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Assessing the population equivalent and performance of wastewater treatment through the ratios of pharmaceuticals and personal care products present in a river basin: Application to the River Thames basin, UK

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Abstract

The quality of surface waters in lowland rivers is largely dependent on the efficiency of wastewater treatment. Even in the developed countries, there have been difficulties in evaluating the effectiveness of wastewater management and the proportion of wastewater content (WWC) in the river, as well as in estimating the contributing human population. This study aimed to develop a

wastewater quality and quantity assessment based on the occurrence of pharmaceuticals in the receiving waters. A survey of 53 pharmaceuticals in 324 samples (river water and influent and effluent of sewage (wastewater) treatment plants) was carried out in southern England in the River Thames catchment over four years. Carbamazepine was selected as stable marker and from its concentration WWC in the rivers and cumulative human populations along the catchment were estimated. The estimated population had a strong relationship (R^2 = 0.94) with that reported by the local water company. The concentration ratio of the labile marker caffeine to carbamazepine indicated the efficiency of wastewater treatment in the different treatment systems (i.e. trickling filter or activated sludge) and in the receiving waters. The ratio in some river samples revealed unexpected discharges of untreated or poorly treated wastewater, with a total concentration of the analytes five times higher than that in treated wastewater (up to 20 µg/L). Such information could be valuable to estimate the discharge or occurrence of not only non-targeted chemicals, but also pathogens within the basin.

Highlights:

- Sixty pharmaceuticals were monitored in the River Thames basin over 4 years.
- Wastewater quality and quantity assessment based on the occurrence were studied.
- Carbamazepine (CBZ) was selected to assess the population and wastewater content of rivers.

- Coffeine (CFF) was selected to estimate the effectiveness of wastewater treatment.
- CFF/CBZ revealed unexpected discharges of untreated or poorly treated wastewater.

Keywords

Sewage/wastewater epidemiology

Molecular marker approach

Cumulative population

Drug ingredient residue

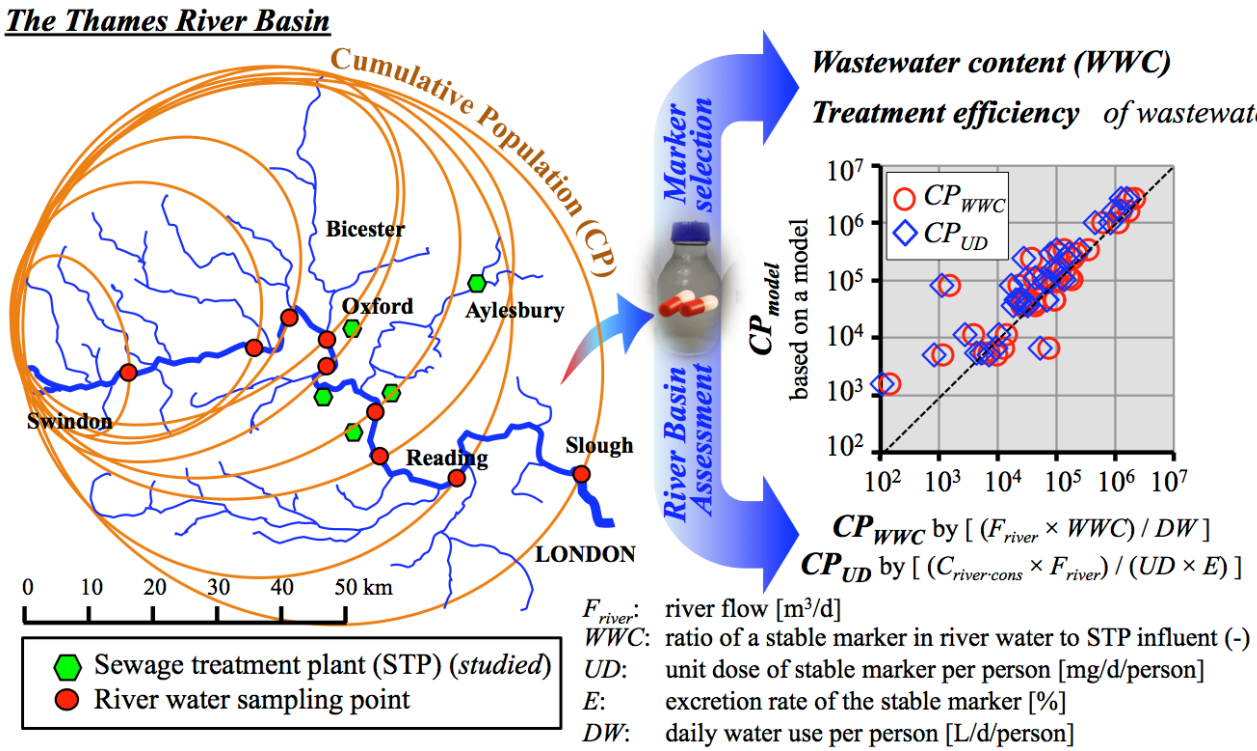
Wastewater content

Efficiency of wastewater treatment

Abbreviations

STP – sewage treatment plant, AS – activated sludge, TF – trickling filter, PPCPs – pharmaceuticals and personal care products, CBZ – carbamazepine, CFF – caffeine, NPX – naproxen, PMD – primidone, CTT – crotamiton, CP – cumulative population, WWC – wastewater content, UD – unit dose per person, DW – daily water use per person,

Graphical Abstract



1. Introduction

The quality of surface waters in lowland rivers of densely populated developed countries is largely dependent on the efficiency of wastewater treatment. Several modelling approaches have been developed to assist in the water quality management of river basins[1]. However, even in developed countries, it is difficult to gauge the effectiveness of sewage treatment; this is where a molecular marker approach can be helpful[2]. Markers can identify and distinguish specific pollution sources as well as tracing their transport and fate pathways. Molecular markers have been studied over two decades, and their effectiveness has been tested through comparison with microbial markers (e.g. *E. coli*). The major weaknesses of microbial markers are the time needed for measurement and difficulties of correlation of the recovery rate in the treatment and measurement of samples[3, 4]. The number of pathogens found in the environment can be connected to the number of infected patients but it does not have a linear relation with either the number of patients or wastewater content (WWC) in the receiving waters. Some pharmaceuticals and personal care products (PPCPs) have been proposed as ideal markers because of their relatively constant consumption and ubiquitous occurrence in the environment[3, 5], while not occurring naturally.

