Estimating removals of contaminants of emerging concern from wastewater treatment plants: The critical role of wastewater hydrodynamics

Zeina Baalbaki [a], Tamanna Sultana [b], Chris Metcalfe [b], Viviane Yargeau [a,*]

[a] Department of Chemical Engineering, McGill University, 3610 University St., Montreal, QC, H3A 0C5, Canada

[b] Water Quality Centre, Trent University, 1600 West Bank Drive Peterborough, ON, K9J 7B8, Canada

*Corresponding author. Tel.:+1 514 398 2273; fax: +1 514 398 6678; E-mail address: viviane.yargeau@mcgill.ca (V. Yargeau).

Abstract

Accurate data are needed to evaluate the capacity of wastewater treatments plants (WWTPs) to remove contaminants of emerging concern (CECs). The variability of CEC removals reported in the literature has raised questions about the methods used to estimate removals. In this study, we used the recently proposed "fractionated approach" to account for the influence of hydrodynamics in WWTPs and applied this method for estimating removals of 23 target CECs. Data on the conductivity and temperature of wastewater at two WWTPs were used to determine the hydraulic model that best described the flow regime of treatment units. Composite samples (24-h) were collected at different stages of treatment over successive days. The concentrations of the target compounds in wastewater were determined by liquid chromatography with mass spectrometry. Different hydraulic models were necessary to define the hydrodynamics at the two WWTPs, resulting in different load fractions to be used in calculations of removals. For WWTP A, that has a primary clarifier, all target CECs, except triclosan, were poorly removed during this step at efficiencies <30%. On the other hand, the activated sludge treatment unit at both WWTPs removed most target CECs at >70%. This study expanded the application of the fractionated approach to compare the hydraulics of two treatment trains of different configurations, including primary and secondary treatment. It demonstrated the sensitivity of the method to account for variations between the different treatment units. Reliable removals of an extended list of CECs in primary and secondary treatment were also provided in this study.

Keywords: Micropollutants, Fractionated approach, Hydraulic model, Removal efficiency, Activated sludge, Primary clarification

1. Introduction:

Contaminants of emerging concern (CECs), which include pharmaceuticals and personal care products (PPCPs), drugs of abuse and steroid hormones have been detected globally in the aquatic environment. The sources of these CECs are industrial and domestic wastewater, including hospital effluents (Luo et al., 2014). There is evidence that discharges of CECs into surface waters may have adverse effects on aquatic organisms (Gay et al., 2016; Kidd et al., 2007; Purdom et al., 1994). In addition, CECs may contaminate sources of drinking water (Kleywegt et al., 2011; Metcalfe et al., 2014; Rodayan et al., 2015), although it is not clear whether the low concentrations typically detected in drinking water are a threat to human health (Schwab et al., 2005; Webb et al., 2003).

Municipal wastewater treatment plants (WWTPs) are not designed to remove micropollutants, and many CECs are not removed effectively during treatment (Luo et al., 2014; Onesios et al., 2009; Verlicchi et al., 2012). Most WWTPs in North America treat sewage using secondary treatment technologies, after which wastewater is discharged to the aquatic environment, sometimes without a disinfection step prior to discharge. Removals of CECs reported in literature vary significantly for given compounds, even for WWTPs with comparable treatment processes, which raises questions about the reliability of the data. Ort et al. (2010) reviewed the available data on removals of CECs in WWTPs and concluded that the sampling schemes typically used are inadequate for accurately estimating these removals. "Negative" removals, or concentrations of CECs in final treated effluents that are higher than concentrations in untreated influent are frequently observed, especially for recalcitrant compounds, such as carbamazepine (Yang et al., 2011; Zorita et al., 2009). Some of these negative removals have been attributed to de-conjugation of Phase II metabolites during biological treatment, resulting in generation of the unconjugated parent compound (Ternes, 1998), as well as to desorption from sludge and particulate matter during treatment (Clara et al., 2005; Ternes, 1998).

The negative and variable removals of CECs estimated for WWTPs may be due to sampling schemes that do not account for the fact that the daily incoming load is distributed over multiple days in the effluent due to the residence time distribution (RTD) of wastewater. Majewsky et al. (2011) recommended an approach to sampling and removal calculations within WWTPs, described as the "the fractionated approach", that compares the mass load of the output stream with a reference mass load of the input stream that takes into account the RTD. This approach requires a more intensive sampling strategy over several days, together with hydraulic modelling of the WWTP, utilizing conductivity as a tracer of the RTD (Majewsky et al., 2011). Ahnert et al. (2010) proposed that also temperature could be used as a tracer to determine the RTD within WWTPs. A subsequent study by Majewsky et al. (2013) using the fractionated approach generated superior estimates of the removals of five pharmaceuticals and four pesticides in a WWTP with activated sludge treatment.

Our subsequent application of the fractionation approach for the removal of drugs of abuse in an activated sludge unit of a WWTP demonstrated the value of this method over conventional approaches for removal calculations (Rodayan et al., 2014a). As a further advance over our previous study, the goal of the present study was to evaluate the capacity of the fractionated approach to estimate CEC removals from the aqueous phase in two WWTPs with different treatment configurations and RTDs. The treatment processes monitored at the two WWTPs were the activated sludge units, as well as a primary clarifier at one of the WWTPs. We applied this method to obtain reliable estimates of removals of 23 target CECs that have been widely detected in domestic wastewater, including several drugs of abuse and pharmaceuticals, two steroid hormones (i.e. estrone, androstenedione), a stimulant (i.e. caffeine), an artificial sweetener (i.e. sucralose) and an antibacterial compound found in personal care products (i.e. triclosan). Wastewater samples were analyzed for CECs using liquid chromatography with high-resolution mass spectrometry (LC-HRMS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2. Materials and Methods:

2.1 CECs, chemicals and reagents

Several of the target compounds in this project were selected according to the criteria identified by Dickenson et al. (2011) in a study that illustrated the value of monitoring a small number of CECs in wastewater treatment facilities in the U.S. Sucralose has been widely detected in drinking water systems in the USA and because of its persistence and ubiquitous presence at high concentrations, has been proposed as a tracer of wastewater contamination (Mawhinney et al., 2011). Our previous studies have shown that drugs of abuse, including cocaine and its major metabolite (i.e. benzoylecgonine), amphetamines and opioid drugs are widely distributed in wastewater in Canada (Metcalfe et al., 2010; Rodayan et al., 2015). For methadone and cocaine, the fate of the metabolites (i.e. EEDP and benzoylecgonine, respectively) was also taken into account. The target CECs selected for monitoring in the two WWTPs are listed in Table 1.

Table 1 Target compounds and their physicochemical properties, internal standards, LODs & LOQs and class for extraction and analysis.

