Occurrence, Source Apportionment, and Risk Assessment of Antibiotics in the Zhuozhang River, China: A Specific Investigation in Water-Scarce and Human Activity-Intensive Regions

Juping Yan [a], Xiayang Wu [a], Ke Dong [a], Zhiyuan Zhang [a], Xuejun Sun [b,e,*], Shaopeng Gao [c], Jinxian Liu [b,d,e], Baofeng Chai [b,d,e]

[a] School of Environmental and Resource, Taiyuan University of Science and Technology, Taiyuan 030024, China

[b] School of Environmental and Resource Sciences, Shanxi University, Taiyuan 030006, China

[c] State Key Laboratory of Tibetan Plateau Earth System, Resources and Environment (TPESRE), Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing, 100101, China

[d] Institute of the Loess Plateau, Shanxi University, Taiyuan, 030006, China

[e] Key Laboratory of Ecological Restoration of the Loess Plateau in Shanxi Province, Shanxi University, Taiyuan, 030006, China

* Corresponding author.

(Xuejun Sun)

E-mail: sunxuejun@sxu.edu.cn

Tel.: +86-0351-7010600

Fax: +86-0351-7010600

Contents

Text S1. Validation of the detection methods. 3

Figure S1. Chromatograms of internal standard and samples of three representative compounds of SAs, MLs, and QNs. 4

Table S1. Detailed information of the antibiotics in this paper. 5

Table S2. The PNEC, EC50, NOEC, and AF values were collected from previous studies to identify the sensitive aquatic organisms in this paper 8

Figure S2. The detection rate of 31 antibiotics in the Zhuozhang River 13

Text S1. Validation of the detection methods.

The chromatographic method used in this study followed the method of Lu (2024). This method was developed and validated in a previously published study. According to the results of the validation experiment in the study, as cited in the References, the concentrations of the target pollutants in water bodies did not affect the treatment efficacy of the method, as demonstrated by investigating the recovery rates at low (1 ng/L), medium (5 ng/L), and high (10 ng/L) concentrations of antibiotics. The average recovery of antibiotics at low (1 ng/L), medium (5 ng/L), and high (10 ng/L) concentrations was 95 ± 38%, 82 ± 34%, and 90 ± 20%, respectively. The result of the validation experiment demonstrates applicability across different concentrations of water samples.

Reference

Lu Zijun, High-throughput screening of new pollutants in water based on dispersive solid-phase extraction [D], Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (P17-P30).

