), [b] (ChemSpider, 2015), [c](PubChem, 2015), [d](Bonnet et al., 2010), [e](Vasskog et al., 2006), [f](Wishart et al., 2006), n.a. not available

3.1.2 Target PFASs

During analysis concentrations of 26 PFASs were determined (*Table* 2). They are divided into subclasses, the perfluoroalkyl carboxylates (PFCAs), characterized by their carboxylic group ($-COO^-$) and the perfluoroalkane (-alkyl) sulfonates (PFSAs), characterized by their sulfonic group ($-SO_3^-$). Some PFCAs and PFSAs are refered to as long chain PFASs, defined by their perfluorocarbon chain length of $\geq C_7$ and $\geq C_6$ (Ahrens and Bundschuh, 2014). Other important subclasses are perfluorooctanesulfonamides (FOSAs), perfluorooctane sulfonamidoethanols (FOSEs), perfluoroalkyl sulfonamidoacetic acids (FOSAAs) and fluorotelomer sulfonates (FTSAs), all can transform into PFCAs and PFSAs (Buck et al., 2011). For the anaerobic degradation experiment of the spiked latrine, the following 14 PFASs were spiked: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFHxS, PFOS and FOSA.

Table 2. Target list of PFASs. PFASs (n=26) analyzed and their therapeutic group (marked bold), the corresponding internal standard (IS) used for quantification and their physicochemical properties: molecular weight (MW), logarithmic dissociation constant (pk_a), logarithmic octanol-water distribution coefficient (K_{ow}) and the organic carbon-water partition coefficient K_{oc}.

Acronym	Compound	Chemical Formular	MW (g mol^{-1})	pK_a	Log K_{OW}	Log K_{oc}	Corresponding IS
PFCAs							
PFBA	Perfluorobutanoate	$C_3F_7CO_2^-$	213.04	0.05	2.82[d]	n.a.	$^{13}C_4$ PFBA
PFPeA	Perfluoropentanoate	$C_4F_9CO_2^-$	263.05	-0.10[a]	3.43[d]	n.a.	$^{13}C_2$ PFHxA
PFHxA	Perfluorohexanoate	$C_5F_{11}CO_2^-$	313.06	-0.17[a]	4.06[d]	1.91[c]	$^{13}C_2$ PFHxA
PFHpA	Perfluoroheptanoate	$C_6F_{13}CO_2^-$	363.07	-0.20[a]	4.67[d]	2.19[c]	$^{13}C_4$ PFOA
PFOA	Perfluorooctanoate	$C_7F_{15}CO_2^-$	413.08	-0.21[a]	5.30[d]	2.31[c]	$^{13}C_4$ PFOA
PFNA	Perfluorononanoate	$C_8F_{17}CO_2^-$	463.09	-0.21[a]	5.92[d]	2.33[c]	$^{13}C_5$ PFNA
PFDA	Perfluorodecanoate	$C_9F_{19}CO_2^-$	513.10	-0.22[a]	6.50[d]	3.17[c]	$^{13}C_2$ PFDA
PFUnDA	perfluoroundecanoate	$C_{10}F_{21}CO_2^-$	563.11	-0.22[a]	7.15[d]	3.30[b]	$^{13}C_2$ PFUnDA

PFDoDA	perfluorododecanoate	$C_{11}F_{23}CO_2^-$	613.12	-0.22[a]	7.77[d]	n.a.	$^{13}C_2$ PFDoDA
PFTriDA	perfluorotridecanoate	$C_{12}F_{25}CO_2^-$	663.13	-0.22[a]	8.25[d]	n.a.	$^{13}C_2$ PFDoDA
PFTeDA	perfluorotetradecanoate	$C_{13}F_{27}CO_2^-$	713.14	-0.22[a]	8.90[d]	n.a.	$^{13}C_2$ PFDoDA
PFHxDA	perfluorohexadecanoate	$C_{15}F_{31}CO_2^-$	813.16	-0.22[a]	n.a.	n.a.	$^{13}C_2$ PFDoDA
PFOcDA	perfluorooctadecanoate	$C_{17}F_{35}CO_2^-$	913.18	-0.22[a]	n.a.	n.a.	$^{13}C_2$ PFDoDA
PFSAs							
PFBS	perfluorobutane sulfonate	$C_4F_9SO_3^-$	299.05	0.14[a]	3.90[d]	n.a.	$^{18}O_2$ PFHxS
PFHxS	perfluorohexane sulfonate	$C_6F_{13}SO_3^-$	399.07	0.14[a]	5.17[d]	2.7[c]	$^{18}O_2$ PFHxS
PFOS	perfluorooctane sulfonate	$C_8F_{17}SO_3^-$	499.09	0.14[a]	6.43[d]	3.34[c]	$^{13}C_4$ PFOS
PFDS	perfluorodecane sulfonate	$C_{10}F_{21}SO_3^-$	599.11	0.14[a]	7.66[d]	3.53[c]	$^{13}C_4$ PFOS
FOSAs							
FOSA	perfluorooctanesulfonamide	$C_8F_{17}SO_2NH_2$	499.12	5.56[a]	5.62[d]	n.a.	$^{13}C_8$-FOSA
N-MeFOSA	*N*-methylperfluorooctan-sulfonamide	$C_8F_{17}SO_2N(CH_3)H$	513.14	7.69[a]	6.07[d]	n.a.	d_3-N-MeFOSA
N-EtFOSA	*N*-ethylperfluorooctane-sulfonamide	$C_8F_{17}SO_2N(CH_2CH_3)H$	527.17	7.91[a]	6.71[d]	n.a.	d_5-N-EtFOSA
FOSEs							
N-MeFOSE	*N*-methylperfluorooctane-sulfonamido-ethanol	$C_8F_{17}SO_2N(CH_3)CH_2$-CH_2OH	557.19	14.40[a]	6.00[d]	n.a.	d_7-N-MeFOSE
N-EtFOSE	*N*-ethylperfluorooctane-sulfonamido-ethanol	$C_8F_{17}SO_2N(C_2H_5)CH_2$-$CH_2OH$	571.22	14.40[a]	6.52[d]	n.a.	d_9-N-EtFOSE
FOSAAs							
FOSAA	Perfluorooctanesulfonamido-acetic acid	$C_8F_{17}SO_2N(CH_3)CH_2$-CO_2H	557.15	n.a.	n.a.	n.a.	d_3-N-MeFOSA
N-MeFOSAA	*N*-methylperfluorooctane-sulfonamidoacetic acid	$C_8F_{17}SO_2N(CH_2CO_2H)H$	571.18	n.a.	n.a.	3.11[b]	d_3-N-MeFOSAA
N-EtFOSAA	*N*-ethylperfluorooctane-sulfonamidoacetic acid	$C_8F_{17}SO_2N(C_2H_5)CH_2$-$CO_2H$	585.20	n.a.	n.a.	3.23[b]	d_5-N-EtFOSAA
FTSAs							
6:2 FTSA	6:2 fluorotelomer sulfonate	$C_8H_4F_{13}SO_3H$	428.13	n.a.	4.44[d]	n.a.	$^{13}C_4$ PFOS

[a](Ahrens et al., 2012), [b](Higgins and Luthy, 2006), [c](Sepulvado et al., 2011), n.a. not available

3.2 Sampling

3.2.1 Latrine

The latrine used for the anaerobic degradation experiments was sampled by the Läk project members at the Swedish Institute of Agricultural and Environmental Engineering (JTI) on the 22nd of August 2014 at Salmunge waste plant in Norrtälje, Sweden. Latrine collected from subscribers is stored in two concrete basins with a volume of 115.5 m^3, whereas one of the basins is used less frequently as a backup when the first one is overloaded. The main basin contained approximately 60 m^3 when sampling was performed. A stirrer placed in the middle of the pool was active during sampling and 20 hours prior to the start of the sampling. Samples were collected in metal buckets between 11:00 and 12:30 on 22nd of August from the main container at two positions: close to the middle near the stirrer and close to the short side of the pool and at two depths (surface and 0.2 m from bottom using a pump). From each sampling point, 10 L latrine was collected, resulting in a total amount of 40 L. The latrine was mixed in a plastic container and stirred vigorously for approximately 5 minutes using a concrete stirrer (Meec tools 480/800 rpm) in order to homogenize the material and avoid sedimentation when transferring into smaller bottles. The bottles were sealed, wrapped with aluminum foil and frozen at -22 °C.

As the latrine was to be used for the anaerobic degradation experiments, it was stored 24 hours in the fridge to unfreeze.

3.2.2 Blackwater

Sampling of blackwater was also carried out by the Läk project members at JTI. Blackwater samples were taken from the Hölö recycling plant close to the municipality of Södertälje, Sweden. The treatment plant processes blackwater from approximately 600 subscribers in two reactors (32 m^3). The blackwater is regularly collected from the households in closed collection tanks with connected toilet streams from primarily low-flush toilets/vacuum toilets. It is transported to the facility by sewer cleaning units and stored in pre-storage tank (200 m^3) until treatment. Up to 1500 m^3 blackwater is treated at the facility per year. The recycling plant performs the treatment in two steps, first wet composting and second sanitation with urea in both reactors. Samples were collected before treatment (3rd December) and after the two different treatments; wet composting (15th December) and urea treatment (22nd December). In the reactor, sludge is recirculated from the bottom to the top for sufficient mixing and to prevent scum creation at the reactors head (*Figure* 5). Samples were taken from a tap on the circulation pipe at the bottom of both reactors before liquid composting (day 0), after wet composting (day 12) and after the urea treatment (day 19). At day 0 additional samples as wet controls were collected and stored in closed containers for the time of wet composting (12 days) and after urea treatment (19 days) at 6.5 °C. This experiment was designed to determine whether target compounds (PhACs and PFASs) are significantly degraded during blackwater storage in the basins, before treatment.

3.3 Treatment Techniques

3.3.1 Latrine Anaerobic Degradation

The purposes of anaerobic degradation is to reduce pathogens (such as viruses) and/or to produce fuels from biological waste. During the treatment various microorganisms, e.g. acidogenics and methanogenic bacteria, break down and convert organic material to energy in the absence of oxygen. It can be distinguished between four steps. The first step is hydrolysis, where biological decomposition of organic matter (break down of polymers to monomers) and solubilization of insoluble organic matter take place. The second and third steps are acidogenesis and acetogenesis, where bacteria transform the products of the first reaction to acids, hydrogen and carbon dioxide. The final step is called methanogenesis. During that process microorganisms convert hydrogen and acetic acids to methane and carbon dioxide (Gavala et al., 2003).

The latrine anaerobic degradation experiments were accomplished by the project partners at JTI as part of Ingela Filipssons master thesis. Two experiments were performed in parallel; one mesophilic at 37 °C and one thermophilic at 52 °C. The temperature is determining which microorganisms are present. In a range of 30 to 35 °C mesophiles and in a range of 50 to 55 °C thermophiles are the primary microorganisms (Dumontet et al., 1999). Mesophilic degradation reduces 50-99 % of viruses in sludge and is considered to be more stable than thermophilic degradation (Bertucci et al., 1988). Thermophilic degradation on the other hand, is more effective with a virus inactivation of 99 % at 50 °C, however the microbial community is more sensitive to environmental changes (Dumontet et al., 1999, Sanders et al., 1979).

Degradation experiments were done at laboratory-scale and were conducted in glass bottles using latrine as substrate, with or without addition of selected PhACs. The reason to perform two experiments using spiked and un-spiked latrine was to ensure the detection of selected target pharmaceuticals in latrine and to assess their possible degradation during anaerobic treatment. Regarding the PFAS degradation experiments, only the spiked experiment was conducted, since low environmental concentration were expected. The spiked solutions contained 8 PhACs and 14 PFASs (see lists in 3.1.1 and 3.1.2). For the anaerobic degradation experiments, besides latrine, sewage sludge was used as an inoculum. Inoculation is already processed sewage sludge containing suitable microorganisms for the degradation process. The sewage sludge used was from two full-scale digester facilities: Kungsängsverket WWTP in Uppsala (for the mesophilic experiments) and Kävlinge WWTP (for the thermophilic degradation). The amounts of inoculum added during the experiments were adjusted so that the mass of organic material in the inoculation was three times higher than in the untreated latrine (ratio 3:1) in order to have optimal conditions. However, during the thermophilic experiment the inoculation had so low volatile solids (VS) content that the estimated inoculum did not fit into the bottle. Therefore, the ratio 2.7:1 was chosen in the thermophilic experiments. The batch reactors were filled with inoculation, untreated latrine and water, reaching a total weight of 600 g. The bottles were gas proof sealed and washed with nitrogen gas to get an oxygen free environment. Additionally they were covered with aluminum foil so no light could reach the bottles, as this might affect the degradation of organic substances. The reactors were incubated at 37 or 52 °C and placed on a shaking table allowing constant mixing of 130 rpm for a duration of 61 days for mesophilic and 59 days for the thermophilic degradation. At different time points of the treatment, bottles were collected for this analysis in order to assess the degradation of target compounds over time (*Table* 3). The volume of gas produced and methane concentration were noted over time. For each time point, the content of the bottle was centrifuged to separate solid material from the liquid phase and stored in the freezer until further analysis. The latrine anaerobic degradation experiment described above and measurements of methane concentration were conducted by JTI.

Figure 4. Batch experiment (Filipsson, 2015).

For the analysis of latrine, a total of 67 liquid and solid samples (134 including duplicates) were analyzed for PhACs and 23 liquid and solid samples (46 with duplicates) were analyzed for PFASs. Only the spiked samples were analyzed in the PFAS analysis, due to time and sample limitations.

A summary of substrate content of latrine samples and days of sampling during treatment is shown in *Table* 3. The raw latrine material itself and the sewage sludge inoculums used for the degradation experiments were analyzed separately, to investigate the background levels of target

compounds. Mesophilic samples were collected on day 0, 14, 30 and at the end of treatment day 61. Thermophilic samples were sampled at day 0, 7, 21, 30 and 59. At the initial time (day 0) and the final day of treatment (day 61 and 59) two samples instead of one were collected, for having a higher data accuracy when determing the removal from before and after treatment and to reduce the number of samples. Two replicates were analyzed for each sample.

Table 3. Overview of analyzed latrine samples for PhACs: substrate in different mixtures, number of liquid and solid samples, replicates, temperature of treatments and samples according to the day of treatment (mark with ×).

		No. of liquid samples	**No. of solid samples**	**Number of replicates**	**T**	**Days**						
Reactor	**Mesophilic**					0	7	14	21	30	59	61
A1	Latrine + inoculum	4	4	2	37°C	×		×		×		×
A2	Latrine + inoculum	2	2	2		×						×
B1	Latrine + inoculum + spiked	4	4	2	37°C	×		×		×		×
B2	Latrine + inoculum + spiked	1	1	2								×
C1	Inoculum	2	2	2	37°C	×						×
	Thermopilic											
A1	Latrine + inoculum	5	5	2	52°C	×	×		×	×	×	
A2	Latrine + inoculum	2	2	2		×					×	
B1	Latrine + inoculum + spiked	5	5	2	52°C	×	×		×	×	×	
B2	Latrine + inoculum + spiked	2	2	2		×					×	
C1	Inoculum	2	2	2	52°C	×					×	
C2	Inoculum	2	2	2		×					×	
D	Untreated latrine	1	2	2	15°C	×						
E	Blank	1	1	2								
	Σ Samples	33	34									
	Total number*	66	68									

*Including dupilicates

For PFASs, only the samples corresponding to the spiked latrine experiments were analyzed, since PFAS concentrations are expected to be low in raw latrine (*Table* 4). To know the initial concentration in the raw material, also the untreated latrine sample was analyzed. In total 11 liquid and 12 solid samples were probed for PFASs.

Table 4. Overview of analyzed latrine samples for PFAS: substrate in different mixtures, number of liquid and solid samples, replicates, temperature of treatments and samples according to the day of treatment (mark with ×).

		No. of liquid samples	**No. of solid samples**	**Number of replicates**	**T**	**Days**						
Reactor	**Mesophilic**					0	7	14	21	30	59	61
B1	Latrine + inoculum + spiked	4	4	2	37°C	×		×		×		×
	Thermopilic											
B1	Latrine + inoculum + spiked	5	5	2	52°C	×	×		×	×	×	
D	Untreated latrine	1	1	2	15°C	×						
E	Blank	1	2	2								
	Σ Samples	11	12									
	Total number*	22	24									

*Including duplicates

3.3.2 Blackwater Treatment

The focus of the blackwater treatment is to sanitize blackwater without extensive loss of nutrients. Treatment reduces contamination and pathogens, together with the degradation of organic material and minimization of odor. Efficient treatment is important because the treated blackwater is aimed to be applied as fertilizer on agricultural fields.

Before processing, the blackwater is stored in a pre-storage tank. The treatment consists of two steps. The first one is wet composting, where blackwater is oxidized due to aeration and constant mixing (aerobic treatment) for about 7-12 days (*Figure 5*). The process itself does not sanitize the substrate, however the biological activity increases the temperature to 40 °C in about one week. The heat is produced by mesophilic microbes using easily available organic matter as energy source (exothermic reaction) (Dumontet et al., 1999). Oxygen is essential for the microbes therefore aeration and constant mixing is important.

The second step is sanitation with urea. Here 0.5 % urea is added to the substrate and it is constantly mixed for seven days. Urea is a nitrogen compound (a carbonyl group attached to two amine groups) formed in the liver and therefore naturally occurring in urine. The urea in the blackwater is supplemented with additional urea to have a higher sanitation effect. In the reactor, urea is degraded by hydrolysis due to the enzyme urease, which is naturally found in feces, to ammonia and carbon dioxide (Equation 1 and 2). Both products have disinfectant properties towards pathogenic microorganisms (Fidjeland et al., 2013, Vinnerås, 2002).

Ammonia occurs in two forms, ammonia (NH_3 unionized) and the ammonium ion (NH_4^+), both form equlibrium. The ammonium ion is available for plant uptake and is an important nutrient, whereas NH_3 has sanitation properties to prevent proliferation of pathogens (Vinnerås, 2002, Nordin, 2010). High temperature (> 20 °C) and high pH (~9) shift the equilibrium towards the side of uncharged ammonia (NH_3), having a sanitation effect (Vinnerås, 2002, Nordin, 2010).

$$CO(NH_2)_2 + 2\ H_2O \rightarrow H_2CO_3 + NH_3 \quad (1)$$

$$NH_3(aq) + H_2O(l) \leftrightarrow NH_4^+(aq) + OH^-(aq) \quad (2)$$

After treatment the substrate is transferred to the post-storage tank until application on farmland. Treated blackwater is stored for about six months before it is used on the fields.