Molecular markers have been widely used as qualitative indicators of faecal pollution of surface and ground waters[3, 5] and as indicators of combined sewer overflow in a coastal area[6]. However, molecular markers have rarely been used in a quantitative way, although carbamazepine (CBZ)

concentrations were used to estimate leakage of sewage from pipes by comparing concentrations in an aquifer with those in sewage[7]. Buerge et al.[8] evaluated caffeine (CFF) as a quantitative marker for the estimation of wastewater burden in lakes in Switzerland and demonstrated the discharge of untreated domestic wastewater as combined sewer overflow to the lakes. Other quantitative approaches used the concentrations in raw sewage to determine trends in the abuse of illicit drugs[9] and to estimate the number of influenza patients from concentrations of antiviral drugs during pandemics and influenza seasons[10]. This approach is called "sewage/wastewater epidemiology"[11]. Nakada et al.[12] found a strong relationship between the population in river basins and the flux of the antipruritic crotamiton (R^2 = 0.85) and the antiepileptic CBZ (R^2 = 0.84) in 37 major rivers in Japan. More recently, O'Brien et al.[13] found the artificial sweetener acesulfame and the anti-epileptic gabapentin effective in estimating the population (the "*de facto* population") connected to sewage treatment plants (STPs) in Australia on a census day (the "*de jure* population"). The results revealed that such stable substances can be used as indicators of populations in river basins, as well as of the proportion of treated or untreated wastewater (i.e. WWC) in a river. To the best of our knowledge, however, no research has yet used these approaches to reveal the effectiveness of sewage management and population in the basin at the same time.

Several different PPCP markers have been suggested. The selection of markers depends on the project objectives, and suitable markers have to be evaluated in each country because of differences

in drug usage, sewage treatment systems, and climate[3]. Although the concentration of substances in the environment is governed not only by the burden of pollution but also by sewage treatment, dilution and natural attenuation, a ratio of two markers (one labile and one stable) can normalize the reductions[7, 14-16]. The ratios of environmental samples when compared with those of raw (untreated) and treated wastewater can be used for to estimate the treatment efficiency in receiving water samples.

The *de facto* population connected to an STP may not coincide with the determined *de jure* population[11, 13]. It is because the former is population equivalent and accounts for tourist and the difference between daytime and nighttime populations, while the later is based on the latest census. The estimated *de facto* population is a useful starter in estimating the loads of chemicals (organic substances, nutrients and metals) and pathogens from daily life. In addition, river flow rates and daily discharge volumes from STPs are not easily available in real time, especially in countries where sewage works have been privatized, such as the UK. Decentralized, small-scale and unstaffed STPs may also make it difficult to estimate the population. Although the human population can be determined based on the latest census data, the quantitative molecular marker approach can estimate the *de facto* population cumulatively along a river before a census. Therefore, the marker approach is effective not only as a faecal pollution indicator for developing countries, but also for estimating *de fact* populations in developed countries where sewage treatment is common. We have used this

approach in estimating cumulative population (CP), WWC, and degree of treatment of wastewater in the River Thames basin, the largest catchment in England.

Kasprzyk-Hordern et al.[17, 18] monitored PPCPs in small rivers in Wales and at the STPs in the catchments and estimated faecal pollution qualitatively[19]. Ashton et al.[20] and Ellis[21] investigated the occurrence of 12 PPCPs in STPs in England and in small streams receiving effluents. Stuart et al.[22] monitored almost 1000 compounds, including PPCPs, in ground water by a semi-quantitative analytical method, and measured surface waters collected at one point each on the River Thames and the River Lambourn for reference. However, there has been no study of the occurrence of PPCPs at multiple points in the River Thames basin. This study provides a first database of 53 PPCPs measured in the River Thames basin. The main aim of this study was to select appropriate markers that could be used to estimate the content of sewage effluent (the WWC), treatment efficiency of effluent (i.e. effectiveness of local sewage treatment) and the contributing human population in the receiving river at a certain point, without modelling. Such information is valuable in estimating the discharge or occurrence of not only the measured chemicals but also other chemicals and pathogens derived from our daily life.

2. Materials and Methods

2.1. Site description and sampling

Surface water samples were taken from nine monitoring sites along the River Thames and from 16 of its major tributaries in August–September 2014 and in January and August 2015 (Figure 1). The distance between the most upstream site (Hannington Wick) and the most downstream site (Runnymede), 34 km upstream of the tidal limit, is approximately 175 km. Details of the river water sampling sites are given elsewhere by Bowes et al.[23] and in Table S1 and Figure S1 of the supplementary material. Raw sewage and final effluent samples were also taken at five STPs in the basin in June 2012, August 2013, August–September 2014, and January and August 2015 (Table S1). Three plants (A, D and O; Table S1) use activated sludge (AS), and two smaller plants (B and C) use a trickling filters (TF) as secondary treatment. The sampling details and characteristics of the sampling sites are summarized in Table S2. The influent, effluent and surface water samples at sites 11 to 13 (site 11 is from the Thames immediately upstream of the small tributary Littlemore Brook carrying the effluent from STP O, site 12 is Littlemore Brook 1.6 km downstream of the STP and site 13 is the Thames 9.1 km downstream of the brook) were collected hourly for 24 h by using autosamplers (ISCO Avalanche, ISCO 6712, Hach Sigma SD 900 or Bühler Montec Xian 1000). For the effluent of STP O and surface water samples (site 11 to 13) collected in 2012 the 24 hourly samples were combined to 12 two-hourly samples and subjected to the PPCP analysis separately. All

other surface water samples from sites 11-13 and STP influent and effluent samples were collected as 24-h composite samples by combining the 24 hourly samples. At the other river sites, grab samples were collected. All samples were collected in duplicate a few days apart during each sampling campaign.

