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Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water

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ABSTRACT

Various pharmaceutical residues are being discharged from wastewater treatment works (WTW) effluents, the impact of which on river water quality is of high relevance to environmental risk assessment. The concentrations of eleven pharmaceutical compounds were determined in three WTWs in England, and the river Ouse receiving effluents from Scaynes Hill WTW. Results show that five compounds propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac were detected in all wastewater and river water samples, with carbamazepine showing the highest concentrations (up to 2336 ng L^{-1}) in WTW influent. Different compounds were removed to different extent in the WTWs, varying from 43 to 92%, with the highest performance obtained by the WTW with tertiary treatment (sand filtration). The pharmaceutical residues from Scaynes Hill WTW were eventually discharged into the river Ouse, causing an elevation in their concentrations downstream of the outfall. This was confirmed by the good agreement between measured concentrations and those predicted by a simple dilution model.

1. Introduction

Pharmaceuticals are a class of emerging environmental pollutants that are widely used both in human and veterinary medicine. They are known to be ubiquitous in the environment, as many pharmaceuticals have been detected in wastewater treatment works (WTW) effluents, surface water and groundwater worldwide [1–4]. There is limited data available on bioaccumulation of drug residues in organisms [3] and only a few specific cases have emerged to date showing the serious impact pharmaceuticals can have on wildlife. In India and Pakistan, a common vulture species suffered a severe population collapse, which was suggested to be caused by an analgesic and anti-inflammatory drug, diclofenac. The drug was regularly used for veterinary medication and residues entered the vultures as they fed on dead domestic livestock, causing renal failure and resulting in an over 95% decline in some populations since early 1990s [5]. Another study found the same drug to cause vitellogenin induction in male Japanese medaka (fish) at environmentally relevant concentrations of just 1 μg L^{-1} [6]. Although evidence is limited, it is clear that pharmaceuticals have the potential to cause serious harm to wildlife and also to humans.

The main route to the environment for pharmaceuticals is through discharged effluent from WTW as a result of excretion from humans and animals, as well as from domestic disposal of medicinal products [3]. The concerning issue with pharmaceuticals is not their acute toxic effects but their chronic toxicity. These compounds are commonly present at low levels throughout the lifecycle of many aquatic organisms and are particularly important for those living in waters receiving sewage effluent (e.g. rivers). These chemicals are persistent and/or biologically active and designed to target a specific metabolic or molecular pathway. Pharmaceuticals generally are biologically active compounds that are intended not to be easily biodegradable and are often water soluble and therefore can be found in wastewaters and can easily end up in natural waters [7]. Potentially, they could have a similar function or cause side effects in non-target organisms as they do in their intended users. A good understanding of the pharmaceutical concentrations present in treated sewage effluents and their receiving river water and the rate of removal of these compounds during WTW is a necessity for improving the knowledge of their fate in the environment. This work aims to determine the concentrations of a range of pharmaceuticals in the different stages of treatment in three WTWs in the UK (Table 1), to identify the most effective treatment technology for degrading pharmaceutical residues. Secondly the concentrations of pharmaceuticals in the river Ouse close to Scaynes Hill WTW will be compared against their concentrations in effluent, so as to assess the importance of WTW as a source of pharmaceuticals in rivers.

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Table 1
Operational characteristics of the UK WTWs used in this study.

WTW	County	Treatment technology	Population covered	Population equivalent (PE)	Typical flow rate ($L s^{-1}$)
Scaynes Hill	West Sussex	Primary sedimentation, then lagoon. Secondary treatment only.	22,000	162,619	230
Manor Farm Road	Berkshire	Primary sedimentation, then two stages of biological trickling filters, finally two stages of humus tanks. Secondary treatment only.	75,000	146,678	926
Basingstoke	Hampshire	Primary sedimentation, then activated sludge process, post-sedimentation, finally sand filtration (tertiary treatment).	32,000	50,738	405

Finally, the potential toxicological impacts of pharmaceuticals on the aquatic organisms in the river Ouse will be evaluated.

