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JTI – Institutet för jordbruks- och miljöteknik

Läkemedel i källsorterat klosettvatten och latrin

– behandling och risker

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ingår i SP-koncernen

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Pharmaceuticals in blackwater and fecal sludge

– Treatments and risks

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Preface

This project was funded by the Swedish Agency for Marine and Water Management (grant 1:12 Arrangements for marine and water environment), Stockholm County Council environmental grant, the Federation of Swedish Farmers (LRF) and Telge Nät. It was a colaboration project between JTI, SLU and SP Process Development (SPPD) with David Eveborn and Lotta Levén from JTI as project managers. Other participants in the project were Emelie Ljung from JTI; Meritxell Gros Calvo, Lutz Ahrens and Karin Wiberg from the Department of Aquatic Sciences and Assessment (SLU); Sahar Dalahmeh and Håkan Jönsson from the Department of Energy and Technology (SLU) and Göran Lundin (SPPD). The development of analytical methods and the analysis were perfomed by Meritxell Gros Calvo and Göran Lundin. The risk analysis was made by Sahar Dalahmeh.

Within this project, pharmaceuticals in source separated, treated and recycled toilet waste (fecal sludge and blackwater) were measured before and after treatment. The treatment efficiency and the risks for humans and the environment were then assessed using these data. Facilities used in the project included Salmunge waste plant, Norrtälje municipality and the blackwater hygienization plant at Nackunga gård, in Hölö managed by Telge Nät. Special thanks to Jan-Christer Carlsson, at Nackunga gård, Hölö, who took part in the sampling of the blackwater. We would also like to acknowledge the master students Ingela Filipsson and Alina Koch, who made their mater theses within this project. Valuable aspects for future research and development directions were provided from stakeholders at a workshop arranged by the project consortium.

Uppsala in May 2016

Anders Hartman
Executive Director at JTI – Swedish Institute of Agricultural and Environmental Engineering

Sammanfattning

Det finns växtnäringsämnen i källsorterat toalettavfall, till exempel klosettvatten och latrin från torrtoaletter. Om dessa fraktioner behandlas genom stabilisering och hygienisering och används som gödningsmedel på åkermark bidrar det till att kretsloppet av växtnäring sluts. För den fortsatta utvecklingen av de källsorterande avloppssystemen är det viktigt att kartlägga vilka mängder av läkemedel som skulle spridas i jordbruket vid användning av källsorterade gödselmedel från avlopp.

Syftet med studien var att undersöka läkemedelsrester i klosettvatten och latrin, före och efter behandling och lagring, och beräkna vilka läkemedelsmängder som skulle spridas i jordbruket genom dessa avfallsfraktioner jämfört med dagens användning av avloppsslam. För att även få en uppfattning om eventuella risker förknippade med spridning av dessa avfallsfraktioner simulerades upptag av läkemedel i olika grödor, ackumulering i mark och läckage till mark och vatten.

Före behandling hade latrin och klosettvatten upp till hundra gånger högre koncentration av läkemedel än det avloppsvatten som kommer in till kommunala reningsverk. När klosettvatten behandlades med våtkompostering, i närvaro av syre, och därefter med ammoniak minskade läkemedelsresterna mer än vid syrefri rötning av latrin eller vid behandling i reningsverk. Minskningen varierade kraftigt mellan olika läkemedelssubstanser. Trots att läkemedelshalterna reducerades väsentligt i det våtkomposterade och ammoniakbehandlade klosettvattnet innehöll det fortfarande upp till tjugo gånger högre halter av vissa substanser än slam från reningsverk. Jordbruksstrategin vid gödsling med klosettvatten (ett kvävegödselmedel) skiljer sig från den som används för slam (ett fosforgödselmedel), vilket resulterar i att läkemedelsdosen vid spridning av klosettvatten blir likvärdig med dosen vid gödsling med slam från reningsverk.

Projektets modellberäkningar tyder på att huvuddelen av läkemedlen till allra största delen bryts ner inom ett år. Det förekommer bara en viss ackumulering av läkemedel i jorden, och det sker försumbara upptag i vete och morötter. Det beräknade dagliga intaget av läkemedel genom förtäring av vete och morot som gödslats med klosettvatten var mycket lågt. För att nå en totalmängd motsvarande den lägsta dagliga dos som förskrivs medicinskt av läkemedlet losartan skulle en vuxen behöva äta gödslat vete eller gödslade morötter i minst 21 000 år. Enligt simuleringarna är således exponering via intag av grödor odlade på en klosettvattengödslad åker försumbar.

Källsorterande och näringsåterförande avloppslösningar har möjlighet att avsevärt förbättra återvinningen av näringsämnen i samhället. Behandlingstekniken för läkemedelsreduktion behöver dock förbättras liksom kunskapen om vad som händer i miljön med fokus på upptag i växter, nedbrytning, transport och spridning.

Fördjupad sammanfattning

Introduktion

Svårnedbrytbara organiska föroreningar, inklusive läkemedelsrester, som på olika sätt når miljön utgör en potentiell risk för människa och miljö. Läkemedel som vi konsumerar passerar kroppen och når i de flesta fall ett kommunalt avloppsreningsverk. I avloppsreningsverken reduceras ett fåtal substanser helt, medan de flesta endast reduceras delvis. Större delen av de substanser som inte reduceras hamnar i den akvatiska miljön, där de riskerar att störa ekosystemen på olika sätt (Wahlberg et al., 2010). Några av de mest kända negativa effekterna är hormonella störningar hos högre organismer (fisk, groddjur m.fl.) och bioackumulering i akvatiska organismer, samt antibiotikaresistens och gentoxicitet (Isidori et al., 2009; Figueira et al., 2011; Ragugnetti et al., 2011; Schultz et al., 2011; Sponchiado et al., 2011; Huerta et al., 2013; Novo et al., 2013; Rodriguez-Mozaz et al., 2015). På grund av reningsverkens begränsade förmåga att bryta ned läkemedel utreds och implementeras idag olika avancerade reningstekniker för att reducera läkemedel, däribland behandling med ozon och/eller kolfilter.

På ett antal platser i Sverige har källsorterade och näringsåterförande avloppslösningar byggts eller planeras att byggas (Sylwan et al., 2014). I system med klosettvattensortering eller latrininsamling kan alla dess växtnäringsämnen (kväve, fosfor, kalium etc.) nyttiggöras genom att hela det insamlade toalettavfallet (latrinen eller klosettvattnet) behandlas genom hygienisering och stabilisering, för att sedan spridas på åkermark som gödsel. Därmed minskar behovet av mineralgödsel och risken för spridning av patogener. Jämfört med konventionella system avlastar dessa system vattenmiljön från såväl huvuddelen av växtnäring som läkemedelsrester (Jönsson m.fl., 2005; Butkovskyi m.fl., 2015). Dessa hamnar istället i markmiljön. Andra fördelar med återföring av klosettvatten som gödselmedel är att det innehåller mera växtnäring, framförallt kväve och kalium, samtidigt som tungmetallhalterna är lägre jämfört med i avloppsslam (Tervahauta et al., 2014). Detta beror på att bl.a. BDT- (bad-, disk- och tvätt-) vatten, dagvatten och avloppsvatten från olika verksamheter inte ingår i källsorterat toalettavfall. Källsorterande systemen är dock förenat med stora utmaningar, vilka främst varit av social, juridisk, ekonomisk och teknisk karaktär.

För den fortsatta utvecklingen av de källsorterade avloppssystemen, är det av stor vikt att kartlägga vilka mängder av läkemedel som skulle spridas i jordbruket vid användning av källsorterade gödselmedel från avlopp. Kunskapen om läkemedelsförekomst i källsorterade avloppsfraktioner är bristfällig, liksom kunskapen om vilken reducerande effekt som erhålls i de behandlings- och hanteringsprocesser som används idag. För att få en första uppfattning om vilka risker som användningen av källsorterade och behandlade gödselprodukter kan ge upphov till, är det också viktigt att undersöka flöden av läkemedlen till marken via gödselprodukterna, och att jämföra dessa flöden och risker med de som finns för konventionella avloppssystem genom utsläpp av renat avloppsvatten och spridning av slam. I detta projekt har vi strävat efter att fylla de nyss nämnda kunskapsluckorna.

Material och metoder

Fokus i projektet har legat på de två källsorterade avloppsfraktionerna latrin och klosettvatten, som provtagits före och efter behandling och analyserats med avseende på läkemedelsrester. För gödsling med klosettvatten simulerades också spridningsgivor, upptaget av läkemedel i gröda, liksom ackumuleringen i marken och spridning från den ytliga jordprofilen (0-70 cm) till underliggande jord och vatten.

Latrinen hämtades från en anläggning i Norrtälje kommun och klosettavloppsvattnet från Telge Näts behandlingsanläggning i Hölö, Södertälje kommun. Behandlingen av klosettavloppsvattnet skedde i två steg med våtkompostering (tillgång till syre) följt av ammoniakbehandling via ureatillsats (0,5 % av våtvikt), satsvis i två parallella reaktorer (R1 och R2). Prov togs efter varje steg i behandlingen för att kunna utreda effekten av våtkompostering respektive ammoniakbehandling på läkemedlen. För att undersöka effekterna av efterlagring av behandlat klosettvatten lagrades prover i kylskåp vid +6 °C under 6 månader. Effekten av rötning (syrefria förhållanden) undersöktes genom satsvisa utrötningsförsök av latrin vid mesofil (+37 °C) och termofil (+52 °C) temperatur.

Ett urval av läkemedelssubstanser gjordes baserat på försäljningsmängder i Sverige, förekomst i avloppsvatten och slam från reningsverk samt terapeutiskt grupptillhörighet. Sammanlagt analyserades 44 läkemedelssubstanser från olika terapeutiska grupper (antibiotika, antidepressiva, antiinflammatoriska, betablockerande, stimulerande substanser o.s.v.). Exempel på välkända läkemedel som ingick i studien är paracetamol, diklofenak, ibuprofen och naproxen (antiinflammatoriska och smärtlindrande) samt trimetoprim och sulfametoxazol (antibiotika).

Metodutveckling och analys av de utvalda läkemedelssubstanserna i latrin och klosettvatten genomfördes på SLU och SPPD. Proverna separerades i en vätskefas och en fast fas genom centrifugering. Varje fas analyserades var för sig. Proverna analyserades med vätskekromatografi följt av masspektrometri (UPLC-MS/MS och UHPLC-QTOF). Endast läkemedlets grundform, d.v.s. varken eventuella konjugerade former eller nedbrytningsprodukter, analyserades i detta projekt. De obehandlade avloppsfraktionerna samt behandlat och efterlagrat (6 månader) klosettvatten karaktäriserades även med avseende på närings- och metallinnehåll.

Det är förbjudet att gödsla såväl vall som morötter med avloppsslam, men för att undersöka hur stort upptaget av läkemedel i grödor gödslade med behandlat klosettvatten kunde bli som mest, simulerades detta med modellen BASL4 (Biosolids Amended Soil Level 4 model; Hughes och Mackay, 2011) för de för Sverige relevanta grödor som modellen är utvecklad för, gräs och morötter. Modellen simulerade även ackumulering av läkemedlen i jorden. Utifrån upptaget i gräs beräknades även potentiellt upptag i vetekärna. Intaget av valda läkemedel via vete och morötter gödslade med klosettvatten beräknades och jämfördes med acceptabelt dagligt intag av dessa läkemedel. Det acceptabla intaget beräknades som den minsta terapeutiska dosen dividerat med en säkerhetsfaktor på 10 000. Säkerhetsfaktorn användes för att ta hänsyn till (1) att den minsta terapeutiska dosen inte är en dos utan effekt (no effect dose), (2) läkemedel med cellgiftseffekt, (3) vissa läkemedel har mindre skillnad mellan den terapeutiska dosen och dosen utan effekt än andra läkemedel och (4) andra okända eller icke förutsedda effekter (DEFRA, 2007; NHMRCA, 2008).

Resultat och diskussion

De uppmätta koncentrationerna i klosettvatten var i nivå med tidigare studier av klosettvatten i Sverige och andra länder (De Graaf et al., 2011; Butkovskyi et al., 2015; Palm Cousins och Magnér, 2014). Största delen av de utvalda substanserna återfanns i vätskefasen. Ibuprofen och naproxen var de substanser som återfanns i högst koncentrationer i latrin och klosettvatten (~100 µg/L resp. ~70 µg/L). Även metoprolol, losartan, valsartan, furosemid och hydroklortiazid uppmättes i högre koncentrationer (~10 till ~30 µg/L). För vissa substanser var andelen i fast fas betydande, vilket visar på vikten av att analysera båda faserna när man studerar läkemedelssubstanser i miljön.

De höga koncentrationerna av vissa läkemedelssubstanser kan kopplas till försäljningsmängder och substansernas olika farmakokinetiska egenskaper, d.v.s. om de utsöndras i oförändrad eller konjugerad form via urin eller fekalier. Eftersom latrinen och klosettvattnet inte är utspätt med t.ex. BDT-vatten, dagvatten och avloppsvatten från olika verksamheter, är koncentrationerna av de flesta läkemedel väsentligt högre (upp till två tiopotenser) än i inkommande vatten till reningsverk.

De flesta substanser påverkades varken av rötning vid mesofil eller termofil temperatur. Endast tre substanser (acetaminofen, naproxen och koffein) minskade signifikant både vid mesofil och termofil temperatur, medan ytterligare fem substanser, såsom atenolol, metoprolol, irbesartan, hydroklortiazid och bezafibrat, minskade signifikant enbart vid termofil temperatur. För några substanser noterades en ökning i uppmätta koncentrationen, t.ex. för atorvastatin, hydroklortiazid, amitriptylin och bisoprolol. En hypotes är att läkemedelssubstanser som konjugerats i kroppen, d.v.s. fått en hydrofil del påkopplad på molekylen, kan återgå till sin ursprungsform vid rötningen och att de därmed bli detekterade igen. En annan möjlig förklaring är att minskningen av antalet partiklar och förändringar i de kemiska egenskaperna under rötningen underlättade extraktion av läkemedelssubstanserna från den fasta fasen.

Vid våtkompostering av klosettvatten följt av behandling med 0,5 % urea erhölls i genomsnitt en större procentuell reduktion jämfört med rötning och den reduktion mellan inflöde och utflöde som rapporterats för ett stort antal reningsverk i Europa, med minst två behandlingssteg inklusive aktiv slam process (Deblonde et al., 2011). Variationen var dock stor beroende på substans. Reduceringseffekten vid ammoniakbehandlingen var begränsad medan koncentrationerna av 13 substanser (kodein, atenolol, metoprolol, propranolol, citalopram, valsartan, candesartan, hydroklortiazid, atorvastatin, lidokain, diklofenak, ibuprofen, och koffein) minskade signifikant vid våtkompostering. Störst reduktion visade kodein och ibuprofen (100 %). Endast en substans, fluoxetin, ökade signifikant i koncentration under behandlingen i båda de undersökta våtkomposteringsreaktorerna, medan acetaminofen endast ökade i reaktor R1. Reduktionen såväl som uppmätta koncentrationer före och efter behandling stämde väl överrens med tidigare resultat från samma behandlingsanläggning (Palm Cousins och Magnér, 2014). Under den sex månader långa efterlagringen kunde ytterligare reduktion endast noteras för valsartan i prov från reaktor R1och av propranolol i prov från R2. Trots att halterna av läkemedel reducerades väsentligt i det våtkomposterade och ammoniakbehandlade klosettvattnet innehöll det fortfarande betydande halter av vissa substanser. Jämfört med slam kunde halterna vara upp till 20 ggr högre.

Det är intressant, men svårt, att rättvisande jämföra tillförseln av läkemedel via gödsling med klosettvatten med den via gödsling med avloppsslam. Svårigheten är att den ur odlingssynpunkt viktigaste växtnäringen i klosettvatten är mineralkväve. I marken är mineralkväve lättrörligt, och därför gödslar man lämpligen med den mängd klosettvatten som tillför den mängd mineralkväve som man tror årets gröda minst kommer att behöva. Överskott av mineralkväve på hösten förloras nämligen till stor del under senhöst, vinter och vår. Avloppsslam är däremot huvudsakligen ett fosforgödselmedel. I marken är fosfor svårrörligt och kan därför förrådsgödslas, vilket innebär att man vid ett tillfälle kan gödsla med tillräckligt med fosfor för att täcka behovet hos fler års kommande grödor, även om detta ökar risken för förluster via erosion etc. När man gödslar med avloppsslam tillför man ofta den största tillåtna givan, en 5-årsgiva. Ovanstående innebär att en gröda som gödslats med klosettvatten bara tillförts läkemedel från en 1-årsgiva med klosettvatten, samt de läkemedel som eventuellt finns kvar i marken från tidigare års gödslingar, medan grödan direkt efter en gödsling med avloppsslam tillförts läkemedel motsvarande en 5-årsgiva med avloppsslam. Å andra sidan tillförs vid gödsling med klosettvatten varje följande års gröda en ny dos läkemedel, medan de vid gödsling med avloppsslam inte tillförs något nytt läkemedel under de kommande fyra åren, utan de grödorna exponeras bara för det som finns kvar i marken.

Att jämföra mängden läkemedel som tillförs med en gödsling med avloppsslam (en 5-årsgiva) med den som tillförs med en gödsling med klosettvatten (en 1-årsgiva) är därför relevant, eftersom det är dessa mängder av nytillförda läkemedel som den kommande grödan exponeras för. Av de 17 läkemedelssubstanser som det vid jämförelsen fanns data på, beräknas en 1-årsgiva av klosettvatten tillföra större mängder av tre läkemedelssubstanser (metoprolol, oxazepam och naproxen), medan 5-årsgivan av slam beräknas tillföra större mängder av nio läkemedelssubstanser. För fem substanser (atenolol, amitriptylin, ibuprofen, diklofenak och bisoprolol) skulle det tillföras lika stora mängder oberoende om klosettvatten eller slam används som gödningsmedel. Att jämföra den totala tillförseln till marken under 5 års gödsling med klosettvatten med den med en 5-årsgiva med slam är också relevant, och den visar att marken beräknas tillföras större mängder av åtta läkemedelssubstanser med klosettvatten (atenolol, metoprolol, amitriptylin, oxazepam, naproxen, ibuprofen, diklofenak och bisoprolol), medan slammet tillförde större mängder av 7 substanser (kodein, ciprofloxacin, karbamezepin, citalopram, ketoconazol, atorvastatin, fluoxetin).

Beräkningsmodellen BASL4 är framtagen för att simulera vad som händer med miljöföroreningar som sprids med avloppsslam på mark. Med modellen kan upptag i gröda simuleras, liksom nedbrytning i matjord och i underliggande jord (alv) ned till 70 cm samt läckage till mark och vatten under 70 cm. BASL4-modellen har hittills bara verifierats för bekämpningsmedel (t.ex. DDT och 2,4-D) och andra väl karaktäriserade organiska föroreningar som t.ex. bensen. De ämnesspecifika kemiska parametrarna för de olika läkemedelssubstanserna som behövs som indata för simuleringarna var inte tillgängliga i litteraturen, utan de fick beräknas och uppskattas i projektet. Beräknade och uppskattade värden adderar till den allmänna modellosäkerheten, och därför blir sammanlagda osäkerheten för de simulerade resultaten stor. De predikterade värdena ger därför endast en grov indikation på storleksordningen på förväntat upptag i växer och läckage till mark och vatten under 70 cm.

Simuleringarna med BASL4 indikerade att många läkemedel efter 3 års gödsling med behandlat klosettvatten skulle nå liknande halter (högst ca 10 ng/g) i alven (20-70 cm djup) som i matjorden (0-20 cm djup). Simuleringarna indikerade också att huvuddelen av läkemedlen till stor del bryts ner (>70 %) under den modellerade tidsperioden. För två läkemedel, furosimid och diklofenak, beräknades >40 % av tillförd mängd att läcka till mark och vatten djupare än 70 cm, medan för de flesta övriga läkemedel beräknades läckaget till <20 %, och ofta <10 %.

Utifrån data i litteraturen och de simulerade halterna i gräs beräknades möjliga halter av läkemedel i skördat vete medan halterna i gödslade morötter kunde simuleras direkt i modellen. Genom att multiplicera dessa livsmedelshalter med vuxnas respektive barns genomsnittliga dagliga intag av morötter och vete beräknades dagligt intag av de olika läkemedelssubstanserna. Dessa intag användes för att beräkna hur lång tid man skulle behöva konsumera morötter respektive vete innan den totalt konsumerade mängden skulle motsvara den minsta terapeutiska dygnsdosen av dessa substanser. Resultaten visar att man skulle behöva konsumera gödslade morötter respektive gödslat vete under mycket lång tid (21 000 år eller mer för vuxna) innan det samlade intaget ens skulle motsvara den minsta terapeutiska dygnsdosen för någon av substanserna.

En fördel med källsorterade avloppssystem är således att man får bättre kontroll över läkemedelsflöden, och man kan därmed undvika onödig exponering för dessa miljöfarliga substanser, både för akvatisk miljö och för människa, även om de återvunna gödselmedlen inte används till energi- eller fodergrödor, utan till livsmedelsgrödor. Modellsimuleringarna är baserade på mycket osäkra simuleringar, men så intressanta att fortsatta simuleringar baserade på enskilda substanser bör göras liksom mätningar på gödslade grödor.

Sammantaget har källsorterade avloppssystem fördelen att kraftigt kunna förbättra samhällets växtnäringskretslopp. Växtnäringskretsloppet för avloppssystem med urinsortering eller klosettvattensortering är, när den fraktionen används som gödsel, väsentligt bättre än för dagens konventionella avloppssystem. Dessa källsorterande system återför 50-80 % av hushållsavloppets kväve och kalium och 60-90 % av dess fosfor, i former som är lätt tillgängliga för grödan. Det konventionella systemet återför, när allt slam används som gödsel, runt 20 % av kvävet, 6 % av kaliumet och 95 % av fosforn till åkermark, men såväl kvävet som fosforn är i former som inte är direkt tillgängliga för grödan. Dock måste behandlingstekniken för läkemedel förbättras, och det behövs en bättre kunskap kring vad som händer med läkemedlen i miljön när det gäller upptag i växter, transport och nedbrytning i jord och grundvatten för att bättre kunna uppskatta fördelar och risker med detta system.

Summary

Source separated toilet waste, such as blackwater (toilet wastewater) and fecal sludge (i.e. waste from dry toilets), contains nutrients. If this waste is stabilized, sanitized, and used as a fertilizer on arable land, it can help to close the plant nutrient loop. However, when these waste fractions are used as fertilizer, pharmaceuticals can also be released. Because of this, it is important to identify and quantify the pharmaceuticals that might be spread on arable land when changing from conventional wastewater systems to source separated, nutrient recycling systems.

This project focused on pharmaceuticals in blackwater and fecal sludge, before and after treatment (liquid composting, ammonia treatment or anaerobic digestion) and post-storage. Furthermore, estimation of the amount of pharmaceutical possibly spread on arable land when using these waste fractions for fertilizer compared to the current use of sewage sludge were investigated. To determine potential risks, uptake of pharmaceuticals in different crops, accumulation in the soil, and leaching into soil and water were simulated. The choice of pharmaceuticals studied in this project was based on their use in Sweden, and on their presence in wastewater and sludge from large scale wastewater treatment plants (WWTPs).

Blackwater and fecal sludge had higher concentrations of pharmaceuticals (up to 100 times) than influent to large scale WWTPs. This is because mixed wastewater (influent) is diluted compared to blackwater and fecal sludge. Aerobic liquid composting and ammonia treatment of blackwater showed better pharmaceutical removal efficiencies than anaerobic digestion of fecal sludge. However, the effect of ammonia treatment on pharmaceutical reduction was limited. There was no significant difference in the reduction of pharmaceuticals between mesophilic and thermophilic anaerobic digestion.

Pharmaceutical reduction in aerobic liquid composting was better than that reported for a large number of European WWTPs. However, pharmaceutical concentrations were still up to 20 times higher in treated and stored blackwater than in sludge from WWTPs. Despite this, and because the strategy for fertilization with blackwater (a nitrogen fertilizer) is different from that used for sewage sludge (a phosphorus fertilizer), pharmaceutical doses were similar for spreading with blackwater or sewage sludge.

Project model calculations suggest that to a large extent pharmaceuticals broke down within a year. There was also only a low accumulation of pharmaceuticals in the soil, and negligible uptake in wheat and carrots. The estimated daily intake of pharmaceuticals by ingestion of wheat and carrots fertilized with blackwater was therefore very low. To reach an amount equivalent to the minimum therapeutic daily dose for the pharmaceutical losartan, adults would need to eat fertilized wheat or carrots for at least 21,000 years. As such, and according to the simulations, exposure via ingestion of crops grown on a blackwater fertilized arable land is negligible.

Source separated systems have the possibility to significantly improve nutrient recycling. However, the treatment technologies need to be improved regarding pharmaceutical reduction. Moreover, a better understanding of the environmental fate of pharmaceuticals in plants, soil, and groundwater is needed, to be able to more accurately estimate the risks of these systems.

1. Introduction

Among the vast array of contaminants of anthropogenic origin reaching our water bodies, pharmaceutically active compounds have currently one of the largest known inputs into the environment (Gros et al., 2012). After intake, pharmaceuticals may undergo metabolic transformations within the human body. Both the non-metabolized parent drug and metabolites are excreted with urine and feces, and in the urban society, the substances are largely led to wastewater treatment plants (WWTPs). Several studies have shown that most pharmaceuticals are not completely removed during conventional wastewater treatment (Jelic et al., 2011; Radjenovic et al., 2007; Joss et al. 2005). They are therefore discharged into receiving water bodies, such as ground water, rivers, lakes and seas, which constitute habitats for aquatic organisms and may be used as sources for drinking water production.