Type and Subtype	Compound	CAS No	Molecular Formula	Molecular Weight	pKa[a,b]	Log Kow[a,b]	Class	Internal Standard	LOD, LOQ (ng/L)
Antibiotics	Trimethoprim	738-70-5	$C_{10}H_{11}N_3O_3S$	290.3	6.8	0.91	B	Trimethoprim-13C3	7,22
	Sulfamethoxazole	723-46-6	$C_{14}H_{18}N_4O_3$	253.3	5.7	0.89	B	Sulfamethoxazole-13C6	4,14
Anti-inflammatory/analgesics	Acetaminophen	103-90-2	$C_8H_9NO_2$	151.2	9.0	0.46	B	Acetaminophen -D3	11,35
	Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	8.2	1.14[a]	A	Codeine-D3	8,28
	Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.2	4.9	3.97	B	Ibuprofen-13C3	6,21
	Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.3	4.2	3.18	B	naproxen-13c1-d3	4,14
Antiepileptic	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	263.3	3.2	2.45	B	Carbamazepine-D10	2,5
Antilipidemic (lipid regulator)	Gemfibrozil	25812-30-0	$C_{15}H_{22}O_3$	250.3	4.7	4.77	B	Gemfibrozil-D6	5,16
Dopamine uptake inhibitor (cocaine and its metabolite and amphetamine-type stimulants)	Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.4	8.6	2.3	A	Cocaine-D3	38,126
	Benzoylecgonine (cocaine metabolite)	519-09-5	$C_{16}H_{19}NO_4$	289.3	NA	-1.32	A	Benzoylecgonine-D3	6,21
	Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1	1.76	A	Amphetamine-D5	6,28
	Ephedrine	299-42-3	$C_{10}H_{15}NO$	165.2	9.7	1.13	A	Ephedrine-D3	11,37
	MDA	4764-17-4	$C_{10}H_{13}NO_2$	179.2	9.7	1.64	A	MDA-D5	12,41
Opioids	Dihydrocodeine	125-28-0	$C_{18}H_{23}NO_3$	301.4	8.8	1.49[c]	A	Dihydrocodeine-D6	12,41
	Methadone	76-99-3	$C_{21}H_{27}NO$	309.4	8.9	3.93	A	Methadone-D9	6,20
	EDDP (methadone's metabolite)	30223-73-5	$C_{20}H_{23}N$	291.4	9.6	4.94	A	EDDP-D3	8,26
	Morphine	57-27-2	$C_{17}H_{19}NO_3$	285.3	9.9	0.96	A	Morphine-D3	7,25
	Tramadol	27203-92-5	$C_{16}H_{25}NO_2$	263.4	9.4	2.63	A	Tramadol-13C-D3	11,36
Disinfectants/antiseptics	Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$	289.6	7.9	4.76	B	Triclosan-13C12	6,19
Steroid hormones	Androstenedione	63-05-8	$C_{19}H_{26}O_2$	286.4	NA[e]	2.75	B	Androstene-3,17-dione-2,3,4-13C3	2,5
	Estrone	53-16-7	$C_{18}H_{22}O_2$	270.4	10.3	3.13	B	Estrone-3,4-13C2	2,5
Stimulant	Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	14.0	-0.07	B	Caffeine-13C3	4,14
Artificial sweetener	Sucralose	56038−13−2	$C_{12}H_{19}Cl_3O_8$	397.6	NA[e]	-1[d]	B	Sucralose-D6	7,22

[a] The PubChem Project National Center for Biotechnology Information (2004)

[b] Ratola et al. (2012)

[c] ChemIDPLUS U.S. National Library of Medicine (2005)

[d] Subedi and Kannan (2014a)

[e] NA: not available

The target CECs are grouped into two classes according to their chemical properties and the methods of extraction and analysis. Class A compounds are drugs of abuse and their metabolites, which are all weak bases, and Class B compounds are all other analytes, which are either weak acids, phenolics or neutral compounds. Class A compounds and their deuterated stable isotope surrogates were purchased from Cerilliant (Round Rock, TX, USA). PPCPs and steroids from the Class B group were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada) and their stable isotope surrogates were purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada), except for androstenedione, for which a deuterated surrogate was provided by Cerilliant. All standards and stock solutions were stored in amber glass vials at -20°C. Methanol, acetonitrile, acetone, formic acid, ammonium hydroxide and sulphuric acid were purchased from Fisher Scientific (Ottawa, ON, Canada). Ultrapure water was generated using a Milli-Q water purification system from Millipore (Bedford, MA, USA).

2.2 WWTPs characteristics

Two WWTPs that both utilize activated sludge (secondary) treatment, but with different treatment trains were selected for this study. WWTP A, which has a design capacity of 68,200 m^3/d, receives wastewater that is primarily domestic at an average flow of 43,146 m^3/d. This WWTP serves a population of 78,700. The treatment train includes aerated grit tanks, primary clarifiers, and two activated sludge trains, each of which contains two three-pass integrated-film activated sludge (IFAS) bioreactors and two secondary clarifiers. WWTP A employs UV for disinfection, after which the effluent is discharged to a nearby river. Table S1 summarizes the main characteristics of these WWTPs, including the solids retention time (SRT), hydraulic retention time (HRT), and the temperature conditions during sampling.

WWTP B, which has a design capacity of 105,000 m^3/d, receives an average wastewater flow of 50,200 m^3/d and serves a population of 54,000. The influent flow is composed of 26% industrial wastewater, with the remaining amount being domestic wastewater. Unlike WWTP A, this plant contains no primary clarifier and the raw sewage undergoes preliminary treatment by screens, followed by activated sludge treatment. The activated sludge treatment comprises four step-feed aeration tanks followed by four secondary clarifiers, and the effluent is discharged into a nearby river without disinfection.

2.3 Hydraulic model

2.3.1 Electro-conductivity and temperature measurements

The electrical conductivity and temperature of the wastewater were monitored with HOBO conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada), which were deployed to take high-frequency measurements (every five minutes). At WWTP A, the probes were deployed at the exit of the grit removal, primary clarifier, aeration tank and secondary clarifier points over two weeks prior to the sampling campaign. For WWTP B, the probes were deployed at the exit of screens, aeration tank and secondary clarifier points in the treatment train over four days, as an earlier calibration of the plant was available. A universal optic-USB base station and Onset HOBOware Pro Version 3.2.2 software (Hoskin Scientific, St-Laurent, QC, Canada) were used for transferring and reading the data, respectively. Conductivity was utilized as a tracer for the investigation of the residence time distribution in WWTP A. However, due to several outliers in the conductivity data for WWTP B, temperature was used as the tracer for residence time in that plant.

2.3.2 Hydraulic model calibration and load fractions

WEST modelling and simulation software (Mike Powered by DHI, Hørsholm, Denmark) was used to determine the hydraulic model that best describes the mixing regimes of the treatment units of the two WWTPs, as described by Majewsky et al. (2011). Briefly, the hydraulic model was built using the actual flow conditions and volumes obtained from the WWTP operators, in addition to the actual conductivity (i.e. for WWTP A) and temperature (i.e. for WWTP B) profiles as input. Calibration of each treatment unit was performed by varying the number of aeration tanks in series and the feed layer of the clarifiers. The best-fit hydraulic model was defined to be the model that minimizes the root mean square error (RMSE) between measured and simulated output profiles. Model simulations were run using the best-fit hydraulic model with a 24-h step input of the soluble inert component available in WEST, along with the actual flow rates to represent a hypothetical CEC affected by hydraulics only (i.e. inert). The output of the model was used to determine the load fractions, represented as f_i, that best describe the output CEC load exiting the treatment unit on a given day (i.e. day i) as fractions of the incoming loads entering on each of the previous days assuming there is no biodegradation, as described by Majewsky et al. (2011).

2.4 Wastewater sampling

Since obtaining an hourly resolution of the CEC removals is not crucial for the present study, 24-h composite samples were collected in both the influent and the effluent simultaneously over multiple days. Collecting effluent samples over multiple consecutive days eliminates the need for shifting the effluent sampling by an optimum offset after the beginning of the influent sampling. The hydraulic models selected for the two WWTPs under study indicated that in order to measure most of the influent load material that exits on a single day in the effluent, sampling periods of three and four consecutive days are required at WWTP A & WWTP B, respectively. The explanation for this approach will be described further in the results section. Samples (24-h composite) were collected from the WWTP A at the exit of the grit removal, primary clarifier, aeration tank and secondary clarifier points in the treatment train on September 10-12, 2013. For WWTP B, samples were collected at the exit of screens, aeration tank and secondary clarifier points in the treatment train on September 23-27, 2013. Dry weather conditions prevailed during both sampling periods. The 24-h composite samples were collected using ISCO 6712 samplers (Avensys, St-Laurent, QC, Canada) equipped with 24 collection bottles and packed with ice. These samplers were programmed to collect equal-volume time-proportional samples every 15 minutes. Samples were then placed in 1-L amber HDPE bottles (Fisher Scientific, Ottawa, ON, Canada) and stored at -20°C until extracted.