2. Experimental

2.1. Chemicals and materials

All the solvents used including methanol and acetonitrile, purchased from Rathburn, were of distilled-in-glass grade. Formic acid was of high performance liquid chromatography (HPLC) grade. Propranolol, sulfamethoxazole, meberverine, thioridazine, carbamazepine, tamoxifen, mecoprop, indomethacine, diclofenac, meclofenamic acid and monensin were purchased from Sigma, UK. These target compounds were chosen based on their high risk characterisation ratio [8], quantity of chemicals used per year, reported occurrence worldwide, and availability of an analytical method. The pharmaceutical internal standards (diuron-d_6 and ^{13}C-phenacetin) were supplied by Cambridge Isotope Laboratories, USA. Stock solutions of all standards ($1000 mg L^{-1}$) were prepared from which working standards solutions ($10 mg L^{-1}$) were made. All standards and internal standards were prepared in methanol and stored in a freezer at −18 °C. Ultrapure water was from a Maxima Unit supplied by USF Elga, UK. Sodium azide, silica gel (0.063–0.2 mm) and aluminium oxide (0.05–0.15 mm, neutral) were purchased from Sigma–Aldrich Company Ltd., UK. The Oasis® HLB solid-phase extraction (SPE) cartridges (6 mL/200 mg) were obtained from Waters Ltd., UK.

2.2. Sampling and samples treatment

Samples were taken at the three WTWs used for this study. For the Scaynes Hill WTW, samples were taken at the influent inlet (influent, IN), after primary treatment (humus tank, HU), after secondary treatment (lagoon, LA), and at the effluent pipe (effluent, EF) in November 2006. For the Manor Farm Road WTW, samples taken included raw wastewater, and effluents of primary sedimentation and humus tanks. For the Basingstoke WTW, samples were taken at the influent, after sedimentation, after activated sludge process and after sand filtration. In addition, water samples were taken from the river Ouse upstream of the Scaynes Hill WTW (UW), and downstream from the WTW (DW) to assess the impact of WTW effluent on downstream water quality. Sodium azide (10 mL, 2 M) was added to each sample on site as a general biocide to eliminate bacteria and prevent sample degradation during storage and processing. The samples were stored in a refrigerator below 4 °C until filtration and extraction. Along with the water samples, a series of measurements were taken for the water quality including pH (7.0–8.2), conductivity (328–1042 μS), dissolved oxygen ($1.0–10.9 mg L^{-1}$), temperature (8.2–15.2 °C) and redox potential (−280–134 mV). The samples (1 L) were filtered under vacuum through pre-ashed glass-fibre filters (Whatman, GF/F). The filtrates were spiked with 100 ng of internal standards.

The discharge flow rates of effluent from the Scaynes Hill WTW for the period when sampling was taking place, were obtained from the operator (Southern Water), which varied between 73 and $76 L s^{-1}$. The flow rates for the River Ouse were obtained from the Environment Agency, which were measured at Ardingly Weir, approximately 9 km upstream of the outfall, and at Gold Bridge in Newick, approximately 8 km downstream of the outfall.

2.3. Sample extraction and clean-up

Filtered water samples were extracted using a SPE system from Supelco, following an established procedure [9]. The Oasis HLB (Waters) cartridges were conditioned with 10 mL of methanol, followed by ultrapure water (3 mL × 10 mL) at a rate of 1–2 $mL min^{-1}$. Then, water samples were at a flow rate of 5–10 $mL min^{-1}$. Afterwards the cartridges were dried for 30 min under full vacuum, with the analytes being eluted to 20 mL glass vials from the sorbents with 10 mL of methanol. The solvent was reduced to 0.1 mL under gentle N_2 flow.

Due to the complex nature of the wastewater samples, an additional clean-up step was required to remove the interfering species and particulate matter that could block and damage the HPLC column, and produce false mass spectrometry (MS) signals in the samples. All wastewater extracts were treated with silica:alumina (1:1) columns after N_2 blow-down. Glass columns (5 mL) were filled with ashed and deactivated silica–alumina (1:1) powder with ashed quartz wool used as stoppers at the top and bottom of the column, to which samples were added and eluted with 10 mL of methanol. N_2 blow-down was used again to reduce the sample size back to 100 μL. All the sample extracts were transferred to VectaSpin Micro centrifuge filters (0.2 μm, Whatman) and centrifuged at 7000 rpm for 10 min in order to further remove particulate matter. The extracts were further concentrated under N_2 blow-down to 100 μL ready for analysis.

2.4. Sample analyses

Liquid chromatography–tandem mass spectrometry (LC–MS–MS) coupled with electrospray ionisation was used for sample analysis, following a method developed by Zhang and Zhou [9]. The LC separation was carried out with a Waters 2695 HPLC separations module, manufactured by Waters Corporation (Milford, MA, USA), which was fitted with a Waters Symmetry C_{18} column (4.6 mm × 75 mm, with particle size 3.5 μm). The mobile phase comprised eluent A (with 0.1% formic acid in ultrapure water), solvent B (acetonitrile) and eluent C (Methanol). Flow rate was 0.2 $mL min^{-1}$ and the elution started with 10% of eluent B, followed by a 25 min gradient to 80% of eluent B and a 3 min gradient to 100% of eluent B, and then changed to 100% of eluent C within 8 min, held for 10 min and then returned back to the initial conditions within 4 min. The system re-equilibration time was 10 min and the sample injection volume was 10 μL.