The reduction of pharmaceuticals in WWTPs varies depending on compound. For about 50% of the pharmaceuticals found in wastewater, their reduction is negligible in conventional wastewater treatment systems in Sweden, while the rest is highly or moderately removed (Hörsing et al., 2014). Of the remaining substances, most are found in the effluent. On mass basis less than 15% of the incoming amount has been found in the produced sludge (Wahlberg et al., 2010). To reduce the pharmaceutical contamination from WWTPs, efforts have been made in testing and developing advanced wastewater treatment technologies, such as membrane bioreactors (Radjenovic et al., 2007; Clara et al., 2005), advanced oxidation processes (AOPs) and ozone and active carbon (Huber et al., 2003; Flyborg et al., 2010; Klavarioti et al., 2013; Ek et al., 2014) for their reduction.

At WWTPs, the sludge is often stabilized by anaerobic digestion (Wahlberg et al., 2010; Samaras et al., 2014). In some countries, including Sweden, WWTP stabilized sludge is used in agriculture as fertilizer and soil amendment (Jelic et al., 2011). Roughly one quarter of the treated sludge was spread on agricultural land in Sweden in 2012 (Paulsson, 2014; SCB, 2014). In the EU, more than 40% of the produced stabilized sludge is used for agricultural purposes (Kelessidis and Stasinakis, 2012). Sludge is also used for soil quality improvements and land-fill covering (Wahlberg et al., 2010). Since pharmaceuticals have been found in sludge (Haglund, 2013; Hörsing et al., 2011) pharmaceuticals will also end up in the terrestrial environment when sludge is used as fertilizer on arable land.

Pharmaceuticals have been designed to produce specific biological effects on humans and organisms. Since many organisms have similar receptors as humans, different unwanted environmental effects are to be expected. Some of the most well-known adverse effects that they might have are the development of antibiotic resistance (Figueira et al., 2011; Rodriguez-Mozaz et al., 2015; Novo et al., 2013), genotoxicity (Ragugnetti et al., 2011; Sponchiado et al., 2011), endocrine disruption (Isidori et al., 2009) and the potential to bioconcentrate/bioaccumulate in aquatic organisms, particularly in fish (Schultz et al., 2011; Huerta et al., 2013).

Source separation and use of urine and feces as fertilizer have the potential to minimize the discharge of pharmaceuticals to water environments since most of the pharmaceuticals are in the source separated fraction, which is spread on land (Butkovskyi et al., 2015). Because of the source separation, the only effluent to water bodies containing pharmaceuticals will be greywater, which already before

treatment have low concentrations of pharmaceuticals (Jönsson et al., 2005). This radically changes the flows of pharmaceuticals to the environment. Blackwater and source separated urine are rich in nitrogen, phosphorus, potassium, sulphur, and organic matter, and low in heavy metals (Tervahauta et al., 2014). Thus, these fractions can be used as fertilizers as well as soil conditioners, which after appropriate treatment and sanitation will be safe from hygienic point of view. The hygienization minimize the risk of spreading pathogens to the environment. Using them as fertilizers will contribute to closing the nutrient cycles, to decrease the required nutrient reduction in, and nutrient emissions from, the wastewater system and to decreasing the demand of chemical fertilizers by the agricultural sector (Winker et al., 2009; Jönsson et al., 2004; Spångberg et al., 2014; Jönsson and Vinnerås, 2013). Also waste production will be minimized, as the sludge production in WWTPs will be significantly reduced. With well-designed and run source separating and nutrient recycling systems, the energy use and the global warming impact will decrease significantly compared to conventional systems with enhanced removal of nitrogen and phosphorus at a WWTP and use of chemical fertilizer in agriculture, and many studies indicate that at society level the impact changes from a net use to a net generation of high value energy (Spångberg et al., 2014; Kretsloppskontoret, 2008; Hellström et al., 2005: Tidåker et al., 2007; Jönsson et al., 2005; Jönsson, 2002). Source separating systems have however so far often suffered from large challenges, mainly of social, legal, economic and technical nature.

Recycling of source separated urine and blackwater is already implemented in several Swedish municipalities and is becoming more common, but the risks associated with these activities are not sufficiently known (Vinnerås & Jönsson, 2013). Prevalence, fate and risks posed by pharmaceuticals and other organic contaminants need to be further investigated, understood and evaluated.

1.2 Objectives

The aim of the current study was to evaluate the prevalence and fate of pharmaceuticals in untreated and treated source separated toilet fractions and to preliminary assesses risks when these fractions are used as fertilizers. Thus, the project was aimed to create a scientific background for future research on risks and risk prevention associated with pharmaceuticals in source separated waste fractions. The starting point was a Swedish perspective, with focus on source separated toilet fractions containing both urine and feces (blackwater and fecal sludge) that are treated with full- or demo-scale methods. The specific goals of the project were to:

- determine the concentrations of pharmaceuticals in untreated blackwater and fecal sludge,
- assess the removal of pharmaceuticals during the treatment of the source separated toilet fractions using i) anaerobic digestion for fecal sludge and ii) combined liquid composting (auto thermal aerobic digestion) and ammonia treatment through urea addition for blackwater,
- assess the application rate of pharmaceuticals on arable land when fertilizing with blackwater and compare these rates with application rates of pharmaceuticals when municipal sewage sludge is spread as fertilizer,

- provide a preliminary assessment of pharmaceutical accumulation in soil and plants, estimate human intake of pharmaceuticals via ingestion of crops fertilized with blackwater and compare these intakes with intake of pharmaceuticals through other food products, and
- identify the need for future research and development within the field.

2. Background information

2.1 Source separation and treatments

When applying source separation, urine, feces or a combination of these human excreta is separated from the total wastewater flow directly at the source (toilet). Water from kitchen, bath and laundry (greywater) is managed separately. Source separation keeps most of the nutrients in more concentrated and less polluted fractions, which usually is an advantage during treatment and when nutrient recycling is pursued. There are different techniques used for source separation of sewage fractions: dry toilets, urine or blackwater separation, all described below.

The interest for source separated systems is growing in both small scale decentralized systems and large scale centralized municipal wastewater management. These systems are promoted by municipal organizations and motivated by an endeavor towards more sustainable systems (Sylwan et al., 2014). Separation and separate collection of the blackwater at the house in environmentally sensitive areas outside of the piped network is commonly found today. Municipal management and recycling as fertilizer of blackwater from closed septic tanks exist today in Södertälje, Norrtälje, Uddevalla and Eskilstuna, where the blackwater is collected at the house and transported to a treatment facility (Fig. 1). More municipalities have the ambition to establish similar recycling systems, e.g. Knivsta and Haninge. In some cases, organic waste is planned to be collected together with the blackwater.

Separated toilet fractions can be treated and recycled in different ways. A major reason to treat the waste before agricultural use is to eliminate pathogens and thus reduce the risks of spreading diseases. Anaerobic digestion, liquid composting, ammonia treatment and long-time storage are some suggested alternatives (Jönsson et al., 2013; Kjerstadius et al., 2012a). Membrane technologies and nutrient precipitation (e.g. struvite) are examples on technologies to concentrate the fertilizer and provide a more portable product (Kjerstadius et al., 2012b). However, these processes usually lead to a secondary liquid residue to handle.

2.1.1 Dry toilets

In dry toilets (i.e. without flush water) the separated waste fractions consist of urine, feces, toilet paper, some addition of sawdust, peat moss or other carbon rich material with water binding capacity and any menstruation hygiene material. This fraction is called fecal sludge. Dry toilet systems today almost solely exist in vacation houses in Sweden. The dry solutions can be combined with urine separation, which is quite common (Kvarnström et al., 2006), as it improves the function. In this context, the separated urine is usually handled locally in the

garden and the greywater is treated in simple compact biological filters (usually soil beds). The fecal sludge
is either transported to a central treatment facility or managed by on-site composting, inside or outside of the toilet.

In a visionary perspective, it might be possible to separate toilet waste with technologies that eliminates wastewater dilution partially or completely with continued user comfort. In that scenario, the toilet waste would have a dry matter content close to 5% (Jönsson et al., 2005) and could be treated by e.g. anaerobic digestion in a conventional Continuously Stirred Tank Reactor (CSTR) to produce biogas. Anaerobic digestion (AD) at mesophilic temperature (35-40 °C) is the most common biological treatment of sewage sludge in conventional WWTPs in Sweden, 125 out of 137 WWTPs run the AD-process at mesophilic temperature and the rest at thermophilic temperature (Paulsson, 2014). The use of thermophilic (50-60 °C) anaerobic digestion would simultaneously hygienize the toilet waste. Nutrients would then be possible to recycle without any further dilution. In view of this, this project evaluated the potential for degradation of pharmaceuticals in non-diluted toilet fractions by anaerobic digestion. Fecal sludge was the toilet waste used in the anaerobic digestion experiment as it was collected without water dilution. However, the fecal sludge from most dry toilets contains relatively small amounts of urine in relation to feces, as many households at least partly dispose the urine on-site, due to the collection cost they have to pay for the fecal sludge.

The fecal sludge used in the project was collected at Salmunge waste plant in Norrtälje municipality, which has a unique solution for dry toilet users. Within the municipality, there are about 30 000 vacation houses. Many of the houses are located close to sensitive water environments. The use of sanitary systems with wastewater discharge has therefore been restricted, and blackwater systems with closed holding tanks as well as dry toilets have long been promoted and are commonly found. About 14% of the vacation houses are subscribers of fecal sludge receptacles. With this subscription, the municipality takes care of collection and disposal of fecal sludge from the subscribers (Holm et al., 2009). Norrtälje also receives and treats fecal sludge from some surrounding municipalities as well as from the island Gotland. The fecal sludge samples were taken at different depths in the storage basin at the Salmunge facility.

2.1.2 Urine separation

A more modern approach to source separation has been to apply urine separation by special urine-separating water closets. The urine contains over 60% of the phosphorus and 80% of the nitrogen in human excreta (Jönsson et al., 2005). This means recycling of the nutrients in urine closes the loop for most of the nutrients in municipal wastewater.

Urine can be collected locally in tanks and is sometimes centrally stored and managed. In urine separating systems, greywater and feces are usually treated in an ordinary wastewater treatment process. The source separated urine has a high hygienic quality and the high pH can eliminate most of the biological contaminants through storage (Jönsson et al., 2013). During the 90s, urine separation was tested in several pilot and full scale applications in Sweden (e.g. Kullön and

Understenshöjden). The outcomes and the experiences from these projects differ. It seems that technical and social issues related to the separation and collection system were recurring challenges for larger scale implementation. The fraction of urine from urine separation was not included in this study.

2.1.3 Blackwater separation

The limited success of urine separation systems in Sweden has increased the interest for blackwater (feces, urine, toilet paper and flush water) systems. Blackwater solutions are now increasingly proposed in environmentally oriented urban housing projects in Sweden. In blackwater systems the outlet from the water closet (blackwater) is kept separate from the greywater (Fig 1). In order to get reasonable concentration of nutrients in the blackwater, low-flush toilets is a necessity. Depending on collection and treatment strategy often extremely low-flush toilets can be necessary which means that one cannot rely on ordinary gravity powered transport. Commonly the extremely low flush toilet systems use vacuum technology. The blackwater is collected locally in closed septic tanks (Eveborn et al., 2007). The tanks are emptied regularly and transported to a treatment facility for hygienization.

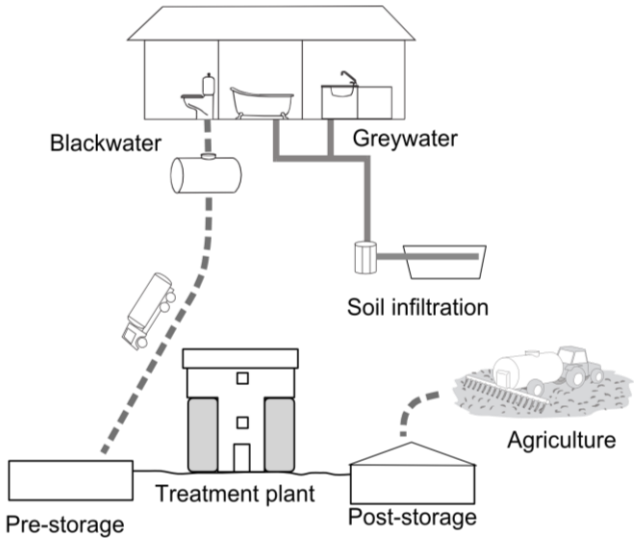


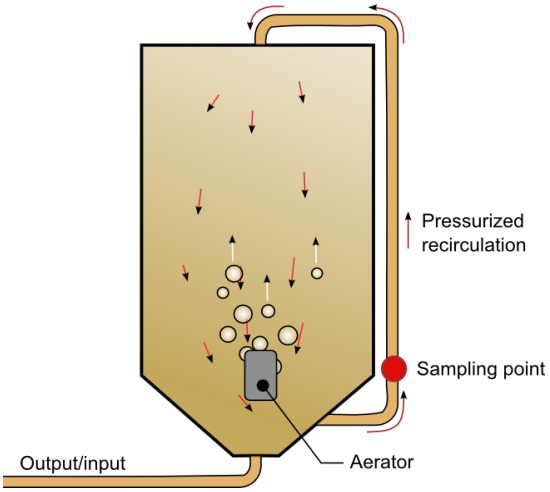
Figure 1. Principle for blackwater separation in rural areas with blackwater collected in closed septic tanks.

The studied blackwater system is located in Södertälje municipality. The region has several sensitive and nutrient overloaded water-bodies including parts of the lake Mälaren and costal bays of the Baltic Sea. In order to decrease the nutrient load that can be attributed to onsite wastewater treatment systems, the municipality has built a treatment plant for blackwater and invited private home owners to install source separated sewer systems with collection of blackwater in closed holding tanks (Fig. 1).

The hygienization plant is located at Nackunga gård, Hölö (close to Södertälje). It is built and managed by Telge Nät (a municipal company) and operated by a local farmer. Blackwater from holding tanks in the area (today about 1500 units)

is regularly emptied and transported to the treatment facility by a vacuum truck. The facility receives and processes about 1500 m^3 blackwater yearly. The hygienized blackwater is spread as fertilizer on arable land close to the treatment facility with a conventional manure slurry spreader.

The facility has two sealed concreate basins of 200 m^3 for pre-storage. Blackwater is batch processed in two parallel reactors (R1 and R2) with a capacity of 32 m^3 each. The treatment process aims to reduce pathogens and to stabilize the substrate (degrade easily degradable organic substances and minimize odor problems). It includes two treatment steps. In the first step, the blackwater is oxidized in a liquid compost reactor (aeration is performed during constant mixing; Fig. 2). The aerobic degradation induce a temperature increase and thereby a thermal treatment. The liquid composting (also named auto thermal aerobic digestion) has been described in in several reviews (e.g. Juteau, 2006; Layden et al., 2007). At the Hölö plant, the temperature is raised in the substrate to about +40 °C during the composting process. In the second step, an ammonia-based treatment (Vinnerås, 2007) is applied. The aeration is turned off when urea is added to the substrate, and the volume is constantly mixed for about 7 days (Fig. 2). The reason for the urea-based treatment is that there is a low concentration of easily degradable organics available in the blackwater. Thus, the microorganisms would not be able to raise the temperature enough for sufficient pathogen reduction without addition of external energy in the form of more organics or external heat (Eveborn et al., 2007). On the other hand, the increased temperature of the substrate (around +40°C) reduces the amount of urea and the time needed for the ammonia-based hygienization (Magri et al., 2015). The post-storage basin has a volume of 1500 m^3. In the current study, samples were taken before treatment, after liquid composting, and after ammonia treatment as well as after post storage for six months.



Process scheme

Phase	Description
1	Reactor is filled up with substrate from storage tank
2	Liquid composting until temperature in reactor reaches +40 °C.
3	Addition of urea and continous mixing for 7 days
4	Reactor is emtied and the treated substrate is discharged to a post storage tank

Figure 2. Description of the Hölö treatment plant and its treatment process. Illustration: David Eveborn.

2.3 Target pharmaceuticals

2.3.1 Consumption and prescription

The target pharmaceuticals were selected based on their consumption and prescription patterns in Sweden (Socialstyrelsen, 2015a; ehälsomyndigheten, 2014). Some of the most consumed pharmaceuticals in Sweden in 2013 were the anti-inflammatory drugs acetaminophen (paracetamol), diclofenac, ibuprofen and naproxen. According to a report from the Swedish Prescribed Drug Register and from the Swedish eHealth Agency, paracetamol (acetaminophen) was also one of the most frequently dispensed drugs in Sweden in 2014 (Socialstyrelsen, 2015b). According to the same report, the number of patients with at least one dispensed prescription of naproxen increased in Sweden compared to 2013. On the other hand, Symbocort™, used to treat asthma and chronic obstructive pulmonary disease with budesonide as an active principle, was one of the most sold preparations in 2013, according to a report from eHälsomyndigheten (2014)

Trimethoprim and sulfamethoxazole are highly used antibiotics. In several medications, they are normally present as a combination of both substances, known as trimethoprim/sulfamethoxazole or co-trimoxazole, and they are used to treat a wide range of infections. Trimethoprim/sulfamethoxazole appears in the list of essential medicines of the World Health Organization (WHO), which lists the most important medications needed in a basic health system (WHO, 2015). Only in 2014, in Uppsala and Stockholm Counties, 6 prescriptions per 1000 inhabitants of trimethoprim/ sulfamethoxazole were dispensed. Azithromycin, clarithromycin and roxithromycin belong to the group of macrolide antibiotics, which are widely used for the treatment of several infections, such as respiratory, gastrointestinal, skin, urinary and soft tissue infections. In 2014, ciprofloxacin and norfloxacin were the most widely prescribed fluoroquinolone antibiotics in Stockholm County, with a total of 21.05 prescriptions per1000 inhabitants for ciprofloxacin. The number of prescriptions in Stockholm County for clarithromycin and azithromycin were 1.5 and 3 prescriptions per 1000 inhabitants, respectively.

For anti-hypertensives, amlodipine was the most widely prescribed anti-hypertensive drug (156 prescriptions per 1000 inhabitants), followed by candesartan (75 prescriptions per 1000 inhabitants), losartan (68 prescriptions per 1000 inhabitants) and ramipril (47 prescriptions per 1000 inhabitants) in Stockholm County in 2014. In addition, most of the target compounds included in this study (losartan, valsartan, candesartan, ramipril and amlodipine) are included in the group of recommended drugs to treat heart and vascular conditions in adults in Uppsala County in the period from 2014 to 2015.

Regarding anti-depressants, citalopram was the most widely prescribed antidepressant in Stockholm County in 2014, with 121 prescriptions per1000 inhabitants, followed by venlafaxine (34 prescriptions per1000 inhabitants) and by the anti-epileptics carbamazepine and lamotrigine and the anti-depressant fluoxetine, with a total number of 27, 28 and 23 prescriptions per 1000 inhabitants, respectively.

Concerning β-blockers, metoprolol was included in the list of the most widely sold preparations in 2013 and it was also the most widely sold substance in Stockholm County in 2014 (265 prescriptions per 1000 inhabitants), followed

by other β-blocking agents, such as bisoprolol (69 prescriptions per 1000 inhabitants), propranolol (22 prescriptions per 1000 inhabitants) and sotalol (4 prescriptions per 1000 inhabitants).

Other widely consumed pharmaceuticals include the lipid regulator atorvastatin, the diuretics furosemide and hydrochlorothiazide and the antihistaminic cetirizine. Atorvastatin is one of the most widely consumed statin drugs worldwide, and it is used to reduce high cholesterol levels (Walley et al., 2005). The number of prescriptions of furosemide in Stockholm County in 2014 were quite significant (170 prescriptions/1000 inhabitants), while the consumption of hydrochlorothiazide and cetirizine was also quite remarkable, according to the number of dispensed drugs (16 and 19 prescriptions per 1000 inhabitants, respectively).

2.3.2 Occurrence and effects

The occurrence of pharmaceuticals in wastewater has been widely reported (Fent et al., 2006; Loos et al., 2009; Rodriguez-Mozaz et al., 2010). Generally, the concentration levels detected in the aquatic environment are in the ng/L to μg/L range (Gros et al., 2010; Zucato et al., 2006). Some studies have already highlighted possible environmental risks and toxic effects to non-target organisms (Corcoran et al., 2010; Pal et al., 2010). However, further efforts are still needed to thoroughly evaluate their impact toxicity to the ecosystem.

Recent studies pointed out the uptake of certain pharmaceuticals by fish (Huerta et al., 2013; Subedi et al., 2012; Ramirez et al., 2009) and by river biofilm and macroinvertebrates of different taxonomic groups (Ruhi et al., 2015). Huerta et al. (2013) detected diclofenac, propranolol, sotalol, citalopram and venlafaxine in fish homogenate samples, from different fish species in Mediterranean rivers, while carbamazepine was detected in fish liver as well (Huerta et al., 2013). In a study conducted in the United States, diltiazem and carbamazepine were detected in fish fillets from sewage water effluent-dominated sites and fluoxetine was found in fish liver tissue (Ramirez et al., 2009). When pharmaceuticals are taken up by aquatic organisms, such as fish, it is expected that these substances will target similar systems as in mammals and therefore have similar effects (Corcoran et al., 2010). Some examples are the exposure to the nonsteroidal anti-inflammatory drug (NSDAIDs) ibuprofen, which showed to alter the pattern of spawning in Japanese medaka fish at concentrations of μg/L (Flippin et al., 2007). On the other hand, diclofenac has been associated with renal failure in Asian vultures and in the serious decline of their population (Oaks et al. 2004). Some studies have also reported histological changes in the liver, kidney and gills of fish (Schwaiger et al., 2004; Triebskorn et al., 2004; Mehinto et al., 2010) and it has been proven that environmentally relevant concentrations can affect hepatic gene expression (Cuklev et al., 2011). Exposure of fish to the antidepressant fluoxetine has shown to have several behavioral and reproductive effects, such as the decrease of territorial behavior (Perreault et al., 2003), reduce their ability to capture preys (Gaworecki et al., 2008), decrease their feeding rates (Stanley et al., 2007), increase estradiol levels (Brooks et al., 2003), induce oocyte maturation (Iwamatsu et al., 1993) and affect testis morphology (Schultz et al. 2011). Antifungal agents, such as ketoconazole, has been shown to induce reproductive alterations in fish, such as decrease the egg production (Ankley et al., 2006) and alter the production of some steroids (Hinfray et al., 2004). Regarding β-blocker

agents, some studies have shown that exposure to propranolol affects fish growth (Huggett et al., 2002; Owen et al., 2007) and similar effects have been observed for atenolol (Winter et al., 2006). Another relevant study showed that the benzodiazepine drug oxazepam altered behavior and feeding rates of the wild European perch (*Perca fluviatilis*) at concentrations normally found in the environment (Brodin et al., 2013).

Concerning antibiotics, the most severe effect associated with their occurrence in the environment is the development of antibiotic resistance. There are several studies that have already demonstrated that municipal wastewaters are significant sources of antibiotic resistance genes (ARGs) in freshwater ecosystems (Rodriguez-Mozaz et al., 2015; Berglund et al., 2015), which is serious since a considerable amount of the drinking water in both Sweden and the world, is produced from surface water. In addition, it is known that drinking water produced from surface water often contains trace levels of pharmaceuticals (Fick et al, 2011).

On the other hand, several investigations have pointed out that, in animal manure amended agricultural soils, ARGs may spread among soil bacteria through vertical (generation) or horizontal transfer (conjugation, transduction, transformation and transposition) (Heuer et al., 2011; Fletcher et al., 2015). Besides their persistence in agricultural soils, pharmaceuticals and ARGs may leach to groundwater and/or contaminate surface waters via surface run-off (Chee-Sanford et al., 2009). The contamination of groundwater bodies by pharmaceuticals and antibiotic resistant bacteria would be a serious environmental problem because, in Sweden and many other countries, groundwater is the main source for drinking water production. Transference to humans via drinking water consumption would be a serious public health issue since the effectiveness of antimicrobial therapies might be compromised by the appearance of bacteria that become resistant to most antibiotics. On the other hand, the pollution of groundwater bodies with pharmaceuticals would also lead to a serious environmental problem, since bioremediation of contaminated groundwater wells is expensive and difficult. Some studies have already detected antibiotic residues in groundwater wells from agricultural areas where either animal manure and/or sewage sludge are applied as fertilizers or that they have been irrigated with wastewater effluent (Garcia-Galan et al., 2011; Gibson et al. 2010).

3. Methodology

3.1 Target compounds and their impact in the environment

In our study, 44 pharmaceuticals were analyzed. Target pharmaceuticals (Table 1) belong to different therapeutic groups, such as analgesics and anti-inflammatories, antibiotics, antihypertensive drugs, antidepressants, antihistamines, anti-diabetics, anti-ulcer and antifungal agents, beta blockers, diuretics, lipid regulators and local anesthetics. Pharmaceuticals were selected based on their high consumption in Sweden in 2014 as well as on their ubiquity in Swedish urban wastewater effluents and sewage sludge (Lindberg et al., 2014; Zorita et al., 2009; Hörsing et al., 2011; Socialstyrelsen, 2015a; ehälsomyndigheten, 2014). No metabolites have been studied for the selected compounds.

