Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters

Pablo Gago-Ferrero *, Meritxell Gros, Lutz Ahrens, Karin Wiberg

Dept. of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Box 7050, SE-75007 Uppsala, Sweden

HIGHLIGHTS

- First study evaluating the impact of OSSFs with respect to the discharge of MPs into surface water
- High concentrations of MPs were detected at sites exclusively affected by OSSFs
- Different composition profiles were observed at sites affected by WWTPs and OSSFs
- OSSFs were shown to be a heterogeneous emission sources of MPs
- Photodegradation reduced the levels of specific MPs

GRAPHICAL ABSTRACT
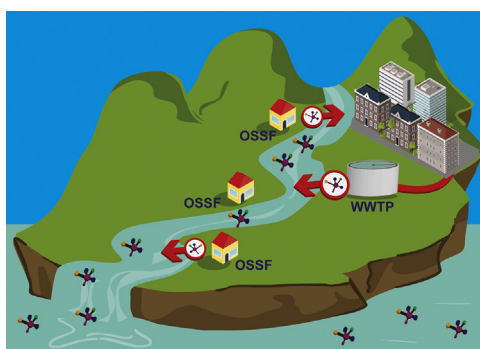
ARTICLE INFO

ABSTRACT

One of the main risks associated with effluents from both wastewater treatment plants (WWTPs) and on-site sewage treatment facilities (OSSFs) is the release of micropollutants (MPs) in receiving water bodies. However, the impact of MPs present in the effluents of OSSFs in the aquatic environment has not been studied so far. The current study evaluates the impact of the effluents of OSSFs and small-to-large scale WWTPs on natural waters. The discharge of 74 MPs was assessed including pharmaceuticals, personal care products, pesticides, artificial sweeteners and perfluoroalkyl substances (PFASs). The sampling was carried out within a Swedish catchment and included three sites that are exclusively affected by OSSFs and other sites that are mainly affected by WWTPs or a mixture of sources (7 sites, 28 samples). Results show that although OSSFs serve a much smaller total number of people, the MPs emitted from OSSFs reached the aquatic environment in significant quantities (concentrations of >150 ng L^{-1} of $\sum$ MPs). The composition profiles for sites affected by WWTPs were similar and were dominated by sucralose (27% of the $\sum$ MPs), caffeine (27% of the $\sum$ MPs), lamotrigine (10% of the $\sum$ MPs), desvenlafaxine (5% of the $\sum$ MPs), and diclofenac (4% of the $\sum$ MPs). In contrast, the sites affected by OSSFs showed high variability, exhibiting a different profile from those affected by WWTPs and also from each other, demonstrating that OSSFs are not homogeneous sources of MPs. Some specific compounds, such as diethyltoluamide (DEET) and caffeine, were proportionally much more important at sites affected by OSSFs than at sites affected by WWTPs (representing a much higher percentage of the $\sum$ MPs in the OSSFs). In contrast, PFASs did not show high concentration variation among the different sampling sites and the composition profiles were relatively similar, indicating that these substances follow different routes of entry into the aquatic environment.

© 2017 Elsevier B.V. All rights reserved.

* Corresponding author.
  E-mail address: pablo.gago.ferrero@slu.se (P. Gago-Ferrero).

1. Introduction

Emissions of organic micropollutants (MPs) into the aquatic environment are of concern for water quality and may trigger unwanted ecological effects. MPs originate from different point and diffuse sources and enter water bodies via different flow paths (Eggen et al., 2014). Previous studies have shown that discharges from wastewater treatment plants (WWTPs), in which MPs are not or only partially removed, is one major pathways of MPs to the aquatic environment (Luo et al., 2014). In sparsely populated areas, where the establishment of conventional WWTPs for the sanitation of wastewater is not feasible, the use of more cost-effective small-scale on-site sewage treatment facilities (OSSFs) is widespread. This is an important issue in Sweden, where approximately 700,000 private households are currently not connected to public WWTPs. The most common types of OSSFs used in Sweden are infiltration beds (30%) and soil beds (14%) (Olshammar et al., 2015). In a general extent, in these systems wastewater coming out of a sedimentation tank is purified by passing through natural soil layers and the treated water is drained to surface or groundwater. OSSFs have three main goals: (1) reducing the number of pathogens, (2) removing nutrients (mainly nitrogen and phosphorus), which otherwise may lead to eutrophication of the catchment area, and (3) reducing the biochemical oxygen demand (BOD). However, these treatments do not target on the removal of MPs. It is noteworthy that a large percentage of facilities (56%) still work with inadequate treatments, using septic tanks without any other treatment.

With respect to research into the environmental impact of OSSFs, most of the efforts have been devoted to the study of the efficiency of phosphorus elimination (Gustafsson et al., 2008; Cucarella and Renman, 2009; Renman and Renman, 2010; Eveborn et al., 2012; Nilsson et al., 2013). Nevertheless, the number of studies focusing on the removal efficiency of MPs is scarce. Some works focused on the elimination of selected MPs by using laboratory scale column-based experiments (Teerlink et al. 2012), while others evaluated the removal of a limited number of MPs in real OSSFs (Huntsman et al., 2006; Godfrey et al., 2007; Carrara et al., 2008; Matamoros et al., 2009; Conn et al., 2010a; Conn et al., 2010b; Stanford and Weinberg, 2010; Garcia et al., 2013; Du et al., 2014). Recently, Gros et al., (2017) and Blum et al., (2017) showed that the removal efficiency for a large set of MPs was similar between different OSSFs and WWTPs (Gros et al., 2017; Blum et al., 2017). However, there are no consistent studies focusing on the impact of OSSF discharges in the receiving aquatic environment.

The present study aimed to evaluate the impact of OSSFs and small-to-large scale WWTPs with respect to the discharge of MPs in the aquatic environment. For this purpose, a field study was conducted in a Swedish catchment, where the sampling locations were selected to be (i) exclusively affected by OSSFs, (ii) affected mainly by WWTPs and (iii) affected by different sources of contamination. A target analysis approach based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) was used to determine and quantify 74 MPs including pharmaceuticals, personal care products, pesticides, artificial sweeteners, and perfluoroalkyl substances (PFASs), among others. These analytes were selected in order to have a representative set of MPs with different physicochemical properties and uses. Moreover, the selected substances are ubiquitous compounds in the environment and are widely used in household and domestic items, being potential candidates to be detected in urban wastewaters. In addition, the samples were collected during four sampling campaigns over one year to investigate seasonal variations. Ultimately, the composition profiles for the different categories of studied MPs were compared to evaluate the impact of OSSF and WWTP discharges. The composition profiles of the field samples were also compared with those of the WWTP effluents to better understand compositional differences and to determine the source pathways of the MPs. To the author's best knowledge, this is the first study performing a consistent evaluation of the impact of MPs from OSSFs discharges.