The MS–MS analyses were completed with a Micromass Quattro triple-quadrupole mass spectrometer equipped with a Z-spray electrospray interface. The analyses were done in the positive ion mode. The parameters for the analysis were: electrospray source block and desolvation temperature were 100 and 300 °C, respectively; capillary and cone voltages were 3.0 kV and 30 V, respectively; argon collision gas 3.6×10^{-3} mbar; cone nitrogen gas flow and desolvation gas: 25 and 550 L h^{-1}. Following the selection of the precursor ions, product ions were obtained at a series of collision energies and were selected according to the fragmentation that produced the highest abundance of fragment ions. The optimal collision energy, cone voltage and transitions chosen for the multiple reaction monitoring (MRM) experiment were optimised and a dwell time of 100 ms was used. The mass spectrometer was operated in MRM mode with unit mass resolution on both mass analysers.

2.5. Analytical quality controls

All data were subject to strict quality control procedures, including the analysis of procedural blanks and spiked samples with each set of samples analysed. None of the target compounds were detected in the procedural blanks. Spiked water samples (100 ng of each target compound) in river (Ouse), influent and effluent matrices (Scaynes Hill WTW) were determined with good precision and recoveries. The limit of detection (LOD), mean recovery and relative standard deviation (RSD) of our analytical method for pharmaceuticals in water have been reported [9]. Briefly, the LOD of target compounds ranged from 1 to 288 pg L^{-1} in river water, and between 0.05 and 5 ng L^{-1} in wastewater samples. The recovery of most compounds is high (71–95%) except for tamoxifen (52%) and thioridazine (9%) in river water, and from 73 to 107% (except tamoxifen at 55% and thioridazine at 11%) in effluent, and 66–115% (except tamoxifen at 48% and thioridazine at 15%) in influent samples. The precision is also good, with RSD < 20% for all compounds. The internal standards diuron-d_6 and ^{13}C-phenacetin were used to compensate for losses involved in the sample extraction and work-up, to further improve the analytical quality.

3. Results and discussion

3.1. Pharmaceuticals in WTWs

The target pharmaceutical compounds were analysed in wastewater from Scaynes Hill WTW daily. Of the eleven compounds, meberverine, thioridazine, mecoprop and meclofenamic acid were all below their LOD in both wastewater and river water samples, suggesting their limited use in the UK. Tamoxifen was detected in 100% of wastewater samples, at 0.1–1.3 ng L^{-1} in influent and 0.1–0.5 ng L^{-1} in effluent samples, although it was not found in river samples. In comparison, the remaining five compounds (propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac) were detected in all water and wastewater samples (Fig. 1), suggesting their widespread and frequent use, and some level of persistence in the environment. According to the National Health Service, the quantity of the five substances dispensed in England in 2006, in primary care (excluding hospitals and retailers), varied from approximately 1 ton for sulfamethoxazole and indomethacine to 40 ton for carbamazepine. Similar to Scaynes Hill WTW, the five compounds were detected in all samples in Manor Farm Road WTW. Their concentrations varied from 65 to 1237 ng L^{-1} in influent, and from 27 to 345 ng L^{-1} in effluent. Slightly higher concentrations of these compounds were found in Basingstoke WTW, with their concentrations ranging from 124 to 1833 ng L^{-1} in influent, although similar concentrations (14–233 ng L^{-1}) were observed in effluent samples.

As shown in Table 2, the concentrations of propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac varied greatly from 24 to 2336 ng L^{-1} in influent, such a major difference in concentrations between different compounds has also been reported by Bendz et al. [10] in Källby WTW in Sweden. The concentration of propranolol (100–1090 ng L^{-1}) is similar to 542 ng L^{-1}