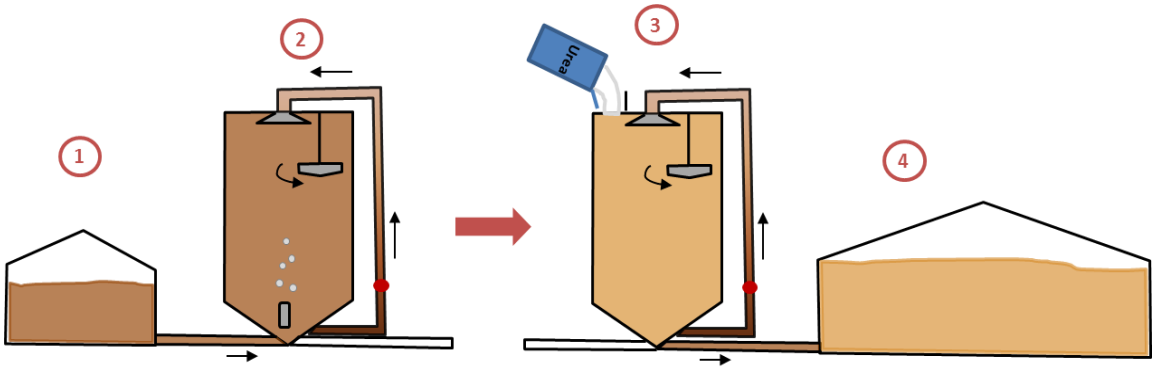

Figure 5. Scheme of the blackwater treatment in four steps: 1) The pre-storage tank where the blackwater is kept until treatment, 2) Wet composting with aeration and constant mixing, 3) Urea sanitation with 0.5 % urea and 4) Post-storage tank. Samples have been collected from the first three steps, the red mark on the circulation pipe is the tap where samples from the reactor have been taken.

For the analysis of blackwater 22 liquid and solid samples (44 including duplicates) were analyzed for PhACs and three liquid and one solid samples (7 including duplicates) were analyzed for PFASs. The experimental setup and the summary of substrate content of samples and days of sampling during treatment can be seen in *Table* 5 and *Table 6*. Samples from three stages of treatment have been collected, untreated blackwater from the pre-storage tank at day 0, after wet composting at day 12 and after urea treatment at day 19. Samples from both reactors were analyzed, but only values determined for reactor one were considered in the results due to

a irregular treatment process in reator two (due to a broken circulation pump). Additionally controls have been collected at day 0. Those were stored for the time of wet composting (wet control 1) and for the whole treatment (wet control 2) at 6.5 °C in order to see potential degradation during storage compared to the treatment.

Table 5. Overview of analyzed blackwater samples for PhACs from reactor 1 (R1) and reactor 2 (R2): substrate in different mixtures, number of liquid and solid samples, replicates, temperature of treatments and samples according to the day of treatment (mark with ×).

		No. of liquid samples	**No. of solid samples**	**Number of replicates**	**Days**		
Reactor					0	12	19
R1	Untreated	1	1	2	×		
R2	Untreated	1	1	2	×		
R1	Wet control 1	1	1	2	×		
R2	Wet control 1	1	1	2	×		
R1	Wet control 2	1	1	2	×		
R2	Wet control 2	1	1	2	×		
R1	After wet composting	1	1	2		×	
R2	After wet composting	1	1	2		×	
R1	After urea treatment	1	1	2			×
R2	After urea treatment	1	1	2			×
	Blank	1	1	2			
	Σ Samples	11	11				
	Total number*	22	22				

*Including duplicates

For the PFAS analysis samples three liquid sample from reactor one and only one solid sample with no duplicate were analyzed, due to limited sample volumes (*Table* 6).

Table 6. Overview of analyzed blackwater samples for PFAS from reactor 1 (R1): substrate in different mixtures, number of liquid and solid samples, replicates, temperature of treatments and samples according to the day of treatment (mark with ×).

		No. of liquid samples	**No. of solid samples**	**Number of replicates**	**Days**		
Reactor	**Mesophilic**				0	12	19
R1	Untreated	1	1	2/1	×		
R1	After wet composting	1	-	2		×	
R1	After urea treatment	1	-	2			×
	Σ Samples	3	1				
	Total number*	6	1				

*Including duplicates

3.4 Chemicals

During the lab analysis the following chemicals were used: Milli-Q Gradient (Merck Millipore, France); glacial acetic acid 100 %, acetonitrile (ACN) > 99.9 %, methanol (MeOH) > 99.5 % (Merck KGaA, Darmstadt, Germany); Na_2EDTA 0.1 M solution (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany); ethanol (EtOH) > 99.8 %, formic acid 98 % (Sigma Aldrich, Stockholm, Sweden); ammonium acetate ≥ 99.0 % (Sigma-Aldrich, Netherlands); ammonium formate 99.0 % (Sigma-Aldrich, US); 0.1 % ammonium hydroxide 25.0 % (Sigma-Aldrich, Spain); ammonia > 99.9 %, hydrochloric acid 30.0 % (HCl), sodium hydroxide (NaOH), magnesium sulfate 98.5-101.5 % ($MgSO_4$), sodium acetate 99.8 % (NaOC) (1.5 g) + $MgSO_4$ (6 g). Additionally primary secondary amine SPE Bulk Sorbent (toluene, acetophenone, dimethyl phthalate, and benzyl

alcohol) (Agilent Technologies, Lake Forest, CA, USA) and supelclean ENVI Carb 120/400 (Bellefonte, PA, USA) were used.

3.4.1 Chemicals used in the PhAC Analysis

The analytical standards for PhACs had a purity of > 95 %, as well as the isotopically labeled standards. The following target pharmaceuticals were purchased from Sigma-Aldrich: amitriptyline (as hydrochloride salt), atenolol, azithromycin, bezafibrate, carbamazepine, ciprofloxacin, citalopram (as hydrobromide salt), clarithromycin, fluoxetine (as hydrochloride salt), furosemide, hydrochlorothiazide, irbesartan, lamotrigine, lidocaine, losartan (as potassium salt), metoprolol (as tartrate salt), norfloxacin, propranolol (as hydrochloride salt), ranitidine (as hydrochloride salt), sotalol (as hydrochloride salt), sulfamethoxazole, trimethoprim, valsartan and venlafaxine (as hydrochloride salt). Other pharmaceuticals were acquired from Cerilliant and purchased through Sigma-Aldrich as a 1 mg mL^{-1} solution and diluted in an appropriate solvent. These substances were: atorvastatin (as atorvastatin calcium solution), codeine, diazepam, diltiazem (diltiazem hydrochloride solution, as free base) and oxazepam.

For the isotopically labeled standards some substances were purchased from Sigma-Aldrich, such as carbamazepine-d_{10} (as 100 µg mL^{-1} solution), venlafaxine-d_6 hydrochloride solution (100 µg mL^{-1} as free base) trimethoprim-d_9, codeine-d_3 (as 1 mg/mL solution), diazepam-d_5 (as 1 mg/mL solution), fluoxetine-d_5 (as 1 mg/mL solution), ofloxacin-d_3, atenolol-d_7 and lidocaine-d_{10}. Other substances such as. atorvastatin-d_5 (as Na salt), azithromycin-d_3, bezafibrate-d_4, ciprofloxacin-d_8, hydrochlorothiazide-$^{13}C,d_2$, bisoprolol-d_5, diltiazem-d_4, metronidazole-d_4, furosemide-d_5, ranitidine-d_6, irbesartan-d_7 and sulfamethoxazole-d_4 were purchased from Toronto Research Chemicals (TRC). The substances acquired as solids were dissolved in methanol (MeOH) (at a concentration of 1 mg mL^{-1}), except for ciprofloxacin, ofloxacin and norfloxacin which were prepared in MeOH adding 100 µL of NaOH 1 M. When prepared, the standards were stored at −20 °C. For the analysis of pharmaceuticals in water samples, working standard solutions were prepared in methanol/water (10:90, v/v), whereas for the analysis in solid samples, working solutions were prepared in methanol/water (30:70, v/v). A mixture containing all isotopically labeled internal standards was prepared in pure methanol. Internal standards were used for internal standard calibration and quantification.

3.4.2 Chemicals used in the PFAS Analysis

The following PFASs were included in the spiking solutions with a concentration of 484.1 mg mL^{-1} for each PFAS with high purity (95-99 %): PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFHxS, PFOS and FOSA purchased from Sigma-Aldrich (Sweden).

The internal standard (IS) mix containing $^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{3}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, all with a concentration of 20 pg μL^{-1}, and $^{13}C_8$-FOSA, d_3-N-MeFOSAA, d_5-N-EtFOSAA, d_3-N-MeFOSA, d_5-N-EtFOSA, d_7-N-MeFOSE and d_9-N-EtFOSE, all with a concentration of 50 pg μL^{-1}, purchased from Wellington Laboratories (Canada), was added to all samples. The injection standard (InjS) consisted of $^{13}C_8$ PFOA (Wellington Laboratories Canada).

3.5 Pharmaceutical Analysis

3.5.1 PhAC Analysis of Liquid Samples

The liquid samples of latrine and blackwater were filtered through glass microfiber filters (0.7 μm, Ø 47 mm, GE Healthcare, Life Science, Whatman^{TM}). 200 mL of each sample were used for the analysis (100 mL were used for each replicate and two replicates per sample were analyzed). For the analysis of the 'latrine spiked' samples, 50 mL per replicate were utilized. Whereas for the 'untreated latrine' 25 mL of sample were extracted, which were afterwards diluted with Millipore water to a final volume of 50 mL. After every sample the filtration equipment was rinsed two to three times with distilled water and two times with ethanol. Samples were filtered from assumed low concentrations (i.e. day 61) to high concentrations (i.e. day 0) in order to avoid potential cross-contamination.

Pharmaceuticals were extracted by solid-phase extraction (SPE), using Oasis HLB cartridges (6cc, 200 mg, Waters Corporation). Prior to SPE, samples were spiked with 50 μL of the isotopically labelled standard mixture (1 ng μL^{-1} mix) at room temperature. Afterwards 3 mL of a Na_2EDTA 0.1 M solution was added to achieve a final concentration of 0.1 % (g solute g^{-1} solution in the water). For the spiked experiments and the analysis of untreated latrine, where 50 mL were extracted, 1.5 mL of a Na_2EDTA 0.1 M solution were added. Finally each sample's pH was adjusted with concentrated formic acid (98 %) to a range of 2.7-3.3. SPE cartridges were pre-conditioned with 6 mL methanol and 6 mL acidified Millipore water (pH 2.7-3.0). The samples were loaded approximately at 1 mL min^{-1}. After the loading, the cartridges were rinsed with about 4 mL Millipore water (pH 2.7-3.0) and then centrifuged to remove water excess at 3500 rpm for 5 min. For the elution, 4 mL MeOH + 4 mL MeOH were used and the eluate was collected in glass tubes. After the elution the samples were concentrated using nitrogen evaporation to complete dryness. Afterwards the samples were reconstituted with 100 μL MeOH and 900 μL of Millipore water (10:90 v/v). Prior to instrumental analysis, extracts were filtered with regenerated cellulose syringe filters (0.2 μm, 17 mm Scantect Nordic). The 'latrine spiked' and 'untreated latrine' extracts were filtered by 0.45 μm filters.

3.5.2 PhAC Analysis of Solid Samples

For the analysis of the solid phase, samples were first freeze dried for 3-5 days. Afterwards, they were grinded with porcelain mortar and pestle to homogenize the samples. Subsequently 1 g of each homogenized sample was weighed and transferred into 50 mL polypropylene centrifuge (PP) tubes. 50 μL of a mixture containing the isotopically labelled standards at 1 ng μL^{-1} was added. Samples were mixed with vortex for 30 s so that the sludge had sufficient contact with the isotopically labelled standards. After that, 7.5 mL of a 0.1 M Na_2EDTA solution were added and the samples were vortexed for 30 s followed by the addition of 7.5 mL acetonitrile (ACN) containing acetic acid (1 % v/v) and subsequent samples were vortexed for 30 s. Then 1.5 g sodium acetate and 6 g $MgSO_4$ salts were added (pre-packed QuEchERs extract pouches, AOAC method, Bond Elut, Agilent Technologies). The samples were immediately shaken by hand for 30 s to avoid coagulation of $MgSO_4$, and vortexed for 1 min for homogenization. Samples were centrifuged at 3500 rpm for 15 min. After centrifugation, about 6 mL of the supernatant (ACN layer) was transferred to 15 mL PP tubes containing 900 mg $MgSO_4$ (SampliQ Anhydrous Magnesium Sulfate for QuEchERs, Agilent Technologies) and 150 mg PSA (SPE bulk sorbent, Agilent Technologies). The tubes were manually shaken for 30 s, vortexed for 1 min and

centrifuged at 3500 rpm for 15 min. After that, the ACN layer, approximately 5 mL, was transferred into glass tubes and evaporated to ~200 μL using nitrogen evaporation. The remaining extracts were transferred to 1 mL amber glass HPLC vials. The glass tubes were two times rinsed with ACN (400-800 μL). Then the extracts were frozen for one hour and then centrifuged at 3500 rpm for 5 min as an extra sample clean-up step. After that the extracts were transferred into a new 1 mL amber glass HPLC vial and concentrated to dryness using nitrogen evaporation. Finally they were reconstituted with 300 μL MeOH + 700 μL Millipore water. Prior to instrumental analysis extracts were filtered through regenerated cellulose syringe filters (0.22 μm).

3.5.3 Instrumental Analysis of PhACs

Pharmaceuticals were analyzed using an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, USA) coupled to a quadrupole-time-of-flight (QTOF) mass spectrometer (QTOF Xevo G2S, Waters Corporation, Manchester, UK). For a detailed description of the instrumental analysis see Appendix A.

3.5.4 Quality Control and Quality Assurance

Recoveries of target pharmaceuticals in aqueous latrine samples and blackwater ranged from 57 % to 170 % and relative standard deviations were not higher than 30 % (*Table* D1). Recoveries for target pharmaceuticals in latrine and blackwater solid samples ranged from 70 % to 160 %, except for clarithromycin and valsartan, whose recovery was around 50 % and 60 %, respectively. Method extraction blanks were examined and no pharmaceuticals were detected in the blank samples. For each sample one duplicate was analyzed.

Method detection limits (MDL) and method quantification limits (MQL) were determined as the minimum detectable amount of analytes with a signal-to-noise of 3 and 10, respectively (*Table* C1). MDL and MQLs have been calculated as the average of those estimated in real samples and in the spiked samples. MDLs in aqueous latrine samples and in blackwater ranged from approximately 5 to 120 ng L^{-1} whereas MQLs ranged from around 10 to 400 ng L^{-1}. In solid samples, MDLs ranged approximately from 3 to 150 ng g^{-1} d.w. and MQLs from 10 to 500 ng g^{-1} d.w.. Quantification of target analytes was performed by the internal standard approach. Calibration standards were measured at the beginning and at the end of each sequence, and one calibration standard was measured repeatedly throughout the sequence to check for signal stability and as quality control.

3.6 PFAS Analysis

3.6.1 PFAS Analysis of Liquid Samples

For the PFAS analysis 100 mL per sample was filtered through glass microfiber filters (0.7 μm, Ø 47 mm, GE Healthcare, Life Science, Whatman™) and 50 mL per replicate were analyzed. The filtrates were spiked with 100 μL isotopically labelled standard mixture (20 pg μL^{-1}) at room temperature and then shaken. For the SPE the reservoir and devices were rinsed 3 times with MeOH. The Oasis WAX 6cc cartridges (Waters Corporation, Milford, MA, USA) were preconditioned with first 4 mL 0.1 % ammonium hydroxide in MeOH, followed by 4 mL MeOH and 4 mL Millipore water. The cartridges were loaded with 50 mL sample at approximately 1 drop per second. After that 4 mL 25 mM ammonium acetate buffer in Millipore water was used

to wash the cartridges, followed by centrifugation at 3000 rpm for 2 min in order to remove water. The extracts were eluted with 4 mL MeOH and 4+4 mL 0.1 % ammonium hydroxide and were reduced under nitrogen stream to 1 mL. Finally 10 μL InjS (200 pg μL^{-1}) was added and the samples were vortexed prior to instrumental analysis.

3.6.2 PFAS Analysis of Solid Samples

For the solid extraction, 0.5 g of dried and homogenized of each solid sample was weighed in a 50 mL PP-tube with exception of the blackwater solid sample for which 1 g was taken since it was not spiked and lower PFAS concentrations were expected. 2 mL of a 100 mM sodium hydroxide (NaOH) in 80 %/ 20 % in methanol/Millipore water solution was added to all samples and then they were soaked for 30 min. Afterwards, 20 mL MeOH and 100 µL of PFAS's isotopically labelled standard mixture was added. The samples were placed on a wrist-action shaker at 200 rpm for 60 min. After the samples have been shaken, they were centrifuged at 3000 rpm for 5 min for phase separation. The supernatants were then decanted into a new PP-tube. 0.1 mL 4 M hydrochloric acid was added and the sample shaken by hand and centrifuged by at 3000 rpm for 5 min. One-eighth of each sample (4.15 mL) was transferred into a 15 mL PP-tube and concentrated under the nitrogen stream to 1 mL. Afterwards the samples were decant into a prepared 1.7 mL Eppendorf centrifuge tube with 25 mg ENVI-Carb and 50 µL glacial acetic acid (Eppendorf). The tubes were tightly closed, vortexed and centrifuged at 4000 rpm for 15 min. Finally 0.5 mL extract was transferred into a 1 mL glass-vial and 10 μL InjS (200 pg μL^{-1}) was added.

3.6.3 Instrumental Analysis of PFASs

The instrumental analysis was conducted using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS, for details of the method see (Ahrens et al., 2009a).

3.6.4 Quality Control and Quality Assurance

Recoveries of target PFASs were calculated with the concentration of internal standard measured in each sample divided by the average of pure IS sample in the liquid phase (n=28) and the solid phase (n=25). The recoveries in liquid latrine and blackwater samples ranged from 30 % to 170 %, excluding 'untreated latrine' (*Table* D2). Recoveries for target PFASs in latrine and blackwater solid samples ranged from 39 % to 188 %, excluding MeFOSA (day 59, *Table* D2). Extracts of 'untreated latrine' that were excluded had extreme high organic matter content which made them cloudy and therefore recoveries varied substantially (2.6 % to 127 %, n=2). The recovery value of MeFOSA in the solid phase is an outlier with 206 %.

