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Pharmaceuticals in source separated sanitation systems: Fecal sludge and blackwater treatment

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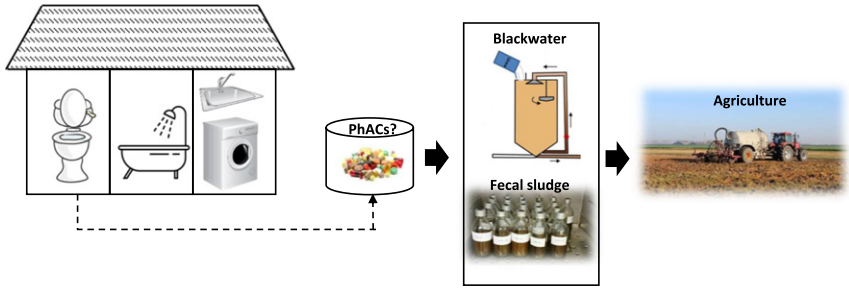
HIGHLIGHTS

- PhACs quantified for the first time in fecal sludge
- PhACs are partially degraded in fecal sludge and blackwater treatments.
- Similar PhACs reduction rates achieved in different fecal sludge AD conditions
- Most PhACs are removed during the blackwater wet composting process.
- In blackwater treatment urea addition has a minor effect in PhACs reduction.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated, for the first time, the occurrence and fate of 29 multiple-class pharmaceuticals (PhACs) in two source separated sanitation systems based on: (i) batch experiments for the anaerobic digestion (AD) of fecal sludge under mesophilic (37 °C) and thermophilic (52 °C) conditions, and (ii) a full-scale blackwater treatment plant using wet composting and sanitation with urea addition. Results revealed high concentrations of PhACs in raw fecal sludge and blackwater samples, with concentrations up to hundreds of μg L^{-1} and μg kg^{-1} dry weight (dw) in liquid and solid fractions, respectively. For mesophilic and thermophilic treatments in the batch experiments, average PhACs removal rates of 31% and 45%, respectively, were observed. The average removal efficiency was slightly better for the full-scale blackwater treatment, with 49% average removal, and few compounds, such as atenolol, valsartan and hydrochlorothiazide, showed almost complete degradation. In the AD treatments, no significant differences were observed between mesophilic and thermophilic conditions. For the full-scale blackwater treatment, the aerobic wet composting step proved to be the most efficient in PhACs reduction, while urea addition had an almost negligible effect for most PhACs, except for citalopram, venlafaxine, oxazepam, valsartan and atorvastatin, for which minor reductions (on average 25%) were observed. Even though both treatment systems reduced initial PhACs loads considerably, significant PhAC concentrations remained in the treated effluents, indicating that fecal sludge and blackwater fertilizations could be a relevant vector for dissemination of PhACs into agricultural fields and thus the environment.

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1. Introduction

Urban wastewater management has started to change during the late 20th century in order to face new demands from society such as the reuse and recovery of nutrients present in wastewater and in controlling greenhouse gases emissions (Skambraks et al., 2017). Nutrient recovery from wastewater could have a direct impact in reducing the dependence on chemical fertilizers, decreasing the discharge of nutrients into the environment and reducing climate change impacts (McConville et al., 2017). Among nutrient recovery schemes, source separation is a promising approach to address most of these challenges. In these systems, domestic wastewater is fractionated into blackwater (urine, feces, toilet paper and flush water) and greywater (wastewater from bath, laundry and kitchen) directly at the source (Otterpohl et al., 2003; Kujawa-Roeleveld and Zeeman, 2006; Kjerstadius et al., 2015).

Most of the nutrients (e.g. nitrogen and phosphorous) found in wastewater come from human urine and feces. Thus, after appropriate treatment and sanitation, blackwater could be converted into a valuable nutrient-rich bio-fertilizer to be reused in agricultural fields (Jönsson, 2002). Nevertheless, an issue that raises concern is the levels of pathogens and organic micropollutants, especially pharmaceuticals (PhACs), present in blackwater fractions (McConville et al., 2017), and its reuse might thus be an important contamination pathway to the environment. Once applied as bio-fertilizer in agricultural areas, and depending on their properties, some of the PhACs will degrade (Xu et al., 2009; Walters et al., 2010; Grossberger et al., 2014) while others might accumulate in soils, be taken up by crops or leach to surface and groundwater bodies, as has been widely reported by the reuse of other organic fertilizers, such as sewage sludge or animal manure (Tanoue et al., 2012; Carter et al., 2014; Verlicchi and Zambello, 2015; Thasho and Cho, 2016; Bourdat-Deschamps et al., 2017; Boy-Roura et al., 2018; Ivanová et al., 2018). Thus, blackwater treatment is recommended in order to avoid potential environmental and human health risks (Larsen et al., 2009). Some of the most common blackwater treatments used nowadays include aerobic and anaerobic biological processes and membrane bioreactors, among others (Chaggu et al., 2007; Luostarinen et al., 2007; Murat Hocaoglu et al., 2011; Jin et al., 2018).

The number of pilot areas with source separation systems is growing in Northern Europe, especially in the Netherlands and Sweden (McConville et al., 2017). In Sweden, these systems are mostly applied in areas that are not connected to public wastewater treatment plants (WWTPs) and that rely on on-site wastewater treatment facilities (Blum et al., 2017; Gros et al., 2017). Indeed, approximately 9% of the population have permanent dwellings with on-site systems and around 2% are based on source separated systems (Ek et al., 2011). It is estimated that there are several tens of thousands of blackwater separation systems in densely populated rural areas (Vinnerås and Jönsson, 2013). In addition, source separation is also common in summer houses, most often as part of dry toilet systems (McConville et al., 2017) and latrine pits (fecal sludge) commonly used also in national parks and roadside facilities. Even though some municipalities are already using source separated fractions as bio-fertilizers in crop farming (Eveborn et al., 2007), little is still known about the potential environmental risks associated with this agricultural practice. Most research on the recovery of nutrients from blackwater or fecal sludge studies the stabilization and sanitation of this waste stream (Vinnerås, 2007; Butkovskyi et al., 2016; Mulec et al., 2016; Rogers et al., 2018; Thostenson et al., 2018) or the production of electrical energy (Vogl et al., 2016), while a limited number of papers investigate the fate of micropollutants, such as PhACs, during treatment (de Graaff et al., 2011; Bischel et al., 2015; Butkovskyi et al., 2015; 2017). Blackwater and fecal sludge treatments, which have been investigated for the reduction of micropollutants, include upflow anaerobic sludge bed reactors (UASB) and composting (Butkovskyi et al., 2016), UASB followed by oxygen-limited autotrophic nitrification-denitrification and struvite precipitation (Butkovskyi et al., 2015) and a combination of aerobic and nitritation-anammox treatments (de Graaff et al., 2011).