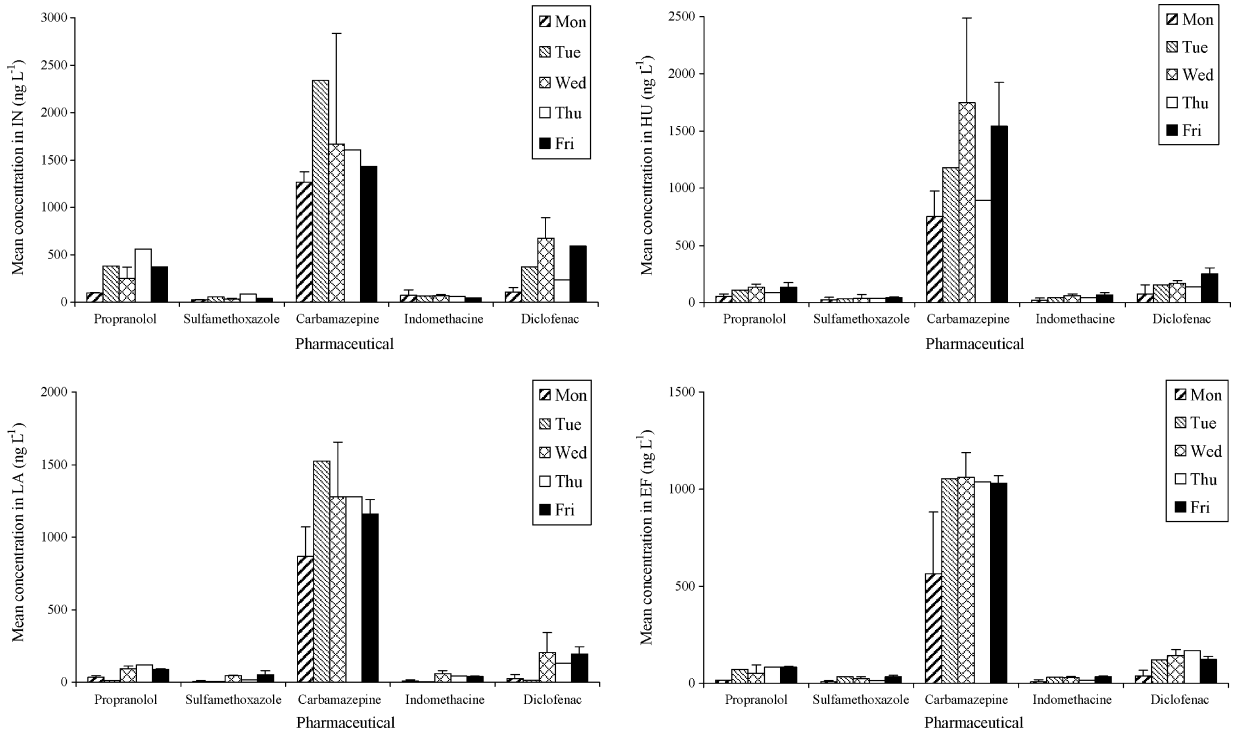

Fig. 1. The daily trend of pharmaceutical concentrations through the different treatment stages in Scaynes Hill WTW. IN, influent; HU, humus tank; LA, lagoon; EF, effluent.

Table 2
Pharmaceuticals concentrations (single value, min–max, or mean ± SD) in wastewater samples worldwide.

Pharmaceutical	WTW	Influent (ng L^{-1})	Effluent (ng L^{-1})	Apparent removal (%)	Reference
Propranolol	5 WTWs, England		16–284		[8]
	Cilfynydd, Wales	542	388	28%	[11]
	Källby, Sweden	50	30	32%	[10]
	7 WTWs, New Mexico, USA		32–77		[17]
	Sheffield Park WTW, England	100–1090	20–72	80–90%	[16]
	3 WTWs, England		16–135		This study
Sulfamethoxazole	8 WTWs, Canada		Up to 871		[12]
	5 WTWs, England		<50–132		[8]
	Källby, Sweden	20	70	0%	[10]
	Cilfynydd, Wales	<3	12	0%	[11]
	Ohio, USA	14–261	79–472		[13]
	7 WTWs, New Mexico, USA		98–2200	53–82%	[17]
	Sheffield Park WTW, England	24–181	12–25		[16]
	3 WTWs, England		8–37		This study
Carbamazepine	Källby, Sweden	1680	1180	30%	[10]
	Cilfynydd, Wales	2593	3117	0%	[11]
	Ohio, USA	25–51	34–111		[13]
	7WTWs, New Mexico, USA		70–800	43–54%	[17]
	Sheffield Park WTW, England	1237–2336	399–652		[16]
	3 WTWs, England		233–1061		This study
Tamoxifen	5 WTWs, England		42		[8]
	3 WTWs, England	0.2–1.5	0.2–0.7	32–45%	This study
Indomethacine	Sheffield Park WTW, England		6–9		[16]
	3 WTWs, England	46–124	9–35	61–89%	This study
Diclofenac	49 WTWs, Germany		Up to 2100		[1]
	5 WTWs, England		<20–2349		[8]
	Källby, Sweden	160	120	22%	[10]
	Soseigawa municipal, Japan	251 ± 100	145 ± 32		[14]
	Cilfynydd, Wales	70	123	0%	[11]
	Ohio, USA	<1–14	8–32		[13]
	Sheffield Park WTW, England		49–85	70–92%	[16]
	3 WTWs, England	107–981	37–176		This study