Table 1. Target pharmaceuticals, classified by therapeutic group and by where the analysis was done

Target pharmaceuticals analyzed by SLU			
Therapeutic group	Compound	Therapeutic group	Compound
Analgesics	Codeine	Anti-depressants	Carbamazepine
β-blockers	Atenolol		Citalopram
	Sotalol		Diazepam
	Metoprolol		Lamotrigine
	Propranolol		Oxazepam
Antibiotics	Azithromycin		Venlafaxine
	Clarithromycin		Fuoxetine
	Norfloxacin		Amitryptiline
	Ciprofloxacin	Anti-ulcer agent	Ranitidine
	Ofloxacin	Anti-fungal agents	Climbazole
	Sulfamethoxazole		Ketoconazole
	Trimethoprim	Local anesthetic	Lidocaine
Anti-hypertensives	Losartan	Diuretics	Furosemide
	Valsartan		Hydrochlorothiazide
	Irbesartan	Lipid regulators	Atorvastatin
	Diltiazem		Bezafibrate

Target pharmaceuticals analyzed by SPPD			
Therapeutic group	Compound	Therapeutic group	Compound
Analgesics and anti-inflammatories	Ibuprofen	Anti-diabetic	Saxagliptine
	Naproxen	Antibiotic	Sulfamethoxazole
	Diclofenac	Anti-histamine	Cetirizine
	Acetaminophen	Anti-depressant	Carbamazepine
	Budesonide		Fluoxetine
Anti-hypertensives	Candesartan	Diuretic	Furosemide
	Ramipril	β-blocker	Bisoprolol
	Amlodipine	Stimulant	Caffeine
Lipid regulator	Atorvastatin		

3.2 Sampling

The project focused on systems that separate urine and feces in a combined product (blackwater or fecal sludge) and use a management strategy that use the complete volume of collected toilet waste back to agriculture as fertilizer.

3.2.1 Fecal sludge collection

Samples of fecal sludge were collected from Salmunge waste plant, Norrtälje municipality in the end of August 2014. At Salmunge, fecal sludge receptacles are emptied by an automatic emptying station, which empties and roughly washes the containers (a minor volume of water is thereby added to the substrate). The fecal sludge is stored in two concrete basins, where the main one has a stirrer (Fig. 3). The backup basin is used when the main basin is full. Fecal sludge samples were taken at two positions (A and B, Fig. 3) and at two depths at each position in the

main basin (surface and 0.2 m from bottom for sample point A, surface and 0.2 m from the permanent bottom sediment which means about 0.5 m from the bottom for sample point B). The stirrer had been running for about 20 hours before sampling.

Surface samples were taken by use of a stainless steel bucket (10 L). Bottom samples were taken by use of a submersible sewage pump that was lowered down to the bottom/permanent bottom sediment in the main basin and then raised 0.2 m before the pump was started. The sampled substrate was transferred to polypropylene (PP) buckets. Totally about 40 L of untreated fecal sludge was collected, about 10 L from each sampling point.

Samples were transported to Uppsala by car and stored in a refrigerated room (+5-9°C) for three days until the sample preparation was done.

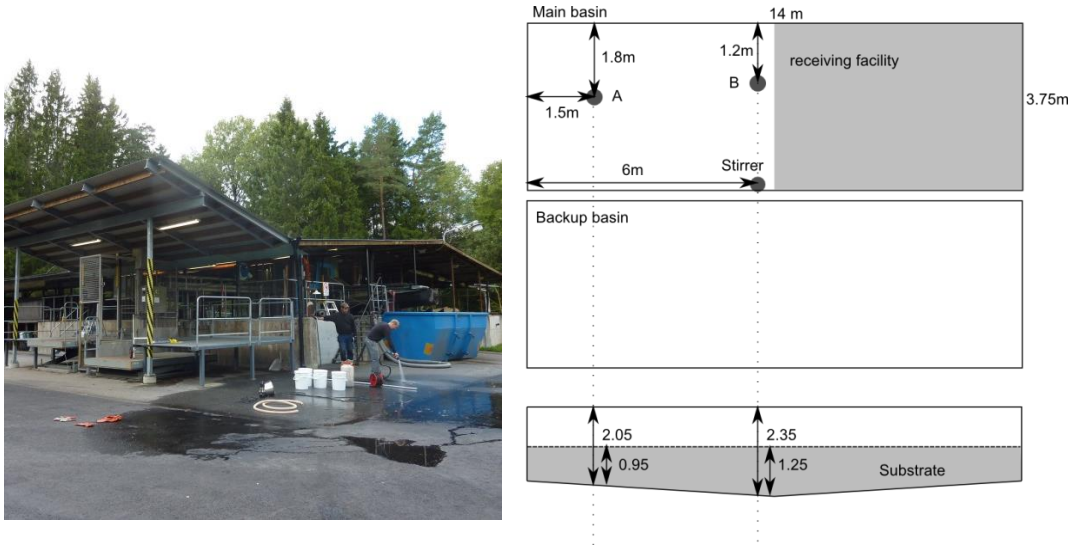


Figure 3. Salmunge waste plant (left) and to the right plan and section drawings of the fecal sludge basins. Sample locations are notated A and B. In the plan drawing the gray surface denotes the area (above the basin) that is used for the receiving facility. In the section drawing the gray area illustrates the sludge level. Photo: JTI

3.2.2 Blackwater collection

Samples of blackwater were collected from the treatment plant at Nackunga gård, Hölö, Södertälje in December 2014. At the Hölö treatment plant, blackwater is treated by a combined process of (i) liquid composting and (ii) ammonia treatment through urea addition (Fig. 2). The plant consists of two reactors (R1 and R2) which are replicates of each other and are operated similarly (see 3.3.2).

Blackwater samples were taken from the tap at the circulation pipe (Fig. 2). The circulation pump is continuously in operation during treatment which enables a homogeneous mixture of the substrate. Before sampling, about 2 L of blackwater was discarded to be sure that there was no standing blackwater left in the tap pipe. The substrate was transferred to a10-25 L polyethylene (PE) bucket. The procedure was repeated for both reactors at the treatment plant (R1 and R2). About 10-25 L of blackwater from each reactor and sampling occasion were collected (Table 3). Samples were transported to Uppsala by car. When the transport and sample

preparation could not be done the same day, the samples were stored in a refrigerated room (+5-9 °C) until the next day.

3.2.3 Sample preparation

The collected bulk samples of fecal sludge were mixed in a large plastic bucket with a concrete mixer (Meec tools 480/800rpm) at The Swedish Institute of Agricultural and Environmental Engineering, Uppsala (JTI). For the blackwater samples, the mixing procedure was done by shaking the plastic buckets several times. Immediately after the mixing procedure, the samples were poured into smaller ethanol-cleaned polyethylene bottles (volume 1000 mL and 1500 mL). The bottles were wrapped with aluminum foil to prevent the degradation of pharmaceuticals by light. Some of the subsamples were then sent for characterization analysis at an accredited laboratory (see 3.4.2), kept refrigerated for control storage (see 3.3.1 and 3.3.2) or prepared for pharmaceutical analysis (see 3.4.1). The untreated fecal sludge was frozen (−20°C) before put in the fridge for control storage or used for future analyzes and experiment (anaerobic digestion).

3.3 Experimental design

3.3.1 Treatment of fecal sludge – anaerobic digestion

Anaerobic batch digestion experiments were performed under controlled conditions in laboratory glass bottles with fecal sludge waste as substrate, with and without addition of selected pharmaceuticals (atenolol, metoprolol, propranolol, ciprofloxacin, sulfamethoxazole, trimethoprim, carbamazepine, furosemide and diclofenac). The method used, the biochemical methane production (BMP) analysis, is described in detail by Westerholm et al. (2012). A brief description is presented below (see Operational conditions and sample collection).

Spiking of fecal sludge

Fecal sludge was spiked in a plastic bottle with an appropriate volume of a methanol solution containing target pharmaceuticals and then mixed by manual shaking for 20 minutes. This spiked fecal sludge was added to a triplicate of bottles in the BMP test. Fecal sludge was spiked so that the final concentration of the added part of each spiked target pharmaceutical was 35 ng/mL.

Operational conditions and sample collection

Two parallel experiments were performed, one in mesophilic temperature at +37 ºC and one in thermophilic temperature at +52 ºC. The inocula for the anaerobic digestion were collected from the mesophilic anaerobic bioreactor in Kungsängsverket, Uppsala, for the mesophilic experiment and from the thermophilic reactor in Kävlinge sewage plant for the thermophilic. Before the experiment was started, the inoculum was degassed for a week at +37 °C or +52 ºC for the mesophilic and thermophilic inoculum, respectively. Dry matter (DM) and volatile solids (VS) of substrate and inocula were measured in triplicate samples with standard methods (SS028113). Glass bottles with the approximate total volume of 1100 mL were filled with inoculum, tap water and substrate to a

final volume of 600 mL liquid volume while flushed with N_2-gas. Each bottle was loaded with 3 g VS/L from the fecal sludge. A fecal sludge to inoculum ratio of 1:3 was used calculated on VS. After filling, the bottles were sealed with a rubber stopper and aluminum-caps and covered with aluminum foil. Incubation was conducted on shake tables (130 rpm) at +37 °C or +52 ºC up to 60 days. The gas production was monitored (Table 2 and Fig. 4). Two parallel bottles with fecal sludge samples were collected each time for pharmaceutical analysis during the mesophilic (day 0, 30 and 61) and thermophilic (day 0, 30 and 59) treatment. The samples were then prepared for pharmaceutical analyzes (see 3.4.2 and 3.4.3).

Table 2. Details about samples from the anaerobic digestion of fecal sludge (n=3-7)

Sample	Description	Temp. (°C)	Incubation (days)	Gas production (NmL CH_4/gVS)	Methane (%)
UL	Untreated fecal sludge	-	-	-	-
ML0	Mesophilic AD of fecal sludge	37	0	0	-
MSL0	Mesophilic AD of spiked fecal sludge	37	0	0	-
ML30	Mesophilic AD of fecal sludge	37	30	221	58
MSL30	Mesophilic AD of spiked fecal sludge	37	30	246	59
ML60	Mesophilic AD of fecal sludge	37	61	254	59
MSL60	Mesophilic AD of spiked fecal sludge	37	61	272	59
TL0	Thermophilic AD of fecal sludge	52	0	0	-
TSL0	Thermophilic AD of spiked fecal sludge	52	0	0	-
TL30	Thermophilic AD of fecal sludge	52	30	230	58
TSL30	Thermophilic AD of spiked fecal sludge	52	30	239	59
TL60	Thermophilic AD of fecal sludge	52	59	257	60
TSL60	Thermophilic AD of spiked fecal sludge	52	59	264	60

Figure 4. Bottles on the shake table at +37 ºC and the gas chromatograph used for methane analysis to the left. To the right, monitoring of gas pressure and methane production.

Control storage of fecal sludge

As a quality control, duplicate samples of fecal sludge (UL) collected from Salmunge waste plant (2 x 1000 mL) were stored in a fridge (temperature +6.5 ± 1.3 °C) for 30 days and 60 days (similar to the sample times during the anaerobic treatment). The caps of the bottles were unscrewed and placed loosely on top of the bottles to allow for some aeration. By the storage of these samples, degradation occurring without treatment could be investigated. After the control storage, the samples were prepared for pharmaceutical analyzes (see 3.4.2 and 3.4.3).

3.3.2 Blackwater treatment

A sampling program was designed in order to investigate the degradation of pharmaceuticals in blackwater at different process stages in the treatment plant, at Hölö, Södertälje (Fig. 3). Two single batches (from reactor R1 and R2) were followed during one treatment period. Samples were taken at phase one (untreated blackwater), in the end of phase two (liquid composted substrate) and in the end of phase three (liquid composted and ammonia treated blackwater). In addition, a storage experiment was performed (to simulate storage of the treated blackwater in the post-storage container) and a control line, similar to the one for the anaerobic treatment, was setup in order to monitor natural degradation during the treatment period.

Operational conditions at Hölö treatment plant and sample collection

The stirrer in the pre-storage was started about two hours before filling each reactor with 32 m^3 of blackwater. After collection of the untreated blackwater samples, the liquid composting process was started. After 12 days of liquid composting the temperature had reached +41 °C in R1 and sampling was performed (Table 3). Urea was added and a new sample was collected after 6 days of ammonia treatment. The temperature had then reached +43 °C. In the second reactor (R2), the temperature

rise was slower. However, the sample occasions were synchronized which means that temperatures and treatment times differed between the two parallel evaluations (Table 3). It took 19 days to reach +40.5 °C in R2 and in the last sampling the ammonia treatment had lasted for 2.5 days. The samples were then prepared for pharmaceutical analyses (see 3.4.2 and 3.4.3).

Table 3. Details about samples from the treatment of blackwater

Sample	Description	Temp. (°C)	Period of liquid composting (days)	Period of ammonia treatment (days)
UR1	Untreated R1	-	-	-
UR2	Untreated R2	-	-	-
WR1	Liquid composted R1	41	12	-
WR2	Liquid composted R2	35	12	-
WUR1	Liquid composted and ammonia treated R1	43	12	6
WUR2	Liquid composted and ammonia treated R2	41	19	3

Controls and post storage of blackwater

Samples of untreated blackwater (UR1 and UR2) and treated blackwater (WUR1 and WUR2) were stored in a fridge (temperature +6.5 ± 1.3°C). Storage was performed in bottles with 1000 mL of sample. The caps to the bottles were unscrewed and placed loosely on top of the bottles to allow some aeration.

Untreated (control) blackwater samples were stored for 12 and 19 days (similar to the process phases in the Hölö treatment plant). In these samples, degradation of pharmaceutical during storage (without treatment) was investigated. Treated blackwater were stored for a period of 3 and 6 months to mimic the post-storage at the Hölö treatment plant where treated substrate may be stored up to about a half year before agricultural use. After the control storage, the samples were prepared for pharmaceutical analyses (see 3.4.2 and 3.4.3).

3.4 Analysis

3.4.1 Characterization of fecal sludge and blackwater samples

Samples of untreated fecal sludge (UL), untreated blackwater (UR1 and UR2) and post stored blackwater samples (6 months of post storage) were sent to an accredited laboratory (ALcontrol Laboratories) for characterization analysis. The analysis included the following parameters: dry matter (DM), loss on ignition, ignition residue, pH, tot-N, NH_4-N, COD_{Cr}, tot-P, Pb, Cd, Cu, Cr, Hg, Ni, Zn, Ag, Sn, K. Also TOC was analyzed for the liquid composted and ammonia treated blackwater after 6 months of post storage. All values are presented in table 1 in appendix.

Samples of treated blackwater (WR1, WR2, WUR1 and WUR2) were sent to ALcontrol Laboratories for analysis of TS, loss on ignition, ignition residue and TOC. These samples were stored frozen before analysis.

3.4.2 Separation of solid and liquid phases of blackwater and fecal sludge samples

For blackwater, 1.5 L of sample (distributed in six pre-weighted empty 250 mL containers) was centrifuged in a Beckman Coulter J26XPi centrifuge at 10000 rpm (15 344 G) for 10 min, at 15 °C. Before centrifugation, the full 250 mL containers were weighted. After centrifugation, the supernatant (liquid phase) was decanted to 1L polypropylene bottles, pre-rinsed with ethanol, whereas the remaining solid residue was transferred with a spatula to 50 mL polypropylene containers. Before transferring the solids to containers, the centrifugation bottles were also weighted. The centrifugation of the inoculum for the anaerobic digestion process, the untreated fecal sludge (UL) and the treated fecal sludge followed the same procedure as blackwater except that the temperature in the centrifuge was set to +10 °C. After centrifugation, samples were frozen at -20ºC until analysis.

3.4.3 Analysis of pharmaceuticals

Pharmaceuticals were analyzed at the Department of Aquatic Sciences and Assessment (IVM), Swedish University of Agricultural Sciences (SLU), in Uppsala and SP Process Development (SPPD) laboratories in Södertälje (Table 1). Concentration levels of pharmaceuticals were measured in both the liquid and solid phases, in order to investigate the partitioning and distribution of target compounds between the two matrices. For the extraction of target pharmaceuticals in the liquid phase, two extraction methods were used (each lab used one extraction method) whereas for the analysis in the solid phase, one single method was used. The analyses were done in duplicate. Detailed information about the analytical methods is given in the following sections.

Sample preparation and extraction of pharmaceuticals – liquid phase

Method A (pharmaceuticals analyzed at SLU)

The liquid samples were filtered through glass fiber filters (0.7 µm, GF/F, Whatman) to remove particulate matter. The liquid fractions of the blackwater samples were extracted and pre-concentrated by solid phase extraction (SPE) using Oasis HLB cartridges (200 mg, 6cc, Waters). For un-spiked fecal sludge and blackwater samples, 100 mL were extracted whereas for spiked fecal sludge, only 50 mL were used. Prior to SPE, samples were spiked with 50 µL of a 1 ng/µL isotopically labelled internal standard (IS) mixture and an adequate volume of a Na_2EDTA solution (0.1 M) in order to reach a concentration of 0.1% (g solute/g solution) in the samples. The IS Mix included the following IS pharmaceuticals: codeine-d_3, atenolol-d_7, bisoprolol-d_7, azithromycin-d_3, trimethoprim-d_9, ofloxacin-d_3, ciprofloxacin-d_8, sulfamethoxazole-d_4, carbamazepine-d_{10}, venlafaxine-d_6, diazepam-d_5, fluoxetine-d_5, irbesartan-d_7, diltiazem-d_4, furosemide-d_5, hydrochlorothiazide-^{13}C,d_2 atorvastatin-d_5, bezafibrate-d_4, ranitidine-d_6 and lidocaine-d_{10}. The sample pH was then adjusted to 3 using formic acid. Cartridges were conditioned with 6 mL pure methanol followed by 6 mL acidified Millipore water (pH = 3). Samples were loaded at a flow rate of approximately 1 mL/min. Cartridges were washed with Millipore water (pH = 3) and they were centrifuged at 3500 rpm for 5 min to remove excess of water. Analytes were eluted with pure methanol (4x4 mL). Extracts were evaporated until dryness under a N_2 stream and

they were reconstituted with 100 µL methanol and 900 µL Millipore water. Prior to instrumental analysis, extracts were filtered through 0.2 µm regenerated cellulose filters.

Method B (pharmaceuticals analyzed at SPPD)

The standard addition method was used for quantification. Sample preparation was done as follows: 40 mL of both fecal sludge and blackwater was measured followed by the addition of 40 mg Na_2EDTA. These 40 mL were then spiked with 40 uL of an isotopically labelled internal standard mixture. The IS Mix included the following concentration (mg/mL) of IS pharmaceuticals: fluoxetine 1.58, atorvastatine 1.95, bezafibrate 0.89, acetaminophen 19.1, carbamazepine 1.1, ibuprofen 5.8, ceterizine 0.78, bisoprolol 1.9 and caffeine 3.2. Samples were centrifuged at 4000 rpm for 30 minutes at 20°C. An equal amount (40 mL) of phosphoric acid 4% was added, and the resulting sample was then split into four 20 mL sub-samples. Three out of these four sub-samples were spiked with increasing concentrations of a standard mixture, containing target pharmaceuticals; the remaining sub-sample was left unspiked. Spiking levels varied depending on the expected concentrations in the un-spiked sample. Samples were extracted by SPE, using Oasis HLB cartridges (200 mg, 6cc, Waters). Prior to extraction, cartridges were conditioned with 3 mL methanol followed by 3 mL pure water (HPLC quality). Samples were loaded at a rate of approx. 1 mL/min and, when sample loading was finished, cartridges were washed with 5 mL pure water. Elution was done with 5 mL pure methanol. Extracts were evaporated gently to dryness under N_2 stream at approximately +40°C, and they were re-dissolved with 300 µL pure methanol.

Sample preparation and extraction of pharmaceuticals – solid phase

The same extraction method was used for all pharmaceuticals in the solid phase. Solid samples were freeze dried and kept at -20°C until analysis. Prior to analysis, samples were thawed, homogenized and ground using mortar and pestle. The analytical method used was adapted from the one described by Peysson et al. (2013), who analyzed a wide range of multiple-class pharmaceuticals in sewage sludge samples. One g of homogenized sample was weighed in 50 mL polypropylene centrifuge tubes. Samples were spiked with 50µ appropriate amount of a 1 ng/µL isotopically labelled internal standard solution. Afterwards, 7.5 mL of a 0.1 M Na_2EDTA solution followed by 7.5 mL of AcN containing 1% acetic acid were added, with 30 s vortex shaking between each addition. A commercially available buffer salt, consisting of 1.5g NaOC and 6 g $MgSO_4$ was added and the tubes were immediately manually shaken for 30 s to prevent coagulation of $MgSO_4$ and samples were swirled in a vortex for 1 min. Extracts were then centrifuged at 3500 rpm during 15 min. A 6 mL sub-sample of the supernatant (AcN phase) was transferred to another polypropylene tube containing 150 mg primary secondary amine (PSA) and 900 mg $MgSO_4$. The tubes were manually shaken for 30 s and swirled in a vortex mixer for 1 min. Extracts were centrifuged again at 3500 rpm during 15 min and the supernatant was transferred to a glass tube. Extracts were concentrated under N_2 stream until approximately 1 mL AcN. Extracts were kept in the freezer at –20 °C for one hour and afterwards they were centrifuged at 3500 rpm for 5 min. Extracts were transferred to an HPLC amber glass vial and they were afterwards evaporated to complete dryness under N_2

stream. Extracts were reconstituted by adding 300 µL methanol and 700 µL Millipore water. Prior to instrumental analysis, extracts were filtered through 0.2µm regenerated cellulose syringe filters.

3.4.4 Instrumental analysis

Analysis by UHPLC-QTOF (pharmaceuticals analyzed at SLU)

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) was used for the analysis of pharmaceuticals. Chromatographic separation was achieved at a flow rate of 0.5 mL min^{-1}, by chromatographic gradient, and using an Acquity HSS T3 column (100 mm x 2.1mm i.d., 1.8 µm particle size), for the compounds analyzed under positive electrospray ionization (PI) and an Acquity BEH C18 column (50 mm × 2.1 mm i.d., 1.7 µm particle size) for the ones analyzed under negative electrospray ionization (NI). The mobile phases used in PI mode were A) 5mM ammonium formate buffer with 0.01% formic acid and B) acetonitrile with 0.01% formic acid and in NI mode they were A) 5mM ammonium acetate buffer with 0.01% ammonia and B) acetonitrile with 0.01% ammonia. 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 30.000 at full width half maximum (FWHM) at m/z 556. MS data were acquired over an 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. 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-propanol/water. For automated accurate mass measurements, the lock-spray probe was employed, using as lock mass leucine encephalin solution (2 mg/mL) in ACN/water (50/50) with 0.1% formic acid, 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. The criteria used for a positive identification of target pharmaceuticals in the samples was based on: a) the accurate mass measurements of the precursor ion ($[M+H]+$ for PI mode and [M-H]- in NI mode) in the LE function, with an error below 5 ppm, b) the presence of at least one characteristic product ion in the HE function and the exact mass of these fragment ions, with a 5 ppm tolerance, and c) the UHPLC retention time of the compound compared to that of a standard (±2%).

Analysis by UPLC-MS/MS (QqQ) (pharmaceuticals analyzed at SLU)

Instrumental analysis was done with a Waters Ultra- Performance Liquid Chromatograph (UPLC) coupled to a Waters Quattro micro mass spectrometer. In order to separate chromatographically and quantify the acidic, basic and neutral compounds, two methods were developed. Acidic pharmaceuticals were analyzed

under negative electrospray ionization. Chromatographic separation was achieved with a Waters HSS T3 1.8 µm, 2.1 x 150 mm column. Oven temperature was set to +40 °C and the injection volume was 5 µL. Mobile phases for the gradient elution were: A) 0.1% acetic acid in ultrapure water (ELGA) and B) 0.1% acetic acid in acetonitrile (gradient grade) using a flow rate of 0.5 mL/min. The gradient profile was as follows: 75% A, at 0 minutes, hold for one minute and increase to 90% B in seven minutes. The total chromatographic time with reaching back to initial conditions and equilibration was 11 minutes. ESI-capillary was set to 2,8 kV, the desolvation temperature at 320 °C with a flow of nitrogen of 700 L/h and the ion source was set to 110 °C. Basic compounds were analyzed under positive electrospray ionization. Chromatographic separation was achieved with a Waters C18 CSH 1.7 µm 2.1 x 100 mm column and the oven temperature was set to 50 °C. Mobile phases used were: A) 3 mM ammonium formate in ELGA water B) 3 mM ammonium formate in acetonitrile/methanol 90/10 using a flow rate 0.5 mL/min. The injection volume was 5 µL. The gradient profile used was: 88% A at 0 minute, hold for one minute and then increase to 86% B. Total chromatographic time 10 minutes. ESI-capillary voltage was set to 3 kV. Ion source temperature, desolvation temperature and flow rate are the same as for negative ionization. Target pharmaceuticals were identified and quantified using the Selected Reaction Monitoring Mode (SRM), using a dwell time of 0.3 seconds.

3.5 Risk analysis

3.5.1 Approach and methods

For the analyses of the risks associated with fertilization with blackwater, a brainstorming session with a group of stakeholders was held to discuss and decide: (1) types of recipients of pharmaceuticals (soil, surface water or ground water), (2) types of crops in which uptake of pharmaceuticals are of importance as animal feed and human food on the Swedish market (grain/cereals, vegetables, forage or root crops) and (3) classes of pharmaceuticals which might pose risks for humans and the environment. The stakeholders group included representatives from: KRAV (Lars Hällbom), Arla Food (Anna Karin Modin Edman), Stockholm County Council (Börje Wreden), Telge Nät AB (Anna Calo), and the blackwater treatment facility in Hölö, Södertälje (Jan-Christer Carlsson). Based on this brainstorming, it was decided to focus on the soil environment, assess the potential for accumulation of pharmaceuticals in soil and uptake by wheat, forage crops (Swedish ‘vall’), root crops, and rape seed (if possible). This decision was taken even though it can be discussed whether the use for forage crops or root crops are legally allowed, as such use of sewage sludge is not legally allowed. Thus, a worst case approach was preferred by the stakeholder group. In line with this, representative compounds of each therapeutic group were selected. Some of these substances were detected at high concentrations in the liquid phase. These compounds were ciprofloxacin, carbamazepine, oxazepam, venlafaxine, metoprolol, hydrochlorothiazide, losartan, furosemide and diclofenac were selected representing beta-blockers, antibiotics, anti-inflammatories, anti-depressants and anti-hypertensives. Hormones were also recommended, but could not be included among the selected pharmaceuticals, because they were not analyzed in our study.

