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Linking changes in antibiotic effluent concentrations to flow, removal and consumption in four different UK sewage treatment plants over four years

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Abstract

The arrival and discharge of seven antibiotics were monitored at two trickling filter sewage treatment plants of 6,000 and 11,000 population equivalents (PE) and two activated sludge plants of 33,000 and 162,000 PE in Southern England. The investigation consisted of 24 h composite samples taken on two separate days every summer from 2012 to 2015 and in the winter of 2015 (January) from influent and effluent. The average influent concentrations generally matched predictions based on England-wide prescription data for trimethoprim, sulfamethoxazole, azithromycin, oxytetracycline and levofloxacin (within 3-fold), but were 3-10 times less for clarithromycin, whilst tetracycline influent concentrations were 5-17 times greater than expected. Over the four years, effluent concentrations at a single sewage plant varied by up to 16-fold for clarithromycin, 10-fold for levofloxacin and sulfamethoxazole, 7-fold for oxytetracycline, 6-fold for tetracycline, 4-fold for azithromycin and 3-fold for trimethoprim. The study attempted to identify the principal reasons for this variation in effluent concentration. By measuring carbamazepine and using it as a conservative indicator of transport through the treatment process, it was found that flow and hence concentration could alter by up to 5-fold. Measuring influent and effluent concentrations allowed assessments to be made of removal efficiency. In the two activated sludge plants, antibiotic removal rates were similar for the tested antibiotics but could vary by several-fold at the trickling filter plants. However, for clarithromycin and levofloxacin the variations in effluent concentration were above that which could be explained by either flow and/or removal alone so here year on year changes in consumption are likely to have played a role.

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Highlights

- Concentrations of 7 antibiotics were measured in 4 sewage treatment plants (STPs)
- Prescription data was used to predict concentrations in influent and effluent
- Measured concentrations and removal rates in STPs were very variable
- Average measured values were in line with predictions for 5 of 7 antibiotics
- Changes in flow, removal rates and consumption influenced effluent levels

Capsule

Considerable variation in effluent concentration for 7 antibiotics in 4 sewage treatment plants over 4 years was observed. This variation was driven by changes in wastewater flow, removal rates and local drug consumption

Keywords

antibiotics
sulfamethoxazole
clarithromycin
trimethoprim
tetracycline
effluent

Abbreviations

TRIM – trimethoprim, SMX – sulfamethoxazole, CBZ – Carbamazepine, CLAR – clarithromycin, AZO – azithromycin, TET – tetracycline, OXY – oxytetracycline, LEVO – levofloxacin, STP – sewage treatment plant, AS – activated sludge, TF – trickling filter

1. Introduction

Prioritisation exercises for pharmaceuticals in the environment typically list antibiotics as one of the groups of highest concern (Besse and Garric, 2008; Christensen et al., 2009; Cooper et al., 2008). Their high ranking is linked not only to their high levels of consumption and toxicity to aquatic wildlife but also to concerns over possible links to antibiotic resistance which could have consequences for mankind (Ågerstrand et al., 2015). This was prompted by the co-occurrence of antibiotics and antibiotic resistance genes in some river environments (Amos et al., 2015; Huerta et al., 2013; Marti et al., 2013; Marti et al., 2014; Rodriguez-Mozaz et al., 2015). Geographic based modelling has been used as part of the risk assessment process for antibiotics (Johnson et al., 2015; Singer et al., 2014). If we should wish to remove more antibiotics in sewage treatment, it will be

important to identify the factors associated with good performance. Currently this is difficult due to the surprisingly wide variety in effluent concentrations and apparent removal rates of similar antibiotics found in the literature.

There have been a number of studies which have examined temporal changes in antibiotic loadings in sewage treatment plants (STPs) ranging from daily (Coutu et al., 2013; Singer et al., 2014) to seasonal (Gracia-Lor et al., 2012). Probably the most notable observation has been a seasonal increase in consumption for some antibiotics associated with winter. For example, in the Czech Republic and Switzerland, both clarithromycin and trimethoprim consumption doubled in winter (Coutu et al., 2013; Golovko et al., 2014; McArdell et al., 2003). For sulfamethoxazole, concentrations in influent rose by one-quarter to one-third in winter in the Czech Republic, Greece and China (Golovko et al., 2014; Kosma et al., 2014; Zhang et al., 2015), but in Portugal consumption was higher in spring than summer for the two antibiotics studied (azithromycin and ciprofloxacin) (Pereira et al., 2015). Sometimes the observed loadings (consumption) appear to be a lot higher than might have been expected from reviewing national or regional prescription data (Singer et al., 2014).

There have not been many studies reviewing the performance of different sewage treatment types with respect to antibiotics, but in a Chinese study a membrane bioreactor gave better removal performance for trimethoprim than a conventional activated sludge (AS) plant (Sui et al., 2011). In the UK there did not appear to be a consistent trend in performance between activated sludge plants or trickling filters with respect to oxytetracycline removal (Gardner et al., 2013).

Regarding seasonal changes in sewage treatment removal performance, for the Czech Republic it appeared that clarithromycin and trimethoprim removal improved by 20% in summer compared to winter, whilst levofloxacin and sulfamethoxazole improved by 10% (Golovko et al., 2014). In a Chinese study, trimethoprim removal performance improved from 30 to 80% in the Beijing summer, which might have been temperature related (Sui et al., 2011). In Portugal, higher azithromycin and ciprofloxacin removal was reported in summer compared to spring (80% versus 50%).