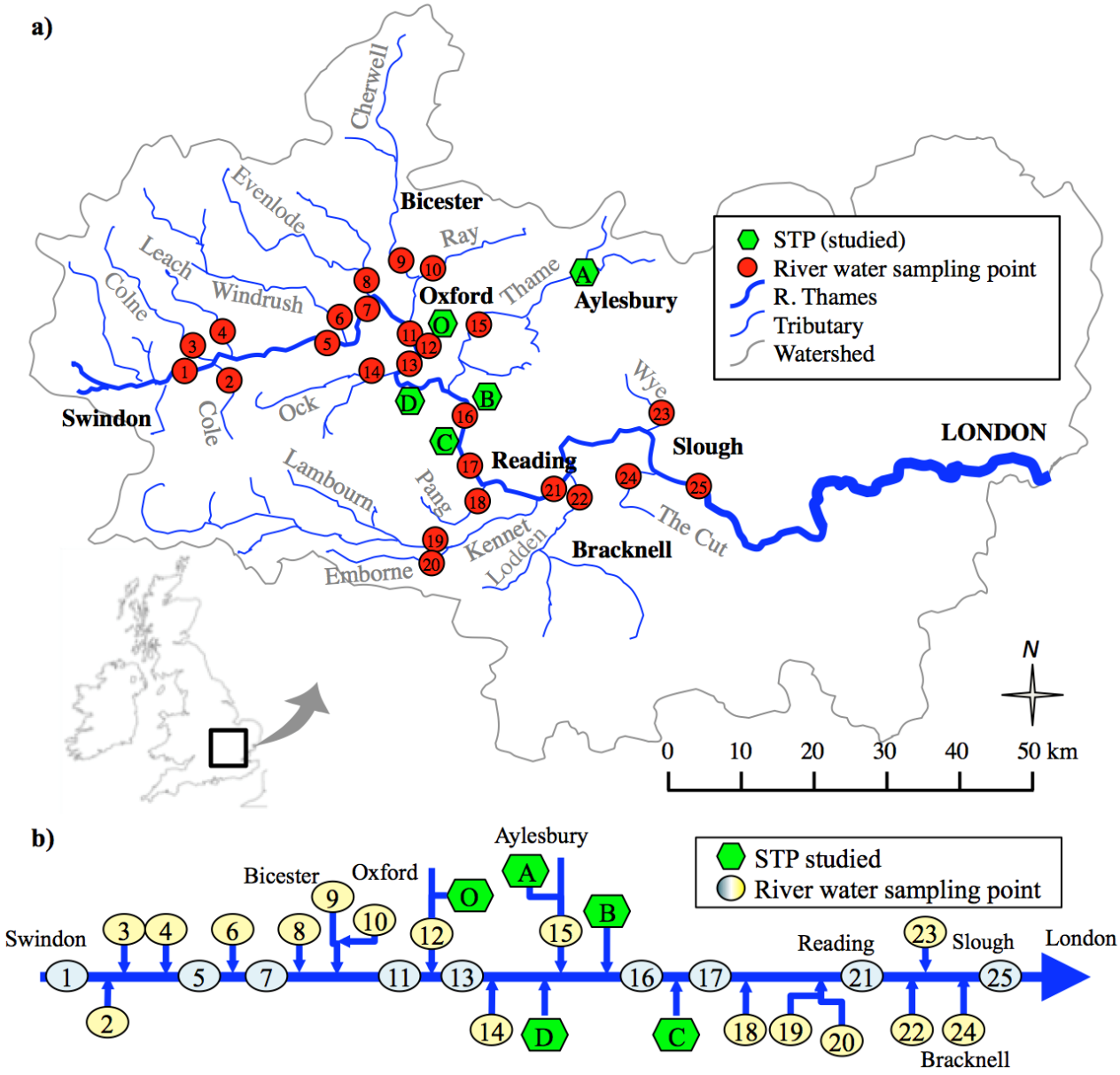


Figure 1. Map of the Thames River basin, showing location of sewage treatment plants (STP) studied and river water sampling sites geographically (a) and schematically (b)

2.2. Sample treatment

The samples were collected in glass bottles for the grab samples and plastic bottles or buckets for the hourly or composite samples. One g/L ascorbic acid was added immediately (added to the bottle or bucket before the sample in the case of the automatic samplers) to reduce degradation of the sample and samples were kept in the dark. On return to the laboratory, they were refrigerated and within one day of collection, the samples were processed. Details on the experimental conditions, source of chemicals and the performance on the analytical method applied can be found elsewhere[23]. In brief, a suitable volume (100 mL for influent, 200 mL for effluent or 500 mL for river water) was spiked with 1.0 g/L EDTA and a surrogate standard mixture (contains 1.0 mg/L of each pharmaceutical)[24], filtered through a glass fibre filter (GF/B, 1.0 μm, Whitman, UK) and the PPCPs in the dissolved phase concentrated by solid-phase extraction through Oasis HLB cartridges (500 mg in 6 cc, Waters, Japan). The cartridges containing the sample concentrate and surrogate standards were then stored in a refrigerator for up to a few weeks and extracted with 6 mL of methanol before being measured by ultra-performance liquid chromatography / tandem mass spectrometry (LC-MS/MS) and quantified by the alternative surrogate method[24]. The results are reported after a three-tiered assessment to decide whether the result was below the quantification limit or not reliable owing to low recovery of the surrogate[25].

2.3. Selection of appropriate markers

The method for the selection of markers is shown in Figure 2. **Step 1** (STP survey) involves regular monitoring of PPCPs at the STPs. From the data, average concentrations in influent (C_{inf}) and effluent (C_{eff}), the frequency of detection, seasonal fluctuation or stability of concentrations, and removal rate (*eq. 1*) were calculated. Values of the population connected to each STP ($P_{connect}$) and flow of discharged effluent (F_{dis}) make it possible to calculate the *de facto* per person usage of water (*eq. 2*) and drug usage rate (*eq. 3*), although we did not have the real-time data on $P_{connect}$ and F_{dis} during each survey. **Step 2** (river survey) involves monitoring of PPCPs in the river basin. In **Step 3** (sorting), the selection of labile and stable markers was based on removal rates at STPs and the frequency of detection in all sample categories. Stability toward photo- and/or biodegradation in the environment[26] and regional difference of C_{inf} in the same survey were also taken into consideration. The selection thresholds for labile markers was a removal rate >80% on average and close to 0% for stable markers. In **Step 4** (assessment), the WWC [%] within surface waters and the efficiency of wastewater treatment in STPs were calculated. The WWC can be estimated from the concentration of a stable PPCP in the sample compared with that in wastewater (*eq. 4*). The efficiency of wastewater treatment[14], can be evaluated from the loss of a labile marker (CFF in this study) relative to that of a stable marker (CBZ) in the sample (*eq. 5*). The treatment efficiency can also distinguish whether effluent in the receiving water was treated or untreated by comparison with the ratio in the

