Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Assessing pharmaceutical removal and reduction in toxicity provided by advanced wastewater treatment systems

Luisa F. Angeles [a], Rachel A. Mullen[a], Irvin Huang[d], Christopher Wilson [b], Wendell Khunjar [c], Howard I. Sirotkin[e], Anne E. McElroy[d, *], and Diana S. Aga [a, *]

[a] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo NY 14260

[b] Hampton Roads Sanitation District, Virginia Beach, VA

[c] Hazen and Sawyer, P.C.

[d] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY

[e] Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY

*Email of corresponding authors:

anne.mcelroy@stonybrook.edu

dianaaga@buffalo.edu

Chemicals and Reagents

Acetaminophen, acetaminophen-d4, acetyl-sulfamethoxazole, acetyl-SMX-d4, azithromycin, caffeine, carbamazepine, clarithromycin, erythromycin, naproxen, norfloxacin, oxolinic acid, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxazole-d4, roxithromycin, tilmicosin, trimethoprim, trimethoprim-d9, and tylosin were purchased from Sigma Aldrich. Erythromycin-H_2O-13C,d3, ciprofloxacin, desvenlafaxine, and diclofenac, were obtained from Cambridge Isotopes (Tewksbury, MA). Spiramycin and sulfathiazole were purchased from ICN Biomedicals, Inc. Carbamazepine-d10, ciprofloxacin-d8 and caffeine-d3 were purchased from CDN Isotopes (Quebec, Canada). Diphenhydramine-d3, bupropion HCl, bupropion-d9 HCl, citalopram HBr, citalopram-d6 HBr, paroxetine maleate, paroxetine-d6 maleate, venlafaxine, venlafaxine-d6, desvenlafaxine, desvenlafaxine-d6, sertraline HCl, sertraline-d3 HCl, norfluoxetine oxalate, and norfluoxetine-d6 oxalate, were obtained from Cerilliant (Sigma-Aldrich, St Louis, MO).

Methanol and acetonitrile were of LC-MS and HPLC grade quality and obtained from Burdick & Jackson (Morristown, NJ). ACS grade phosphoric acid was obtained from J.T. Baker (Philipsburg, NJ). Nylon filters, 47 mm in diameter with a pore size of 0.45 um, were obtained from VWR (Radnor, PA). The Oasis™ hydrophilic-lipophilic balance (HLB) SPE 500 mg cartridges were purchased from Waters (Milford, MA). Barnstead NANOpure™ water system (Waltham, MA) was used for the water in all experiments.