2. Materials and methods

2.1. Chemicals and reagents

In total, 74 substances were evaluated including 49 pharmaceuticals of major consumption in Sweden and covering different therapeutic groups (viz. antibiotics, NSAIDs, antilipidemics, antiepileptics, antidiabetics, β-blocking agents, antifungals, analgesics, antihypertensives, diuretics, antiulcers, anesthetics, benzodiazepines, and antidepressants); 14 per- and polyfluoroalkyl substances (PFASs); 5 personal care products; 3 pesticides; one artificial sweetener; one illicit drug and one stimulant. Target analyte names, CAS numbers, molecular formulas, molecular weights and log K_{OW} values are shown in Table SI-1-1 in the Supporting Information (SI). All analytical standards used for quantification were of high purity grade (>95%) and purchased from Sigma-Aldrich (Sweden) except pesticides, which were acquired from Teknolab AB (Kungsbacka, Sweden). Isotopically labeled standards (IS) for PFASs, pesticides and pharmaceuticals and personal care products (PPCPs) were purchased from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), and Sigma-Aldrich and Toronto Research Chemicals (Toronto, Canada), respectively. More information about standards, chemicals and reagents used can be found in the supplementary material.

For chemical analysis, gradient grade methanol (MeOH), acetonitrile (AcN) and ethyl acetate (EA) 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 (500 mg, 6 cm^3) from Waters Corporation (Milford, USA). Glass fiber filters (Whatman™, 1.2 μm and 0.7 μm) were purchased from Sigma-Aldrich (Sweden).

2.2. Sampling design and sample collection

Surface water samples were collected at 7 different locations in the Fyris River catchment in the vicinity of Uppsala (Sweden) to evaluate the impact of OSSFs and WWTPs in the receiving natural water (Fig. 1, Table SI-2-2 in the SI). In order to evaluate seasonal variations, four sampling campaigns were carried out during one year including November 2014, March 2015, June 2015, and September 2015 (Campaigns 1, 2, 3 and 4, respectively, abbreviated C1, C2, C3 and C4). Sampling dates, sampling coordinates and physicochemical water parameters (temperature, pH, conductivity, concentration of several ions, total organic carbon (TOC) and dissolved organic carbon (DOC)) are reported in Table SI-2-1a and SI-2-1b in the SI. Fig. 1 schematically shows the sampling area as well as the sampling sites. Sampling site 1 (S1) is located in a small stream in the catchment of Fyris River. The only known source for MPs at this site is the upstream input of ~660 population equivalents (PE) from a number of OSSFs. Sampling site 2 (S2) is located approx. 9 km downstream of Site 1, right after the village Björklinge (~3300 inhabitants) and the discharge of its small-scale WWTP (3700 PE). The wastewater treatment steps include mechanical treatment and primary sedimentation, biological treatment by infiltration into a bed filled with plastic media, chemical treatment by addition of iron chloride and a final sedimentation treatment to remove particulate matter. Sampling site 3 (S3) is located in a small tributary to Fyris River that receives the input of ~560 PE from OSSFs. Sampling site 4 (S4) is located in the Sävja River, a tributary to Fyris River, and the site is affected by ~8400 PE from OSSFs. Sampling site 5 is a key sampling location, since it is located in Fyris River just downstream of the large-scale WWTP of Uppsala (Kungsängsverket, 172,000 PE). The wastewater treatment steps at this plant include mechanical treatment and primary sedimentation, biological treatment using activated sewage sludge with
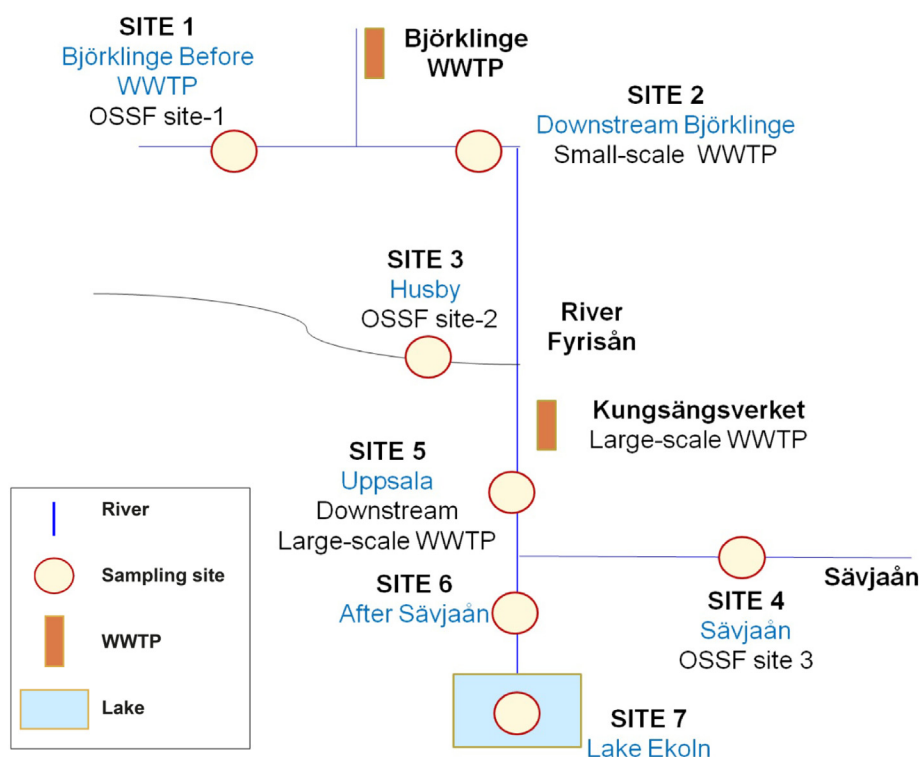
Fig. 1. Sampling locations. *WWTP*: waste water treatment plant; *OSSF*: on-site sewage treatment facilities.

nitrogen removal, chemical treatment by addition of iron chloride and a final lamella sedimentation treatment to remove particulate matter. Sampling site 6 (S6) is located in the Fyris River ~2.5 km downstream from S5, and downstream of the joint of Fyris and t Sävja River. Finally, Sampling site 7 (S7) is located in Lake Ekoln, 7 km downstream from sampling site 5, where the dilution effect is expected due to the higher water volume of the lake. The lake may receive direct inputs from boats and other aquatic recreational activities. The total water fluxes ($m^3 s^{-1}$) at the selected sampling sites for each campaign are summarized in Table SI-2-3 in the SI. Grab water samples of 0.5 L were collected two times per site in polypropylene bottles with two weeks apart. The first samples were stored in a freezer (-20 °C), and after the second sampling, the two samples were merged to a 1-L sample. However, the pooling of the two samples can only account partly for the variability of the MP concentrations in surface water over time. In addition, effluent wastewater from the WWTPs of Uppsala and Björklinge were sampled (24 h-composite samples). Three field blanks (bottles opened and closed but not filled in the field) and three method blanks, consisting of 1 L Milli-Q water were also analyzed to check for any background levels of the analytes.

2.3. Statistical analysis

Similarity between the different sampling sites was investigated by cluster analysis based on MPs' compositional profiles (Past 3.10, Hammer et al., 2001). The paired group (UPGMA) clustering algorithm and Chord similarity index was chosen for evaluating the similarity of MPs' composition profiles in sampling sites.