STP influent. A high ratio indicates the presence of untreated or very poorly treated sewage, meaning both CFF and CBZ remain and CFF occurs at much higher concentrations than CBZ. Low ratios mean the sewage has been treated efficiently and most of the labile CFF has been removed while most of the stable CBZ still remains. The *CP* at any point along the river can be calculated in two ways: CP_{UD} (*eq. 6*) is based on the concentration of a stable marker ($C_{river \cdot stable}$) and daily river flow at individual points (F_{river}), the per-person unit-dose (*UD*) of the stable PPCP in the UK and the excretion rate (*E*) of the stable marker; CP_{WWC} (*eq. 7*) is based on F_{river} and WWC at individual points, and the per-person daily water use (*DW*) [m^3/d/person] in the study area. The numerator of *eq. 7* implies the proportion of wastewater [m^3/d] in river water at the point. In **Step 5** (validation), CP_{UD} and CP_{WWC} were compared with that calculated in elsewhere[22] by using a model (Low Flows 2000 Water Quality eXtension (LF2000WQX))[26] (CP_{model}) based on data provided by the water company in 2005.

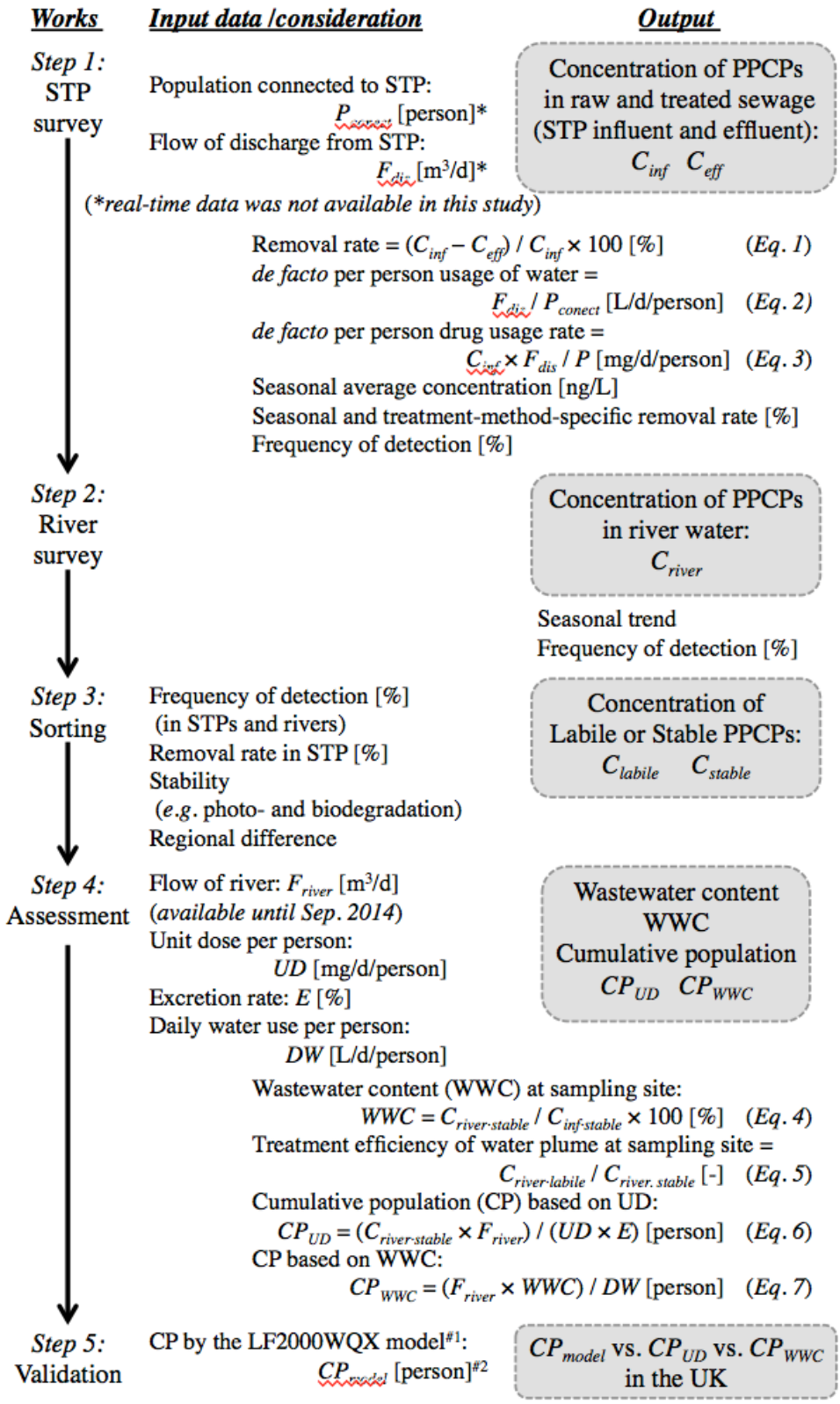


Figure 2. Workflow of appropriate PPCP marker selection and assessment of a river basin.

#1 Williams et al., 2009.

#2 Bowes et al., 2013.

3. Results and Discussion

3.1. Summary results of the occurrence of PPCPs in the River Thames basin

Fifty-two out of the 53 targeted PPCPs were detected in at least one sample (Table S3) and higher concentrations were detected in sewage samples than in river water samples in general. The mean concentrations of seventeen PPCPs in the river water and sewage samples were at concentrations comparable with the mean values reported in the UK[16,20], but in that study a few values in Wales[16] were a few ten times higher than the means. These were the antibiotic trimethoprim and calcium channel blocker diltiazem in the sewage samples, and beta-adrenoceptor blocker atenolol in both the river water and sewage samples.

