



Fate and mass balance of contaminants of emerging concern during wastewater treatment determined using the fractionated approach



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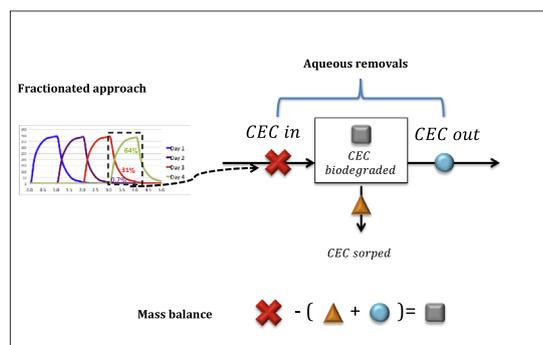
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HIGHLIGHTS

- Fractionated approach was used to calculate removal of 26 CECs in multiple units.
- Removals were the highest in secondary treatment and <70% in tertiary treatment.
- Mass balancing allowed to identify contribution of different pathways to removal.
- Degradation of some contaminants was observed in the primary clarifier.
- Formation of metabolites explained some negative removals.

GRAPHICAL ABSTRACT



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ABSTRACT

Contaminants of emerging concern (CECs) are often poorly removed from wastewater using conventional treatment technologies and there is limited understanding of their fate during treatment. Inappropriate sampling strategies lead to inaccuracies in estimating removals of CECs. In this study, we used the “fractionated approach” that accounts for the residence time distribution (RTD) in treatment units to investigate the fate of 26 target CECs in a municipal wastewater treatment plant (WWTP) that includes primary, secondary and tertiary treatment steps. Prior hydraulic calibration of each treatment unit was performed. Wastewater and sludge samples were collected at different locations along the treatment train and the concentrations of target CECs were measured by liquid chromatography mass spectrometry. The most substantial aqueous removal occurred during activated sludge treatment (up to 99%). Removals were <50% in the primary clarifier and tertiary rotating biological contactors (RBCs) and up to 70% by sand filtration. Mass balance calculations demonstrated that (bio)degradation accounted for up to 50% of the removal in the primary clarifier and 100% in activated sludge. Removal by sorption to primary and secondary sludge was minimal for most CECs. Analysis of the selected metabolites demonstrated that negative removals obtained could be explained by transformations between the parent compound and their metabolites. This study contributes to the growing literature by applying the fractionated approach to calculate removal of different types of CECs across each wastewater treatment step. An additional level of understanding of the fate of CECs was provided by mass balance calculations in primary and secondary treatments.

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1. Introduction

Contaminants of emerging concern (CECs), including pharmaceuticals and personal care products (PPCPs), drugs of abuse (DOAs), hormones and stimulants are present in the aquatic environment (Blair et al., 2013; Hughes et al., 2013; Rodayan et al., 2015), and these CECs were found to have adverse effects on aquatic organisms (Gay et al., 2015; Kidd et al., 2007; Kümmerer, 2008; Purdom et al., 1994). Discharges of wastewater treatment plants (WWTPs) have been identified as the primary source for CECs introduced into surface waters (Luo et al., 2014). Therefore, accurate determination of the fate of CECs during wastewater treatment is required in order to facilitate risk assessments and to identify strategies to improve their removal.

Unexplained temporal variations in the removals of CECs in activated sludge units and in some cases negative removals (i.e. higher concentration in the effluents than in the influents) have often been observed in WWTPs, which has raised questions about the appropriateness of the protocols for sampling wastewater (Majewsky et al., 2011; Ort et al., 2010). In a critical review, Ort et al. (2010) suggested that sampling strategies that fail to account for the residence time distribution (RTD), which affects the transport of CECs in treatment units, could result in a mismatch between the sampled influent and effluent. This mismatch results in biased and unreliable data on removal efficiencies. Majewsky et al. (2011) proposed a strategy for sampling and removal calculation referred to as the “fractionated approach” to account for the RTD within the units of the WWTPs and match the mass loads of the influent with the effluent. This approach requires prior hydraulic modelling of the WWTP followed by a tailored sampling strategy where composite samples are collected on several consecutive days. The fractionated approach has shown promise for evaluating the removal of pharmaceuticals, pesticides and drugs of abuse during activated sludge treatment (Majewsky et al., 2013; Rodayan et al., 2014a). However, the fractionated approach has not yet been applied to a complete WWTP treatment train to identify and quantify the mechanisms of CEC removal at each stage of treatment.

To date, most studies on the removal of CECs did not investigate the contribution of different removal mechanisms, as they ignored the distribution of CECs between the aqueous and the particulate compartments (Petrie et al., 2015). A limited number of previous studies have taken a mass balance approach to determine removals of CECs in conventional primary and secondary treatment steps through the processes of adsorption and (bio)degradation that were the major removal mechanisms for the CECs investigated (Carballa et al., 2007; Gao et al., 2012; Heidler and Halden, 2008; Jelic et al., 2011; Petrie et al., 2014; Wick et al., 2009; Winkler et al., 2007). The majority of these studies were based on analysis of CECs in grab or 24-h composite wastewater samples collected simultaneously from different treatment stages, while a small number of studies accounted for the hydraulic retention time in the sampling campaign but did not consider the wastewater residence time distribution.

In a recent review, Petrie et al. (2015) identified inadequate sampling approaches, a lack of understanding of adsorption of CECs onto particulates in WWTPs, as well as a lack of data on the fate of the metabolites of pharmaceuticals as information gaps that lead to misrepresentative data of CEC removals. In the present study, advanced sampling strategies that account for the hydraulic behaviour of treatment units were combined with mass balance analysis to monitor the fate of 26 target compounds in a WWTP in order to gain insights into the mechanisms of removal of CECs. The target CECs were contaminants that were reported to be present in surface water or persistent during wastewater treatment. The list included pharmaceuticals and some of their metabolites, hormones, drugs of abuse, a stimulant (i.e. caffeine), an artificial sweetener (i.e. sucralose) and an antibacterial agent (i.e. triclosan). The WWTP monitored in this study employs primary treatment, secondary treatment by activated sludge, tertiary treatment by both rotating biological contactors (RBCs) and sand filtration and disinfection

by chlorination/dechlorination. This study contributes to the literature on the use of the fractionated approach for reliable determination of CEC removals, and for the first time, uses this approach to estimate removals during the tertiary treatment steps with RBCs and sand filtration. Further, the predominant removal mechanisms during primary and secondary treatment were identified by the mass balance analysis.

2. Materials and methods

2.1. Chemicals and other supplies

The target CECs included several pharmaceuticals identified by Dickenson et al. (2011) in a study that illustrated the value of monitoring a small number of CECs in WWTPs in the USA. Sucralose was added to the list as a tracer of wastewater contamination due to its persistence and ubiquitous presence at high concentrations (Mawhinney et al., 2011). The drugs of abuse were selected based upon our previous studies indicating their presence in wastewaters in Canada (Metcalf et al., 2010; Rodayan et al., 2014a). Androstenedione was selected as a model androgen because of the lack of data in the literature investigating its fate (Esperanza et al., 2007), and estrone was selected as a model estrogen because of its widespread occurrence in wastewater (Servos et al., 2005). To illustrate the importance of including metabolites in mass balance calculations, two metabolites of carbamazepine, rac *trans*-10,11-dihydro-10,11-dihydroxy carbamazepine (CBZ-DiOH) and carbamazepine 10,11-epoxide (CBZ-EP) were monitored because these compounds have been previously detected in wastewater and sludge (Hummel et al., 2006; Miao and Metcalfe, 2003; Miao et al., 2005). Other metabolites monitored in this study included EDDP, the primary metabolite of methadone, and benzoylecgonine, the primary metabolite of cocaine.

The target CECs, along with their physicochemical properties and the suppliers from which they were purchased are listed in Table 1. The analytes were classified as Class A and B compounds (Table 1) according to the extraction and analysis procedures. Class B compounds are drugs of abuse and some of their metabolites, which are mainly weak bases, in addition to carbamazepine and its two metabolites. Class A compounds are all other target compounds that are weak acids, neutral or phenolic compounds. The internal standards used for each compound are listed in Table 1. 10,11-Dihydrocarbamazepine was used as a surrogate for CBZ-DiOH, as performed in previous studies (Leclercq et al., 2009; Miao and Metcalfe, 2003). All analytical standards and stock solutions were stored in amber glass vials at $-20\text{ }^{\circ}\text{C}$.

