Occurrence, source estimation and risk assessment of pharmaceuticals in the Chaobai River characterized by adjacent land use

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Text S1 Chemicals and reagents used for the analysis

Ultrapure water was made by a Milli-Q water purification system (Advantage A10, Millipore, USA). HPLC-grade methanol, acetonitrile, dichloromethane, and acetone were from Fisher Scientific (Geel, Belgium), and formic acid (HPLC grade, >99%) were from Dikma Technologies, Inc. (Lake Forest, USA). Analytical grade Na_2EDTA, citric acid monohydrate, NaOH, Na_2HPO_4, H_2SO_4, and HCl were purchased from Beijing Chemical Reagents Company (Beijing, China). Na_2EDTA-McIlvaine buffer was prepared by dissolving 21.00 g citric acid monohydrate, 17.75 g Na_2HPO_4, and 60.50 g $Na_2EDTA{\cdot}2H_2O$ in 1.625 L of ultrapure water, with pH adjusted to 4.00.

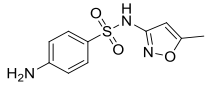
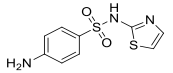
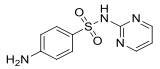
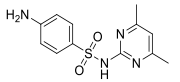
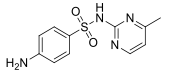
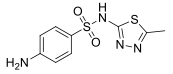
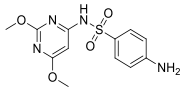
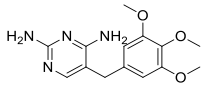
Text S2 Experimental procedures and instrumental parameters

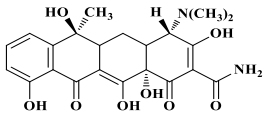
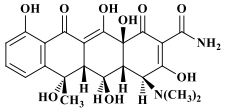
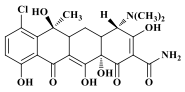
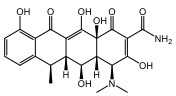
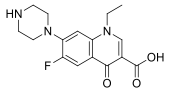
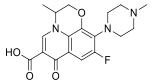
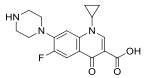
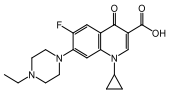
The target pharmaceuticals in river water samples were extracted by solid phase extraction (SPE) using Oasis HLB cartridges (500 mg, 6 mL, Waters, Milford, MA, USA). The cartridge was preconditioned with 5 mL each of methanol, HCl (0.5 mol L^{-1}) and ultrapure water sequentially. One liter of water sample was spiked with internal standards and extracted by the HLB cartridge at a flow rate of approximately 3 mL min^{-1}. Afterwards, the cartridge was rinsed with 5 mL each of 5% methanol aqueous solution and ultrapure water, dried under vacuum, and then eluted with 10 mL methanol sequentially. The eluate was dried under a gentle stream of N_2 and dissolved with a mixture of 400 μL methanol and 600 μL ultrapure water. The extract was centrifuged at 10,000 r min^{-1} for 8 min (Centrifuge 5418, Eppendorf, Hamburg, Germany), and the supernatant was filtered through 0.2 μm PES filters (PALL, USA) for UPLC–MS/MS analysis.

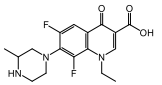
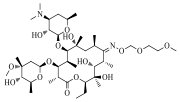
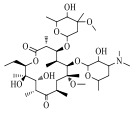
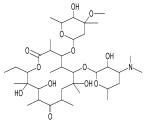
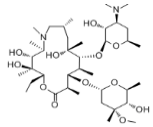
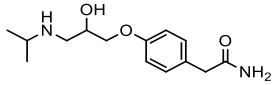
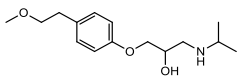
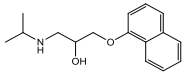
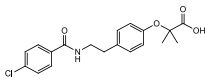
The chromatographic separation of target pharmaceuticals was performed on an Agilent 1290 ultra-performance liquid chromatography (UPLC) equipped with an Agilent Zorbax SB-C18 column (100 mm × 2.1 mm, 1.8 μm). The column was maintained at 30℃ and the injection volume was 5 μL. Ultrapure water containing 0.2% formic acid (v/v) (A) and acetonitrile (B) were used as the mobile phases at a total flow rate of 0.2 mL min^{-1}. The gradient elution program (time in min, % mobile phase B) was set as follows: (0, 5), (2, 5), (5, 13), (8, 15), (13, 20), (18, 30), (25, 60), (27, 100), (30, 100), (30.1, 5), and (33, 5). An Agilent 6420 Triple Quad mass spectrometer (MS), equipped with an electrospray ionization (ESI) source and operated in the positive ion mode, was employed to analyze the target compounds. The MS system was operated under the following conditions: capillary voltage 4.0 kV, drying gas temperature 350 °C, drying gas flow rate 10 L min^{-1}, and nebulizing gas pressure 40 psi. For each compound, the operational parameters, recovery and LOQ are listed in Table S2.