These studies imply consumption for some antibiotics could increase between two and four-fold in winter and removal performance decline by more than 2/3. However, this may not always lead to higher antibiotic concentrations in the river. Many Western countries experience their

highest river flows in winter, so that dilution might rise 30-fold (Johnson, 2010) which more than compensates for relatively minor changes in seasonal antibiotic use or removal efficiency.

Changes in effluent concentrations of antibiotics over several years at the same STP has not been examined before. Nor have there been serious attempts to disentangle the reasons for variations in effluent concentrations over long time-scales. A better understanding of why there are differences in antibiotic discharge between STPs would assist both risk assessment and a strategy to improve their removal from wastewater.

Following the development of a method, which permitted the simultaneous analysis of several pharmaceuticals in wastewater including some key antibiotics, a study was prepared to look at several antibiotics in use in the UK. Sulfamethoxazole (SMX) is used to treat infections such as urinary tract, inner ear, prostitis and bronchitis. It is one of the sulphonamide group which entered the market in the 1970s and inhibits an enzyme involved in the synthesis of tetrahydrafolic acid (part of the thymidine metabolic pathway in DNA synthesis) (Seydel et al., 1972). Trimethoprim (TRIM) is used to treat a number of infections including those of the urinary and respiratory tract and belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors. TRIM acts by targeting an enzyme involved in the tetrahydrafolic acid pathway and so SMX and TRIM have often been used together in therapy since the late 1960s (Burchall, 1973; Seydel et al., 1972). Clarithromycin (CLAR) is used to treat infections such as skin, throat and pneumonia. Azithromycin (AZO) has been used to treat throat, intestinal and sexually transmitted infections. Both of these are macrolide antibiotics which came on the market in the early 1990s and bind to the microbial 50s ribosome sub-unit thereby inhibiting protein synthesis (Piscitelli et al., 1992; Retsema et al., 1987). Tetracycline (TET) is often used in treating skin infections and Lyme disease whilst oxytetracycline (OXY) has been used to treat skin, chest and genital infections. These two antibiotics have been in use since the 1960s and target the microbial 30s ribosomal sub-unit to inhibit protein synthesis (Gale, 1963). Levofloxacin (LEVO) has been used to treat a range of infections including intestinal, pneumonia, urinary tract and prostitis. It belongs to the fluoroquinolone group of antibiotics, which became widely used from 1990, and function by inhibiting the DNA gyrase and topoisomerase IV enzymes (Drlica and Zhao, 1997; Hooper et al., 1987).

By looking at 4 different sewage treatment plants over 4 years, sampling on two occasions each year through taking 24 h composite samples, this study attempted to address the following questions:

How variable are antibiotic effluent concentrations over four years within four different UK sewage treatment plants? To what extent are antibiotic influent and effluent concentrations predictable based on National consumption rates? To what extent does sewage treatment type influence removal performance? How important are flow, removal performance or changes in drug consumption in the variability of antibiotic effluent concentrations?

2. Materials and methods

2.1. Sampling approach at the treatment plants and flow assessment

Four separate STPs in Southern England, UK were examined (Table 1). Two plants were of the activated sludge (AS) type (Ox and Did) and two smaller plants of the trickling filter (TF) type (Ben and Cho). In the UK, AS plants have a hydraulic residence time in the region of 10-14 h and TF plants only 0.5 h. The AS plants handle the most wastewater but TFs are the most numerous (Johnson et al., 2007). A 24 hr composite sample was collected by combining hourly samples using an autosampler (Isco Avalanche, Isco 6712, Hach Sigma SD 900 or Bühler Montec Xian 1000). Each treatment plant was sampled, using composite samplers, on two separate occasions during each sampling campaign in June 2012, August 2013, August 2014 and January and August 2015. There have been issues with insufficient or inappropriate sample frequency leading to a number of misinterpretations in understanding the fate of pharmaceuticals in treatment plants (Ort et al., 2010). One example is that in periods of high rainfall both rainwater and elevated groundwater can enter the sewer system, which could have the effect of greatly diluting concentrations of chemicals derived from local households. However, these sampling periods did not occur during periods of high rainfall in the region (Table S1). In addition, this study used carbamazepine as a form of conservative tracer to help avoid misinterpretations of the relative influence of flow versus other losses as described further below.

Table 1 Sewage treatment plants sampled in the survey

Type	Tertiary treatment	Name	Human PE (k)	Ave. dry weather flow (m^3/d)	L/person
AS	None	Ox	162.8	38,000	233
AS	Sand filter	Did	31.7	8,000	252
TF	Particle filter	Cho	11.3	2,406	213
TF	Nitrifying Fixed bed bioreactor & Particle filter	Ben	5.9	1,368	232

AS Activated Sludge
TF Biological filter (trickling filter)
Human PE human population equivalent = head of population connected to the sewage plant
Values from the database for the LF2000WQX model (Williams et al., 2009)

Unfortunately, daily flow values at the STPs were not available, only the consented flow (Table 1). In the UK, sewage treatment is managed by private companies and there is no requirement to make such information publically available. However, the conservative pharmaceutical carbamazepine (CBZ) makes a very suitable indicator for changes in flow. It is very widely prescribed for epilepsy patients, who take this medication at the same dose throughout the year, and its remarkable persistence has been noted before (Clara et al., 2004; Nakada et al., 2008). Measurements taken in this study showed no decline between the influent and effluent (Tables S4 and S6).