In this study, we investigated, to the best of our knowledge for the first time, the occurrence and removal of 29 multiple-class PhACs of major use in two different source separated sanitation treatment systems: (i) anaerobic digestion (AD) of fecal sludge (latrine), using batch experiments under mesophilic and thermophilic conditions and (ii) a full-scale blackwater treatment plant based on wet (aerobic) composting followed by ammonia treatment (urea addition) for sanitation of pathogens. Analytical methods were developed for the analysis of PhACs in both solid and liquid fractions of fecal sludge and blackwater, and quantification of target compounds was based on ultra-high-performance-liquid chromatography (UHPLC) followed by high resolution mass spectrometry (HRMS). In addition to the analysis of PhACs, the production of biogas was recorded in the anaerobic batch experiments. The results derived from this study provide valuable information about the performance of these source separated sanitation treatment techniques and will be helpful in future assessments for enhancing the removal of micropollutants and ensure a safe reuse of these waste streams.

2. Materials and methods

2.1. Chemicals and reagents

In total 29 PhACs were analyzed. Standards were purchased from Sigma-Aldrich (Sweden) for the PhACs amitriptyline (as hydrochloride salt), atenolol, azithromycin, bezafibrate, carbamazepine, ciprofloxacin, citalopram (as hydrobromide salt), clarithromycin, fluoxetine (as hydrochloride salt), furosemide, hydrochlorothiazide, irbesartan, lamotrigine, lidocaine, losartan (as potassium salt), metoprolol (as tartrate salt), norfloxacin, propranolol (as hydrochloride salt), ofloxacin, sotalol (as hydrochloride salt), sulfamethoxazole, trimethoprim, valsartan and venlafaxine (as hydrochloride salt). Other PhACs, such as atorvastatin (as atorvastatin calcium solution), codeine, diazepam, diltiazem and oxazepam were acquired as a 1 mg mL^{-1} solution in methanol from Cerilliant and purchased through Sigma-Aldrich (Sweden). All analytical standards were of high purity grade (>95%). The isotopically labeled substances (IS) atorvastatin-d_5 (as calcium salt), carbamazepine-d_{10} (100 μg mL^{-1} solution), codeine-d_3 (1 mg mL^{-1} solution), citalopram-d_6 (as HBr solution at 100 μg mL^{-1}), diazepam-d_5 (1 mg mL^{-1} solution), fluoxetine-d_5 (1 mg mL^{-1} solution), lamotrigine-^{13}C-$^{15}N_4$ (500 μg mL^{-1} solution), lidocaine-d_{10}, ofloxacin-d_3, trimethoprim-d_9 and venlafaxine-d_6 (100 μg mL^{-1} HCl solution, free base) were acquired from Sigma-Aldrich. Atenolol-d_7, azithromycin-d_3, bezafibrate-d_4, bisoprolol-d_5, ciprofloxacin-d_8, hydrochlorothiazide-^{13}C-d_2, diltiazem-d_4 (as hydrochloride salt), furosemide-d_5 irbesartan-d_7 and sulfamethoxazole-d_4 were purchased from Toronto Research Chemicals (TRC) (details in Table S1 in Supplementary material (SM)). For chemical analysis, HPLC grade methanol (MeOH) and acetonitrile (ACN), were purchased from Merck (Darmstadt, Germany), whereas formic acid 98% (FA), ammonium formate, 25% ammonia solution and ammonium acetate were acquired from Sigma-Aldrich (Sweden). Ultrapure water was produced by a Milli-Q Advantage Ultrapure Water purification system (Millipore, Billercia, MA) and filtered through a 0.22 μm Millipak Express membrane. The solid phase extraction (SPE) cartridges used were Oasis HLB (200 mg, 6 cm^3) from Waters Corporation (Milford, USA). Glass fiber filters (Whatman™, 0.7 μm) were purchased from Sigma-Aldrich (Sweden). Pre-packed Bond Elut QuEChERS extract pouches (1.5 g sodium acetate and 6 g $MgSO_4$) were acquired from Agilent Technologies (Sweden). SampliQ Anydrous $MgSO_4$ for QuEChERS and PSA (SPE bulk sorbent) were also acquired from Agilent Technologies (Sweden).

2.2. Treatment techniques

2.2.1. Fecal sludge anaerobic digestion

The fecal sludge (latrine) used for the anaerobic digestion (AD) experiments was sampled in August 2014 at Salmunge waste plant in Norrtälje, Sweden. The fecal sludge collected from private houses is stored in two concrete basins (each one 116 m^3), where the second is used as a backup. The main basin contained approximately 60 m^3 when sampling was performed. A stirrer placed in the middle of the pool was active 20 h prior to and during sampling. Samples were collected from the main basin in metal buckets at two positions: close to the middle, near the stirrer, and close to the short side of the pool, and at two depths (surface and 0.2 m from bottom using a pump). From each sampling point, 10 L fecal sludge was collected, resulting in a total amount of 40 L. Sludge was afterwards mixed in a polypropylene container and stirred vigorously for approximately 5 min using a concrete stirrer (Meec tools 480/800 rpm) in order to homogenize the material and avoid sedimentation when transferring into smaller bottles. The bottles were sealed, wrapped with aluminum foil and transported refrigerated to the lab for use in the anaerobic digestion experiments.

Anaerobic batch digestion experiments were performed under controlled conditions in laboratory glass bottles, using the collected fecal sludge waste as substrate. Two parallel experiments were performed in triplicate under (i) mesophilic conditions (37 °C) and (ii) thermophilic conditions (52 °C). As inocula for the experiments, sludge from the mesophilic reactor at Kungsängsverket WWTP in Uppsala and from the thermophilic reactor at Kävlinge WWTP in Lund were used for the two treatments. Before the experiments, the inoculum was degassed for a week at 37 °C or 52 °C, respectively. Dry matter (DM) and volatile solids (VS) of substrate and both inocula were measured in triplicate using standardized methods (Table S2). Glass bottles with a total volume of 1.1 L were filled with inoculum, tap water and substrate (fecal sludge) to a final volume of 600 mL, while flushed with N_2-gas. Each bottle was loaded with 3 g VS/L of fecal sludge. A fecal sludge to inoculum mass ratio of 1:3 was used and calculated based on the VS. Bottles were sealed with a rubber stopper and aluminum-caps and were covered with aluminum foil. Incubation was conducted on a shaker (130 rpm) at 37 °C or 52 °C for 61 days for mesophilic conditions and 59 days for thermophilic, respectively. PhACs were analyzed in the raw fecal sludge (latrine) used for the AD experiments and at specific times along the treatment experiment in order to assess the degradation of target compounds over time (Table 1). Methane production was also monitored at specific times along the experiment by gas chromatography (GC), and results are summarized in Table 1. Additionally, for both treatments, control samples were prepared for PhAC analysis consisting of bottles filled with only inocula and tap water.