Methanol, acetonitrile, and water of liquid chromatography–mass spectrometry (LC–MS) grade, as well as other chemicals used for sample preparation were purchased from Fisher Scientific (Ottawa, ON, Canada). Ultrapure water was generated using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Study site

The study site is a municipal WWTP located in Guelph, ON, Canada, serving a population of approximately 134,894, having a design capacity of 64,000 m³/d and receiving an average incoming flow rate of 50,755 m³/d of domestic, commercial and industrial wastewater. The WWTP (Fig. 1) provides preliminary treatment by screening and aerated grit removal, after which the load is split into four activated sludge lines. Each of the lines consists of two primary clarifiers, two aeration tanks and one or two secondary clarifiers in parallel. The WWTP provides tertiary treatment for the recombined effluent of the lines by rotating biological contactors (RBCs), followed by sand filtration. The tertiary treatment step was added to the treatment sequence in order to meet regulations concerning the levels of TSS (i.e. 10 mg/L) and ammonia nitrogen (i.e. 3.4 mg/L) in the effluent. For disinfection, chlorine is added to wastewater after the RBCs as 12% sodium hypochlorite at an average

Table 1

Target CECs and their chemical and physical characteristics, internal standards, class (determining the corresponding extraction and analysis methods), LODs and LOQs in aqueous and biosolids samples and the supplier of the compounds and their surrogates.

Type	Subtype	Compound	Log K_{ow}^a	pKa ^a	Internal standard (surrogate)	Class	Aqueous LOD, LOQ (ng/L) ^d	Biosolids LOD, LOQ (ng/L) ^d	Company ^e compound, surrogate	
Pharmaceuticals	Antibiotics	Trimethoprim	0.91	6.8	Trimethoprim-13C3	A	7, 22	3, 10	S, I	
		Sulfamethoxazole	0.89	5.7	Sulfamethoxazole-13C6	A	4, 14	2, 9	S, I	
	Analgesics	Codeine	1.14	8.2	Codeine-d3	B	6, 18	3, 9	C, C	
		Ibuprofen	3.97	4.9	Ibuprofen-13C3	A	6, 21	10, 32	S, I	
	Antiepileptic and metabolites	Naproxen	Naproxen	3.18	4.2	Naproxen-13C1-d3	A	4, 14	6, 19	S, I
			Carbamazepine	2.45	3.2	Carbamazepine-d10	B	2, 5	2, 6	C, C
rac <i>trans</i> -10,11-Dihydro-10,11-dihydroxy carbamazepine (CMZ-DiOH)		0.13	N.A.	10,11-Dihydrocarbamazepine	B	4, 13	3, 9	T, T		
Carbamazepine 10,11-epoxide (CMZ-EP)		1.26	N.A.	Carbamazepine 10,11-epoxide-d8	B	2, 7	1, 5	T, T		
Drugs of abuse	Cocaine and metabolite	Benzoylcgonine (cocaine's metabolite)	-1.32	N.A.	Benzoylcgonine-d3	B	4, 14	2, 5	C, C	
		Cocaine	2.3	8.6	Cocaine-d3	B	26, 84	11, 36	C, C	
	Amphetamines	Amphetamine	1.76	10.1	Amphetamine-d5	B	4, 13	1, 4	C, C	
		Methamphetamine	2.07	10.2	Methamphetamine-d9	B	7, 23	1, 4	C, C	
		EDDP (methadone's metabolite)	4.94	9.6	EDDP-d3	B	5, 18	4, 15	C, C	
	Opioids	Ephedrine	1.13	9.7	Ephedrine-d3	B	7, 25	3, 9	C, C	
		Dihydrocodeine	1.49	8.8	Dihydrocodeine-d6	B	8, 22	5, 14	C, C	
		Fentanyl	4.05	8.6	Fentanyl-d5	B	6, 20	3, 8	C, C	
		Ketamine	2.18	7.5	Ketamine-d4	B	8, 27	2, 8	C, C	
		Methadone	3.93	8.9	Methadone-d9	B	4, 13	1, 4	C, C	
		Morphine (codeine metabolite)	0.89	9.9	Morphine-d3	B	5, 17	10, 32	C, C	
		Oxycodone	0.66	8.3	Oxycodone-d3	B	10, 22	3, 7	C, C	
	Personal care product	Antibacterial	Tramadol	2.63	9.4	Tramadol-d3	B	7, 24	1, 3	C, C
			Triclosan	4.76	7.9	Triclosan-13C12	A	6, 19	6, 19	K, M
	Steroid hormones		Androstenedione	2.75	NA	Androstene-3,17-dione-2,3,4-13C3	A	2, 5	7, 25	S, C
Estrone			3.13	10.3	Estrone-3,4-13C2	A	2, 5	6, 19	S, I	
Nervous stimulant		Caffeine	-0.07	14	Caffeine-13C3	A	4, 14	5, 16	S, I	
Artificial sweetener		Sucralose	-1 ^b	11.8 ^c	Sucralose-d6	A	7, 22	9, 30	S, T	

^a National Center for Biotechnology Information (2004).

^b Subedi and Kannan (2014).

^c Buseti et al. (2015).

^d LODs and LOQs were obtained based on standard deviation of y-intercept of measured concentrations of serial dilutions.

^e Companies: S: Sigma-Aldrich Canada (Oakville, ON, Canada), I: C/D/N Isotopes (Pointe-Claire, QC, Canada), C: Cerilliant Corporation (Round Rock, Tex, USA), M: Cambridge isotope Laboratories (Tewksbury, MA, USA), T: Toronto Research Chemicals (North York, ON, Canada), K: KICTeam (Langley, BC, Canada).

chlorine dosage of 1760 kg/d. The disinfected de-chlorinated final effluent is discharged into the Speed River. Table 2 summarizes the main characteristics of line 1 in the WWTP since only line 1 was investigated in the present study.

2.3. Hydraulic model

Electrical conductivity was utilized as a tracer for the investigation of the residence time distribution as proposed earlier (Ahnert et al., 2010;

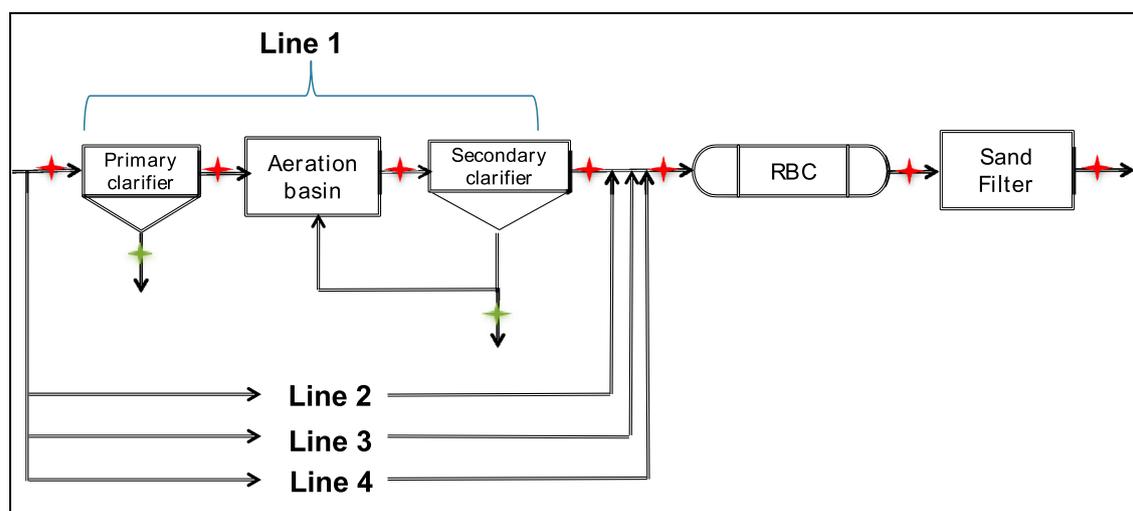


Fig. 1. Schematic of the WWTP. Lines 1–4 correspond to the four lines of primary and secondary treatment. Red marks represent locations where conductivity probes were deployed and aqueous samples were collected. Green marks represent locations where sludge samples were collected. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

Main characteristics of the WWTP, including hydraulic retention time (HRT), solids retention time (SRT) of treatment units in line 1, average temperature and pH during sampling campaigns.