2.4. Sample preparation

Samples were filtered through 1.2 μm and 0.7 μm glass fiber filters (Whatman™) and then spiked with the IS mixtures. All IS were spiked to a final concentration of 50 ng L^{-1} except the ones used for PFASs quantification, for which a final concentration of 8 ng L^{-1} was reached. Thereafter, 500 mL was extracted by solid phase extraction (SPE) using

Oasis HLB (500 mg, 6 cm^3, 60 μm) cartridges. The cartridges were conditioned with 6 mL methanol followed by 6 mL Milli-Q. During extraction, samples were loaded onto the cartridges at a flow rate of approximately 2.5 mL min^{-1}. After sample loading, cartridges were rinsed with 6 mL Milli-Q water, dried under vacuum for 20 min and centrifuged to remove water excess at 3500 rpm for 5 min. For the elution, 2 times 4 mL methanol were used followed by 5 min of vacuum. Eluates from the cartridges were pooled and collected in the same glass tubes. Extracts were gently evaporated to a volume of 200 μL under a stream of nitrogen and transferred to chromatographic vials. Then, the extracts were evaporated to dryness and reconstituted with 1 mL of water/methanol (80:20).

2.5. Instrumental analysis

Analysis were carried out using an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, USA) coupled to a quadrupole-time-of-flight (QTOF) mass spectrometer (QTOF Xevo G2S, Waters Corporation, Manchester, UK). Extracts were analyzed in both positive and negative electrospray ionization mode (PI and NI, respectively). Chromatographic separation was performed on an Acquity HSS T3 column (100 mm × 2.1 mm, 1.8 μm) in PI mode and on an Acquity BEH C18 column (50 mm × 2.1 mm, 1.7 μm) in NI mode. Both columns were purchased from Waters Corporation (Manchester, UK) and were preceded by a guard column of the same packaging material. For PI mode, the aqueous phase consisted of 5 mM ammonium formate buffer with 0.01% formic acid and the organic phase was acetonitrile with 0.01% formic acid. For NI mode, the aqueous phase consisted of 5 mM ammonium acetate buffer with 0.01% ammonia and the organic phase was acetonitrile with 0.01% ammonia.

The adopted elution gradient for both ionization modes started with 5% of organic phase for 0.5 min, increasing to 95% by 16 min, and then to 99% in the following 0.1 min. These almost pure organic conditions were kept constant for 3 min, and then initial conditions were restored and kept for 2 min. The total run time was 21 min in both modes. The chromatographic flow rate was 0.5 mL min^{-1} and the injection volume was

5 μL. The column temperature was set to 40 °C and the sample manager temperature was 15 °C. The resolution of the TOF mass spectrometer was 30,000 at full width and 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 kV and 0.4 kV were used in PI and NI modes, respectively. A cone voltage of 30 V was applied, the desolvation gas flow rate was set at 700 L h^{-1} and the cone gas flow was set to 25 L h^{-1}. The desolvation temperature was set to 450 °C and the source temperature to 120 °C. Two acquisition functions with different collision energies were created: the low energy (LE) function with a collision energy of 4 eV, and the high energy (HE) function with a collision energy ramp ranging from 10 to 45 eV. Calibration of the mass-axis from m/z 100 to 1200 was conducted daily with a 0.5 mM sodium formate solution prepared in 90:10 (v/v) 2-propranolol/water. For automated accurate mass measurement, the lock-spray probe was employed (10 μL min^{-1}), using a lock mass leucine encephalin solution (2 mg mL^{-1}) in ACN/water (50:50) with 0.1% formic acid.

2.6. Quality assurance and quality control

Background contamination in the laboratory is a common problem observed in the determination of MPs, especially for PFASs. Several measures were taken in order to minimize this problem. All glassware used was previously washed and heated overnight at 450 °C and further sequentially rinsed with HPLC grade water, ethanol and acetone. Furthermore, gloves were worn during sample preparation; separate solvents and only previously unopened packages of solvents, chemicals and other supplies, and glassware were used. Since many of the compounds analyzed undergo photo-degradation and the samples may suffer the exposure to light during the procedure, all samples and stock standard solutions were in amber glass bottles and stored in the dark.

Quality parameters of the analytical methods, including recovery efficiency, method precision, method limits of detection (MLODs) and method limit of quantification (MLOQs) are summarized in Table S5, in the SI. Recoveries were determined by spiking a known concentration of target analytes and comparing the concentrations before and after the whole SPE-HPLC-MS/MS process, calculated by internal standard. Surface and wastewater samples can contain the target MPs. Therefore, blanks (non-spiked samples) were also analyzed and the levels found subtracted from those obtained from spiked samples. Method and field blanks were used to evaluate potential background levels of target analytes and to determine MLODs and MLOQs. Some PFASs were detected in the blank samples. In these cases, MDLs and MQLs were calculated from the blanks (average of the concentrations detected in blanks + 3 × standard deviation or the lowest calibration point when compounds were not detected in the blanks as described by Gros et al., (2017). For the rest of compounds, MLODs and MLOQs were calculated as from the signal-to-noise ratio (S/N = 3 and S/N = 10, respectively) of real samples. Method precision was determined as relative standard deviation (%RSD) from the recovery experiments. Nine-point calibration curves (0.025–250 ng mL^{-1}) were generated using linear regression analysis. The linearity was qualified by linear correlation coefficient, r^2 for the range of concentrations present in the samples for each compound. The calibration curves obtained were linear with $r^2 > 0.99$ in all cases.

Quantification was based on peak areas and was performed by the internal standard calibration approach. For each compound, its corresponding deuterated compound was used for quantification, except for those substances whose labeled analogue was not available. In this case, the most similar deuterated compound, in terms of chemical structure and chromatographic retention time, was used as internal standard (IS). Table SI-3-1 shows the IS used for the quantification of each compound. The identification and confirmation criteria for the analysis of the target compounds was based on the Commission Decision 2002/657/EC.

3. Results and discussion

In order to perform a comprehensive discussion of the obtained results, the evaluated MPs were divided in five categories, where the first three categories include only pharmaceuticals: (I) antihypertensives and B-blocking agents; (II) antidepressants, benzodiazepines and antiepileptics; (III) other pharmaceuticals, including antibiotics, analgesics, NSAIDs and antifungals, among others; (IV) PFASs and (V) "other substances" including illicit drugs, artificial sweeteners, pesticide, stimulants and personal care products.

3.1. Impact of OSSFs and WWTPs on the occurrence of MPs

Residues representing the five compound categories were detected in all the analyzed samples, demonstrating a strong presence of MPs in the study area. The specific concentrations for each compound in each sample can be found in Table SI-4-1a and Table SI-4-1b, in the SI, along with the MLODs, MLOQs and frequencies of detection. In total, 44 of the 74 target substances were determined in at least one sampling site, and 10 compounds were determined in at least 80% of the analyzed samples (i.e. sucralose, tramadol, atenolol, lamotrigine, caffeine, perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS)). Some compounds were found at particularly high concentration levels including sucralose (up to 3000 ng L^{-1}), lamotrigine (up to 530 ng L^{-1}), caffeine (up to 340 ng L^{-1}), desvenlafaxine (up to 260 ng L^{-1}), diclofenac (up to 260 ng L^{-1}), valsartan (up to 230 ng L^{-1}), tramadol (up to 240 ng L^{-1}), and hydrochlorothiazide (up to 220 ng L^{-1}), among others. These results are in the same range of concentrations as those found in previous studies focusing on natural water impacted by discharges from sewage facilities (Osorio et al., 2012; Alygizakis et al., 2016; König et al., 2017).