2.2 Analytical approach

The method of pharmaceutical analysis described elsewhere (Narumiya et al., 2013) was used with minor modifications. Briefly, water samples were taken in polyethylene bottles or buckets, to which 1 g/L ascorbic acid had been added as a preservative. Immediately after being taken, the samples were filtered through glass fibre filters (GF/B, 1.0 µm, Whatman, UK) and EDTA (to be approximately 1 g/L) and a 50 µL of surrogate mixture (1 mg/L of each isotope-labelled pharmaceutical dissolved in methanol) were added into the filtrate. The antibiotics were concentrated by solid-phase extraction in an OASIS HLB cartridge (200 mg, 6 cm^3, Waters, MA) within 1 d after sampling. The cartridges were kept at 4 °C in darkness and transported to Kyoto University, Japan, where the compounds retained in the cartridge were eluted with 6mL of methanol after being dried for 1 h under gentle air pressure in a glass manifold. The eluents were

dried with nitrogen gas and dissolved in 1 mL of 0.1% formic acid and methanol (85:15, v/v). The antibiotics were measured by ultra-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) and the values quantified were correlated with the recovery rate of the surrogate (Narumiya et al., 2013). Antibiotic concentrations were reported after a three-tiered assessment (Kuroda et al., 2015) as follows. First, the concentration in the sample in the injection vial had to be more than three times higher than that in the running blank for each sampling day; otherwise, the concentration was reported as below the limit of quantification (<LOQ). Second, the signal to noise ratio (S/N) had to be more than 10; otherwise, the concentration was also reported as <LOQ. Finally, the recovery of surrogate compounds had to be more than 30%; otherwise, the concentration was reported as LR (low recovery). For running blank samples, 100 - 500 mL of ultrapure water (Milli-Q waters) was analysed in each week during the sampling period. The LOQ was set at the larger value of either three times of the running blank concentration or the sample concentration with a signal to noise ratio of 10.

2.3 Predicting antibiotic per capita consumption, influent and effluent concentrations

It is possible to access English data on prescriptions from the Health and Social Care Information website for pharmaceutical consumption http://www.hscic.gov.uk/searchcatalogue?productid=17711. In theory, this data should be a reasonable guide, since in the UK antibiotics are only obtained by prescription from a medical practice. This can be used to calculate per capita consumption using the population data for that year obtained from the Office for National statistics http://www.ons.gov.uk/ons/rel/pop-estimate/population-estimates-for-uk--england-and-wales--scotland-and-northern-ireland/2013/index.html To predict influent concentrations, the amount of intact parent compound excreted by patients needs to be found from the medical literature. This will have some limitations as it is not always clear whether a study refers to a conjugated or free compound, and faecal excretion is often not reported. In this study a mean as well as highest and lowest excretion value was recorded (Table S2). From this, a probable influent concentration range can be predicted using the mean wastewater discharge per capita of 233 L/d for these STPs (Table 1). Removal rates for sewage

treatment can also be compiled from the literature and are reported as a weighted mean, highest and lowest removal (Table S3).

To summarise, the influent concentrations are predicted by taking the drug consumption per capita for a nation less that prevented from being excreted as the free parent compound. For the effluent concentration the value is modified by that removed in sewage treatment. So, for example, the effluent concentration (*W*, in ng/L) is derived as follows:

$$W = \frac{(C \times E \times (1-R))}{D}$$

Where *C* is the substance consumption (ng/cap/d); *E* is the substance amount not excreted (ng/cap/d); *R* is the amount of the drug that is prevented from escaping into sewage effluent (ng/cap/d); and *D* is the volume of wastewater (L/cap/d).

The mean excretion and mean sewage removal rate can be combined to report the expected effluent concentration, whilst the lowest excretion rate and highest sewage removal rate predict the best possible effluent concentration and finally, the highest excretion rate and lowest sewage removal rate predict the worst possible effluent concentration.

3. Results and discussion

In this discussion of the results, it is important to distinguish between observations of effluent concentration, which is about exposure, and an explanation of the factors which might be driving that variation in effluent concentration. Recording the differences in effluent concentration has a value in its own right since it is important to assess and predict environmental exposure as accurately as we can. Exposure models need corroboration with measured data. Then there is the issue of trying to identify the principal cause behind that variability. There are three major candidates to explain the variability in effluent exposure, these are; that changes in flow (dilution) is the driving variable in the STP, that the quantity of drugs arriving at the STP is changing (human consumption), or that removal within the STP is fluctuating. As tools to examine why there are

differences in effluent concentration, we can compare with the influent concentration (to give an indication of removal) and with a conservative indicator to tell us about changes in flow. Carbamazepine (CBZ) is a suitable conservative indicator, because the molecule is very resistant to degradation and as an epilepsy drug it is consumed at constant rates throughout the year (Clara et al., 2004; Nakada et al., 2008).

