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

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Abstract: In the Belgrade region, direct discharges of untreated wastewater into the Sava and Danube Rivers have a detrimental impact on water quality. This study investigates this impact by investigating the presence of pharmaceuticals in Belgrade's wastewater and corresponding surface water, alongside the associated environmental risk. A highly sensitive and selective LC-MS/MS method was developed, validated and applied to the analysis of water samples. Thirteen out of seventeen target pharmaceuticals were detected in wastewater, while nine compounds were found in surface water, with diclofenac reaching the highest concentration (1.1 μg L^{-1}). Metoprolol, carbamazepine, and diclofenac were the most prevalent in both wastewater and surface water samples. Risk assessment indicated that diclofenac and azithromycin posed a high environmental risk (RQ > 1), while mixture toxicity assessment suggested a cumulative hazard at all sampling sites. Additionally, the study evaluated pharmaceutical removal efficiencies in two wastewater treatment plants in Serbia, revealing variable efficiencies and even negative removal rates for some compounds, highlighting the inadequacy of conventional treatment plants in effectively eliminating these substances. The results emphasize the urgency of regulatory actions and the need for adequate treatment technologies to reduce pharmaceutical pollution in aquatic environments.

Keywords: drugs; emerging pollutants; urban wastewater; water quality; WWTP; ecological assessment.

INTRODUCTION

Pharmaceuticals are increasingly recognized as significant environmental contaminants due to their rising production and widespread use, posing potential risks to aquatic ecosystems. Municipal sewage discharge is a major source of these

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contaminants. Pharmaceuticals enter wastewater through multiple pathways: (i) excretion of parent compounds and metabolites following human consumption; (ii) improper disposal of unused drugs; (iii) hospital and healthcare facility discharges; and (iv) pharmaceutical manufacturing effluents.[1–4] These originate either as pharmaceutical waste (unused drugs disposed through drains) or human metabolites (excreted after therapeutic use). Human excretion is the predominant pathway, with 30-90% of administered doses excreted as parent compounds or metabolites.[5] Following administration, many pharmaceuticals undergo hepatic metabolism and are conjugated to facilitate excretion. The concern is that certain metabolites may retain or exceed parent compound toxicity and persistence.[6] However, current environmental risk assessment studies are predominantly focused on parent compounds. Numerous studies have confirmed the widespread occurrence of pharmaceuticals in wastewater and surface waters at concentrations capable of disrupting aquatic organisms and potentially affecting human health.[7–11]

This issue is particularly concerning in the Republic of Serbia, as less than 21% of the population is connected to wastewater treatment plants (WWTPs).[12] Belgrade, the capital, exemplifies this issue, as it completely lacks treatment facilities, resulting in the direct discharge of raw sewage into the Sava and Danube Rivers. Furthermore, existing WWTPs are often insufficient for removing pharmaceuticals,[13] allowing these contaminants to enter surface water, groundwater, and even drinking water.[14–16] Ongoing research is necessary to better understand the fate of pharmaceutical pollutants, their impact on aquatic life, and the potential risks they pose to ecosystem stability.

This study aimed to: (i) develop and validate a fast, simple, and sensitive liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for the determination of selected pharmaceuticals in wastewater; (ii) determine the concentrations of selected contaminants in Belgrade urban wastewater directly discharged to the Sava and the Danube Rivers, as well as in corresponding river water; (iii) assess the environmental risk posed by contaminants detected in river water; and (iv) evaluate the removal efficiency of the analyzed pharmaceuticals in two WWTPs. The pharmaceuticals selected for the study are among the most commonly used and/or frequently detected in the investigated area.

EXPERIMENTAL

Chemicals and reagents

High-purity (>95%) analytical standards of seventeen selected pharmaceuticals were obtained from domestic pharmaceutical companies (Hemofarm, STADA Group, Vršac, Serbia, and Zorka-Pharma, Šabac, Serbia). These included: antibiotics (trimethoprim, sulfamethoxazole, azithromycin, erythromycin); the drugs for the treatment of cardiovascular diseases (metoprolol, bisoprolol, enalapril, cilazapril, amlodipine, atorvastatin, clopidogrel,

simvastatin); anxiolytic drugs (bromazepam, carbamazepine, lorazepam, diazepam); and one non-steroidal anti-inflammatory drug (diclofenac).