2.2.2. Blackwater treatment

Blackwater samples were taken from the full-scale treatment plant at Nackunga gård, Hölö (Södertälje, Sweden) in December 2014. The plant processes blackwater from approximately 1500 subscribers in two batch fed 32 m^3 reactors (R1 and R2), which operate in parallel. The degradation of PhACs was studied during one batch in the two reactors (R1 and R2). The treatment consists of two steps. The first step is wet composting where blackwater is mineralized due to aeration and constant mixing (aerobic treatment) for about 7–12 days. At the end of the aerobic treatment the temperature of the substrate should have raised to about 40 °C. The increase in temperature is attributed to mesophilic microbes which use the available organic matter as energy source (Dumontet et al., 1999). In the second step, which is facilitated by the temperature increase, the substrate is sanitized with urea, which is a nitrogenous compound (a carbonyl group attached to two amine groups) formed in the liver and therefore, naturally occurring in urine. In this process step, the urea in the blackwater is supplemented with 0.5% additional urea, added to the substrate, which is constantly mixed for approximately 7 days (no aeration is performed during urea treatment) to have higher sanitation effect. In the reactor, urea is degraded by hydrolysis due to the enzyme urease, naturally found in feces, to ammonia and carbon dioxide and both products have disinfectant properties towards pathogenic microorganisms (Nordin et al., 2009; Fidjeland et al., 2013).

Samples were collected at different stages of the treatment, including: (i) untreated blackwater, (ii) after the wet composting process, and (iii) after the ammonia treatment (addition of urea) (Fig. 1). For the wet composting process, samples were collected after 12 days of aeration. The temperature in the reactors had then reached 41 °C and 35 °C (R1 and R2, respectively). For reactor R2 it took additional 6 days to finalize the wet composting process and reach 40 °C. In the end, final samples were collected after 6 days (R1) and 3 days (R2) of urea treatment. The temperature had then reached 43 °C and 41 °C (R1 and R2, respectively). For each treatment step, samples were taken from a sampling tap located on a continuously operated circulation loop bringing the substrate from bottom to the top of the reactor. The circulation loop provided a homogenous mixture of the substrate and the samples. About 10–25 L of blackwater from each reactor and sampling occasion were collected in a polyethylene bucket, which were then transferred to polyethylene bottles. After collection, samples were transported to the laboratory and were kept at 4 °C until sample preparation. Samples (1000 mL) of un-treated blackwater from R1 and R2, respectively, were stored in a fridge at 6.5 °C ± 1.3 °C. Untreated blackwater samples were stored for 12 and 19 days, respectively, which was like the process phases in the full-scale blackwater treatment plant, to determine whether target PhACs were degraded due to other processes not associated with the reactor treatment. Furthermore, treated blackwater was stored for a period of 3 and 6 months respectively (same conditions as above), to assess any potential degradation of PhACs during post-storage, before its application as fertilizer in agricultural fields (Fig. 1).

Table 1
Summary of the experiments performed during the anaerobic digestion of fecal sludge and samples analyzed as well as the gas and methane production in each of the experiments.

Experiment	Temperature (°C)	Incubation (days)	Gas production (NmL CH_4/g VS)	% methane
Mesophilic	37	0	0	–
	37	14	–	–
	37	30	221	58
	37	61	254	59
Thermophilic	52	0	0	–
	52	21	–	–
	52	30	230	58
	52	59	257	60

2.3. Characterization of fecal sludge and blackwater and PhACs analysis

2.3.1. Chemical characterization of fecal sludge and blackwater

Samples of untreated fecal sludge and blackwater were analyzed for dry matter (DM), volatile solids (VS), pH, total nitrogen, ammonium nitrogen (N-NH_4), chemical oxygen demand (COD), total phosphorous (P), potassium (K) and metals (Pb, Cr, Cd, Cu, Zn, Hg, Ni, Ag and Sn). All analyses were performed using standardized methods, and results are presented in Table 2 (for details about analytical methods, see the Supplementary material).

2.3.2. Sample pre-treatment for PhAC analysis

Raw fecal sludge samples (used in the AD experiments) and blackwater samples were centrifuged in order to analyze the liquid and solid fractions separately. For fecal sludge and blackwater, 1.5 L of sample (distributed in six pre-weighted empty 250 mL containers) were centrifuged in a Beckman Coulter J26XPi centrifuge at 10,000 rpm for 10 min, at 15 °C. After centrifugation, the supernatant (liquid fraction) was decanted to 1 L polypropylene bottles, pre-rinsed with ethanol,

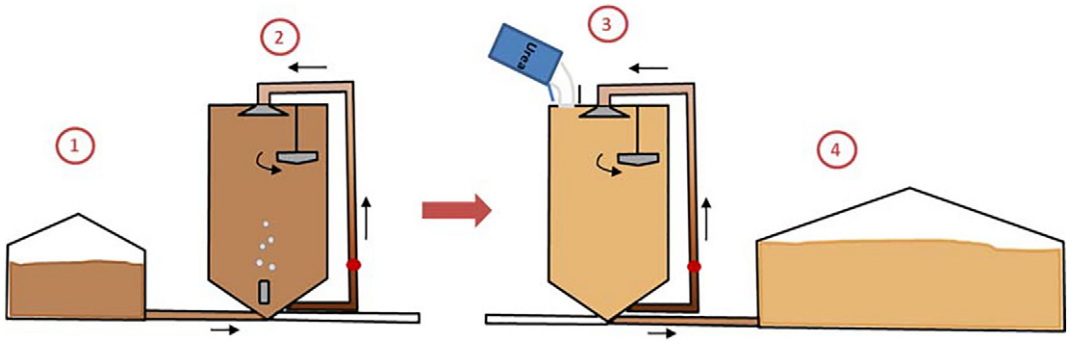

Fig. 1. Scheme of the blackwater treatment: 1) the pre-storage tank where the blackwater is stored until treatment; 2) wet composting process with aeration and constant mixing; 3) sanitation by addition of 0.5% urea; 4) post-storage of the treated blackwater.

whereas the remaining solid residue was transferred with a spatula to 50 mL polypropylene containers. The samples taken at the start and at different time points during the AD experiment followed the same pre-treatment procedure as raw fecal sludge and blackwater. After centrifugation, solid and liquid fractions were frozen at −20 °C until analysis.