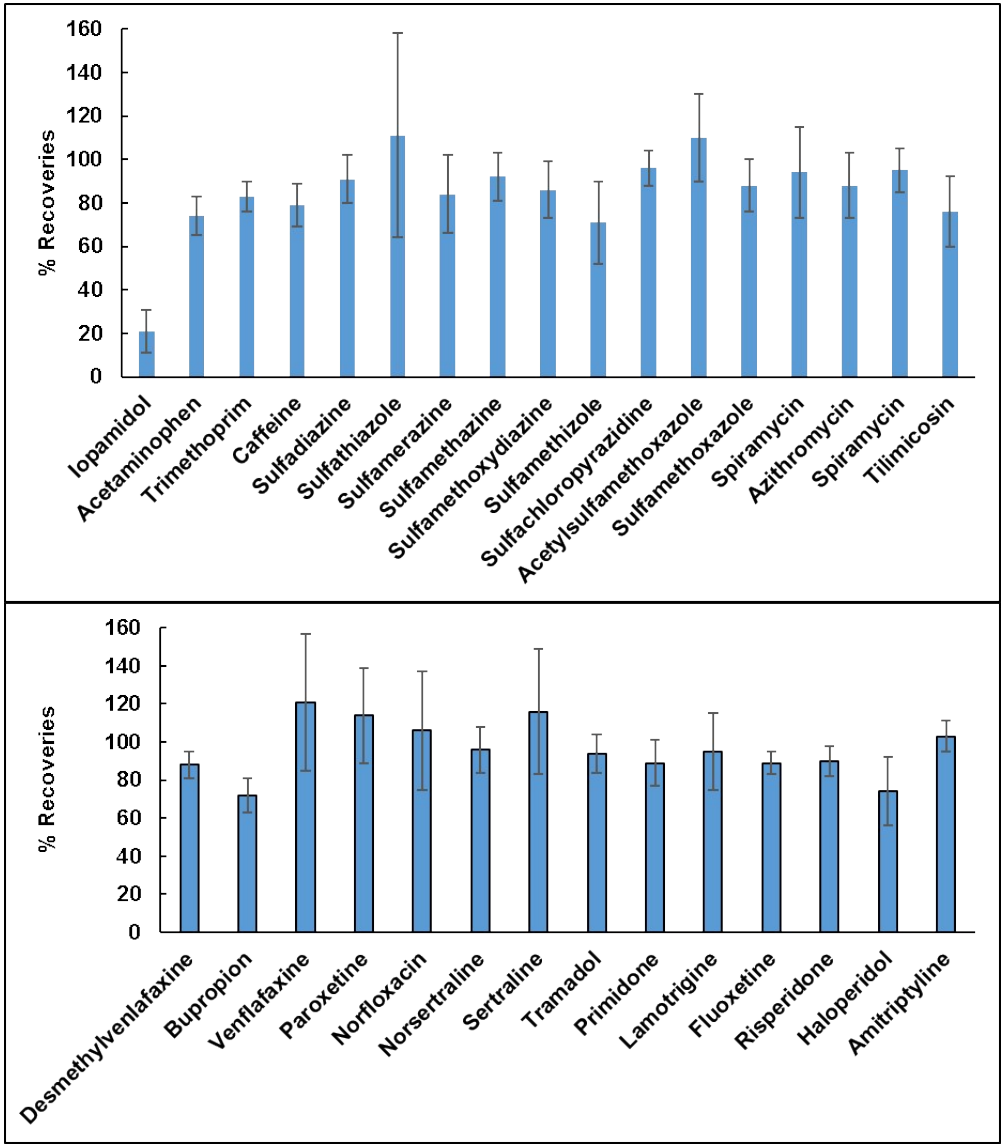

Figure S1. Method recoveries of target pharmaceuticals arranged according to elution in the chromatographic column (n=3); error bars are standard deviation.

Table S1. Nitrate levels in the seven WWTPs in this study

WWTP	Nitrate (mg/L)
1	1.5 - 3
2	1.5 - 3
3	< 3
4	0.98
5	1.1
6	2.6
7	< 3

Table S2. Selected reaction monitoring (SRM) transitions for the different target analytes including retention time and instrument limit of detection (iLOD).

Analytes	Retention time	Precursor ion	Tube lens voltage	Quantitative ion (CE)	Qualitative ion (CE)	iLOD, µg/L
ANTIDEPRESSANTS						
amitriptyline	20.06	278	90	233 (18)	91 (30)	0.71
bupropion	15.30	240	58	131 (27)	184 (12)	0.34
citalopram	18.46	325	112	109 (29)	262 (20)	0.12
haloperidol	18.65	376	103	123 (35)	165 (22)	0.82
lamotrigine	13.48	256	140	159 (28)	166 (24)	1.33
norfluoxetine	20.4	296	44	134 (5)	-	0.45
paroxetine	19.42	330	100	151 (23)	192 (20)	0.39
primidone	16.17	219	83	91 (27)	162 (13)	1.69
risperidone	14.71	411	108	191 (28)	110 (47)	2.41
sertraline	21.1	306	46	159 (28)	275 (11)	0.28
venlafaxine	16.2	27	67	58 (17)	121 (29)	0.52
MACROLIDES						
anhydro erythromycin	20.67	717	89	158 (28)	558 (16)	0.74
azithromycin	15.67	750	116	158 (36)	591 (25)	0.81
clarithromycin	21.68	749	105	158 (26)	591 (16)	0.49
erythromycin	21.3	735	95	576 (18)	158 (29)	7.07
roxithromycin	22.09	838	110	76 (18)	158 (32)	0.33
spiramycin I	15.58	422	66	101 (16)	174 (18)	1.34
spiramycin II	15.98	443	65	101 (16)	174 (20)	0.75
spiramycin III	16.79	450	67	101 (17)	174 (20)	1.39
tilmicosin	17.24	870	187	174 (40)	670 (39)	0.76
tylosin	20.11	917	138	132 (35)	174 (36)	0.25
PHARMACEUTICALS AND PERSONAL CARE PRODUCTS						
acetaminophen	5.73	152	59	65 (30)	110 (15)	7.21
caffeine	13.76	195	71	110 (25)	138 (19)	0.23
carbamazepine	21.86	237	97	193 (33)	194 (19)	0.15
diclofenac	27.50	296	53	214 (33)	250 (13)	0.17
iopamidol	2.55	778	142	387 (40)	559 (22)	1.06
trimethoprim	9.1	291	94	230 (23)	261 (24)	0.76
QUINOLONES						
ciprofloxacin	13.58	332	78	231 (35)	288 (16)	1.12
enrofloxacin	14.13	360	81	245 (25)	316 (18)	2.57
norfloxacin	13.18	320	70	233 (23)	276 (16)	0.67
oxolinic acid	19.5	262	57	160 (27)	216 (28)	1.21
sarafloxacin	14.95	386	85	299 (26)	342 (17)	0.74