2.5 Sample preparation

Prior to extraction, wastewater samples were filtered using 1-μm glass-fiber filter (Fisher Scientific, Ottawa, ON, Canada). All compounds were extracted using solid phase extraction (SPE) techniques using Oasis cartridges purchased from Waters (Milford, MA, USA). Sample volumes extracted were 100 mL for untreated wastewater influent and 200 mL for all other wastewater samples. The extraction method for Class A compounds was described by Metcalfe et al. (2010) and modified by Yargeau et al. (2014). For Class B compounds, the extraction method was previously described by Li et al. (2010) and Metcalfe et al. (2014). Table S2 summarizes the methods and the instruments used for extracting the two classes of compounds. Prior to extraction, samples were spiked with 100 μL of 500 ng/mL of the stable isotope internal standards listed in Table 1. Recoveries of Class A compounds by the SPE procedure were in the range of 55% to 110%, while for Class B compounds, SPE recoveries ranged from 71% to 130%, as reported by Li et al. (2010).

2.6 Analysis

Analysis of the Class A compounds was conducted by LC-HRMS using an Accela LC system coupled to a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was achieved using the method described by (Rodayan et al., 2014b). Ionization was operated using heated electrospray ionization source (HESI) in positive mode using the method described in (Rodayan et al., 2014a). Acquisition was performed in full scan mode (50–400 m/z) at high resolution (RFWHM = 41,000). The ions of interest were extracted using an m/z window of ±0.01.

Class B compounds were analyzed by LC-MS/MS using an Agilent 1100 HPLC (Mississauga, ON, Canada) coupled to a Q-Trap 5500 instrument (AB Sciex, Concord, ON, Canada) with a turbo spray ionization source. The chromatographic method was previously described by Metcalfe et al. (2014). Acquisition was performed using the precursor and product ion transitions for multiple reaction monitoring (MRM) of the target analytes and their corresponding deuterated surrogates. The MRM transitions for the target compounds are also listed in the same study (Metcalfe et al., 2014). Negative ionization mode was used for the analysis of sucralose, ibuprofen, gemfibrozil, estrone, naproxen, and triclosan, whereas, positive ionization mode was used for carbamazepine, sulfamethoxazole, androstenedione, acetaminophen and caffeine.

A linear calibration curve of seven concentration points from 5 ng/mL to 150 ng/mL and from 0.78 ng/mL to 200 ng/mL was used for quantification for compounds of class A and B, respectively. To quantify target compounds with concentrations beyond these limits, dilutions were performed on the sample prior to analysis until the concentration of the target compound was within the concentrations range of the calibration curve. The Limits of Detection (LODs) were determined based upon analysis of serial dilutions of standards of each target analyte. The limits of quantification (LOQs) were defined as 3.3xLODs. The LODs and LOQs for each target compound are listed in Table 1.

2.7 Removal calculations

The measured concentrations of CECs at the influent of each treatment unit, along with the load fractions were used to calculate a reference influent load, using Eq. 1. This reference load was compared to the effluent load of the treatment unit that is based on one day in order to obtain more reliable removal calculations, using the so-called fractionated approach (Eq. 2). Considering that samples from the effluent of each treatment unit were collected on multiple consecutive days, it was possible to calculate CEC removal values using different effluent loads as the basis for calculations. This is opposed to the commonly used day-by-day approach that calculates the removal by comparing input and output loads from the same day. In the present study, concentrations of CECs used for calculations of loads were based on the dissolved portion only of the CECs in the water phase.

$$L_{ref} = \sum_{i=1}^{i=n} f_i * L_{inf,\,i} \tag{1}$$

$$R = \frac{L_{ref} - L_{eff}}{L_{ref}} * 100\% \tag{2}$$

- L_{ref}: Reference mass load of the contaminant in the aqueous phase of the input stream (mg/d) based on several days of sampling (from the ith to the nth). Where "n" is the number of days of sampling.
- $L_{inf,\,i}$: Mass load of the contaminant in the input stream on the ith day of sampling or "day i" (mg/d)
- f_i : Load fraction or fraction of the incoming load on the ith day of sampling (day i) that is contained in the effluent load on the nth day of sampling (day n)
- L_{eff} : Mass load of the contaminant in the effluent on nth day of sampling (mg/d)
- R: Removal rate of contaminant based on the fractionated approach

3. Results and Discussions

3.1 Hydraulic model

3.1.1 Calibration

Conductivity and temperature were considered to be the conservative tracers defined within the WEST software. However, in reality, the measured conductivity of wastewater exhibited a reduction following the aeration tanks and a slight increase following primary and secondary clarification, as shown in Figure 1. As a result, it was necessary to rescale the predicted effluent conductivity to account for the actual decline or increase in this parameter observed within the WWTPs. This correction was necessary prior to evaluating the fit of the models, as it decreased the bias of the RMSE between predicted and measured conductivity due to the natural changes of the measured conductivity. The correction was based on the calculated percentage change of the average measured conductivity between the influent and the effluent of each treatment unit. The correction was assumed to be constant throughout the monitoring period. The effluent conductivity predicted by the model was multiplied by the observed percentage change to obtain a rescaled predicted conductivity. The best-fit models for WWTP A and WWTP B were determined based on the corrected predicted conductivity. The natural change of temperature through treatment units, on the other hand, was not significant and did not require correction.

3.1.2 Model selection

For WWTP A, the primary and secondary clarifiers were each modelled by a single clarifier, with a volume equivalent to the actual total volume. The 10-layered model of the clarifier assumes that the influent of the clarifier enters in the mid layer. Adjusting the feed layer to one layer below the mid layer and one layer above it for primary and secondary clarifiers respectively was shown to improve the fit of the hydraulic models. The model produced predictions of effluent conductivity that are in agreement with the observed conductivity, as illustrated in Figure 1(a) (dashed curves). With regards to the aeration tanks, increasing the number of aeration tanks in series to five tanks with the same actual total volume proved to improve the fit of the hydraulic model. Generally speaking, a model describing a WWTP with a large number of tanks in series exhibits a behaviour that approaches a “plug” flow (Levenspiel, 1972). WWTP A employs three-pass aeration tanks with a feed that enters the first pass, which is well represented by the semi-plug flow behaviour.

For WWTP B, the best-fit hydraulic model that minimizes the RMSE between predicted and observed temperature for the aeration tanks was observed to be a single aeration tank. Unlike aeration in WWTP A, step-feed operation is employed at the WWTP B. That is, the feed is entering at different points of the multi-pass aeration tanks, resulting in more homogeneous content in the aeration tank and making the system behave as a mixed tank. The effluent temperature profiles in Figure 1(b) show a wider residence time distribution (i.e. more broadening of the peaks) than profiles in Figure 1(a), suggesting that substantial mixing occurs in the activated sludge unit of WWTP B when compared to that of WWTP A. Similarly to WWTP A, the secondary clarifier was represented by a single clarifier with the actual total volume.