found in Cilfynydd WTW in Wales [11]. The concentrations of sulfamethoxazole (24–181 ng L^{-1}) and diclofenac (107–981 ng L^{-1}) are also comparable to those reported in WTW effluents in Canada [12], Ohio, USA [13] and Soseigawa, Japan [14]. Carbamazepine levels are similar to those found in Källby WTW, Sweden and Cilfynydd WTW in Wales, but significantly higher than those in Ohio, USA (Table 2).

With each stage of wastewater treatment in Scaynes Hill WTW, the concentrations of the 5 compounds showed a gradual decrease (Fig. 1). Similar to influent, the concentrations of individual compounds in the WTW effluent also varied significantly during the five day sampling period, with RSD values varying from 23% for carbamazepine to 50% for sulfamethoxazole. However, Jones et al. [15] found that the pharmaceuticals (paracetamol, salbutamol, ibuprofen and mefenamic acid) entering and leaving an activated sludge WTW did not show major changes. Zhang et al. [16] also found that the same 5 compounds being studied here did not vary significantly in their concentrations in effluent from Sheffield Park WTW, West Sussex, UK.

Overall, the concentrations of propranolol in the effluents from the three WTWs (16–135 ng L^{-1}) are comparable to 16–388 ng L^{-1} detected in other UK WTWs, 30 ng L^{-1} in Källby WTW in Sweden, and 32–77 ng L^{-1} in New Mexico, USA (Table 2). Sulfamethoxazole was detected in 100% of effluent samples at the concentrations of 8–37 ng L^{-1}. In comparison, sulfamethoxazole was only detected in 9% of effluent samples in other UK WTWs [8], albeit at similar concentrations (<50–132 ng L^{-1}). Similar concentrations of sulfamethoxazole (70 ng L^{-1}) were observed at Källby WTW in Sweden [10], but significantly higher concentrations (up to 2200 ng L^{-1}) have been determined in New Mexico, USA [17]. Carbamazepine was the dominating compound in terms of abundance in all stages of the wastewater treatment, consistent with similar findings in Sheffield Park WTW [16]. Its concentrations in effluent varied between 233 and 1061 ng L^{-1}, similar to 1180 ng L^{-1} found in Källby WTW in Sweden [10] and <1–6300 ng L^{-1} being reported in WTW effluents worldwide [18]. Tamoxifen were detected at very low level (0.1–0.7 ng L^{-1}), which are significantly lower than those found in other UK WTWs at up to 42 ng L^{-1} [8].

3.2. Removal of pharmaceuticals during WTW processes

As the wastewater was passed through the WTWs there was typically a gradual reduction in the concentrations of pharmaceutical compounds being observed, as shown in Fig. 2 for Scaynes Hill WTW. For example, primary sedimentation (humus tank) removed 24% of indomethacine, 26% of carbamazepine, 28% of sulfamethoxazole, 60% of diclofenac and 69% of propranolol. By passing through a lagoon, a further reduction in concentration of between 0% for carbamazepine and 26% for indomethacine was made, suggesting that lagoon is a relatively inefficient secondary treatment method for pharmaceuticals. The overall removal efficiency for the pharmaceuticals was calculated from the following formula:

$$\%\ \text{Apparent removal} = \frac{100(C_{IN} - C_{EF})}{C_{IN}} \quad (1)$$

where C_{IN} and C_{EF} are the daily pharmaceutical concentrations in the influent and effluent, respectively.

As shown in Table 3, the overall removal efficiency varied highly between compounds and between WTWs. A clear feature common to all three plants is that the lowest removal was found for carbamazepine, varying from 43 to 54%, no matter which treatment processes were used. Secondly, the use of tertiary treatment at Basingstoke WTW did show an improvement in the removal of all 5 compounds (from 54 to 92%) over the other two plants, suggesting that pharmaceutical residues can be removed more completely by