Fig. 2 shows the total concentrations of MPs at the seven sampling sites during the four sampling campaigns. In all sampling campaigns, the most contaminated site was S5, reaching a total concentration of MPs up to 7000 ng L^{-1} (Campaign 4, September 2015). This sampling site is located just downstream of the large WWTP of Uppsala (172,000 PE). Therefore, it is not surprising to detect high concentrations of MPs at this site, being due to high consumption of products containing the target analytes by a large population in combination with poor capacity to degrade these compounds in conventional wastewater treatment (Eggen et al., 2014). Site S6, located 2.5 km downstream from the WWTP of Uppsala, was also heavily contaminated with up to 2200 ng L^{-1} (September 2015). This site is basically affected by the substances discharged by the WWTP, as the composition profile is almost identical to the one determined at S5 (see Section 3.3). There was a strong dilution gradient from site S5 to S7 (from downstream of the WWTP to Lake Ekoln; ~7 km from S5 to S7) with decreasing concentrations by an average attenuation factor of 37 for *antihypertensives and B-blocking agents*; 9 for *antidepressants, benzodiazepines and antiepileptics*; 14 for *other pharmaceuticals*, 7 for *other substances* and only 1.5 for *PFASs*, which can be mainly explained by a dilution effect, adsorption to sediments and transformation reactions of the target compounds. The dilution effect can be explained by the fact that site S7 at the lake is impacted by diffuse sources (i.e. run-off and atmospheric deposition) from a much larger, low populated, catchment area than the river resulting in decreasing concentrations for most MPs. Large differences in the different categories of MPs are mainly explained by degradation processes (see Section 3.2). However, the really low dilution effect of PFASs (always with an attenuation factor < 2) is likely due to the fact that WWTPs are important as point sources of PFASs (Ahrens, 2011), but diffuse sources such as atmospheric deposition, runoff and storm water along with e.g. near-shore OSSFs are making up a significant contribution to the PFAS levels in the lake. Another sampling site with high levels of pollution was the one located just downstream of the WWTP of
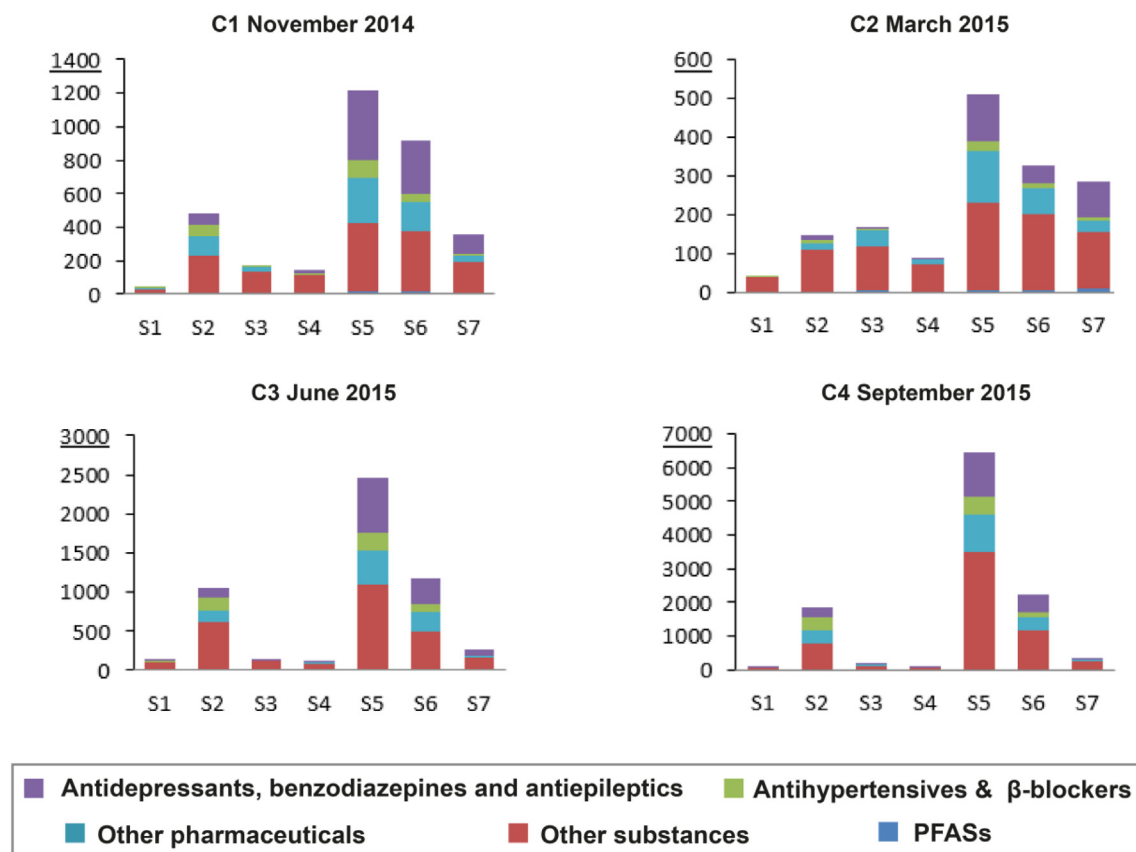
Fig. 2. Levels of the different categories of evaluated micropollutants detected in the evaluated area during the four sampling campaigns carried out in different seasons (ng L^{-1}).

Björklinge (S2). Total concentrations of up to 1800 ng L^{-1} were detected at this site (September 2015). At the rest of the sampling sites, which basically were affected only by emissions from OSSFs, the total concentrations of MPs were lower but still significant, with total maximum levels of 120 ng L^{-1} (S1), 170 ng L^{-1} (S3) and 140 ng L^{-1} (S4). PFAS concentrations followed a different trend than the rest of compounds. Firstly, the total concentrations for these compounds did not show such extreme differences among the different sampling locations as for the other MPs categories. Secondly, there were no clear trends in the levels of PFAS contamination between the sites affected by WWTPs (i.e. S2 and S5) and those affected by OSSFs (i.e. S3 and S4). This can be illustrated by site S2 (affected by Björklinge WWTP). As mentioned before, this site showed high concentrations for all the evaluated MPs but the average $\sum$ PFASs was only 2.5 ng L^{-1}. In contrast, S3 and S4 (normally with low levels of MPs) showed higher average $\sum$ PFAS levels, viz. 7.8 and 6.2 ng L^{-1}, respectively. It is also interesting that there was in general no decrease in $\sum$ PFAS concentrations from site S6 to site S7 in the lake. This shows that PFASs have many sources and that the entry of PFASs into surface waters follows different paths than the rest of the studied MPs. Greater persistence of these compounds also contributes to this behavior.