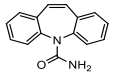
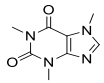
Table S1

Physicochemical properties of target pharmaceuticals and internal standards.

Category	Compound	Acronym	CAS No.	Formula	MW	pK_a[a]	logK_{ow}[a]	Structure
Sulfonamides (SAs)	Sulfamethoxazole	SMX	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	1.85 ± 0.30 5.60 ± 0.04	0.89	
	Sulfathiazole	STZ	72-14-0	$C_9H_9N_3O_2S_2$	255.32	2.01 ± 0.30 7.11 ± 0.09	0.05	
	Sulfadiazine	SDZ	68-35-9	$C_{10}H_{10}N_4O_2S$	250.30	1.57 ± 0.10 6.50 ± 0.30	–0.12	
	Sulfamethazine	SMN	57-68-1	$C_{12}H_{14}N_4O_2S$	278.33	2.07 ± 0.30 7.49 ± 0.13	0.80	
	Sulfamerazine	SMR	127-79-7	$C_{11}H_{12}N_4O_2S$	264.30	2.82 ± 0.31 6.84 ± 0.30	0.34	
	Sulfamethizole	SML	144-82-1	$C_9H_{10}N_4O_2S_2$	270.33	1.86 ± 0.30 5.29 ± 0.04	0.51	
	Sulfadimethoxine	SDM	122-11-2	$C_{12}H_{14}N_4O_4S$	310.33	2.13 ± 0.30 6.08 ± 0.09	1.48	
	Trimethoprim	TMP	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	7.20 ± 0.17	0.79	

Tetracyclines (TCs)	Tetracycline	TCN	60-54-8	$C_{22}H_{24}N_2O_8$	444.44	3.30 ± 0.10 7.70 ± 0.15 9.50 ± 0.15	–1.47	
	Oxytetracycline	OTC	79-57-2	$C_{22}H_{24}N_2O_9$	460.43	3.27 ± 0.10 7.32 ± 0.04 9.11 ± 0.10	–1.5	
	Chlortetracycline	CTC	64-72-2	$C_{22}H_{23}ClN_2O_8$	478.9	3.30 ± 0.40 7.40 ± 0.13 9.27 ± 0.50	–0.33	
	Doxycycline	DOX	564-25-0	$C_{22}H_{24}N_2O_8$	444.43	3.02±0.30 7.97±0.15 9.15±0.30	–0.72	
Fluoroquinolones (FQs)	Norfloxacin	NOR	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.33	3.59 ± 0.70 8.38 ± 0.25	1.48	
	Ofloxacin	OFL	82419-36-1	$C_{18}H_{20}FN_3O_4$	362.15	2.27 ± 0.40 6.41 ± 0.30	1.41	
	Ciprofloxacin	CIP	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.34	2.68 ± 0.20 8.38 ± 0.25	1.31	
	Enrofloxacin	ENR	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.4	3.85 ± 0.30 6.19 ± 0.18	NA [b]	

	Lomefloxacin	LOM	98079-51-7	$C_{17}H_{19}F_2N_3O_3$	351.35	5.64 8.47	–0.39	
Macrolides (MLs)	Roxithromycin	ROX	80214-83-1	$C_{41}H_{76}N_2O_{15}$	837.53	9.17 ± 0.30	3.73	
	Clarithromycin	CLA	81103-11-9	$C_{38}H_{69}NO_{13}$	747.95	7.25	3.16	
	Erythromycin	ERY	114-07-8	$C_{37}H_{67}NO_{13}$	733.93	8.90 ± 0.15	2.83	
	Azithromycin	AZN	83905-01-5	$C_{38}H_{72}N_2O_{12}$	748.98	7.34	4.02	
β-blockers	Atenolol	ATE	29122-68-7	$C_{14}H_{22}N_2O_3$	266.34	9.17 ± 0.38	0.10	
	Metoprolol	MET	37350-58-6	$C_{15}H_{25}NO_3$	267.36	9.18 ± 0.38	1.79	
	Propranolol	PROP	525-66-6	$C_{16}H_{21}NO_2$	259.35	9.45 ± 0.03	3.10	
Lipid regulators	Bezafibrate	BF	41859-67-0	$C_{19}H_{20}ClNO_4$	361.82	3.29 ± 0.10	3.46	