SULFONAMIDES						
acetyl sulfamethoxazole	18.01	296	73	134 (24)	198 (17)	0.35
sulfachloropyridazine	15.54	285	61	108 (25)	156 (15)	0.77
sulfadiazine	6.82	251	60	92 (28)	156 (15)	0.2
sulfadimethoxine	18.74	311	73	108 (30)	156 (21)	0.27
sulfamerazine	10.78	265	65	156 (16)	172 (17)	0.28
sulfamethazine	13.33	279	70	124 (26)	186 (17)	0.78
sulfamethizole	14.07	271	60	92 (27)	156 (14)	0.14
sulfamethoxazole	16.03	254	54	92 (26)	156 (16)	0.2
sulfamethoxydiazine	13.98	281	65	92 (29)	108 (26)	0.46
sulfathiazole	8.96	256	59	92 (27)	156 (15)	0.18
SURROGATES						
13C1,d3-anhydroerythromycin	20.67	721	147	562 (16)	162 (28)	n/a
13C1,d3-erythromycin	21.3	739	131	580 (16)	162 (29)	n/a
d10-carbamazepine	21.86	247	113	202 (35)	204 (22)	n/a
d3-caffeine	13.76	198	83	110 (22)	138 (19)	n/a
d4-acetaminophen	5.73	156	94	69 (29)	114 (17)	n/a
d4-acetylsulfamethoxazole	18.01	300	105	112 (34)	138 (26)	n/a
d4-sulfamethoxazole	16.03	258	102	112 (26)	160 (16)	n/a
d6 -venlafaxine	16.2	284	91	58 (18)	121 (29)	n/a
d6-bupropion	15.30	249	87	131 (28)	185 (13)	n/a
d6-citalopram	18.46	331	112	109 (29)	262 (20)	n/a
d6-norfluoxetine	20.4	302	67	140 (5)	-	n/a
d6-norsertraline	21.81	281	152	123 (41)	159 (20)	n/a
d6-paroxetine	19.42	336	130	76 (32)	198 (21)	n/a
d8-ciprofloxacin	13.58	340	106	235 (38)	296 (17)	n/a
d9-trimethoprim	9.1	300	108	234 (24)	264 (25)	n/a

Table S3. Thermo TSQ Quantum Ultra triple quadrupole mass spectrometer settings

Spray voltage	3000 V
Ion sweep gas pressure	0 AU
Vaporizer temperature	350°C
Sheath gas pressure	40 AU
Auxiliary gas pressure	35 AU
Capillary temperature	325°C
Collision gas pressure	1.5 mTorr
Cycle time	0.300 s
Q1 peak width	0.70 FWHM

Table S4A. Pharmaceutical concentrations (ng/L) in wastewater treatment plants 1-3 effluents, where n.d. stands for not detected

	WWTP 1		WWTP 2			WWTP 3				
	STE	GAC	STE	MBBR	MFB	STE	FSE	Ozonation	BAF	GAC
acetaminophen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
acetylsulfamethoxazole	128	n.d.	37	28	28	785	707	n.d.	146	23
amitriptyline	38	9	25	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
anhydro erythromycin	23	n.d.	22	22	n.d.	47	44	n.d.	n.d.	n.d.
azithromycin	185	n.d.	22	57	n.d.	639	340	n.d.	n.d.	n.d.
bupropion	290	n.d.	246	218	181	502	409	49	46	n.d.
caffeine	60	n.d.	16	16	11	733	76	n.d.	42	n.d.
carbamazepine	177	2	125	130	138	204	n.d.	n.d.	7	n.d.
ciprofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	231	153	n.d.	n.d.	n.d.
citalopram	252	n.d.	178	173	n.d.	166	151	n.d.	n.d.	n.d.
clarithromycin	29	n.d.	16	35	1	54	68	n.d.	n.d.	n.d.
diclofenac	273	n.d.	76	92	n.d.	170	n.d.	n.d.	n.d.	n.d.
erythromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
fluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
haloperidol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
lamotrigine	579	n.d.	422	451	189	n.d.	n.d.	n.d.	n.d.	n.d.
naproxen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
norfloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
norfluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
oxolinic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
primidone	n.d.	n.d.	n.d.	n.d.	n.d.	156	150	97	87	n.d.
risperidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
roxithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sertraline	90	n.d.	55	48	n.d.	87	30	n.d.	n.d.	n.d.
spiramycin I	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
spiramycin II	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
spiramycin III	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

sulfachloropyrazidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfadiazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamerazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamethazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamethoxazole	406	18	283	256	38	14	n.d.	n.d.	n.d.	n.d.
tilimicosin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trimethoprim	35	n.d.	69	52	n.d.	636	n.d.	n.d.	6	n.d.
tylosin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
venlafaxine	668	n.d.	373	358	n.d.	1284	946	n.d.	37	n.d.

STE – secondary treatment effluent, MBBR – moving bed biofilm reactor, FSE – floc/sed effluent, BAF – biologically activated filtration, GAC – granular activated carbon

Table S4B. Pharmaceutical concentrations (ng/L) in wastewater treatment plants 4-7 effluents, where n.d. stands for not detected

	WWTP 4			WWTP 5		WWTP 6		WWTP 7		
	PTI	PTE	SBR/UV	PTI	MBR/UV	PTI	SBR/CL	PTI	AS	NAS
acetaminophen	25662	18595	n.d.	38144	n.d.	23530	n.d.	547	n.d.	n.d.
acetylsulfamethoxazole	346	397	n.d.	n.d.	n.d.	2656	54	131	101	140
amitriptyline	24	27	n.d.	13	n.d.	159	10	7	5	5
anhydro erythromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2	n.d.
azithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	441	175	14	16	8
bupropion	93	103	133	129	42	363	240	45	37	35
caffeine	86601	101390	n.d.	102179	n.d.	78510	331	13026	37	96
carbamazepine	97	98	121	96	116	317	297	75	85	74
ciprofloxacin	1226	1310	85	593	88	2670	522	420	255	127
citalopram	146	132	129	99	84	241	155	53	46	47
clarithromycin	13	22	n.d.	115	n.d.	57	46	17	14	13
diclofenac	74	82	45	50	137	31	65	18	13	15
erythromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
fluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
haloperidol	n.d.	n.d.	n.d.	n.d.	n.d.	7	n.d.	n.d.	n.d.	n.d.
lamotrigine	n.d.	n.d.	n.d.	n.d.	n.d.	2000	1402	433	345	294
naproxen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
norfloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
norfluoxetine	n.d.	n.d.	n.d.	70	n.d.	64	n.d.	n.d.	n.d.	n.d.
oxolinic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	24	n.d.	n.d.	n.d.	n.d.
primidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
risperidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
roxithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sertraline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	24	16	17
spiramycin I	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

spiramycin II	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
spiramycin III	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfachloropyrazidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfadiazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamerazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamethazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamethoxazole	75	82	n.d.	n.d.	35	n.d.	50	16	n.d.	12
tilmicosin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trimethoprim	146	139	40	69	83	548	32	108	95	78
tylosin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
venlafaxine	358	293	241	388	242	672	510	111	138	114

PTI – primary treatment influent, PTE – primary treatment effluent, CL – chlorination

Table S5. Removal of pharmaceuticals in each stage of the treatment process in the seven WWTPs in this study