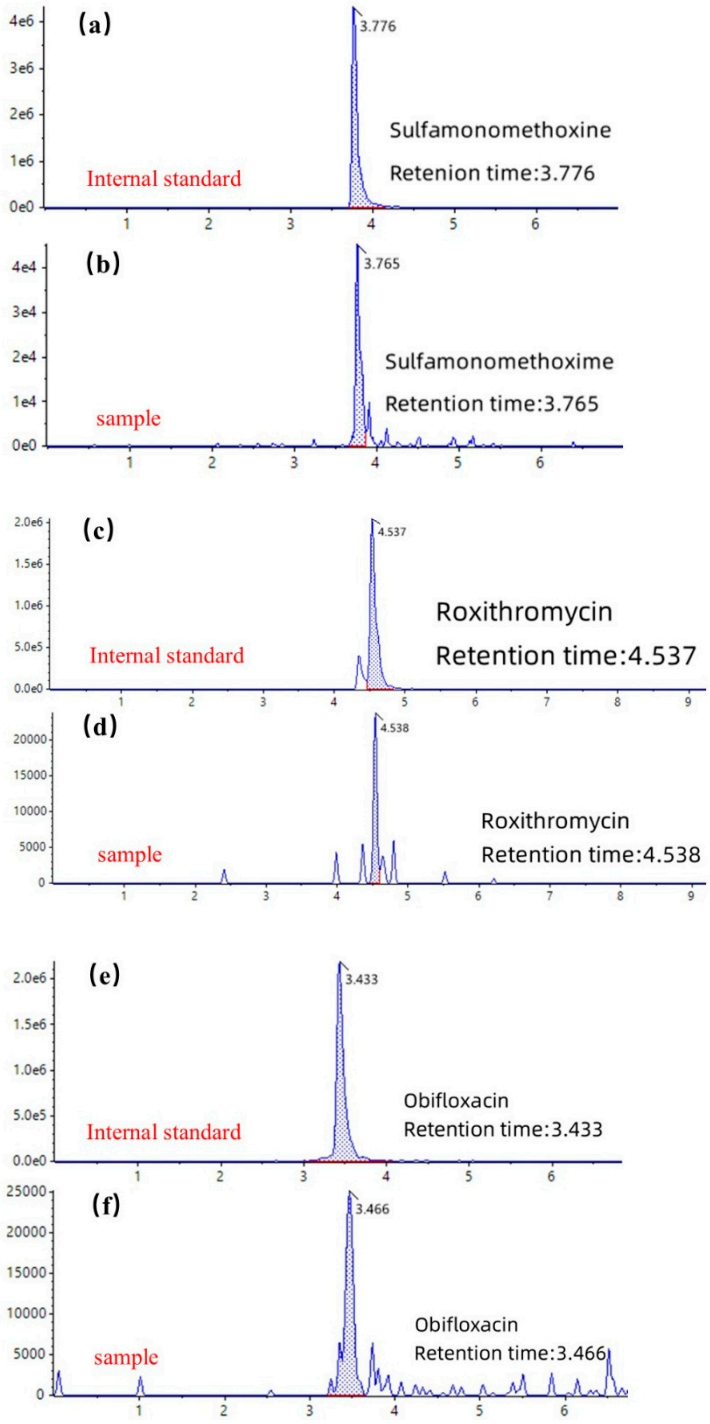

Figure S1. Chromatograms of internal standard and samples of three representative compounds of SAs, MLs, and QNs.

Table S1. Detailed information of the antibiotics in this paper.

Antibiotics	abbreviation	formula	CAS	Purity	Production Company	Recovery (%)	Limit of quantification (ng/L)	Limit of Detection (ng/L)	Q1 Mass	Q3 Mass	Retention time
Enrofloxacin	ENR	$C_{19}H_{22}FN_3O_3$	93106-60-6	98.0%	Alta Scientific Co., Ltd	80.9	0.3	0.1	360	316.1	3.98
									360	245.1	
Nalidixic acid	NA	$C_{12}H_{12}N_2O_3$	389-08-2	99.9%	Alta Scientific Co., Ltd	69.1	0.3	0.1	233	215	4.41
									233	187	
Sparfloxacin	SPA	$C_{19}H_{22}F_2N_4O_3$	111542-93-9	99.9%	Alta Scientific Co., Ltd	68.2	0.3	0.1	393	349.2	4.04
									393	292.2	
Sulfadiazine	SDZ	$C_{10}H_{10}N_4O_2S$	68-35-9	98.0%	Alta Scientific Co., Ltd	123	0.6	0.2	250.4	156	2.65
									250.4	184.2	
Sulfamethazine	SMZ	C12H14N4O2S	57-68-1	98.0%	Alta Scientific Co., Ltd	104	0.3	0.1	279.1	186.1	4.03
									279.1	156	
Sulfamethoxazole	SMX	$C_{10}H_{11}N_3O_3S$	723-46-6	98.0%	Alta Scientific Co., Ltd	94.3	0.06	0.02	254.2	156	4.21
									254.2	92.1	
Sulfaquinoxaline	SQX	$C_{14}H_{12}N_4O_2S$	59-40-5	98.0%	Alta Scientific Co., Ltd	106	0.03	0.01	301.1	156	4.33
									301.1	108	
Sulfisoxazole	SSZ	$C_{11}H_{13}N_3O_3S$	127-69-5	98.7%	Alta Scientific Co., Ltd	64.4	0.3	0.1	268	156	3.98
									268	113	
Sulfadoxine	SDM	$C_{12}H_{14}N_4O_4S$	2447-57-6	98.4%	Alta Scientific Co., Ltd	88.3	0.06	0.02	311.1	156.1	4.34
									311.1	108.2	
Oxolinic acid	OA	$C_{13}H_{11}NO_5$	14698-29-4	95.0%	Alta Scientific Co., Ltd	122	0.06	0.02	262	244.1	4.52
									262	216.1	
Flumequin	FMQ	$C_{14}H_{12}FNO_3$	42835-25-6	98.9%	Alta Scientific	130	0.3	0.1	262.1	244.1	4.51