3.1 Comparison of measured antibiotic concentrations with those predicted by national consumption

A fundamental part of predicting antibiotic concentrations in sewage is the use of national consumption statistics followed by an assessment of patient excretion. In this study, this approach gave acceptable predictions for most antibiotics (Table 2). However, assuming the excretion values were not erroneous (Table S2), this region appears to consume significantly less CLAR and more TET than expected for England as a whole.

Table 2. Consumption, predicted influent and effluent concentrations of the selected antibiotics together with measured influent and effluent concentrations. Numbers in parentheses are highest and lowest expected, or highest and lowest measured.

	TRIM	SMX	CLAR	AZO	TET	OXY	LEVO
Influent							
2014 National use (kg/yr)	11,599	2,255	18,796	2,579	903	16,473	116
Individual (mg/cap/d)	0.590	0.115	0.955	0.131	0.046	0.837	0.006
Expected (ng/L)[a]	1163 (1087-1517)	89 (49-148)	2049 (1517-2459)	225 (67-264)	118 (128-138)	1796 (1078-2515)	21 (17-24)
Measured Ox (ng/L)	733 (574-1022)	230 (128-356)	533 (247-773)	368 (214-507)	1660[c]	827[c]	28 (<LOQ-56)
Measured Did (ng/L)	575 (313-939)	138 (29-244)	454 (331-579)	207 (77-292)	1887 (747-3028)	1099 (598-1600)	22 (<LOQ-79)
Measured Cho (ng/L)	588 (202-1582)	163 (62-318)	421 (123-641)	222 (105-359)	1166 (845-1486)	492 (112-872)	1.8[d] (<LOQ 11)
Measured Ben (ng/L)	327 (88-529)	32 (11-53)	184 (<LOQ-497)	150 (108-213)	564[c]	1406[c]	40 (<LOQ -119)
Comment on influents	1.6-3.6 x less than expected ≈as expected	≈as expected	3.8-11 x less than expected, perhaps due to low consumption	≈as expected	4.8-16x more than expected	1.3-3.6 x less than expected ≈as expected	≈as expected
Effluent							
Expected (ng/L)[b]	885 (338-1504)	46 (12-147)	1517 (136-2435)	164 (30-264)	54 (1.2-131)	395 (97-1886)	11 (4.3-18)
Measured Ox (ng/L)	359 (294-455)	88 (59-141)	98 (24-377)	156 (69-264)	45 (25-84)	32 (17-40)	5.3 (<LOQ-16)
Measured Did (ng/L)	243 (160-305)	92 (24-140)	104 (40-221)	110 (74-215)	119 (77-173)	84 (43-144)	5.9 (<LOQ-11)
Measured Cho (ng/L)	208 (128-321)	128 (61-227)	181 (92-338)	91 (48-135)	98 (58-174)	56 (21-146)	2.1 (<LOQ-4.5)
Measured Ben (ng/L)	161 (87-254)	27 (<LOQ-55)	152 (64-334)	76 (35-133)	133 (42-239)	191 (99-602)	11 (<LOQ-47)
Comment on effluents	2.5-5.5 x less than expected, mainly due to low influent conc.	Variable, but ≈as expected	8.4-16 x less than expected mainly due to low influent conc.	≈as predicted	Variation between STPs but closer to expectation than influent	Less than expected, high variation between STPs	≈as expected

[a] Calculated with local wastewater discharge of 233 L/cap/d and range of excretion proportions [weighted average (min-max)] given in table S1

[b] Calculated with STP removal rates given in table S2: expected value=average excretion with average removal, range=best case (lowest excretion with highest removal) to worst case (highest excretion with lowest removal)l

[c] Only one valid measurement

[d] Very uncertain average, because 5 of 6 values were <LOQ (set to 0 for the calculation)

250 *3.2 Variation in carbamazepine concentrations and the relationship to STP flow*

251 In the absence of metered flow values for the STPs, changes in CBZ concentration were used
252 as an indicative marker of changes of flow within the sewage treatment system. To serve as a
253 useful conservative marker it should be readily detectable at all the plants at similar concentrations
254 proportional to the human population. This also assumes that a high proportion of the population
255 consistently consumes and excretes the drug. In fact, the measurements were broadly similar at all
256 sites with an influent summer average of 613 ng/L of CBZ. The compound also did demonstrate a
257 conservative nature as the effluent concentration was between 94 and 144% of the influent
258 concentration across the different STPs over the 4 years. The variation over all years sampled in the
259 influent CBZ concentration was 2-3 fold at Ox and Ben with 4-fold at Did and 5-fold at Cho. Thus,
260 up to a 5-fold variation in influent antibiotic concentration over all years sampled at a STP could be
261 considered 'natural' and potentially linked to flow. It was noticeable that the January CBZ
262 concentration was half that of the summer average. This would be consistent with a doubling of
263 sewer flow associated with an ingress of rain or groundwater into the system in winter.
264
265 *3.3 Variation in antibiotic influent and effluent concentrations*