2.3.3. Analysis of PhACs in the liquid fractions

Prior to analysis, AD and blackwater liquid fractions were filtered through glass fiber filters (0.7 μm, GF/F, Whatman), while for raw fecal sludge liquid fraction, 2.7 μm followed by 0.7 μm glass fiber filters were used. For analysis of AD and blackwater samples, 100 mL of the filtrate was measured and extracted whereas for raw fecal sludge, 25 mL was diluted to 50 mL with MilliQ water. Samples were spiked with 50 μL of a 1 ng μL^{-1} isotopically labeled internal standard (IS) mixture and an adequate volume of a Na_2EDTA solution (0.1 M) was added to reach a concentration of 0.1% (g solute g^{-1} solution) in the samples. Sample pH was then adjusted to 3 using formic acid. Samples were extracted and pre-concentrated by solid phase extraction (SPE) using Oasis HLB cartridges (200 mg, 6 cm^3). The cartridges were conditioned with 6 mL pure methanol followed by 6 mL acidified Millipore water (pH = 3 with formic acid). Samples were loaded at a flow rate of approximately 1 mL min^{-1}. Cartridges were washed with Millipore water (pH = 3) and centrifuged at 3500 rpm for 5 min to remove excess of water. Analytes were eluted with pure methanol (4 × 2 mL). Extracts were evaporated until dryness under a gentle N_2 stream and then reconstituted with methanol/HPLC grade water (10:90, v/v). Prior to instrumental analysis, blackwater extracts were filtered through 0.2 μm regenerated cellulose (RC) syringe filters, while for AD and untreated latrine extracts, 0.45 μm RC filters were used.

Table 2
Chemical characterization of untreated fecal sludge (US) and blackwater samples, taken from Reactor 1 (R1) and Reactor 2 (R2).

Parameters	Untreated fecal sludge	Untreated blackwater	
	US	R1	R2
Dry matter (DM) (mg L^{-1})	76,000	4400	3600
Volatile solids (VS) (mg L^{-1} ash)	10,640	1500	1300
pH	6.7	8.3	8.2
Total N (mg L^{-1})	3724	710	700
N-NH_4 (mg L^{-1})	2432	520	510
P (mg L^{-1})	988	120	130
COD (mg L^{-1})	1600[a]	5400	5300
Pb (μg L^{-1})	<2.0[b]	36	37
Cd (μg L^{-1})	0.33[b]	2.0	1.9
Cu (μg L^{-1})	2280	1100	1000
Cr (μg L^{-1})	3.5[b]	38	28
Hg (μg L^{-1})	0.11[b]	1.3	0.21
Ni (μg L^{-1})	4.1[b]	49	50
Zn (μg L^{-1})	230[b]	2400	2400
Ag (μg L^{-1})	<1.0[b]	1.4	3.0
Sn (μg L^{-1})	5.6[b]	58	58
K (μg L^{-1})	1064	160	150

[a] g kg−1 DM.
[b] mg kg−1 DM.

2.3.4. Analysis of PhACs in the solid fractions

Prior to analysis, solid fractions were freeze dried for 3–5 days and then homogenized by grinding with mortar and pestle. The analytical method was adapted from the one described by Peysson (2013) for the analysis of PhACs in sewage sludge by using the quick, easy, cheap, effective, rugged and safe (QuEChERS) method. Briefly, 1 g of homogenized sample was weighted in 50 mL polypropylene centrifuge tubes and 50 μL of the IS mixture (1 ng μL^{-1}) was added. Samples were mixed with a vortex mixer for 30 s, and thereafter 7.5 mL of a 0.1 M Na_2EDTA solution were added. Samples were vortexed for 30 s, 7.5 mL ACN containing acetic acid (1% v/v) were added, and samples were vortexed again for 30 s. Then, 1.5 g sodium acetate and 6 g $MgSO_4$ pre-packed QuEChERS salts were added. The samples were immediately shaken by hand and centrifuged at 3500 rpm during 5 min. Approximately 6 mL of the supernatant (ACN layer) was transferred to 15 mL polypropylene tubes containing pre-weighted 900 mg $MgSO_4$ and 150 mg PSA sorbents. The tubes were manually shaken for 30 s, vortexed for 1 min and centrifuged at 3500 rpm for 15 min. After that, the ACN layer, approximately 5 mL, was transferred into glass tubes and evaporated to ~200 μL using nitrogen evaporation. The remaining extracts were transferred to 1 mL amber glass HPLC vials. The extracts were frozen at −20 °C for 1 h and then centrifuged at 3500 rpm for 5 min as an extra sample clean-up step. After that, the extracts were transferred into another 1 mL amber glass HPLC vial and concentrated to dryness using a gentle N_2 stream. Finally, extracts were reconstituted with methanol/HPLC grade water (30:70, v/v). Prior to instrumental analysis extracts were filtered through RC syringe filters (0.22 μm).

2.3.5. Instrumental analysis

An Acquity ultra-high-performance-liquid chromatography (UHPLC) 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 PhACs. For the compounds analyzed under positive electrospray ionization (PI), chromatographic separation was achieved using an Acquity HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm particle size), while for the compounds analyzed under negative ionization (NI), an Acquity BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm particle size) was used. The operating flow rate for PI and NI was 0.5 mL min^{-1}. The mobile phases used in PI mode were A) 5 mM ammonium formate buffer with 0.01% formic acid and B) ACN with 0.01% formic acid, while in NI mode A) 5 mM ammonium acetate buffer with 0.01% ammonia and B) ACN with 0.01% ammonia were used. 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 MS 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 at a scan time of 0.25 s. Capillary voltages of 0.35 and 0.4 kV were used in PI and NI 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^{-1}) 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 PhACs 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%).

2.3.6. Quality assurance, quality control and statistical analysis

Relative recoveries were determined by spiking AD and blackwater (liquid and solid fractions) in triplicate, with a known concentration of target analytes, and comparing the theoretical concentrations with those achieved after the whole analytical process, calculated using the internal standard calibration. Since liquid and solid samples can contain target PhACs, blanks (non-spiked samples) were also analyzed, and the levels found were subtracted from those obtained from spiked samples. Recoveries of target PhACs in aqueous fecal sludge AD samples and blackwater ranged from 57% to 170% and relative standard deviations were <30% (Table S3 in SM). Recoveries in solid samples ranged from 70% to 160%, except for clarithromycin and valsartan, whose recovery was around 50% and 60%, respectively (Table S3 in SM). No target compounds were detected in the method extraction blanks. Method detection limits (MDL) and quantification limits (MQL) were determined as the minimum detectable amount of analyte with a signal-to-noise of 3 and 10, respectively (Table S4 in SM). MDLs and MQLs were calculated as the average of those estimated in real samples and in the spiked samples used to calculate recoveries. MDLs in aqueous AD samples and in blackwater ranged from approximately 5 to 120 ng L^{-1}, whereas MQLs ranged from around 10 to 400 ng L^{-1}. In solid samples, MDLs ranged approximately from 3 to 150 μg kg^{-1} dw and MQLs from 10 to 500 μg kg^{-1} dw. Quantification of target analytes was performed by linear regression calibration curves using the internal standard approach, to account for possible matrix effects. Calibration standards were measured at the beginning and at the end of each sequence, and one calibration standard was measured repeatedly throughout the sequence to check for signal stability and as quality control. Independent two samples *t*-tests were performed to assess for differences in compounds concentration in the samples taken at the beginning and at the end of the AD experiments and blackwater treatment. *t*-Tests were performed at a 95% confidence level, using SPSS software, version 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Occurrence of PhACs in untreated fecal sludge and blackwater