Characteristic		
HRT (h)	Primary clarifier (line 1)	3.72
	Aeration tanks (line 1)	6.3
	Secondary clarifier (line 1)	2.9
	RBCs	0.8
	Sand filter	0.4
SRT (days) ^a	(Line 1)	7.76
Average T (°C)		20
Average pH		8.03

^a The SRT was calculated by dividing total solids in the activated sludge unit of line 1 (volume times sludge concentration) by the wasted sludge (waste flow times waste sludge concentration).

Majewsky et al., 2011). HOB0 conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada) were used to collect electrical conductivity and temperature data (one reading per minute) for hydraulic model calibration. The probes were deployed over 3 weeks (June 12–July 8, 2014) before and after each treatment unit (red marks in Fig. 1). A universal optic-USB base station and Onset HOBOWare Pro Version 3.2.2 software (Hoskin Scientific, St-Laurent, QC, Canada) were used for data transfer and read out, respectively.

The hydraulic model was created in the simulation software WEST (Mike Powered by DHI, Hørsholm, Denmark). Each part of the treatment plant was modelled separately, using the measured electrical conductivity at the entrance of each treatment step as tracer input for the respective hydraulic model, along with the actual flow conditions and tank volumes. A good fit between the simulated and measured electrical conductivities at the exit of each treatment step was obtained by varying the number of tanks in series (aeration tanks) and the number of layers and feed layer (clarifiers) which determine the flow regime (i.e. from plug flow to fully mixed). The best-fit model was determined on the basis of minimizing the root mean square error (RMSE) and visual inspection of the output graphs. Simulations were run with the calibrated hydraulic model of each treatment unit employing a 24-h step increase of inert tracer as input, along with the actual flow rates during the sampling period. The output was used to obtain the load fractions that describe how the material in the effluent of each treatment stage on a certain day is composed out of fractions of the influent to that treatment step over several days, as illustrated by Majewsky et al. (2011).

2.4. Wastewater sampling

Wastewater was collected before and after each treatment unit at the locations shown in Fig. 1 (red marks). The number of sampling days was based on the best-fit hydraulic model that indicated that the effluent of the activated sludge unit on a given day is composed of influent material entering the WWTP over four days, which will be elaborated upon in the results section. The samples collected during the first sampling campaign (July 21–25, 2014) were analyzed for the Class A compounds, while the samples collected during the second campaign (June 16–20, 2015) were analyzed for the Class B compounds. The samples were collected as 24-h composite using onsite Hach Sigma samplers that collect flow-proportional samples and refrigerate them at 4 °C. This is with the exception of the effluent of the RBC, where ISCO 6712 samplers (Avensys, St-Laurent, QC, Canada) equipped with 24 bottles and packed with ice replaced daily were used to collect 24-h time-proportional composite samples. Both samplers collected samples every 15 min. Primary and secondary waste sludge samples were collected as grab samples over the four days (green marks in Fig. 1). At the end of each day of the sampling campaign, the collected aqueous and sludge samples were transferred into 1-L amber HDPE bottles (Fisher Scientific) and stored at –20 °C until extraction was performed (within 3 weeks).

2.5. Sample preparation

2.5.1. Wastewater samples

Wastewater samples were thawed and filtered using 1- μ m glass-fiber filter (Fisher Scientific) prior to extraction. Volumes of 100 mL of raw wastewater influent and 200 mL of all other sample matrixes were spiked with the appropriate internal standards (listed in Table 1). Solid phase extraction (SPE) was performed using two different methods. Class A compounds were extracted with Oasis MAX anion exchange cartridges (Waters Corporation), as described by Metcalfe et al. (2014). Class B compounds were extracted with Oasis MCX cation exchange cartridges, as described by Yargeau et al. (2014). Both methods and instruments used are summarized in the Supplementary material (Table S1). SPE recoveries of target compounds ranged from 71% to 130% for Class A compounds (average recovery of 75%) and from 60% to 100% for Class B compounds (average recovery of 78%). Glass containers pre-cleaned with hexanes and acetone were used for all sample preparation and analysis experiments.

2.5.2. Sludge samples

Approximately 1 g of freeze-dried sludge was placed in accelerated solvent extraction (ASE) stainless steel cells and spiked with internal surrogates (Table 1, 100 ng/g) before extraction. Extraction of sludge was conducted by pressurized liquid extraction using a Dionex ASE 350 accelerated solvent extraction system (Thermo Fisher Scientific, Waltham, MA, USA), followed by SPE clean up based on the methods summarized in the Supplementary material (Table S1). For the Class A compounds, the two extraction methods used were described by Edwards et al. (2009) as the neutral drug method and the acidic drug method. However, the neutral drugs method with acetone and water (3:7) as the ASE extraction solvent (Table S1) gave the highest recoveries in the sludge matrix for this study. For Class B compounds (mainly drugs of abuse), the extraction method that achieved the highest recoveries was the one suggested for the beta-blocker atenolol in the same study by Edwards et al. (2009) and is also summarized in Table S1. All sludge samples were extracted in triplicates and the extraction efficiencies of target compounds were >70%.

2.6. Analysis

Analysis of the Class A compounds was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) using an Agilent 1100 HPLC (Mississauga, ON, Canada) coupled to a Q-Trap 5500 instrument (AB Sciex, Concord, ON, Canada) operated with a turbospray ionization source. The Class A target compounds of all samples were separated chromatographically using the method described by Metcalfe et al. (2014). The analytes were measured in either negative or positive ion mode, depending on the compound. Acquisition was performed using the precursor and product ion transitions for multiple reaction monitoring (MRM) of the target compounds and their corresponding deuterated surrogates. The MRM transitions for the target compounds are listed elsewhere (Metcalfe et al., 2014; Thompson et al., 2011).

Analysis of the Class B compounds was conducted by liquid chromatography with high-resolution mass spectrometry (LC-HRMS) using an Accela LC system coupled to a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation and analysis in positive ion mode was achieved using the methods described by Rodayan et al. (2014b). Acquisition was performed in full scan mode (50–400 m/z) at high resolution (R_{FWHM} = 41,000). The ion of interest was extracted using an m/z window of ± 0.01 . Linear calibration curves of nine points were used for quantification of the concentrations of the target compounds of both classes. Recoveries of the internal standards were used to adjust the concentrations of all target analytes.

2.7. CEC removals

The equations used for the calculations of CEC removals from the aqueous phase are presented in Eqs. (1)–(3) and Fig. S1 in the Supplementary material. Calculation of the CEC removals was first based on the CEC load in the aqueous phase only of the input and output streams of each treatment unit, as described in Eq. (1). To account for the residence time distribution, a fractionated input load of the CEC that corresponds to the output load on the fourth day of sampling was calculated (using Eq. (2)), as proposed by Majewsky et al. (2011). The term “input reference load” will be used throughout the paper to refer to the incoming fractionated load of each treatment unit. The input reference load was compared to the output load to obtain estimates of the CEC removals per treatment unit (Eq. (3)).

$$L_{aq} = Q * C_{aq} \quad (1)$$

$$L_{aq,ref} = \sum_{i=1}^{i=4} f_i * L_{aq,in,i} \quad (2)$$

$$R = \frac{L_{aq,ref} - L_{aq,out}}{L_{aq,ref}} * 100\% \quad (3)$$

where L_{aq} : Load of the contaminant in the aqueous phase of a specific stream (mg/d), C_{aq} : Concentration of the contaminant in the aqueous phase of a specific stream (mg/L), Q : Flow rate of the corresponding stream (L/d), $L_{aq,ref}$ (mg/d): Input reference mass load of the contaminant in the aqueous phase of the input stream based on several days of sampling, $L_{aq,in,i}$ (mg/d): Mass load of the contaminant in the input stream on the i^{th} day of sampling, f_i : Fraction of incoming contaminant load on the i^{th} day of sampling that is contained in the outgoing load on the last day of sampling, $L_{aq,out}$ (mg/d): Mass load of the CEC in the aqueous phase of the output stream on the last day of sampling, R (%): Removal of CEC from the aqueous phase in a specific treatment unit using the fractionated approach.