Based on the data of the present study and assuming that the average of the four grab samples taken at site S6 are representative, the estimated average input fluxes of the studied MPs into the Lake Ekoln, which collects the water from the Fyris River catchment, are as follows: 240 g day^{-1} for antidepressants, benzodiazepines and antiepileptics; 72 g day^{-1} for antihypertensives and β-blockers; 190 g day^{-1} for other studied drugs; 10 g day^{-1} for PFASs and 420 g day^{-1} for other evaluated substances (Fig. SI-4-1, in the SI). The differences among the input fluxes for the different categories of substances in the four sampling campaigns are discussed in Section 3.2.

3.2. Seasonal trends of MPs

The sampling sites that are directly affected by WWTP discharges (viz. S2, S5 and S6) with the exception of S7 (for which the dilution effect is pronounced) followed a clear pattern regarding concentration of MPs during the four studied sampling campaigns. The total concentrations ($\sum$ MPs) for these sites ranged from 480 to 1200 ng L^{-1} in the first campaign carried out in November 2014. Concentrations dropped to between 150 and 510 ng L^{-1} in the second campaign (March 2015). Subsequently, these concentrations strongly increased reaching values between 1100 and 2500 ng L^{-1} in the third campaign (June 2015) to keep increasing and reach levels between 1800 and 6400 ng L^{-1} during the fourth campaign (September 2015). Moreover, as can be observed in Fig. 3 and Fig. SI-5-1 (SI), the relative proportions between the different types of pollutants appear to be relatively stable (see Section 3.3). However, the sampling sites affected by OSSFs (i.e. sites S1, S3 or S4) did not follow this trend with $\sum$ MP levels of 40–180 ng L^{-1} (Campaign 1), 41–170 ng L^{-1} (Campaign 2), 120–140 ng L^{-1} (Campaign 3) and 63–150 ng L^{-1} (Campaign 4). The water fluxes changed consistently at all the evaluated points. Therefore, those differences are due to changes in the consumption of the substances evaluated in the communities served by OSSFs during the different seasons. Another reason is that the wastewater from the OSSFs reaching the receiving stream may be affected with the seasons (e.g. dryer soil), which can affect transport. The significant lower levels obtained in March are due to the ongoing spring flood.

When focusing on the differences in the total $\sum$ MP input fluxes to Lake Ekoln (calculated based on the data obtained at the sites S5 and S6) for each campaign, it can be concluded that the levels remained relatively stable over a year, viz. between 1800 and 1900 g day^{-1}. Only campaign C1 showed a lower input flux (1400 g day^{-1}); however,
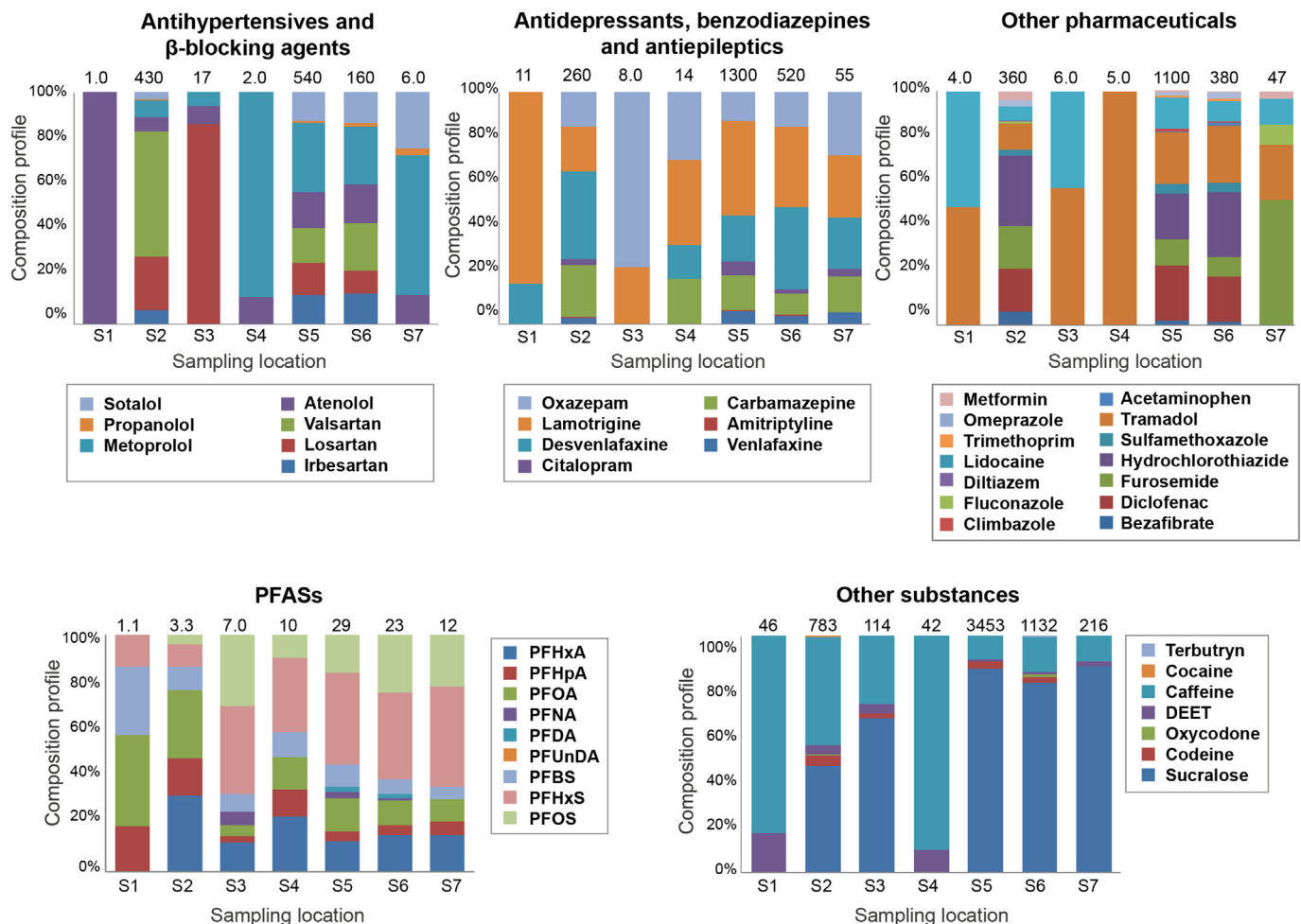
Fig. 3. Composition profiles (%) for the evaluated groups of substances during the 4th sampling campaign (C4, September 2015). The total concentration of all the compounds belonging to each category is listed on the top of each bar (ng L^{-1}).

this value is biased by the lower levels of sucralose detected in this specific campaign. Therefore, results indicate lower consumption and emission of sucralose during March 2014 in relation to the other sampling periods. The $\sum$ MP input fluxes for antidepressants, benzodiazepines and antiepileptics remained stable during the four sampling campaigns. In contrast, the $\sum$ input fluxes for antihypertensives and β-blocking agents experienced strong variations. During the sampling campaigns C3 and C4 (carried out in summer and early fall), the values increased 100% in comparison to those obtained for C1 and C2 (carried out late fall and winter, the dark and cold season; from 89 and 71 g day^{-1} to 160 and 150 g day^{-1}, respectively). Antidepressants and benzodiazepines are compounds associated to a continuous consumption all the year round. Therefore, relatively constant fluxes were expected. However, antihypertensives and β-blocking agents are related to a population of higher age and with a more intermittent or less constant consumption throughout the year. This trend has been observed in other studies (González-Moreno et al., 2014). The category of PFASs also experienced strong variations with $\sum$ PFAS input fluxes ranging from 12 g day^{-1} (C2) to 40 (C4) g day^{-1}, but without following a clear pattern.