Antiepileptics	Carbamazepine	CBZ	298-46-4	$C_{15}H_{12}N_2O$	236.27	13.94 ± 0.20	2.67	
Stimulants	Caffeine	CAF	58-08-2	$C_8H_{10}N_4O_2$	194.19	10.40	–0.13	
Internal standards (ISs)	Demeclocycline	DMC	127-33-3	$C_{21}H_{21}ClN_2O_8$	464.85			
	Sulfamethazine -$^{13}C_6$	SMN-$^{13}C_6$	1189426-16-1	$C_6{}^{13}C_6H_{14}N_4O_2S$	293.30			
	Ofloxacin-D_3	OFL-D_3	1173147-91-5	$C_{18}H_{17}D_3FN_3O_4$	364.39			
	Caffeine-$^{13}C_3$	CAF-$^{13}C_3$	78072-66-9	$^{13}C_3C_5H_{10}N_4O_2$	197.19			

[a] Ben et al., 2018. [b] NA: not available.

Table S2

Operational parameters of tandem MS and quality assurance and quality control of target pharmaceuticals in river water.

Compound	Precursor ion (m/z)	Product ions (m/z) (Collision energy)	Fragmentor (V)	Recovery (RSD) (%) [a]	LOQ [b] (ng/L)	%ME [c]	Coefficient of correlation (r^2) [d]
SMX	254.3	91.9 (25); 156.0 (15)	105	89 (4)	1.4	96	0.999
STZ	256.3	92.1 (25); 156.1 (10)	100	87 (8)	1.4	92	0.999
SDZ	251.2	156.0 (12); 92.1 (25)	105	102 (4)	1.3	102	0.999
SMN	279.3	186 (10); 92 (35)	115	102 (2)	1.1	99	0.999
SMR	265.3	92 (40); 156 (15)	110	96 (5)	1.6	106	0.999
SML	271.3	155.9 (10); 92.2 (30)	100	79 (7)	1.0	100	0.999
SDM	311.0	92 (30); 156 (20)	110	116 (10)	0.6	99	0.999
TMP	291.3	230.1 (20); 123.1 (40)	135	116 (3)	0.7	95	0.999
TCN	445.3	410.2 (15); 154 (25)	120	55 (4)	1.4	107	0.993
OTC	461.3	426.2 (15); 442.9 (10)	135	120 (6)	0.9	95	0.991
CTC	479.2	154 (20); 444 (10)	130	86 (1)	1.0	87	0.995
DOX	445.2	428.1 (15); 154 (32)	125	84 (5)	1.0	90	0.998
NOR	320.2	233.1 (30); 275.9 (15)	135	93 (9)	1.1	95	0.997
OFL	362.3	318 (20); 261.2 (35)	135	142 (11)	1.0	110	0.996
CIP	332.2	288.1 (15); 188.9 (30)	135	94 (5)	0.8	108	0.998
ENR	360.1	342.2 (18); 316.2 (15)	140	85 (2)	0.6	118	0.999
LOM	352.2	308.1 (15); 265.1 (25)	125	106 (2)	0.5	103	0.999
ROX	837.6	679.3 (12); 158.2 (30)	160	105 (7)	0.6	95	0.999
CLA	748.6	158.2 (35); 590.2 (15)	165	98 (6)	0.7	92	0.999
ERY	716.4	558.2 (15); 158 (35)	160	79 (5)	0.9	100	0.998
AZN	749.6	591.5 (30); 158 (40)	160	107 (7)	1.8	103	0.995

ATE	267.3	145.0 (28); 74.1 (30)	100	89 (5)	0.3	98	0.994
MET	268.3	56.2 (35); 116.1 (25)	115	98 (3)	1.1	96	0.999
PROP	260.3	116.2 (18); 74.2 (25)	105	88 (4)	0.7	99	0.996
BF	362.2	316.2 (10); 139.1 (30)	110	109 (8)	0.4	101	0.999
CBZ	237.2	194.2 (20); 179.0 (35)	110	85 (9)	0.4	93	0.999
CAF	195.1	138.0 (20); 110.1 (30)	110	91 (1)	1.4	100	0.999

[a] The recovery for each target pharmaceutical was determined according to the following procedure: a reference sample (raw river water) and three samples spiked with a concentration of 0.5 μg L^{-1} of the target pharmaceutical were simultaneously run through the SPE process and instrumental analysis. Then the target pharmaceutical was quantified by the internal standard method, in which an 8-point calibration curve was established with mixed pharmaceutical standards (i.e., 1 to 1000 μg L^{-1}) and the related internal standard (i.e., 1000 μg L^{-1}) in Milli-Q water. The recovery (%) was calculated by the ratio of the difference value between the mean concentration in spiked samples and the concentration in reference sample to the known spiked concentration (i.e., 0.5 μg L^{-1}). (Please see Table S2).