The individual stock standard solutions of pharmaceuticals were prepared in methanol at a concentration of 100 mg L^{-1}. The working standard solutions were prepared by mixing the appropriate amounts of the individual stock standard solutions and diluting them with methanol. All solutions were stored at 4 °C. For the pH adjustment of the water samples, concentrated acetic acid and ammonia were used. All solvents used were HPLC-grade from J.T. Baker (Center Valley, USA) or Sigma-Aldrich (St. Louis, USA), and all reagents were of analytical grade. Deionized water was obtained using the GenPure ultrapure water system (TKA, Niederelbert, Germany).

Sample preparation

In this study, we modified a previously developed sample preparation method[17] for determining pharmaceuticals in surface water and groundwater samples by replacing the methanol-dichloromethane elution solvent with a safer methanol-ethyl acetate mixture. The modified method, briefly described below, was validated for wastewater samples.

Waters (Milford, MA, USA) Oasis hydrophilic-lipophilic balance (HLB, 200 mg/6 mL) cartridges were utilized for the solid-phase extraction (SPE) of wastewater samples. Prior to the SPE procedure, wastewater samples were filtered through < 1 μm glass fibre filters (Whatman GmbH, Dassel, Germany). SPE sorbent was preconditioned with 5 mL of a methanol-ethyl acetate (1:1) mixture, followed by 5 mL of deionized water and 5 mL of deionized water with its pH adjusted to 6.0. The wastewater sample (100 mL), adjusted to pH 6.0, was then passed through the cartridge, and the sorbent was dried by vacuum suction for 10 min. Afterwards, analytes were eluted using 15 mL of a methanol-ethyl acetate (1:1) mixture, and the extract was evaporated under the nitrogen stream and reconstituted into 1 mL with methanol. The extract was filtered through a 0.45 μm polyvinylidene difluoride (PVDF) filter from Roth (Karlsruhe, Germany) and analyzed.

LC–MS/MS analysis

Water sample extracts were analyzed using LC–MS/MS based on the method developed by Grujić *et al.*[18], which was further expanded to include drugs for the treatment of cardiovascular diseases, allowing the determination of 17 pharmaceuticals. Briefly, a Surveyor LC system (Thermo Fisher Scientific, Waltham, MA, USA) was used for the separation of the analytes on the reversed-phase Zorbax Eclipse® XDB-C18 column (75 mm × 4.6 mm i.d., 3.5 μm particle size; Agilent Technologies, Santa Clara, USA). A pre-column (12.5 mm × 4.6 mm i.d., 5 μm particle size; Agilent Technologies, Santa Clara, USA) was installed in front of the separation column. The mobile phase consisted of water (A), methanol (B), and 10% acetic acid (C). The gradient changed as follows (Fig. S-I, Supplementary Material): 0 min, A 65%, B 33%, C 2%; 12 min, B 98%, C 2%; 18 min, B 98%, C 2%; 18.01 min, B 100%. The initial conditions were then re-established and held for 10 min. The flow rate of the mobile phase was 0.5 mL min^{-1}. An aliquot of 10 μL of the final extract was injected into the LC system. The LC system was coupled with an LCQ Advantage quadrupole ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, USA). The electrospray ionization (ESI) technique was used, and all analytes were analyzed in the positive ionization mode. The optimal source parameters were as follows: source voltage (4.5 kV), sheath gas (25 au, *i.e.*, 25 arbitrary units), and capillary temperature (290 °C). Fragmentation reactions used for identification and quantification, as well as the confirmation of selected analytes, are shown in Table S-I (Supplementary Material).

The standard addition method was used to analyze the collected wastewater samples, compensating for matrix effects and incomplete analyte extraction. Each water sample was divided into six aliquots: four aliquots were spiked with a working standard solution at concentrations of 50–1000 ng L^{-1}, resulting in final extract concentrations of 5–100 µg L^{-1}, and the remaining two were not spiked.

Method validation

The linearity of the analytical response was studied using the matrix-matched standards (MMS) prepared at five concentration levels (5–100 µg L^{-1}). MMS were prepared by adding working standard solutions of the pharmaceuticals to the blank extracts obtained after the SPE procedure. The extraction recoveries and the repeatability of the method, expressed as the relative standard deviation (RSD), were determined by analyzing three replicate wastewater samples spiked at two concentration levels (100 and 1000 ng L^{-1}). The limits of detection (LODs) and quantification (LOQs) were determined as the minimum detectable amount of analyte with a signal-to-noise (S/N) ratio of 3 and 10, respectively [19], using spiked samples at a concentration of 50 ng L^{-1}.