Blanks were used in order to see potential contamination during the extraction and to determine MDLs. The blanks average concentrations were 0.30 ng L^{-1} (n=2) for the liquid phase and 0.63 ng g^{-1} d.w. for the solid phase (n=4). For each sample, except for untreated solid blackwater sample, duplicate samples were analyzed. MDLs and MQLs have been calculated from the sum of the mean of the blanks plus three times the determined standard derivation (SD). MDLs in liquid latrine and blackwater samples ranged from approximately 0.3 to 1.0 ng L^{-1}, whereas MQLs ranged from around 0.7 to 3.3 ng L^{-1}. In solid samples, MDLs ranged from 0.1 to 5.6 ng g^{-1} d.w. and MQLs from 0.3 to 18.5 ng g^{-1} d.w.

4 Results and Discussion

4.1 Occurrence and Removal of PhACs

In all three treatments 25 out of the 29 target compounds were detected. Compounds not detected were, norfloxacin, fluoxetine, diltiazem (detected only in one sample of untreated latrine) and ranitidine. In the mesophilic and thermophilic treatment samples, 24 PhACs were found (excluding diazepam and the four before mentioned PhACs). In the blackwater 19 of the 29 compounds were detected, ruling out sotalol, clarithromycin, sulfamethoxazole, trimethoprim, irbesartan, bezafibrate, norfloxacin, fluoxetine, diltiazem and ranitidine.

Initial concentrations of the source material, 'untreated latrine' and inoculum, and latrine before treatment (day 0) in liquid and solid phase of mesophilic and thermophilic treatment are summarized in *Table* B1. It can be seen that most β-blockers, antidepressants, antihypertensives and diuretics were detected in high concentrations in all substrates. The highest concentrations in the liquid phase of 'untreated latrine' were valsartan (180 μg L^{-1}), metoprolol (48 μg L^{-1}) and losartan (32 μg L^{-1}). In the samples before treatment (day 0) the following three PhACs were detected with the highest concentrations (mesophilic); valsartan (3 μg L^{-1}), losartan (34 μg L^{-1}) and furosemide (11 μg L^{-1}). The thermophilic values are similar. 'Untreated latrine' as the source material shows the highest concentrations, the inoculum lower ones, since it is treated sewage sludge and the concentrations before mesophilic and thermophilic treatment lay in between. This is expected, since the latrine was a mixture of the 'untreated latrine' and inoculum at a ratio 1:3 (untreated latrine:inoculum). Under the detection limit or moderate concentrations (in range of 0.0061 to 1.2 μg L^{-1}) were detected for antibiotics, the analgesic codeine, the lipid regulators and the local anesthetic lidocaine. The same pattern can be seen for the solid phase. In 'untreated latrine' the compounds with the highest concentrations were losartan (7.4 μg g^{-1} d.w.), metoprolol (1.3 μg g^{-1} d.w.) and valsartan (0.12 μg g d.w.), whereas for the latrine before treatment, losartan (1.3 μg g^{-1} d.w.), citalopram (0.7 μg g^{-1} d.w.) and metoprolol (0.7 μg g^{-1} d.w.), had the highest concentrations. Amitriptyline was only detected in the solid phase whereas lipid regulators, atorvastatin and bezafibrate were only found in the liquid phase. For atorvastatin, however, the analytical method used did not perform well and this would explain why it is only detected in the liquid phase.

Environmental concentrations measured in the blackwater analysis were similar, but had in general lower levels than the ones measured for the anaerobic degradation experiments (*Table* B2). The β-blockers, antidepressants, antihypertensives and diuretics show the highest concentrations during all treatment steps in solid and liquid phase. In the liquid phase the highest concentrations were found for both diuretics furosemide (40 μg L^{-1} day 19) and hydrochlorothiazide (14 μg L^{-1} day 0). Antibiotics, the analgesic codeine, the lipid regulators and the local anesthetic lidocaine were measured at low levels, under the detection limits or at moderate levels in range of 13 to 1,600 ng L^{-1}. The three compounds with highest concentrations in the solid phase were propranolol (2.4 μg g^{-1} d.w. day 0), oxazepam (1.6 μg g^{-1} d.w. day 0) and citalopram (1.1 μg g^{-1} d.w. day 0).

Environmental concentrations after all treatments remained within high μg L^{-1} and μg g^{-1} d.w. in the liquid and solid phase. Compared to conventional wastewater, the concentrations in latrine and blackwater were higher (Gracia-Lor et al., 2010, Radjenovic et al., 2009, Jelic et al., 2011). Latrine and blackwater are up to 25 times higher concentrated than WWTP influents. Therefore,

it was expected to detect higher concentrations (de Graaff et al., 2011). PhACs with highest consumtion in Sweden (mentioned in chapter 2.1), such as metoprolol (to ~119,000 patients), oxazepam (~53,000), citalopram (~48,000) and valsartan (~39,000) were the ones measured with high concentrations in latrine and blackwater.

High concentrations in the liquid phase of all treatments show that most PhACs tend to partition to the liquid phase. The concentrations measured in the solid phase, however, were also substantial (*Table* B1 and *Table* B2), especially for the β-blockers (metoprolol, propranolol), antidepressants (citalopram and carbamazepine) and antihypertensives (losartan). This insight stresses the need of the evaluation of both phases when assessing the fate of PhACs. Additionally the high concentrations of PhACs in the latrine and blackwater could be explained by their high usage and consumption and their behavior (e.g. their pharmacokinetics and their behavior during treatment) (Halling-Sorensen et al., 1998). For instance, the anti-hypertensive drug valsartan is mostly excreted as non-metabolite (81.5 % of a dose in excreta) (Waldmeier et al., 1997), whereas propranolol can be excreted as a metabolite, that even has the same toxicity as non-metabolized propranolol (Nałęcz-Jawecki et al., 2008, Celiz et al., 2009). Other pharmaceuticals that produce bioactive metabolites are for example acetaminophen, carbamazepine and diclofenac. Metabolites were not investigated in this study, therefore concentration values might be underestimated (Celiz et al., 2009).

For blackwater, detected concentrations are similar than those reported elsewhere (de Graaff et al., 2011, Winker et al., 2008). The study by Graaff et al. (2001) analyzed blackwater during anaerobic treatment and found similar concentrations of metoprolol (45 μg L^{-1}), propranolol (1 µg L^{-1}), and carbamazepine (1.1 μg L^{-1}). Winker et al. (2008) tested urine for several PhACs in Germany and revealed higher concentrations for bezafibrate (368 μg L^{-1}) and carbamazepine (62.1 μg L^{-1}). Bezafibrate was not detected and lower concentrations were determined for carbamazepine (up to 2.4 μg L^{-1}) in this study. Variations in detected compounds are expected, since blackwater was taken in different countries with different usage and consumption patterns and at different times of the year.

4.1.1 Removal Efficiency

The analysis of the anaerobic degradation experiments of the spiked experiment revealed that 18 (62 %) in the mesophilic and 16 (55 %) of the PhACs in the thermophilic experiment showed removal (*Figure* 6). Whereas in both experiments 17 % of the compounds showed an increase of concentrations after treatment and 21 % (mesophilic) and 28 % (thermophilic) of the PhACs have not been detected. The removal efficiency, for each PhAC, was determined by the sum of the amounts detected in the liquid and solid phase, respectively expressed as ng. In the mesophilic experiment 56 % of the 18 PhACs with removal show high degradation (70-100 %). Low removal rates (0-30 %) were dominating in the thermophilic experiment. Compounds that were removed completely in the mesophilic spiked experiment (n=10) were: codeine, atenolol, azithromycin, ciprofloxacin, sulfamethoxazole, trimethoprim, oxazepam, irbesartan, hydrochlorothiazide and bezafibrate. For the thermophilic spiked experiment five PhACs were removed completely; sulfamethoxazole, trimethoprim, irbesartan, hydrochlorothiazide and bezafibrate. The average removal, considering increased and PhACs with removal, is for the mesophilic experiment 45 % and for the thermophilic 31 %. In general both treatments show a similar distribution, but the removal efficiency of the compounds with removal is higher for mesophilic conditions.

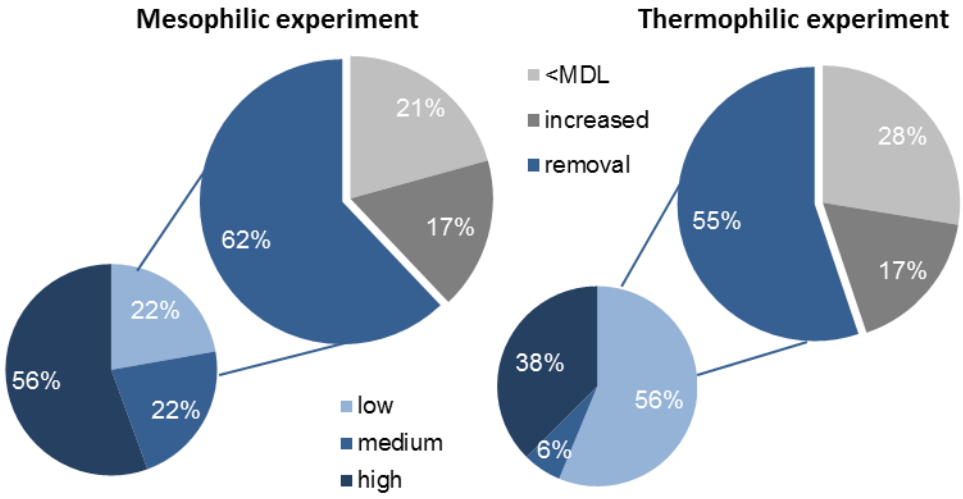

Figure 6. Removal overview of anaerobic degradation. PhACs after the mesophilic (61 days) and thermophilic (59 days) treatment of spiked latrine in percent. The bigger pie charts show the percentages of PhACs below the method detection limit (< MDL in light grey), of PhACs where the concentration after the treatment has increased (dark grey) and of the PhACs that showed removal from before to after the treatment (blue). The smaller pie charts present the degree of removal; low removal (0-30 %, light blue), medium (30-70 %, blue) and high removal rates (70-100 %, dark blue).

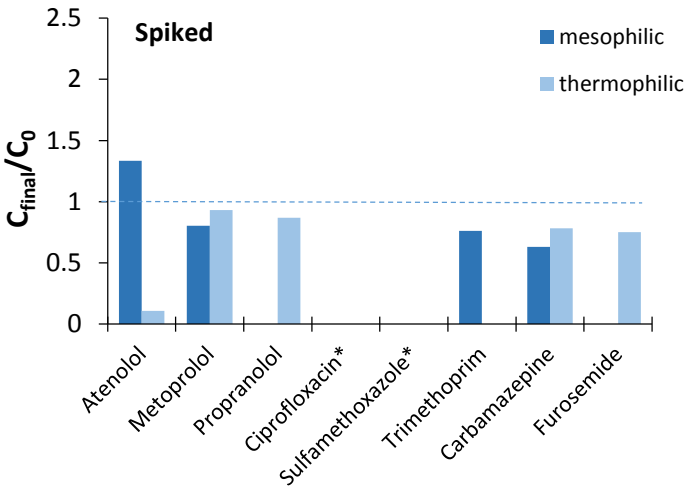

Figure 7. Removal of spiked PhACs in latrine after mesophilic (37 °C, 61 days) and thermophilic (52 °C, 59 days) of anaerobic treatment. Values above 1 indicate an increase and below 1 indicate a removal of PhACs during the treatment (* = completely removed). The change of PhACs concentration during treatment was calculated as C_{61d}/C_0, for mesophilic, and as C_{59d}/C_0, for thermophilic. "C" is expressed in ng and is the sum between the amounts detected in the solid and liquid phase.

Figure 7 shows the removal rates of the spiked target pharmaceuticals under mesophilic and thermophilic conditions. In the spiked samples it can be observed that ciprofloxacin and sulfamethoxazole were completely removed in both treatments, as supported by other studies (Malmborg and Magnér, 2015, Carballa et al., 2007, Narumiya et al., 2013). Under mesophilic conditions the following additional compounds have been completely removed, propranolol and furosemide; and under thermophilic conditions atenolol (~90 %) and trimethoprim. Metoprolol

and carbamazepine show low removal, which is in accordance to other studies (Narumiya et al., 2013, Malmborg and Magnér, 2015).

Changes of each PhAC after mesophilic and thermophilic treatment (non-spiked) and blackwater treatment (from reactor 1) can be seen in *Figure* 8. During the mesophilic experiment β-blockers (except propranolol) and antidepressants (except citalopram and amitriptyline) show substantial removals. Six PhACs show increased concentrations (propranol, azithromycin, citalopram, amitriptyline, hydrochlorothiazide and atorvastatin). Under thermophilic conditions all β-blockers show significant removals, as well as most antidepressants. Three compounds had increased concentrations losartan, valsartan and furosemide. In both treatments all antibiotics were either not detected or completely removed (except clarithromycin has a removal of about 10 %, thermophilic). This is in good agreement with Carballa et al. (2007) who evaluated the anaerobic degradation of pharmaceuticals during anaerobic treatment of sewage sludge. Several studies assessed the removal rate of PhACs during anaerobic treatment (see 2.1.2). Suarez et al. (2010) found that carbamazepine, diazepam, sulfamethoxazole and trimethoprim were persitent. Compared to this study carbamazepine had a ~30 % removal and sulfamethoxazole and trimethoprim were either not detected or completely removed. Our results, however, are supported by Narumiya et al. (2013), who reported that carbamazepine showed no degradation and sulfamethoxazole and trimethoprim were almost completely degraded (> 90 %).

Another study determined substantial removal for atenolol, trimethoprim, oxazepam, furosemide and hydrochlorothiazide (Malmborg and Magnér, 2015). That can be confirmed for atenolol, trimethoprim, oxazepam and hydrochlorothiazide in the thermophilic experiment, but not for furosemide and hydrochlorothiazide in the mesophilic experiment.

Different removal rates have been assessed for antidepressant citalopram, moderate removal of ~40 % (Suarez et al., 2010), high removal 85 % (Bergersen et al., 2012) and ~20 % removal after thermophilic and ~150 % increased concentration after mesophilic treatment in this study. Reasons for different results between studies could be different experimental designs (batch vs continuous reactor, latrine vs sewage sludge), different types of microorganisms, as well as analytical uncertainties and other unknown factors.

The thermophilic experiment showed slightly higher removals than the mesophilic experiment. These results are contradictory with the spiked tests, where the mesophilic experiment revealed better removal rates (see *Figure* 7). But in general, both mesophilic and thermophilic experiments show similar results, indicating that the temperature difference of 15°C might have no influence on the removal of PhACs.

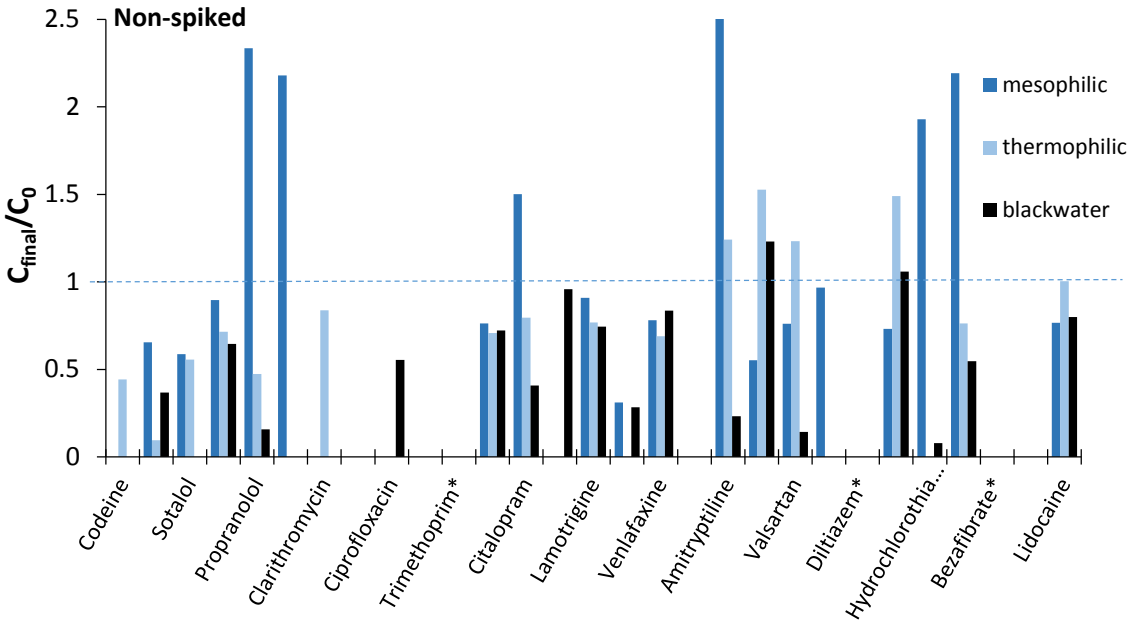

Figure 8. Removal of non-piked PhACs in latrine after mesophilic (37 °C, 61 days) and thermophilic (52 °C, 59 days) of anaerobic treatment and PhACs in blackwater after treatment. Values above 1 indicate an increase and below 1 indicate a removal of PhACs during the treatment (* = not detected or completely removed). The change of PhACs concentration during treatment was calculated as C_{61d}/C_0, for mesophilic, and as C_{59d}/C_0, for thermophilic. "C" is expressed in ng and is the sum between the amounts detected in the solid and liquid phase.

The blackwater treatment results reveal that 16 (55 %) of the 29 PhACs showed removal (*Figure* 9). However, 34 % of the compounds were under the MDL and 10 % had increased concentrations. The removal rate between low, medium and high removal is evenly distributed. Two compounds (10 %) had slightly increased concentration after treatment (losartan and furosemide, see *Figure* 8). Completely removed were codeine and azithromycin, whereas propranolol, valsartan, amitriptyline, oxazepam and hydrochlorothiazide show high removals (84-91 %). Diazepam, venlafaxine and lidocaine show low removal and the remaining compounds were moderately removed. The average removal for all PhACs in the blackwater treatment is 49 %. De Graaff et al. (2011) determined removal rates for inter alia trimethoprim, metoprolol, propranolol and carbamazepine during anaerobic treatment of blackwater. In that study, trimethoprim was either not detected or showed high removal, whereas propranolol and carbamazepine were rather resistant, which is in accordance with this study. Metoprolol on the other hand, showed a removal of 67 % in De Graaff's study and 35 % in this study.

Potential explanations to the increase in concentration of some PhACs in the treatments could be the transformation of metabolites to the parent compound or the reduced amounts of particles to which the compound can be adsorbed (which would increase the extraction efficiency). Out of 29 target PhACs analyzed, 14 and 16 in the anaerobic degradation treatment and 17 compounds of the blackwater treatment remained in the sludge after treatment with some removal or even increased concentrations. The overall conclusion is that both source separated treatments, anaerobic degradation of latrine and wet composting with urea sanitation of blackwater, are not sufficient to removal of pharmaceutical residues.