Mass balances were then carried out across the primary and biological treatment steps based on the total contaminant load in both aqueous and particulate phases, according to Eqs. (4)–(7). The total load of the CEC on each day was obtained in the input stream ($L_{tot,in}$), output stream ($L_{tot,out}$) and sludge stream ($L_{tot,sludge}$) by summing particulate and dissolved loads, as shown in Eq. (4). The input reference load was also obtained by the fractionated approach (Eq. (6)). In both the primary and biological treatment stages, the primary mechanisms of removal of the studied CECs are biodegradation and sorption to solids (Andersen et al., 2005; Li and Zhang, 2010; Radjenović et al., 2009; Verlicchi et al., 2012). Abiotic removal due to hydrolysis or photolysis was previously investigated for some of the studied CECs and reported to be negligible (Li and Zhang, 2010; Pèrez et al., 2005). Volatilization is also expected to be limited, due to the low Henry constants of the studied CECs (Gao et al., 2012). Therefore, the difference between the total incoming load and the outgoing load (in the output stream or sorbed to sludge) was assumed to be the load that was lost due to (bio)degradation (L_{deg}), as shown in Eq. (7). It should be kept in mind that this load could also include experimental or modelling errors that could cause a bias in the results.

$$L_{tot} = L_{aq} + L_s \quad (4)$$

$$L_s = Q * C_s * TSS \quad (5)$$

$$L_{tot,ref} = \sum_{i=1}^{i=4} f_i * L_{tot,in,i} \quad (6)$$

$$L_{deg} = L_{tot,ref} - L_{tot,out} - L_{tot,sludge} \quad (7)$$

where L_{tot} : Mass load of the contaminant in the liquid and particulate phase of a certain stream (mg/d), L_s : Mass load of contaminant measured in the particulate phase of a stream (mg/d), C_s : Concentration of

contaminant measured in the particulate phase of a stream on a dry weight basis (mg/g) and TSS: Total suspended solids in the corresponding stream (g/L), which was collected by the WWTP operators following the APHA/AWWA protocol (Rice et al., 2012). L_{aq} is computed using Eq. (1), except in the sludge stream where the volume fraction of solids is more significant, so $L_{aq,sludge} = Q_{sludge} * C_{aq,sludge} * f_w, f_w$: Volume fraction of water in sludge, L_{deg} : Mass load of the contaminant that was (bio)degraded (mg/d).

The measured CEC concentrations were used to calculate the K_d value, defined by Eq. (8), to represent the partitioning of CECs between the dry solids and the aqueous phase for primary and secondary waste sludge, separately. Error bars for loads, removals and K_d values were obtained based on standard deviations from lab triplicates using the propagation of error formulas (Ku, 1966). Investigating the statistical significance of the difference between the incoming and the outgoing loads (Section 3.7) was performed using the unequal-variance two-sample t -test with a confidence level of 95%.

$$\text{Log } K_d = \log \left(\frac{C_s}{C_{aq}} \right) \quad (8)$$

3. Results and discussion

3.1. Calibration of hydraulic model and load fractions

The hydraulic mixing of the aeration tanks in line 1 was best described by three continuous-stirred tanks (CSTs) in series, all having equal volume and each representing perfect mixing. This is consistent with previous studies where the hydrodynamics of the aeration tanks in the activated sludge process were represented by a number of perfectly mixed tanks in series (Majewsky et al., 2011; Rodayan et al., 2014a). The primary and secondary clarifiers were both best modelled with a 10-layer settling tank and the 5th layer as feed layer. Fig. S2 illustrates that the simulated and the measured output conductivity profiles were in good agreement for all treatment steps, verifying the goodness of the fit of the obtained hydraulic model. The RBC hydraulic behaviour was modelled by a single CST and the sand filter as a series of 2 CSTs. The volumes for the RBCs and sand filters were adjusted until the simulated effluent matched the actual hydraulic behaviour, as it was not possible to estimate the actual hydraulic volume. The obtained volume was small, leading to low retention times, consistent with data obtained from operators at the WWTP. The absence of significant shifts or damping in the dynamics (Fig. S2) demonstrates the minimal mixing occurring in both the RBCs and the sand filters due to their very short HRTs (see Table 2).

The obtained load fractions for the removal calculations are summarized in Table 3. For a selected treatment unit, load fractions represent the fractions of CEC incoming load on different days that make their way to the output on the last day of sampling, assuming no removal. Hence, these fractions do not necessarily add up to 100%. For both the primary and secondary clarifier the effluent was comprised of 92% of CEC load in the influent of the last day of sampling and only 8% of the

Table 3

Load fractions for each treatment unit (f_i) describing the fraction of incoming CEC load on day i that is contained in the output of day 4 (last day of sampling) assuming no removal (including hydraulics effect only).

Treatment unit	Load fractions (%)			
	f_1	f_2	f_3	f_4
Primary clarifier	0	0	8	92
Aeration tanks	0	0	12	73
Secondary clarifier	0	0	8	92
Activated sludge (combination)	4E–02	1	36	64
RBCs	0	0	4	96
Sand filter	0	0	3	97

day before. By contrast, the effluent of the aeration tank was composed of 73% of the CEC load on the same day and 12% from the day before, which can be related to the higher degree of mixing that occurs in the aeration tanks. The effluent from the activated sludge unit had even more distributed load fractions (64% from the last day and 36% from the previous day, 1% of three days ago and 0.04% of four days ago) due to the recycling of sludge. The load fractions of the activated sludge unit indicated the need for four days of sampling. For the RBCs and sand filters, only 4% and 3% respectively of CEC load on a previous day was present in the effluent on a given day, which is attributed to their low HRTs (Table 2), resulting in low mixing.

3.2. Concentrations of CECs in wastewater

The concentrations of the CECs at the different treatment steps are shown in Table 4. Most of the target compounds were detected in the influent to the primary clarifier, except for the prescription opioid drugs, fentanyl and ketamine. The lowest concentration of a target compound detected in the influent was observed for estrone (7 ng/L) and the highest mean concentration was for caffeine (28,960 ng/L). Among all target pharmaceuticals, ibuprofen, was present at the highest levels in influent wastewater. Selected metabolites of cocaine, methadone and carbamazepine were monitored as well. In the case of cocaine, only 1–9% is excreted unchanged from the human body, while 35–54% is excreted as benzoylecgonine (Ratola et al., 2012). The ratio between benzoylecgonine and cocaine was calculated to be 1.5. Despite the fact that the majority of studies reported values in the range of 3.1–3.5, lower values similar to the present study were reported in some studies (Bones et al., 2007), suggesting that some cocaine is being discharged into the sewage system without consumption (Karolak et al., 2010; Ratola et al., 2012).

Table 4 shows that the CEC concentrations in the combined secondary effluent (i.e. combined output from all the lines, Fig. 1) were

generally in the same ranges as the concentrations in the secondary effluent (i.e. from line 1), indicating that in terms of CEC removal, line 1 had a similar efficiency at removing CECs as the other three lines combined. In the final effluent, some target compounds, nine out of 26 CECs were not detected at concentrations above the limits of detection (LODs) or limits of quantification (LOQs). The highest effluent levels were observed for sucralose (3476 ng/L) followed closely by caffeine (2015 ng/L), unlike in the influent where caffeine had levels one order of magnitude higher than sucralose.

3.3. Removals by primary clarifier

3.3.1. Aqueous phase

The CEC removals (%) based on aqueous phase data at each treatment step were estimated using the fractionated approach, as well as concentrations at the input and output streams (Fig. 2). The results obtained in the present study are generally comparable to results from the literature summarized in the Supplemental material (Table S2). Poor removals from the aqueous phase of wastewater (<40%) were observed for all target CECs in the primary clarifier, with the highest removals observed for tramadol and codeine (Fig. 2). Negative removals in the primary clarifier were observed for a number of CECs, namely oxycodone, naproxen, estrone, EDDP and dihydrocodeine (Fig. 2). Some of these CECs were previously reported to have negative removals (Table S2).