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, Supplementary Material). A map with marked sampling sites (WW1–WW7) is shown in Fig. 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 hours using automatic samplers and combined into composite samples. Corresponding surface water samples were collected at eight sampling sites downstream from wastewater discharge. Three samples were collected from the Sava River (SW1–SW3, Fig. 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 equivalent (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 by 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.

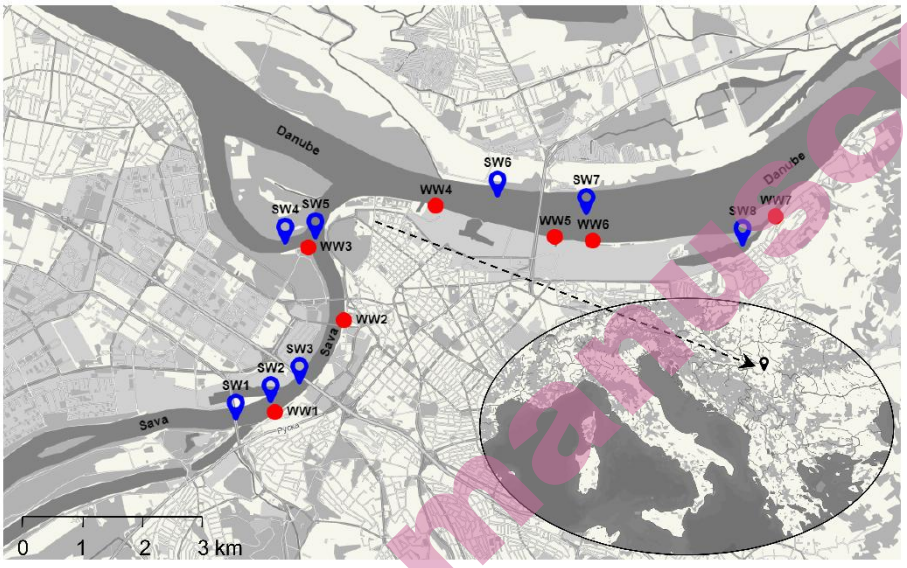

Fig. 1. Sampling sites of wastewater (WW) and corresponding surface water (SW) in the Sava and the Danube rivers in Belgrade (Serbia).

Risk assessment

The risk quotient (RQ) approach was used to assess the environmental risk of pharmaceuticals detected in surface water. Risk quotients were calculated using the equation:

$$RQ = \frac{MEC}{PNEC} \quad (1)$$

where MEC is the measured environmental concentration and PNEC is the predicted no-effect concentration of the detected compounds.[21] PNEC values were obtained from the NORMAN database,[22] using the lowest available values for each compound (Table S-III, Supplementary Material). The risk to aquatic life may increase when pharmaceuticals are present in mixtures, as each compound contributes to toxicity, even if individual concentrations are below their PNEC values. Therefore, to fully estimate the risks to aquatic life arising from exposure to pharmaceuticals, the combined effects of their mixtures were considered. Risk at each sampling site was evaluated using the concentration addition concept,[23,24] with RQ_{mix} calculated as:

$$RQ_{mix} = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} \quad (2)$$

Based on RQ values, the environmental risk was classified as: high ($RQ \geq 1$), medium ($0.1 \geq RQ > 1$), and low ($RQ < 0.1$).[25]

RESULTS AND DISCUSSION

Method validation

The developed analytical method was validated for wastewater samples, with linearity, recovery, repeatability, detection limits, and quantification limits assessed (Table S-IV, Supplementary Material). Linearity was evaluated using

MMS (5–100 µg L^{-1}), corresponding to initial sample concentrations of 50–1000 ng L^{-1}. Determination coefficients (R^2) ranged from 0.990 to 0.999 for all tested analytes, indicating adequate linearity. The recoveries ranged from 70% to 120% for most analytes, except for azithromycin and erythromycin (55–62%), likely due to pH adjustment to 6.0. Our previous study showed that pH values of 3.0 and 7.5 were optimal for azithromycin and erythromycin, respectively.[18] However, pH 6.0 provided the best overall recoveries, making it the most suitable choice for simultaneous analysis. The resulting RSD values were all below 20%. Low limits of detection (3.3–50.0 ng L^{-1}) and quantification (11.1–166.7 ng L^{-1}) were obtained, demonstrating the method's sensitivity, which is in agreement with values reported in the literature[19], and its suitability for determining trace levels of selected analytes in wastewater samples.