266 Measurements in the influent proved somewhat challenging with some recovery problems
267 in this matrix (Table S4), thus the averages shown in figure 1 are sometimes from a smaller number
268 of successful measurements. Typically, influent concentrations for these different antibiotics can
269 double (or halve) depending on the year (Table S5) but the differences can be quite large for some,
270 such as 8-fold for OXY and TRIM at Cho compared to only 5-fold for CBZ at this plant. Similarly, an
271 8-fold variation in influent concentrations of SMX were observed at Did over 3 years compared to a
272 4-fold variation in CBZ. This compares with a 3-5-fold seasonal variation in antibiotic loading over
273 the course of one year at a Swiss STP (Coutu et al., 2013).
274

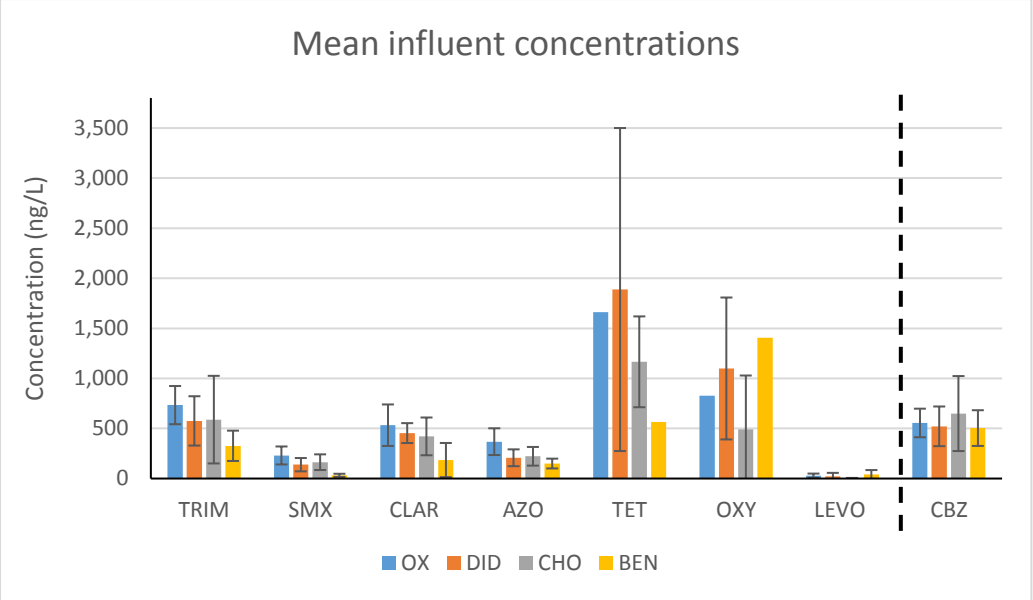

Figure 1. Antibiotic and carbamazepine influent concentrations (mean and SD) at the different plants over four years. Note for each STP the number of available influent values were for TRIM n=6-10, for SMX n=7-10, for CLAR n=5-7, for AZO n=4-6, for TET n=1-2, for OXY n=1-2, for LEVO n=1-5 and for CBZ n=5-10 (full details in Table S4).

It was planned to take two separate composite samples for both influent and effluent at each STP a few days apart. However, autosamplers failed on some occasions whilst on others the recovery was too low (less than 30%) and so the measurement was not included. The biggest problems with recovery were for TET and OXY. But, for most years, at each location, the results were from two measurements taken on separate days (Table S4).

Over the four years of sampling, effluent concentrations at an STP could vary by up to 16-fold for CLAR, 10-fold for SMX and LEVO, 7-fold for OXY, 6-fold for TET, 4-fold for AZO and 3-fold for TRIM (Table S5). In comparison the effluent concentration of the conservative pharmaceutical CBZ varied by no more than 4-fold at any STP (Table S5). For most antibiotics the highest relative variability in effluent concentrations were found at the smallest works, the trickling filter plant at Ben (6000 PE) (Fig. 2).