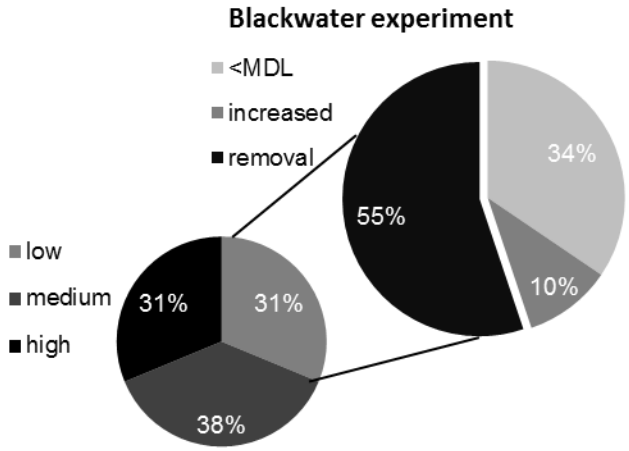

Figure 9. Removal overview of blackwater treatment (19 days) in percent. The bigger pie chart shows the percentages of PhACs below the method detection limit (< MDL in light grey), of PhACs where the concentration after the treatment has increased (dark grey) and of the PhACs that showed removal (black). The smaller pie chart presents the degree of removal; low removal (0-30 %, light grey), medium (30-70 %, grey) and high removal rates (70-100 %, black).

4.1.2 Temporal Changes of PhACs during Treatments

When comparing the changes of the PhACs during the mesophilic and thermophilic treatment along the different time points, it can be seen that the individual compounds change their behavior during the treatment (*Figure* 10, values from the spiked experiment). Atenolol degraded at the beginning of both treatments, already reacting to the hydrolysis step of anaerobic treatment. Azithromycin shows the same behavior in the thermophilic treatment and a slower degradation in the mesophilic treatment, but with a complete removal at the end of treatment. Metoprolol was found to have low degradation rates in aforementioned results, which can be observed here for the thermophilic treatment but not in the mesophilic. The majority of compounds show low variation during the different steps of anaerobic degradation and slight drops in concentrations were observed. In the thermophilic treatment a slight increase in concentrations for some compounds at day seven can be seen. A possible explanation could be the reduction of particles to which the compound can be adsorbed due to the biological decomposition of organic matter (hydrolysis step), which would increase the extraction efficiency. Additionally the variation could be due to analytical errors, since the variation has not been observed in the non-spiked experiment and the standard deviation for some values were higher. Valsartan in the mesophilic and amitriptyline in the thermophilic treatment deviate from the general pattern, because their concentration after the treatment was determined much higher than before the treatment.

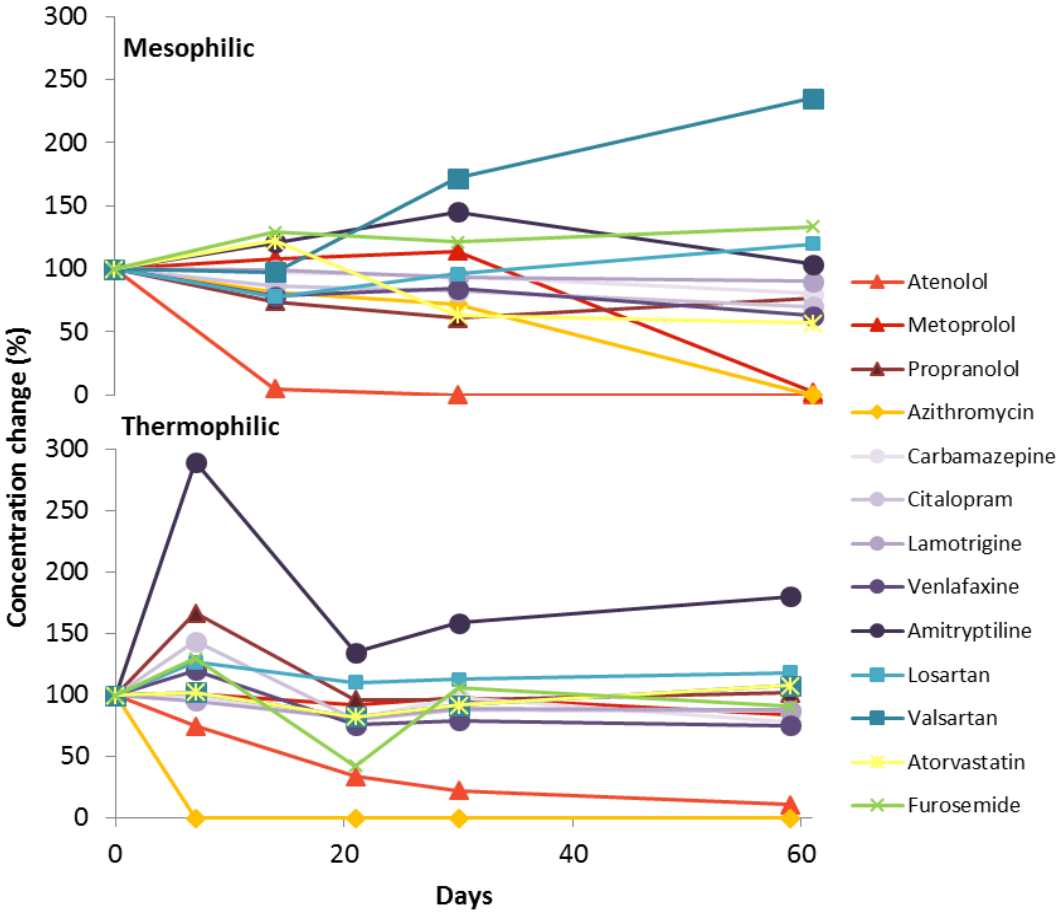

Figure 10. Changes of individual PhACs over time in latrine during anaerobic mesophilic and thermophilic treatment. Values above 100 % indicate an increase and below indicate a removal of PhACs during the treatment.

The blackwater treatment reveals a clear trend for most compounds (*Figure* 11 upper graph). The concentrations dropped during the wet composting (the first 12 days) and remained constant or increase slightly during the urea treatment. Therefore, it can be assumed that urea treatment has no or even a negative effect due to some increased concentrations on the removal of PhACs (the same pattern could be observed in the values from reactor two). Significant decreased concentrations can be seen for atenolol, propranolol, azithromycin, citalopram, amitriptyline and valsartan. In order to ensure that during blackwater storage no degradation occurs, a control sequence was performed along the blackwater treatment (*Figure* 11 lower graph). The control samples were stored at 6.5 °C in the dark under anaerobic conditions for 12 and 19 days. The analysis revealed for most compounds low to no change in concentrations, as expected, and an increase for some due to aforementioned possible reasons. The low effect of the urea treatment on the degradation of pharmaceuticals is supported by a study adding urea to digested, dewatered sludge as a sanitation technology (Malmborg and Magnér, 2015).

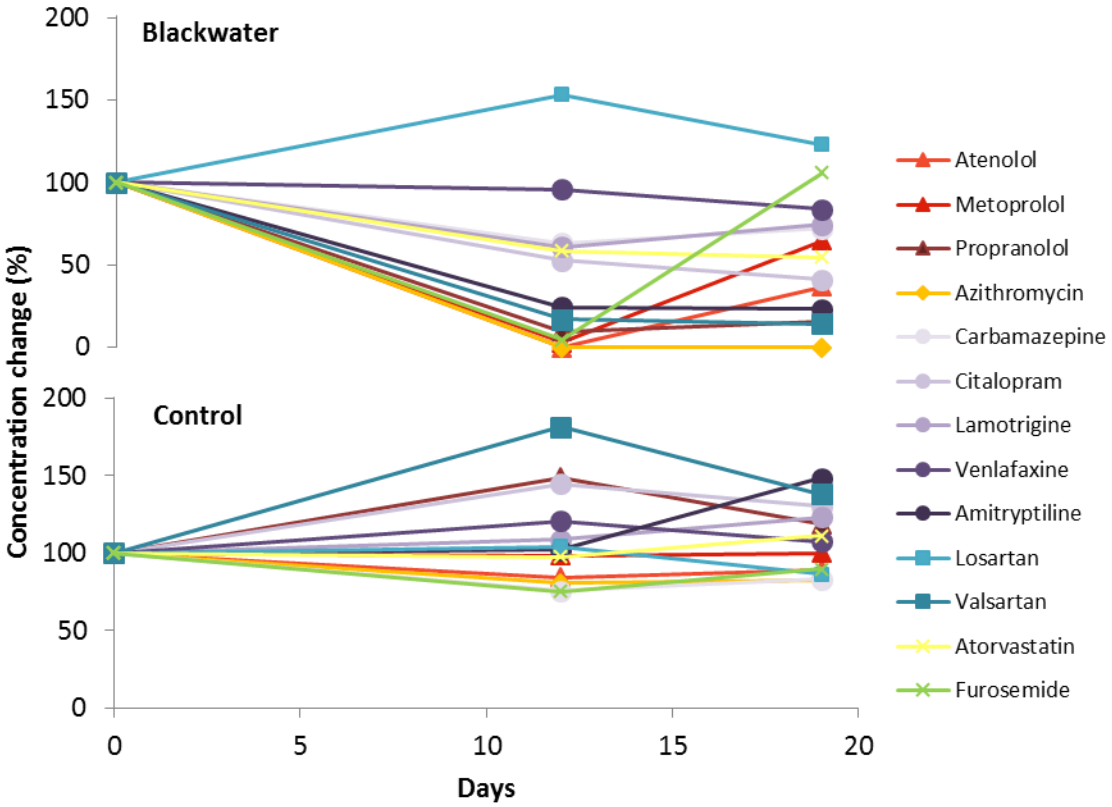

Figure 11. Changes of individual PhACs over time in blackwater during treatment with wet composting (day 0-12) and urea sanitation (day 12-19, upper graph). Control samples were stored at 6.5 °C in the dark under anaerobic conditions for 12 and 19 days, to determine whether target compounds could be degraded during blackwater storage in the basins (lower graph). Values above 100 % indicate an increase and below indicate a removal of PhACs during the treatment.

4.1.3 Sorption Behavior of PhACs between Liquid and Solid Phase

All treatments showed average removal between 30 and 50 %, but substantial concentrations still remain in the treated fraction. The percentage of how much of each PhACs has been removed or how much remained in the liquid or solid phase after the treatment is presented in the distribution charts (*Figure* 12, *Figure* 13 and *Figure* 14). The mesophilic and thermophilic distribution charts are distinguished between the spiked and non-spiked experiment.

As previously discussed, PhACs tend to partition to the liquid phase, but the percentage of the PhACs in the solid phase is still significant. Codeine, amitriptyline and citalopram for example were only detected in the solid phase of the mesophilic and thermophilic experiment and venlafaxine was found in the solid fraction in high percentage. The β-blockers also seem to have the tendency to occur mainly in the solid phase, despite their removal. However, the antihypertensives, losartan, valsartan and irbesartan, the diuretics, furosemide and hydrochlorothiazide and the lipid regulator atorvastatin tend to only partition to the liquid phase. Due to the limitation of the method atorvastatin could only be analyzed in the liquid phase. Antibiotics were either not detected or completely removed, only clarithromycin showed low removal in the thermophilic treatment and occurred only in the liquid phase. The antidepressants occured in the liquid and solid phases in both treatments. When comparing the spiked with the non-spiked experiment it becomes clear that more compounds were detected in the spiked one,

as expected. The spiked experiment was conducted to ensure the detection for certain PhACs (the eight which were spiked are marked with #) and to determine their fate during the treatments. The three antibiotics, ciprofloxacin, sulfamethoxazole and trimethoprim, for example were not detected in the non-spiked experiment, but in the spiked one and show complete removals. The remaining five compounds that have been spiked show similar distribution in both experiments, in the mesophilic and thermophilic treatments. The results prove that the distribution of the compounds was comparable between the spiked and the non-spiked experiment.

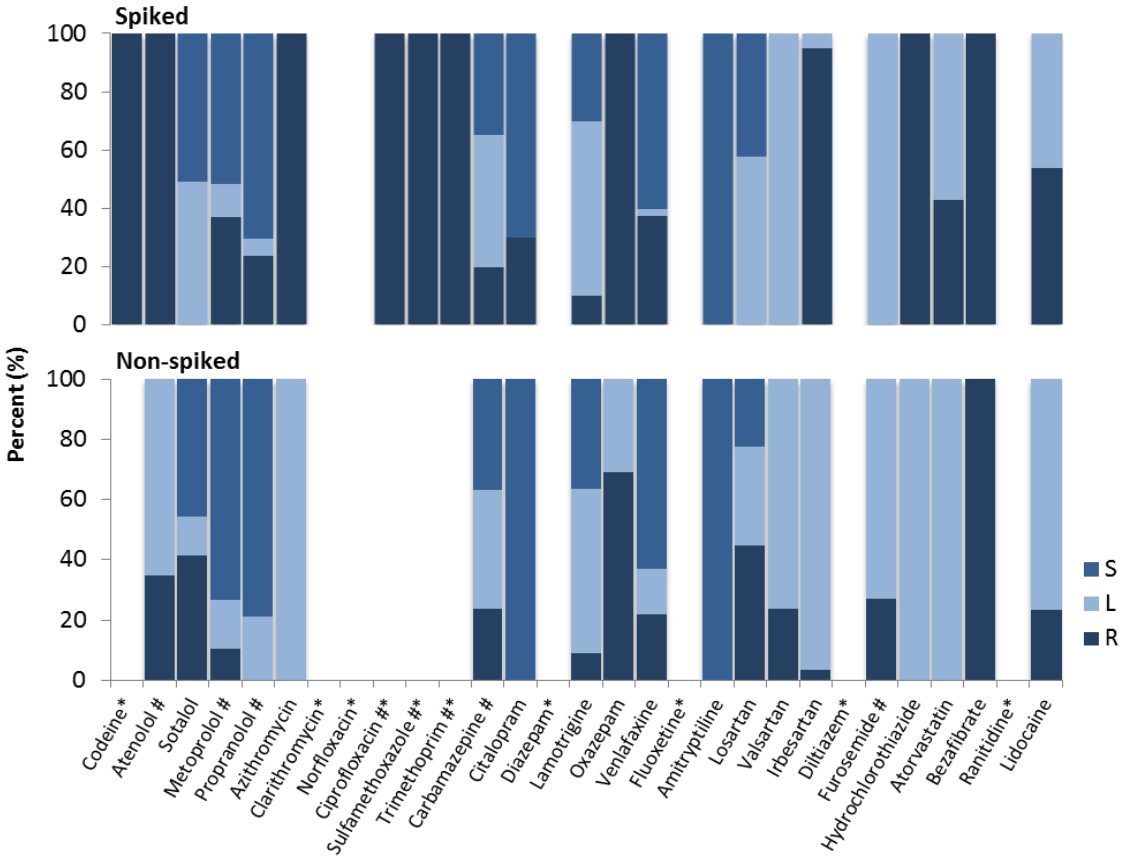

Figure 12. Distribution of PhACs after mesophilic treatment in the solid phase (blue), liquid phase (light blue) or removed (dark blue) in latrine spiked (upper graph, # = spiked compounds) and in non-spiked latrine (lower graph) in percent (* = completely removed or not detected).

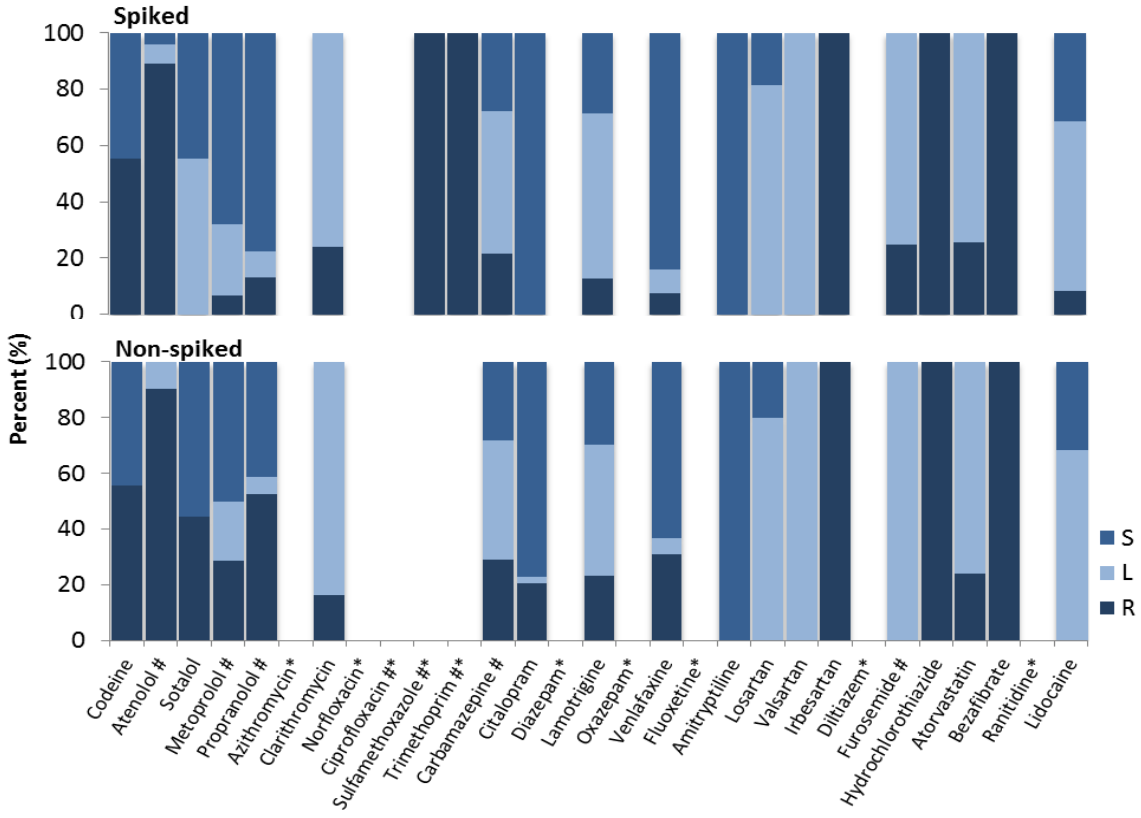

Figure 13. Distribution of PhACs after thermophilic treatment in the solid phase (blue), liquid phase (light blue) or removed (dark blue) in latrine spiked (upper graph, # = spiked compounds) and in non-spiked latrine (lower graph) in percent (* = completely removed or not detected).

The distribution chart for blackwater illustrates that higher concentrations of each compound were found in the liquid phase (*Figure* 14). Mostly the eight antidepressants have been found in the solid phase, except for fluoxetine which was in general not detected. Amitriptyline was almost completely partitioned in the solid phase, as was already seen in the anaerobic degradation experiments. It should be mentioned that after the wet composting, the treated blackwater had low amounts of solids and therefore, it was expected that there is lower capacity for PhACs to partition to the solid phase.