3.2. Efficiency of removal of the PPCPs detected

When concentrations in both influent and effluent of STPs were available, the rate of removal by the AS and TF STPs was calculated by season (Table S4). In the winter survey, the removal rate could be calculated only for 4 data sets observed at 2 AS and 2 TF STPs mainly due to problems with the auto-sampler trouble, although a total of 11 samples (5 influent and 6 effluent) were collected. Generally speaking, however, the highest rate of removal was in the order of AS STPs in summer > AS STPs in winter ≈ TF STPs in summer > TF STPs in winter. For instance, the anti-

inflammatory naproxen (NPX) was removed at rates of 98% ± 1%, 93% ± 3%, 77% ± 8%, and 71% ± 4%, respectively. A lower efficiency of removal by TF than AS STPs was also observed in Wales[27], although seasonal differences were not discussed.

3.3. Selection of PPCP markers for assessment of the River Thames basin

On the basis of the average concentrations of each PPCP in the samples, the frequency of detection (Table S3) and seasonal removal rates at STPs (Table S4), 4 stable and 4 labile PPCP marker candidates were selected (Table 1). The frequency of detections at measurable concentrations was relatively high even after dilution in river water. The low detection rates for influent samples in summer 2013 were due to low recovery rates of surrogates for the anti-epileptics CBZ and primidone (PMD).

Among the stable PPCP candidates, CBZ had the highest frequency of detection in both STP influent and effluent samples, the highest concentration on average and the lowest coefficient of variation, followed by PMD and CTT. The neuroleptic agent sulpiride showed higher coefficients of variation in the STP samples, implying its unsuitability as a marker in the UK. CBZ and PMD are used daily to treat chronic epilepsy [28]. Therefore, the seasonal difference in their concentrations in STP influents, implying changes in dilution of the influent, may be caused by infiltration of groundwater and surface run-off into the sewer system, especially during periods of high river flow

(discussed below). CBZ, PMD and CTT can be considered as stable with no detectable degradation in river water after 5-days incubation[26], whilst another report gives an estimated half-life of over 28-days for CBZ[6].

Among the candidates for labile markers, CFF had the highest concentration in all samples, followed by NPX, theophylline (used to treat lung diseases), and the β1 receptor antagonist atenolol. Because of their high concentrations and frequencies of detection, CFF and NPX were selected as potential labile markers. The half-life of CFF in natural sunlight was reported as about 12-d[8], and 1.4-h was reported for NPX[29], although the value is governed by the differences in water quality (e.g. suspended solid, dissolved organic matter and nitrate) and location of the experiment (i.e. latitude which governs sunlight intensity and water temperature).

Table 1. Frequency of detection and concentration of potential markers in influent, effluent and river water samples in each survey.

PPCPs	Concentration in Influent																	
	Summer, 2012 (n=6)			Summer, 2013 (n=9)			Summer, 2014 (n=7)			Winter, 2015 (n=5)			Summer, 2015 (n=8)			Summer, All (n=30)		
	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)
Stable PPCPs																		
Carbamazepine	100	440	14	0	-	-	100	618	33	100	282	27	100	790	26	70	633	35
Crotamiton	83	100	22	100	248	38	100	213	35	100	153	17	100	234	61	97	210	51
Primidone	100	65	35	0	-	-	100	109	41	100	72	16	88	147	42	67	110	51
Sulpiride	83	116	74	100	70	88	100	68	92	100	33	76	88	54	85	93	74	86
Labile PPCPs																		
Caffeine	100	31,600	38	56	31,200	23	100	54,200	32	100	26,000	18	100	48,900	32	87	42,900	39
Theophylline	100	2,800	14	44	3,430	17	100	3,360	32	100	2,220	31	88	2,510	28	80	2,990	27
Naproxen	100	3,800	40	44	5,340	11	100	8,920	31	100	4,420	24	100	6,000	26	83	6,180	43
Atenolol	100	1,680	13	100	2,220	21	100	1,770	24	100	1,110	17	100	1,540	27	100	1,830	26
	Concentration in Effluent																	
	Summer, 2012 (n=28)			Summer, 2013 (n=10)			Summer, 2014 (n=5)			Winter, 2015 (n=6)			Summer, 2015 (n=8)			Summer, All (n=51)		
	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)
Stable PPCPs																		
Carbamazepine	100	565	12	80	711	20	100	556	23	100	274	8	100	876	26	96	639	27
Crotamiton	100	107	16	100	239	18	100	211	23	100	136	22	100	274	32	100	169	50
Primidone	100	49	41	40	193	25	80	130	43	100	76	17	100	138	33	86	86	70
Sulpiride	93	175	13	100	82	37	100	65	72	100	53	94	88	66	78	94	128	49
Labile PPCPs																		
Caffeine	43	496	139	70	647	110	100	1,060	145	100	957	121	75	1,370	101	59	800	129
Theophylline	100	39	127	80	113	65	100	111	93	100	141	106	100	98	81	96	68	107
Naproxen	100	256	154	60	808	61	80	1,110	67	100	780	67	100	577	97	90	458	118
Atenolol	100	413	21	90	236	45	60	299	39	100	388	34	100	206	40	94	338	40
	Concentration in River water																	
	Summer, 2012 (n=73)			Summer, 2013 (n=8)			Summer, 2014 (n=52)			Winter, 2015 (n=47)			Summer, 2015 (n=52)			Summer, All (n=185)		
	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)	Freq (%)	Average (ng/L)	cv (%)
Stable PPCPs																		
Carbamazepine	100	176	120	88	209	69	98	112	108	98	21	120	85	175	87	97	169	107
Crotamiton	99	35	127	100	76	107	85	64	86	86	17	154	85	61	78	92	55	96
Primidone	90	15	107	50	15	44	90	23	77	93	4	87	61	33	61	84	22	86
Sulpiride	99	58	130	75	39	100	85	21	95	93	5	134	64	23	68	88	40	138
Labile PPCPs																		
Caffeine	100	735	222	75	118	26	100	156	82	100	131	58	97	189	64	98	400	268
Theophylline	100	43	188	88	17	70	100	22	91	98	9	67	97	22	79	99	31	176
Naproxen	97	67	133	75	53	88	88	55	195	91	35	122	75	49	33	88	53	157
Atenolol	99	130	129	88	77	159	88	22	155	95	14	128	58	19	133	86	73	175

Abbreviation: pharmaceuticals and personal care products (PPCPs), frequency of detection (Freq.), and coefficient of variation (cv).