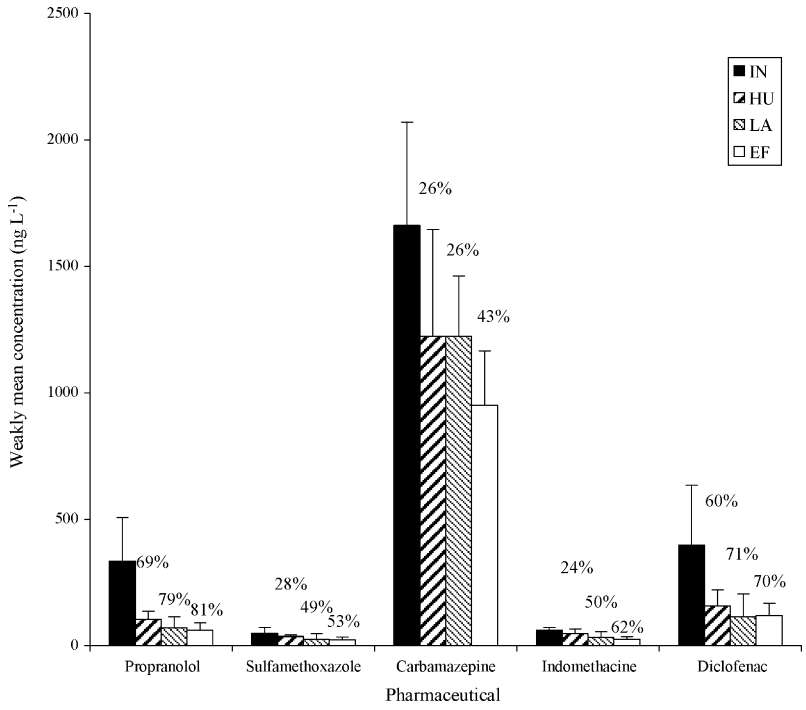

Fig. 2. Removal of five pharmaceutical compounds in the different stages of treatment in Scaynes Hill WTW. Concentrations shown are the weekly mean in influent (IN), humus tank (HU), lagoon (LA) and effluent (EF).

investment in tertiary treatment. At Scaynes Hill WTW, the removal efficiency ranged from 43% for carbamazepine to 81% for propranolol. The mean concentration of propranolol was reduced from 334 ng L^{-1} entering the works to 62 ng L^{-1} in the final effluent; a reduction of 81%. Similarly, the treatment works removed 61% of indomethacine present in the raw influent.

Lower removal efficiency of 22–32% has been reported for propranolol, carbamazepine and diclofenac at Källby WTW in Sweden [10]. Many other previous studies have shown that the reduction of pharmaceutical compounds in WTWs is often incomplete. In Brazil, removal for polar pharmaceutical compounds varied from 12 to 90% [19]. In Germany, reported reduction ranged from 10 to 90% [1], depending on the nature of the compounds. These reductions occurred in common tertiary treatment WTWs, consisting of preliminary clarification followed by aeration and then finally endpoint clarification. To achieve non-detectable concentrations of pharmaceutical residues, additional advanced treatment by oxidation (e.g. ozonation at 10–15 mg L^{-1}), activated carbon or membrane filtration is needed [20].

A further complication with pharmaceuticals is that although they may be removed by processes such as sedimentation and sand filtration, they are only temporarily stored in the sand particles by partitioning into the sludge component of the processes, which may be eventually sprayed in landfill sites, incinerated or amended to agricultural soils, posing potential threats to the environment. Only a complete degradation will provide a lasting solution to preventing pharmaceutical exposure to the environment. In addition, as no measurement was made of pharmaceutical concentration in sediments and sludge, the data did not reflect a full mass balance. Further work should also include the determination of pharmaceuticals in the particulate phase.

3.3. Pharmaceuticals in the River Ouse

In addition to sampling in Scaynes Hill WTW, the concentrations of the pharmaceutical compounds in the river Ouse close to effluent discharge were measured. River water was sampled both upstream and downstream of the WTW, to identify a potential source–sink relationship. Six compounds including meberverine, thioridazine, tamoxifen, thioridazine, monensin and meclofenamic acid were on average below the limit of detection at both river sites. The other compounds propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac were found in 100% of river samples (Fig. 3a and b), consistent with their widespread occurrence in the WTW. Their concentrations in river water were found to vary daily over the sampling period, with the exception of propranolol in upstream, confirming the need for regular sampling and analysis in order to monitor pharmaceutical concentrations in rivers.

Similar to wastewater samples, the highest concentrations were obtained for carbamazepine at 46–67 ng L^{-1} in upstream, to 167–334 ng L^{-1} in downstream. Significantly higher concentrations at up to 1100 ng L^{-1} have been detected in surface waters

Table 3
Weekly mean pharmaceutical concentrations in the influent and effluent of WTWs and their apparent removal.