Apart from the general trends, there are particular behaviors of some specific compounds that draw attention. High levels of hydrochlorothiazide (values above 100 ng L^{-1}) at the sites S2, S5 and S6 (sites downstream of WWTPs) were observed at different seasons. However, hydrochlorothiazide could not be detected at S7 in September 2015 (C4), while the highest concentrations for this compound at sites S2, S5 and S6 were found in C4 (Table SI-5B in the SI). One possible explanation is the photodegradation that hydrochlorothiazide undergoes in

water due to exposure to sunlight (Brigante et al., 2005). At the site located in the lake (S7), the concentration of hydrochlorothiazide was higher in the campaigns C1 and C2 (carried out in winter). The concentration decreased in the campaign C3 (June) and the compound was undetectable in the campaign C4 (September). Photodegradation occurs mainly during the period of highest impact of sunlight. The same behavior was observed for various compounds included in the present study: lamotrigine showed a reduction of 75% between the concentration measured at C4 and the average concentration detected in C1 and C2; sotalol showed a 75% decrease, carbamazepine 58%, venlafaxine 57%, desvenlafaxine 38% and lamotrigine 29%. The accelerated photodegradation of the target compounds in natural water is attributed mainly to the formation of hydroxyl radicals through photochemical reactions (Rúa-Gómez and Püttmann, 2013). Nevertheless, taking into account both degradation and the changes in the inputs of the different MPs, the overall $\sum$ MP levels at sampling point S7 were maintained in the range 270 to 360 ng L^{-1} during the four campaigns, showing a much lower variability than at the rest of the sites.

Another compound with an interesting seasonal trend was the insect repellent diethyltoluamide (DEET). This substance was found at very low levels in the sampling campaigns C1 and C2 (average concentration 1.0 ng L^{-1}), carried out during fall-winter. However, in sampling campaign C3 (early spring), a large increase in the concentration of this compound was observed (average concentration 8.0 ng L^{-1}), but limited to the sampling sites associated to WWTPs. The reason can be possibly found in the indoor consumption of this product during that season. During the sampling campaign C4 (early fall), DEET was present at even

higher levels (average concentration 13 ng L^{-1}). The increase was particularly pronounced at the locations associated with OSSF emissions (S1, S3 or S4). In these cases the increase was >400% in comparison with the previous campaigns. This might be explained by a higher use of DEET due to the mosquito season, which is particularly noticed in areas located far out from main urban areas.

3.3. Composition profiles of MPs in surface water and corresponding WWTPs

In addition to the changes in the total concentrations, the specific variation of each analyte and their relative concentration in the different categories of evaluated substances were carefully studied. Fig. 3 graphically shows the specific composition profile of each sampling site (expressed in%) in the fourth sampling campaign (Sept 2015). These graphs are also available for the other three sampling campaigns in Fig. SI-5-1, in the SI.

One of the most relevant conclusions that can be drawn from the pharmaceutical charts (categories I, II and III) is the similarity in the composition profile between the sampling sites S2, S5 and S6 of the same campaign. The profiles of these sites that are directly affected by WWTP discharges (i.e. S2, S5 and S6) are much more homogeneous than those affected by OSSFs (i.e. S1, S3 and S4). This assertion is confirmed by observing the cluster analysis carried out for these categories (Fig. SI-5-2), where sampling sites S5, S6 and S7 (the latter is located in the lake and receives the input from the previous ones) are closely related. The next site in proximity is S2, which is affected by a different WWTP. In contrast, the behavior for sites affected by OSSFs is much more erratic.

In the graphs corresponding to the category (I): antihypertensives and β-blocking agents of the sampling campaign C4 (Fig. 3), it can be seen that the composition profile is very similar for site S5 and S6 (affected by Uppsala WWTP), with high concentrations of irbesartan, losartan, valsartan, atenolol, metoprolol and, to a lesser extent, propranolol and sotalol. Sampling site S7, located 7 km further down (in the lake), maintains the same proportions for the detected compounds but with much lower concentration levels. The composition profile seems at first sight different due to the absence of valsartan, losartan and irbesartan (all of them with a similar chemical structure). However, the high detection limits (10–20 ng L^{-1}) of these compounds may be the cause of their non-detection. By obviating these three substances, the composition profile is maintained and the dilution effect is the main factor for the drastic reduction of the concentrations. The sampling site S2, affected by Björklinge WWTP, showed a similar composition profile but the percentage of valsartan was higher (54%) compared to sites S5 and S6 (16% and 20%, respectively). It is noteworthy that valsartan was not determined at any of the sampling sites directly affected by OSSFs (i.e. S1, S3 and S4), which might be because of the high detection limit obtained for this compound (20 ng L^{-1}). On the other hand, the composition profiles of the sites affected by OSSFs were totally different from those affected by WWTPs as well as between themselves. Sampling site S1 generally did not contain any antihypertensives and β-blocking agents. Only atenolol was detected at a concentration of 1.0 ng L^{-1}. At site S3 and S4, metoprolol and atenolol were detected at low concentrations (<2.0 ng L^{-1}). Apart from those substances, the relatively high concentration of losartan (representing >80% of the $\sum$ category (I)) at site S3 is noteworthy. This compound was not present at this site in any of the other sampling campaigns, so its presence may be related to a temporary consumption in one or a few of the households connected to a OSSF that affect this sampling site. The other three sampling campaigns showed very similar composition profiles for antihypertensives and β-blocking agents for the sites affected by WWTPs, whereas the variability for those affected by OSSFs was much higher. This statement is applicable to the rest of the substances except the ones showing clear seasonal trends (discussed in Section 3.2.).

In the category (II): antidepressants, benzodiazepines and antiepileptics, seven compounds were detected: oxazepam, lamotrigine, desvenlafaxine, citalopram, carbamazepine, amitriptyline and venlafaxine. Fig. 3 shows the composition profiles for the studied sites, which follow a trend similar to those described for antihypertensives and beta-blocking agents. It can be observed that these profiles were very similar at sites S5 and S6 with lamotrigine (35–40% of the $\sum$ category (II)) and desvenlafaxine (20–35% of the $\sum$ category (II)) as the dominant compounds. It is interesting that the profile remains almost the same at site S7 associated to the lake, although with much lower levels. Site S2 also shows a similar profile, although with a lower contribution of lamotrigine (19% of the $\sum$ category (II)) and a higher contribution of carbamazepine (23% of the $\sum$ category (II)), a recalcitrant compound (Rodríguez-Rodríguez et al., 2012) that is present in all the samples except site S1. Sites associated with OSSFs showed lower concentrations (and also higher variability). It is noteworthy in the case of site S4 that, unlike other categories of substances, it showed a composition profile very similar to those sites related to WWTPs (i.e. S2, S5 or S6). Apart from this, fourteen additional pharmaceuticals included in the category (III): other drugs were detected: metformin, omeprazole, trimethoprim, lidocaine, diltiazem, fluconazole, climbazole, acetaminophen, tramadol, sulfamethoxazole, hydrochlorothiazide, furosemide, diclofenac and bezafibrate. Of note are the high levels of hydrochlorothiazide (20–28% of the $\sum$ Other-drugs) and tramadol (22–24%) at the sites S2, S5 and S6 at different seasons. The relationships between the composition profiles showed similar trends to those followed for the rest of pharmaceuticals: high similarity at sites affected by WWTPs and higher variability for the ones affected by OSSFs.