3.3.2. Primary sludge

Concentrations of CECs in primary sludge, along with estimated log K_d values for the CECs in primary sludge are summarized in Table 5. The average concentration of the selected CECs in the solids of the primary sludge was the highest for caffeine, followed by triclosan and codeine; however, triclosan was found to have the highest log K_d value (Table 5). High concentrations in primary sludge can be attributed

Table 4
Concentrations (ng/L \pm standard deviation) of target CECs in line 1 of the WWTP at the influent to the primary clarifier (primary influent), effluent of the primary clarifier (primary effluent), effluent of the secondary clarifier (secondary effluent), as well as the combined secondary effluents of all lines (1–4), and effluent of RBCs (RBCs effluent) and sand filter effluent. Standard deviation was based on 3 replicates of sample preparation and analysis.

Compound	Primary influent (line 1)	Primary effluent (line 1)	Secondary effluent (line 1)	Combined secondary effluent	RBCs effluent	Sand filter effluent
Androstenedione	92 \pm 5	65 \pm 2	42 \pm 2	41 \pm 2	40 \pm 1	44 \pm 1
Estrone	7 \pm 3	8 \pm 2	5 \pm 1	7 \pm 3	5 \pm 1	6 \pm 2
Trimethoprim	25 \pm 2	25 \pm 3	37 \pm 3	29 \pm 3	22 \pm 2	<LOD
Sulfamethoxazole	33 \pm 5	31 \pm 5	43 \pm 16	47 \pm 21	37 \pm 6	<LOQ
Ibuprofen	3644 \pm 105	1690 \pm 90	<LOQ	<LOQ	<LOQ	<LOQ
Naproxen	471 \pm 36	504 \pm 35	36 \pm 5	20 \pm 3	18 \pm 2	<LOQ
Triclosan	166 \pm 26	132 \pm 11	50 \pm 2	31 \pm 4	42 \pm 3	29 \pm 2
Caffeine	28,960 \pm 4658	28,123 \pm 4750	1756 \pm 226	2148 \pm 127	2245 \pm 194	2015 \pm 270
Sucralose	2437 \pm 220	2635 \pm 166	4591 \pm 291	3178 \pm 84	3175 \pm 209	3476 \pm 129
Cocaine	361 \pm 18	287 \pm 30	111 \pm 26	97 \pm 21	<LOQ	<LOQ
Benzoylecgonine	524 \pm 14	415 \pm 18	83 \pm 5	58 \pm 4	50 \pm 2	47 \pm 3
Amphetamine	101 \pm 2	77 \pm 3	<LOQ	<LOD	<LOD	<LOD
Methamphetamine	300 \pm 7	213 \pm 10	47 \pm 5	43 \pm 2	23 \pm 0.2	<LOD
EDDP	249 \pm 29	299 \pm 21	231 \pm 55	156 \pm 7	169 \pm 15	156 \pm 4
Ephedrine	1616 \pm 62	1053 \pm 61	111 \pm 8	86 \pm 8	72 \pm 1	72 \pm 3
Codeine	2116 \pm 92	1204 \pm 40	1281 \pm 109	901 \pm 16	529 \pm 15	130 \pm 5
Dihydrocodeine	324 \pm 23	459 \pm 23	22 \pm 1	39 \pm 3	28 \pm 1	27 \pm 1
Methadone	123 \pm 3	98 \pm 3	48 \pm 4	54 \pm 2	44 \pm 1	42 \pm 2
Morphine	295 \pm 12	338 \pm 61	68 \pm 19	24 \pm 3	23 \pm 4	21 \pm 5
Oxycodone	126 \pm 10	137 \pm 24	64 \pm 3	46 \pm 3	35 \pm 3	27 \pm 7
Tramadol	174 \pm 6	116 \pm 8	177 \pm 11	105 \pm 5	125 \pm 7	105 \pm 5
Ketamine	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ
Fentanyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	591 \pm 24	416 \pm 20	606 \pm 28	588 \pm 17	532 \pm 8	519 \pm 13
CMZ-DIOH	1074 \pm 54	702 \pm 40	553 \pm 51	650 \pm 26	703 \pm 21	648 \pm 49
CMZ-EP	192 \pm 36	258 \pm 40	295 \pm 41	315 \pm 44	385 \pm 62	119 \pm 32

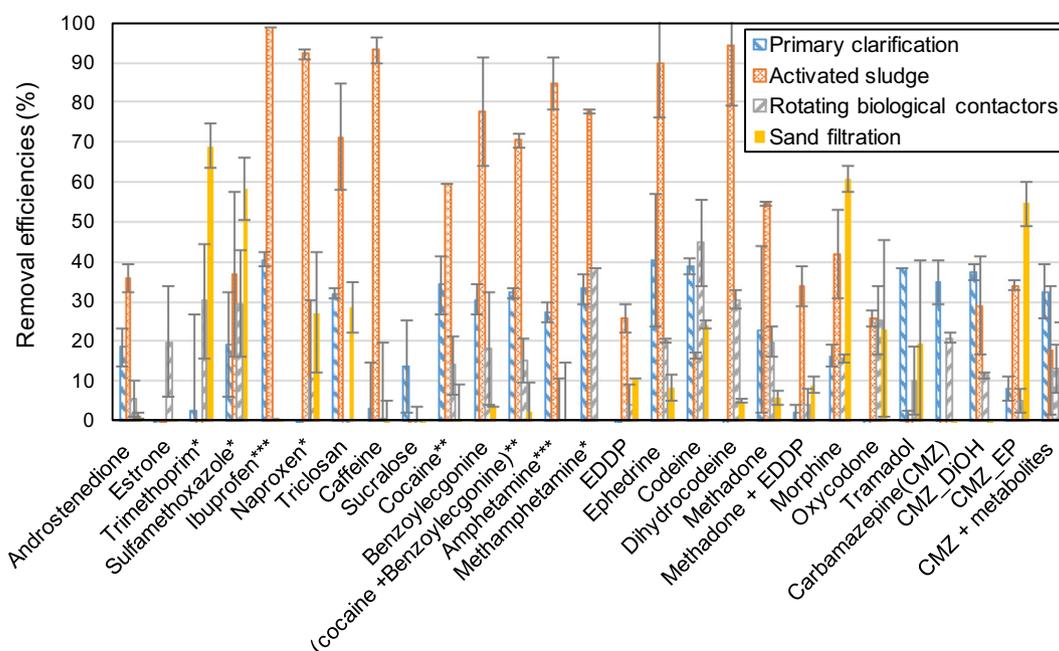


Fig. 2. Estimated removal efficiencies (%) of target CECs. The removal efficiency was based on the incoming load to each treatment unit. (***, **, * denote compounds that were <LOD or <LOQ in the effluent of the activated sludge, RBC and sand filter, respectively, so removal was based on LOD (for <LOD) or LOQ (for <LOQ) and may be higher than reported here. Absence of a column indicates that either the removal was negative or the compound was <LOD or <LOQ in the influent to the treatment unit).

to high incoming concentrations (i.e. codeine and caffeine) in the wastewater, or high partitioning to sludge because of high hydrophobicity (i.e. triclosan, Table 1). Some CECs (i.e. naproxen, sucralose and ketamine) were not detected in the solids of primary sludge (Table 5), which is consistent with previous studies (Brorström-Lunden et al., 2008; Jelic et al., 2011; Radjenović et al., 2009). The data reported in the present study on the levels drugs of abuse in primary and secondary waste sludge are valuable additions to the limited research in this area (Subedi and Kannan, 2015). The estimated $\log K_d$ values for drugs of abuse indicate that these compounds are poorly sorbed to particulates, and the concentrations detected in the aqueous phase of untreated wastewater can thus be used to estimate community drug consumption using the “sewage epidemiology” approach (Castiglioni et al., 2006).

The total input reference mass load of the CECs to the primary clarifier was assigned to different fate pathways, and the percentage of each pathway was calculated (Fig. 3). According to data obtained from operators at the WWTP, the primary clarifier removes 84% of TSS on average. This is higher than normal and can be attributed to the high HRT of 3.72 h as opposed to the usual HRT of 2–3 h in primary clarifiers under dry weather conditions. Also, the chemical addition of ferric chloride for phosphorus removal enhances the settling of solids, contributing to the high TSS reduction. Despite the high TSS reduction, the fraction of CECs that is removed with sludge was <5% of the total incoming load for 15 out of 22 compounds (Fig. 3) owing to their hydrophilic properties (Table 1). This fraction was previously reported to be <0.1% for ibuprofen, naproxen, sulfamethoxazole and caffeine in primary treatment (Carballa et al., 2007; Gao et al., 2012). Ternes et al. (2004) suggested that compounds with $K_d < 500$ L/kg or $\log K_d < 2.70$ often have a negligible fraction sorbed to sludge. In the present study, the estimated $\log K_d$ values for androstenedione, trimethoprim, triclosan and carbamazepine were above this threshold (Table 5), and these compounds were observed to be the only four compounds with more than 10% of their incoming load sorbed to primary sludge.