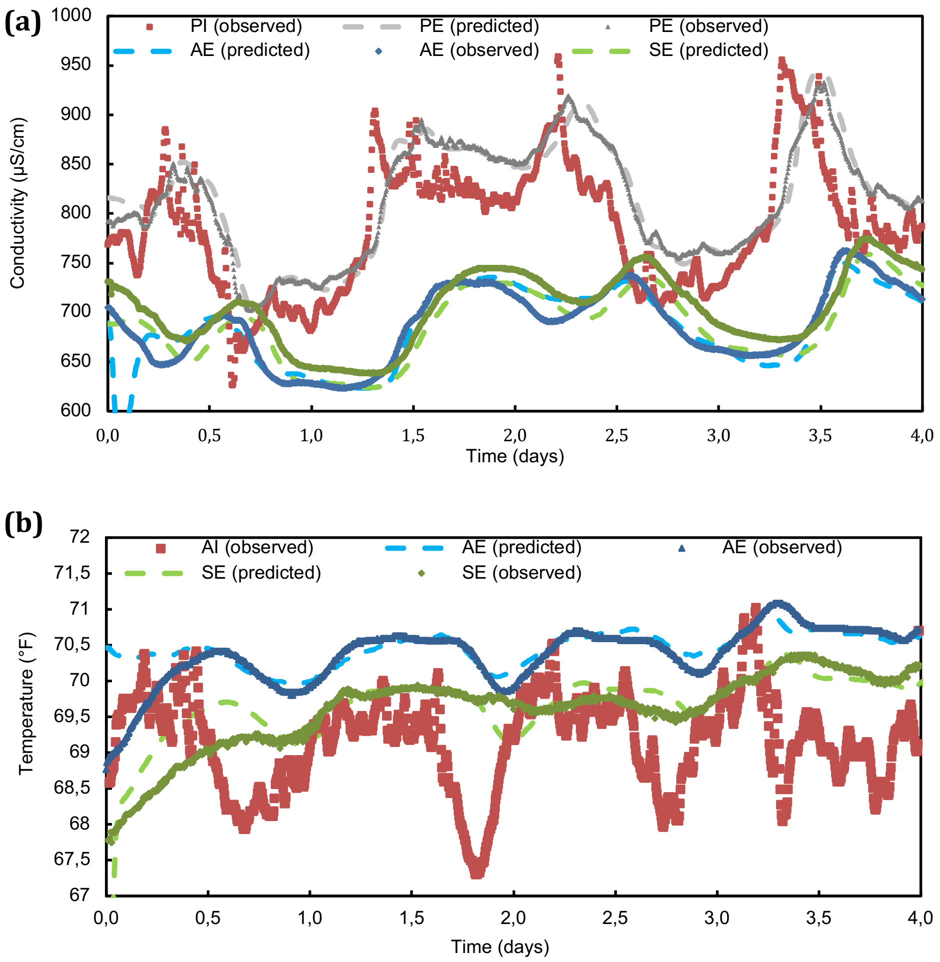

Figure 1. Observed (recorded) and predicted (simulated) conductivity trends in (a) WWTP A and (b) WWTP B at different locations (PI: influent to primary clarification, PE: effluent of primary clarification, AE: effluent of aeration, SE: effluent of secondary clarification)

3.1.3 Load fractions

Table 2 summarizes the load fractions determined for each treatment unit where, as previously explained, "f_i" represents the load fractions that correspond tothe 4th, 3rd or 2nd sampling day, depending on which day of the output was taken as a basis. It is important to note that the load fractions based on the effluent on a single day do not necessarily add up to 100%, since each fraction is normalized against the influent load of a different sampling day (Eq. 1). As shown in Table 2, the effluent of the activated sludge unit of WWTP A on the last day of sampling (Day 3) contained influent material from the same and two previous days. This explains the need for three days of sampling at WWTP A to characterize the reference load that corresponds to the effluent load on the last day. The load fractions for the activated sludge unit of WWTP B were more widely distributed than those from WWTP A, as shown in Table 2, so four days of sampling were required. Figure 2 provides extra illustration of the response loads obtained from model simulations of the activated sludge treatment trains and used to calculate load fractions. Comparing the activated sludge unit to the primary clarifier, the load fractions obtained from the activated sludge are distributed over a larger number of days than the primary clarifier (i.e. three days), but with low contribution from the first sampling day of sampling (i.e. load fraction of 0.3%). This is linked to the higher HRT and the greater extent of mixing that occurs in the activated sludge unit as compared to the primary clarifier. The load fractions were also evaluated for aeration tanks and secondary clarifier, separately. The secondary clarifier of WWTP A showed more distribution of load fractions over the days than that of WWTP B, while the opposite was observed for aeration tanks.

Table 2 Load fractions of influent loads composing the effluent on a given day for the different treatment units in WWTP A and WWTP B. “fi” denotes the fraction of influent load entering on day i that is contained in effluent of day 4, 3 or 2 (second column)

WWTP	Based on	Load fractions of primary clarifier				Load fractions of aeration tanks				Load fractions of secondary clarifier				Load fractions of activated sludge			
		f1	f2	f3	f4	f1	f2	f3	f4	f1	f2	f3	f4	f1	f2	f3	f4
WWTP A	Day 3 effluent	0.0	9.9	90.1	-	0.0	10.5	90.1	-	0.0	12.4	88.2	-	0.3	31.2	69.1	-
	Day 2 effluent	10.2	90.1	-	-	10.6	89.5	-	-	9.7	90.0	-	-	32.9	68.5	-	-
WWTP B	Day 4 effluent	-	-	-	-	0.0	1.0	28.4	70.7	0.0	0.0	3.1	97.3	0.5	4.8	38.8	55.0
	Day 3 effluent	-	-	-	-	0.7	25.7	70.5	-	0.0	2.8	96.9	-	3.8	35.5	55.1	-

The main factors causing the variations in load fractions between the different treatment units and the similar units between the two WWTPs (Table 2) are variations in volumes and flow rates, as well as the differences in the modes of operations. The different load fractions that are used to calculate the reference influent load (Eq. 1) eventually affect the calculation of the CEC removals (Eq. 2). Furthermore, due to the daily variation in the influent flow rates, as well as daily variations in the diurnal pattern of flow, slight daily variations are observed in the curves representing RTD of activated sludge on different days, as shown in Figure 2.

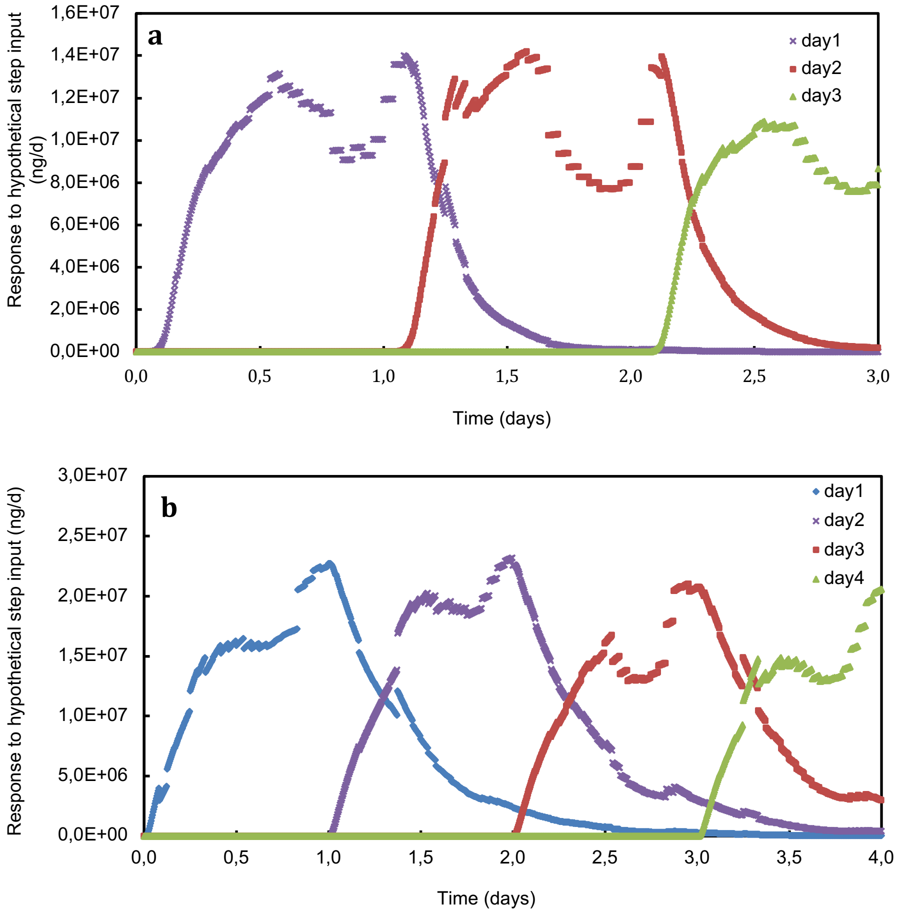

Figure 2. Simulated response to a hypothetical CEC 24-h step input: (a) Input applied on first, second and third days of sampling separately to the best-fit hydraulic model of the activated sludge unit in WWTP A; (b) Input applied on first, second, third and fourth sampling days separately to the best-fit hydraulic model of the activated sludge unit in WWTP B.