Pharmaceuticals	Scaynes Hill WTW			Manor Farm Road WTW			Basingstoke WTW		
	C_{IN} (ng L^{-1})	C_{EF} (ng L^{-1})	Removal (%)	C_{IN} (ng L^{-1})	C_{EF} (ng L^{-1})	Removal (%)	C_{IN} (ng L^{-1})	C_{EF} (ng L^{-1})	Removal (%)
Propranolol	334	62	81.4	690	135	80.4	1090	110	89.9
Sulfamethoxazole	49	23	52.7	110	37	66.4	181	32	82.3
Carbamazepine	1662	950	42.8	1237	637	48.5	1833	837	54.3
Indomethacine	62	24	60.8	65	19	70.8	124	14	88.7
Diclofenac	397	119	70.1	782	176	77.5	981	78	92.0

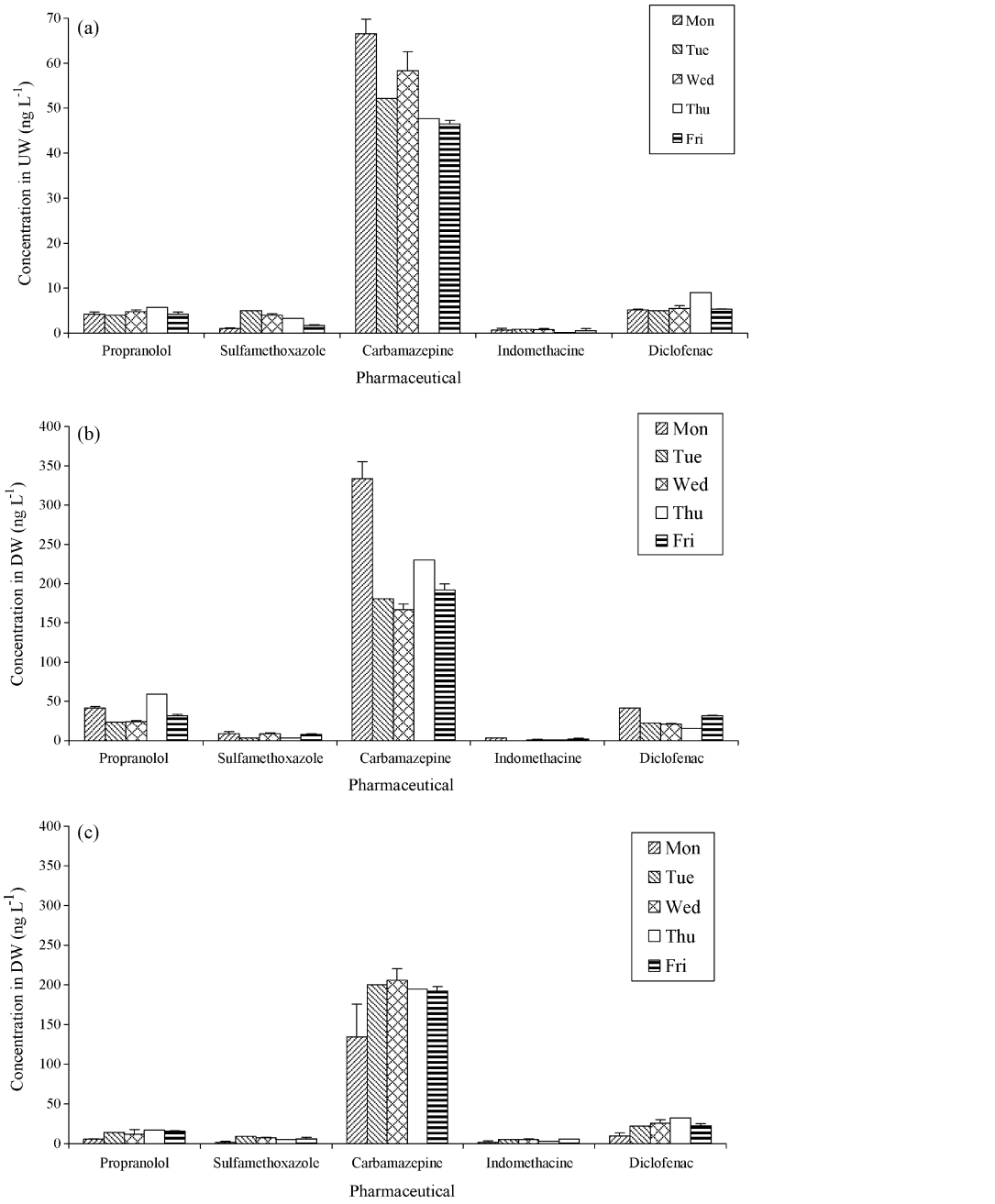

Fig. 3. Daily variation of pharmaceutical concentrations in the upstream (a) and downstream (b) of Scaynes Hill WTW outfall in the River Ouse. Concentrations in the downstream were also predicted (c) using Eq. (2). UW, upstream; DW, downstream.