Despite the generally low biological activity of primary clarifiers, some of the removal during primary clarification can be attributed to (bio)degradation, as shown in Fig. 3. The percentage of incoming load degraded during primary clarification was as high as 40–54% for a

Table 5

Average concentrations of target CECs in primary and activated sludge and the range of concentrations on the four days of sampling represented as average (lowest–highest), along with the estimated $\log K_d \pm$ standard deviation in primary and activated sludge.

Compound	Average concentrations (lowest–highest) (ng/L)		$\log K_d \pm$ standard deviation (log L/kg)	
	Primary sludge	Activated sludge	Primary clarifier	Secondary clarifier
Androstenedione	98 (85–113)	39 (15–65)	3.20 ± 0.19	2.92 ± 0.37
Estrone	<LOD	28 (<LOD–53)	<LOD	3.65 ± 0.32
Trimethoprim	31 (24–43)	154 (92–198)	3.07 ± 0.19	3.59 ± 0.18
Sulfamethoxazole	12 (6–17)	<LOQ	2.15 ± 0.49	<LOQ
Ibuprofen ^a	196 (73–496)	39 (21–59)	2.27 ± 0.64	3.36 ± 0.38
Naproxen	<LOD	23 (<LOQ–25)	<LOD	2.95 ± 0.32
Triclosan	599 (340–909)	1335 (1199–1377)	3.68 ± 0.11	4.49 ± 0.27
Caffeine	2828 (1536–4716)	776 (674–953)	2.10 ± 0.37	2.73 ± 0.31
Sucralose	<LOD	152 (110–218)	<LOD	1.53 ± 0.19
Cocaine	27 (16–35)	<LOD	1.93 ± 0.18	<LOD
Benzoyllecgonine	5 (<LOD–8)	<LOQ	1.40 ± 0.07	<LOQ
Amphetamine ^a	20 (7–37)	13 (5–20)	2.29 ± 0.38	3.09 ± 0.33
Methamphetamine	14 (11–17)	4 (<LOD–4)	1.83 ± 0.02	1.92
EDDP	89 (73–109)	65 (44–95)	2.46 ± 0.1	2.44 ± 0.17
Ephedrine	47 (22–69)	26 (<LOQ–55)	1.60 ± 0.48	2.16 ± 0.5
Codeine	202 (77–305)	36 (<LOQ–53)	2.17 ± 0.26	1.36 ± 0.4
Dihydrocodeine	29 (10–44)	41 (18–56)	1.71 ± 0.08	3.20 ± 0.31
Methadone	49 (7–125)	46 (<LOQ–125)	2.57	2.55 ± 0.71
Morphine	96 (40–171)	76 (37–117)	2.61 ± 0.4	2.99 ± 0.29
Oxycodone	25 (9–42)	8 (<LOD–13)	2.09 ± 0.45	1.97 ± 0.44
Tramadol	92 (21–232)	7 (<LOD–11)	2.58 ± 0.5	1.49 ± 0.36
Ketamine	<LOQ	<LOQ	<LOQ	<LOQ
Fentanyl	18 (11–27)	23 (15–31)	N.A.	N.A.
Carbamazepine ^b	49 (32–78)	27 (22–34)	2.75 ± 0.21	2.29 ± 0.04
CMZ-DiOH	<LOD	<LOD	<LOD	<LOD
CMZ-EP	<LOD	<LOD	<LOD	<LOD

^a Denotes compounds that were <LOD or LOQ in the secondary effluent, indicating that the obtained $\log K_d$ in the secondary clarifier might not be accurate as it was based on the LOD or LOQ of the compound.

^b Carbamazepine solids analysis was based on the 2014 sampling campaign.

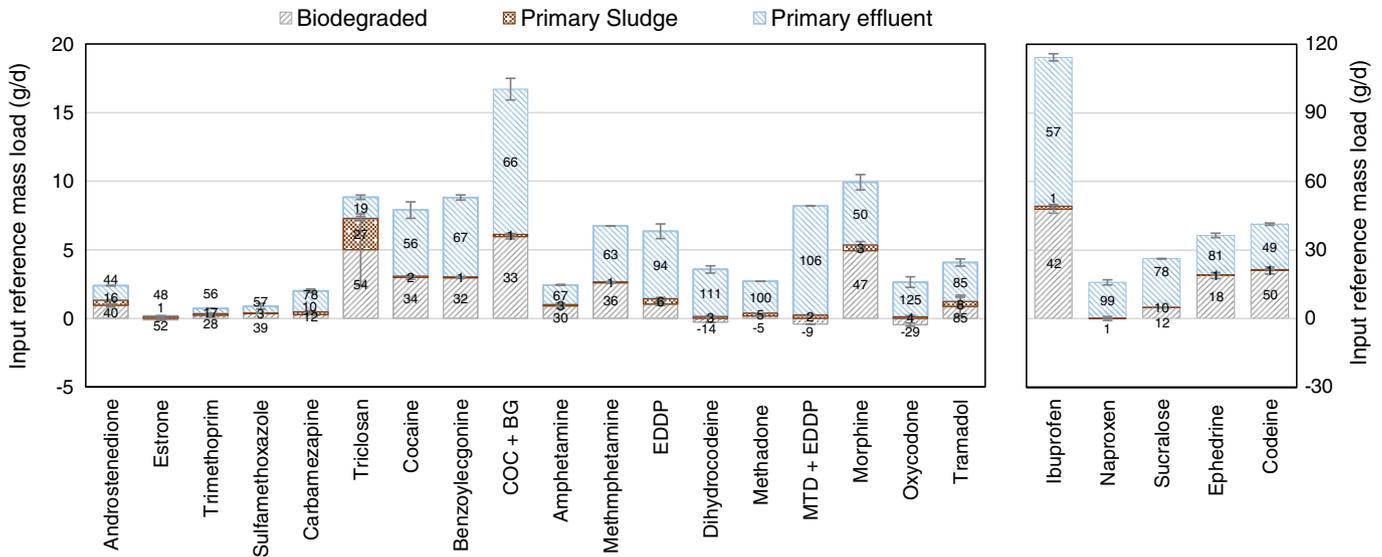


Fig. 3. Input reference mass loads (g/d) of target CECs into the primary clarifier assigned into three main fate pathways: (bio)degraded, discharged with primary sludge and discharged with the primary effluent with the % contribution of each pathway indicated in the corresponding column (COC: cocaine, BG: benzoyllecgonine, MTD: methadone). For caffeine (not shown due to the high mass loads): 14% biodegraded, 2% sorbed to primary sludge and 84% in the effluent.

number of the investigated CECs (i.e. ibuprofen, morphine, codeine, triclosan and ephedrine). Considering the small error bars of the mass loads in Fig. 3 (except for triclosan), this high (bio)degradation in the primary clarifier is unlikely attributed to measurement bias caused by sample preparation and analysis. McCall et al. (2016) showed in a review article that several studies reported the formation of transformation products of drugs of abuse in the sewers (HRT 30 min to 12 h) under different conditions, and Heuett et al. (2015) reported the detection of transformation products of drugs of abuse in influent sewage to a WWTP. The degradation of some pharmaceuticals and formation of transformation products in a real sewer pipe was also reported by Jelic et al. (2015). Having similar conditions as in sewers, primary clarifiers could also allow (bio)degradation of CECs. In addition, the primary clarifier under study has a higher HRT

(3.72 h) than usual, which could explain the high observed CEC (bio)degradation. In fact, most of the compounds with the highest (bio)degradation in the primary clarifier also showed high (bio)degradation potential in the activated sludge (Fig. 4). It should be noted that the sum of the percentages removed by (bio)degradation and removed with sludge (Fig. 3) is not equivalent to the percentage removal from the aqueous phase calculated previously (Fig. 2). This is explained by the fact that the input reference mass load used in the mass balance calculations includes not only CEC loads in the aqueous phase, but also in the particulate phase. The CEC concentrations in the suspended particulate phase of primary inlet load were assumed to be equal to those in primary sludge, making the assumption that equilibrium partitioning of CECs between the particulate and aqueous phases takes place before the primary clarifier.