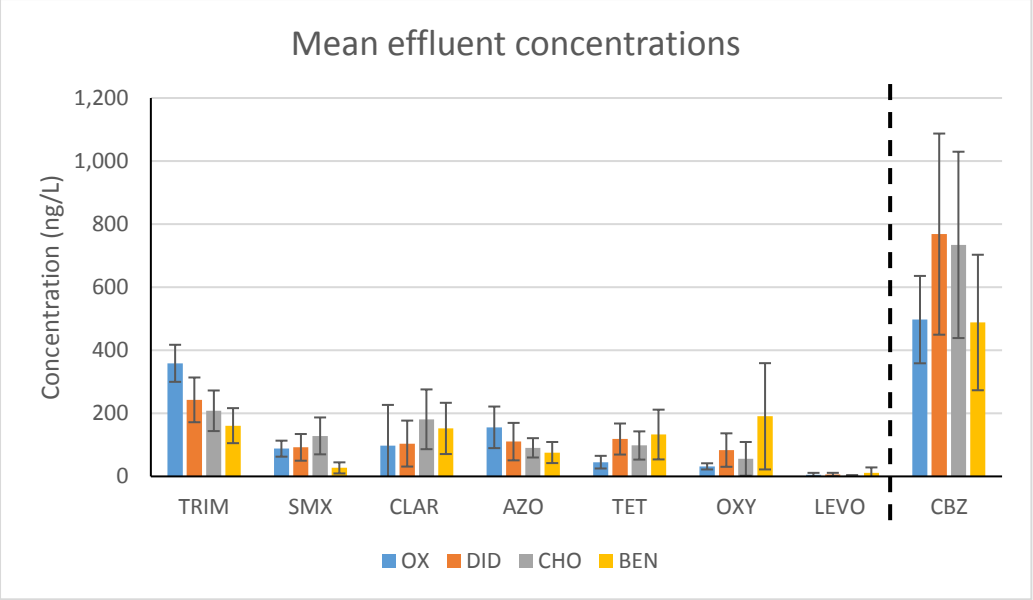

Figure 2. Antibiotic and carbamazepine effluent concentrations (mean and SD) at the different plants over four years. Note for each STP the number of available effluent values were for TRIM n=6-10, for SMX n=5-9, for CLAR n=5-10, for AZO n=5-9, for TET n=3-8, for OXY n=3-8, for LEVO n=3-6 and for CBZ n=5-10 (full details in Table S4).

3.4 The role played by changes in STP flow on antibiotic concentrations

The anti-epileptic pharmaceutical CBZ was selected as a conservative indicator and thus reveal the impact of changes in wastewater flow entering the treatment plants. Thus, an increase in CBZ concentration was interpreted as a reduction in the quantity of wastewater in the sewer (i.e. reduced dilution) whilst a big reduction of CBZ concentration would most likely reflect an ingress of rain or groundwater causing dilution in the system. As an example, for the biggest STP, Ox, it will be noted that a 26% drop in CBZ concentration which occurred in January 2015 was mirrored by most antibiotics where we have data (Fig. 3). A 1/3 increase in CBZ concentration in August 2015 was not matched by SMX or CLAR. Indeed the ratio of CLAR to CBZ is unstable indicating changes in prescription/use is most likely driving the variability in influent concentration for this antibiotic.

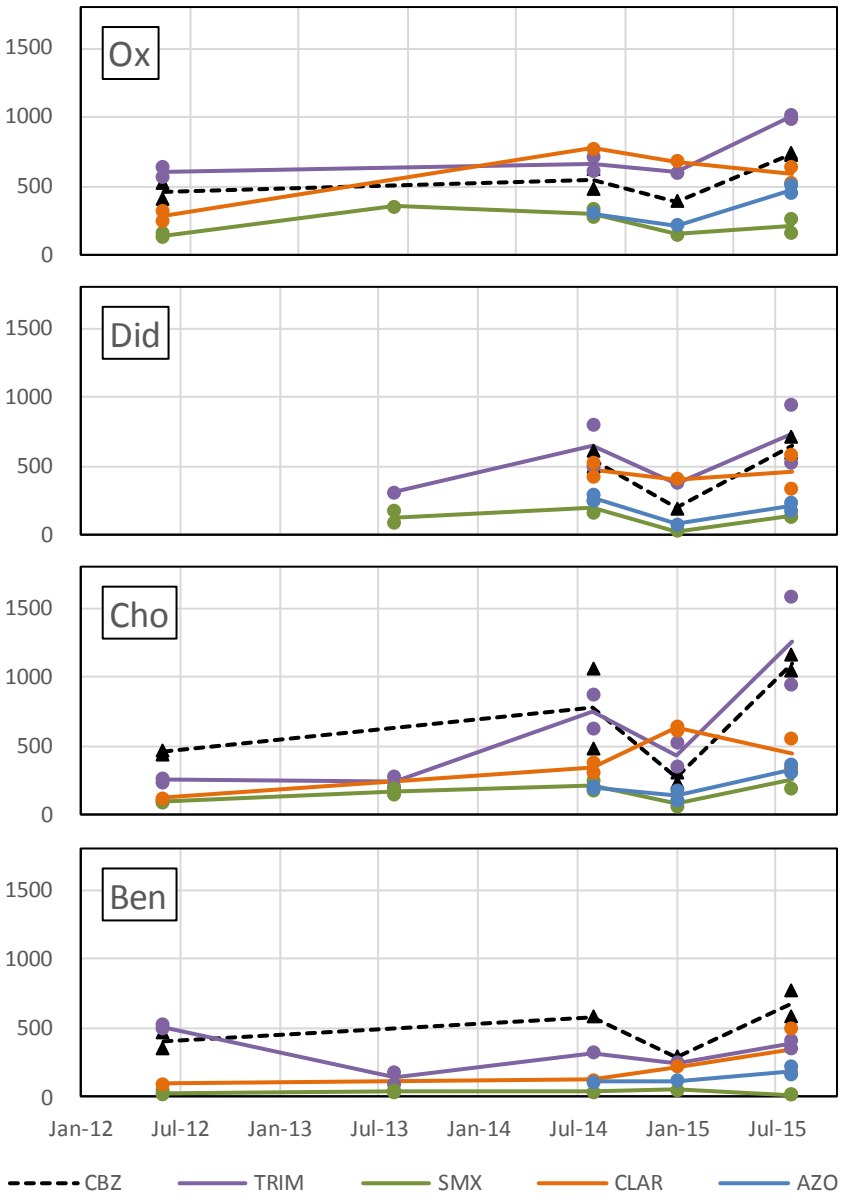

Figure 3. Comparison of some antibiotic influent concentrations (ng/l) against carbamazepine (CBZ) over four years (All data is plotted and the line is the average).