The concentrations of PhACs detected in untreated fecal sludge and blackwater samples are summarized in Table 3. For liquid fractions, 19 out of the 29 monitored PhACs were detected in blackwater, while 11 substances were found in fecal sludge. For the solids, 15 and 16 out of the 29 targeted PhACs were present in blackwater and fecal sludge solid fractions, respectively (Table 3). Identified compounds included the following therapeutic groups: analgesics (codeine), β-blocking agents (atenolol, sotalol, metoprolol, propranolol), psychiatric drugs (carbamazepine, citalopram, diazepam, lamotrigine, oxazepam, venlafaxine, amitryptiline), antihypertensives (losartan, valsartan, irbesartan, diltiazem), diuretics (furosemide, hydrochlorothiazide), lipid regulators (atorvastatin) and a local anesthetic (lidocaine). In general, concentrations detected were within 1.6 and 180 μg L^{-1} and from 0.043 to 31 μg L^{-1} for fecal sludge and blackwater liquid fractions, respectively, while for solid fractions concentrations ranged from 76 to 7400 μg kg^{-1} dw and from 61 to 2400 μg kg^{-1} dw for fecal sludge and blackwater solid fractions, respectively. The compounds found at the highest concentrations, in both blackwater and fecal sludge liquid fractions (~5 μg L^{-1}), were metoprolol, propranolol (in blackwater), carbamazepine (in fecal sludge), lamotrigine (in blackwater), venlafaxine, losartan, valsartan, furosemide and hydrochlorothiazide. For solid fractions, the substances detected at the highest concentrations (>500 μg kg^{-1} dw in at least one of the samples) were propranolol, citalopram, oxazepam, venlafaxine, losartan and hydrochlorothiazide, for blackwater, and atenolol, metoprolol, carbamazepine, venlafaxine, losartan, irbesartan, furosemide and hydrochlorothiazide for fecal sludge. Results also indicate that most PhACs primarily partition to the liquid phase, in both blackwater and fecal sludge. Nevertheless, the distribution in the solid phase is also significant for some substances (e.g. carbamazepine, citalopram, diazepam, oxazepam and amitryptiline.), indicating that both solid and liquid phases should be evaluated when studying the occurrence and fate of PhACs in blackwater and fecal sludge.

The concentrations detected in the liquid fractions (blackwater and fecal sludge) were higher than those reported for urban influent wastewater samples (Gros et al., 2010; Behera et al., 2011; Jelic et al., 2011; Collado et al., 2014), where levels rarely reach high μg L^{-1} levels (e.g. 10 μg L^{-1}). This is expected, since source separated fractions are about 25 times more concentrated than wastewater samples from conventional domestic WWTPs (de Graaff et al., 2011). Concentrations detected in solid fractions were similar to those reported for sewage sludge (Radjenović et al., 2009; McClellan and Halden, 2010; Martín et al., 2012; Narumiya et al., 2013; Boix et al., 2016). In general terms, the concentrations detected in blackwater are in good agreement with those previously reported in other studies. Bischel et al. (2015) analyzed 12 PhACs in source separated urine and detected concentrations ranging from <3 to 120 μg L^{-1} for hydrochlorothiazide and from <1 to 300 μg L^{-1} for atenolol. Butkovskyi et al. (2015) determined the occurrence of 14 multiple class PhACs in an UASB reactor in the Netherlands and found high PhACs levels exceeding 100 μg L^{-1} for hydrochlorothiazide, metoprolol and ciprofloxacin in untreated blackwater. In a more recent study, the same authors (Butkovskyi et al., 2017) detected concentrations of 15 ± 6.9 μg L^{-1} for oxazepam, 300 ± 54 μg L^{-1} for metoprolol and 200 ± 40 μg L^{-1} for hydrochlorothiazide in blackwater samples from a demonstration site in the Netherlands, based on blackwater and greywater separation. Finally, de Graaff et al. (2011) evaluated the occurrence and removal of PhACs during blackwater anaerobic treatment followed by a nitritation-anammox process and found high average concentrations of metoprolol (45 μg L^{-1}), propranolol (1.0 μg L^{-1}) and carbamazepine (1.1 μg L^{-1}) in untreated blackwater samples.

3.2. Reduction of PhACs in source separated sanitation treatment systems

3.2.1. Fecal sludge anaerobic digestion

The matrix analyzed in the AD experiments was a mixture of fecal sludge and inocula from the biogas reactors treating sludge from WWTP. Table S5 in SM shows the concentration of the PhACs detected

Table 3
Concentrations (mean; standard deviation in brackets, $n = 3$) of the PhACs detected in the liquid and solid fractions of untreated fecal sludge (US) and blackwater samples taken from Reactor 1 (R1) and Reactor 2 (R2), respectively.