Blackwater is mostly spread onto fields after liquid composting, hygienization and storage for up to six months. However, plant uptake and soil accumulation of pharmaceuticals were simulated for the concentrations that were measured in blackwater directly after liquid composting and ammonia treatment, prior to storage, in order to analyze a worst case scenario.

The behavior of the selected pharmaceuticals upon blackwater application as well as uptake of pharmaceuticals by crops were modelled using Biosolids Amended Soil Level 4 model (BASL4) (Hughes & Mackay, 2011). BASL4 uses the fugacity approach to model the dynamic fate of organic chemicals. Transport and loss processes such as chemical degradation, volatilization, leaching, diffusion, sorbed phase transport due to bioturbation, and the degradation of the organic matter present in the soil and amendment are quantified. Based on these quantifications, the model generates predicted environmental concentrations (PECs) of the chemicals in soil, soil pore water, air, plants and soil macro flora. The possibility to validate the performance of BASL4 for simulations of pharmaceuticals in soil and plant is limited due to scarcity of suitable experimental and field measurement data. It is also well known that fugacity model predictions do not take into account for all processes determining the fate of environmental pollutants, e.g. binding to mineral surfaces, and may therefore occasionally yield significantly biased predictions.

Yet, the model has been tested for other organic pollutants e.g. DDT, benzene, and 2,4-D, showing reasonable agreement between the predicted and the observed levels (Hughes & Mackay, 2011). Despite the absence of appropriate validation data for pharmaceuticals, BASL4 was chosen as the best available tool for the purpose of the project, allowing rough estimates for soil accumulation and plant uptake of pharmaceuticals in soil fertilized with blackwater. The model was downloaded for free from the Canadian Environmental Modelling Centre website (www.trentu.ca/cemc).

The BASL4 model was built to determine concentrations of chemicals in roots and leaves in root crops (carrots) and leafy crops (grasses).The focus in this study was on uptake of pharmaceuticals by wheat, rape seeds and root crops (crops in which PPCPs can reach to human body by direct consumption) and by wheat and forage crops (in which PPCPs can reach human being by consuming milk or meat of animals fed with these crops). Since the model cannot predict concentrations in wheat grains, it was decided to calculate uptake of PPCP in grass, which was thought to be representative for forage grass (In Swedish: vall) and also for the wheat crop but at early stages before kernels develop. Translocation of PPCPs from wheat leafs to kernels was then discussed in the view of the available literature.

Chemicals with low K_{OW} (i.e., log $K_{OW} < 1$) are generally too lipophobic to enter the root system from soil pore water and those with high K_{OW} (log $K_{OW} > 2.5$) are sparingly soluble in plant xylem and phloem fluids (Duarte-Davidson and Jones (1996). The latter, are therefore most likely to be found sorbed to the lipid-like material of the plant, e.g. the waxy cuticle of a leaf or the lipid content of a root surface. Since carrot roots have relatively high lipid contents, they will give worst case scenario concentrations of the more hydrophobic substances in plants in general. Several studies of organic chemical uptake in plants suggest that high K_{OW} compounds present in soil remain bound to the soil organic matter (OM) and

are usually not found in significant quantities in plants other than in the root peel of some relatively high-lipid-content tubers, such as carrots (Duarte-Davidson & Jones, 1996; O'Connor, 1996; Wild & Jones, 1995).

Accumulation of pharmaceuticals in soil and plant was simulated for a blackwater application period of 3 years, during which the treated blackwater was assumed to be applied once every year. Figure 5 shows schematic diagram of the modelled blackwater fertilization scenario.

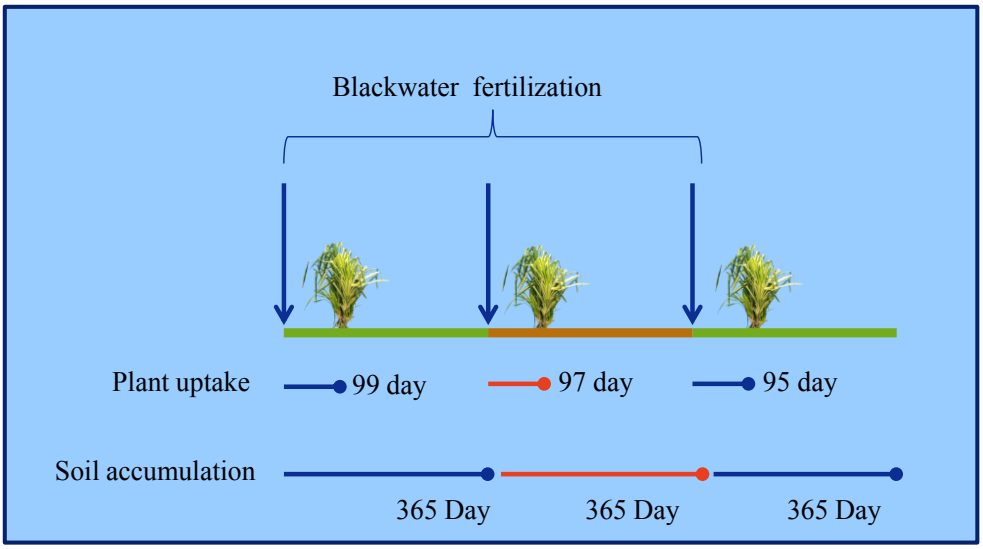


Figure 5. Blackwater application scheme and times, marked by dots, at which the plant uptake and soil accumulation were simulated using BASL4.

Calculations of the application loads of pharmaceuticals and modelling of pharmaceuticals accumulation in soil and plants is given in the following sections.

3.5.2 Calculations of application loads of pharmaceuticals in soil

The concentrations of the PPCPs applied onto the soil were estimated using the following equations:

Total concentration of pharmaceuticals in the treated blackwater = concentations of pharmceutical in solid fraction $\left(\frac{\text{ng}}{\text{g}}\right) \times \text{DM} \left(\frac{\text{g}}{\text{L}}\right) +$ concentrations of pharmaceuticals in liquid fractions $\left(\frac{\text{ng}}{\text{L}}\right)$

Eq.1

PPCPs application rate (µg/ ha) = blackwater application rate (m^3/ha) x Concentration of PPCPs in the blackwater (µg/m^3)

Eq. 2

Blackwater application rate (m^3/ha) = blackwater application rate (kg/ha) ÷ blackwater density (kg/m^3)

(Eq.3)

PPCPs application rate (kg PPCPs / m^3 soil) = application rate of PPCP (kg PPCPs/ha) ÷ (soil depth (m) x 10000 m^2/ha)

Eq. 4

Concentrations of PPCPs in soils (kg PPCPs/kg soil) = PPCPs application rate (kg PPCPs / m^3 soil) ÷ Density of soil (kg/m^3)

Eq.5

Blackwater application rate was assumed to be 40 ton/ha, which corresponds to about 90 kg N/ha, which is the same as commonly applied in Hölö (Personal communication with Jan Christer Carlsson – Nackunga gård, Hölö, 12th March 2015). Due to low content of total solid in the treated blackwater, its density was assumed to be the same as for water (1000 kg/m^3). The soil density was assumed to be (1440 kg/m^3) (database for Ultuna soil plot trial 1956-2009).

3.5.3 Modeling of pharmaceuticals accumulation in soil and plant

Input data into BASL4 model

PPCPs Properties

The following characteristics of the PPCPs compounds were needed to characterize the concentration changes over time, the evaporation, and the uptake of the PPCPs in the soils:

- Molar mass (g/mole)
- Degradation half-life in soil, which is the time during which 50% of the compound degrades in soil (days)
- Water solubility (mg/L)
- Vapor pressure (Pa)
- Octanol-water partitioning coefficient (K_{OW})
- Mineral matter-water partitioning coefficient (K_{MW}; L/kg)
- Organic carbon- water partitioning coefficient (K_{OC}; L/kg)

All compound characteristics were obtained from the *ChemSpider* database (Chemspider, 2015) except the degradation half-life in soil mineral matter-water partitioning coefficient, and the organic carbon–water partitioning coefficient. Degradation half-life was obtained from experimental data from literature (Table 4). The solid partitioning coefficient (K_d) values of the selected compounds were determined according to (Tolls, 2001), thus estimated using Equation 6, and were compared with other data obtained from available literature (Table 4).

$$K_d \text{ (L/kg)} = 1000 \times \frac{\text{concentration in the solid phase (dry matter) } (\frac{ng}{g})}{\text{concentration in the liquid phase } (\frac{ng}{L})}$$

Eq.6

The organic carbon-water portioning coefficient (K_{OC}) was estimated using Equation 7 below

$$K_{OC} \text{ (L/kg)} = K_d \times \frac{TOC}{DM}$$

Eq.7

Where TOC is the total organic carbon of the blackwater (mg/L) and the DM is the total dry matter in the blackwater (mg/L).

Mineral matter-water partitioning coefficients (K_{MW}) were approximated using Equation 8 based on minerals and metals in urine and black water as obtained from Jönsson (2000).

$$K_{MW}\ (L/kg) = K_d \times \frac{\sum[NH4]+[NO3]+[TP]+[TS]+[TK]+[CO3]+[Cu]+[Zn]+[Pb]+[Cd]+[Hg]+[Cr]+[Ni]+[Zn] \text{ in urine and feces}}{\sum DM \text{ in urine} + DM \text{ in faeces}}$$

Eq.8

Table 4. Properties of selected compounds including K_{OW} (octanol-water partitioning coefficient), K_d (solids-water partitioning coefficient), K_{OC} (organic carbon-water partitioning coefficient), and K_{MW} (mineral matter-water partitioning coefficient)

Compound	Molar weight (g/mole)	Half- life in soil (days)	Water solubility at 25 °C (mg/L)	Vapor Pressure (Pa)	Log K_{OW}	Estimated K_d in this study (L/kg)	Estimated K_d in other studies (L/kg)	Estimated K_{OC} (L/kg)[n]	Estimated Log $K_{OC\text{-}soil}$	Estimated K_{MW} (L/kg)[m]
Ciprofloxacin	331.341	1155–3466	11480	3.8×10^{-11}	0.28		430[c]	98.9	1.55	400
Carbamazepine	236.269	462–533[a]	17.66	1.3×10^{-5}	2.45	126	20.1[d]	29	3.588	117
Oxazepam	286.713	75	179.1	5.55×10^{-10}	2.24	205	1100 [e]	47.2	2.761	191
Venlafaxine	277.402	120	266.7	3.28×10^{-5}	3.28[o]	92	100 [e]	21.2	3.166	86
Metoprolol	267.364	75	4777	3.84×10^{-5}	1.88	88	15[f]	20.2	1.794	82
Hydrochloro-thiazide	297.739	9-11[b]	1292	2.38×10^{-8}	-0.07	83	4366[j]	0.5	1.901	1.9
Sucralose	397.64	75	22750	4.33×10^{-11}	-1	36	0.2 [h]	8.3	1	33
Losartan	422,911	75	0.938	7.25×10^{-16}	4.01	30	15.1[i]	6.9	5.148	28
Furosemide	330.75	120	149.3	4.08×10^{-9}	2.03	1	158 [k]	3.5	2.043	14
Diclofenac	296.149	3-20[p]	4.518	8.19×10^{-6}	4.51	16	16 [m]	3.68	2.921	15

[a](Walters et al., 2010), [b] digested sludge (Lin & Gan, 2011); [c]Kd in soil (Tolls, 2001); [d]Digested sludge (Carballa et al., 2008); [e]Adsorption coeeficient using linear model for primary sludge (Hörsing et al., 2011); [f] Digested sludge(Scheurer et al., 2010): [h] Assumed based on the fact that Sucralose is a very hydrophilic and persistence to soil sorption(Hoque et al., 2014); [i]Kd in soil (Eriksen et al., 2009); [j]Estuary (Lara-Martín et al., 2014); [l]K_d in primary sludge (Ternes et al., 2004); [k]Kd in sludge (Thomas et al., 2010); [m] K_{MW} was estimated as 0.93 x K_d using Eq. 8; [n]K_{OC} was estimated as 0.23 x K_d using Eq. 7 [p] (Al-Rajab et al., 2010).

Soil properties

The following soil properties were used as input data in the model.

Table 5. Soil properties

Parameter	Value	Unit	Source	Comment
Density of organic matter	1000	kg/m^3	BASL4	This value lies within the range reported in the literature.
Density of inorganic matter	2500	kg/m^3	BASL4	This value lies within the range reported in the literature.
Air boundary layer thickness	4.75	mm	BASL4	Very little data found about this parameter. Thus we adopted the default value of BASL4.
Molecular diffusivity- air	0.018	m^2/h	BASL4	Chemical-specific property. Difficult to find data about this parameter, and thus we adopted the default value in BASL4.
Molecular diffusivity- water	1.8×10^{-6}	m^2/h	BASL4	Chemical-specific property. Difficult to find data about this parameter and thus adopted the default value in BASL4.
Leaching rate	5	mm/day	Estimated based on hydraulic conductivity	Site specific. The value used represent the 35% of the saturated hydraulic conductivity measured by Myrbeck et al. (2012).
Bioturbation rate	0.3	cm/year	BASL4	-
Bioavailability factor	1	-	BASL4	-

Table 6. Model parameters used for two-layers soil

Soil Layer parameter	Unit	Layer 1	Layer 2	Reference/ comment
Soil depth	m	0.20	0.50	SLU, Dept. of Soil and Environment
Diffusion distance	m	0.125	0.325	BASL4
Volume fraction of air		0.10	0.09	Andersson and Wiklert (1977)
Volume fraction of water		0.39	0.36	Andersson and Wiklert (1977)
Mass of organic matter	g OC/g soil solids	0.035	0.025	Organic content in the upper layer (0-20 cm) of the agricultural soils in the study area ranges from 3 to 5% (Jordbruksverket, 2015)
Fraction of fast degraded organic matter	g/g OC	0.53	0.53	(Andrén & Kätterer, 1997)
Fraction of slowly degraded organic matter	g/g OC	0.47	0.47	(Andrén & Kätterer, 1997)
Fraction of organic carbon/ organic matter	DOC/ g OM	0.58	0.58	(Gerzabek et al., 2006)
Degradation half-life of fast degrading organic carbon	days	60	60	BASL4
Degradation half-life of slow degrading organic carbon	days	25550	25550	Assumed value

Sludge properties and application modes

The dry matter (DM), loss on ignition (LOI) and total organic carbon (TOC) of the untreated blackwater and blackwater after liquid composting and ammonia treatment are summarized in Table 7. The fractions of fast and slow degrading organic carbon in the liquid composted and ammonia treated blackwater used as fertilizer are given in Table 8.

Surface application of sludge onto the top soil is the most common mode of application in Sweden. Thus, the blackwater was assumed to be applied on the surface layer of the soil (the top 20 cm of the soil profile), followed by one plowing event (Table 8). Blackwater amendment was calculated based on dry content of the organic matter, and the chemical concentration in the amendment was specified in units of mg/kg dry amendment (Table 9). This simplification was thought to be acceptable because the soil layer into which the amendment is applied is mixed, and the water content of the blackwater and soil probably adjust to a common value within some hours after application. The slight increase in

the soil's water retention capacity with increased organic matter was ignored. Layer depths change as a result of changes in the quantity of organic matter present, but these changes are considered sufficiently small that diffusion distances remain constant. Leaching and diffusion rates thus remain unchanged throughout the simulation. The model did not simulate runoff.

Table 7. The composition of untreated blackwater (UR) as well as liquid composted and ammonia treated blackwater (WUR) for the two reactors as well as the average for the two

	Parameter	Reactor 1 (mg/L)	Reactor 2 (mg/L)	Average (mg/L)
UR	DM	4400	3600	4000
	Loss of ignition	2900	2300	2600
	TOC	Not measured	Not measured	
WUR	DM	2100	2300	2200
	TOC	1100	1200	1150

Table 8. Blackwater (WUR) application modes in the BASL4 simulation

Parameter	Value	Comment
Number of blackwater applications	3	
Time of application (days)	1, 365, 731	Blackwater application assumed to be done once per year at the beginning of growing season.
Method of application	Surface application	
Fraction of fast degrading organic carbon in blackwater	0.78	It is assumed that the fast degrading organic carbon to the slow degrading organic carbon will approximate the fraction of BOD/COD of black-water. The BOD/COD of the blackwater was obtained from Jönsson et al (2003) for untreated blackwater.
Fraction of slow degrading OC in blackwater	0.22	Calculated as 1 minus fraction of fast degrading organic carbon in blackwater.
Number of ploughing events per year	1	
Time of ploughing (days)	2, 366, 732	It is assumed that soil ploughing is done 1day after blackwater application.
Total simulated time (h)	26280	3 year period

Table 9. Concentration of pharmaceuticals (mg/kg dw) and application rates (kg/ha) in the applied 40 ton (Mg) of blackwater after liquid composting and ammonia treatment

	Concentration (mg/kg dw)	Application rates (g/ha)
Ciprofloxacin	0.3412	0. 0300
Carbamazepine	1.238	0. 1089
Oxazepam	2.4543	0. 2160
Venlafaxine	3.0998	0. 2728
Metoprolol	2.8194	0. 2481
Hydrochlorothiazide	1.0093	0. 0888
Losartan	5.5906	0. 4920
Furosemide	28.919	2.5449
Diclofenac	1.5686	0. 1380

3.5.4 Risk assessment of consumption of contaminated crops by human

The estimated daily intake (EDI) of pharmaceuticals through consumption of wheat and carrot fertilized with blackwater was determined by multiplying the daily consumption of the wheat and carrot with the corresponding simulated concentration of each pharmaceutical as obtained from BASL 4 using wet weight (ww) basis. Since BASL 4 estimate the concentration of the pharmaceuticals in wheat grass, a bioaccumulation factor of 0.3 were assumed to estimate the pharmaceuticals concentrations in wheat grains. The 0.3 bioaccumulation factor was adapted from Eggen et al. (2011) for barley.

The EDI due to consumption wheat and carrot fertilized with treated blackwater was calculated as a fraction of, and thus compared with the acceptable daily intake (ADI), which is the amount of pharmaceutical that can be consumed daily during the person life span without having adverse effects as reported by Prosser and Sibley (2015).

Ideally, the ADI should be calculated using the "No observable adverse effects" level (NOAEL) (NHMRCA, 2008). Due to the absence of the NOAEL for the selected compounds, the ADI per kg was calculated by dividing the lowest therapeutic dose for adults (mg/day) by a safety factor and average body weight of 76 kg. This approach was followed by DEFRA (2007) and Prosser et al. (2015) using an overall factor of safety of 1000. The 1000 factor of safety comprised of 10 for differences in response between humans, 10 for the lowest therapeutic dose not being a no-effect level, 10 for cytotoxic drugs; due to the higher level of toxicity associated with these compounds (DEFRA, 2007; NHMRCA, 2008). In the current study, we added an additional safety factor of 10 to account for any other unknown or unpredicted effects, thus the overall safety factor in this study was 10 000.

To make the low simulated intakes a little bit easier to grasp, and to compare them with intakes that the population presently are exposed to, the simulated EDIs through consumption of wheat and carrot were compared to EDI due to drinking tap water in Stockholm or eating perch fish from River Fyris downstream Kungsängsverket, Uppsala, using pharmaceutical concentrations reported by Fick et al. (2011).

To assess whether the hazard is acceptable or not, usually a hazard quotient and a hazard index are defined. The hazard quotient is the ratio of the expected estimated daily intake to the acceptable daily intake for kids and adults, whilst the hazard index is the sum of the hazard quotients for a pollutant from different pathways. Besides consumption of wheat and carrot, intake of pharmaceuticals may occur through e.g. consumption of other matrices such as milk, water, fish and meat, inhalation and/or skin application. Therefore, we only adapted a hazard quotient to assess the hazard. For the purpose of this study, compound which showed a hazard quotient <1 were considered to have tolerable hazard and no further assessment was necessary (European Agency for the Evaluation of Medicinal Products, 2001; Sanderson et al., 2004) for these.

For the purpose of the aforementioned calculations, the following basic data were used: the daily consumption of wheat (wheat flour and pasta) and fish were 45 g, and 20 g/day/person (Jordbruksverket, 2009) for adults; Children were assumed to consume 70% of these amounts; The daily consumption of carrot was 12 g for adults and 11 g for children (Beckman, 2015); Average body weight of adult and children were 76.6 kg and 18 kg (Beckman, 2015).

4. Results and discussion

4.1 Occurrence of pharmaceuticals in blackwater and fecal sludge

Tables 10 and 11 show the concentrations of the pharmaceuticals detected in untreated fecal sludge and blackwater samples. Results are given for both the liquid and solid phases. Three pharmaceuticals were measured by both SPPD and SLU (sulfamethoxazole, carbamazepine and furosemide) for comparison. Concentrations in the liquid phase were in good agreement between the two laboratories. However, some differences were observed in the solid phase (see tables 10 and 11). It is difficult to explain the reason for this discrepancy. However, differences are not un-expected because of the complexity of the samples and because different instrumental analytical methods were used for the analysis In general, concentrations fall within the high μg/L and μg/kg dry weight (dw) range for liquid and solids, respectively. The concentrations are higher than those reported for urban influent wastewater and sewage sludge (Gros et al., 2010; Jelic et al., 2011; Radjenovic et al., 2009), where levels rarely reach high μg/L levels (i.e. 10 μg/L) for wastewater. This is expected, since blackwater and fecal sludge are about 25 times more concentrated than waste water and sludge from conventional domestic WWTPs (de Graaffet al., 2011).

Most of the target compounds tend to partition to the liquid phase, in both blackwater and fecal sludge. The substances that were found at the highest concentrations are ibuprofen (~100 μg/L in blackwater and fecal sludge), naproxen (~70 μg/L in blackwater and fecal sludge), metoprolol (~10 μg/L in blackwater and ~48 μg/L in fecal sludge), losartan (~10 μg/L in blackwater and 32 μg/L in fecal sludge), valsartan (~12 μg/L in blackwater and ~100 μg/L in fecal sludge), furosemide (~30 μg/L in blackwater and ~10 μg/L in fecal sludge) and hydrochlorothiazide (~14 μg/L in blackwater and ~32 μg/L in fecal sludge). However, concentrations found in the solids were also significant, especially for propranolol, oxazepam, citalopram, amitriptyline and venlafaxine in blackwater, and for atenolol, metoprolol, losartan, carbamazepine, losartan, irbesartan and hydrochlorothiazide in fecal sludge (Table 10 and 11). These results show that both

solid and liquid phases have to be evaluated when studying the occurrence and fate of pharmaceuticals in blackwater and fecal sludge. High levels of certain pharmaceuticals in both fecal sludge and blackwater could be explained by their consumption (see 3.1) and by their pharmacokinetic behavior. For instance, for atenolol, a large percentage of an administered dose is excreted via urine and feces, whereas for metoprolol the main route of excretion is urine and only about 5% of an administered dose is excreted as the unchanged parent compound (Reeves et al., 1978; Regårdh et al., 1980). For valsartan, fecal excretion is predominant (86% of a dose) and it is largely excreted as non-metabolized drug (81.5% of a dose in the excreta) (Waldmeier et al. 1997). Irbesartan is also significantly excreted via feces (Chando et al., 1998), whereas hydrochlorothiazide and furosemide are excreted via urine in large proportion as an unchanged drug (Beermann et al., 1976; Calesnick et al., 1966). For analgesics and anti-inflammatories, naproxen is mainly excreted via urine and a small proportion (1-2%) is also excreted in feces (Runkel et al., 1972).

Concentrations detected in blackwater in our study match quite well with those reported by other authors. De Graaff et al. (2011), who evaluated the occurrence and removal of pharmaceuticals during blackwater anaerobic treatment followed by a nitritation-annamox process in the Netherlands, found high concentrations of metoprolol (45 μg/L), ibuprofen (147 μg/L), propranolol (1 μg/L), carbamazepine (1.1 μg/L) and cetirizine (1.4 μg/L) in untreated blackwater samples. Winker et al. (2008) analyzed several pharmaceuticals in urine samples in Germany and they detected even higher mean concentration levels of carbamazepine (24 μg/L), diclofenac (20 μg/L) and ibuprofen (446 μg/L), respectively. Butkovskyi et al. (2015) determined the occurrence and removal of 14 multiple class pharmaceuticals in an up-flow anaerobic sludge blanket (UASB) reactor in the Netherlands and found high pharmaceutical levels in the untreated blackwater influent samples, with values exceeding 100 μg/L for naproxen, ibuprofen, hydrochlorothiazide, metoprolol and ciprofloxacin. Concentrations for acetaminophen (paracetamol) ranged from 2.7 to 7.0 mg/L. Within a Swedish context, previous results that report concentrations of several pharmaceuticals in untreated black-water samples from the treatment plant at Hölö are in quite good agreement with the findings in our study (Palm Cousins and Magnér, 2014). Pharmaceutical concentrations detected were quite similar for certain compounds, such as metoprolol (15 μg/L), furosemide (28 μg/L) and ibuprofen (160 μg/L), among others. However, for other substances, Palm Cousins and Magnér (2014) measured higher concentrations, e.g. for citalopram, which was detected at 2.9 μg/L and ciprofloxacin, which was found at 6.1 μg/L. Some deviations in concentration levels are expected, since samples were taken at different periods of the year. Hence, differences could be attributed to the fact that the blackwater of each batch is made up from black water from different households, and to changes in health and pharmaceutical usage by the population.