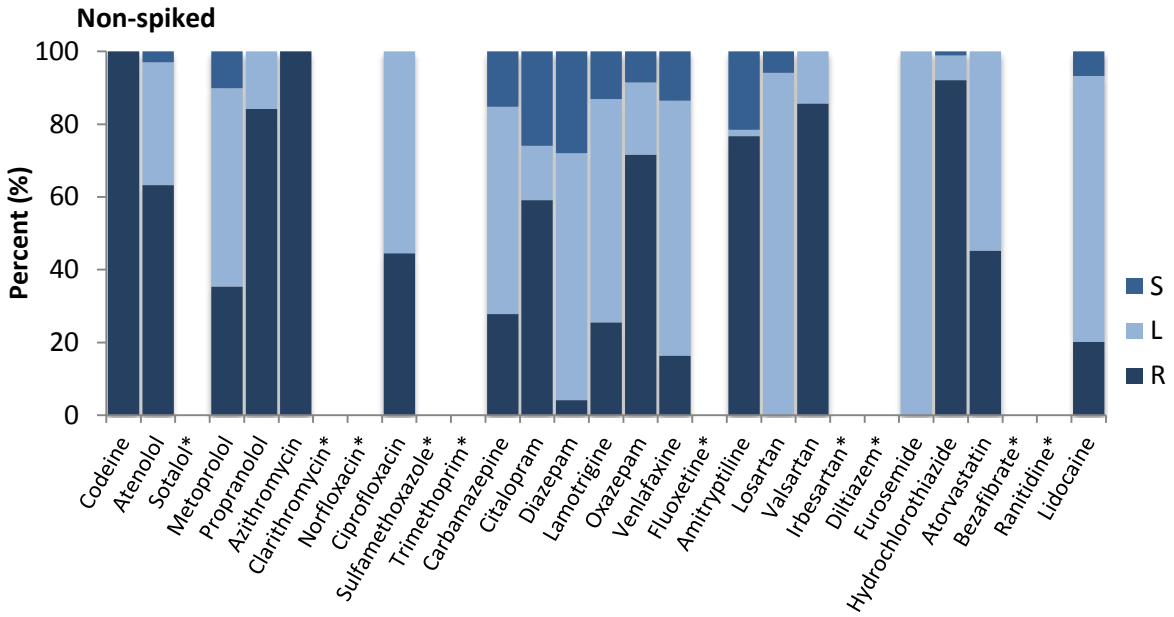

Figure 14. Distribution of PhACs after treatment (19 days) in the solid phase (blue), liquid phase (light blue) or removed (dark blue) in blackwater (non-spiked) in percent (* = not detected).

The physicochemical properties of the PhACs can help to explain the distribution between the liquid and solid phase, such as the octanol-water partition coefficient K_{ow}, the organic carbon-water partition coefficient K_{oc}, the logarithmic acid dissociation constant pK_a and the solid-water distribution coefficient K_d, which influence the distribution of pharmaceuticals (*Table 1*, the coefficient values were foun in literature). K_d values were calculated from results and are displayed in *Table* E1, logarithmic values in *Table 7* and a comparison to literature values in *Table 8.*

Codeine, amitriptyline, citalopram, venlafaxine plus β-blockers atenolol, sotalol, metoprolol and propranolol were most detected in the solid phase despite removal. Those compounds, excluding atenolol and sotalol, have high K_{oc} values ranging from 1.79 to 5.70. High K_{oc} values mean a larger hydrophobicity of the compound and hence tend to be distributed in the solid phase, which is in accordance to the results. Additionally, those compounds have all a pK_a around 9, that means that they are weaker acids (stronger base), which indicates lower ionic interactions with the positively charged sludge. Since the treatment's pH was neutral (~8), specific ionic interactions for non-ionized molecules could be neglected (Ternes et al., 2004). However, atenolol and sotalol have low K_{oc} values, but high pK_a and moderate K_d values (*Table 7)*, and this may explain their proneness to be found in the solid phase.

On the other hand, losartan and valsartan have high K_{oc} values indicating that they are prone to be found in the solid phase, but they have low pK_a and low K_d values which may explain their tendency for the liquid phase (*Table* 7). The example of atenolol and sotalol, and losartan and valsartan indicate that the pK_a and K_d coefficients are important parameters to take into account when evaluating the partition of pharmaceuticals between liquid and solid phase. A statistical correlation, however, should be conducted to underline that statement.

Table 7 shows that citalopram and oxazepam occur primarly in the solid phase in the anaerobic degradation experiments and citalopram and amitriptyline in the blackwater experiment, which

can be seen in the distribution charts. Some PhACs revealed low tendency to sorb onto the solid phase (losartan, irbesartan, furosemide, hydrochlorothiazide and lidocaine). When comparing the K_d values from before the treatment and after the treatment, no substantial changes could be observed. Especially during the blackwater treatment changes were expected due to differences of pH during treatment (pH before treatment: 9.4, after wet composting: 5.4 and after treatment: 9.1). During the wet composting the pH dropped to 5.4 and several studies have reported that changes in pH changes the K_d values (Narumiya et al., 2013, Ternes et al., 2004, Carballa et al., 2008). However, this behavior was not observed in this study.

Table 7. The logarithmic K_d values of PhACs before and after treatment during the mesophilic and thermophilic treatment and K_d values before, after wet composting (day 12) and after urea treatment (day 19) during blackwater treatment (n.d. = not detected).

	log K_d						
	Mesophilic		**Thermophilic**		**Blackwater**		
Compound	Day 0	Day 61	Day 0	Day 59	Day 0	Day 12	Day 19
Analgesics							
Codeine	n.d.	n.d.	n.d.	n.d.	1.8	n.d.	n.d.
β-blockers							
Atenolol	1.8	n.d.	2.2	1.6	0.8	n.d.	1.6
Sotalol	1.9	1.7	1.8	1.7	n.d.	n.d.	n.d.
Metoprolol	2.3	2.4	2.4	2.2	1.6	1.8	1.9
Propranolol	2.3	2.8	2.6	2.7	2.7	3.5	n.d.
Antibiotics							
Sulfamethoxazole	n.d.	n.d.	-0.4	n.d.	n.d.	n.d.	n.d.
Trimethoprim	2.0	n.d.	2.1	n.d.	n.d.	n.d.	n.d.
Antidepressants							
Carbamazepine	1.7	1.6	1.8	1.5	1.7	2.2	2.1
Citalopram	3.8	n.d.	3.1	n.d.	3.5	3.2	2.9
Diazepam	n.d.	n.d.	n.d.	n.d.	2.1	n.d.	2.3
Lamotrigine	1.5	1.4	2.0	1.4	1.7	2.4	2.0
Oxazepam	3.4	n.d.	n.d.	n.d.	2.5	2.4	2.3
Venlafaxine	2.7	3.1	2.6	2.7	2.0	2.1	2.0
Amitryptiline	n.d.	n.d.	n.d.	n.d.	4.2	3.8	3.8
Antihypertensives							
Losartan	2.0	1.6	1.6	1.1	1.8	1.5	1.5
Irbesartan	1.9	n.d.	1.8	n.d.	n.d.	n.d.	n.d.
Diuretics							
Furosemide	n.d.	n.d.	n.d.	n.d.	0.7	n.d.	n.d.
Hydrochlorothiazide	1.3	n.d.	2.0	n.d.	1.6	n.d.	1.9
Local anesthetic							
Lidocaine	1.5	n.d.	1.4	1.5	1.3	1.9	1.6

Not detected: azithromycin, clarithromycin, norfloxacin, ciprofloxacin, fluoxetine, atorvastatin, bezafibrate and ranitidine

When comparing the K_d values of selected PhACs with values reported in other studies no great correspondence can be seen (*Table* 8). It should be emphasized that these K_d values are from conventional wastewater treatment and are given only as rough estimates, taking into account for non-homogenicity of sewage sludge and a possible non-equilibrium state in the samples (Radjenovic et al., 2009). K_d values determined for carbamazepine, however, are for example in good agreement with those found in the literature. K_d values are rather low indicating that sorption is not the main removal pathway and if the biodegradation is negligible, carbamazepine passes through the treatments in the liquid phase with significant amounts (Ternes et al., 2004). This can be confirmed also from other previous studies (Heberer, 2002, Ternes, 1998).

Ternes et al. (2004) determined that compounds with a K_d value greater than 500 kg L^{-1} greatly sorb to the solids. According to Jones et al. (2006) sorption could be a possible removal pathway during treatment; this might apply to citalopram, oxazepam, amitriptyline and propranolol since high K_d values were determined. In general it can be concluded that biodegradation rather than sorption is a major removal pathway in the overall fate of PhACs during source separated treatment (Ternes et al., 2004, Radjenovic et al., 2009).

Table 8. Comparison of calculated K_d values and literature K_d values for selected PhACs in liters per kilograms. Calculated K_d values of latrine and blackwater are displayed from before and after mesophilic, thermophilic and blackwater treatment. Literature values are conventional secondary sludge and conventional digested sludge. Values are present with their standard derivation.The COD corresponds to the organic content in the biomass (Maurer et al., 2007).

	K_d (L kg^{-1}) ± SD						Literature K_d *values* (L kg^{-1}) ± SD	
	Mesophilic		**Thermophilic**		**Blackwater**		**Secondary sludge**	**Digested**
Compound	Day 0	Day 61	Day 0	Day 59	Day 0	Day 19		
β-blockers								
Atenolol	62±3.8	n.d.	160±12	36	6.7±1.5	41±3.5	64±88[a] 0.21[e] <40 L kg^{-1}_{COD}[f]	
Metoprolol	180±13	240±22	270±17	150±35	41±4.0	88±4.0	1.0±23 L kg^{-1}_{COD}[f]	
Propranolol	210±18	650±38	430±140	460±170	500±100	n.d.	366±138[a] 320±58 L kg^{-1}_{COD}[f]	
Antibiotics								
Sulfamethoxazole	1.1±0.6	n.d.	0.4	n.d.	n.d.	n.d.	77±60[a] 114-400[d]	5.8-61.5[c]
Trimethoprim	100±1	n.d.	140	n.d.	n.d.	n.d.	253±37[a] 157-375[d]	
Antidepressants								
Carbamazepine	51±2.6	40±2.3	63±12	31±10	55±0.2	130±10	135±39[a] 1.3±0.5[b]	20.2-56.4[c]
Diazepam	n.d.	n.d.	n.d.	n.d.	130±7.1	200±3.4	21±8[b]	

[a](Radjenovic et al., 2009), [b](Ternes et al., 2004), [c](Carballa et al., 2008), [d](Göbel et al., 2005), [e](Jones et al., 2002) and [f](Maurer et al., 2007), n.d. = not detected

4.1.4 Summary of Latrine Anaerobic treatment and Blackwater Treatment of PhACs

Environmental concentrations of all samples ranged up to high μg L^{-1} and μg g^{-1} d.w. in the liquid and solid phase. In the anaerobic degradation treatment concentrations of many PhACs were higher in latrine compared to concentrations found in conventional WWTP effluents, due to lower dilution. Moreover no influence of temperature on the removal of PhACs could be seen under mesophilic (37 °C) and thermophilic (52 °C) conditions, which was previously reported (Carballa et al., 2007, Malmborg and Magnér, 2015). The spiked experiment showed slightly higher removal under mesophilic conditions whereas in the non-spiked experiment, the thermophilic treatment showed higher removal, but with no substantial differences. Average to low removal rates were observed, but only some showed complete removal (antibiotics n=4). For a few compounds, concentrations even increased during treatment (17 %), which makes them persistent during treatment. The average removal of the mesophilic treatment was 45 % and for the thermophilic 31 %. Malmborg and Magnér (2015) found that anaerobic degradation was the most efficient technique for the removal of a wide spectrum of PhACs and other substances compared to six other sanitation techniques. The average removal in that study, however, was ~30 %, which is lower than determined in this study. In general it can be concluded that anaerobic degradation does not efficiently remove pharmaceuticals from latrine and that

additional advanced treatments are unavoidable or efforts to find a better suitable removal technique need to be made, as stated also by Graaff et al. (2011).

The wet composting and urea sanitation of blackwater revealed slightly higher removal of PhACs with an average of 49 %. But still substantial concentrations remained in the treated fraction, ruling out most antibiotics because they were completely removed (n=4), except ciprofloxacin (to 50 % removed). The study from Cousins and Magnér (2014), which also analyzed blackwater from Hölö, determined 70 % removal for 31 detected PhACs. The aerobic wet composting step was determined to be the most efficient step of the removal, whereas urea sanitation showed low to no effect (*Figure* 11). In general it can be concluded that blackwater sanitation is not sufficient enough in the removal of PhACs in blackwater.

When comparing the distribution of PhACs of the remaining fraction between the liquid and solid phase in all treatments, most PhACs have a higher tendency to partition onto the liquid phase (e.g. antihypertensives, diuretics and lipid regulators), but also some substances are prone to partition onto the solid phase (e.g. β-blockers, antidepressants and some antihypertensives). These results stress the need of evaluating both phases when assessing the fate of PhACs. pK_a and K_d coefficients were found to be important variables when understanding compounds partitioning.

Statements about the risks of the PhACs entering the environment via the treated sludge application on farmland cannot be made in this study, further research is needed. The environmental risk assessment by Verlicchi et al. (2012), where potentially high risks to aquatic organisms for 67 PhACs were determined, can give an idea of potential risks of some PhACs (12 of them analyzed in this study, see chapter 2.1.2). Compounds which were determined to pose high risks are three antibiotics (azithromycin, clarithromycin and sulfamethoxazole) and two antidepressants (diazepam and fluoxetine). Different fates have been determined in this study for those compounds: fluoxetine was not detected; sulfamethoxazole was completely removed in the thermophilic experiment and not detected in the other two; azithromycin showed increased concentration in mesophilic (~230 %), no detection in thermophilic latrine and complete removal in blackwater; clarithromycin showed low removal in the thermophilic treatment (~10%) and was not detected in the other treatments; and diazepam was detected in blackwater with a removal of ~5 %. Consequently azithromycin, clarithromycin and diazepam could pose potential risks if the treated latrine or blackwater would get in contact with aquatic organisms.

4.2 Occurrence and Removal of PFASs

19 out of 26 PFASs were detected in both anaerobic degradation experiments, including the 14 spiked PFASs (*Table* B3 and *Table* B4). Not detected were PFOcDA, PFDS, N-MeFOSA, N-EtFOSA, N-MeFOSE, N-EtFOSE and FOSAA.

Next to the spiked compounds five non-spiked PFASs were detected in the anaerobic degradation experiments, 6:2 FTSA, PFTriDA, PFHxDA, N-MeFOSAA and N-EtFOSAA. 6:2 FTSA was only found in liquid phase under mesophilic conditions with a maximum concentration of 19 ng L^{-1} (*Table* B3). Whereas PFTriDA, PFHxDA, N-MeFOSAA and N-EtFOSAA were detected only in solid phases with low concentrations, that ranged from the MDL to 11 ng g^{-1} d.w. (N-EtFOSAA mesophilic day 30 and 61, *Table* B4 and for MDL values see *Table* C1). None of those five PFASs were detected in the 'untreated latrine', indicating that

the PFASs were introduced via the inoculum, which was mixed with the 'untreated latrine' before treatment. Inoculum from two different WWTPs were used; Kungsängsverket WWTP (for the mesophilic experiments) and Kävlinge WWTP (for the thermophilic experiment). In the case of 6:2 FTSA which was only found in the mesophilic experiment it can be assumed that only the inoculum from the Kungsängsverket WWTP contained 6:2 FTSA. Regarding the 'untreated latrine' four other compounds were found; PFPeA, PFHxA, PFHpA and FOSA in the range of 1.8 (FOSA) and 380 ng g^{-1} d.w. (PFPeA) and none were found in the solid phase.

The blackwater experiment revealed very low concentrations. Seven compounds were detected by the method, but only 5 of them have concentrations higher than their MQL, which were PFOA (4.3 ng L^{-1}), PFHxS (6 ng L^{-1}) and FOSA (2.6 ng L^{-1}) in the liquid phase and MeFOSAA (0.4 ng g^{-1} d.w.) and EtFOSAA (1.1 ng g^{-1} d.w.) in the solid phase. The few detected PFASs and their low concentrations indicate that latrine and blackwater are no substantial sources for PFASs. Several studies reported high concentrations up to some hundreds ng L^{-1} and some thousands in ng g^{-1} d.w. in conventional wastewater (Arvaniti and Stasinakis, 2015, Arvaniti et al., 2012, Gomez-Canela et al., 2012). No data about PFASs fates in source separated systems are reported so far. High concentration in conventional wastewater and low in the latrine and blackwater shows that different types of wastewater have different compositions of PFASs. In this case PFASs in conventional wastewater are probably not introduced by blackwater, indicating that source separation could be beneficial for accurate and PFAS specific treatments. It has been estimated that 85 % of indirect emissions of PFASs are a consequence of the use and disposal of consumers products, such as household products, paper and textile (Paul et al., 2009). That indicates that greywater might be a main sources of PFASs in conventional wastewater. Nonetheless further research is needed in order to determine the major source of PFASs in wastewater.

4.2.1 Removal Efficiency

In the mesophilic experiment 23 % of the PFASs showed a low removal (0-30 % removal rate), 27 % were below the method detection limit and half showed increased concentrations (*Figure* 15). Whereas in the thermophilic experiment 50 % of the PFASs had removals of 0-30 %. In both anaerobic degradation treatments no PFASs were removed completely. The average removal of detected PFASs for the mesophilic treatment is -24 %, which means that more PFAS's concentrations have increased than decreased. For the thermophilic treatment an average of 4 % removal was determined.