3.5. Variation in antibiotic removal performance of STPs

The AS pair of Ox and Did were not consistently better than the TF pair of Cho and Ben at removing antibiotics from the waste stream (Fig 4, Table S6), but performed on average better than the trickling filter types for two (CLAR and SMX) of the four antibiotics for which sufficient data were available for a robust comparison. The mean removals of CLAR and SMX were 79% (SD 18%) and 40% (SD 22%) respectively for the combined AS plants compared to 36% (SD 26%) and 15% (SD 40%) for the two TFs (Table S6). For TRIM there was little difference in removals between the ASPs

(47% (SD 15%) compared to 44% (SD 25%) at the TFs. For AZO at the ASPs 34% (SD 21%) was removed compared to 42% (SD 31%) at the TFs. For the remaining antibiotics LEVO, OXY and TET only 1-3 valid pairs of influent and effluent concentrations were available for each pair of sewage works making any comparisons unreliable. A previous study of the removal of trickling filter plants reported only 3 to -23% removal for SMX and 40% removal for TRIM (Kasprzyk-Hordern et al., 2009). The winter period of January 2015 did not produce noticeably worse antibiotic removal performance than the summer samplings.

Both of the TFs (Ben and Cho) showed considerable variation in antibiotic removal performance from year to year (Fig. 4). Occasionally, a higher antibiotic concentration was found in the effluent than influent of those TF plants (TRIM in 2013 in Cho and LEVO in 2012 and SMX in summer 2015 in Ben). Analysing 24-hour composite samples reduces the effects of short term variations compared to grab-samples, but temporal changes in the influent concentrations could still lead to a certain level of sample mis-match, especially if an unusually high influent concentration preceded the initiation of sampling or a high load was just about to leave in the effluent. It is also possible that higher than expected effluent concentrations were due to the delayed release of the parent molecule from a conjugate without further significant removal. Good performance in removal of one antibiotic in one year did not necessarily translate to good removal for another antibiotic. In other words performance was quite unpredictable in all senses for these TF plants.

In contrast, the AS plant Ox showed remarkable removal consistency for all antibiotics in each year with the exception of TRIM in summer 2012 and CLAR in winter 2015 (Fig. 4). The highest removals (84-97%) were for TET and OXY.

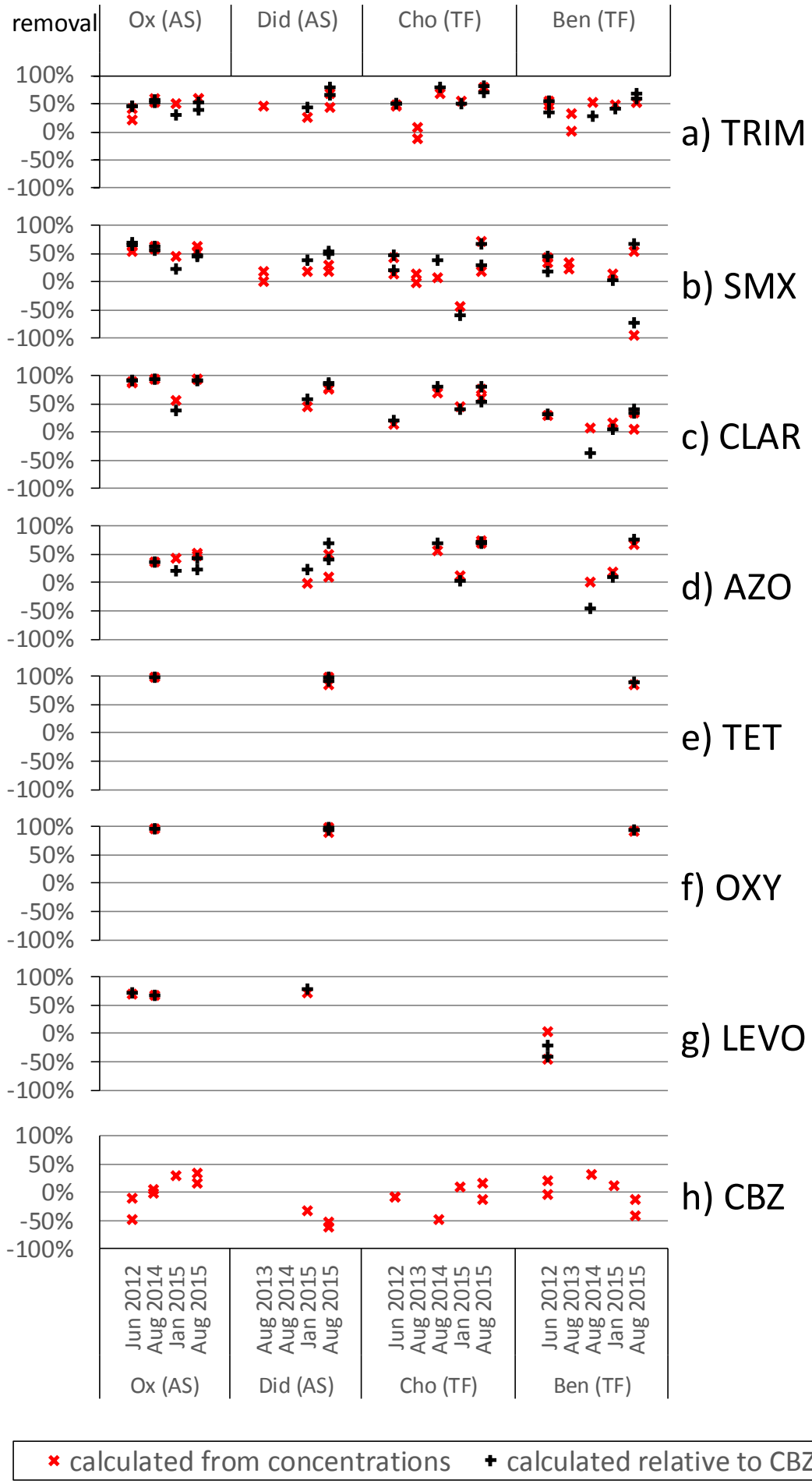
removal
Ox (AS)
Did (AS)
Cho (TF)
Ben (TF)
100%
50%
0%
-50%
-100%
a) TRIM
b) SMX
c) CLAR
d) AZO
e) TET
f) OXY
g) LEVO
h) CBZ
Jun 2012
Aug 2013
Aug 2014
Jan 2015
Aug 2015
Ox (AS)
Did (AS)
Cho (TF)
Ben (TF)
calculated from concentrations
calculated relative to CBZ