3.4. Estimating treatment efficiency of wastewater

A ratio of the labile and stable markers (CFF/CBZ) was used to estimate the extent to which the wastewater present in the river had been successfully treated. The ratios in influent were around 100 irrespective of treatment method (AS or TF) and season. On the other hand, the ratios in effluent showed differences between treatment methods and seasons, and dropped by one (TF in winter) to three (AF in summer) orders of magnitude compared to influent (Figure 3a). It was deduced that the treatment efficiencies were lower in winter than summer in both AS and TF STPs, and the lower value of the ratio indicates higher efficiency of wastewater treatment. The ratio also had a relationship with the total concentration of PPCPs detected (Figure S2).

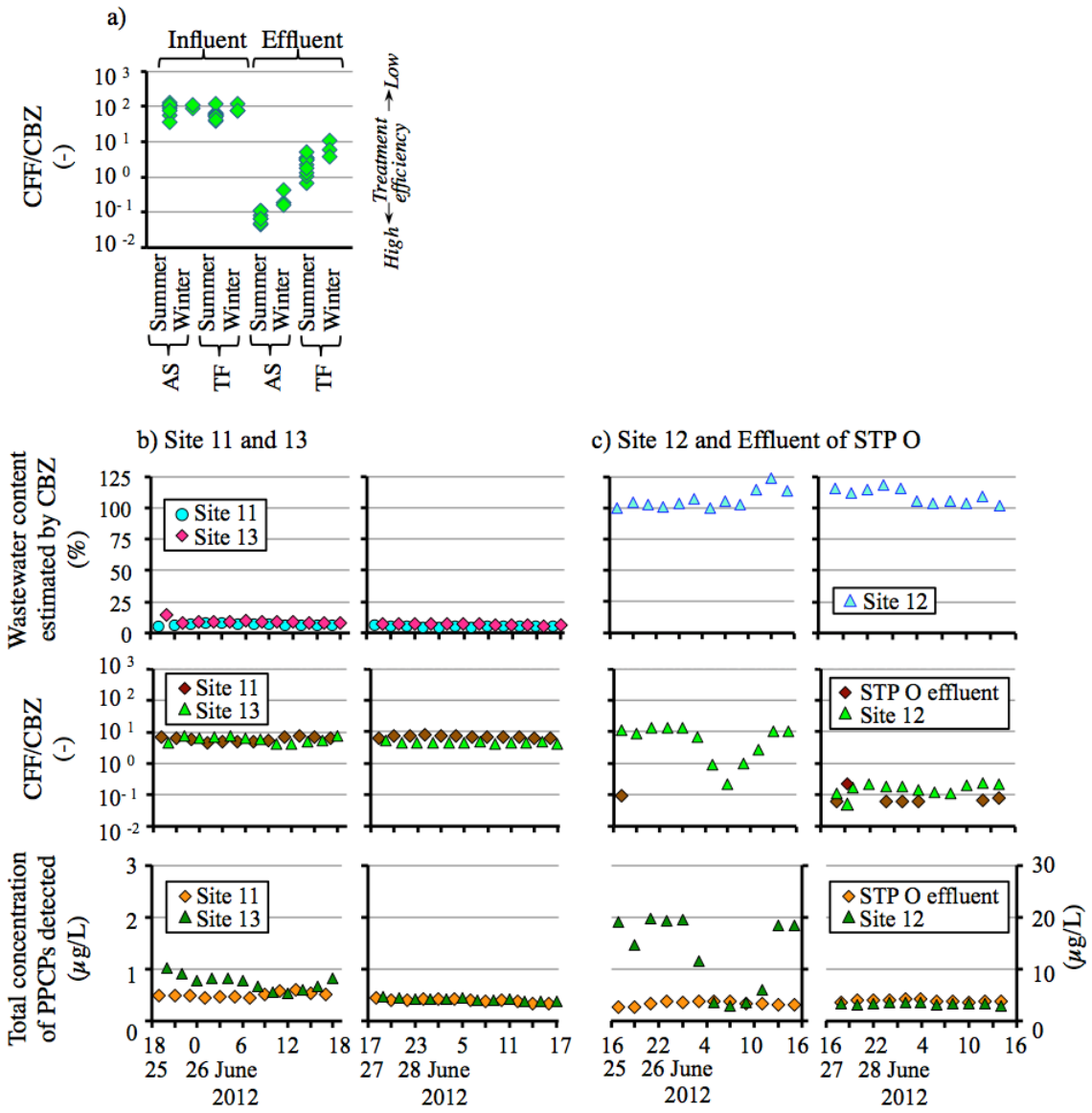


Figure 3. Ratio of CFF/CBZ in influent and effluent in AS STPs and TF STPs in summer and winter (a), and time series of wastewater content (top row), ratio of CFF to CBZ indicating treatment efficiency (middle row) and total PPCP concentration detected (bottom row) at (b) sampling site 11 and 13 on the Thames and (c) site 12 on Littlemore brook and the outlet of STP O.

AS: activated sludge, TF: trickling filter

3.5. Diurnal variation of indicators estimating WWC and treatment efficiency

At sites 11 and 13 on the Thames and site 12 on Littlemore Brook, surface water samples were collected hourly for 48 h and combined as two-hourly samples in 2012, and analysed for PPCPs. The WWC in the river was calculated (Figure 2; eq. 4) at individual sites. The average CBZ (stable marker) concentration in influent in 2012 (440 ng/L, Table 1) was used for the estimation. Diurnal variation of WWC in June 2012 was stable and was estimated to be 5.8 ± 1.0% at site 11 in the Thames upstream of Littlemore Brook, 8.0 ± 1.8% at site 13 downstream of the brook and 108 ± 7% for 48 in Littlemore Brook where the effluent dominated the flow (site 12) (Figure 3b and c, top row).