As with total concentrations, the composition profiles of the category (IV): PFASs followed a completely different trend than those showed by the other categories of substances studied. The composition profiles of the sites affected by WWTPs did not show similarities among themselves (the profiles at points S2 and S5 are totally different from each other) (Fig. SI-5-1 in the SI). Sampling locations S3, S4, S5, S6 and S7 showed similar composition profiles, dominated mainly by PFHxS (30–40% of the $\sum$ PFASs) and PFOS (16–34%), although these sites are located in different rivers (S3 and S4 in tributaries to Fyris River (S5 and S6)) and in the lake (S7), having different affectations. This indicates that diffuse sources, such as precipitation and surface run-off, are important pathways for PFASs (Ahrens et al., 2013) into the aqueous environment. Sampling sites S1 and S2 showed a completely different composition profile, which can be explained by the concentrations being close to the MLODs (Table SI-4-1a, SI). In all cases, the profiles were stable during the different sampling campaigns. This reasoning is in agreement with the cluster analysis (Fig. SI-5-2), showing that sites S5, S6 and S7 are very closely related but the rest of the sites did not follow a clear pattern.

In the graphic for the category (V): other substances, it can be observed that in all cases the main component is either the artificial sweetener sucralose or the stimulant caffeine. At the sampling location directly affected by the large WWTP of Uppsala (S5) and the following sites located downstream (S6 and S7), sucralose was by far the compound present at the highest concentrations. The results indicate a high input of sucralose and caffeine from the effluents of the WWTPs to the river (concentrations up to 3000 and 460 ng L^{-1}, respectively), which is in agreement with previous studies (Loos et al., 2009; Luo et al., 2014). This fact is due to both a large consumption of these substances and a poor degradation rate in humans, in the environment and during the applied conventional wastewater treatment (Scheurer et al., 2009; Pasquini et al., 2014; Luo et al., 2014). In contrast, at the sampling location S2 (affected by the WWTP of Björklinge) a higher proportion of caffeine than sucralose was observed for all sampling campaigns (Fig. 3 and Fig. SI-5-1, SI). This fact is quite surprising since the proportion of sucralose in the effluents of the WWTP of Björklinge is much higher than the one of caffeine (79% $\sum$ category (V) and 11%, respectively) (Fig. SI-5-3, SI). This indicates that a large part of the

present caffeine is not related to the WWTP effluents. The main cause of the high levels of this compound might be high inputs from OSSFs. This fact is reflected in the cluster analysis (Fig. SI-5-2), where it can be observed that S2 is much more closer to the sites affected by OSSFs than to S5, S6 and S7 (affected by WWTPs) that are very closely related among them. It is also noteworthy that the compound codeine was present in an appreciable percentage (2–5% of the $\sum$ category (V)) in the majority of the samples (68%), reflecting a spread consumption of this substance.

The composition profiles of WWTP effluents of Björklinge and Uppsala, which directly affect sampling locations S2 and S5, respectively, are shown in Fig. SI-5-3 in the SI. The composition profiles for each pair (S2/ WWTP Björklinge and S5/WWTP Uppsala) for almost all categories of studied substances were very similar for all seasons. However, there were some exceptions. For example, as already pointed out, there was a notable difference between S2 and the Björklinge effluent with respect to caffeine with 45% and 10%, respectively of the $\sum$ category (V). As in previous cases, PFAS composition profiles did not follow the general trend and showed more notable differences between the corresponding pairs in several cases (e.g. PFOS or PFHxA), indicating that WWTP effluents are not the only significant sources of these substances in natural waters. The wastewaters from the OSSFs are generally infiltrated through a porous media and can thus be influenced by adsorption. This mechanism can be partially responsible for the differences observed between areas mainly impacted by WWTPs and OSSFs.

4. Conclusions

In this study, the impact of OSSFs in natural waters with respect to the presence of MPs was evaluated for the first time. The impact from OSSFs was also compared to the ones related to WWTPs present in the area. The results showed high levels of MPs, e.g., >10% of the evaluated MPs were detected at levels above 200 ng L^{-1}. In total 44 out of the 74 evaluated MPs were detected, 10 of them with detection frequencies of ≥80%, showing a widespread distribution of MPs in the aquatic environment. As expected, the sampling locations affected by small to large scale WWTPs showed larger total concentrations of MPs than those affected exclusively by OSSFs. However, it should also be noted that although OSSFs serve comparatively a much smaller total number of people, MPs reach the aquatic environment in significant quantities ($\sum$ MPs > 150 ng L^{-1}). An exception to the general trend was found for PFASs, whose concentrations were not significantly different between sites affected by WWTPs and OSSFs.

The composition profiles at sampling sites affected by WWTPs were similar to each other (and also similar to those of upstream WWTP effluents). In contrast, the sampling sites affected by OSSFs showed a much broader range of profiles. They were different both from those affected by WWTPs and also from each other, indicating that OSSFs are not homogeneous sources of MPs. Seasonal variations also play a relevant role in the presence of MPs in the aquatic environment due to different factors such as photodegradation caused by solar radiation (e.g. hydrochlorothiazide), changes in the consumption/use of various substances (such as the insect repellent DEET, which is mainly used in summer), and changes in water mass fluxes. As in the case of total concentrations, the PFAS profiles were different to those of the corresponding WWTP effluents, and this leads to the conclusion that these substances have other sources (e.g. diffuse sources) and follow different entry pathways into natural waters than the rest of MPs studied.

Overall, it can be affirmed that OSSFs are relevant input sources of MPs into the aquatic environment. Due to the fact that OSSFs are diffuse sources of contamination, their discharges may seriously affect surface and ground waters and even drinking water supplies (Godfrey et al., 2007). It is also relevant that depending on the use of chemicals in the households, OSSFs' impact may lead to spikes in the levels of MPs in natural waters that may cause ecotoxicological effects as it has been reported for incidental spills (Thompson et al., 2016). Therefore, devoting

higher attention to the efficiency of wastewater treatment carried out at OSSFs as well as putting more efforts in the evaluation of their environmental impact are desirable actions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx. doi.org/10.1016/j.scitotenv.2017.05.258.

References

Ahrens, L., 2011. Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate. J. Environ. Monit. 13 (1), 20–31.

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci. Total Environ. 541, 1097–1105.