Figure 4. Differences in removal performance of antibiotics at the different treatment plants over four years. Results were calculated from valid duplicate or single paired 24 h composite influent and effluent samples. AS: activated sludge process, TF: trickling filter.

3.6 Attempting to attribute variation in effluent antibiotic concentration

As discussed previously, we may attribute variability in antibiotic effluent concentration to to changes in wastewater dilution, differences in removal rates (treatment), or to changes in drug consumption. Changes in drug consumption rates may be inferred if variability in dilution or STP removal are insufficient to account for changes in effluent concentration. Changes in flow within the STP are assessed by differences in CBZ and could account for some of the variation in every case. This was particularly notable as higher flows in January 2015 and lower than usual flows in August 2015. Secondly there is the inconsistency in the efficiency of sewage treatment (removal) most notably in the trickling filter plant Ben (Table 3). Where neither of these two factors are sufficient to explain the variability in effluent concentrations, a change in the local drug consumption may be inferred. Thanks to its greater stability of performance and flow at the largest STP studied (Ox) it is possible to attribute differences in CLAR and LEVO effluent concentrations there largely to changes in local drug consumption. For CLAR the influent concentrations during the winter sampling where higher than expected compared to CBZ (fig. 3) at all four STPs making it likely that increased prescription in winter played a role here. Similarly, much of the changes in LEVO at Ben may be attributable to consumption changes. Ben is the smallest STP studied and LEVO the rarest drug, meaning that if there are only a few people taking it at any one time, so variability can be introduced just by a single person starting or stopping taking it. However, the influence of sewage treatment performance for this antibiotic cannot be ruled out because there is insufficient data to quantify the performance and its variability (fig. 4 and table S6).

371 **Table 3. Analysis of the largest recorded incidences of variation in antibiotic effluent**
372 **concentration**
373

STP	Antibiotic	Change in effluent concentration	Change in carbamazepine dilution marker in effluent	STP removal performance	Likely attribution of variability
Ox	CLAR	16-fold	2-fold	50-95% (2-fold)	Largely consumption changes
	LEVO	5-fold		Consistent 75%	Largely consumption changes
Did	CLAR	5-fold	4-fold	50-75% (1.5-fold)	Instability in flow and removal
	SMX	6-fold		0-30% (several-fold)	Instability in flow and removal
Cho	OXY	7-fold	5-fold	Variability unknown	Changes in flow plus unknown
Ben	CLAR	5-fold	4-fold	5-25% (5-fold)	Instability in flow and removal
	SMX	10-fold		20-50% (2.5-fold)	Changes in local flow, removal and consumption combined
	LEVO	10-fold		0%	Largely a consumption issue with a smaller influence of changes in local flow
	OXY	6-fold		Variability unknown	Changes in flow plus unknown
	TET	6-fold		Variability unknown	Changes in flow plus unknown

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375

376 ## 4. Conclusions

377 The average influent concentrations of the four sewage plants fell within a maximum factor
378 of 4 of average predictions based on England-wide prescription data for SMX, TRIM, AZO, OXY, and
379 LEVO but were 4-11 times less for CLAR whilst TET influent concentrations were 5-16 times greater
380 than expected. For the two trickling filter plants, removal rates were very variable both year on
381 year and without consistency between antibiotics. The large activated sludge plant (Ox) showed
382 more consistent removal and better performance than the other plants for CLAR and SMX. Over
383 the four years, sewage effluent concentrations varied up to 16-fold CLAR, 10-fold for LEVO and
384 SMX, 7-fold for oxytetracycline OXY, 6-fold for TET, 4-fold for AZO and 3-fold for TRIM.

Changes in flow and removal performance within the STPs were clearly playing an important role in this variability of effluent concentration. However, these were not sufficient to account for all the variability in some cases for CLAR and LEVO and here year on year and seasonal changes in prescriptions and regional prescription preferences are likely to have played a role.

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