Therapeutic group	Compound	Fecal sludge		Blackwater			
		Liquid (μg L^{-1})	Solid (μg kg^{-1} dw)	Liquid R1 (μg L^{-1})	Liquid R2 (μg L^{-1})	Solid R1 (μg kg^{-1} dw)	Solid R2 (μg kg^{-1} dw)
Analgesics	Codeine	ND	140 (±30)	1.6 (±0.12)	1.2 (±0.12)	90 (±30)	61 (±8)
β-Blockers	Atenolol	1.7 (±0.10)	2400 (±500)	4.7 (±1.4)	5.2 (±1.4)	<MQL	<MQL
	Sotalol	ND	130 (±30)	ND	ND	ND	ND
	Metoprolol	48 (±3)	1250 (±160)	9.5 (±1.3)	11.3 (±1.2)	380 (±23)	314 (±13)
	Propranolol	0.73 (±0.09)	350 (±90)	4.8 (±1.4)	6.5 (±1.3)	2400 (±240)	2000 (±500)
Antibiotics	Azithromycin	ND	ND	<MQL	<MQL	ND	ND
	Ciprofloxacin	ND	ND	<MQL	<MQL	–	–
Antidepressants	Carbamazepine	16 (±3)	1540 (±170)	3.4 (±1.1)	2.3 (±1.1)	180 (±1.4)	120 (±30)
	Citalopram	ND	300 (±80)	0.31 (±0.02)	0.31 (±0.04)	940 (±40)	800 (±50)
	Diazepam	ND	ND	0.048 (±0.004)	0.043 (±0.004)	<MQL	<MQL
	Lamotrigine	1.6 (±0.3)	430 (±70)	7.3 (±1.2)	8.6 (±1.7)	340 (±50)	230 (±50)
	Oxazepam	ND	380 (±130)	4.8 (±0.8)	4.6 (±1.1)	1600 (±400)	1200 (±500)
	Venlafaxine	12 (±4)	630 (±70)	6.4 (±1.4)	7.5 (±1.4)	710 (±80)	540 (±50)
	Amitryptiline	ND	ND	<MQL	<MQL	430 (±60)	380 (±80)
Antihypertensives	Losartan	32 (±4)	7400 (±1800)	10 (±0.3)	11 (±0.02)	680 (±130)	510 (±40)
	Valsartan	180 (±90)	120 (±50)	12 (±0.5)	11 (±0.24)	ND	ND
	Irbesartan	ND	1200 (±300)	ND	ND	ND	ND
	Diltiazem	ND	76 (±12)	ND	ND	ND	ND
Diuretics	Furosemide	10 (±1.3)	570 (±60)	37 (±7)	34 (±7)	200 (±22)	300 (±70)
	Hydrochlorothiazide	27 (±12)	1090 (±120)	14 (±4)	15 (±0.6)	514 (±23)	400 (±10)
Lipid regulators	Atorvastatin	ND	ND	0.72 (±0.05)	0.70 (±0.03)	–	–
Local anaesthetic	Lidocaine	1.0 (±0.1)	ND	0.65 (±0.03)	0.59 (±0.01)	13 (±2.4)	9.5 (±0.1)

ND: non detected; MQL: method quantification limit.

in the inocula used in the AD experiments. Results of Tables 3 and S5 indicate that fecal sludge is the major contributor of most PhACs detected in the samples used for the AD experiments. Nevertheless, for metoprolol, carbamazepine, lamotrigine, losartan, valsartan and furosemide, the contribution of the inocula is remarkably high. Furthermore, the use of different inocula for mesophilic and thermophilic experiments could explain the differences in the substances detected in each experiment and their concentrations. Out of the 29 PhACs analyzed, 17 substances were detected in the mesophilic and 18 in the thermophilic experiment. Oxazepam was only detected in the mesophilic experiments, while sotalol and clarithromycin were only found in the thermophilic samples.

To calculate removal rates of PhACs in both mesophilic and thermophilic treatments, the concentrations used were those obtained considering both liquid and solid fractions. It should be noted that, for solid samples, concentrations were transformed to μg L^{-1} using the percentage of total solids. For mesophilic experiments (Fig. 2), only two compounds, oxazepam and losartan, showed a reduction of ≥50% during AD treatment, while seven compounds, including atenolol, metoprolol, carbamazepine, lamotrigine, venlafaxine, valsartan and lidocaine, showed reduction rates between 10 and 37%. Remaining PhACs were poorly removed (<10%). In the thermophilic treatment (Fig. 3), irbesartan, hydrochlorothiazide and bezafibrate were completely removed, followed by atenolol with 90% reduction, and propranolol with 50% reduction. Most of the other detected PhACs showed removal rates between 20 and 46%. These results indicate that most PhACs are relatively unaffected by AD. Furthermore, no significant differences were observed between mesophilic and thermophilic conditions ($p < 0.05$, t-test), except for selected substances, which is in good agreement with other studies (Carballa et al., 2007; Samaras et al., 2014; Kjerstadius et al., 2015; Malmborg and Magnér, 2015).

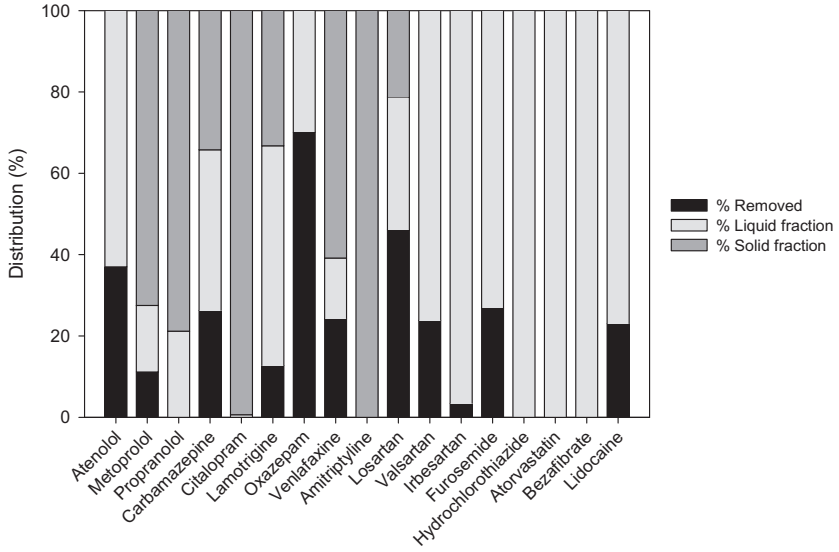

Fig. 2. Mass fractions of the identified PhACs in the liquid phase (light grey bars; %), solid phase (dark grey bars; %) and the percentage of PhACs removed during treatment (black bars; %) after 61 days of mesophilic anaerobic digestion experiments at 37 °C.

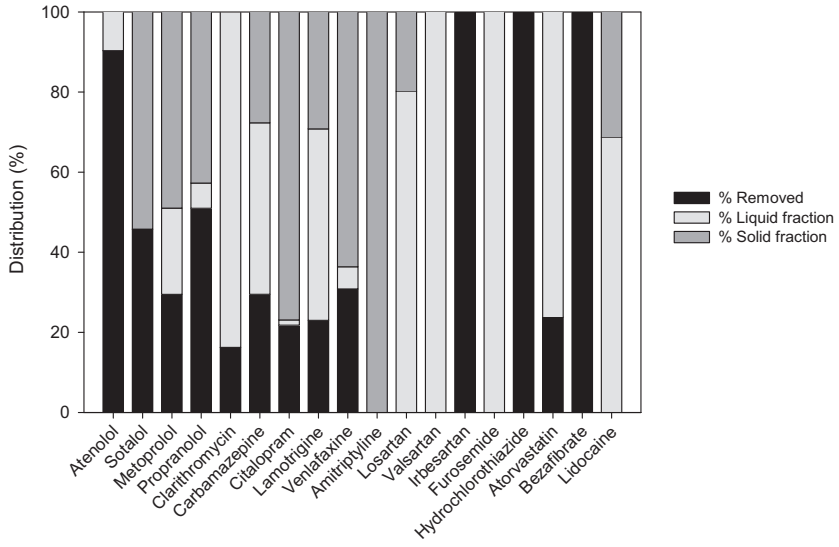

Fig. 3. Mass fractions of identified PhACs in the liquid (light grey bars; %), solid phase (dark grey bars; %) and the percentage of PhACs removed during treatment (black bars; %) after 59 days of thermophilic anaerobic digestion experiments at 52 °C.