					Co., Ltd				262.1	202.1	
Cinoxacin	CIN	C12H10N2O5	28657-80-9	99.9%	Alta Scientific Co., Ltd	83.4	0.06	0.02	263.1 263.1	217.1 245	4.19
Sulfabenzamide	SB	C13H12N2O3S	127-71-9	98.0%	Alta Scientific Co., Ltd	68.2	0.21	0.07	277.1 277.1	156 108.2	3.98
Sulfamethoxypyridazine	STD	C11H12N4O3S	80-35-3	99.0%	Alta Scientific Co., Ltd	103	0.3	0.1	281 281	156 126.1	4.04
Sulfamonomethoxine	SMM	$C_{11}H_{12}N_4O_3S$	1220-83-3	98.0%	Alta Scientific Co., Ltd	107	0.3	0.1	281.1 281.1	156 126.1	4.04
Sulfaphenazole	SPP	$C_{15}H_{14}N_4O_2S$	526-08-9	99.8%	Alta Scientific Co., Ltd	124	0.3	0.1	315 315	156 108	4.36
Lomefloxacin	LOM	$C_{17}H_{19}F_2N_3O_3$	98079-51-7	99.0%	Alta Scientific Co., Ltd	59.6	0.6	0.2	352.3 352.3	265.2 308.3	3.95
Ofloxacin	OFL	$C_{18}H_{20}FN_3O_4$	82419-36-1	98.0%	Alta Scientific Co., Ltd	69.8	0.6	0.2	362.2 362.2	318.1 261.1	3.91
Marbofloxacin	MAR	C17H18FNO3S	873945-29-0	99.0%	Alta Scientific Co., Ltd	77.8	0.6	0.2	363.1 363.1	320 345.1	3.87
Fleroxacin	FLX	$C_{17}H_{18}F_3N_3O_3$	79660-72-3	98.0%	Alta Scientific Co., Ltd	73.6	0.15	0.05	370 370	326.1 269.2	3.27
Sarafloxacin	SAL	$C_{20}H_{17}F_2N_3O_3$	98105-99-8	98.0%	Alta Scientific Co., Ltd	85.7	1.5	0.5	386.1 386.1	341.8 298.8	4.04
Orbifloxacin	OBI	$C_{19}H_{20}F_3N_3O_3$	113617-63-3	99.9%	Alta Scientific Co., Ltd	97	0.3	0.1	396.2 396.2	352.1 295	3.43
Difloxacin	DFL	$C_{21}H_{19}F_2N_3O_3$	98106-17-3	99.5%	Alta Scientific Co., Ltd	80.4	0.45	0.15	400.1 400.1	355.7 298.9	4.05
Oleandomycin	OLE	C35H61NO12	3922-90-5	98.2%	Alta Scientific Co., Ltd	121	0.03	0.02	688.4 688.4	158.2 544.3	4.24
Erythromycin	ERY	$C_{37}H_{67}NO_{13}$	114-07-8	98.0%	Alta Scientific Co., Ltd	100	0.3	0.1	734.4 734.4	576.2 158.1	4.11