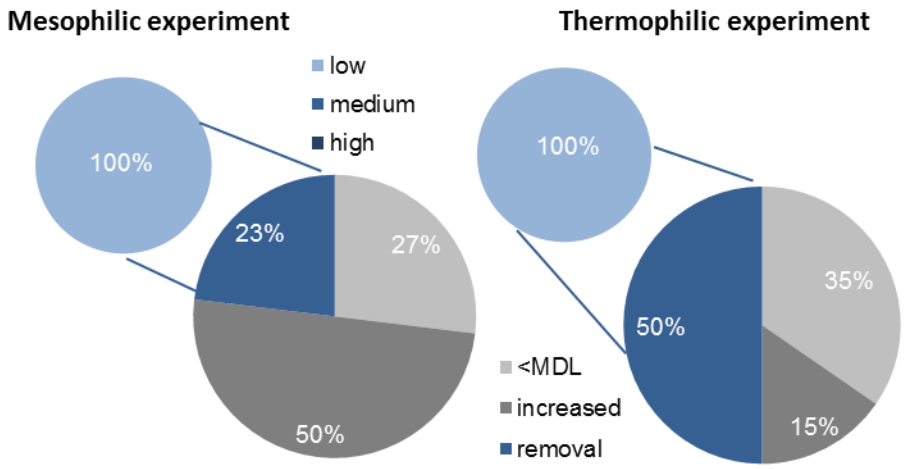

Figure 15. Removal overview after the mesophilic (61 days) and thermophilic (59 days) treatment of spiked latrine in percent. The bigger pie charts show the percentages of PFASs below the method detection limit (< MDL in light grey), of PFASs where the concentration after the treatment has increased (dark grey) and of the PFASs that showed removal (blue). The smaller pie chart presents the degree of removal; low removal (0-30 %, light blue), medium (30-70 %, blue) and high removal rates (70-100 %, dark blue).

Different removal efficiencies were determined between the mesophilic and thermophilic treatment (*Figure* 16). Most compounds (50 %) in the mesophilic treatment have increased concentrations after the treatment. This is in accordance with several studies, where concentrations of specific PFASs in treated wastewater were found higher than in the raw sewage (Arvaniti et al., 2012, Stasinakis et al., 2013, Yu et al., 2009), indicating biodegradation of PFAS precursor compounds (Arvaniti and Stasinakis, 2015). Most studies, however, reported no consistent removal during secondary biological treatments (Arvaniti et al., 2012, Schultz et al., 2006). The mesophilic experiment showed six PFAS with removal between 0.5 % (PFUnDA) and 19 % (PFDoDA).

Most compounds in the thermophilic treatment displayed removal after treatment ranging from 9 % (PFDoDA , PFTeDA and PFBS) to 28 % (PFBA), three had increased concentrations (PFTriDA 47 %, PFOS 36 % and MeFOSAA 43 %). Especially PFOS has been reported to have increased concentrations after treatment, Yu et al. (2009) determined a mean increase of 95 % in one of the conventional activated sludge process investigated. PFTriDA, PFHxDA and MeFOSAA, as well as other compounds, had low concentrations (< 4.3 ng L^{-1} in the liquid phase and < 2.7 ng g^{-1} d.w. in the solid phase) or were below the method detection limit and therefore have higher analytical uncertainty (*Table* B3 and *Table* B4). Thus, the results for these compounds should be interpreted with caution.

No significant influence of anaerobic degradation on the removal of PFASs could be determined. When comparing the mesophilic and thermophilic treatments, it can be seen that more PFASs showed removal as well as slightly higher removal rates in the thermophilic treatment. It is possible that different microbial communities in the treatments affected the degradation of PFASs. Or possibly temperature might have had an effect, since the thermophilic treatment was conducted 15 °C warmer than the mesophilic treatment. PFASs, however, are extremely persistent and more investigations are needed.

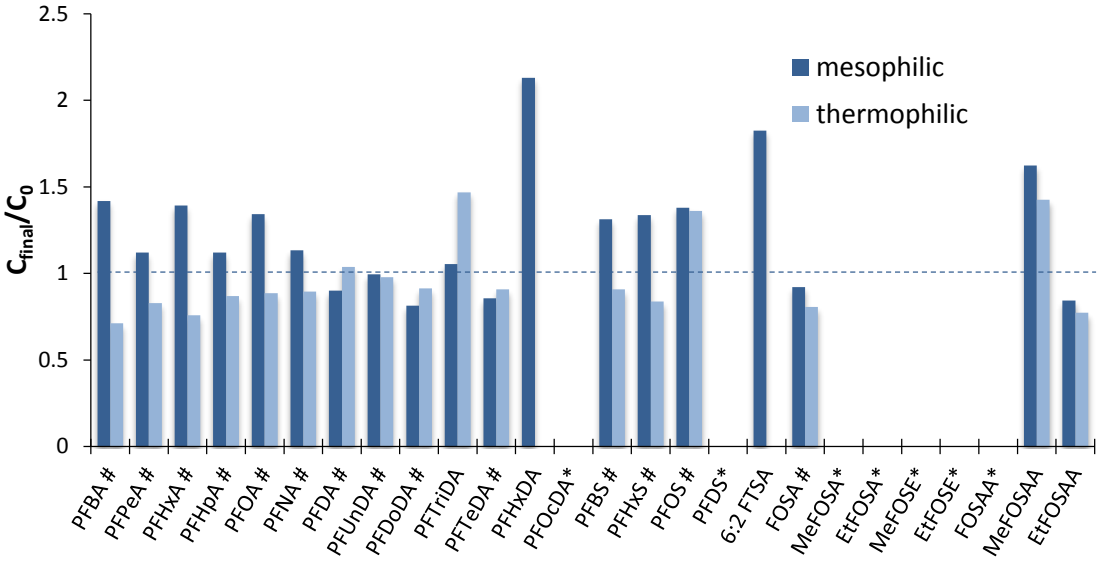

Figure 16. Removal of PFASs in latrine after mesophilic (37 °C, 61 days) and thermophilic (52 °C, 59 days) of anaerobic treatment. Values above 1 indicate an increase and below 1 indicate a removal of PFASs during the treatment (# = spiked PFASs, * = not detected). The change of PFASs concentration during treatment was calculated as C_{61d}/C_0, for mesophilic, and as C_{59d}/C_0, for thermophilic, the concentration were the sum of the liquid and solid phase in ng. "C" is expressed in ng and is the sum between the amounts detected in the solid and liquid phase.

Regarding the blackwater treatment, 73 % of the compounds were not detected (*Figure* 17). The seven PFASs detected (PFHxA, PFOA, PFHxS, PFOS, FOSA, MeFOSAA and EtFOSAA), however, showed an average removal rate of 96 %. It should be stressed that the values display only the liquid phase. Moreover the results should be interpreted with caution, since the measured values are very low indicating a high analytical uncertainty. No comparable values were found in literature. Therefore no concrete statement should be made for the removal efficiencies of PFASs during blackwater treatment. In conclusion, the results lead to the assumption that blackwater is no major source of PFASs and therefore do not represent a major threat to the environment.

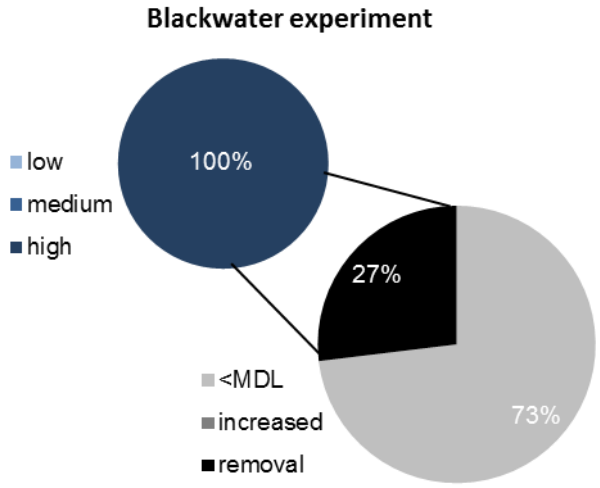

Figure 17. Removal overview after blackwater treatment (19 days) in percent. The bigger pie chart shows the percentages of PFASs below the method detection limit (< MDL in light grey), of PFASs where the concentration after the treatment has increased (dark grey) and of the PFASs that showed removal (black). The smaller pie chart

presents the degree of removal; low removal (0-30 %, light grey), medium (30-70 %, grey) and high removal rates (70-100 %, black).

4.2.2 Temporal Changes of PFASs during Treatments

No large variation at the different days of treatment could be seen during the spiked mesophilic anaerobic degradation (*Figure* 18). Some compounds have increased concentrations towards the end of treatment, indicating that biodegradation probably caused formation of the precursor compounds. Consequently PFASs were not consistently removed along the treatment. The thermophilic experiment disclosed at the beginning of treatment increased concentrations for most compounds (day 7), dropped concentrations at day 21 and mostly consistent concentrations towards the end of treatment. A possible reasons for the peak at the beginning of treatment could be that the anaerobic degradation might have been at an early stage, indicating that the different stages of the anaerobic degradation process might influence the degradation or capacity of sorption of PFASs in the latrine.

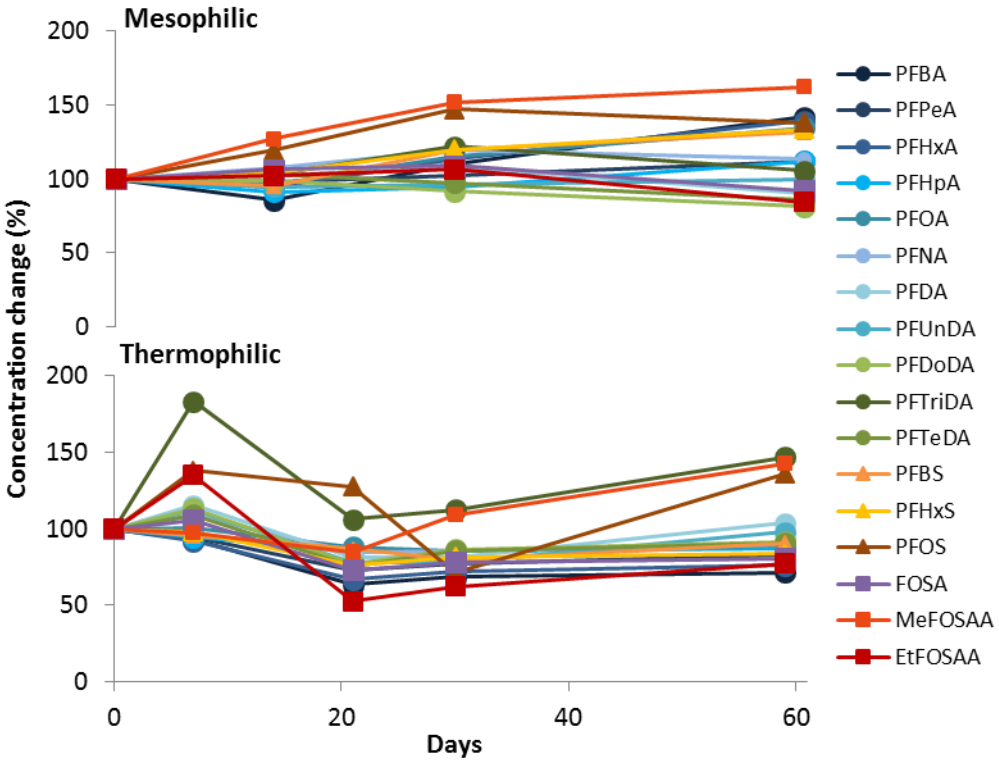

Figure 18. Changes of individual PFASs over time in latrine during anaerobic mesophilic and thermophilic treatment. Values above 100 % indicate an increase and below indicate a removal of PFASs during the treatment.

4.2.3 Sorption Behavior of PFASs between Liquid and Solid Phase

The distribution between the liquid and solid phase and the removal of each PFAS in percentage can be seen in *Figure* 19. The mesophilic and thermophilic treatments demonstrate same distributions pattern. It is clear to see that the longer the perfluorocarbon chain of PFCAs and PFSAs the more they tend to sorb onto the solid phase. The PFCAs that mainly tend to distribute in the liquid phase were PFBA, PFPeA, PFHxA, PFHpA, PFOA with perfluorocarbon chains

shorter than C_8. The same was observed for the PFSAs, PFBS and PFHxS with a short chain length ($<C_7$) occurred in the liquid phase. On the other hand, exclusively found in the solid phase were long-chained PFASs (PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxDA, MeFOSAA and EtFOSAA). The occurrence of PFDoA in the solid phase is supported by Arvaniti and Stasinakis (2015). This distribution pattern whereby short-chain PFSAs and PFCAs occur mostly in the liquid phase and long-chained ones tend to partition onto particles was previously stated by Ahrens and Bundschuh (2014). The results point out that the analysis of both phases is of importance in order to avoid underestimations.

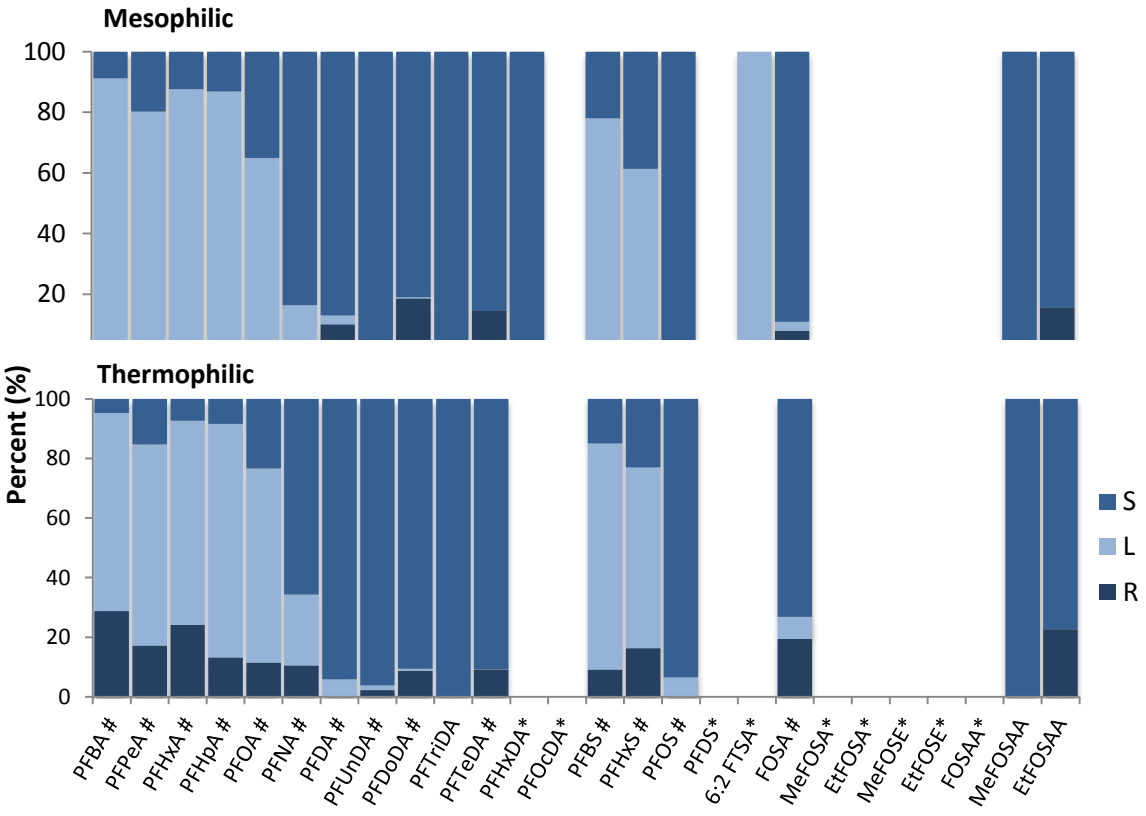

Figure 19. Distribution of PFASs in the solid phase (blue), liquid phase (light blue) or removed (dark blue) in latrine spiked (# = spiked compounds) after mesophilic treatment (37 °C, 61 days, upper graph) and in latrine spiked (# = spiked compounds) after thermophilic treatment (52 °C, 59 days, upper graph)in percent (* = not detected).

The target PFASs are characterized by relatively high values of octanol-water partition coefficient K_{ow}, ranging between 2.82 and 8.90 (log K_{ow}) and organic carbon-water partition coefficient K_{oc}, 1.91–3.53 (log K_{oc}) compared to the values from the PhACs (see *Table* 2). The long-chained PFCAs and PFSAs have higher values than the short-chained ones. Additionally the acid dissociation constant pK_a is low for PFCAs and PFSAs, which makes them strong acids compared to FOSAs and FOSEs which have higher pK_a values. Since PFCAs and PFSAs have extreme pK_a values (-0.22 to 0.14, see *Table* 2), they exists as anions at pH 8 in anaerobic degradation. Sludge in general is positively charged and the lower the pH, the more cations could react with the anions (high ionic interactions), thus higher the sorption onto the solid phase (Arvaniti et al., 2014). But since the pH was more or less consistent and neutral during the

anaerobic degradation low ionic interactions can be assumed. Regarding the solid-water distribution coefficient (K_d) high values were determined for long-chained PFCAs (PFNA, PFDA, PFUnDA, PDoDA and PFTeDA) as well as for PFOS and FOSA, in the range of 2.3 and 4.9 log K_d (*Figure* 20). Those high values indicate that the compounds highly sorbed onto solids (*Figure 12*, *Figure 13* and *Figure 14*). In general, K_d values ranged from 3.8 to 86,000 L kg^{-1} (log 0.58 to 4.9). Sorption behaviors of conventional primary sludge were reported by Arvaniti and Stasinakis (2015), were K_d values ranged from 330 to 6,015 L kg^{-1}. Another study analyzed secondary anaerobically digested sludge and determined values between 162 and 11,770 L kg^{-1} (Arvaniti et al., 2014). Subsequently higher K_d values were determined in the latrine of the study and secondary sludge in Arvaniti's study, both treated with anaerobic degradation. Possible explanations for deviations of values between the studies could be different experimental designs (batch vs. full-scale experiments), different chemical characteristics of sludge used (liquid/solid content, organic carbon, cations) and different analytical parameters of methods used (Arvaniti et al., 2014).

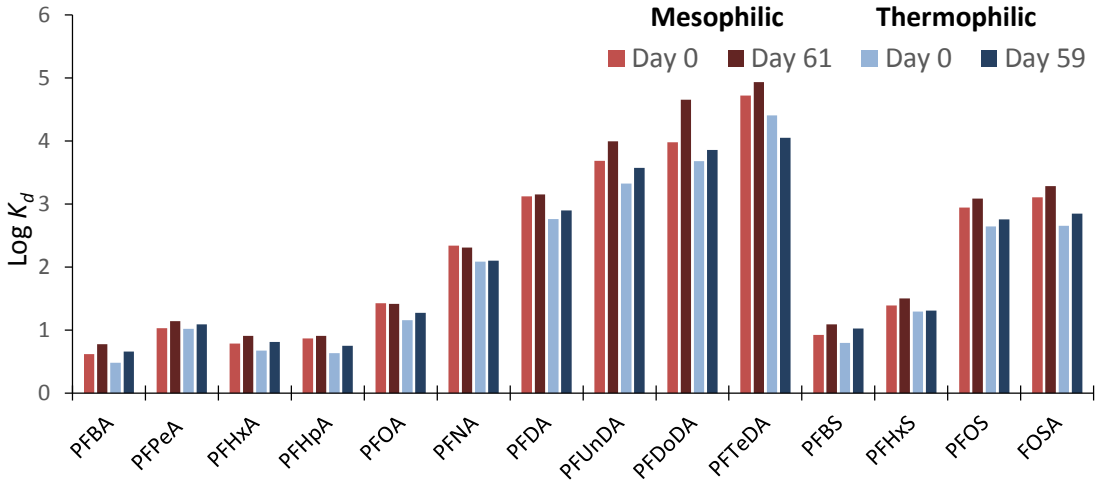

Figure 20. Sorption behavior of PFASs during mesophilic and thermophilic treatment at initial (day 0) and final time (day 61 and 59) expressed in logarithmic K_d values.