Removal rates observed in our study match quite well with previous AD experiments showing a removal of 45–50% for furosemide, 11–85% for citalopram, and 72–85% for oxazepam during mesophilic and thermophilic conditions (Bergersen et al., 2012; Butkovskyi et al., 2015; Malmborg and Magnér, 2015). Furthermore, atenolol has shown to be biotransformed during AD (Inyang et al., 2016), and irbesartan was notably degraded during AD of sewage sludge (Boix et al., 2016). For other commonly detected PhACs, such as carbamazepine and propranolol (mesophilic conditions), no significant degradation was observed in this study (Figs. 2 and 3), which is also in good agreement with earlier studies, where these substances were shown to be unaffected by AD in both fecal and sewage sludge (Carballa et al., 2007; de Graaff et al., 2011; Narumiya et al., 2013; Malmborg and Magnér, 2015; Boix et al., 2016; Falås et al., 2016). Few compounds showed a significant increase ($p < 0.05$; t-test) in concentrations at either mesophilic (citalopram, atorvastatin, hydrochlorothiazide and amitriptyline) or thermophilic temperature (amitriptyline, losartan). 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) (Evgenidou et al., 2015; Jelic et al., 2015). Other explanations could be changes in the chemical conditions of fecal sludge during degradation and a reduction of the number of particles to which the substance can be adsorbed, influencing the efficiency of the extraction of the PhACs.

Figs. 2 and 3 also show the distribution of detected compounds after treatment between liquid and solid fecal sludge fractions. In general, PhACs are more prone to be found in the liquid phase. However, some substances, such as propranolol, citalopram, venlafaxine and amitriptyline partition to a greater 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. The distribution of PhACs between both fractions could be explained by their physico-chemical properties such as the octanol-water partition coefficient (K_{ow}) and the organic carbon-water partition coefficient (K_{oc}), which influence the partitioning of PhACs. Metoprolol, propranolol, citalopram, venlafaxine and amitriptyline have quite high log K_{ow} values ranging from 1.9 to 4.9 as well as high log K_{oc} values ranging from 1.79 to 5.70 (Table S1 in SM). High K_{ow} and K_{oc} values indicate high tendency to be distributed to the solid phase because it represents the hydrophobic and organic carbon rich fraction. Substances that show high K_{oc} levels would be more likely to be detected in the solid phase. Interestingly, other studies reported a positive correlation between hydrophobicity and persistence of PhACs during AD of sewage sludge (Malmborg and Magnér, 2015).

3.2.2. Wet composting and ammonia treatment

In the samples from the two aerobic reactors, 17 out of the 29 targeted PhACs were detected after wet composting and ammonia treatment. As depicted in Fig. 4, both reactors showed a significant overall reduction for 8 PhACs (viz. atenolol, metoprolol, propranolol, citalopram, valsartan, hydrochlorothiazide, atorvastatin and lidocaine ($p < 0.05$, t-test)). In general, Reactor 2 (R2) showed a factor of 1.5 to 2.6 (depending of the compounds) higher removal rates than Reactor 1 (R1), except for citalopram, amitriptyline, oxazepam and hydrochlorothiazide (Fig. 4). The higher removal efficiency in R2 may be attributed to the longer wet composting time, as a result of a slower temperature increase (see Section 2.2.2). Indeed, the residence time is known to have an effect on the degradation of PhACs, and previous studies reported higher reduction efficiencies with longer retention times (Hörsing et al., 2011). In general, the degree of PhACs reduction

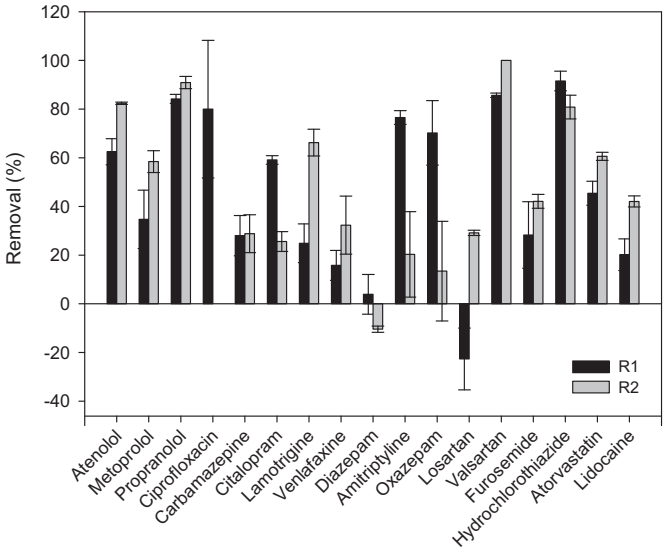

Fig. 4. Overall PhACs removal during blackwater treatment, including wet composting and urea treatment processes.

varied between the different compounds (Fig. 4). Most PhACs showed overall removal rates in both reactors from approximately 30 to 80%, including substances such as atenolol, metoprolol, citalopram, furosemide and atorvastatin, while six compounds (carbamazepine, lamotrigine, venlafaxine, lidocaine, diazepam and losartan) presented some or even no reduction during treatment (<50%). Only three PhACs, namely propranolol, valsartan and hydrochlorothiazide, showed high overall removal rates during treatment (>80%). Comparing the performance of wet composting and urea addition, most PhACs were reduced during the wet composting process (on average 53%, considering all compounds in both reactors), while ammonia treatment showed further reduction (on average 25%) for just a minor number of compounds, in both reactors (citalopram, venlafaxine, oxazepam, valsartan and atorvastatin). The low influence of ammonia treatment on the degradation of PhACs is in good agreement with a previous study where urea was added to digested and dewatered sewage sludge as a sanitation technology (Malmborg and Magnér, 2015).

Even though blackwater treatment showed moderate to high removal efficiencies for most target PhACs, high concentrations were still present in the treated effluents (Table S6 in SM). These levels are higher than those observed in urban wastewater effluents (Deblonde et al., 2011; Jelic et al., 2011; Al Aukidy et al., 2012; Jelic et al., 2012; Collado et al., 2014; Čelić et al., 2019). For example, furosemide showed concentrations up to 40 μg L^{-1} in R1 and 20 μg L^{-1} in R2, and losartan had concentrations up to 16 μg L^{-1} in R1 and 8.8 μg L^{-1} in R2 (Table S6 in SM). These concentrations are from one up to two orders of magnitude higher than those observed in wastewater effluents. Finally, the treated blackwater was stored at 6 °C for 3 and 6 months in order to assess whether PhACs were degraded during the post-storage period, before its application as fertilizer in crop fields. Results showed that, except valsartan and propranolol, no PhAC degraded further during this post-storage.