Wastewater and surface water sample analysis

The validated method was used to analyze selected pharmaceuticals in urban wastewater samples discharged into the Sava and Danube rivers in Belgrade, as well as in the corresponding surface water. As shown in Table I, 13 out of 17 target pharmaceuticals were detected in urban wastewater, with concentrations ranging from 15 ng L^{-1} (cilazapril) to 1.2 µg L^{-1} (sulfamethoxazole). The most ubiquitous drugs were metoprolol, bisoprolol, enalapril, carbamazepine, and diclofenac, detected at all sampling sites, confirming their widespread use and persistence in wastewater. Sampling site WW1 had the most detected compounds (13 out of 17), likely because it is the primary discharge point for Belgrade's wastewater.

TABLE I. Pharmaceuticals detected in Belgrade urban wastewater at seven sampling sites (WW1–WW7) (n = 2).

Pharmaceuticals	Concentration ± SD / ng L^{-1}						
	WW1	WW2	WW3	WW4	WW5	WW6	WW7
Trimethoprim	122 ± 12	53 ± 5	–	–	482 ± 72	–	–
Metoprolol	290 ± 22	154 ± 13	434 ± 58	36 ± 3	305 ± 32	143 ± 20	294 ± 8
Sulfamethoxazole	122 ± 19	122 ± 9	–	–	1184 ± 152	–	–
Azithromycin	318 ± 48	102 ± 0	–	–	–	–	–
Bisoprolol	63 ± 11	46 ± 4	118 ± 22	83 ± 7	49 ± 8	35 ± 4	76 ± 9
Enalapril	155 ± 12	128 ± 26	240 ± 51	284 ± 44	174 ± 17	164 ± 20	208 ± 34
Cilazapril	15 ± 2	89 ± 16	22 ± 4	–	–	–	–
Erythromycin	133 ± 22	266 ± 34	347 ± 22	–	–	–	–
Carbamazepine	274 ± 44	313 ± 30	449 ± 14	263 ± 8	127 ± 25	190 ± 49	253 ± 41
Lorazepam	171 ± 24	84 ± 3	318 ± 19	167 ± 33	77 ± 10	105 ± 22	–
Diazepam	163 ± 22		–	–	–	–	–
Atorvastatin	71 ± 5	25 ± 4	–	–	–	–	–
Diclofenac	442 ± 73	253 ± 11	58 ± 5	471 ± 70	782 ± 156	479 ± 105	310 ± 71

Pharmaceuticals in untreated wastewater have been studied across the Western Balkans, with Petrović *et al.*[9] detecting trimethoprim, sulfamethoxazole, carbamazepine, lorazepam, and atorvastatin in Novi Sad at concentrations similar to ours, with carbamazepine being among the most abundant in both studies. However, diclofenac and metoprolol concentrations were higher in their study, with diclofenac exceeding 1 µg L^{-1}. Terzić *et al.*[26] investigated 44 pharmaceuticals in wastewater from Bosnia and Herzegovina, Croatia, and Serbia, reporting a wide concentration range for many compounds. For pharmaceuticals detected in both studies, the concentrations measured in our samples fell within the ranges reported.

In the corresponding surface water samples, 9 out of 17 target pharmaceuticals were detected (Table II), with concentrations ranging from 19 ng L^{-1} (bisoprolol) to 1.1 µg L^{-1} (diclofenac). The most prevalent drugs in the surface water samples were metoprolol, carbamazepine, and diclofenac, detected at all sites. At sampling site SW8, 9 out of 17 substances were detected, with notably higher concentrations, likely due to its location in a small bay with limited water circulation.

TABLE II. Pharmaceuticals detected in Belgrade surface water at eight sampling sites (SW1–SW8) (n = 2).

Pharmaceuticals	Concentration ± SD / ng L^{-1}							
	SW1	SW2	SW3	SW4	SW5	SW6	SW7	SW8
Trimethoprim	145 ± 11	–	–	–	–	–	–	334 ± 54
Metoprolol	95 ± 3	107 ± 13	53 ± 8	308 ± 50	82 ± 14	78 ± 11	132 ± 12	1012 ± 150
Sulfamethoxazole	–	–	–	–	–			127 ± 7
Azithromycin	–	–						58 ± 1
Bisoprolol				33 ± 2			19 ± 2	661 ± 124
Enalapril	–	–	–		–		–	1099 ± 8
Carbamazepine	20 ± 2	20 ± 2	23 ± 1	38 ± 7	24 ± 4	32 ± 5	56 ± 8	552 ± 29
Diazepam	–	–	–	–		–	–	176 ± 18
Diclofenac	316 ± 47	157 ± 2	267 ± 50	225 ± 45	375 ± 75	1087 ± 123	251 ± 50	950 ± 176