in Germany [2,18]. The lowest concentrations were shown by indomethacine at 0.2–0.9 ng L^{-1} in upstream, to 0.1–3 ng L^{-1} in downstream. Overall, a clear elevation in pharmaceutical concentrations is observed in the downstream over upstream, indicating that the Scaynes Hill WTW is a source of pharmaceutical inputs to the river Ouse.

To make a more quantitative estimation of the WTW as a source of pharmaceuticals in the river Ouse, the concentrations of pharmaceuticals in the downstream of effluent discharge site were estimated using a simple dilution model assuming the mass balance being observed:

$$C_{DW} = \frac{C_{UW} \times V_{UW} + C_{EF} \times V_{EF}}{V_{DW}} \quad (2)$$

where C_{DW} and C_{UW} are pharmaceutical concentrations in downstream and upstream, while V_{UW}, V_{DW} and V_{EF} are the flow rates in upstream, downstream and effluent, respectively.

As shown in Fig. 3c, the predicted pharmaceutical concentrations in downstream site closely resembled those being measured (Fig. 3b). Statistical analysis showed that the prediction underestimated measured values by 26%. But if one of the data points (i.e. carbamazepine on Monday) was excluded in the statistical analysis, then the underestimation from prediction was reduced to only 2.6%, with a r^2 value of 0.932, and a P value <0.001, suggesting a significant relationship.

A further comparison was made between the weekly mean pharmaceutical concentrations in the effluent and the receiving river water. It is clear that for all compounds, their concentrations

were higher in the downstream than in the upstream, and the highest concentrations were always found in effluent. For example, the mean concentration of propranolol in the effluent was 62 ng L^{-1}. In comparison, lower concentrations were detected in the receiving river in downstream (36 ng L^{-1}) and in upstream (4 ng L^{-1}). The same trends were observable for other compounds (sulfamethoxazole, carbamazepine, indomethacine and diclofenac) when their concentrations were compared, further confirming the WTW as a key source of pharmaceuticals into river Ouse.

3.4. Risk assessment of pharmaceutical compounds

Safety threshold values for pharmaceutical compounds are limited and often related to single compound-single organism toxicity studies. Many pharmaceutical compounds have not yet been studied as extensively as others and reliable toxicity data are limited to acute effects only. Cleuvers [21] studied the toxicity of a number of compounds to *Daphnia magna* including diclofenac, carbamazepine and propranolol. The EC_{50} values were found to be 68, 72 and 7.5 mg L^{-1} respectively, which are substantially higher in comparison to the concentrations measured in this study at ng L^{-1} range. Nevertheless, it must be noted that the impact of a mixture of these chemicals could prove more toxic than the individual compounds alone. For example, Flaherty and Dodson [22] found that pharmaceutical mixtures behaved unpredictably and caused serious side effects such as deformities and increased mortality in *D. magna*.

Due to low pharmaceutical concentrations found in natural waters, their impact in causing chronic toxicity to aquatic populations close to sewage effluents is of more importance. Recently when studying cytological effects of pharmaceuticals in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*), Triebskorn et al. [23] determined that the lowest observed effect concentrations (LOEC) for carbamazepine and diclofenac were 1 μg L^{-1}. Although the highest pharmaceutical concentration (334 ng L^{-1} of carbamazepine) in the river Ouse is still lower than its LOEC, the safety margin becomes relatively constrained. Furthermore, due to the more significant impacts from mixtures of pollutants and potential persistence of such chemicals, it is prudent that these chemicals should be monitored regularly.

4. Conclusions

Five pharmaceutical compounds propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac were frequently detected in wastewater and river water samples, suggesting their widespread use and some degree of persistence. Pharmaceuticals were found to vary in concentrations, with carbamazepine being the most abundant. During wastewater treatment, all compounds were found to show concentration decline from influent to effluent, with removal efficiency from 43 to 92%. These compounds were also found in both the upstream and downstream of the effluent outfall at Scaynes Hill WTW, with concentrations elevated at the outfall. Through a simple dilution model, the WTW was shown to be a key source of pharmaceuticals in the river Ouse. Further research is needed to assess potential bioaccumulation of pharmaceuticals in aquatic organisms and resulting chronic toxic effects.

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