Therefore, as shown in Table 2, slightly different load fractions obtained for each treatment unit when different days are taken as the basis of the effluent load for removal calculation. Seasonal changes in flow rates would also be expected to have a significant effect on these calculations. This exercise illustrates the sensitivity of the fractionated approach to changes in the operating conditions within the WWTP. Eliminating this bias in CEC removal estimates by accounting for hydraulics is essential, especially when comparing the efficiency of different treatment stages or trains in removing CECs. The CEC removal efficiencies in primary and secondary treatment obtained by applying the fractionated approach are discussed in sections 3.3 and 3.4.

3.2 Concentrations of target contaminants

The average measured concentrations and the calculated mass loads of CECs in the influent and effluent streams of both WWTPs are summarized in Table 3. In the influent streams of both WWTPs, the average concentrations of the target CECs ranged from 6 ng/L for estrone to 4,400 ng/L for sucralose at WWTP A and from 7 ng/L for estrone to 40,800 ng/L for caffeine at WWTP B. Trimethoprim was not detected in the influent of both WWTPs, while MDA and dihydrocodeine were not detected in the influent of WWTP A, but were detected in the influent of WWTP B. Codeine, morphine and sulfamethoxazole were not detected in the influent of WWTP B only (Table 3). Table 3 also presents the mass loads of the target CECs contained in the wastewater for each city. The differences in mass loads between the two cities might be explained by differences in the population characteristics and demographics influencing the patterns of use of these CECs. In general, no significant variations were observed for any of the CECs in the influent of WWTP A during the sampling period. The ratios of benzoylecgonine to cocaine concentrations were calculated to be 4.1 in WWTP A and 3.1 in WWTP B. According to Ratola et al. (2012), the majority of studies report a ratio of 3.1-3.5 in wastewater. It has been suggested that a ratio of benzoylecgonine to cocaine lower than 5 indicates that some cocaine was being discharged directly into the sewage without being consumed and metabolized (Karolak et al., 2010).

After the secondary treatment step in both WWTPs investigated, some compounds were <LOD or <LOQ, although they were detected in the influent, including sulfamethoxazole, cocaine and androstenedione for WWTP A and acetaminophen, ibuprofen, benzoylecgonine, amphetamine, MDA, dihydrocodeine, estrone and triclosan for WWTP B. The concentrations of sucralose were the highest of all target CECs in the effluent of both WWTPs, with average concentrations of around 5600 ng/L and 1100 ng/L in WWTP A and B, respectively.

Table 3 Mean concentration (ng/L ± SD) and mean mass loads (mg/d ± SD) of target CECs in the influent to the primary treatment and effluent of the secondary treatment at WWTP A and WWTP B. SD is standard deviation based on different days samples and their replicates (n=9 for WWTP A and n=12 for WWTP B)

	Concentrations (ng/L ± STD)				Mass loads (g/d ± STD)			
	WWTP A		WWTP B		WWTP A		WWTP B	
	Influent	Effluent [c]	Influent	Effluent	Influent	Effluent [c]	Influent	Effluent
Trimethoprim	<LOQ [b]	<LOD [a]	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
Sulfamethoxazole	16 ± 6	<LOD	<LOD	<LOD	0.620 ± 0.224	<LOD	<LOD	<LOD
Acetaminophen	NA	NA	7475 ± 1012	<LOD	NA	NA	447 ± 61	<LOD
Codeine	2518 ± 77	1278 ± 30	<LOD	<LOD	98.9 ± 3	50.1 ± 1.2	<LOD	<LOD
Ibuprofen	2488 ± 100	114 ± 7	249 ± 13	<LOQ	97.6 ± 3.9	4.46 ± 0.27	14.7 ± 0.8	<LOQ
Naproxen	2140 ± 91	343 ± 19	237 ± 15	63 ± 3	84.1 ± 3.5	13.6 ± 0.737	14.0 ± 0.9	3.73 ± 0.18
Carbamazepine	90 ± 2	91 ± 5	23 ± 8	26 ± 3	3.53 ± 0.09	3.59 ± 0.20	1.39 ± 0.46	1.54 ± 0.16
Gemfibrozil	19 ± 0.5	22 ± 2	<LOQ	18 ± 3	0.727 ± 0.018	0.859 ± 0.068	<LOQ	1.04 ± 0.19
Benzoylecgonine	1165 ± 35	112 ± 3	554 ± 29	<LOD	45.7 ± 1.3	4.40 ± 0.13	32.2 ± 1.6	<LOD
Cocaine	284 ± 16	< LOD	181 ± 24	133 ± 12	8.14 ± 0.75	<LOD	10.8 ± 1.3	7.96 ± 0.69
Amphetamine	178 ± 5	21 ± 0.5	56 ± 8	<LOD	7.03 ± 0.19	0.825 ± 0.019	3.30 ± 0.45	<LOD
EEDP	428 ± 29	448 ± 15	155 ± 18	14 ± 4	16.8 ± 1.2	17.6 ± 0.6	9.14 ± 1.01	0.862 ± 0.217
Ephedrine	1672 ± 49	88 ± 5	902 ± 70	106 ± 6	65.6 ± 1.9	3.47 ± 0.20	53.5 ± 3.9	6.23 ± 0.344
MDA	<LOD	<LOD	41 ± 5	<LOD	<LOD	<LOD	2.44 ± 0.30	<LOD
Dihydrocodeine	<LOD	<LOD	467 ± 66	<LOD	<LOD	<LOD	28.4 ± 3.7	<LOD
Methadone	170 ± 4	168 ± 6	18 ± 2	11 ± 0.3	6.67 ± 0.17	6.58 ± 0.25	1.09 ± 0.13	0.645 ± 0.016
Morphine	259 ± 18	33 ± 2	<LOD	<LOD	10.2 ± 0.7	1.29 ± 0.08	<LOD	<LOD
Tramadol	189 ± 7	286 ± 16	43 ± 6	77 ± 3	7.45 ± 0.27	11.2 ± 0.6	2.54 ± 0.34	4.53 ± 0.17
Androstenedione	32 ± 1	<LOQ	55 ± 7	15 ± 5 [d]	1.27 ± 0.05	<LOQ	3.25 ± 0.39	0.297 ± 0.159
Estrone	6 ± 1	5 ± 1	7 ± 2	<LOQ	0.239 ± 0.041	0.196 ± 0.035	0.393 ± 0.121	<LOQ
Triclosan	281 ± 8	28 ± 5	27 ± 3	<LOQ	10.9 ± 0.3	1.08 ± 0.20	1.57 ± 0.18	<LOQ
Caffeine	NA	NA	40899 ± 6558	73 ± 21	NA	NA	2434 ± 391	4.24 ± 1.25
Sucralose	4362 ± 223	5578 ± 351	3866 ± 305	1097 ± 147	170 ± 9	218 ± 14	228 ± 14.5	65.0 ± 8.83

[a,b] <LOD and <LOQ: indicates that the compound concentrations were below the limits of detection or quantification, respectively

[c] refers to the secondary effluent of WWTP A before the UV disinfection

[d] Androstenedione’s concentration in the effluent was based on a single day, as on the rest of the days, the concentration was <LOQ.