In a review paper,[27] the reported concentration ranges of pharmaceuticals in the Lower Danube River basin were: carbamazepine (3.94–945 ng/L), diclofenac (0.8–255 ng/L), sulfamethoxazole (30–204 ng/L), and trimethoprim (0.8–223 ng/L). Our results align with these ranges, except for diclofenac, which showed significantly higher concentrations at two sampling sites. Similarly elevated diclofenac levels were reported in the Danube near Budapest, Hungary (up to 931 ng/L).[28]

In most cases, the same compounds were found in both surface water samples and wastewater discharged into them, indicating the impact of Belgrade's municipal wastewater on surface water quality. The concentrations of most detected compounds were significantly lower in river water. However, for some compounds, such as diclofenac, similar or even higher concentrations were observed in surface water compared to wastewater. The highest detected

diclofenac concentration in river water exceeded those in wastewater, likely due to its high environmental persistence.[29] Metoprolol, diclofenac, and carbamazepine were the most frequently detected analytes in both sample types.

Hazard characterization of detected pharmaceuticals

Hazard identification was conducted for each detected pharmaceutical according to the REACH/CLP criteria, evaluating five key hazard classes: persistence, bioaccumulation, toxicity, mobility, and endocrine disruption.[30] A comprehensive summary of the hazard characterization for all detected pharmaceuticals is provided in Table S-V (Supplementary Material). The hazard assessment revealed that several detected pharmaceuticals exhibited concerning profiles: carbamazepine and erythromycin showed high persistence and toxicity, while azithromycin showed moderate-to-high persistence combined with high toxicity. Multiple pharmaceuticals (metoprolol, sulfamethoxazole, carbamazepine, erythromycin) demonstrated high mobility, raising concerns about their potential for widespread environmental distribution. Additionally, various compounds, including metoprolol, sulfamethoxazole, azithromycin, erythromycin, carbamazepine, lorazepam, diazepam, and diclofenac, showed potential endocrine disrupting activity. These hazard characteristics justify inclusion of the detected pharmaceuticals in the risk assessment.

Risk assessment

The environmental risk associated with analytes detected in surface water samples was assessed, with the obtained RQs presented in Fig. 2A. Based on the calculated RQ values, bisoprolol posed a low risk (RQ < 0.1), while most of the other detected analytes posed a moderate risk (0.1 ≤ RQ ≤ 1). However, diclofenac posed a high risk, with RQ values exceeding 1 at all sampling sites, reaching a maximum of 27.17 at site SW6. Azithromycin also exceeded the high-risk threshold (RQ > 1) at one site (RQ = 3.05 at SW8). Both diclofenac and azithromycin have been proposed for inclusion in the EU Priority Substances List in the field of water policy,[31] due to their widespread presence in water bodies and harmful effects on aquatic organisms. Other studies,[32–34] have also reported high risks for these substances, highlighting the need for further attention and regulatory consideration.

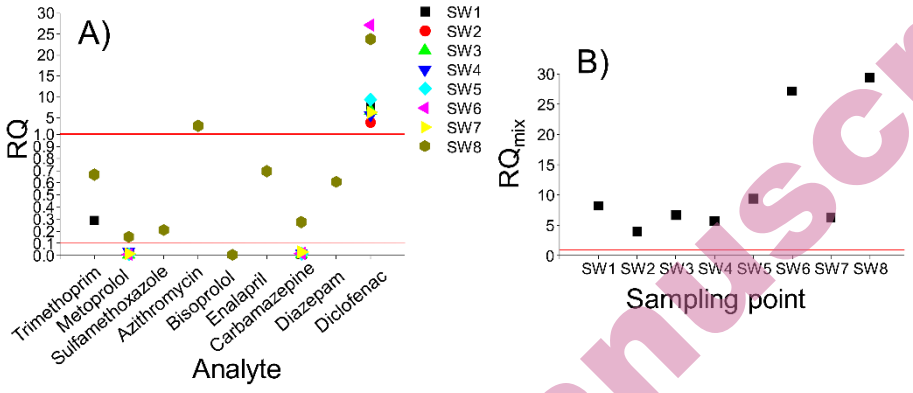

Fig. 2. (A) Risk quotients (RQs) for individual compounds at each surface water site; (B) Risk quotients for the compound mixture (RQ_{mix}) at each site.