[b] LOQs of target pharmaceuticals in river water were determined according to the EURACHEM methods, which consisted in replicate (n=10) injections of a very low (but detectable) standard in the matrix. Standard deviation σ of the areas was calculated and multiplied by 10. The obtained 10σ was transformed in concentration (i.e. LOQ) by using the calibration curve (in the lower range).

[c] The matrix effect (%ME) for each pharmaceutical, which represents how the matrix in river water may interfere with the analyte's signal, is calculated by the ratio of the difference value between the detected concentration of spiked post-extracted reference river water sample and un-spiked extracted reference river water to the detected concentration of spiked Mill-Q water sample." The values of %ME have been also added in Table S2, which were in the range of 87–118%, falling within the acceptable 70–120% range.

Table S3

Standard deviation and predicted no effect concentration of target pharmaceuticals

Compound	Primary TMoA[a]	TMoA Standard deviation σ [b]	PNEC [c] (ng L^{-1})
SDZ	bactericides	1.43	135
SMX	bactericides	1.43	27
TMP	bactericides	1.43	2600
TCN	bactericides	1.43	90
OTC	bactericides	1.43	170
CTC	bactericides	1.43	50
NOR	bactericides	1.43	2000
CIP	bactericides	1.43	5
ENR	bactericides	1.43	31000
LOM	bactericides	1.43	106
CLA	bactericides	1.43	70
ERY-H2O	bactericides	1.43	20
ATE	β-blockers	1.34	30000
MET	β-blockers	1.34	7900
PROP	β-blockers	1.34	244
BF	lipid regulators	2.02	1873
CBZ	antiepileptics	1.56	13800
CAF	stimulants	2.20	46000

[a] TMoA: toxic mode of action (Munz et al., 2017). [b] Standard deviation σ was calculated over all log transformed toxicity data which serves as the slop of log–normal SSD for individual substance. [c] PNEC: predicted no effect concentration (Ben et al., 2018).

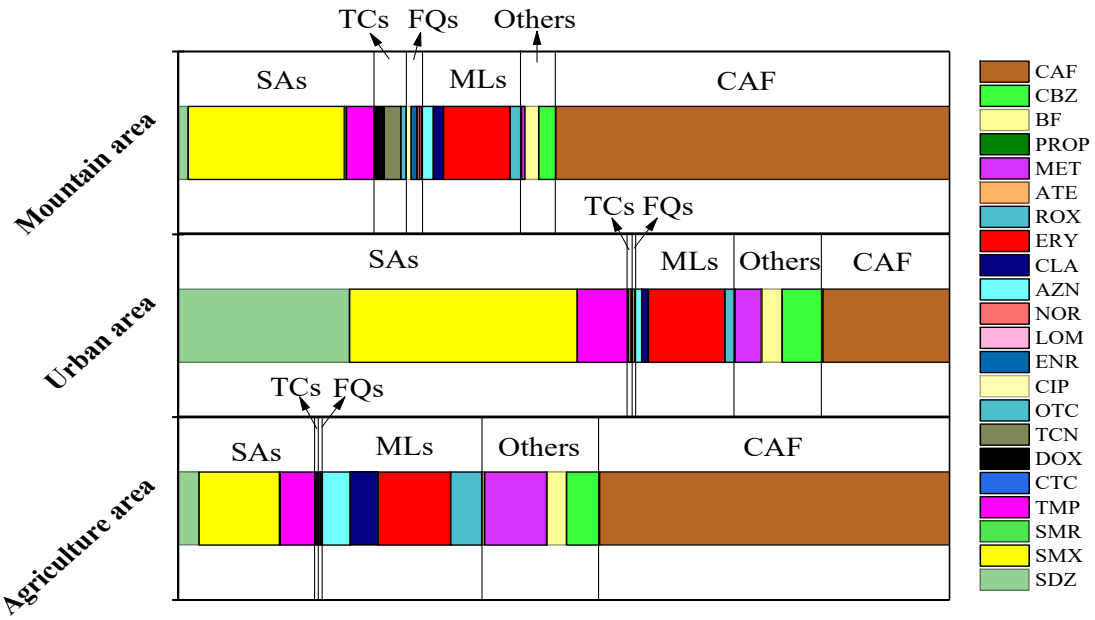


Fig. S1 Composition profiles of target pharmaceuticals at the sampling sites from mountain, urban and agriculture area. For each area, the summed concentrations of the pharmaceuticals belonging to a specific category were calculated. The figure presents the proportion of concentration of each category in the total concentration.

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