3.3 Aqueous removal of CECs during primary clarification

Removals of CECs during primary clarification were only determined for WWTP A, since WWTP B does not have a primary clarifier unit. The mass loads of the influent to the primary clarifier of WWTP A were calculated using the fractionated approach and using both day 2 and day 3 effluent as the basis of the calculation. Results for reference (influent) and effluent mass loads of all CECs are presented in Figure S1, and average removal efficiencies in the primary treatment process are summarized in Table 4. The reference mass loads were generally higher when based on day 2 effluent than when based on day 3 effluent, which can be explained by the higher concentrations of most CECs observed in the influent on the second day of sampling.

On the other hand, the effluent mass loads observed on the second and the third days of sampling (day 2 and day 3) were comparable.

Triclosan was estimated to have the highest removal (94%) in the primary clarifier, which is in agreement with previously reported results (Behera et al., 2011; Blair et al., 2013; Winkler et al., 2007). The high removals in the primary clarifier are likely due to the high log K_{ow} of this compound (i.e. 4.76), which contributes to partitioning to sludge during the primary treatment step (Rogers, 1996). In addition to adsorption, some biodegradation of CECs is expected to take place in the primary clarifier, contributing to their removal from the aqueous phase (Jones et al., 2005; Petrie et al., 2014). Androstenedione, carbamazepine, gemfibrozil, ibuprofen, naproxen and sucralose had poor removals of 10-20% in the primary clarifier. Poor removal of these compounds during primary clarification was also reported in previous studies (Behera et al., 2011; Blair et al., 2013; Ternes & Joss, 2006; Zorita et al., 2009). It is notable that androstenedione, which is an intermediate in the biosynthesis of testosterone, was poorly removed by primary clarification. There are only limited monitoring data for this compound in the literature (Esperanza et al., 2007). In the case of ibuprofen and naproxen that have similar pK_a (<4) and log K_{ow} values (3-4), their removals in the primary clarifier were almost equivalent at 13-15%. Estrone, which is a metabolite of estradiol, showed negative removal in the primary clarifier. Negative removals of estrone during primary clarification have also been reported by others (Andersen et al., 2003; Behera et al., 2011; Carballa et al., 2004; Kirk et al., 2002; Ternes et al., 1999). These observations were attributed to the transformation of estradiol to estrone during primary clarification (Andersen et al., 2003; Carballa et al., 2004; Verlicchi et al., 2012).

Because of the anaerobic conditions and short residence times in the primary clarifier, biodegradation of CECs is likely low. Photolytic degradation is also expected to be low in the primary clarifier because of short residence times. Also, since the target compounds have low volatility, the removal of the target CECs in the primary clarifier is likely due to the sorption to primary sludge. Among the drugs of abuse monitored, the highest removal in the primary clarifier was 15% for tramadol, which can be explained by the relatively high hydrophobicity of tramadol compared to the other drugs of abuse monitored, resulting in a higher tendency to sorb to sludge (Table 1). Some target compounds, namely methadone, EDDP, ephedrine and cocaine showed negative removals in the primary clarifier, even using the fractionated approach. Subedi and Kannan (2014b) reported negative removals of methadone and its metabolite, EDDP during a primary treatment process, consistent with the observations in the present study. Since sorption and desorption are likely the primary processes affecting the CEC concentrations in the primary clarifier, it is possible that desorption of these compounds from particulate material in the raw wastewater into the aqueous phase takes place during primary clarification. The trends in the removal of drugs of abuse during primary clarification are in agreement with the limited literature available for these compounds (Rodayan et al., 2014a; Subedi & Kannan, 2014b).

Overall, these results demonstrate that primary clarification has limited capacity for removing many of the target CECs, with 14 out of the 17 compounds showing removals < 20% during this step in the treatment train at WWTP A. This is consistent with these compounds being relatively hydrophilic and having a low tendency to partition onto solids due to their low log K_{ow} (<3) and low pK_a.

It is important to emphasize that this study has focused on the CEC removal from the aqueous phase (i.e. removal of the dissolved proportion of the CEC) without accounting for the CEC load sorbed to particulate material. In fact, Miège et al. (2009) reported that only 15 of 117 peer-reviewed articles examining CEC fate in WWTPs measured the CEC concentrations in sludge and only one considered the CEC concentration in both aqueous and particulate phases for removal calculations. Some sorption of CECs onto filters is expected to take place during sample preparation. However, a previous study by Miao and Metcalfe (2007) evaluated the proportion of CECs sorbed to suspended particulates by analyzing the particulate material removed by centrifugation prior to SPE. This study concluded that the proportion of the CEC load retained on particulates is negligible compared to the amount dissolved in the wastewater for most water soluble CECs (Miao & Metcalfe, 2007). Thus, the adsorption of CECs on filters that takes place during sample preparation introduces negligible bias to the calculation of removals during wastewater treatment.

3.4 Aqueous removal of CECs during activated sludge treatment

As with the primary clarifier, the matching mass loads of CECs in the aqueous phase of the influent and effluent of the activated sludge unit were calculated, as illustrated in Figure S2 and Figure S3 for WWTP A and WWTP B, respectively. In WWTP A, the effluent mass loads used for calculating removals were based on both the second and third sampling days (as for the primary clarifier), while for WWTP B, it was based on the fourth and third sampling days. The average removals of target compounds in the activated sludge treatment step for the two WWTPs are also presented in Table 4.

The majority of the removals for most of the target compounds took place in the activated sludge step, when compared to the low removals in the primary clarifier of WWTP A (Table 4). Amphetamine and benzoylecgonine exhibited removals of >90% during activated sludge treatment, consistent with previous research on the removal of these two compounds in WWTPs with activated sludge treatment (Bijlsma et al., 2012; Postigo et al., 2010; Rodayan et al., 2014a). Acetaminophen and caffeine were shown to undergo substantial removal (WWTP B) of almost 100%, probably due to biodegradation, as suggested in previous studies (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Yang et al., 2011). Acetaminophen and caffeine were also shown to undergo substantial removal (WWTP B) of almost 100%, which is in agreement with previous studies (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Yang et al., 2011). Removals of 92-95% and 72-77% were observed for ibuprofen and naproxen, respectively in the activated sludge process for both WWTPs, which is in good agreement with previous findings (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Sim et al., 2010; Yang et al., 2011). Removals of androstenedione were >93% in WWTP A and 74% in WWTP B. Almost complete removal of androstenedione from wastewater was previously reported by Esperanza et al. (2007).

Low removals of <40% were observed for estrone and carbamazepine during the activated sludge treatment at both WWTPs, indicating that these compounds are persistent. These observations for carbamazepine are consistent with the many studies showing that carbamazepine is very persistent in WWTPs (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Miège et al., 2009; Yang et al., 2011). Estrone was previously reported to be persistent in some studies (Castiglioni et al., 2006; Zorita et al., 2009), although other studies reported high removal (Behera et al., 2011; Miège et al., 2009). The wide range of the CEC removal efficiencies reported in the literature illustrate the value of the fractionation method for generating reliable and consistent data on removals of CECs.

Table 4 Removals (%) of target compounds in primary clarifier and activated sludge treatment steps in WWTP B and WWTP A

Compound	Removal in primary clarifier (%)	Removal in Activated sludge (%)	
	WWTP A	WWTP A	WWTP B
Trimethoprim	<LOD[a]	<LOQ[b]	<LOD[a]
Sulfamethoxazole	16[c]	71[c]	<LOD[a]
Acetaminophen	NA[d]	NA[d]	100
Codeine	1	54	<LOD[a]
Ibuprofen	15	95	92
Naproxen	14	77	72
Carbamazepine	13	15	7
Gemfibrozil	20	-39	-37
Benzoylecgonine (cocaine metabolite)	3	90	99
Cocaine	-4	77[c]	42
Amphetamine	6	91[c]	90[c]
EEDP (methadone's metabolite)	-8	32	93
Ephedrine	-2	95	88
MDA	<LOD[a]	<LOD[a]	75 [c]
Dihydrocodeine	<LOD[a]	<LOD[a]	95 [c]
Methadone	-20	25	44
Morphine (codeine metabolite)	3	91	<LOD[a]
Tramadol	15	-47	-73
Triclosan	94	61	38[c]
Androstenedione	11	93[c]	74
Estrone	-31	34	24[c]
Caffeine	NA[d]	NA[d]	100
Sucralose	20	-50	68

[a,b] <LOD and <LOQ: indicates that the compound concentrations were below the LOD or LOQ in both the influent and effluent and that removals could not be calculated.

