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SUPPLEMENTARY MATERIAL TO

Pharmaceuticals in Belgrade's wastewater: impact on surface waters and environmental risk assessment

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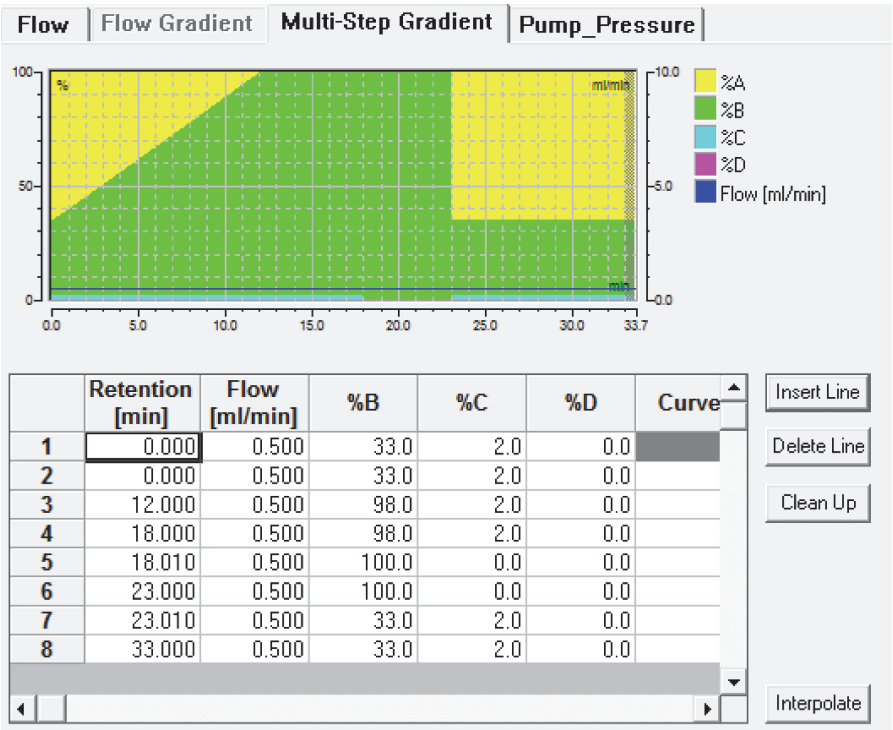

	Retention [min]	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	0.500	33.0	2.0	0.0	
2	0.000	0.500	33.0	2.0	0.0	
3	12.000	0.500	98.0	2.0	0.0	
4	18.000	0.500	98.0	2.0	0.0	
5	18.010	0.500	100.0	0.0	0.0	
6	23.000	0.500	100.0	0.0	0.0	
7	23.010	0.500	33.0	2.0	0.0	
8	33.000	0.500	33.0	2.0	0.0	

Fig. S-I. Mobile phase gradient profile.

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Water sample collection

Wastewater and surface water samples were collected in Belgrade, Serbia, near the confluence of the Sava and the Danube rivers (Table S-II). A map with marked sampling sites (WW1–WW7) is shown in Fig. S-1. Wastewater samples were taken at seven discharge points, which handle about 80 % of Belgrade's untreated wastewater. The Belgrade sewage system consists of 212 km of collectors, 1,439 km of pipe network, 32,750 drains and 53,394 sewage connections.[20] Sample WW1 was taken from the largest sewage canal, serving approximately 500,000 inhabitants. Samples were collected over 24 h using automatic samplers and combined into composite samples. Corresponding surface water samples were collected at eight sampling sites downstream of wastewater discharge. Three samples were collected from the Sava river (SW1–SW3, Fig. S-1), four samples from the Danube river (one before the confluence of the two rivers, SW4; three after the confluence, SW6–SW8), and one at the confluence of the Sava and Danube rivers (SW5). Surface water samples were collected by direct sampling from a boat in the middle of the river flow at a depth of about 50 cm. All water samples were collected in 1 L PET bottles and stored at 4 °C until analysis (usually within 1–2 days after sampling). No precipitation occurred on the day of sampling.

Since Belgrade lacks a WWTP, influent and effluent samples were collected from two WWTPs located in small municipalities in Serbia (Sombor, WWTP1, and Velika Plana, WWTP2). The selected WWTPs provide primary and secondary treatment of wastewater using biologically active sludge. WWTP1 has a treatment capacity of 50,000 population equivalents (PE) or 9,300 m^3 day^{-1}, while WWTP2 has a capacity of 35,000 PE. Composite 24 h samples of influent and effluent wastewater were collected at each WWTP using automatic sampling devices. Water samples were stored in 1 L PET bottles and kept frozen without preservatives until preparation for analysis, which occurred a few days after sampling.