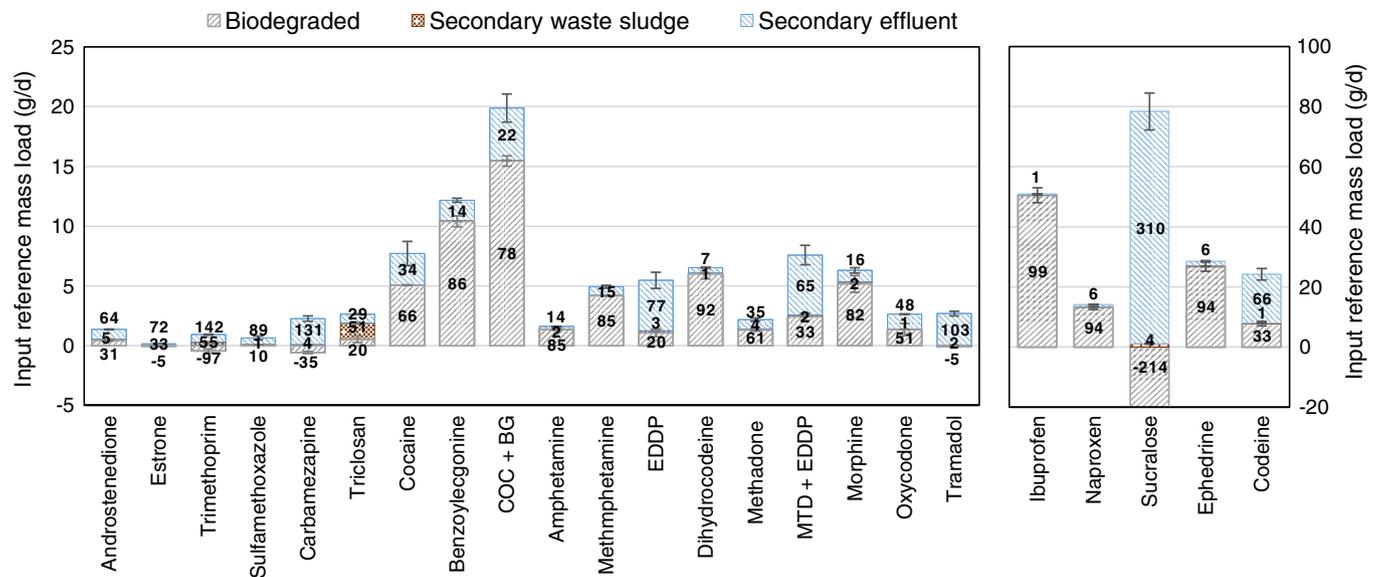


Fig. 4. Input reference mass loads of target CECs into activated sludge assigned into three main fate pathways: (bio)degraded, discharged with waste activated sludge and discharged with the secondary effluent with % of each pathway in columns (COC: cocaine, BG: benzoyllecgonine, MTD: methadone). For ibuprofen and amphetamine, the concentration in the effluent was <LOQ, so the LOQ was used for calculations. For caffeine (not shown due to the high mass loads): 92% biodegraded and 8% in the effluent.

3.4. Removals by activated sludge

3.4.1. Aqueous phase

The estimated removals of CECs (%) during the activated sludge treatment step are illustrated in Fig. 2. In general, for the majority of the compounds, most of the removal takes place in the activated sludge treatment stage when compared to other treatment steps. Among the target compounds, the removals varied from >80% for six target CECs, namely ibuprofen, naproxen, amphetamine, ephedrine, dihydrocodeine and caffeine to negative removals for carbamazepine, tramadol, estrone, sucralose and trimethoprim. The variable data on CEC removal by activated sludge treatment reported in the literature is summarized in the supplementary material (Table S2).

3.4.2. Secondary sludge

The measured concentrations of CECs in the solids of secondary sludge and estimates of the $\log K_d$ values for secondary sludge are summarized in Table 5. Triclosan followed by caffeine and trimethoprim were shown to have the highest average concentrations in secondary sludge. Similar to the analysis of the primary sludge, triclosan had the highest estimated $\log K_d$ value (i.e. 4.49) for secondary sludge. Caffeine and sucralose ($\log K_{ow} - 0.07$ and -1 , respectively), on the other hand, had high concentrations in secondary waste sludge owing to their high loads to the biological treatment (Table 5). The differences in the estimated $\log K_d$ values between primary and secondary waste sludge for each of the CECs can be explained by variable sludge composition (Ternes et al., 2004).

Fig. 4 displays the CEC input reference load to the activated sludge unit assigned to different fate pathways. The reference mass loads were calculated based on the assumption that the CEC concentrations in the suspended solids contained in the output of the primary and secondary clarifiers were the same as those measured in the primary and secondary sludge, respectively. This involves making the assumption that the CEC particulate concentration is uniform throughout the primary and secondary clarifiers. Discharge with the secondary waste sludge was found to be the predominant removal mechanism for trimethoprim, triclosan and estrone, with 55%, 51% and 33% of their input reference load, respectively ending up in the secondary waste sludge. For all the remaining compounds, a low fraction (i.e. <5%) of the input reference load was discharged with secondary waste sludge (Fig. 4), due to their hydrophilic nature ($\log K_{ow}$, Table 1). This is in good agreement with previous research for a number of the target compounds whose fate in activated sludge units has been previously investigated through mass balance (i.e. ibuprofen, naproxen, carbamazepine, sulfamethoxazole and caffeine) (Gao et al., 2012; Joss et al., 2005; Petrie et al., 2014).

(Bio)degradation was the predominant removal mechanism for 17 out of the 22 target CECs. More than 90% of the incoming load was removed by (bio)degradation in the case of ibuprofen, naproxen, ephedrine, dihydrocodeine, as well as caffeine (not shown in Fig. 4). Substantial (bio)degradation of ibuprofen, naproxen and caffeine was also reported by other authors (Carballa et al., 2007; Gao et al., 2012; Joss et al., 2005). For the majority of the compounds studied, the mass fraction removed by (bio)degradation (Fig. 4) and the data on removals from the aqueous phase (Fig. 2) were comparable. This indicates that (bio)degradation is responsible for the majority of the removal of the studied CECs from the aqueous phase in the activated sludge unit, while removal by adsorption is of insignificant importance. By contrast, the fraction of biodegraded material was negative for several CECs, such as carbamazepine, sucralose, trimethoprim and tramadol, which indicated that the mass loads in the secondary output were higher than the incoming loads, consistent with their negative removal from the aqueous phase (Fig. 2). The use of the fractionated approach accounting for RTD of the treatment units limited the bias associated with the sampling strategy and removal calculations but some negative removals were still observed. These negative removals are, therefore, likely due to the presence of conjugated forms of the

compound that transform into the parent compound during treatment, as well as desorption from particulate matter (Jelic et al., 2011; Ternes and Joss, 2006). For carbamazepine and trimethoprim, the absence of removal by biodegradation obtained in this study is in agreement with previous studies (Gao et al., 2012; Joss et al., 2005; Li and Zhang, 2010; Petrie et al., 2014).

3.5. Removals by RBCs

The WWTP was upgraded to include RBCs in order to meet regulations with regards to the ammonia nitrogen levels in the effluent (i.e. maximum concentration of 3.4 mg/L). Although the ammonia nitrogen in the secondary effluent was reported to be <1 mg/L during the summer, indicating full nitrification by the activated sludge process, levels as high as 6.6 mg/L were observed over several days during the winter. The RBCs removed ammonia nitrogen and achieved effluent levels below 2.41 mg/L throughout the year. In spite of their capability to remove ammonia nitrogen, the RBCs were generally ineffective at removing most of the target CECs (i.e. <30%), except for methamphetamine, codeine and dihydrocodeine, as shown in Fig. 2. Additionally, the CECs whose removal calculation had to be based on LOQ or LOD due to the fact that they were below these limits (marked with two stars) could also have high removals. Among the target CECs, low average removals of <15% were observed for 11 of the 26 target CECs. Negative removals at this treatment stage were observed for sucralose and triclosan, indicating the possible de-conjugation of their conjugates during tertiary treatment, as in the activated sludge treatment stage or possible sampling bias. The fate of a limited number of the target compounds (i.e. trimethoprim, sulfamethoxazole, triclosan, ibuprofen, carbamazepine and caffeine) has been investigated before in RBCs, and their obtained removals were higher than in the present study (Batt et al., 2007; Kanda et al., 2003; Thompson et al., 2005; Vasiliadou et al., 2014). This can be explained by the fact that the RBCs are used as tertiary treatment in this study, with a low HRT (i.e. 0.8 h) and little organic material available for biofilm growth, while in the previous studies, RBCs were used for secondary treatment as an alternative to activated sludge treatment. The contribution of the particulate CEC load to the total load in the RBCs and the sand filter is deemed insignificant, since the TSS load entering these units is low (<20 mg/L). Hence, a detailed mass balance was not performed for these units.