Clarithromycin	CLR	$C_{38}H_{69}NO_{13}$	81103-11-9	99.0%	Alta Scientific Co., Ltd	105	0.3	0.1	748.7 748.7	590.4 158.3	4.44
Roxithromycin	RTM	$C_{41}H_{76}N_2O_{15}$	80214-83-1	98.0%	Alta Scientific Co., Ltd	98.2	0.06	0.02	837.6 837.6	679.5 158.1	4.54
Spiramycin	SPI	$C_{43}H_{74}N_2O_{14}$	8025-81-8	96.9%	Alta Scientific Co., Ltd	88.7	0.3	0.1	843.4 843.4	174.1 318.3	4.01
Sulfathiazole	STZ	$C_9H_9N_3O_2S_2$	72-14-0	98.0%	Alta Scientific Co., Ltd	105	0.3	0.1	256 256	156 108	4.01
Tylosin	TYL	$C_{46}H_{77}NO_{17}$	1401-69-0	99.0%	Alta Scientific Co., Ltd	92.6	0.3	0.1	916.6 916.6	174 772.5	4.28
Carbenicillin	CAR	$C_{17}H_{18}N_2O_6S$	4697-36-3	98.0%	Alta Scientific Co., Ltd	75.1	0.6	0.2	411.1 411.1	160 217.1	4.2

Table S2. The PNEC, EC50, NOEC and AF values were collected from previous studies to identify the sensitive aquatic organisms in this paper.

Antibiotics	Non-target organism	Toxicity data ($mg \cdot L^{-1}$)	AF	PNEC ($ng \cdot L^{-1}$)	Reference
SDZ	M. aeruginosa	$EC_{50} = 0.135$	1000	135	[1]
SMZ	Lemna minor	$EC_{50} = 1.277$	1000	1277	[2]
SMX	S. leopoliensis	$EC_{50} = 0.03$	1000	30	[3]
SQX	Daphnia magna	$EC_{50} = 131$	1000	131000	[4]
SSZ	Lemna minor	$EC_{50} = 0.62$	1000	620	[2]
SDM	Lemna gibba	$EC_{50} = 0.445$	1000	445	[5]
SMM	Chlorella vulgaris	$EC_{50} = 5.9$	1000	5900	[6]
STZ	Daphnia magna	$EC_{50} = 8.2$	1000	8200	[7]
STD	Lemna minor	$EC_{50} = 3.82$	1000	3820	[8]
ERY	Anabaena sp.	$EC_{50} = 0.022$	1000	22	[9]
CLR	P. subcapitata	$EC_{50} = 0.002$	1000	2	[10]
RTM	P. subcapitata	NOEC = 0.01	100	1	[11]

SPI	M.aeruginosa	EC_{50} = 0.005	1000	5	[12]
LOM	Fish	EC_{50} = 770	1000	770000	[13]
OFL	M.aeruginosa	EC_{50} = 0.02	1000	20	[14]
MAR	C.dubia	EC_{50} = 17	1000	17000	[15]
SAL	Fish	EC_{50} = 190	1000	190000	[13]
ENR	M.aeruginosa	EC_{50} = 0.05	1000	50	[14]
CIN	Lemna minor	EC_{50} = 0.0671	1000	67.1	[16]
SPA	M.aeruginosa	EC_{50} = 0.015	1000	15	[12]
NA	V. fischeri	EC_{50} = 199.94	1000	199940	[17]
OA	M.aeruginosa	EC_{50} = 0.18	1000	180	[12]
FMQ	P. subcapitata	EC_{50} = 9.3	1000	9300	[18]