A more detailed comparison of calculated and literature K_d values for PFOS, PFOA and PFDA can be seen in *Table* 9. Values show similar correspondence. PFOS and PFDA, both have long chains, were mostly adsorbed to the solid phase and therefore high K_d values were determined as it can be confirmed by other studies (Arvaniti et al., 2012, Arvaniti et al., 2014, Yu et al., 2009).

One exception are lower values (103-209 L kg^{-1}) for PFOS from the study conducted by Ochoa-Herrera and Sierra-Alvarez (2008) of anaerobic granular sludge, which indicate low sorption. Differences in sludge and experimental setup could be reasons, e.g. the study's treatment was conducted at a pH of 7.2 and 30 °C. They also concluded that the type of sludge strongly influences the sorption behavior of PFOS.

Values determined for PFOA are all low, indicating a low tendency to occur in the solid phase, K_d values determined in this study were lowest. Several studies reported also different bioaccumulation potentials between PFOS and PFOA (Giesy and Kannan, 2001, Gebbink et al., 2011). PFOS has a high and PFOA a low potential, which might be explained by the different

functional group (carboxylate) and shorter chain length (C_7) (Ahrens and Bundschuh, 2014, Martin et al., 2003).

Table 9. Comparison of calculated K_d values and their SD for PFOS, PFOA and PFDA during mesophilic and thermophilic treatment (day 0 and 61, 0 and 59) and K_d values of primary and digested sludge's found in literature (in L kg^{-1}).

	Calculated K_d (L kg^{-1}) ± SD				Literature K_d (L kg^{-1}) ± SD	
	Mesophilic		**Thermophilic**		**Primary sludge**	**Digested sludge**
Compound	Day 0	Day 61	Day 0	Day 59		
PFOS	880±160	1,200±140	420±41	5,700±130	894-2237[a]	77-277[d]
					398-948[b]	4,908±1035[c]
					1,289±229[c]	
PFOA	27±4.0	26±2.1	15±1.2	19±2.5	188-597[a]	162±42[c]
					212-2657[b]	
					330±220[c]	
PFDA	1,300±58	1,400±150	580±17	790±110	6,795-18,398[b]	2,589±787[c]
					1601±477[c]	

[a](Yu et al., 2009), [b](Arvaniti et al., 2012), [c](Arvaniti et al., 2014), [d](Ochoa-Herrera and Sierra-Alvarez, 2008)

4.2.4 Summary of Latrine Anaerobic treatment and Blackwater Treatment of PFASs

Low environmental (non-piked) concentrations were found in latrine. Besides spiked compounds five PFASs were detected in latrine with rather low concentrations (up to 19 ng L^{-1} in the liquid and 11 ng g^{-1} d.w in the solid phase). Since they were not detected in the 'untreated latrine' it can be assumed that they were present in the inoculum.

23 % and 50 % of all PFASs in the mesophilic and thermophilic treatment had decreased concentrations with removal rates between 0.5 % (PFUnDA) to 28 % (PFBA). Some compounds showed increased concentrations, indicating potential transformation of precursors compounds. When comparing the mesophilic and thermophilic treatments, it can be seen that more PFASs showed removal as well as slightly higher removal rates in the thermophilic treatment. Further research is needed to determined if temperature might have had an effect and/or the different microbial communities of the mesophilic (37 °C) and thermophilic (52 °C) treatment. In general no significant influence of anaerobic degradation on the removal of PFASs could be determined.

On the topic of the distribution of PFASs between the liquid and solid phase of the treated fraction in latrine a clear trend could be seen, short-chain PFSAs and PFCAs occured mostly in the liquid phase and long-chained ones tend to partition onto solids.

Regarding the blackwater treatment, 73 % of the PFAS's were not detected and the ones detected had very low concentrations. Therefore no conclusions could be made about the removal efficiency of blackwater sanitation. It can be assumed, however, that PFASs occur not significantly in latrine and blackwater and therefore PFASs in thoses source separated wastewaters are no major threat to the environment.

5 Conclusion

The overall aim of this study was to determine the fate and the removal efficiency of 29 PhACs and 26 PFASs during two source separated wastewater treatments: *i)* anaerobic degradation of latrine and *ii)* blackwater treatment. Regarding the fate, high concentrations in latrine and blackwater have been found for many PhACs during the treatment and after. Those were higher than concentrations found in conventional WWTP effluents, due to lower dilution. Low environmental concentrations were found for PFASs in latrine and blackwater, greatly lower than concentrations found in conventional wastewaters, indicating that PFASs in conventional wastewater are probably not introduced by latrine or blackwater. It can be assumed that latrine and blackwater are no major source of PFASs and therefore do not represent a major threat to the environment.

The following assumption for the removal efficiency could be drawn:

1) The removal of target PhACs and PFASs in **latrine** during anaerobic degradation is not efficient. The average removal rates for all detected PhACs determined were 45 % under mesophilic and 31 % under thermophilic conditions and for PFASs even lower with -24 % (negative possibly due to degradation of PFAS precursors) and 4 %, respectively.

2) Regarding the removal efficiency of selected PhACs and PFASs during **blackwater** treatment using aerobic wet composting combined with urea treatment, a higher removal rate was determined for PhACs, 49 %, but still a significant amount remains in the treated fractions. Thus the treatment is not efficient. No conclusions could be made about the fate of PFASs in blackwater.

3) When comparing the performance of the two source separated treatments it can be concluded that both treatments revealed moderate to low removal rates for PhACs and PFASs.

The occurrence of PFASs in latrine and blackwater, however, is low. Therefore their removal might not have to be considered in the source separated treatments. Regarding the PhACs additional advanced treatments might be necessary or efforts to find a better suitable treatment technique need to be made, because the treated end-product of blackwater is reused as fertilizer in agricultural fields. Further research is needed for example to determine the fate of the PhACs in the treated end-product when applied on farmland, their distribution pathways in the environment and their risk for humans and ecosystems.

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8 Appendix

8.1 Appendix A: Instrumental analysis of PhACs

Pharmaceuticals were analyzed using an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, USA) coupled to a quadrupole-time-of-flight (QTOF) mass spectrometer (QTOF Xevo G2S, Waters Corporation, Manchester, UK). Extracts were injected twice, since some compounds were analyzed under positive electrospray ionization (PI) and the others under negative electrospray ionization (NI). Chromatographic separation took place using an Acquity HSS T3 column (100 mm x 2.1 mm i.d., 1.8 µm particle size) for the compounds analyzed by PI and an Acquity BEH C18 column (50 mm × 2.1 mm i.d., 1.7 m particle size) for the substances determined under NI. Both columns were purchased from Waters Corporation. The mobile phases used in PI mode were A) 5 mM ammonium formate buffer with 0.01 % formic acid and B) acetonitrile with 0.01 % formic acid and in NI mode they were A) 5 mM ammonium acetate buffer with 0.01 % ammonia and B) acetonitrile with 0.01 % ammonia. The chromatographic flow rate used was 0.5 mL min^{-1}, the total run time was 21 min in both positive and negative electrospray ionization and the injection volume was 5 µL. The column temperature was set at 40 °C and the sample manager temperature at 15 °C. The resolution of the TOF mass spectrometer was around 30000 at full width and half maximum (FWHM) at m/z 556. MS data were acquired over a m/z range of 100–1200 in a scan time of 0.25 s. Capillary voltages of 0.35 and 0.4 kV were used in positive and negative ionization modes, respectively. A cone voltage of 30 V was applied, the desolvation gas flow rate was set at 700 L h^{-1} and the cone gas flow was set to 25 L h^{-1}. The desolvation temperature was set to 450 °C and the source temperature to 120 °C. Samples were acquired with MS^E experiments in the resolution mode. In this type of experiments, two acquisition functions with different collision energies were created: the low energy (LE) function with a collision energy of 4 eV, and the high energy (HE) function with a collision energy ramp ranging from 10 to 45 eV. Calibration of the mass-axis from m/z 100 to 1200 was conducted daily with a 0.5 mM sodium formate solution prepared in 90:10 (v/v) 2-propranolol/water. For automated accurate mass measurement, the lock-spray probe was employed, using a lock mass leucine encephalin solution (2 mg mL^{-1}) in ACN/water (50/50) with 0.1 % formic acid. The solution was pumped at 10 µL min^{-1} through the lock-spray needle. The leucine encephalin $[M+H]^+$ ion (m/z 556.2766) and its fragment ion (m/z 278.1135) for positive ionization mode, and $[M-H]^-$ ion (m/z 554.2620) and its fragment ion (m/z 236.1041) for negative ionization, were used for recalibrating the mass axis and to ensure a robust accurate mass measurement over time. MS data were determined in a m/z range of 100-1200 in a scan time of 0.25 s.

The data were evaluated using the operating software UNIFI™ (Waters Corporation). For identification of the target pharmaceuticals the following criteria were used: a) the accurate mass measurements of the precursor ion ($[M+H]^+$ for PI mode and $[M-H]^-$ for NI mode) in the LE function, with an error below 5 ppm, b) the presence of at least one characteristic m/z ion in the HE function and the exact mass of these fragment ions and c) the UHPLC retention time of the compound compared to that of a standard (±2 %).

8.2 Appendix B: Overviews of Concentrations

Table B1. Initial concentrations and standard deviations (SD) for all PhACs in the liquid phase in ng L^{-1} and in the solid phase in ng g^{-1} d.w.. The concentrations are given for 'untreated latrine', inoculum under mesophilic and thermophilic conditions and concentrations before treatment (day 0). Concentrations are displayed as averages from duplicates A and B (n.d. = not detected, < MQL = below the method quantification limit).

	Liquid phase (ng L^{-1}) ± SD					Solid phase (ng g^{-1} dw)) ± SD				
Compound	Only latrine	Inoculum mesophilic	Day 0 mesophilic	Inoculum thermophilic	Day 0 thermophilic	Only latrine	Inoculum mesophilic	Day 0 mesophilic	C Inoculum thermophilic	Day 0 thermophilic
Analgesics										
Codeine	n.d.	n.d.	n.d.	n.d.	n.d.	140±35	n.d.	n.d.	<MQL	<MQL
β-blockers										
Atenolol	1,700±100	n.d.	n.d.	n.d.	3,100±300	2,400±102	n.d.	220±37	n.d.	310±45
Sotalol	n.d.	n.d.	<MQL	293	290±21	130±27	n.d.	<MQL	<MQL	<MQL
Metoprolol	48,000±2,600	290±36	1,600±160	1,200±48	2,900	1,300±50	190±2.1	600±15	410±0.08	710±73
Propranolol	730±90	280±70	350±73	160±9.02	470±39	350±92	16±8.7	84±5	25±15	210±95
Antibiotics										
Azithromycin	n.d.	<MQL	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	n.d.	n.d.	n.d.	880±8.4	1,100±110	n.d.	n.d.	n.d.	n.d.	n.d.
Ciprofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trimethoprim	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antidepressants										
Carbamazepine	16,000±2,800	1,400±58	5,400±240	5,600±460	8,700±370	1,500±170	60±2.6	250±0.5	270±29	500±52
Citalopram	n.d.	n.d.	n.d.	150	220	300±74	510±130	730±20	300±160	420±6.8
Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lamotrigine	1,600±270	3,200±440	3,800±180	1,600±92	1,500±160	430±74	<MQL	98±12	75±2.5	140±41
Oxazepam	n.d.	330±0.083	n.d.	n.d.	n.d.	380±130	n.d.	92±17	n.d.	n.d.
Venlafaxine	12,000±3,600	<MQL	<MQL	<MQL	570±8.9	630±69	110±0.95	210±2.4	190±6	250±1.7
Amitryptiline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<MQL	<MQL	<MQL	52±16
Antihypertensives										
Losartan	32,000±4,500	5,700±130	34,000±2,000	7,600±290	15,000±450	7,400±1,800	<MQL	1,300±39	n.d.	310±42
Valsartan	180,000±92,000	2,000±500	36,000±1,500	9,300±460	19,000±4,000	<MQL	n.d.	n.d.	n.d.	n.d.
Irbesartan	n.d.	<MQL	480±61	n.d.	2,600±85	1,200±300	n.d.	150±1.1	n.d.	n.d.
Diltiazem	n.d.	n.d.	n.d.	n.d.	n.d.	76±12	n.d.	n.d.	n.d.	n.d.
Diuretics										
Furosemide	10,300±1,300	1,400±390	11,000±630	2,100±110	2,200±480	590±61	n.d.	n.d.	n.d.	n.d.
Hydrochlorothiazide	27,000±12,000	n.d.	n.d.	n.d.	350±55	1,100±120	n.d.	98±17	n.d.	170±5.7
Lipid regulator										

Atorvastatin	n.d.	<MQL	520±53	<MQL	1,200±160	n.d.	n.d.	n.d.	n.d.	n.d.
Bezafibrate	n.d.	n.d.	n.d.	220±6	670±210	n.d.	n.d.	n.d.	n.d.	n.d.
Local anesthetic										
Lidocaine	n.d.	330±4.6	580±48	201	370±36	n.d.	n.d.	19±1.01	<MQL	11±1.3

Table B2. Initial concentrations and SDs for all PhACs in blackwater in the liquid phase in ng L^{-1} and in the solid phase in ng g^{-1} d.w.. The concentrations are given for the initial time of treatment (day 0), after wet composting (day 12) and after urea treatment (day 19). Concentrations are displayed as averages from duplicates A and B (n.d. = not detected, < MQL = below the method quantification limit).

	Liquid concentration (ng L^{-1})) ± SD			Solid concentration (ng g^{-1} d.w.)) ± SD		
Compound	Day 0	Day 12	Day 19	Day 0	Day 12	Day 19
Analgesics						
Codeine	1,600±120	n.d.	n.d.	90±28	n.d.	n.d.
β-blockers						
Atenolol	4,800±1,400	n.d.	1,700±280	<MQL	n.d.	<MQL
Sotalol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metoprolol	9,500±1,300	302±7.02	6,100±550	380±23	<MQL	540
Propranolol	4,800±1,400	210±2.5	2,400±220	2,400±240	740	n.d.
Antibiotics						
Azithromycin	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ciprofloxacin	1,000±580	n.d.	580	n.d.	n.d.	n.d.
Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trimethoprim	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antidepressants						
Carbamazepine	3,400	2,100±340	2,400±360	180±1.5	320	302
Citalopram	310±20	600±15	670±3.0	940±38	1,100	550
Diazepam	48±4.0	23±0.71	51±1.8	<MQL	n.d.	<MQL
Lamotrigine	7,300±1,200	3,800±60	5,400±400	340±49	930	550
Oxazepam	4,800±800	6,000±700	2,300±820	1,600±370	1,700	470
Venlafaxine	6,400±1,400	7,500±290	6,700±910	710±83	100	620
Amitryptiline	<MQL	41±6.0	33±2.6	430±56	250	200
Antihypertensives						
Losartan	10,000±270	19,000±4,200	15,000±2,700	680±130	660	450
Valsartan	12,000±450	2,000±6,300	1720±42	n.d.	n.d.	n.d.
Irbesartan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diltiazem	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diuretics						
Furosemide	37,000±7,300	1,700±470	40,000±7,100	190±22	n.d.	n.d.
Hydrochlorothiazide	14,000±4,300	n.d.	1,100±320	510±23	45	91
Lipid regulator						
Atorvastatin	720±50	420±90	390±60	n.d.	n.d.	n.d.
Bezafibrate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Local anesthetic						
Lidocaine	650±30	490±9.01	510±61	13±2.4	42	23

Table B3. Initial concentrations and SDs for all PFASs in the liquid phase (in ng L^{-1}). The concentrations are given for untreated latrine and all sampling days of mesophilic and thermophilic treatment. Concentrations are displayed as averages from duplicates A and B (n.d. = not detected, <MQL = below the method quantification limit).

Liquid (ng L^{-1}) ± SD		Mesophilic				Thermophilic				
Acronym	Only latrine	Day 0	Day 14	Day 30	Day 61	Day 0	Day 7	Day 21	Day 30	Day 59
PFCAs										
PFBA	n.d.	7,800±650	6,400±1,900	8,900±75	1,100±24	9,700±2,800	8,800±490	6,500±350	6,700±2100	7,000±46
PFPeA	380±42	3,200±57	3,000±620	3,300±39	3,800±66	3,400±470	3,100±39	2,800±60	2,700±510	2,900±110
PFHxA	36±1.7	5,800±220	5,300±1,400	6,800±5.5	8,500±57	7,200±1,800	6,500±390	5,100±170	5,300±1,500	5,600±110
PFHpA	7.2±0.36	6,800±22	6,100±1,200	6,600±190	7,900±410	8,000±1,400	7,400±230	7,300±170	7,100±1,200	7,200±800
PFOA	n.d.	4,400±5.7	4,400±530	6,000±180	7,500±640	5,800±540	5,500±210	5,600±250	5,300±730	5,300±850
PFNA	n.d.	1,300±59	1,600±37	2,500±130	3,100±300	2,200±7.7	2,200±130	2,700±4.3	2,500±390	2,300±370
PFDA	n.d.	250±7.6	250±9.3	440±29	510±87	490±5.5	430±39	600±4.3	530±86	470±98
PFUnDA	n.d.	70±1.0	35±4.6	57±2.7	57±6.3	140±4.1	100±12	140±7.8	120±17	100±29
PFDoDA	n.d.	36±0.73	7.9±0.56	9.6±0.68	9.1±1.3	58±1.6	45±0.88	55±1.8	40±1.6	47±14
PFTriDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	<MQL	<MQL	<MQL	2.4±0.801	6.4±1.1	13±0.502	10±1.0	10±1.6	19±0.42
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOcDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs										
PFBS	n.d.	3,600±29	3,200±620	4,200±76	4,600±42	4,200±530	3,700±200	3,600±91	3,500±560	3,700±400
PFHxS	n.d.	4,300±160	4,100±770	5,200±41	6,600±870	5,600±650	5,600±91	4,900±210	5,100±810	5,000±380
PFOS	n.d.	260±20	240±7.1	400±16	490±49	450±31	480±67	690±7.1	600±83	540±140
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSAs										
FOSA	1.8±0.302	220±13	170±2.6	270±0.11	300±38	560±5.4	530±68	650±13	560±96	370±99
N-MeFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-EtFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSEs										
N-MeFOSE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-EtFOSE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSAAs										
FOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-MeFOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-EtFOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FTSAs										
6:2 FTSA	n.d.	10±2.01	5.7±2.6	13±1.8	19±6.91	n.d.	n.d.	n.d.	n.d.	n.d.