[c] Denotes that the compound concentrations were either <LOQ or <LOD in the effluent of the corresponding treatment stage and that removal was calculated assuming a concentration equal to LOQ & LOD, respectively, indicating that the removal might actually be higher than the reported values.

[d] NA: indicates compounds that were not analyzed, such as acetaminophen and caffeine that were later added to the list of target CECs and were not quantified in the samples from WWTP A.

Negative removals were observed for tramadol and gemfibrozil in the activated sludge step of both WWTPs under study, which may be due to the presence of conjugated metabolites in the influent of the unit that are converted to the parent compound by the activated sludge treatment, as was suggested by other authors (Jelic et al., 2011; Lishman et al., 2006). There was also negative removal of the artificial sweetener, sucralose, in the activated sludge unit at WWTP A, while a positive removal of 68% was calculated for this compound in WWTP B. Among the limited studies on the fate of sucralose in WWTPs, low removals of sucralose in activated sludge were previously reported (Pasquini et al., 2013; Subedi & Kannan, 2014a), and a number of negative removals of sucralose were observed in Norwegian and Swedish WWTPs (Brorström-Lunden, 2008; Green, 2007). These were linked to the possibility of the presence of conjugated and complex forms of sucralose in the influent load, which might transform back to the parent form during the biological treatment. Negative and variable removals of carbamazepine were obtained in WWTP A and WWTP B using the day-by-day approach of removal calculation (results not shown here); however, the fractionated approach yielded positive and consistent removals.

4. Conclusions

In order to obtain reliable data on the removals of CECs during treatment of wastewater, it is essential to account for mixing regimes that affect the transport of CECs in the WWTP. In this study, we applied the fractionated approach to characterize CEC removals in two WWTPs. Based on the hydraulic modelling using the fractionated approach, WWTP B had a longer HRT, as well as a higher degree of mixing than the activated sludge unit in WWTP A, resulting in the need for data over more sampling days for reliable quantification of removal in WWTP B, as well as different calculations of CEC removal. The removals of target CECs were generally low or negative in the primary clarifier and ranged from negative removals to 100% removal over the entire activated sludge treatment train. This study contributes to advancing the study of the fractionation approach by applying this approach to the primary treatment step and demonstrating that the method is able to detect differences in the hydrodynamics of each of the units investigated at the two treatment plants, resulting in different removal calculations for each unit. Further, the removals estimated for an extended list of target CECs obtained in this study by taking into account hydrodynamics are key for calibrating fate models for CECs. These models can be used to improve our understanding of CEC fate during wastewater treatment and to provide insights for limiting the discharge of contaminants into surface waters.

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Supplementary materials:

Table S1 Summary of the operating characteristics of the WWTPs and average temperatures during the sampling campaigns

Characteristic	Treatment stage	WWTP A	WWTP B
Hydraulic retention time - HRT (h)	Primary clarifier	5.97	-
	Aeration tanks	4.52	12.3
	Secondary clarifier	3.36	10.6
	Total	13.85	22.9
Solid retention time - SRT (days)		10.5	27.6
Average wastewater T (°C)		21	20

Table S2 SPE extraction methods and instruments used for compounds of Class a and Class B

Extraction aspect	Class A	Class B
Instrument	Gilson GX-271 ASPECTM automated instrument	Manual manifold
Cartridge	6 mL-150 mg Oasis MCX cartridges	6 mL-500 mg Oasis MAX cartridges
Sample pH	pH 2.5 using sulfuric acid	pH 8.0 using sodium hydroxide
Cartridge conditioning	6 mL of acetone and 6 mL of water (pH 2.5)	6 mL of methanol, 6 mL of 0.1 M ammonium hydroxide and 6 mL of water (pH 8.0)
Elution	3x3 mL of 5% ammonium hydroxide in methanol, 1 mL/min	2 mL methanol and then 3x3 mL of 2% formic acid in methanol, 1 mL/min
Reconstitution	25% methanol/75% water to a total volume of 0.4 mL	50% methanol/50% water to a total volume of 0.4 mL

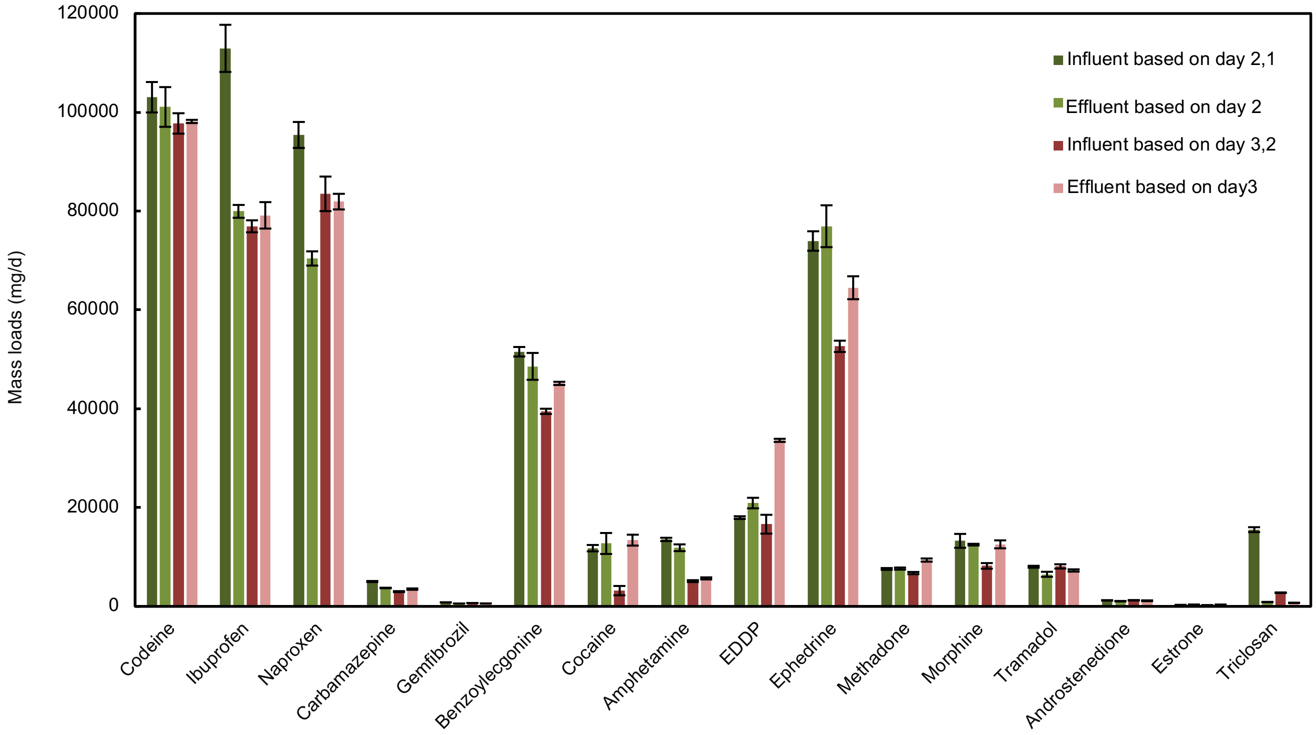

Figure S1. Mass loads of target CECs in influent (based on load fractions of two days) and effluent (load on the last day) of the primary clarifier of the WWTP A. Day 1,2,3 represent first, second and third sampling day, respectively. Error bars = one standard deviation of 3 replicates.