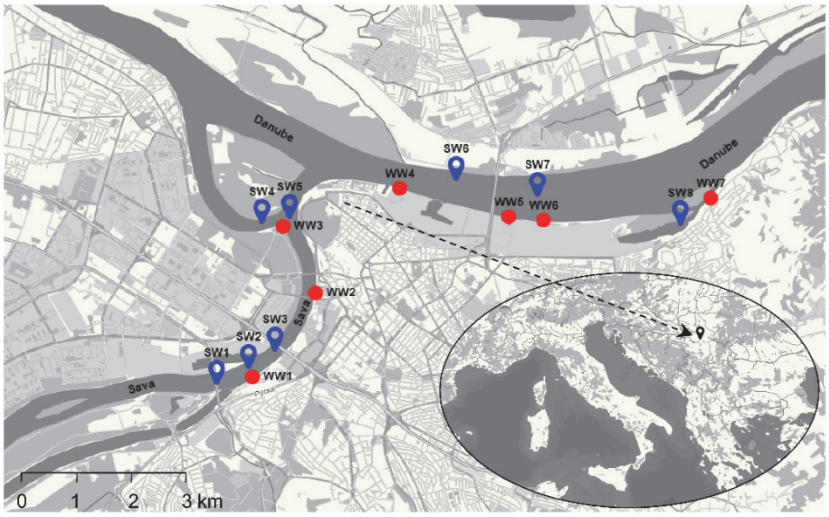

Fig. S-1. Sampling sites of wastewater (WW) and corresponding surface water (SW) in the Sava and the Danube rivers in Belgrade (Serbia).

Table S-I. MS operating parameters for selected pharmaceuticals.

Pharmaceuticals	Precursor ion (*m/z*)	Quantification reaction	Collision energy (%)	Confirmation reaction	Collision energy (%)
Trimethoprim	291 $[M+H]^+$	291→230	44	291→123	44
Metoprolol	268 $[M+H]^+$	268→191	37	268→218	37
Sulfamethoxazole	254 $[M+H]^+$	254→188	34	254→156	34
Azithromycin	749 $[M+H]^+$	749→591	30	591→434	28
Bisoprolol	326 $[M+H]^+$	326→116	31	326→222	31
Enalapril	377 $[M+H]^+$	377→234	30	377→303	30
Cilazapril	418 $[M+H]^+$	418→211	25	211→183	32
Erythromycin	734 $[M+H]^+$	734→576	26	734→716	26
Bromazepam	316 $[M+H]^+$	316→288	36	288→261	35
Amlodipine	409 $[M+H]^+$	409→238	25	409→294	25
Carbamazepine	237 $[M+H]^+$	237→194	34	237→220	34
Lorazepam	321 $[M+H]^+$	321→303	32	303→275	26
Diazepam	285 $[M+H]^+$	285→257	40	257→228	39
Atorvastatin	559 $[M+H]^+$	559→466	25	559→440	25
Diclofenac	296 $[M+H]^+$	296→278	28	278→250	22
Clopidogrel	322 $[M+H]^+$	322→212	28	212→184	23
Simvastatin	419 $[M+H]^+$	419→285	21	419→199	21

Table S-II. The sampling site description.

Sample	Latitude	Longitude	Site description	
Surface water (SW)				
SW1	44.7962	20.4259	The Sava; 4 km before the confluence	
SW2	44.7989	20.4336	The Sava; 3.5 km before the confluence; downstream from the largest WW canal (WW1)	
SW3	44.8019	20.4400	The Sava; 2.7 km before the confluence	
SW4	44.8233	20.4368	The Danube; 500 m before the confluence	
SW5	44.8241	20.4434	The confluence	
SW6	44.8305	20.4828	The Danube; 3.6 km after the confluence	
SW7	44.8279	20.5091	The Danube; 5.6 km after the confluence	
SW8	44.8231	20.5359	The Danube; 8.5 km after the confluence; small bay	
Wastewater (WW)				Number of inhabitants connected to WW canal
WW1	44.7976	20.4346	The largest WW canal; catchment area of 7,277 ha	500,000
WW2	44.8118	20.4494	Catchment area of 113 ha	23,000
WW3	44,8229	20.4419	The second large WW canal; catchment area of 2,620 ha	225,000
WW4	44.8293	20.4694	Catchment area of 92 ha	17,000
WW5	44.8245	20.4953	Catchment area of 1,112 ha	165,000
WW6	44.8239	20.5035	Catchment area of 50 ha	5,000
WW7	44.8277	20.5430	Catchment area of 116 ha	7,000

Table S-III. Lowest PNEC values of detected pharmaceuticals in freshwater obtained from NORMAN database.