	WWTP 1	WWTP 2		WWTP 3				WWTP 4		WWTP 5	WWTP 6	WWTP 7	
	GAC	**MBBR**	**MFB**	**FSE**	**O3**	**BAC**	**GAC**	**PT**	**SBR/UV**	**MBR/UV**	**SBR/CL**	**AS**	**NAS**
acetaminophen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28%	100%	100%	100%	99%	0%
acetylsulfamethoxazole	100%	24%	0%	10%	100%	-100%	85%	-15%	100%	n.d.	98%	23%	-39%
amitriptyline	76%	3%	97%	n.d.	n.d.	n.d.	n.d.	-16%	97%	94%	94%	29%	-11%
anhydro erythromycin	97%	1%	97%	6%	98%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-166%	62%
azithromycin	100%	-100%	99%	47%	100%	n.d.	n.d.	n.d.	n.d.	n.d.	60%	-17%	53%
bupropion	100%	11%	17%	19%	88%	5%	99%	-11%	-29%	67%	34%	19%	6%
caffeine	100%	2%	31%	90%	100%	-100%	99%	-17%	100%	100%	100%	100%	-100%
carbamazepine	99%	-3%	-7%	100%	n.d.	-100%	98%	-1%	-24%	-21%	6%	-14%	12%
ciprofloxacin	n.d.	n.d.	n.d.	34%	99%	n.d.	n.d.	-7%	94%	85%	80%	39%	50%
citalopram	100%	3%	100%	9%	100%	n.d.	n.d.	10%	2%	15%	35%	15%	-4%
clarithromycin	98%	-100%	96%	-26%	99%	n.d.	n.d.	-75%	98%	100%	18%	19%	7%
diclofenac	100%	-21%	100%	100%	n.d.	n.d.	n.d.	-11%	46%	-100%	-100%	26%	-18%
haloperidol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	89%	n.d.	n.d.
lamotrigine	100%	-7%	58%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	30%	20%	15%
norfluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	99%	99%	n.d.	n.d.
paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	98%	n.d.	n.d.
primidone	n.d.	n.d.	n.d.	4%	35%	10%	98%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sertraline	100%	13%	99%	65%	99%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sulfamethoxazole	95%	10%	85%	99%	n.d.	n.d.	n.d.	-10%	100%	-100%	-100%	99%	-100%
tilmicosin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trimethoprim	98%	25%	99%	100%	NA	-100%	87%	5%	71%	-20%	94%	12%	18%
venlafaxine	100%	4%	100%	26%	100%	-100%	99%	18%	18%	38%	24%	-24%	17%

Table S6. t – test assuming unequal variances (α = 0.05) for difference of mean concentration in each stage of the treatment process in WWTP 3

	FSE		O3		BAC		GAC	
	p-value	**P < α = 0.05**	**p-value**	**P < α = 0.05**	**p-value**	**P < α = 0.05**	**p-value**	**P < α = 0.05**
acetylsulfamethoxazole	0.5565	not sig.	0.0013	sig.	0.0132	sig.	0.0233	sig.
anhydro erythromycin	0.6685	not sig.	0.0215	sig.	n/a	-	n/a	-
azithromycin	0.4214	not sig.	0.0026	sig.	n/a	-	n/a	-
bupropion	0.2171	not sig.	0.0000	sig.	0.7792	not sig.	0.0155	sig.
caffeine	0.0069	sig.	0.0035	sig.	0.0103	sig.	0.0103	sig.
carbamazepine	0.0055	sig.	n/a	-	0.0029	sig.	0.0029	sig.
ciprofloxacin	0.0584	not sig.	0.0048	sig.	n/a	-	n/a	-
citalopram	0.2847	not sig.	0.0024	sig.	n/a	-	n/a	-
clarithromycin	0.2618	not sig.	0.0083	sig.	n/a	-	n/a	-
diclofenac	0.0087	sig.	n/a	-	n/a	-	n/a	-
primidone	0.6548	not sig.	0.0100	sig.	0.3100	not sig.	0.0059	sig.
sertraline	0.3104	not sig.	0.0003	sig.	n/a	-	n/a	-
sulfamethoxazole	0.0150	sig.	n/a	-	n/a	-	n/a	-
trimethoprim	0.0125	sig.	n/a	-	0.0184	sig.	0.0184	sig.
venlafaxine	0.2283	not sig.	0.0010	sig.	0.0182	sig.	0.0182	sig.

Table S7. Second – order rate constants for the reactions of pharmaceuticals with ozone and hydroxyl radical

Compound	$k_{O3,app}$ (M^{-1} s^{-1})	$k_{OH,app}$ (M^{-1} s^{-1})
acetylsulfamethoxazole	2.5×10^{2} [a]	6.8×10^{9} [a]
azithromycin	1.1×10^{5} [a]	2.9×10^{9} [a]
bupropion[c]	-	-
caffeine[c]	-	-
ciprofloxacin	1.9×10^{4} [a]	4.1×10^{9} [a]
venlafaxine[c]	-	-
citalopram[c]	-	-
clarithromycin[c]	-	-
primidone	1.0 [b]	8.4×10^{9} [b]
sertraline[c]	-	-
anhydro erythromycin[c]	-	-

[a] Rate constant obtained from Dodd et. Al., 2006. [b] Rate constant obtained from Real et. Al., 2009. [c]Data not available