Table B4. Initial concentrations and SDs for all PFASs in the solid phase (in ng g^{-1} d.w.).The concentrations are given for untreated latrine and all sampling days of mesophilic and thermophilic treatment. Concentrations are displayed as averages from duplicates A and B (n.d. = not detected, < MQL = below the method quantification limit).

Solid (ng g^{-1} d.w.) ± SD		Mesophilic				Thermophilic				
Acronym	Only latrine	Day 0	Day 14	Day 30	Day 61	Day 0	Day 7	Day 21	Day 30	Day 59
PFCAs										
PFBA	n.d.	32±0.51	38±0.48	42±1.1	39±2.2	29±0.49	34±1.6	33±0.79	37±0.51	32±1.4
PFPeA	n.d.	34±0.203	41±0.85	46±3.4	41±0.86	35±1.5	38±0.31	34±2.1	38±1.2	36±0.62
PFHxA	n.d.	35±0.14	43±1.6	47±2.0	43±0.54	34±2.1	38±0.41	35±1.6	39±1.0	36±0.54
PFHpA	n.d.	49±15	49±6.4	55±2.2	53±3.1	35±2.5	43±1.2	49±13	44±0.7	41±4.7
PFOA	n.d.	120±35	110±14	130±5.0	120±9.0	83±4.9	100±1.8	120±29	110±3.5	98±11
PFNA	n.d.	290±39	330±11	370±4.2	350±13	270±4.1	320±5.7	340±19	340±5.5	290±32
PFDA	n.d.	330±19	360±4.4	410±22	380±18	280±13	360±41	380±3.5	380±2.9	370±1.7
PFUnDA	n.d.	340±25	340±7.1	380±1.7	450±61	290±39	370±0.3	400±13	400±26	380±1.7
PFDoDA	n.d.	340±19	360±71	370±17	370±25	280±30	360±0.03	380±25	420±23	340±13
PFTriDA	n.d.	0.5±0.051	0.5±0.2	0.6±0.22	0.6±0.062	<MQL	0.5±0.034	0.4±0.028	0.5±0.96	0.6±0.055
PFTeDA	n.d.	250±27	280±44	290±16	290±5.1	160±35	210±12	230±19	260±6.6	210±13
PFHxDA	n.d.	n.d.	n.d.	n.d.	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.
PFOcDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs										
PFBS	n.d.	30±7.7	39±0.29	51±10	52±5.5	26±2.1	34±9.7	47±41	24±5.4	39±9.0
PFHxS	n.d.	110±11	130±14	150±16	140±9.7	110±15	100±14	110±19	100±6.4	100±2.1
PFOS	n.d.	230±62	290±0.5	390±72	400±52	200±26	270±78	370±320	190±39	310±57
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSAs										
FOSA	n.d.	284±5.0	320±27	360±14	340±23	250±8.6	290±6.8	300±15	320±0.16	260±18
N-MeFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-EtFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSEs										
N-MeFOSE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-EtFOSE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FOSAAs										
FOSAA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
N-MeFOSAA	n.d.	2.0±0.057	2.7±0.11	3.6±0.74	4.3±0.59	0.6±0.14	0.8±0.0041	1.0±0.045	1.3±0.025	1.3±0.38
N-EtFOSAA	n.d.	7.2±0.26	8.4±0.083	11±0.95	11±0.56	2.6±0.049	2.9±0.41	2.8±0.22	3.2±0.29	2.8±0.76
FTSAs										
6:2 FTSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table B5. Initial concentrations and SDs for all PFASs in blackwater in the liquid phase in ng L^{-1} and in the solid phase in ng g^{-1} d.w.. The concentrations are given for the initial time of treatment (day 0), after wet composting (day 12) and after urea treatment (day 19) for the liquid phase and initial time of treatment (day 0) for the solid phase. Concentrations are displayed as averages from duplicates A and B (n.d. = not detected, < MQL = below the method quantification limit).

	Liquid concentration (ng L^{-1}) ± SD			Solid concentration (ng g^{-1} dw) ± SD
Acronym	Day 0	Day 12	Day 19	Day 0
PFCAs				
PFBA	n.d.	n.d.	n.d.	n.d.
PFPeA	n.d.	n.d.	n.d.	n.d.
PFHxA	n.d.	n.d.	<MQL	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.
PFOA	n.d.	n.d.	4.3±0.37	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.
PFTriDA	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.
PFOcDA	n.d.	n.d.	n.d.	n.d.
PFSAs				
PFBS	n.d.	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	6±1.3	n.d.
PFOS	n.d.	n.d.	<MQL.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.
FOSAs				
FOSA	n.d.	n.d.	2.6±0.34	n.d.
N-MeFOSA	n.d.	n.d.	n.d.	n.d.
N-EtFOSA	n.d.	n.d.	n.d.	n.d.
FOSEs				
N-MeFOSE	n.d.	n.d.	n.d.	n.d.
N-EtFOSE	n.d.	n.d.	n.d.	n.d.
FOSAAs				
FOSAA	n.d.	n.d.	n.d.	n.d.
N-MeFOSAA	n.d.	n.d.	n.d.	0.4
N-EtFOSAA	n.d.	n.d.	n.d.	1.1
FTSAs				
6:2 FTSA	n.d.	n.d.	n.d.	n.d.

8.3 Appendix C: MDLs and MQLs

Table C1. Determined values of the method detection limits (MDL) and the method quantification limits (MQL) of each PhACs in the liquid (ng L^{-1}) and solid phase (ng g^{-1} d.w.) for latrine and blackwater as well as for untreated latrine (n.d. = not detected).

	Latrine and Blackwater				Untreated Latrine			
	Liquid (ng L^{-1})		Solid (ng g^{-1} d.w.)		Liquid (ng L^{-1})		Solid (ng g^{-1} d.w.)	
Compound	MDL	MQL	MDL	MQL	MDL	MQL	MDL	MQL
Analgesics								
Codeine	22	75	20	40	67	222	11	40
β-blockers								
Atenolol	69	227	20	80	68	227	27	91
Sotalol	29	100	15	60	150	600	15	60
Metoprolol	4	12	5	20	2	8	6	20
Propranolol	4	13	2.5	10	5	17	2.5	10
Antibiotics								
Azithromycin	69	230	170	560	72	240	167	560
Clarithromycin	6	21	20	40	6	21	20	40
Ciprofloxacin	118	390	n.d.	n.d.	120	400	n.d.	n.d.
Sulfamethoxazole	115	380	10	40	93	310	10	40
Trimethoprim	48	160	29	96	73	240	15	80
Antidepressants								
Carbamazepine	9	29	20	54	14	50	16	54
Citalopram	13	43	23	77	29	97	23	77
Diazepam	2	6	14	47	9	30	14	47
Lamotrigine	2	4	9	34	6	21	9	34
Oxazepam	111	370	20	71	110	360	21	71
Venlafaxine	61	204	5	20	42	140	5	20
Amitryptiline	8	25	5	20	7	22	5	20
Antihypertensives								
Losartan	30	95	44	145	30	95	44	145
Valsartan	100	331	100	500	99	331	100	500
Irbesartan	75	250	20	80	86	290	20	80
Diltiazem	4	13	15	50	13	44	15	50
Diuretics								
Furosemide	5	16	25	100	5	16	25	100
Hydrochlorothiazide	15	50	10	40	15	50	10	40
Lipid regulator								
Atorvastatin	20	62	n.d.	n.d.	160	520	n.d.	n.d.
Bezafibrate	30	100	10	35	34	114	10	35
Local anesthetic								
Lidocaine	25	83	3	6	11	40	2	6

Table C2. Determined values of the method detection limits (MDL) and the method quantification limits (MQL) of each PFAS in the liquid (ng L^{-1}) and solid phase (ng g^{-1} d.w.).

	Liquid (ng L^{-1})		Solid (ng g^{-1} d.w.)	
Acronym	MDL	MQL	MDL	MQL
PFCAs				
PFBA	0.9	2.8	1.9	6.5
PFPeA	1.0	3.3	3.9	13.1
PFHxA	1.0	3.3	1.6	5.4
PFHpA	1.0	3.3	1.6	5.5
PFOA	1.0	3.3	2.6	8.8
PFNA	1.0	3.3	4.1	13.8
PFDA	1.0	3.3	3.5	11.6
PFUnDA	1.0	3.3	4.1	13.7
PFDoDA	1.0	3.3	2.6	8.6
PFTriDA	1.0	3.3	0.1	0.3
PFTeDA	1.0	3.3	0.7	2.3
PFHxDA	1.0	3.3	0.1	0.3
PFOcDA	1.0	3.3	0.1	0.3
PFSAs				
PFBS	0.3	1.0	5.1	16.8
PFHxS	1.0	3.3	5.6	18.5
PFOS	1.0	3.3	4.6	15.3
PFDS	1.0	3.3	0.1	0.3
FOSAs				
FOSA	0.4	1.4	1.3	4.4
N-MeFOSA	1.0	3.3	0.1	0.3
N-EtFOSA	1.0	3.3	0.1	0.5
FOSEs				
N-MeFOSE	1.0	3.3	0.1	0.3
N-EtFOSE	1.0	3.3	0.1	0.3
FOSAAs				
FOSAA	0.2	0.7	0.6	1.9
N-MeFOSAA	1.0	3.3	0.1	0.3
N-EtFOSAA	1.0	3.3	0.1	0.3
FTSAs				
6:2 FTSA	1.0	3.3	0.1	0.3

8.4 Appendix D: Recoveries

Table D1. Relative PhAC recoveries (n=3) calculated with the internal standard in the liquid phase of blackwater and latrine and in the solid phase. The recoveries and their standard deviation (SD) are displayed in percent (n.a.= not available).

	Liquid	Liquid	Solid
Compound	% Rec ± SD blackwater	% Rec ± SD latrine	% Rec ± SD
Analgesics			
Codeine	120±13	90±2.3	120±23
β-blockers			
Atenolol	75±11	74±17	97±21
Sotalol	84±29.82	92±19	160±29
Metoprolol	110±4.2	84±14	71±0.62
Propranolol	80±8.03	77±7.2	120±6.3
Antibiotics			
Azithromycin	140±16	102±3.7	84±15
Clarithromycin	150±10	66±2.0	50±7.6
Ciprofloxacin	57±16	78±1.0	n.a.
Sulfamethoxazole	93±8.2	76±3.2	120±20
Trimethoprim	103±13	130±1.3	150±7.5
Antidepressants			
Carbamazepine	93±7.5	93±8.3	110±9.9
Citalopram	154±20	71±5.3	84±16
Diazepam	98±19	127±0.03	82±2.2
Lamotrigine	84±11	84±8.3	130±12
Oxazepam	80±6.5	77±3.9	150±8.9
Venlafaxine	97±15	90±5.4	109±7.9
Amitryptiline	82±22	84±28	150±7.8
Antihypertensives			
Losartan	106±1.3	74±1.2	80±20
Valsartan	170±73	50±0.25	60±18
Irbesartan	102±13	140±57	100±8.1
Diltiazem	107±5.9	77± 2.3	120±15
Diuretics			
Furosemide	73±2.5	75±6.0	71±9.0
Hydrochlorothiazide	83±6.9	130±0.56	120±4.4
Lipid regulator			
Atorvastatin	100±4.7	102±33	n.a.
Bezafibrate	88±21	78±1.4	103±23
Local anesthetic			
Lidocaine	83± 8.0	90±6.0	97±4.6

Table D2. PFAS recoveries of internal standards calculated with real samples of the liquid phase (n=28) and the solid phase (n=25). The recoveries and their standard deviation (SD) are displayed in percent.

	Liquid	Solid
Internal standards	% Rec ± SD	% Rec ± SD
13C4-PFBA	34±19	75±10
$^{13}C_2$ PFHxA	49±22	82±13
$^{13}C_4$ PFOS	68±12	91±34
$^{18}O_2$ PFHxS	62±15	84±13
$^{13}C_4$ PFOA	54±21	110±17
$^{13}C_5$ PFNA	71±15	78±11
d_3-N-MeFOSAA	120±12	130±24
$^{13}C_2$ PFDA	76±17	90±14
$^{13}C_8$-FOSA	73±8	83±10
d_5-N-EtFOSAA	130±21	140±30
$^{13}C_2$ PFUnDA	87±10	88±17
$^{13}C_2$ PFDoDA	64±17	80±12
d_3-N-MeFOSA	65±15	103±36
d_7-N-MeFOSE	56±12	79±13
d_9-N-EtFOSE	53±14	76±16
d_5-N-EtFOSA	50±13	80±14

8.5 Appendix E: Solid-Water Distribution Coefficients

Table E1. Calculated K_d values (n= 2) with their SD and logarithmic K_d values (n= 2) for each PhAC for the initial day (0) and final day (61, 59 and 19) of mesophilic and thermophilic degradation and blackwater treatment (n.d. = not detected).

	Mesophilic				**Thermophilic**				**Blackwater**			
	K_d (L kg^{-1}) ± SD		log K_d (L kg^{-1})		K_d (L kg^{-1}) ± SD		log K_d (L kg^{-1})		K_d (L kg^{-1}) ± SD		log K_d (L kg^{-1})	
Compound	Day 0	Day 61	Day 0	Day 61	Day 0	Day 59	Day 0	Day 59	Day 0	Day 19	Day 0	Day 19
Analgesics												
Codeine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	59±11	n.d.	1.8	n.d.
β-blockers												
Atenolol	62±3.8	n.d.	1.8	n.d.	160±12	36	2.2	1.6	6.7±1.5	41±3.5	0.8	1.6
Sotalol	86±1.6	54±27	1.9	1.7	64±29	45	1.8	1.7	n.d.	n.d.	n.d.	n.d.
Metoprolol	180±13	240±22	2.3	2.4	270±17	150±35	2.4	2.2	41±4.0	88±4.0	1.6	1.9
Propranolol	210±18	650±38	2.3	2.8	430±140	460±170	2.6	2.7	500±100	n.d.	2.7	n.d.
Antibiotics												
Sulfamethoxazole	1.1±0.6	n.d.	n.d.	n.d.	0.4	n.d.	-0.4	n.d.	n.d.	n.d.	n.d.	n.d.
Trimethoprim	100±1	n.d.	2.0	n.d.	140	n.d.	2.1	n.d.	n.d.	n.d.	n.d.	n.d.
Antidepressants												
Carbamazepine	51±2.6	40±2.3	1.7	1.6	63±12	31±10	1.8	1.5	55±0.2	130±10	1.7	2.1
Citalopram	6,700±600	n.d.	3.8	n.d.	1200	n.d.	3.1	n.d.	3,100±160	820	3.5	2.9
Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	130±7.1	200±3.4	2.1	2.3
Lamotrigine	29±0.8	26±1.0	1.5	1.4	100±7.2	28±7.2	2.0	1.4	47±7.4	100±3.7	1.7	2.0
Oxazepam	2,300±530	n.d.	3.4	n.d.	n.d.	n.d.	n.d.	n.d.	320±66	200±36	2.5	2.3
Venlafaxine	450±3.0	1,300±170	2.7	3.1	400±37	550±74	2.6	2.7	110±19	92±6.2	2.0	2.0
Amitryptiline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16,000±1,900	5,900±230	4.2	3.8
Antihypertensives												
Losartan	110±26	39±4.7	2.0	1.6	40±1.5	13±3.4	1.6	1.1	68±7.6	30±2.7	1.8	1.5
Irbesartan	74±18	n.d.	1.9	n.d.	66	n.d.	1.8	n.d.	n.d.	n.d.	n.d.	n.d.
Diuretics												
Furosemide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.1±0.8	n.d.	0.7	n.d.
Hydrochlorothiazide	21±1.3	n.d.	1.3	n.d.	100±59	n.d.	2.0	n.d.	35.6±6.1	80±11	1.6	1.9
Local anesthetic												
Lidocaine	35±0.9	n.d.	1.5	n.d.	26±2.9	29±4.8	1.4	1.5	20±2.3	44±2.6	1.3	1.6

Azithromycin, clarithromycin, norfloxacin, ciprofloxacin, fluoxetine, diltiazem, atorvastatin, bezafibrate, and ranitidine were not detected

Table E2. Calculated K_d values (n= 2) with their SD and logarithmic K_d values (n= 2) for each PFAS for the initial day (0) and final day (61, 59 and 19) of mesophilic and thermophilic degradation and blackwater treatment (n.d. = not detected).

	Mesophilic				**Thermophilic**			
	Kd (L kg^{-1}) ± SD		log Kd		Kd (L kg^{-1}) ± SD		log Kd	
Compound	Day 0	Day 61	Day0	Day 61	Day 0	Day 59	Day 0	Day 59
PFCAs								
PFBA	4.1±0.21	6.0±0.17	0.62	0.78	3.8±0.73	4.5±0.11	0.58	0.66
PFPeA	11±0.13	14±0.27	1.0	1.1	12±1.2	12±0.34	1.1	1.1
PFHxA	6.1±0.12	8.0±0.078	0.78	0.90	5.7±1.1	6.4±0.11	0.76	0.81
PFHpA	7.3±1.1	8.1±0.44	0.86	0.91	4.9±0.66	5.6±0.64	0.69	0.75
PFOA	27±4.0	26±2.1	1.4	1.4	15±1.2	19±2.5	1.2	1.3
PFNA	220±19	200±14	2.3	2.3	120±1.1	130±17	2.1	2.1
PFDA	1,300±58	1,400±150	3.1	3.2	580±17	790±110	2.8	2.9
PFUnDA	4,800±210	9,900±1,200	3.7	4.0	2,200±180	3,800±550	3.3	3.6
PFDoDA	9,500±370	4,500±4,700	4.0	4.7	4,900±330	7,200±1,200	3.7	3.9
PFTeDA	52,000±5,700	86,000±1,500	4.7	4.9	23,000±4,200	11,000±490	4.4	4.1
PFSAs								
PFBS	8.4±1.1	12±0.72	0.92	1.1	7.0±0.76	11±1.8	0.84	1.0
PFHxS	25±1.8	32±3.2	1.4	1.5	22±2.8	20±0.99	1.3	1.3
PFOS	880±160	1,200±140	2.9	3.1	420±41	5,700±130	2.6	2.8
FOSAs								
FOSA	1,300±49	1,900±180	3.1	3.3	450±9.9	700±120	2.7	2.9