Table10. Concentrations of pharmaceuticals in the liquid and solid phase of blackwater and fecal sludge (n=2)

		Target Pharmaceuticals analyzed at SLU					
Therapeutic group	**Compound**	**Blackwater liquid R1 (µg/L)**	**Blackwater liquid R2 (µg/L)**	**Blackwater solid R1 (µg/kg dw)**	**Blackwater solid R2 (µg/kg dw)**	**Fecal sludge liquid (µg/L)**	**Fecal sludge solid (µg/kg dw)**
Analgesics	Codeine	1.60±0.12	1.23±0.12	90±30	61±8	<LOD	140±30
β-blockers	Atenolol	4.7±1.4	5.2±1.4	<LOQ	<LOQ	1.7±0.10	2400±500
	Sotalol	<LOD	<LOD	<LOD	<LOD	<LOD	130±30
	Metoprolol	9.5±1.3	11.3±1.2	383±23	314±13	48±3	1250±160
	Propranolol	4.8±1.4	6.5±1.3	2380±240	2000±500	0.73±0.09	350±90
Antibiotics	Azithromycin	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD
	Clarithromycin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Norfloxacin	<LOD	<LOD	-	-	<LOD	-
	Ciprofloxacin	1.0±0.6	<LOQ	-	-	<LOD	-
	Ofloxacin	<LOD	<LOD	-	-	<LOD	-
	Sulfamethoxazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Trimethoprim	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anti-hypertensives	Losartan	10±0.3	10.6±0.016	680±130	510±40	32±4	7400±1800
	Valsartan	12.0±0.5	11.4±0.24	<LOD	<LOD	180±90	120±50
	Irbesartan	<LOD	<LOD	<LOD	<LOD	<LOD	1200±300
	Diltiazem	<LOD	<LOD	<LOD	<LOD	<LOD	76±12
Anti-depressants	Carbamazepine	3.4±1.1	2.3±1.1	183.5±1.4	120±30	16±3	1540±170
	Citalopram	0.310±0.020	0.31±0.04	940±40	800±50	<LOD	300±80
	Diazepam	0.048±0.004	0.043±0.004	<LOQ	<LOQ	<LOD	<LOD
	Lamotrigine	7.3±1.2	8.6±1.7	340±50	230±50	1.6±0.3	430±70
	Oxazepam	4.8±0.8	4.6±1.1	1600±400	1200±500	<LOD	380±130
	Venlafaxine	6.4±1.4	7.5±1.4	710±80	540±50	12±4	630±70
	Fuoxetine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Amitriptyline	<LOQ	<LOQ	430±60	380±80	<LOD	<LOD
Anti-ulcer agent	Ranitidine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anti-fungal agents	Climbazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Ketoconazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Local anesthetic	Lidocaine	0.65±0.03	0.59±0.01	12.8±2.4	9.51±0.10	1.0	<LOD
Diuretics	Furosemide	37±7	34±7	203±22	300±70	10.3±1.3	570±60
	Hydrochlorothiazide	14±4	14.5±0.6	514±23	400±100	27±12	1090±120
Lipid regulators	Atorvastatin	0.72±0.05	0.70±0.03	-	-	<LOD	-
	Bezafibrate	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

<LOD: below limit of detection (Appendix, Table 2); <LOQ: below limit of quantification (Appendix, Table 2); dw: dry weight; - no analysis due to limits in extraction method of solid phase.

Table 11. Concentrations of pharmaceuticals in the liquid and solid phases of blackwater and fecal sludge (n=2)

		Target pharmaceuticals analyzed at SPPD					
Therapeutic group	**Compound**	**Blackwater liquid R1 (µg/L)**	**Blackwater liquid R2 (µg/L)**	**Blackwater solid R1 (µg/kg dw)**	**Blackwater solid R2 (µg/kg dw)**	**Fecal sludge liquid (µg/L)**	**Fecal sludge solid (µg/kg d.w.)**
Analgesics and anti-inflammatories	Ibuprofen	76 ± 16	108 ± 35	174 ± 106	25± 15	112 ± 5	151 ± 14
	Naproxen	71 ± 15	26 ± 11	69± 22	40 ± 5	41.4 ± 1.2	105 ± 10
	Diclofenac	3.2 ± 1.4	2.5 ± 1.6	12 ± 8	17 ± 9	2.1 ± 0.2	8 ± 3
	Acetaminophen	<LOD	15 ± 4	16 ± 4	23 ± 7	66.9 ± 1.2	1890 ± 70
	Budesonide	<LOQ	<LOQ	<LOQ	<LOQ	1.6 ± 0.3	<LOQ
Anti-hypertensives	Candesartan	3.7 ± 1.3	7.2 ± 1.3	<LOQ	<LOQ	<LOQ	<LOQ
	Ramipril	2.5 ± 1.9	0.16 ± 0.09	4 ± 4	3 ± 4	0.23 ± 0	<LOQ
	Amlodipine	<LOQ	0.050 ± 0.001	24 ± 14	15 ± 11	0.38 ± 0.02	14 ± 3
Lipid regulators	Atorvastatin	1.8 ± 1.1	1.8 ± 0.9	8.9 ± 0.2	9.1 ± 1.2	3.8 ± 0.2	<LOQ
Anti-diabetic	Saxagliptine	<LOD	<LOD	0.2 ± 0.1	0.11 ± 0.08	<LOQ	<LOQ
Antibiotic	Sulfamethoxazole	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ
Anti-histaminic	Cetirizine	4 ± 3	1.6 ± 0.6	51 ± 3	46 ± 20	0.28 ± 0.03	22.0 ± 0.4
Anti-depressants	Carbamazepine	1.8± 0.1	2.2 ± 1.1	61.6 ± 2.2	64 ± 0	5.02 ± 0.04	315 ± 8
	Fluoxetine	<LOQ	<LOQ	11.1 ± 0.6	8.5 ± 0.3	0.18 ± 0	<LOQ
Diuretic	Furosemide	62 ± 36	36 ± 18	20.0 ± 2.3	25 ± 6	7.47 ± 0.04	64 ± 20
β-blockers	Bisoprolol	0.5 ± 0.4	0.7 ± 0.2	36.7 ± 4.4	45± 15	0.2 ± 0	18.2 ± 0.8
Stimulant	Caffeine	11.3 ± 1.9	24 ± 8	700 ± 68	509 ± 5	3.9 ± 0.1	69± 3

<LOD: below limit of detection (Appendix, Table 3); <LOQ: below limit of quantification (Appendix, Table 3); dw: dry weight.

4.2 Treatment efficiency

4.2.1 Reduction of pharmaceuticals during anaerobic digestion

The samples used for the pharmaceutical analysis in the anaerobic degradation experiments were a mixture of fecal sludge and inoculum from a biogas reactor. The inoculum was taken from two different WWTPs, one mesophilic and one thermophilic. The use of different inocula for the mesophilic and thermophilic experiment could explain the differences in substances detected in each experiment and their concentration. Out of the 44 pharmaceuticals analyzed, 30 substances were found in at least one sample at concentrations above the limit of quantification (LOQ) before treatment in the non-spiked samples. In total, 27 pharmaceuticals were detected in both mesophilic and thermophilic non-spiked samples, and after treatment, 23 were still found (Fig. 6 A and B). Clarithromycin and amlodipine were not detected in mesophilic samples, whereas azithromycin, oxazepam and ramipril were not found in thermophilic samples. For ciprofloxacin and atorvastatin, the concentration before and after treatment were calculated only using the amounts detected in the liquid phase, because the extraction method for the solid phase did not perform well for these two substances. Since ciprofloxacin has a tendency to sorb onto the solid phase the problem with the extraction may be one explanation to why ciprofloxacin is below LOQ in both mesophilic and thermophilic sample although it was added in the spiked samples.

After 60 days of anaerobic digestion of fecal sludge, only naproxen and caffeine were significantly reduced at both temperatures ($p<0.05$; t-test, Fig. 6 B). The high removal of these substances during anaerobic digestion of blackwater or sewage sludge is previously reported (Malmborg and Magnér, 2015; Samaras et al., 2014; de Graaff et al., 2011; Carballa et al., 2007). Carballa et al. (2007) suggest an order of removal for the analgesic and anti-inflammatory compounds, naproxen > diclofenac > ibuprofen. This suggestion is only in partial agreement with the result in our study, since neither diclofenac nor ibuprofen was significantly reduced at any temperature. The added antibiotics (sulfamethoxazole and trimethoprim) were only found before treatment after addition in spiked samples and showed a 100% removal at both mesophilic and thermophilic temperature (Fig. 7), which agrees with previous studies (Malmborg and Magnér, 2015; Narumiya et al. 2013; Carballa et al. 2007). Additional five pharmaceuticals (atenolol, metoprolol, hydrochlorothiazide, irbesartan and bezafibrate were significantly reduced only at thermophilic temperature ($p<0.05$; t-test, Fig. 6 A). The reduction of atenolol, hydrochlorothiazide and bezafibrate at thermophilic temperature is supported by findings in literature (Malmborg and Magnér, 2015; Narumiya et al. 2013). However, when metoprolol and furosemide was added, no reduction was shown at any temperature ($p<0.05$, t-test, Fig. 7). Metoprolol is known to be reduced in a mesophilic UASB reactor treating blackwater (Butkovskyi et al. 2015) and during anaerobic digestion of sewage sludge in semi-continuous laboratory-scale reactors at mesophilic temperature, but not under thermophilic conditions (Malmborg and Magnér, 2015).

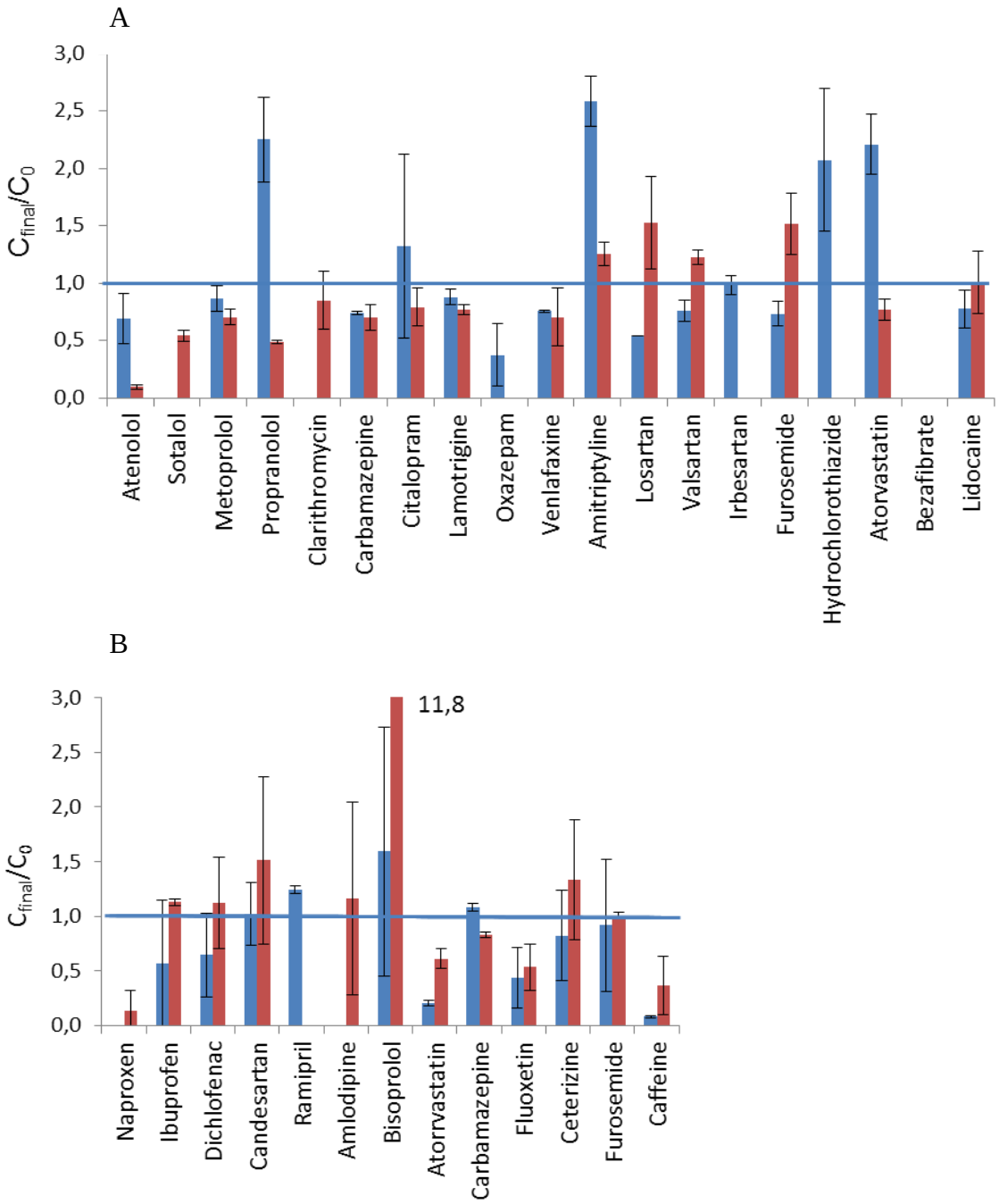


Figure 6. Changes of pharmaceutical concentrations in non-spiked fecal sludge after mesophilic (+37 °C, 61 days, blue bars) and thermophilic (+52 °C, 59 days, red bars) anaerobic treatment (n=2). A). Substances analyzed at SLU and B) substances analyzed at SPPD. Values above 1 indicate an increase in concentration during treatment and below 1 indicate a reduction in concentration during treatment. The change of pharmaceutical 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/L and is the sum between the amounts detected in the solid and liquid phase.

Many compounds were unaffected by the anaerobic treatment although earlier results have revealed reduction. Bergersen et al. (2012), Butkovskyi et al. 2015 and Malmborg and Magnér (2015) showed decrease of e.g. furosemide (45-50%), citalopram (11-85%), fluoxetine (32%), and oxazepam (72-85%) during mesophilic and thermophilic anaerobic digestion of sewage sludge. The difference between studies could be due to e.g. differences in the experimental systems (batch vs

continuous reactor, fecal sludge vs sewage sludge), the active microorganisms or analytical methods. Independent of whether the compound was added or found in the samples originally, carbamazepine and propranolol, were not significantly degraded (Fig. 6 and 7), which is in good agreement with earlier studies where this substances showed to be unaffected by anaerobic digestion (Malmborg and Magnér, 2015; Narumiya et al. 2013; de Graaff et al. 2011; Carballa et al. 2007). A few compounds showed significant increase ($p<0.05$; t-test) at either mesophilic (atorvastatin, hydrochlorothiazide and amitriptyline; Fig. 6 A) or thermophilic temperature (bisoprolol; Fig 6 B).

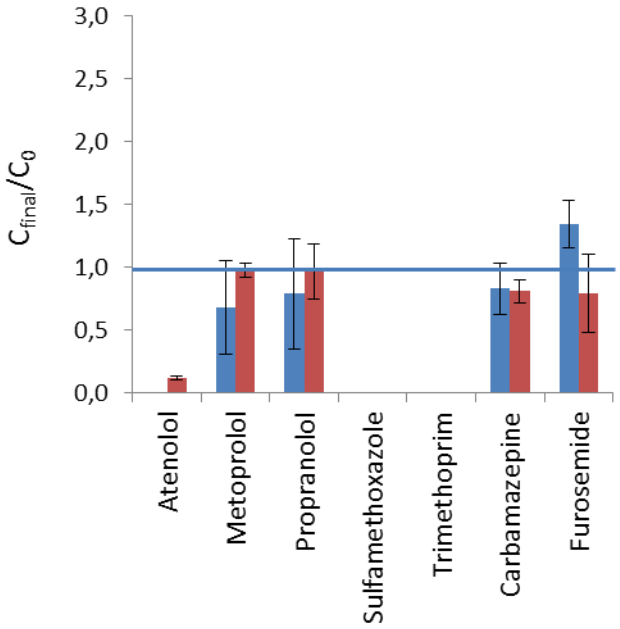


Figure 7. Changes of concentrations of spiked pharmaceuticals in fecal sludge (n=2) after mesophilic (+37 °C, 61 days, blue bars) and thermophilic (+52 °C, 59 days, red bars) anaerobic treatment. Values above 1 indicate an increase in concentration during treatment and below 1 indicate a reduction in concentration during treatment. The change of pharmaceutical 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/L and is the sum between the amounts detected in the solid and liquid phase.

In order to check if the change in concentration was an effect of the anaerobic treatment or just due to degradation over time, control samples were placed at +6 °C for 60 days. No significant degradation was observed in these control samples indicating that the removal observed was an effect of the anaerobic digestion process.

In general, no significant influence of temperature on the removal of pharmaceuticals was shown, which has also been reported previously (Carballa et al., 2007; Malmborg and Magnér, 2015; Kjerstadius et al. 2012). The degree of removal varies considerably from compound to compound. In the anaerobic digestion of fecal sludge some compounds showed significant reduction (Fig. 6 and 7). On the other hand, in some cases, concentrations after treatment were even higher than before treatment. One hypothesis for the increase in concentration of certain compounds could be the transformation of metabolites to the original compounds during treatment (conjugates are cleaved back to the original compound). Other explanations can be changes in the chemical preconditions of fecal sludge during degradation and a reduction of the amount of

particles to which the substance can be adsorbed influencing the efficiency of the extraction of the pharmaceuticals.

4.2.2 Reduction of pharmaceuticals during blackwater treatment

Prior to this project, little was known about the reduction of pharmaceuticals during liquid composting of blackwater. The treatment facility at Hölö is one of few blackwater treatment plants in Sweden. It enables sampling during the whole treatment procedure, after liquid composting and ammonia treatment. As in fecal sludge, concentrations of ciprofloxacin and atorvastatin were calculated by only using the amounts detected in the liquid phase, due to limitations in the extraction in the solid phase.

In the samples from the two aerobic reactors, 32 of the 44 pharmaceuticals analyzed were detected after liquid composting and ammonia treatment (Fig. 8A and B). Both reactors showed significant degradation of 13 substances (i.e. codeine, atenolol, metoprolol, propranolol, citalopram, valsartan, hydrochlorothiazide, atorvastatin, lidocaine, ibuprofen, diclofenac, candesartan and caffeine; $p<0.05$, t-test). Reactor 2 (R2) showed a better removal except for four compounds (i.e. citalopram, amitriptyline, oxazepam and bisoprolol) compared to R1 (Fig. 8 A and B). The higher efficiency in R2 may be due to a longer period of treatment caused by a broken circulation pump (Table 3). The treatment time is known to affect the degradation of pharmaceuticals and has previously been reported for e.g. naproxen (Hörsing et al. 2014). The degree of reduction varied between the different compounds. Ibuprofen and codeine showed 100% reduction in both reactors (Fig. 9). Moreover, only less than 13% was left in average for propranolol, valsartan, hydrochlorothiazide, atorvastatin and caffeine. It was only one of the substances investigated, fluoxetine, that increased significantly in concentration after treatment in both reactors ($p<0.05$, t-test), but for reactor 1 (R1) also acetaminophen increased.

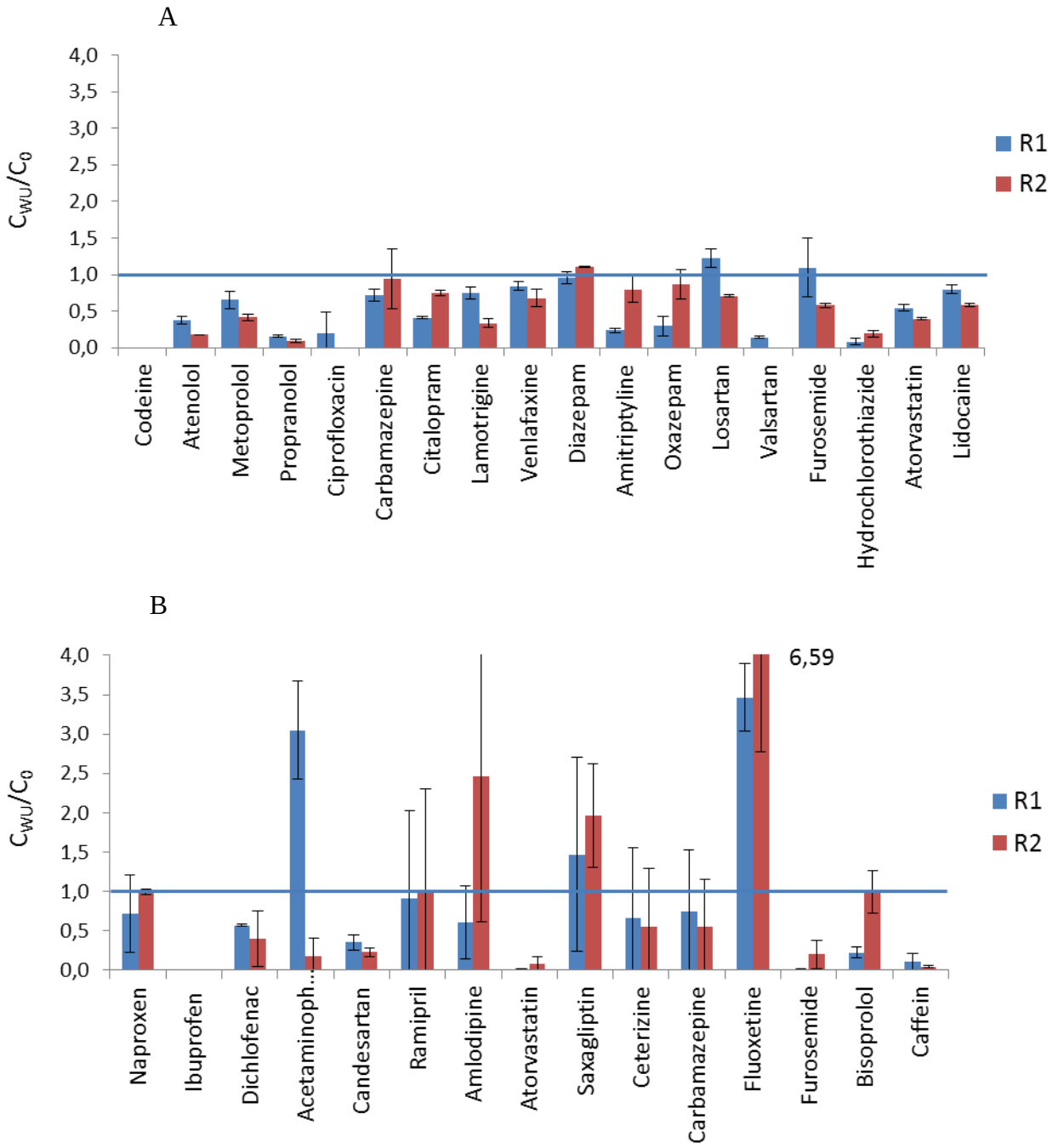


Figure 8. Changes of pharmaceutical concentrations in blackwater treatment (n=2): reactor R1 and R2 after liquid composting and ammonia treatment for A) substances analyzed at SLU, and B) substances analyzed at SPPD. Values above 1 indicate an increase in concentration during treatment and below 1 indicate a reduction in concentration during treatment. Pharmaceuticals "evolution" during treatment was calculated as $C_{treated\ BW}/C_0$. "C" is expressed in ng/L and is the sum between the concentrations detected in the solid and liquid phase.

It was mainly the liquid composting that caused the reduction of pharmaceuticals. The ammonia treatment showed further reduction in just a few compounds in both reactors; codeine, citalopram, valsartan, ibuprofen, diclofenac and atorvastatin (data not shown), while the concentration of oxazepam and candesartan only decreased in R1 and in R2 losartan, lidocaine and candesartan were reduced ($p<0.05$; t-test). At the same time, some compounds showed a small increase in the concentration after addition of urea (atenolol, metoprolol, propranolol, diazepam, amitriptyline,

furosemide, hydrochlorothiazide and caffeine). However, there was still a significant decrease compared to the initial concentration, except for diazepam and furosemide. The small effect of the ammonia 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). Furthermore, the concentrations of pharmaceuticals and their removal rates in blackwater treatment (including liquid composting and ammonia treatment) has been investigated previously in Hölö (Palm Cousins and Magnér, 2014) and showed a good agreement with our study (Fig. 9 and 13).

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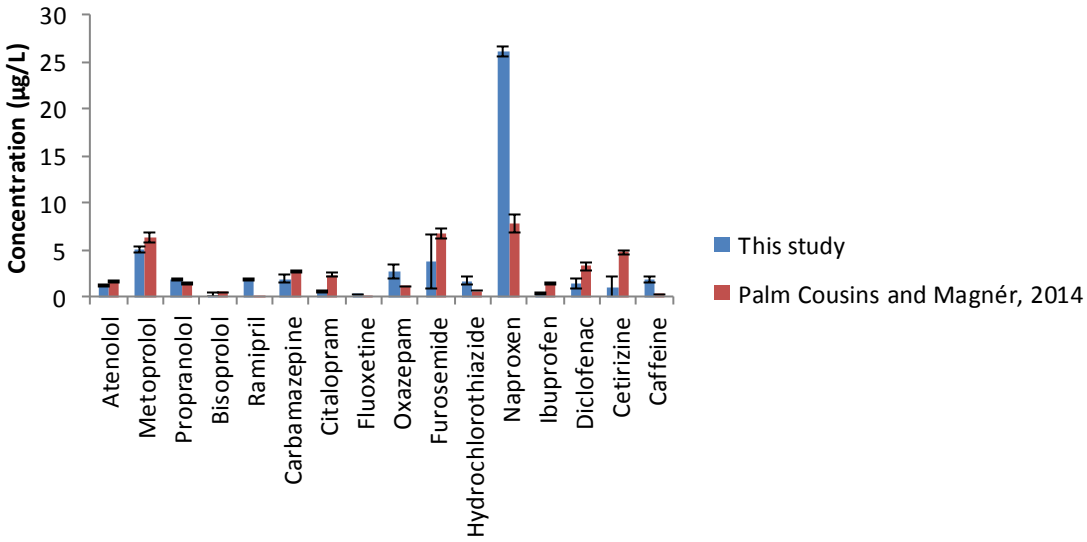


Figure 9. Concentrations of pharmaceuticals in the treated blackwater (liquid composted and ammonia treated) from this study and the previous measurement at Hölö.