Blum, K.M., Andersson, P.L., Renman, G., Ahrens, L., Gros, M., Wiberg, K., Haglund, P., 2017. Non-target screening and prioritization of potentially persistent, bioaccumulating and toxic domestic wastewater contaminants and their removal in on-site and large-scale sewage treatment plants. Sci. Total Environ. 575, 265–275.

Brigante, M., DellaGreca, M., Previtera, L., Rubino, M., Temussi, F., 2005. Degradation of hydrochlorothiazide in water. Environ. Chem. Lett. 2 (4), 195–198.

Carrara, C., Ptacek, C.J., Robertson, W.D., Blowes, D.W., Moncur, M.C., Sverko, E., Backus, S., 2008. Fate of pharmaceutical and trace organic compounds in three septic system plumes, Ontario, Canada. Environ. Sci. Technol. 42, 2805–2811.

Conn, K.E., Lowe, K.S., Drewes, E., Hoppe-Jones, C., Tucholke, M.B., 2010a. Occurrence of pharmaceuticals and consumer product chemicals in raw wastewater and septic tank effluent from single-family homes. Environ. Eng. Sci. 27 (4), 347–356.

Conn, K.E., Siegrist, R.L., Barber, L.B., Meyer, M.T., 2010b. Fate of trace organic compounds during vadose zone soil treatment in an onsite wastewater system. Environ. Toxicol. Chem. 29, 285–293.

Cucarella, V., Renman, G., 2009. Phosphorus sorption capacity of filter materials used for on-site wastewater treatment determined in batch experiments — a comparative study. J. Environ. Qual. 38, 381–392.

Du, B., Price, A.E., Scott, W.C., Kristofco, L.A., Ramírez, A.J., Chambliss, C.K., Yelderman, J.C., Brooks, B.W., 2014. Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent. Sci. Total Environ. 466–467, 976–984.

Eggen, R.I.L., Hollender, J., Joss, A., Schärer, M., Stamm, C., 2014. Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ. Sci. Technol. 48 (14), 7683–7689.

Eveborn, D., Kong, D.G., Gustafsson, J.P., 2012. Wastewater treatment by soil infiltration: long-term phosphorus removal. J. Contam. Hydrol. 140, 24–33.

Garcia, S.N., Clubbs, R.L., Stanley, J.K., Scheffe, B., Yelderman, J.C., Brooks, B.W., 2013. Comparative analysis of effluent water quality from a municipal treatment plant and two on-site wastewater treatment systems. Chemosphere 92, 38–44.

Godfrey, E., Woessner, W.W., Benotti, M.J., 2007. Pharmaceuticals in on-site sewage effluent and groundwater, Western Montana. Ground Water 45, 263–271.

González-Moreno, R., Rodríguez-Mozaz, S., Gros, M., Pérez-Cánovas, E., Barceló, D., León, V.M., 2014. Input of pharmaceuticals through coastal surface watercourses into a Mediterranean lagoon (Mar Menor, SE Spain): sources and seasonal variations. Sci. Total Environ. 490, 59–72.

Gros, M., Blum, K.M., Jernstedt, H., Renman, G., Rodríguez-Mozaz, S., Haglund, P., Andersson, P.L., Wiberg, K., Ahrens, L., 2017. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. J. Hazard. Mater. 238, 37–45.

Gustafsson, J.P., Renman, A., Renman, G., Poll, K., 2008. Phosphate removal by mineral-based sorbents used in filters for small-scale wastewater treatment. Water Res. 42, 189–197.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

Huntsman, B.E., Staples, C.A., Naylor, C.G., Williams, J.B., 2006. Treatability of nonylphenol ethoxylate surfactants in on-site wastewater disposal systems. Water Environ. Res. 78, 2397–2404.

König, M., Escher, B.I., Neale, P.A., Krauss, M., Hilscherová, K., Novák, J., Teodorović, I., Schulze, T., Seidensticker, S., Kamal Hashmi, M.A., Ahlheim, J., Brack, W., 2017. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. Environ. Pollut. 220, 1220–1230.

Loos, R., Gawlik, B.M., Boettcher, K., Locoro, G., Contini, S., Bidoglio, G., 2009. Sucralose screening in European surface waters using a solid-phase extraction-liquid chromatographyetriple quadrupole mass spectrometry method. J. Chromatogr. A 1216 (7), 1126–1131.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641.

Matamoros, V., Arias, C., Brix, H., Bayona, J.M., 2009. Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products. Water Res. 43, 55–62.

Nilsson, C., Renman, G., Westholm, L.J., Renman, A., Drizo, A., 2013. Effect of organic load on phosphorus and bacteria removal from wastewater using alkaline filter materials. Water Res. 47, 6289–6297.

Olshammar, M., Mats, E., Rosenquist, L., Ejhed, H., Sidvall, A., Svanström, S., 2015. SMED-Rapport Nr 166. Uppdatering av kunskapsläget och statistik för små avloppsanläggningar. http://www.smed.se/wp-content/uploads/2015/11/SMED-Rapport-2015-166.pdf.

Osorio, V., Pérez, S., Ginebreda, A., Barceló, D., 2012. Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions. Environ. Sci. Pollut. Res. 19, 1013–1025.

Pasquini, L., Munoz, J.F., Pons, M.N., Yvon, J., Dauchy, X., France, X., Le, N.D., France-Lanord, C., Görner, T., 2014. Occurrence of eight household micropollutants in urban wastewater and their fate in a wastewater treatment plant. Statistical evaluation. Sci. Total Environ. 481 (1), 448–459.

Renman, A., Renman, G., 2010. Long-term phosphate removal by the calcium-silicate material Polonite in wastewater filtration systems. Chemosphere 79, 659–664.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrović, M., Caminal, G., Barceló, D., Vicent, T., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus *Trametes versicolor* in bioslurry reactor. J. Hazard. Mater. 233–234, 235–243.

Rúa-Gómez, P.C., Püttmann, W., 2013. Degradation of lidocaine, tramadol, venlafaxine and the metabolites O-desmethyltramadol and O-desmethylvenlafaxine in surface waters. Chemosphere 90 (6), 1952–1959.

Scheurer, M., Brauch, H.J., Lange, F., 2009. Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). Anal. Bioanal. Chem. 394, 1585–1594.

Stanford, B.D., Weinberg, H.S., 2010. Evaluation of on-site wastewater treatment technology to remove estrogens, nonylphenols and estrogenic activity from wastewater. Environ. Sci. Technol. 44, 2994–3001.

Teerlink, J., Martinez-Hernandez, V., Higgins, C.P., Drewes, J.E., 2012. Removal of trace organic chemicals in onsite wastewater soil treatment units: a laboratory experiment. Water Res. 46, 5174–5184.

Thompson, M.S.A., Bankier, C., Bell, T., Dumbrell, A.J., Gray, C., Ledger, M.E., Lehmann, K., McKew, B.A., Sayer, C.D., Shelley, F., Trimmer, M., Warren, S.L., Woodward, G., 2016. Gene-to-ecosystem impacts of a catastrophic pesticide spill: testing a multilevel bioassessment approach in a river ecosystem. Freshw. Biol. 61, 2037–2050.