3.3. Comparison between treatments

Results derived from this study indicate that blackwater treatment, based on aerobic degradation of PhACs during wet composting for 12 to 19 days followed by ammonia treatment, is slightly more efficient in reducing PhAC levels than anaerobic digestion of fecal sludge and that the efficiency increases with treatment time. The average reduction of PhACs during blackwater treatment was 49%, while for mesophilic and thermophilic anaerobic digestion average removals were 31% and 45%, respectively. Comparing the removal of representative PhACs for each therapeutic group in aerobic, mesophilic anaerobic and thermophilic anaerobic treatments, compounds such as propranolol, citalopram and valsartan showed higher reduction rates in the aerobic treatment (on average, 74%) in comparison to anaerobic digestion (on average 20%), considering both mesophilic and thermophilic conditions. Other compounds, such as the recalcitrant carbamazepine, venlafaxine, oxazepam and hydrochlorothiazide showed similar removal rates in all treatments (from ~30 to 90%). These results are in good agreement with previous studies, where aerobic wastewater treatment showed higher removal efficiencies for PhACs, in comparison with anaerobic conditions (Lahti and Oikari, 2011; Alvarino et al., 2014; Falås et al., 2016). Furthermore, several studies reported non-significant differences between mesophilic and thermophilic anaerobic conditions (Carballa et al., 2007; Samaras et al., 2014; Malmborg and Magnér, 2015; González-Gil et al., 2016).

Comparing the degree of PhACs reduction in blackwater treatment with the removal efficiencies observed in conventional wastewater treatment plants (WWTPs), similar reduction rates were observed for most PhACs (Jelic et al., 2011; Petrovic and Verlicchi, 2014; Voulvoulis et al., 2016), including the β-blocking agents atenolol, metoprolol and propranolol (Jelic et al., 2011; Verlicchi et al., 2012; Collado et al., 2014; Papageorgiou et al., 2016; de Jesus Gaffney et al., 2017), the antibiotic ciprofloxacin (Verlicchi et al., 2012; Golovko et al., 2014; de Jesus Gaffney et al., 2017), the antidepressants venlafaxine, oxazepam and diazepam (Jelic et al., 2011; Verlicchi et al., 2012; Collado et al., 2014; Papageorgiou et al., 2016), the antihypertensives losartan and valsartan (Verlicchi et al., 2012; Gurke et al., 2015), the diuretics furosemide (Verlicchi et al., 2012; Papageorgiou et al., 2016) and the lipid regulators atorvastatin (Collado et al., 2014). Nevertheless, other substances such as the antiepileptic carbamazepine, the antidepressants lamotrigine and citalopram and the diuretic hydrochlorothiazide presented lower reduction rates in WWTPs in comparison with blackwater treatment (Jelic et al., 2011; Golovko et al., 2014; Gurke et al., 2015; Beretsou et al., 2016). Indeed, most studies in the scientific literature have reported negative reduction rates for carbamazepine (due to an increase in concentration after wastewater treatment) (Jelic et al., 2011; Bahlmann et al., 2014). Important is also that the treated fecal sludge and blackwater are used as fertilizers on arable land and thus none of their PhACs are directly emitted to water.

Blackwater treatment with wet composting and urea addition showed similar performances to other blackwater treatments in the reduction of PhACs. Treatments based on UASB followed by oxygen limited autotrophic nitrification-denitrification and struvite precipitation showed, for the liquid fraction, high reduction rates for compounds such as ciprofloxacin (~85%), hydrochlorothiazide (~90%) and oxazepam (~80%), while moderate removal was observed for metoprolol (~40%) (Butkovskyi et al., 2015). Another study based on UASB followed by partial nitritation-anammox showed an overall removal of 56% for metoprolol (de Graaff et al., 2011). On the other hand, urine storage showed no capability to degrade PhACs (Bischel et al., 2015). Regarding AD, a study that investigated the efficiency of several sewage sludge treatment and sanitation processes, including AD, pasteurization, thermal hydrolysis, advanced oxidation processes using Fenton's reaction, ammonia treatment and thermophilic dry digestion, showed that AD was the most efficient treatment for the removal of a wide range of PhACs, compared to the other technologies (Malmborg and Magnér, 2015).

4. Conclusions

In the past decade, domestic wastewater reuse and nutrient recycling have gained more attention as sustainable water cycle management solutions, driven by the increasingly noticeable resource restrictions of the 21st century. In general, source separation and the application of fecal sludge and blackwater as fertilizers on arable land can be beneficial for closing the nutrient loop. Nevertheless, one major issue that poses some concern is the flow of micropollutants, especially PhACs, onto arable fields and possibly further into the environment, which can affect ecosystems and human health. This study confirms that a wide range of PhACs are present in untreated fecal sludge and blackwater and that the treatment technologies studied herein are unable to completely degrade initial PhACs loads. Thus, significant PhACs concentrations still remain in the treated effluents. In general, PhACs removal was higher in the aerobic treatments (blackwater) in comparison with anaerobic digestion processes (fecal sludge). Indeed, no significant differences in PhACs reduction were observed between mesophilic and thermophilic AD conditions while for blackwater treatment most PhACs were removed during the wet composting process, with urea addition having a minor effect on PhACs removal. Furthermore, the potential of wet composting and urea addition in the reduction of PhACs is similar than other state-of-the-art blackwater treatments and the conventional treatments applied in urban WWTP. In addition, in our use case the PhACs loads from source separation systems was similar than those from conventional WWTP (on a per capita basis). The major difference, however, is related to the concentrations at release and the environmental endpoints. For a conventional WWTP the environmental endpoint is typically a water recipient while for source separated systems the endpoint is arable land.

These results point out that further research is required to thoroughly assess the potential environmental and human health risks

associated with fecal sludge and blackwater reuse as fertilizer in crop fields. There is a clear incentive to minimize the spreading of PhACs and antibiotic resistant pathogens and antibiotic resistance genes (ARGs), associated with the occurrence of antibiotics, on agroecosystems. Thus, more research and development is required for an efficient removal of PhACs and ARGs in source separated and nutrient recycling treatment systems. This includes the identification of necessary technical improvements to current state-of-the art sanitation systems, the inclusion of adequate post-treatments or the assessment of advanced novel treatment technologies. Furthermore, a better knowledge on the fate of PhACs and ARGs in soil-plant-groundwater ecosystems is needed in order to estimate any potential human health risks. Questions such as PhACs accumulation and ARGs spread in soils, leaching to surface and groundwater bodies, crop uptake and potential human exposure through dietary ingestion are topics of major concern. Further interesting questions are how the soil and crop type may influence these risks.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135530.

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