The treated blackwater was stored at +6 °C for 6 month in order to study the effect of post-storage. Very few substances were further reduced during storage, only valsartan in reactor R1 and propranolol in R2 decreased compared to the concentrations found after the treatment at Hölö. Even though blackwater treatment showed quite good removal of some target pharmaceuticals, considerably high concentrations of some substances still remain in the treated liquid fraction (Table 12 and 13) compared to effluents of WWTPs (Deblonde et al., 2011; Jelic et al., 2011). Highest concentrations in the liquid phase of blackwater were found for furosemide (up to 30 µg/L), naproxen (~25 µg/L) and losartan (~10 µg/L) and in the solid phase propranolol (~2500 µg/kg dw). Furthermore, compared to the liquid phase of sludge from two Swedish WWTPs the concentrations for metoprolol (~0.6 µg/L), propranolol (~0.2 µg/L), carbamazepine (~3.7 µg/L), lamotrigine (~2.4 µg/L), oxazepam (~0.3 µg/L), losartan (~5.7 µg/L), valsartan (~4.1 µg/L), and furosemide (~1.6 µg/L), were up to 20 times lower than in blackwater .

Table 12. Concentrations in liquid composted and ammonia treated blackwater after 6 month of the post-storage (n=2)

Target Pharmaceuticals – analyzed at SLU

Therapeutic groups	Compounds	Liquid R1 (µg/L)	Liquid R2 (µg/L)	Solid R1 (µg/kg dw)	Solid R2 (µg/kg dw)
Analgesics	Codeine	<LOQ	<LOQ	<LOQ	<LOQ
	Atenolol	1.34±0.05	0.59±0.04	52	48±3
β-blockers	Metoprolol	7.2±0.5	7.892±0.024	320	365±9
	Propranolol	2.6±0.4	0.80±0.02	2200	2900±400
Antibiotics	Azithromycin	<LOQ	<LOQ	<LOQ	<LOQ
	Ciprofloxacin	1.04±0.05	1.06±0.16	180	210±40
Antidepressants	Carbamazepine	2.4±0.4	2.62±0.01	440	820±80
	Citalopram	0.57±0.06	0.37±0.04	360	270±8
	Lamotrigine	6.6±1.1	4.2±0.3	400	800±400
	Venlafaxine	7.0±1.0	8.11±0.04	8.8	10.6±0.3
	Diazepam	0.059±0.010	0.053±0.001	88	340±70
	Amitriptyline	<LOQ	<LOQ	200	1210±240
	Oxazepam	1.44±0.08	5.1±0.4	280	430±230
Antihypertensives	Losartan	11.5±0.5	7.97±0.05	<LOQ	<LOQ
	Valsartan	0.90±0.09	<LOQ	<LOQ	<LOQ
Diuretics	Furosemide	35.4±0.2	24.0±1.9	28	197±15
	Hydrochlorothiazide	1.08±0.06	3.2±0.2	<LOQ	95.00±0.04
Lipid regulator	Atorvastatin	1.03±0.05	1.28±0.07	13	11.0±0.1
Local anesthetic	Lidocaine	0.44±0.01	0.42±0.02	<LOQ	<LOQ

<LOQ: below limit of quantification (Appendix, Table 2)

Table 13. Concentrations in treated blackwater after 6 month of the post-storage (n=2)

Target Pharmaceuticals – analyzed at SPPD

Therapeutic groups	Compounds	Liquid R1 (µg/L)	Liquid R2 (µg/L)	Solid R1 (µg/kg dw)	Solid R2 (µg/kg dw)
Analgesics and anti-inflammatories	Naproxen	26	25	160	47.3±2.7
	Ibuprofen	<LOQ	0.55	15	115±150
	Diclofenac	1.8	0.37	17	8.7±0.6
	Budesonide	<LOQ	<LOQ	<LOQ	<LOQ
	Acetaminophen	0.32	<LOQ	9 .0	5.7±8 .1
Antihypertensives	Candesartan	1.5	1.6	<LOQ	<LOQ
	Ramipril	0.23	0.21	6.5	<LOQ
	Amlodipine	<LOQ	<LOQ	<LOQ	6.7±1 .2
Lipid regulators	Atorvastatin	<LOQ	<LOQ	<LOQ	4.5±6 .4
Antidiabetic	Saxagliptine	<LOQ	<LOQ	<LOQ	<LOQ
Antibiotic	Sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ
Anti-histaminic	Cetirizine	1.5	0.030	52	5.9
Antidepressants	Carbamazepine	0.070	2.4	170	161±13
	Fluoxetine	0.15	0.36	<LOQ	12.4±1 .1
Diuretic	Furosemide	0.57	10	5.8	73±84
β-blockers	Bisoprolol	0.070	1.1	39	54.9±2.9
Stimulant	Caffeine	0.47	0.30	20	23.6±5.9

<LOQ: below limit of quantification (Appendix, Table 2)

4.2.3 Partitioning between liquid and solid phase

Figures 10, 11 and 12 show the percentage of pharmaceuticals that have been removed during mesophilic and thermophilic anaerobic digestion and blackwater treatment as well as the distribution of pharmaceuticals between the solid and liquid phases at the end of the treatment. In general, pharmaceuticals are more prone to be found in the liquid phase. However, some substances sorb significantly to the solid phase. During anaerobic digestion, metoprolol, propranolol, citalopram, venlafaxine and amitriptyline sorbed to a great extent to the solid phase (60-100%), whereas for other substances, namely carbamazepine, lamotrigine and losartan, the fraction of pharmaceutical present in the solids was lower (~20-30%), but yet not negligible (Fig. 10 and 11). For blackwater, carbamazepine, citalopram, amitriptyline, oxazepam, diazepam, amlodipine and saxagliptin showed the highest sorption onto the solid phase (~20-100%), and other pharmaceuticals such as metoprolol, lamotrigine, venlafaxine, lidocaine and bisoprolol also showed some partitioning onto the solids (~10-20%; Fig. 12A and B).

The distribution of pharmaceuticals between both phases could be explained by their physico-chemical properties, being the octanol-water partition coefficient (K_{OW}), the organic carbon-water partition coefficient (K_{OC}) and pK_a (the acid dissociation constant at logarithmic scale, a quantitative measure of the strength of an acid in solution), which are the most important parameters that influence the partitioning

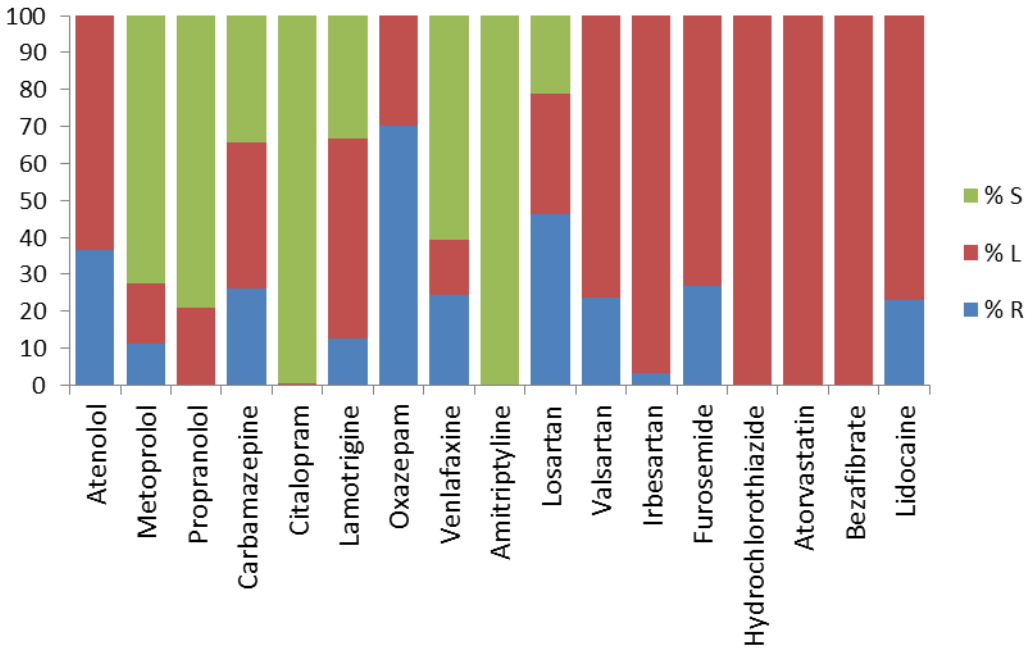
of pharmaceuticals. Metoprolol, propranolol, citalopram, venlafaxine, candesartan, ramipril, amlodipine and amitriptyline have quite high log K_{OW} values (Table 14), from 1.88 up to 4.92, as well as high K_{OC} levels (Table 14). High K_{OW} values indicate larger hydrophobicity of the compounds, being therefore, more prone to be distributed in the solid phase. On the other hand, high K_{OC} values indicate the tendency of a compound to sorb onto organic carbon. Fecal sludge and sewage sludge have high organic matter content, and therefore, substances that show high K_{OC} levels would be more likely to be detected in the solid phase. Most of the substances with major distribution to the solid phase (metoprolol, propranolol, citalopram, amitriptyline and oxazepam) have pK_a values around 9. At pH 8, which was the pH of the anaerobic digestion samples, the compounds are mainly present in their neutral form, increasing their hydrophobicity and hence, their proneness to distribute onto the solid phase. At the present pH (pH~7) the acidic compounds with low pK_a like ibuprofen, diclofenac, naproxen and furosemide are more likely to be in the liquid phase as can be seen in Figure 10 and 11.

Table 14. Target list of pharmaceutically active compounds analyzed and physiochemical properties

Compound	Chemical formula	MW (g mol^{-1})	pK_a	Log $K_{OW,dry}$	Log K_{OC}
Analgesics					
Codeine	$C_{18}H_{21}NO_3$	299.37[a]	8.21[a]	1.19[a]	3.12[b]
Naproxen	$C_{14}H_{14}O_3$	230.09[a*]	4.15[a]	3.18[b]	2.54[b]
Ibuprofen	$C_{13}H_{18}O_2$	206.13[a*]	4.91 [a]	3.97 [b]	2.60 [b]
Diclofenac	$C_{14}H_{11}NO_2Cl_2$	295.02[a*]	4.15 [a]	4.51 [b]	2.92[b]
Budesonid	$C_{25}H_{34}O_6$	430.24[a*]	n.a.	3.98 [b]	1.00 [b]
Acetaminophen	$C_8H_9NO_2$	151.06[a*]	9.38 [a]	0.46 [b]	1.79 [b]
β-blockers					
Atenolol	$C_{14}H_{22}N_2O_3$	266.34[a]	9.60[d]	0.16[a]	2.17[b]
Bisoprolol	$C_{18}H_{31}NO_4$	325.23[a*]	n.a.	1.87 [b]	1.52 [b]
Sotalol	$C_{12}H_{20}N_2O_3S$	272.37[a]	8.20[h]	0.24[a]	1.58[b]
Metoprolol	$C_{15}H_{25}NO_3$	267.37[a]	9.60[d]	1.88[a]	1.79[b]
Propranolol	$C_{16}H_{21}NO_2$	259.35[a]	9.40[a]	3.48[a]	3.09[b]
Antibiotics					
Azithromycin	$C_{38}H_{72}N_2O_{12}$	748.98[a]	8.70[a]	4.02[a]	n.a.
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.95[a]	8.90a	3.16[a]	n.a.
Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.33[a]	6.10/8.75[d]	0.46[d]	1.97[b]
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.34[a]	6.16/8.63[a]	0.28[a]	1.55[b]
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28[a]	5.70[d]	0.89[a]	3.19[b]
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.32[a]	7.12[d]	0.91[a]	2.96[b]
Antidepressants					
Carbamazepine	$C_{15}H_{12}N_2O$	236.27[a]	7.00[d]	2.45[a]	3.59[b]
Citalopram	$C_{20}H_{21}FN_2O$	324.39[a]	9.59e	3.74[a]	4.40[b]
Diazepam	$C_{16}H_{13}ClN_2O$	284.74[a]	3.40[a]	2.82[a]	4.05[b]
Lamotrigine	$C_9H_7Cl_2N_5$	256.09[a]	5.70[c]	0.99[c]	3.13[b]
Oxazepam	$C_{15}H_{11}ClN_2O_2$	286.70[a]	10.90[d]	2.24[a]	3.08[b]
Venlafaxine	$C_{17}H_{27}NO_2$	277.40[a]	10.09[c]	3.28[a]	3.17[b]
Fluoxetine	$C_{17}H_{18}F_3NO$	309.30[a]	10.05[e]	4.05[a]	5.32[b]
Amitryptiline	$C_{20}H_{23}N$	277.40[a]	9.40[a]	4.92[c]	5.70[b]
Antihypertensives					
Losartan	$C_{22}H_{23}ClN_6O$	422.90[a]	5.50[a]	4.01[a]	5.96[b]
Candesartan	$C_{24}H_{20}N_6O_3$	440.16[a*]	2.45 [a]	4.79[b]	5.85 [b]
Ramipril	$C_{23}H_{32}N_2O_5$	416.23[a*]	n.a.	3.32 [b]	3.22 [b]
Amlodipine	$C_{20}H_{25}N_2O_5Cl$	408.15[a*]	8.79 [a]	3.00 [b]	3.52 [b]
Valsartan	$C_{24}H_{29}N_5O_3$	435.52[b]	3.60[c]	4.00[c]	6.01[b]
Irbesartan	$C_{25}H_{28}N_6O$	428.53c	4.08/4.29[c]	5.31[c]	7.94[b]
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.52[a]	8.18/12.86[f]	2.79[a]	3.98[b]
Diuretics					
Furosemide	$C_{12}H_{11}ClN_2O_5S$	330.70[a]	3.80/7.50[c]	2.03[a]	2.28[b]
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	297.70[a]	7.90[a]	-0.07[a]	1.90[b]
Lipid regulator					
Atorvastatin	$C_{33}H_{35}FN_2O_5$	558.60[a]	-2.70/4.33[f]	5.7[c]	n.a.
Bezafibrate	$C_{19}H_{20}ClNO_4$	361.82[a]	-0.84/3.83[f]	4.25[a]	3.17[b]
Anti-ulcer agent					
Ranitidine	$C_{13}H_{22}N_4O_3S$	314.41[a]	8.08[f]	0.27[a]	4.44[b]
Antidiabetic					
Saxagliptine	$C_{18}H_{25}N_3O_2$	315.19[a*]	7.90 [a]	n.a.	n.a.
Local anesthetic					
Lidocaine	$C_{14}H_{22}N_2OCl$	234.34[a]	8.01[a]	2.44[a]	2.96[b]
Antihistaminie					
Ceterizine	$C_{21}H_{25}N_2O_3Cl$	388.16[a*]	2.70 [a]	-0.61[b]	3.85 [b]
Stimulant					
Caffeine	$C_8H_{10}N_4O_2$	194.08[a*]	14.0 [a]	-0.07 [b]	1.00 [b]

[a]ChemIDplus Advanced (2015), [b]chemspider.EPISuite (PCKOCWIN v1.66),
[c]Pubchem.ncbi.nlm.nih.gov (2015), [d](Bonnet et al., 2010), [e](Vasskog et al., 2006), [f]Drugbank.ca (2015), n.a. not available, * monoisotopic mass

A) Non-spiked mesophilic anaerobic digestion (SLU)



B) Non-spiked mesophilic anaerobic digestion (SPPD)

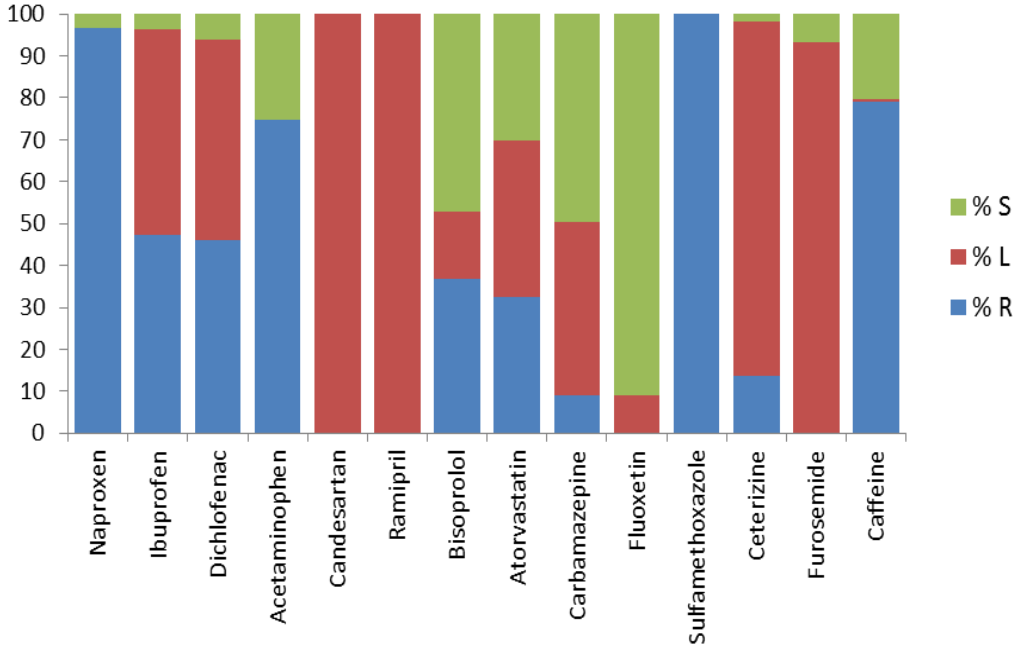
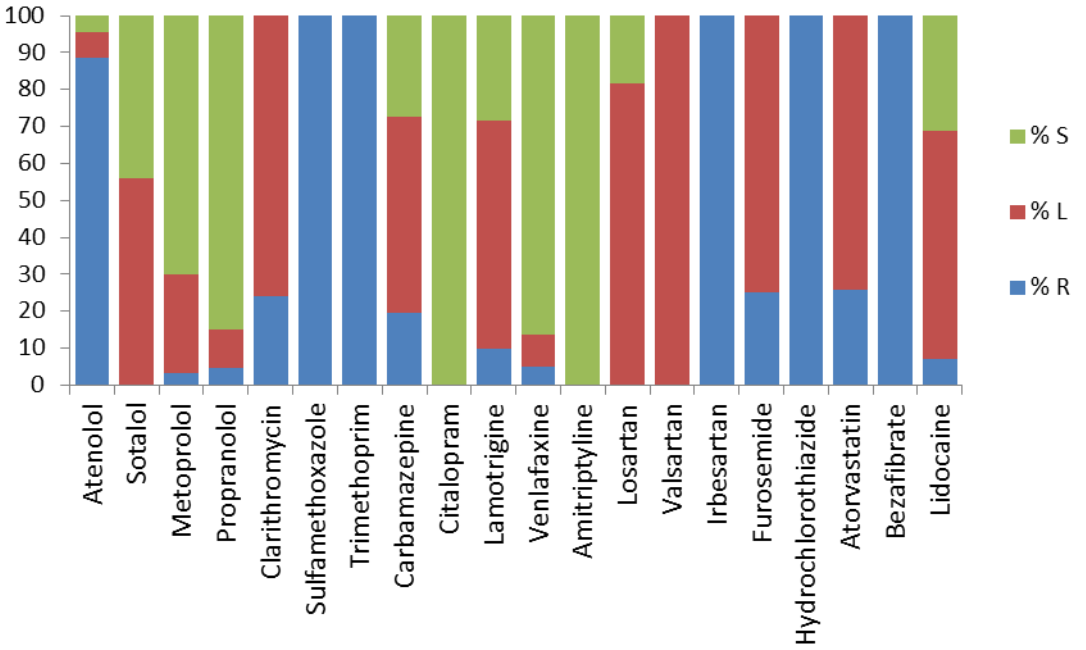


Figure 10. Distribution of pharmaceuticals between the liquid phase (red) and solid phase (green) and percentage of compound removed (blue) in fecal sludge samples after mesophilic treatment (+37°C, 61 days). (% S: fraction of pharmaceutical remaining in the solid phase after treatment; %L: percentage of pharmaceutical remaining in the liquid phase after treatment; %R: percentage of pharmaceutical removed during treatment). Note: Codeine was found in one of the spiked bottle but at levels below the limit of quantification (it is detected but it could not be quantified).

A) Non-spiked thermophilic anaerobic digestion (SLU)



B) Non-spiked thermophilic anaerobic digestion (SPPD)

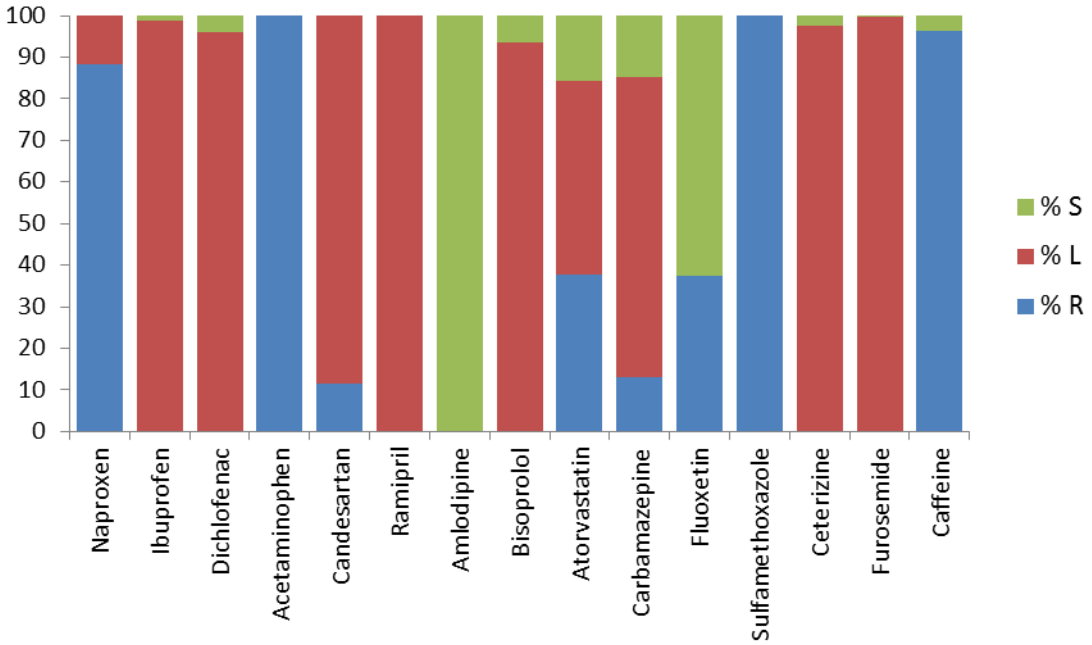
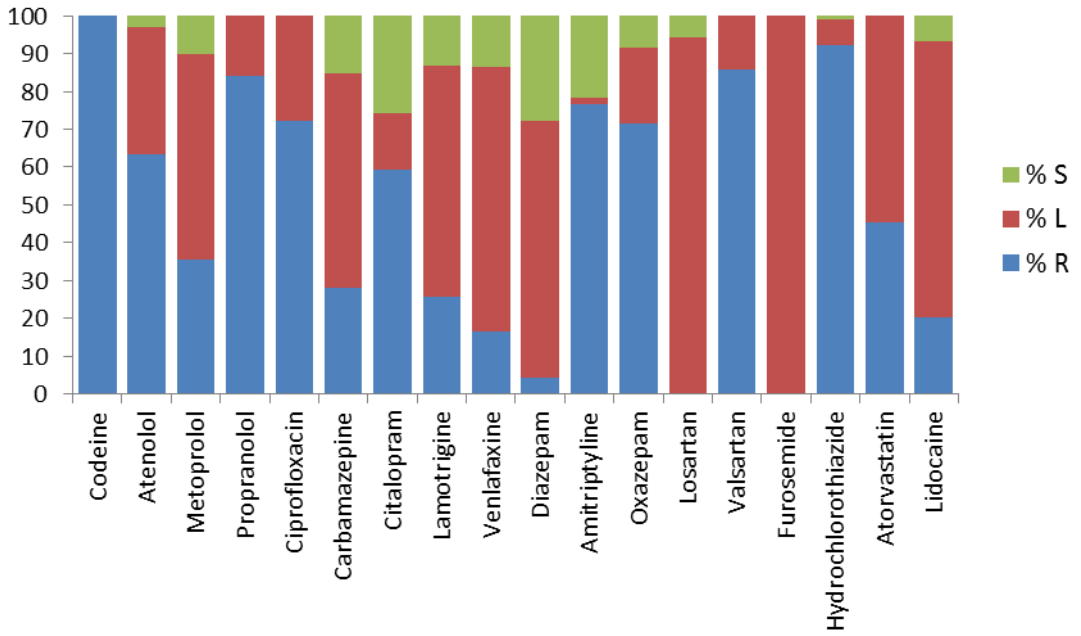


Figure 11. Distribution of pharmaceuticals between the liquid phase (red) and solid phase (green) and percentage of compound removed (blue) in fecal sludge samples after thermophilic treatment (+52°C, 59 days). (% S: fraction of pharmaceutical remaining in the solid phase after treatment; %L: percentage of pharmaceutical remaining in the liquid phase after treatment; %R: percentage of pharmaceutical removed during treatment).