References

1. Lützhoft, H.C.H.; Halling-Sorensen, B.; Jorgensen, S.E. Algal toxicity of antibacterial agents applied in Danish fish farming. *Archives of Environmental Contamination and Toxicology* **1999**, *36*, 1-6, doi:10.1007/s002449900435.
2. Bialk-Bielinska, A.; Stolte, S.; Arning, J.; Uebers, U.; Boeschen, A.; Stepnowski, P.; Matzke, M. Ecotoxicity evaluation of selected sulfonamides. *Chemosphere* **2011**, *85*, 928-933, doi:10.1016/j.chemosphere.2011.06.058.
3. Ferrari, B.; Mons, R.; Vollat, B.; Fraysse, B.; Paxéus, N.; Lo Giudice, R.; Pollio, A.; Garric, J. Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? *Environmental Toxicology and Chemistry* **2004**, *23*, 1344-1354, doi:10.1897/03-246.
4. De Liguoro, M.; Fioretto, B.; Poltronieri, C.; Gallina, G. The toxicity of sulfamethazine to *Daphnia magna* and its additivity to other veterinary sulfonamides and trimethoprim. *Chemosphere* **2009**, *75*, 1519-1524, doi:10.1016/j.chemosphere.2009.02.002.
5. Brain, R.A.; Johnson, D.J.; Richards, S.M.; Sanderson, H.; Sibley, P.K.; Solomon, K.R. Effects of 25 pharmaceutical compounds to *Lemna gibba* using a seven-day static-renewal test. *Environmental Toxicology and Chemistry* **2004**, *23*, 371-382, doi:10.1897/02-576.
6. Yang, Q.; Gao, Y.; Ke, J.; Show, P.L.; Ge, Y.; Liu, Y.; Guo, R.; Chen, J. Antibiotics: An overview on the environmental occurrence, toxicity, degradation, and removal methods. *Bioengineered* **2021**, *12*, 7376-7416, doi:10.1080/21655979.2021.1974657.
7. Jung, J.; Kim, Y.; Kim, J.; Jeong, D.-H.; Choi, K. Environmental levels of ultraviolet light potentiate the toxicity of sulfonamide antibiotics in *Daphnia magna*. *Ecotoxicology* **2008**, *17*, 37-45, doi:10.1007/s10646-007-0174-9.

8. Pro, J.; Ortiz, J.A.; Boleas, S.; Fernández, C.; Carbonell, G.; Tarazona, J.V. Effect assessment of antimicrobial pharmaceuticals on the aquatic plant *Lemna minor*. *Bulletin of Environmental Contamination and Toxicology* **2003**, *70*, 290-295, doi:10.1007/s00128-002-0208-1.
9. Yan, Z.; Yang, H.; Dong, H.; Ma, B.; Sun, H.; Pan, T.; Jiang, R.; Zhou, R.; Shen, J.; Liu, J.; et al. Occurrence and ecological risk assessment of organic micropollutants in the lower reaches of the Yangtze River, China: A case study of water diversion. *Environmental Pollution* **2018**, *239*, 223-232, doi:10.1016/j.envpol.2018.04.023.
10. Isidori, M.; Lavorgna, M.; Nardelli, A.; Pascarella, L.; Parrella, A. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment* **2005**, *346*, 87-98, doi:10.1016/j.scitotenv.2004.11.017.
11. Yang, L.-H.; Ying, G.-G.; Su, H.-C.; Stauber, J.L.; Adams, M.S.; Binet, M.T. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. *Environmental Toxicology and Chemistry* **2008**, *27*, 1201-1208, doi:10.1897/07-471.1.
12. Halling-Sorensen, B. Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere* **2000**, *40*, 731-739, doi:10.1016/s0045-6535(99)00445-2.
13. Xu, M.; Huang, H.; Li, N.; Li, F.; Wang, D.; Luo, Q. Occurrence and ecological risk of pharmaceuticals and personal care products (PPCPs) and pesticides in typical surface watersheds, China. *Ecotoxicology and Environmental Safety* **2019**, *175*, 289-298, doi:10.1016/j.ecoenv.2019.01.131.

14. Robinson, A.A.; Belden, J.B.; Lydy, M.J. Toxicity of fluoroquinolone antibiotics to aquatic organisms. *Environmental Toxicology and Chemistry* **2005**, *24*, 423-430, doi:10.1897/04-210r.1.