The ratio of CFF/CBZ was calculated for site 11, 12, 13 and the outlet of STP O to estimate the treatment efficiency. For most outlet samples (effluent), the exact ratio was however unavailable (CFF/CBZ < 0.1) because the CFF concentration was below the quantification limit due to the high treatment efficiency of AS STP (O). For sites 11 and 13 the ratio was stable below 10 (Figure 3b and c, middle), corresponding to that in TF effluent (Figure 3a). The values observed at site 12 (Littlemore Brook) surprisingly fluctuated from over 10 to 0.1 on Jun 25 and 26 (Figure 3c, middle). However, on Jun 27 and 28 the ratio remained stable at around 0.1, typical of good treatment from AS STPs (Figure 3a). The 0.1 ratio observed on Jun 27 to 28 was reasonable because Littlemore Brook receives effluent from an efficient AS STP (O) predominantly. The ratio revealed that discharge of wastewater without appropriate treatment must have occurred during the afternoon and evening of June 25 and the afternoon of June 26. During these periods, total concentration of PPCPs detected surged to 20 µg/L, which was five to six times higher than those in the effluent of STP O or observed at the site 12 during other periods (Figure 3c, bottom). This event might be due to some discharge of raw sewage, whether from the STP or one of the many other businesses and homes in the area is unclear, but the untreated or poorly treated sewage did not enter via the final effluent channel, because both the

total concentration and the ratio CFF/CBZ in the effluent of the plant were stable between June 25 and 28 (Figure 3c, bottom). At site 13, an increase in the total PPCP concentration from 0.5 to 1.0 µg/L was observed in the afternoons/evenings of June 25 and 26 (Figure 3b, bottom), which may due to the inflow of Littlemore Brook. However, the ratio estimating treatment efficiency at site 13 was stable throughout the sampling period (Figure 3b, middle). This might be because the flow rate of the brook was estimated to be less than 10 % of the main stem (Table S2) and the ratio at Site 11 was already high compared with that of STP effluent.

3.6. Estimation of WWC and treatment efficiency

In the main stem of the River Thames, the estimated WWC ranged from <10% to around 20%, except for the higher values in summer 2015 (Figure 4b). Although frequent rain fell in the study area throughout the year, slightly more in winter than in summer, heavy rainfall was not recorded during the sampling times. The greater dilution in winter might indicate that the flow rate was affected by precipitation further upstream or rising groundwater levels. The relatively low WWC in winter 2015 would be caused by the higher river flow (i.e. high dilution). The calculated WWC was greatest at sampling site 1 on the main river (upper Thames at Hannington, 26 ± 26%) and at tributary sites 10 (the River Ray, 28 ± 17%), 12 (Littlemore Brook, 95 ± 26%), 15 (the River Thame, 34 ± 18%), 22 (the River Loddon, 25 ± 8%) and 24 (The Cut, 50 ± 22%). These sampling points receive effluent from major STPs serving the towns/urban areas of Swindon (Hannington), Bicester (Ray), Oxford (Littlemore Brook), Aylesbury (Thame) and Bracknell (The Cut). The Loddon receives STP effluent from Basingstoke and several STPs along its tributary, the river Blackwater.

Crotamiton (CTT) has been shown to be an effective stable marker in Japan[12]. The WWC estimated by CTT (Figure S3) indicated the same conclusions as those estimated by CBZ. This result implies that CTT would also be an effective marker in the UK, although the frequency of detection and average concentration of CTT in the rivers (87% and 54 ng/L, respectively) were slightly lower than those of CBZ (96% and 122 ng/L).

The ratio of CFF/CBZ was also calculated for the river water samples (Figure 4d-f). In the surface water samples from tributaries and the River Thames, the ratio did not reach that found in the efficiently treated effluents of AS (approx. 0.1), with an exception for the site 12 on Littlemore Brook, where the flow consists mainly of effluent from STP O. This suggests that the flow at the other sampled river sites contains sewage that has not been treated by AS. This may originate from the effluents of TF STPs or from small-scale treatment facilities such

as septic tanks[22].

Both CFF and CBZ have been detected in STPs and the aquatic environment world-wide[7,27,33]. Buerge et al.[7] monitored CFF at 13 STPs in Switzerland and reported the CFF removal efficiency of 99.6 % ± 0.2% (n = 16). They also reported a low biodegradability and moderate photo-degradability (half-lives of about 12 d) of CFF in the receiving water environment, despite higher removal efficiency in the STPs. The CBZ removals observed in around 10 countries were summarized[33] and were mostly below 10%. Although the removals of CFF and CBZ in STP were almost same as the observations in this study, the concentrations in STPs or water environments varied among the countries, which may be due to the differences in usage of the drugs, wastewater treatment systems, dilution factors after discharge of wastewater, and so on. In other words, if the concentrations of both stable and labile PPCPs (e.g., CBZ and CFF, respectively) in STPs and receiving waters are monitored in other areas or countries, the assessment proposed in this study can be applicable.

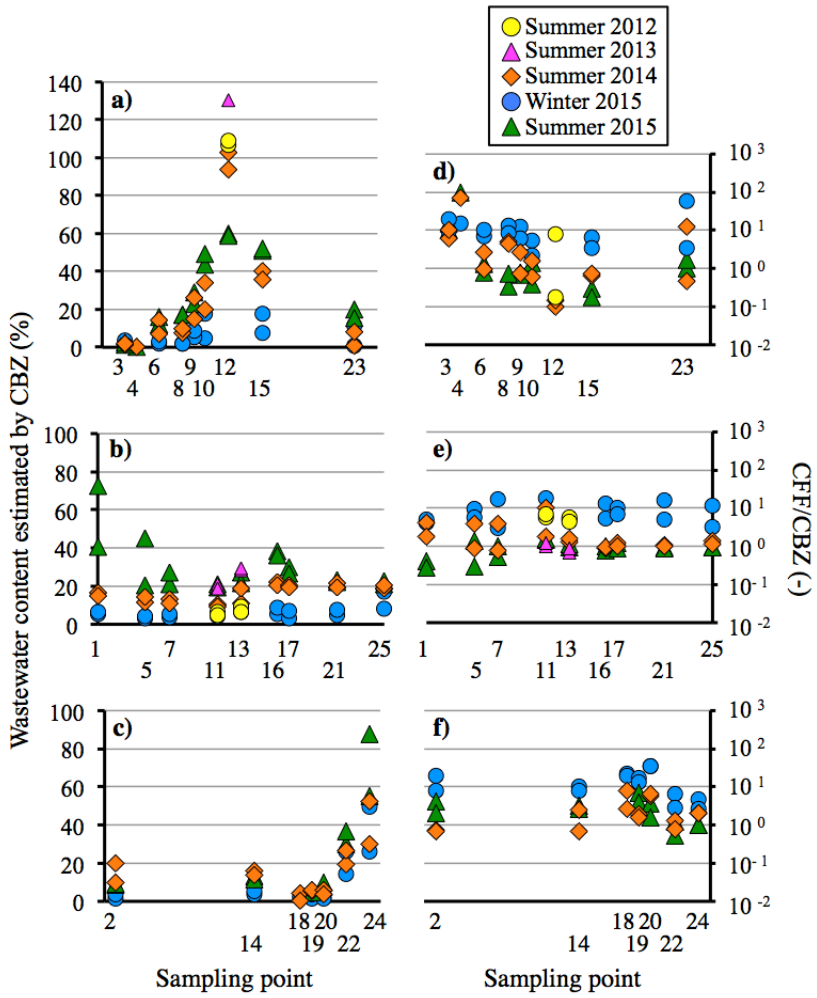


Figure 4. Wastewater content estimated by CBZ and ratio of CFF/CBZ for the estimation of treatment efficiency in the northern tributaries (top: a, d, respectively), the main stem of the Thames, (middle: b, e) and the southern tributaries (bottom: c, f).