Pharmaceuticals	Norman PNEC ID	CAS No.	Taxon. group	Scientific name	Applied AF	Justification	Derivation method	Lowest PNEC freshwater (μg L^{-1})	Ref
Trimethoprim	PNEC-ID-0348354	738-70-5	n.r. [a]	n.r.	0	n.r.	n.r.	0.5	1
Metoprolol	PNEC-ID-0348060	51384-51-1	PI	*Desmodesmus subspicatus*	50	n.r.	deterministic	8.6	2
Sulfamethoxazole	PNEC-ID-0348126	723-46-6	P	*Synechococcus leopoliensis*	10	n.r.	deterministic	0.6	2
Azithromycin	PNEC-ID-0347903	83905-01-5	PI	*Microcystis aeruginosa*	10	n.r.	deterministic	0.019	2
Bisoprolol	PNEC-ID-0348209	66722-44-9	PIV	n.r.	50	n.r.	deterministic	92	3
Enalapril	PNEC-ID-0031812	75847-73-3	fish	-	1000	[b]	deterministic	1.58	4
Carbamazepine	PNEC-ID-0347929	298-46-4	I	*Daphnia pulex*	50	n.r.	deterministic	2	2
Diazepam	PNEC-ID-0257661	439-14-5	F	*Danio rerio*	10	[c]	deterministic	0.29	5
Atorvastatin	PNEC-ID-0348226	134523-00-5	PIV	n.r.	10	n.r.	deterministic	8.5	6
Diclofenac	PNEC-ID-0348270	15307-86-5	n.r.	n.r.	0	n.r.	n.r.	0.04	7

[a] n.r. – not reported
[b] One predicted short-term L(E)C50 from each of three trophic levels (i.e., base set)
[c] Long-term results (e.g., EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels

Table S-IV. Validation parameters of the analytical method: recoveries and relative standard deviations (RSD), limits of detection (LOD) and quantification (LOQ), and linearity correlation coefficient (R^2).

Pharmaceuticals	Recovery, % (RSD, %) Spiking level, ng L^{-1}		LOD, ng L^{-1}	LOQ, ng L^{-1}	R^2
	100	1000			
Trimethoprim	88 (8)	92 (5)	11.0	36.8	0.993
Metoprolol	87 (12)	87 (5)	3.3	11.1	0.993
Sulfamethoxazole	83 (5)	70 (8)	30.0	100.0	0.999
Azithromycin	58 (7)	55 (11)	5.2	17.4	0.999
Bisoprolol	71 (12)	84 (11)	5.2	17.2	0.997
Enalapril	117 (19)	110 (11)	30.0	100.0	0.993
Cilazapril	88 (2)	101 (7)	3.9	13.2	0.997
Erythromycin	56 (17)	62 (10)	4.1	13.7	0.997
Bromazepam	89 (11)	93 (7)	25.0	83.3	0.998
Amlodipine	81 (4)	96 (2)	23.4	77.9	0.998
Carbamazepine	107 (8)	101 (8)	3.6	11.8	0.999
Lorazepam	83 (13)	93 (6)	14.3	47.6	0.996
Diazepam	86 (10)	95 (4)	21.1	70.3	0.990
Atorvastatin	95 (2)	83 (5)	19.6	65.5	0.999
Diclofenac	83 (20)	92 (13)	23.1	76.9	0.993
Clopidogrel	87 (7)	94 (1)	9.3	30.9	0.999
Simvastatin	118 (19)	107 (7)	50.0	166.7	0.998

Table S-V. Hazard identification of detected pharmaceuticals.

Pharmaceutical	Persistence	Bioaccumulation	Toxicity	Mobility	Endocrine Disruption (ED)	Ref.
Trimethoprim	Moderate	Low	Moderate	High	Not reported	8
Metoprolol	Moderate	Low	Low	Moderate–high	Possible functional ED	9-11
Sulfamethoxazole	Moderate	Low	High	Moderate–high	May exhibit endocrine toxicity	12-14
Azithromycin	Moderate–high	Low–moderate	High	Moderate–high	May have endocrine effects	15
Bisoprolol	Moderate	Low	Low	Moderate	Not reported	16,17
Enalapril	Moderate	Low	Low	Moderate	Not classified as ED	18,19
Cilazapril	Moderate	Low	Low	Moderate	Not reported	20
Erythromycin	High	Low	High	High	Potential disruptor	21-23
Carbamazepine	High	Low	High	High	Potential to function as an ED	24-26
Lorazepam	Moderate	Low	Moderate	Moderate	Functional ED uncertainty	27
Diazepam	Moderate–high	Low–moderate	Moderate	Moderate	Potential to interact with endocrine function	28,29
Atorvastatin	Low–moderate	Low	Moderate	Moderate	Not reported	30,31
Diclofenac	Moderate-high	Low	Moderate-high	Moderate-high	Possible ED	32,33

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