Sucralose is not shown in this graph, as it has a significantly higher range of mass loads values

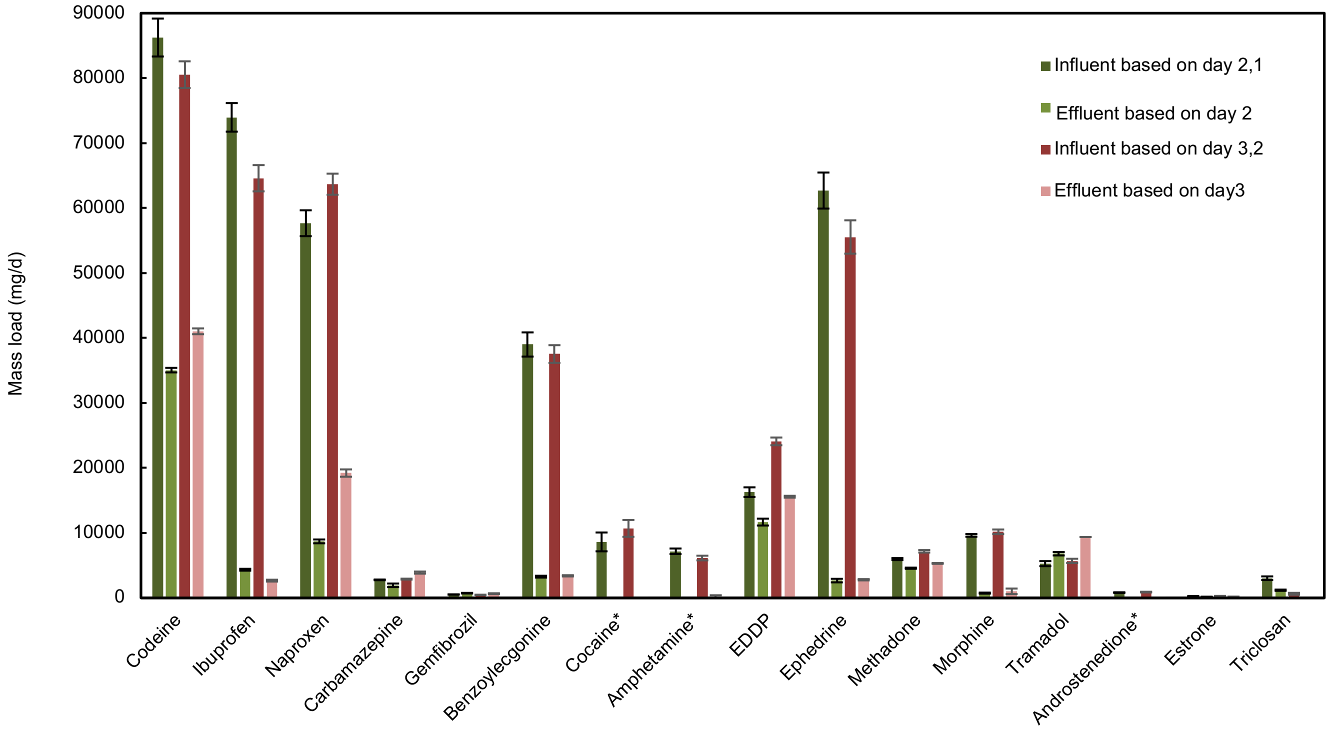

Figure S2. Mass loads of target CECs in influent (based on load fractions of two days) and effluent (load on last day) of the activated sludge unit at the WWTP A. Day 1,2,3 represent first, second and third sampling days, respectively. Error bars = one standard deviation of 3 replicates.

* denotes compounds with effluent concentration < LOD or LOQ, where mass loads were considered 0 in this graph. Sucralose is not shown in this graph, as it has a significantly higher range of mass loads values

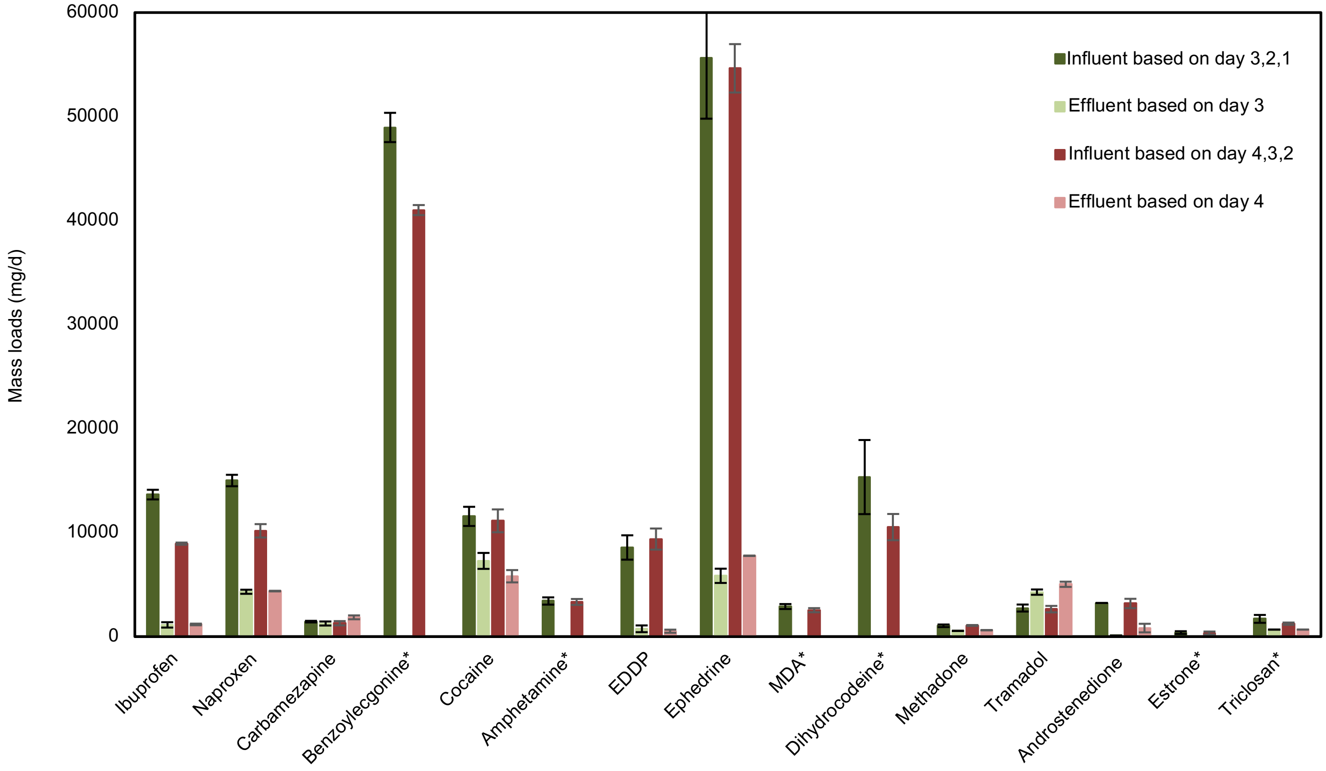

Figure S3. Mass loads of target CECs in influent (based on load fractions of 3 days) and effluent (load on last day) of the activated sludge unit at the WWTP B. Day 1,2,3,4 represent first, second, third and fourth sampling days, respectively. Error bars = one standard deviation of 3 replicates.

* denotes compounds with effluent concentration < LOD or LOQ, where mass loads were considered 0 in this graph.

Caffeine, sucralose and acetaminophen are not shown in this graph, as they have a significantly higher range of mass loads values