3.6. Removals by sand filtration

Similar to the RBCs, the average removal of CECs by sand filtration and chlorination was based on the incoming load in the aqueous phase only, due to the low TSS load. The removal was >50% for only 4 CECs (i.e. trimethoprim, sulfamethoxazole, morphine and CBZ-EP). The incoming loads of trimethoprim and sulfamethoxazole were reduced by more than 69% and 58%, respectively by sand filtration. Data from previous studies reported considerable removal (over 60%) of trimethoprim in sand filters (Göbel et al., 2007; Nakada et al., 2007; Sui et al., 2010), but lower removals (i.e. 20–30%) were reported for sulfamethoxazole (Gao et al., 2012; Göbel et al., 2007; Nakada et al., 2007). With the exception of the four compounds listed above, sand filtration and chlorination were inefficient at removing most of the other target CECs, as <30% of their incoming load was removed (Fig. 2). The observed removal for triclosan was similar to that observed by Nakada et al. (2007) (i.e. 29%). Negative removals (or no removal) were obtained for estrone, sucralose, carbamazepine and CBZ-DiOH during the sand filtration and chlorination steps. For estrone and carbamazepine, the negative removals are in agreement with previous studies (Gao et al., 2012; Nakada et al., 2007; Sui et al., 2010), indicating possible desorption of these compounds during sand filtration. Removal from the aqueous phase in sand filters is attributed to adsorption to solid particles that are retained by the sand filter. However, previous studies also indicated that (bio)degradation may contribute to CEC removal due to the

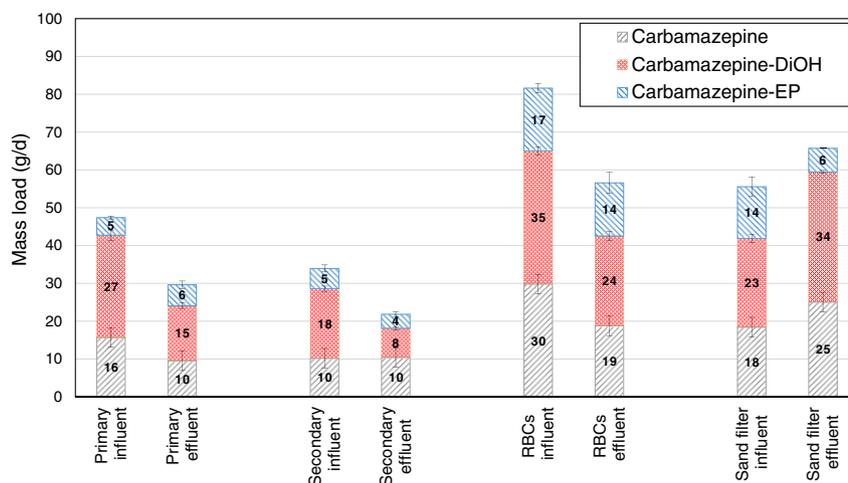


Fig. 5. Mass loads of carbamazepine and its two investigated metabolites (CMZ-DiOH & CMZ-EP) in both the input (reference load) and the output of the primary clarifier, secondary treatment, RBCs and sand filter. The numeric values of the load are presented in the columns.

formation of biofilm on sand particles (Göbel et al., 2007). In the WWTP investigated, chlorine was added right before sand filtration, which makes the growth of a biofilm unlikely.

3.7. Metabolites

The combined loads of parent compounds and their metabolite(s) were considered for some target CECs when calculating removal efficiencies for each treatment stage (Fig. 5) to account for possible inter-transformations. Cocaine and its major metabolite had comparable removals in all treatment units. Although EDDP (methadone's major metabolite) alone exhibited negative removal of -17% in the primary clarifier, the removal efficiency based on combined concentrations of EDDP and its parent compound methadone was above zero, indicating that some of the methadone was possibly converted into EDDP. Similarly, removals based on combined loads were obtained for carbamazepine and its two investigated metabolites, CBZ-DiOH and CBZ-EP. A limited number of studies have investigated the removals of carbamazepine and its major metabolites in WWTPs (Hummel et al., 2006; Leclercq et al., 2009; Miao et al., 2005). Only the study by Miao et al. (2005) examined the concentrations of carbamazepine and its metabolites at each treatment step of the WWTP and observed a decline in the concentration of carbamazepine and its two metabolites in the primary clarifier and an increase in carbamazepine and CBZ-DiOH concentrations by activated sludge. In the present study, the calculation was further refined by considering the input and output mass loadings of carbamazepine and its two major metabolites in each treatment unit taking the RTD into account, as shown in Fig. 5. It should be noted that in the present study, due to use of the fractionated approach, the input reference load a treatment step is not equivalent to the output load of the previous treatment step (Fig. 5).

A decrease was observed in the loads of carbamazepine and CBZ-DiOH during primary clarification ($p < 0.05$), while the variations in the loads of CBZ-EP across the primary clarifier were not statistically different ($p = 0.26$). Similarly, activated sludge was not shown to cause a decrease in the loads of carbamazepine and CBZ-EP ($p = 0.57$ and 0.07 , respectively), but it decreased the loads of CBZ-DiOH ($p < 0.05$) and, thus, the combined load of carbamazepine and its metabolites. This is possibly explained by partial transformation of CBZ-DiOH back into carbamazepine during both primary clarification and activated sludge causing the persistence of carbamazepine by compensating for the (bio)degraded load. Tertiary treatment in the form of RBCs diminished the load of carbamazepine and CBZ-DiOH significantly ($p < 0.05$), but not that of CBZ-EP ($p = 0.25$). Sand filtration and chlorination, on the other hand, resulted in a decrease in the average load of only CBZ-EP,

accompanied with a load increase of both carbamazepine and CBZ-DiOH ($p < 0.05$ for all the compounds). The combined load of carbamazepine and the two metabolites was also increased ($p = 0.03$). This might suggest the transformation of CBZ-EP to both CBZ-DiOH and carbamazepine during the sand filtration step. The two metabolites of carbamazepine were not quantified in sludge samples in the present study. However, Miao et al. (2005) concluded that these compounds are present at low concentrations in biosolids (<8 ng/g).

4. Conclusions

Removal data for the aqueous phase showed that most CECs were poorly removed (i.e. $<40\%$) in the primary clarifier. The greatest removals typically occurred in the activated sludge, while removals were $<50\%$ in the RBCs and ranged from no removal to 70% removal during sand filtration. The mass balances, which looked at dissolved as well as adsorbed CECs provided further insight into the predominant removal mechanisms for CECs during primary and secondary treatment. It showed that not only sorption to the primary sludge, but also (bio)degradation contribute to the removal of some of the target CECs in the primary clarifier. In activated sludge, (bio)degradation was found to be the predominant removal mechanism, with sorption accounting for $<5\%$ for most CECs. The estimated $\log K_d$ values (1.40 to 3.68 in primary sludge and 1.36 to 4.49 in activated sludge) also indicated that most CECs are not significantly removed by partitioning onto sludge, with the exception of triclosan. Accounting for the levels of metabolites of the selected CECs (i.e. carbamazepine, methadone and cocaine) explained some of the negative removals that were observed. The current study expands the understanding of the removal pathways of CECs at different treatment steps, for which limited data is available in the literature. It also takes into account the hydraulics following a novel approach applied for the first time to primary clarification, RBCs and sand filtration to provide reliable data of the CEC removals. Reliable data on the fate of CECs are valuable for the calibration of mathematical fate models that can be used to optimize treatment technologies and reduce discharges of CECs into the aquatic environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.08.073>.

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