A) Blackwater treatment Reactor 1 (SLU)



A) Blackwater treatment Reactor 1 (SPPD)

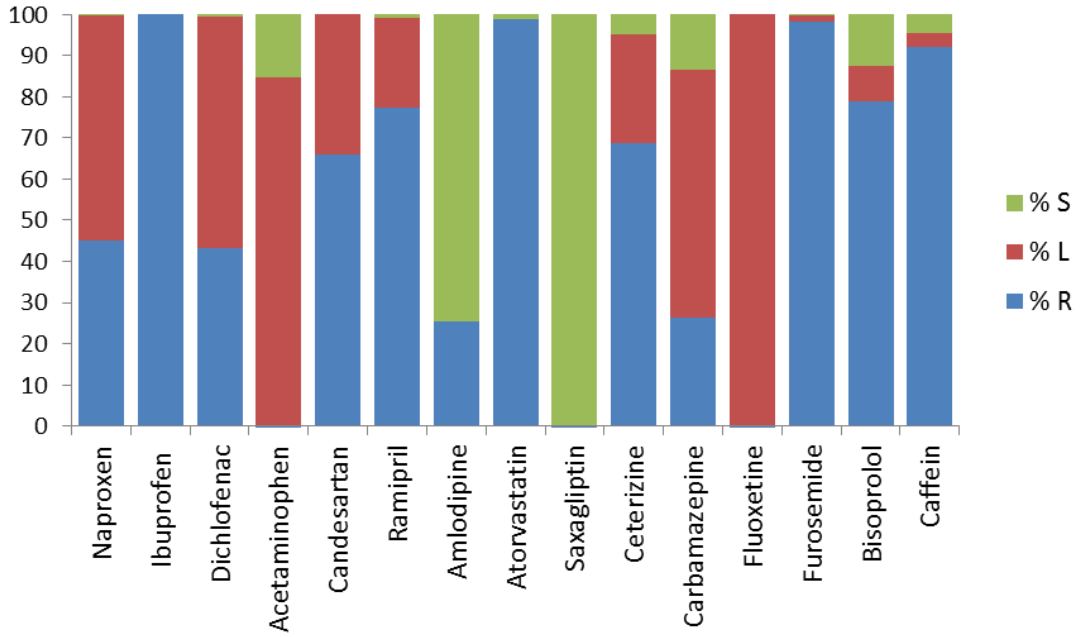
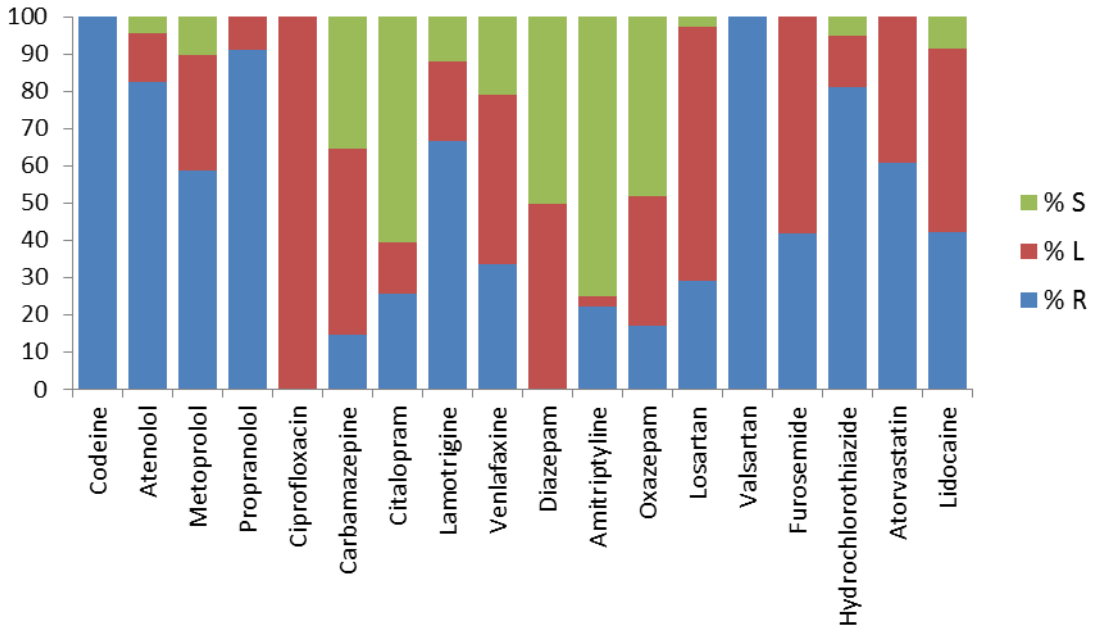


Figure 12 A. Percentage of pharmaceuticals removed (blue) during blackwater treatment (including liquid composting and ammonia treatment) and distribution of pharmaceuticals between the liquid phase (red) and solid phase (green) at the end of the treatment (%S: fraction of pharmaceutical remaining in the solid phase after treatment; %L: fraction of pharmaceutical remaining in the liquid phase after treatment; %R: percentage of pharmaceutical removed during treatment).

B) Blackwater treatment Reactor 2 (SLU)



B) Blackwater treatment Reactor 2 (SPPD)

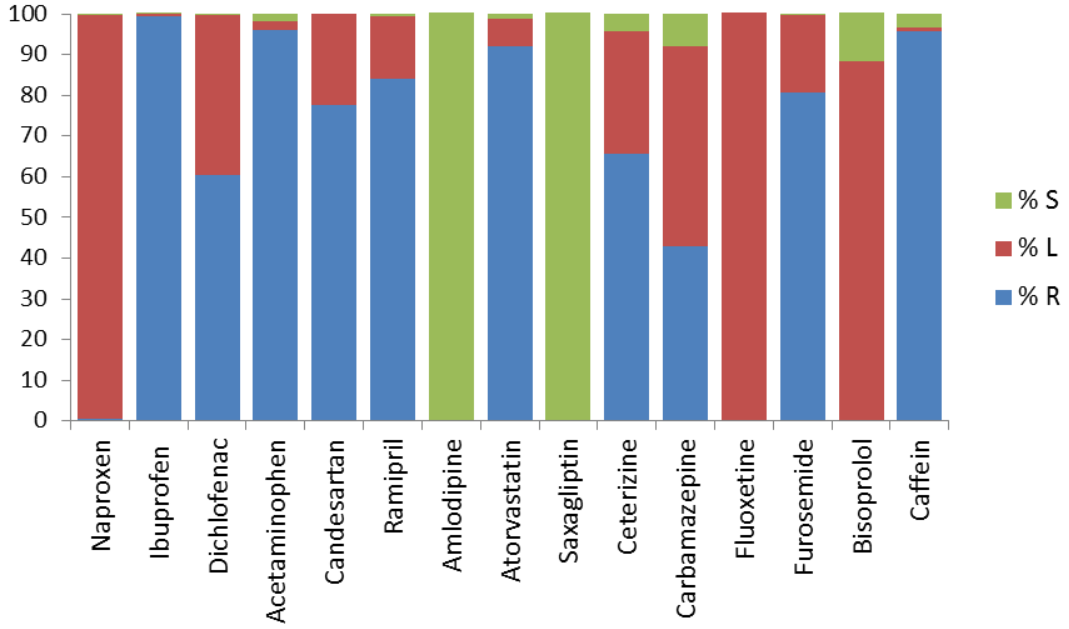


Figure 12 B. Percentage of pharmaceuticals removed (blue) during blackwater treatment (including liquid composting and ammonia treatment) and distribution of pharmaceuticals between the liquid phase (red) and solid phase (green) at the end of the treatment (%S: fraction of pharmaceutical remaining in the solid phase after treatment; %L: fraction of pharmaceutical remaining in the liquid phase after treatment; %R: percentage of pharmaceutical removed during treatment).

4.2.4 Comparison between treatments

In our study, the aerobic degradation of pharmaceuticals during liquid composting and ammonia treatment of blackwater in Hölö revealed better removal efficiency than anaerobic digestion of fecal sludge (average reduction 58% during 21 days compared to 32 % during 60 days calculated on the 29 substances found). In Palm Cousins and Magnér (2014), the removal of pharmaceuticals was on average around 70% for 31 substances found in the treated blackwater from Hölö. Only 15 substances were overlapping between the two studies. It has previously been reported that aerobic treatment is more efficient than anaerobic treatment confirming our result (Naturvårdsverket 2008; Wahlberg et al., 2010).

The degree of reduction of pharmaceuticals in blackwater treatment with liquid composting and ammonia treatment was higher compared to the reduction in conventional wastewater treatment plants in Europe for 6 out of 11 substances analyzed in both treatments (Figure 13). However, fluoxetine and naproxen had higher reduction in WWTPs, while the removal of atenolol, metoprolol and diclofenac were equal to blackwater treatment.

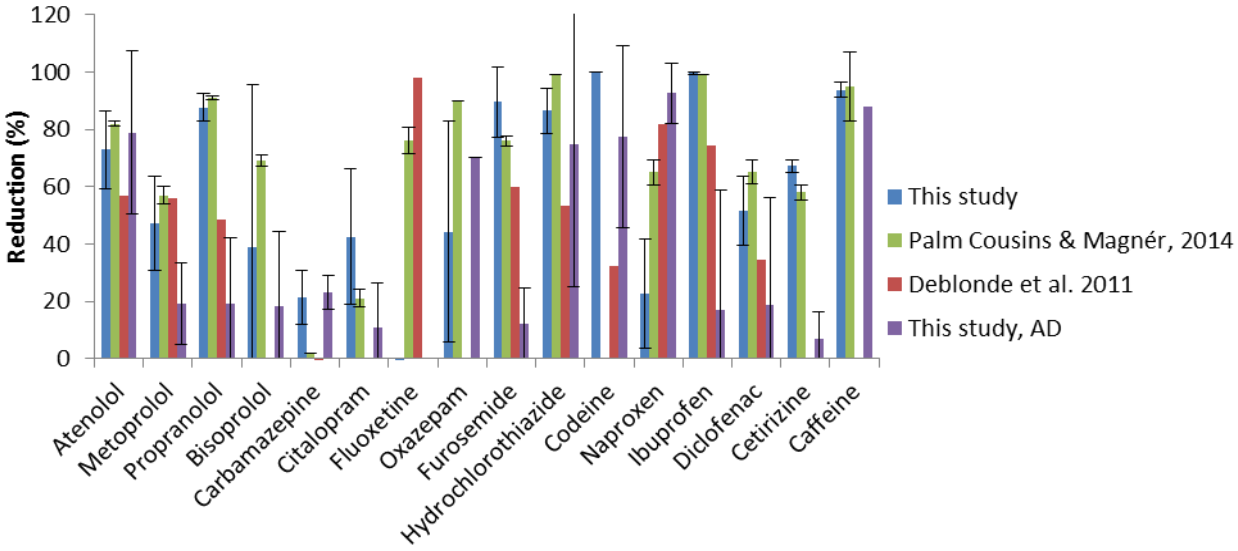


Figure 13. Reduction of pharmaceuticals in the treated blackwater in our study (blue), the previous measurement at Hölö (green), conventional WWTPs in Europe (red) and anaerobic digestion (AD) of fecal sludge in our study (average of mesophilic and thermophilic treatment; purple). The average of reduction in the WWTPs is based on the concentration of pharmaceuticals in effluent compared to influent in a large number of European WWTPs. All WWTPs that are included have primary, secondary (with activated sludge system) and sometimes a tertiary treatment (Deblonde et al., 2011).

4.3 Risk analysis

4.3.1 Pharmaceutical loads on arable land

The concentrations of most of the pharmaceuticals in liquid composted and ammonia treated blackwater (Table 12 and 13) were significantly higher than those disposed in the effluent from WWTPs (Deblonde et al., 2011; Jelic et al., 2011). Blackwater is mostly urine and feces with some flush water, whereas municipal wastewater is a mixture of some blackwater and much larger volumes

of greywater and other additional water (storm water, ground water etc.). The additional greywater and other water result in dilution of the pharmaceuticals in WWTPs. In addition, the concentrations of pharmaceuticals in blackwater include both liquid and solid fraction of the blackwater, whilst, the concentrations of pharmaceuticals in the solid fractions of the municipal wastewater are separated and removed with the sludge.

The estimated amounts of pharmaceuticals reaching soil through fertilization with 40 ton treated blackwater/ha ranged from 0.0005 g/ha of ketoconazole to 2.5 g/ha of furosemide (Table 15). Naproxen, furosemide, amlodipine and budesonide are among the compounds which showed the highest estimated amounts of pharmaceutical applied via blackwater into the top surface soil layer (0-20 cm below surface) as shown in Table 15.

For assessing the risks of pharmaceuticals related to agriculture, it is interesting to compare pharmaceutical application rates due to blackwater fertilization with these due to sewage sludge application. Yet, it is difficult to make a fair comparison between the two types of fertilizers because the agricultural practices for using the blackwater are different from these used for sewage sludge; blackwater is rich in nitrogen and thus usually applied to meet crop demand for nitrogen while sewage sludge is low in nitrogen and high in phosphorus and thus applied as source of phosphorus. Nitrogen fertilizers, such as blackwater, are applied yearly, while phosphorus fertilizers can be applied at larger doses every few years. Thus, sewage sludge is usually applied as a five year dose once every five years. The pharmaceuticals that the crop is exposed to right after an application event thus comes from a one year dose of blackwater or a five year dose of sewage sludge. Over a five year cycle however the soil is exposed to five one-year doses of blackwater of one five-year dose of sewage sludge. Thus, it is of interest to compare the dose of pharmaceuticals from both one and five one year doses of blackwater with that from a five year dose of sewage sludge.

In Table 15 the pharmaceutical dose that the crop is exposed to right after application of blackwater or sewage sludge, i.e. a one-year load of blackwater and a five-year load of sewage sludge, is shown. Out of the 17 substances for which data were found, three substances (metoprolol, oxazepam and naproxen) were applied in larger dose by the blackwater, while nine were applied in larger dose by the sewage sludge and five, in similar dose (atenolol, amitriptyline, ibuprofen, diclofenac and bisoprolol). However, a total of five years of blackwater application compared with one five-year dose of sewage sludge contributed with larger amounts of eight substances (atenolol, metoprolol, amitriptyline, oxazepam, naproxen, ibuprofen, diclofenac and bisoprolol) as compared to seven substances (kodein, ciprofloxacin, carbamazepine, citalopram, ketoconazole, atorvastatin, fluoxetine) for the 5-years sewage sludge application (maximum of 5 ton DM/ ha)) from different WWTPs (Table 15). Ciprofloxacin, citalopram, ketoconazole applications to soil via municipal sewage sludge were 4-7 times higher than those loaded via blackwater (5-years application load), despite that blackwater includes both the liquid and solid fractions of the pharmaceuticals. This comparison could not be done for the rest of the compounds found in the blackwater, because data were lacking for their concentration in sludge in the studied treatment plants (Table 15).

4.3.2 Accumulation of pharmaceuticals in soil and leaching

The model simulations (BASL4) of the pharmaceutical's accumulation in soil showed that at the end of the first year of blackwater fertilization, the concentrations of all of the modeled compounds were low (<10^{-6} g/g dry soil) (Fig. 14). After three years of blackwater application, BASL 4 showed only small accumulations of the selected compounds. Furosemide showed the largest accumulation in the soil (1.2 × 10^{-8} g/g dw) (Fig. 14).

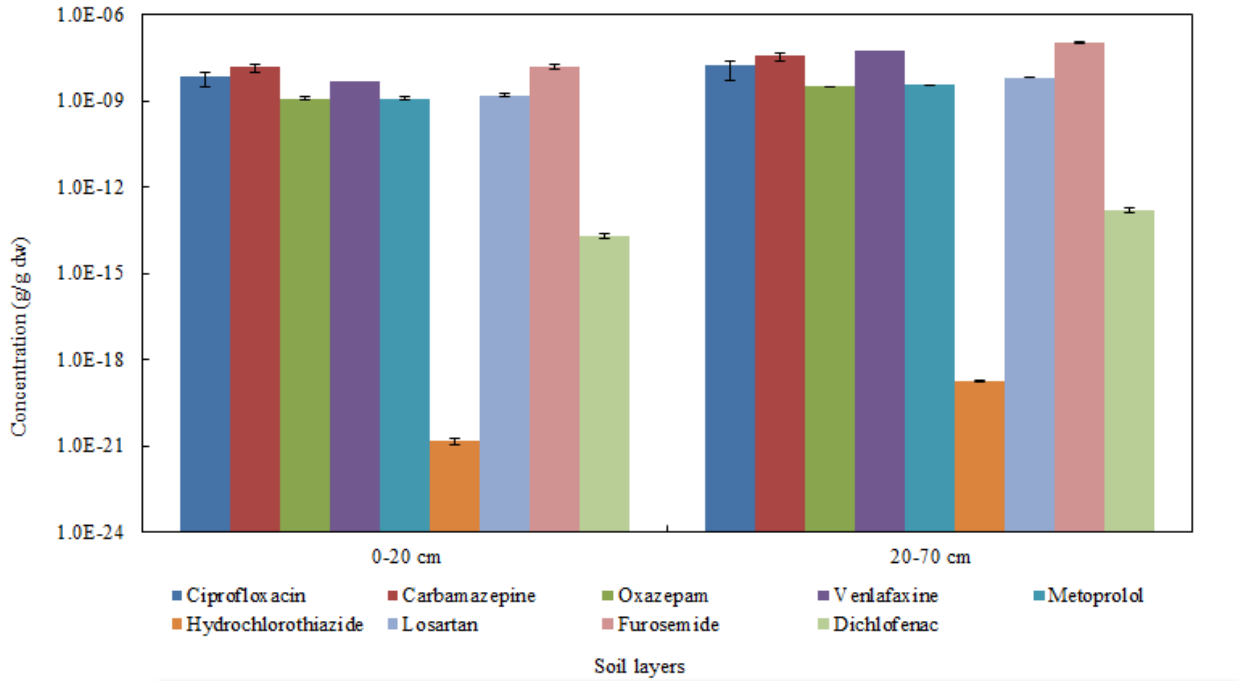


Figure 14. The 3-years average soil concentration (g/g dw) of the selected pharmaceuticals in the top soil layer (0-20 cm below soil surface) and the lower soil layer (20-70 cm), estimated from concentrations in the soil 12 months after each fertilization event,(fertilization was assumed to be done three times with 1 year lapse between each fertilization events). The bars show standard deviation.

For losses of pharmaceuticals from soil due to leaching or degradation, the simulations showed that >70% of the losses were due to degradation for all of the modeled substances except for diclofenac and furosemide, which had losses around 40% due to leaching (Fig. 15). It should however be remembered both that a worst case scenario with 5 mm of leaching per day throughout the year was simulated, while in practice there often is no leaching for the first four months or more after application of blackwater, and that the simulation was largely based on estimated values for physico-chemical properties and landscape/soil/plant-specific parameters, which means that the uncertainties in results are large. For example, the calculations assumed an organic carbon fraction of 3.5% and 2.5% in the soil (upper and lower layer, respectively), while variation in nature is large.

To the best of our knowledge, data on pharmaceutical concentrations in soils fertilized with blackwater nationally and globally is currently not available, and the latest available information about occurrence of pharmaceuticals in Swedish soils was reported in the national screening database of 2006 (IVL, 2006). In this database, ciprofloxacin was reported to be detected at all studied farms, but it was

<10 × 10^{-9} g/g. In the absence of guidelines for pharmaceutical in soils, it is difficult to assess whether the levels of pharmaceuticals from blackwater (Table 15; Fig. 14) are of concern in soil environments, especially as the pharmaceuticals have different characteristics e.g. biodegradability, water solubility and volatilization (Table 4). This also means that the results of the model simulations have not been verified due to absence of real measurements on short and long term accumulation of pharmaceutical in soil.

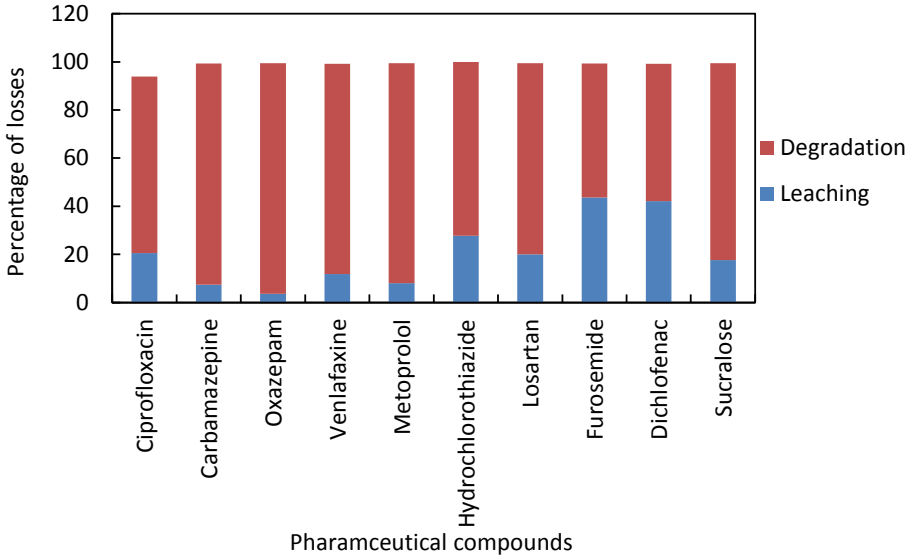


Figure 15. Estimated percentage of losses of selected pharmaceuticals from soil due to degradation and leaching as estimated using BASL4.

The simulated concentrations in soil were higher than the concentrations measured in soils irrigated with treated wastewater. This might be because treated wastewater is a diluted form of blackwater. One study (Grossberger et al. (2014) reported occurrence of carbamazepine (5.67 × 10^{-9} g/g dw), metoprolol (0.91 × 10^{-9} g/g dw), caffeine (1× 10^{-9} g/g dw), lamotrigine (1× 10^{-9} g/g dw) and sulfamethoxazole (0.28 × 10^{-9} g/g dw) in soils from three different locations irrigated with treated wastewater in Israel. Also, Williams and McLain (2012) showed that carbamazepine accumulate in soils (0.18 × 10^{-9} g/g dw), following irrigation and aquifer recharge with treated wastewater and that carbamazepine exhibited significant accumulation over time. It can, however, not be excluded that our higher values are overestimated by the model. Applying blackwater to the field may under certain conditions pose a risk for leaching to surface water and to deeper soils for water soluble and easily mobilized substances. Our model simulations indicated that pharmaceutical concentrations in deeper soil layers (20-50 cm below the surface) are slightly higher than those in the top layer (Fig. 14). If blackwater fertilization is followed by a heavy rain event, migration of e.g. furosemide could be expected in the view of its leaching potential (Fig. 15), and also on hilly fields significant leaching through surface runoff. However, in Sweden the weather in spring and early summer are usually fairly dry with the soil drying up. Thus, the risk of leaching is normally low.

Table 15. Estimated loads of pharmaceuticals (g/ha) in blackwater after liquid composting and ammonia treatment in comparison to those applied via municipal sludge from different wastewater treatment plant. The underlined numbers show that these concentrations are higher in blackwater than in sewage sludge, while the bold numbers show that these concentrations are higher in sewage sludge than in treated blackwater

		Treated blackwater		**5-years application rate of municipal sludge**					
		1- year application rate	**5-years application rate**	**1**	**2**	**3**	**4**	**5**	**1-5**
Class	**Compound**			**Skövde**	**Henriksdal**	**Öhn**	**Kungsäng**	**Visby**	**Average**
Analgesics	Codeine	0.0026	0.013	**0.1450**	**0.0700**	**0.1000**	**0.0475**	**0.0800**	**0.0885**
β-blockers	Atenolol	0.0553	0.2765	0.0650	0.0950	0.0600	0.0950	0.1900	0.1010
	Metoprolol	0.2481	1.2405	0.0250	0.0390	0.0250	0.0270	0.0250	0.0282
	Propranolol	0.0719	0.3595	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
Antibiotics	Azithromycin	0.0061	0.0305	0.0250	0.0280	0.0250	0.0250	0.0250	0.0256
	Ciprofloxacin	0.0300	0.15	**2.2500**	**1.2500**	**0.8500**	**0.3400**	**0.6000**	**1.0580**
Antidepressants	Carbamazepine	0.1089	0.5445	**0.9500**	**1.0000**	**0.6000**	**0.4350**	**0.4450**	**0.6860**
	Citalopram	0.0869	0.4345	**3.8000**	**2.8500**	**3.1500**	**2.3000**	**2.5000**	**2.9200**
	Lamotrigine	0.1931	0.9655	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
	Venlafaxine	0.2728	1.364	0.4300	1.5500	0.7500	0.7000	0.7500	0.8360
	Diazepam	0.0035	0.0175	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
	Amitryptiline	0.0320	0.16	0.0250	S0.0390	0.0250	0.0270	0.0250	0.0282
	Oxazepam	0.2160	1.08	0.2150	0.0900	0.0600	0.0900	0.0700	0.1050
Antihypertensives	Losartan	0.4920	2.46	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
	Valsartan	0.4520	2.26	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
	Amlodipine	1.1225	5.6124	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
Diuretics	Furosemide	2.545	12.725	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
	Hydrochlorothiazide	0.0888	0.444	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
Antifungal agents	Ketoconazole	0.0005	0.0025	**2.5500**	**6.0000**	**5.5000**	**9.0000**	**2.7500**	**5.1600**
Lipid regulator	Atorvastatin	0.0133	0.0665	**0.2500**	**0.2500**	**0.4100**	**0.3350**	**0.2500**	**0.2990**

Class	Compound	Treated blackwater		5-years application rate of municipal sludge					
		1- year application rate	5-years application rate	1	2	3	4	5	1-5
				Skövde	Henriksdal	Öhn	Kungsäng	Visby	Average
Local anesthetic	Lidocaine	0.0164	0.082	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
Sweeter	Saccharin	0.0024	0.012	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
	Sucralose	0.4577	2.2885	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
Analgesics/anti-inflammatories	Naproxen	0.6469	3.2345	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
	Ibuprofen	0.0274	0.137	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
	Diclofenac	0.1402	0.701	0.2950	0.1550	0.0500	0.0500	0.1000	0.1300
	Acetaminophen	0.0211	0.1055	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
β-blockers	Bisoprolol	0.0279	0.1395	0.0500	0.0215	0.0140	0.0270	0.0215	0.0268
Antibiotics	Sulfamethoxazole	0.1781	0.8905	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F	D.N.F
Antidepressant	Fluoxetine	0.0050	0.025	**0.1950**	**0.3950**	**0.2150**	**0.8000**	**0.2750**	**0.3760**

Application loads of pharmaceuticals from municipal sewage sludge were calculated based on pharmaceutical concentrations obtained for five waste water treatment plants as reported in the IVL data base http://www3.ivl.se/miljo/db/IVL_screening_registersida.htm

D.N.F : data not found

4.3.3 Accumulation of pharmaceuticals in crops

The simulated uptake of pharmaceuticals revealed no significant uptake ($<10^{-9}$ g/g) of pharmaceuticals in the roots and leaves of any of the crops, neither of the root crop (carrot) nor of the grass crop during the first 100 days in the growing seasons of the three years period of blackwater fertilization, which was the maximum period that could be simulated with BASL4 (Fig. 16). Cortés et al. (2013) reported that soybean (*Glycine max*) and wheat (*Triticum aestivum*) fertilized with municipal sewage sludge did not show detectable level of diclofenac or ibuprofen, despite that the concentrations of the two substances were 22×10^{-9} and 217×10^{-9} g/g dw, respectively, in the used sludge. It was also concluded that fertilizing soybean and wheat with sludge at double the commonly used application rates did still not result in detected plant uptake (Cortés et al., 2013).