15. Kergaravat, S.V.; Hernandez, S.R.; Maria Gagneten, A. Second-, third- and fourth-generation quinolones: Ecotoxicity effects on *Daphnia* and *Ceriodaphnia* species. *Chemosphere* **2021**, *262*, doi:10.1016/j.chemosphere.2020.127823.

16. Li, M.; Wei, D.; Du, Y. Acute toxicity evaluation for quinolone antibiotics and their chlorination disinfection processes. *Journal of Environmental Sciences* **2014**, *26*, 1837-1842, doi:10.1016/j.jes.2014.06.023.

17. Backhaus, T.; Scholze, M.; Grimme, L.H. The single substance and mixture toxicity of quinolones to the bioluminescent bacterium *Vibrio fischeri*. *Aquatic Toxicology* **2000**, *49*, 49-61, doi:10.1016/s0166-445x(99)00069-7.

18. Munch Christensen, A.; Ingerslev, F.; Baun, A. Ecotoxicity of mixtures of antibiotics used in aquacultures. *Environmental Toxicology and Chemistry* **2006**, *25*, 2208-2215, doi:10.1897/05-415r.1.

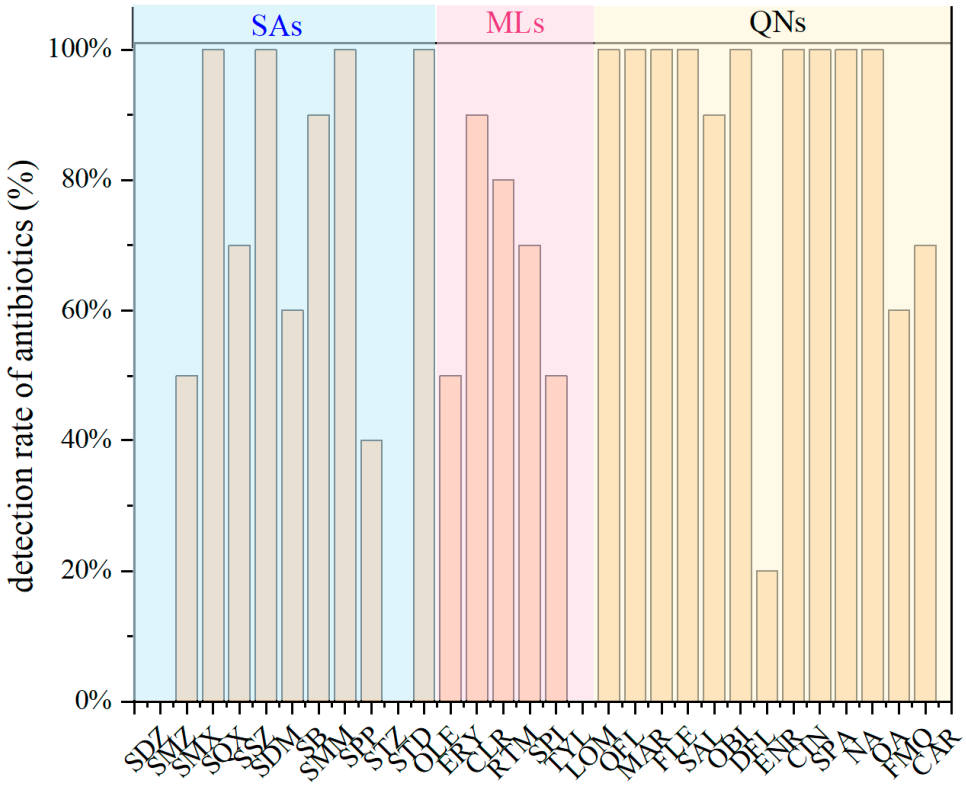

Figure S2. The detection rate of 31 antibiotics in the Zhuozhang River.