3.7. Estimation of CP in the basin and validation

We estimated cumulative human populations along the Thames and its tributaries from the UD of CBZ (UD_{CBZ}) and the excretion rate (E_{CBZ}) (CP_{UD}, eq. 6) and from the WWC calculated from CBZ ($WWC_{CBZ} = C_{CBZ,river}/ C_{CBZ,influent}$), daily river flow ($F_{river}$) and the *DW* ($CP_{WWC}$, eq. 7). This was then compared with that based on the information supplied by the UK Water Industry which is incorporated in the LF2000WQX model (CP_{model})[30] (Figure 5). The population is the equivalent upstream of each sampling point. A consumption value UD_{CBZ} = 2.22 mg per person per day was calculated by dividing the total prescription of CBZ in England [31] by the total population. The total population and *DW* in the area were set as 53.9 million in 2013[32] and as 236 L/person/day (calculated from the average dry weather flows and connected populations: Table S1), respectively. For E_{CBZ}, it is known that the liver metabolizes almost 70% of orally administered CBZ and only a small percentage is excreted in urine[33]. However, the remaining, approximately, 30% of CBZ is unchanged and subsequently discharged with the faeces. Ort et al.[34] defined the rate of CBZ dissolved as 10% of the excretion based on monitoring at STPs in Switzerland, so this value (10%) was used as E_{CBZ}. Daily rive r flow (F_{river}) data was available until September 2014 at the time of writing[35].

There was a strong relationship between CP_{WWC} and CP_{model} on the two sampling dates in the summer 2014 campaign (R^2 = 0.94, Figure 5a). A few outliers were observed for small catchments (<1000 persons) in tributaries. The CP_{WWC} suggested a relatively smaller population than that supplied by the Water Industry in the LF2000WQX model for the catchments, which was based on data obtained in 2005[26].

The CP estimated from UD_{CBZ} (CP_{UD}) had a very similar relationship with CP_{model} (data not shown) as CP_{WWC}. In fact, the relation between CP_{UD} and CP_{WWC} had a very high correlation (R^2=1.0) (Figure 5b), but CP_{UD} suggested a relatively smaller population than that

indicated by CP_{WWC}. This might be due to an overestimation of the excretion rate CBZ (E_{CBZ}) and/or UD_{CBZ}. The overestimation and/or seasonal fluctuation of *DW* can be normalized by the concentration of CBZ in influent. Future work will study the limitations of this approach. The CP values were determined from *eqs. 6* and *7*, and the differences in the equations are the three constants: CBZ concentration in STP influent (618 ng/L, Table 1), UD_{CBZ} and *DW*. This relationship implies that the three parameters relate to the catchment population and each other.

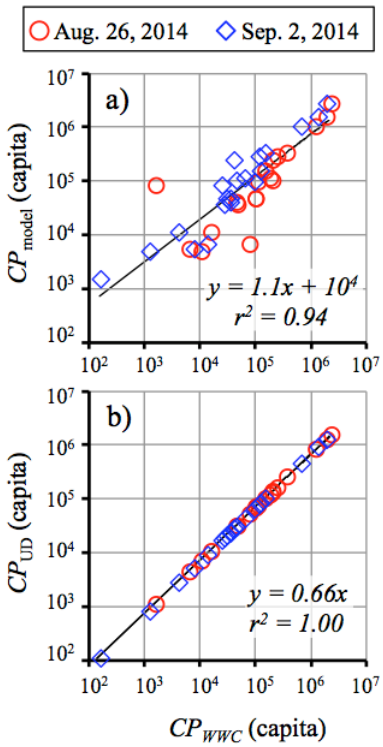


Figure 5. Relationships of cumulative population (*CP*) in the River Thames basin estimated from WWC_{CBZ} (CP_{WWC}) with those estimated by (a) the LF2000WQX model (CP_{model}) and (b) unit dose of CBZ (CP_{UD}).

4. Conclusion

CBZ and CFF were selected as stable and labile marker in the River Thames basin, respectively, through a survey for 53 pharmaceuticals and personal care products in the basin for 4 years. A ratio of CFF to CBZ was used to estimate the efficiency of wastewater treatment and clearly indicated the difference in the efficiency at the different treatment systems (i.e. AS or TF) in summer and winter. The ratio revealed a discharge of wastewater without appropriate treatment in a short period at a small brook. WWC in the river was estimated from the CBZ concentrations in each survey. The estimated WWC were less than 20% at the most sites, except in a dry summer and for the sites (up to 95%) receive effluent from major STPs serving the towns or urban areas. Cumulative human populations along the catchment were estimated from CBZ concentration and the estimated one had a strong relationship (R^2 = 0.94) with that reported by a local water company.

This assessment approach, involving markers, proved useful for estimating WWC, efficiency of wastewater treatment and then the cumulative human population in the River Thames basin. The assessment can be applicable to other areas or countries, if the concentrations of both stable and labile PPCPs in STPs and receiving water environment are monitored. The information was obtained relatively quickly and at lower cost than a census, and could be useful for maintaining public health and safety within the river basin by identifying untreated or poorly treated wastewater, which contains chemicals and pathogens from our daily life.

ASSOCIATED CONTENT

Supplementary material

Details of sewage treatment plants surveyed, information on monitoring site, catchment characterization and sampling, and summary of field measurements, and additional references can be found in the Supplementary material (Figures S1–S3 and Tables S1–S4).

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