Eggen et al. (2011) reported that ciprofloxacin was detected in barley and carrot grown in soil heavily spiked with ciprofloxacin (6500×10^{-9} g/g dw). Bioaccumulation factors of ciprofloxacin from root to aerial part in barely and carrot were 0.3 and 0.07, respectively (Eggen et al., 2011). Sabourin et al. (2012) reported that ciprofloxacin was found at concentration of 2.1×10^{-9} g/g dw in the root of carrot grown in soils fertilized with municipal sewage sludge at rate of 8 ton dry matter/ha; In Sweden, a five year dose corresponds of sewage sludge corresponds to 4-5 ton dry matter/ha. However, as similar concentrations were also detected in controls, these measurements should be disregarded.

Winker et al. (2010) showed that the recalcitrant pharmaceutical carbamazepine was transported into roots and aerial plant parts of ryegrass fertilized with urine spiked with carbamazepine resulting in soil concentration of 32 000 10^{-9} g/g dry soil. The carbamazepine transport into the ryegrass was clearly driven by transpiration. In the same study, uptake of carbamazepine in ryegrass fertilized with non-spiked urine was lower than the detection limit (75×10^{-9} g/g dw) (Winker et al., 2009). In addition, Shenker et al. (2011) reported that carbamazepine was taken up in cucumber grown in hydroponic and greenhouse experiments where different types of soil were irrigated with fresh and treated wastewater. Carbamazepine was taken up by water mass flow, and thus it was translocated and accumulated in the leaves more than in the fruits and roots. The same study (Shenker et al. (2011) also revealed that uptake of carbamazepine by cucumber was negatively correlated to the soil organic matter and concluded high potential for carbamazepine uptake in crops grown in soils poor in organic matter.

Goldstein et al. (2014) reported detection of caffeine, lamotrigine and carbamazepine in cucumber fruit irrigated with treated domestic wastewater in sandy, aeolian and alluvial soils. Carbamazepine was detected at concentrations up to 2.2×10^{-9} g/g dw cucumber fruit (Goldstein et al. 2014). In the same study, carbamazepine was also detected in tomato fruit at concentrations of 1.2×10^{-9} g/g dw tomato.

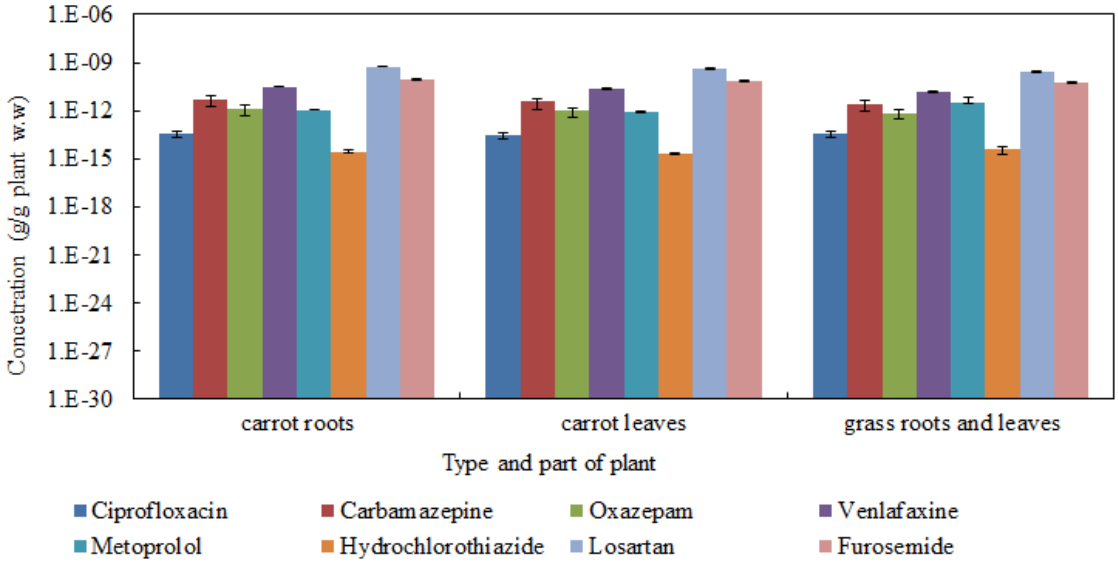


Figure 16. The average concentrations (g/g ww) of selected pharmaceuticals in the roots and leaves of carrot and grass plants after 100 days in the growing season during the first, second and third year of blackwater fertilization with blackwater after liquid composting and ammonia treatment (40 ton/ ha. year), simulated using BASL4 model. The bars show standard deviations.

4.3.4 Hazards of pharmaceuticals compounds on humans

Using the average daily intake of different food stuffs, the calculation of the estimated daily intake (EDI) of ciprofloxacin, carbamazepine, oxazepam, venlafaxine, metoprolol, hydrochlorothaizide, losartan, furosemide and diclofenac in wheat and carrot fertilized with black water showed very low daily intakes (<3 $\times 10^{-9}$ g/day for children and <4 $\times 10^{-9}$ g/day for adults) (Table 16). The estimated daily intakes of the compounds were much lower than the acceptable daily intake (Table 17), and thus the hazard quotient of all of the aforementioned compounds were <<<0.01 in spite of the large safety factor of 10 000 used when calculating the acceptable daily intake from the lowest therapeutic dose. The acceptable daily intake per kg of body weight was in the calculation assumed to be the same for children as for adults. If hazard quotients for the substances are <1, no further risk assessment is needed, according to European Agency for the Evaluation of Medicinal Products (2001) and Sanderson et al. (2004).

It is difficult to grasp how small the simulated intake of pharmaceuticals from fertilized crops is. To illustrate this, we calculated for each pharmaceutical the number of years an adult and a child would need to eat fertilized wheat or carrots to reach the amount that corresponds to the lowest therapeutic dose for one single day. The simulation showed that a one day therapeutic dose would be reached before 100 000 years only for two substances, losartan (an anti-hypertensives) and furosemide (a diuretic) (Table 18).

Table 16. Estimated daily intake of pharmaceuticals via consumption of wheat and carrot and the hazard quotient to an acceptable daily intake calculated as 1/10000 of the lowest therapeutic dose

	Wheat				Carrot			
Compounds	Estimated daily intake for adult (g/day)	Estimated daily intake for children (g/day)	Hazard quotient for adults	Hazard quotient for children	Estimated daily intake for adult (g/day)	Estimated daily intake for children (g/day)	Hazard quotient for adults	Hazard quotient for children
Ciprofloxacin	4.802×10^{-13}	3.41×10^{-13}	9.603×10^{-09}	2.901×10^{-08}	4.44×10^{-13}	4.07×10^{-13}	8.880×10^{-09}	3.464×10^{-08}
Carbamazepine	3.573×10^{-11}	2.54×10^{-11}	1.787×10^{-06}	5.398×10^{-06}	5.864×10^{-11}	5.38×10^{-11}	2.932×10^{-06}	1.144×10^{-05}
Oxazepam	9.855×10^{-12}	7×10^{-12}	3.285×10^{-06}	9.925×10^{-06}	1.532×10^{-11}	1.4×10^{-11}	5.107×10^{-06}	1.992×10^{-05}
Venlafaxine	2.174×10^{-10}	1.54×10^{-10}	2.898×10^{-05}	8.756×10^{-05}	3.836×10^{-10}	3.52×10^{-10}	5.115×10^{-05}	1.995×10^{-04}
Metoprolol	4.063×10^{-11}	2.89×10^{-11}	4.063×10^{-06}	1.228×10^{-05}	1.368×10^{-11}	1.25×10^{-11}	1.368×10^{-06}	5.336×10^{-06}
Hydrochlorothiazide	5.315×10^{-14}	3.77×10^{-14}	1.063×10^{-08}	3.211×10^{-08}	3.248×10^{-14}	2.98×10^{-14}	6.496×10^{-09}	2.534×10^{-08}
Losartan	3.69×10^{-09}	2.62×10^{-09}	7.380×10^{-04}	2.230×10^{-03}	6.612×10^{-09}	6.06×10^{-09}	1.322×10^{-03}	5.159×10^{-03}
Furosemide	7.587×10^{-10}	5.39×10^{-10}	1.897×10^{-04}	5.731×10^{-04}	1.108×10^{-09}	1.02×10^{-09}	2.771×10^{-04}	1.081×10^{-03}
Diclofenac	1.854×10^{-11}	1.32×10^{-11}	1.854×10^{-06}	5.602×10^{-06}	1.672×10^{-11}	1.53×10^{-11}	1.672×10^{-06}	6.522×10^{-06}

Table 17. The lowest therapeutic dose, acceptable daily intake of selected pharmaceuticals. (n.f. not found)

Compounds	No observable adverse effects dose (mg/day)	Lowest therapeutic dose (mg/day)	Acceptable daily intake* per body weight (g/kg per day)	Acceptable daily intake* for adults (g/day)	Acceptable daily intake* for children (g/day)
Ciprofloxacin	n.f.	500	6.53×10^{-07}	5.00×10^{-05}	1.18×10^{-05}
Carbamazepine	n.f.	200	2.61×10^{-07}	2.00×10^{-05}	4.70×10^{-06}
Oxazepam	n.f.	30	3.90×10^{-08}	3.00×10^{-06}	7.00×10^{-07}
Venlafaxine	n.f.	75	9.80×10^{-08}	7.50×10^{-06}	1.76×10^{-06}
Metoprolol	n.f.	100	1.31×10^{-07}	1.00×10^{-05}	2.35×10^{-06}
Hydrochlorothiazide	n.f.	50	6.50×10^{-08}	5.00×10^{-06}	1.17×10^{-06}
Losartan	n.f.	50	6.50×10^{-08}	5.00×10^{-06}	1.17×10^{-06}
Furosemide	n.f.	40	5.20×10^{-08}	4.00×10^{-06}	9.40×10^{-07}
Diclofenac	n.f.	100	1.31×10^{-07}	1.00×10^{-05}	2.35×10^{-06}

These results indicate that backwater fertilization seem to cause an insignificant risk for humans concerning pharmaceuticals intake. In fact, source separation and recycling of blackwater seem to be a way to significantly decrease the hazard due to pharmaceuticals in the environment, especially to aquatic life, and to some extent also to humans.

Table 18. Estimated years until the total amount of consumed pharmaceutical substance via consumption of blackwater fertilized wheat or carrots, equals the amount of a one-day therapeutic dose for an adult and a child, respectively

	Wheat		Carrot	
Compounds	Years to reach one-day dose for adult	Years to reach one-day dose for child	Years to reach one-day dose for adult	Years to reach one-day dose for child
Ciprofloxacin	2 852 990 000	944 247 000	3 085 277 000	790 909 000
Carbamazepine	15 336 000	5 076 000	9 344 000	2 395 000
Oxazepam	8 340 000	2 760 000	5 365 000	1 375 000
Venlafaxine	945 000	313 000	536 000	137 000
Metoprolol	6 743 000	2 232 000	20 027 000	5 134 000
Hydrochlorothiazide	2 577 595 000	853 100 000	4 217 559 000	1 081 169 000
Losartan	37 000	12 000	21 000	5 000
Furosemide	144 000	48 000	99 000	25 000
Diclofenac	14 777 000	4 891 000	16 386 000	4 201 000

In other words, in treated blackwater the pharmaceuticals are concentrated in small volumes of liquid and small fractions of solids. When used as fertilizers on soils, pharmaceuticals are subjected to biological degradation by bacteria, fungi and other macro flora, oxidation, evaporation and leaching. Thus, what is taken up by the plants (as a secondary recipient) seems from these simulations (with reservations for uncertainties) to be such small amounts that any hazardous effects on humans should be very insignificant. Meanwhile, in conventional municipal wastewater treatment effluent, the pharmaceuticals end up in large volumes of water which are disposed to the receiving water, where fish and other aquatic flora are the primary recipients of these chemicals. Fish and other aquatic flora are known to accumulate pharmaceuticals (Ramirez et al., 2009; Flippin et al., 2007; Huerta et al., 2013).

Pharmaceuticals are designed to cure human beings, and they are thoroughly tested to normally not be toxic at their therapeutic doses. Thus, having as low levels of intake as estimated in our study for wheat and carrot, only $1/10^3$-$1/10^8$ of the acceptable daily intake, estimated as $1/10^4$ of the lowest therapeutic dose, is not expected to pose any toxicity what so ever on human beings.

In characterizing hazards of organic pollutants from agriculture which end up in the food chain, it is relevant to compare hazard of pharmaceuticals with those associated with use of herbicides, which are not designed for human consumption. Beckman (2015) reported that dimethoate and prosulfocarb herbicides were detected in apples at concentrations of 100×10^{-9} and 40×10^{-9} g/g respectively. While these apples were not produced in Sweden, they were nonetheless imported

to and consumed in Sweden. Considering that average consumption of apples in Sweden as 45 g/day for children (Beckman, 2015).This means that the daily intake of dimethoate and prosulfocarb herbicides via eating of apples were 4500×10^{-9} and 1800×10^{-9} g/day respectively. These intake levels of herbicide by children are much higher (100-1000 times) than the intake of any of the studied pharmaceuticals via wheat or carrot in this study (Table 16). The aforementioned levels of herbicides are also higher than the maximum allowed residue of dimethoate (20×10^{-9} g/g) and prosulfocarb (10×10^{-9} g/g) in fruits. The intake from apples with the maximum allowed levels would be 900×10^{-9} g/day of dimethoate and 450×10^{-9} g/day of prosulfocarb, as compared to 2.6×10^{-9} for the pharmaceutical substance with the highest simulated concentration, losartan.

It should be pointed out that the above hazard analysis was meant to present the hazards related only to the parent compounds, without recognition neither to the metabolites of the pharmaceuticals or to their biodegradation by-products in soil. In addition, effects of mixture of pharmaceuticals were not evaluated in the current study. This, however, still means that more research is needed in to characterize hazards of metabolites or biodegradation products and mixture of compounds of pharmaceuticals.

4.3.5 Antibiotics toxicity and development of resistant bacteria

Borche (2010) claimed that ciprofloxacin clearly pose an ecotoxicological hazard already at concentrations currently detected in the environment, hence posing an environmental risk to environmental bacteria, which are essential for basic processes such as organic breakdown, nitrification and denitrification. Costanzo et al. (2005) showed that presence of antibiotics e.g. amoxicillin, erythromycin, and clarithromycin significantly decreased denitrification rates by benthic bacteria. However, the antibiotic concentrations studied by Costanzo et al. (2004) were higher than environmentally relevant concentrations. The toxicity of ciprofloxacin to environmental bacteria hinders its biodegradation, leads to long biodegradation half-life and hence to accumulation of this chemical in the environment. Walter et al. (2010) showed that the degradation half-life of ciprofloxacin in soil varies from 1155 to 3466 days, which gives clear evidence about persistence and accumulation of this chemical in soil. Moreover, Liu et al. (2009) showed phytotoxicity by inhibited soil phosphatase activity and significantly affected soil respiration activity at effective concentrations of 13×10^{-6} g/g dw of sulfamethoxazole.

While antibiotics can be toxic at high concentrations, they can also induce antibiotic resistance in bacteria upon long term exposure to low concentrations. Antibiotic resistance might be useful for the bacteria responsible for organic matter degradation, nitrogen transformation, or phosphate accumulation, because such bacteria might help in degrading the antibiotics themselves. Yet, resistance to antibiotics is a problem when development occurs in human or animal pathogenic bacteria, which will compromise human and animal health. Indirect evidence regarding development of antibiotic resistance in the environment was found when sewer receiving hospital wastewater showed an increased prevalence of oxytetracycline resistance bacteria (Guardabassi et al., 1998). Gullberg et al. (2011) showed that long-term exposure to even low levels of antibiotics leads to increased antibiotic resistance in microbial populations. A more recent study

focused on antibiotic resistance towards ampicillin, ciprofloxacin, gentamicin, tetracycline, and chloramphenicol in effluent from wastewater treatment plant in Slovenia (Birošová et al., 2014). The study revealed high concentrations of coliform bacteria, which were resistant to ampicillin and gentamicin, were found in the sludge during winter. The same study claimed that the number of bacteria with high resistance to all tested antibiotics increased in the sludge during the summer.

In several treatment facilities in Sweden, blackwater is sanitized using urea addition (0.5 % for 7 days) followed by 6 months of storage. Under such conditions, wastewater borne pathogens are hygienized. Since hygienization is not 100% efficient in inactivating pathogens, it might be necessary to perform more research about development of antibiotic resistance in human pathogens in fields fertilized with treated blackwater. This risk can be compared with the risks when antibiotics end up in recipient water bodies through effluent from large wastewater treatment plants and with the risk from antibiotics in manure from cows and pigs. Winker et al. (2009) showed e.g. that when using manure from German pigs or cattle, five different antibiotics (tetracycline, oxytetracycline, chlortetracycline, sulfamethazine and sulfadiazine) were applied in larger doses than 80 g/ha. This is about 2500 times higher than the largest dose of antibiotics applied with blackwater in this study (0.03 g/ha of ciprofloxacin, Table 15).

5. Conclusions

- Fecal sludge and blackwater showed comparably high concentrations of many pharmaceuticals. These concentrations were higher than those found in influent to large scale WWTPs, which can be attributed to less dilution.
- The average removal efficiency of pharmaceuticals was more efficient in the aerobic treatment of blackwater by liquid composting and ammonia treatment than in the anaerobic digestion of fecal sludge. In addition, the reduction of pharmaceuticals in the liquid composting was generally more efficient compared to conventional WWTPs. Many substances showed low removal rates, often even negligible, in the ammonia treatment and during six month of post storage. No significant difference in the reduction on average between mesophilic and thermophilic anaerobic digestion was found.
- Considerably high concentrations of some substances still remain in the treated blackwater after combined liquid composting, ammonia treatment and post storage compared to effluents and sludge from most WWTPs.
- The study shows that the theoretical dose of pharmaceuticals on land when fertilizing with blackwater was at comparable level as when sewage sludge was used as fertilizer.
- Based on simulation with the BASL4 model, fertilization with blackwater at 40 ton/ha per year during 3 years seems to cause low accumulation of pharmaceuticals in soil and very low concentrations in wheat and carrot fertilized with the blackwater.

- The estimated daily intakes of pharmaceuticals in a worst case scenario through ingestion of wheat and carrot fertilized with blackwater were insignificant in relation to the acceptable daily intake of pharmaceuticals based on the lowest therapeutic dose. The simulations of the behavior of the substances in the soil are uncertain, but still give a first indication that the risk levels posed by the pharmaceutical in the treated blackwater used as fertilizer ought to be low.

6. Future perspectives

This report provides new insights into recycling of source separated toilet fractions. To further study this area several interesting directions can be taken, which are exemplified below.

In general, source separation and application of toilet waste as fertilizer on arable land is beneficial considering closing of the plant nutrient loop. However, there are a number of concerns that should be further investigated, e.g. evolutionary selective pressure combined with antibiotics in the environment may accelerate the development and spreading of antibiotic-resistant pathogens. Worth noting is that in many countries the large use of antibiotics in animal production might constitute a far larger risk, than fertilization with source separated toilet fractions. There is also the complex interdependence between agribusiness, food industry and consumers to consider. Thus, there is a clear incentive to minimize the spreading of pharmaceuticals on arable land. Therefore, more research and development for efficient removal of pharmaceuticals in source separated and nutrient recycling systems is needed. That includes identification and development of necessary technical improvements, fate and behavior of pharmaceuticals and metabolites during the treatment as well as fate of antibiotic resistant genes.

Furthermore, a better knowledge of the fate of pharmaceuticals in plants, soil and groundwater is needed in order to estimate the risk with this system. Our simulations indicate that a large part of the pharmaceuticals that are spread on the arable land when recycling blackwater is degraded already in the fields and very little is accumulated in soil or taken up by the crop during growth. These results are highly interesting, but uncertain as the model used was not validated for these substances. The simulation model needs to be calibrated, and the results need to be validated and verified. Especially better estimating of the risk of leaching is needed. Questions regarding occurrence and risks associated with pharmaceuticals and their metabolites, leakage of pharmaceuticals to groundwater and run off from agricultural fields fertilized with source separated fractions are of concern. Further interesting questions are how the soil and crop type influence the risks.

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Appendix

Table 1. Characterization of untreated fecal sludge (UL), untreated blackwater (UR1 and UR2) and post stored blackwater samples (6 months of post storage)

	UL	UR1	UR2	WUR1 $_{6month}$	WUR2 $_{6month}$
DM (mg/L)	7.6[a]	4400	3600	2300	2100
Loss of ignition (mg/L)	86[b]	2900	2300	1400	1100
pH	6.7	8.3	8.2	9.1	9.1
N_{tot} (mg/L)	49[c]	710	700	3200	3400
NH_4-N (mg/L)	32[c]	520	510	1200	1500
P (mg/L)	13 [c]	120	130	31	44
COD_{Cr} (mg/L)	1600[c]	5400	5300	820	1400
TOC(mg/L)				370	670
Pb (µg/L)	<2.0[d]	36	37	4.1	12
Cd (µg/L)	0.33[d]	2.0	1.9	0.16	0.73
Cu (µg/L)	30[d]	1100	1000	200	400
Cr (µg/L)	3.5[d]	38	28	<2.5	5.4
Hg (µg/L)	0.11[d]	1.3	0.21	<0.1	0.20
Ni (µg/L)	4.1[d]	49	50	10	17
Zn (µg/L)	230[d]	2400	2400	270	910
Ag (µg/L)	<1.0[d]	1.4	3.0	<0.5	0.64
Sn (µg/L)	5.6[d]	58	58	6.7	15
K (mg/L)	14[c]	160	150	160	150

[a] %; [b] % of DM; [c] g/kg DM; [d] mg/kg DM

Table 2. LOD and LOQ for target pharmaceuticals analyzed at SLU

Therapeutic group	Compound	LOD liquid (ng/L)	LOQ liquid (ng/L)	LOD solid (ng/g)	LOQ solid (ng/g)
Analgesics	Codeine	20	75	20	40
β-blockers	Atenolol	69	227	20	80
	Sotalol	29	100	15	60
	Metoprolol	4	12	5	20
	Propranolol	4	13	2.5	10
Antibiotics	Azithromycin	69	230	170	560
	Clarithromycin	6	21	20	40
	Norfloxacin	128	430	-	-
	Ciprofloxacin	118	390	-	-
	Ofloxacin	44	150	-	-
	Sulfamethoxazole	115	380	10	40
	Trimethoprim	48	160	29	96
Anti-hypertensives	Losartan	30	95	43	140
	Valsartan	100	331	63	210
	Irbesartan	75	250	20	80
	Diltiazem	4	13	15	50
Anti-depressants	Carbamazepine	9	29	20	54
	Citalopram	13	43	23	77
	Diazepam	2	6	5	20
	Lamotrigine	2	4	9	34
	Oxazepam	111	370	20	71
	Venlafaxine	61	204	5	20
	Fuoxetine	10	32	100	500
	Amitriptyline	8	25	5	20
Anti-ulcer agent	Ranitidine	115	115	400	800
Anti-fungal agents	Climbazole	6	20	1.5	5
	Ketoconazole	10	23	2	6
Local anesthetic	Lidocaine	25	83	1.7	5.6
Diuretics	Furosemide	5	16	25	100
	Hydrochlorothiazide	15	50	10	40
Lipid regulators	Atorvastatin	20	62	-	-
	Bezafibrate	30	100	10	35

<LOD: below limit of detection; <LOQ: below limit of quantification; - no analysis due to limits in extraction method of solid phase

Table 3. LOD and LOQ for target pharmaceuticals analyzed at SPPD

Therapeutic group	Compound	LOD liquid (ng/L)	LOD solid (ng/g)
Analgesics and anti-inflammatories	Ibuprofen	118 ± 38	12
	Naproxen	274 ± 124	6.1
	Diclofenac	113 ± 67	5.3
	Acetaminophen	237 ± 17	-
	Budesonide	15	-
Anti-hypertensives	Candesartan	39.7 ± 7.3	-
	Ramipril	2.6 ± 2.3	-
	Amlodipine	8	0.9
Lipid regulators	Atorvastatin	5.3 ± 3.0	0.8
Anti-diabetic	Saxagliptine	10	-
Antibiotic	Sulfamethoxazole	2	-
Anti-histaminic	Cetirizine	1.6 ± 0.3	-
Anti-depressants	Carbamazepine	7.0 ± 0.8	0.2
	Fluoxetine	8.4 ±3.6	-
Diuretic	Furosemide	775 ± 382	1.6
β-blockers	Bisoprolol	2.0 ± 0.6	-
Stimulant	Caffeine	174 ± 33	-

<LOD: below limit of detection; <LOQ: below limit of quantification; - Not investigated

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