The risk of the entire mixture of pharmaceuticals was also assessed at all surface water sampling sites. As shown in Fig. 2B, the RQ_{mix} significantly exceeded the threshold of 1 at all sites, with the highest value of 29.42 observed at site SW8. At this location, as well as at all other sites, diclofenac was the predominant contributor to the high RQ_{mix} value. However, even contaminants that individually do not pose a significant risk can contribute to an increased RQ_{mix} value, and as part of a mixture, they may amplify environmental risk due to cumulative effects. These findings emphasize the need to assess the combined effects of contaminant mixtures, rather than focusing solely on individual compounds.

Pharmaceuticals detected in WWTPs and their removal rates

Pharmaceutical concentrations in wastewater samples from the selected WWTPs in Serbia (Table III) show significant variations in both influent and effluent levels. Among the nine detected pharmaceuticals, the highest influent concentrations were recorded for carbamazepine (2.7 μg L^{-1}) at WWTP1 and diclofenac (5.9 μg L^{-1}) at WWTP2. Generally, influent concentrations exceeded those obtained for untreated wastewater from Belgrade (Table I), particularly for metoprolol, erythromycin, and diclofenac. These variations may stem from differences in wastewater composition, such as a different proportion of domestic effluents.

Our results align with other studies reporting carbamazepine concentrations of up to 2150 ng L^{-1} in wastewater in Spain and 2499 ng L^{-1} in China,[35,36] as well as diclofenac levels reaching 4056 ng L^{-1} in Slovenia.[37] In contrast, Afonso-Olivares *et al.*[38] reported lower concentrations of carbamazepine, diclofenac, and erythromycin compared to our study, while sulfamethoxazole and trimethoprim were found at higher levels. Differences in consumption patterns may account for regional variations in pharmaceutical concentrations.

TABLE III. Pharmaceuticals detected in influent and effluent samples of two WWTPs in Serbia (n = 2).

Pharmaceuticals	Concentration ± SD / ng L^{-1}		RE[a] / %	Concentration ± SD / ng L^{-1}		RE / %
	Influent WWTP1	Effluent WWTP1		Influent WWTP2	Effluent WWTP2	
Trimethoprim	37 ± 5	145 ± 7	-292	142 ± 13	109 ± 22	23
Metoprolol	860 ± 77	11630 ± 15	-1252	644 ± 112	3095 ± 12	-381
Sulfamethoxazole	215 ± 24	151 ± 13	30	385 ± 73	107 ± 16	72
Bisoprolol	78 ± 3	73 ± 9	6	89 ± 5	29 ± 2	67
Enalapril	543 ± 55	105 ± 8	81	868 ± 149	399 ± 58	54
Erythromycin	1448 ± 261	520 ± 78	64	956 ± 2	3833 ± 25	-301
Carbamazepine	2675 ± 589	389 ± 62	85	1368 ± 328	529 ± 74	61
Lorazepam	148 ± 2	242 ± 5	-63	188 ± 13	-	100
Diclofenac	1592 ± 143	919 ± 55	42	5927 ± 948	324 ± 35	95

[a]RE: removal efficiency

All pharmaceuticals detected in influent samples were also present in effluent samples from both WWTPs, except for lorazepam (which was detected in only one effluent), indicating incomplete removal by conventional wastewater treatment. The highest effluent concentrations were observed for metoprolol (11.6 μg L^{-1}) at WWTP1 and erythromycin (3.8 μg L^{-1}) at WWTP2. The removal efficiencies (RE) of pharmaceuticals were evaluated based on influent and effluent concentrations. RE varied significantly, ranging from 6% (bisoprolol) to 85% (carbamazepine) at WWTP1 and 23% (trimethoprim) to 100% (lorazepam) at WWTP2. Gros *et al.*[14] suggest that pharmaceuticals may persist in WWTP effluents due to low adsorption onto activated sludge or slow microbial degradation within the plant's retention time. Consequently, conventional secondary treatment with activated sludge achieves only partial removal, with low RE commonly reported for certain pharmaceuticals.[15,39] Also, several pharmaceuticals (trimethoprim, lorazepam, and metoprolol in WWTP1; erythromycin and metoprolol in WWTP2) exhibited higher concentrations in effluent than in influent samples, resulting in negative RE. This phenomenon, documented in other studies,[14,15,37] may result from pharmaceutical retention within WWTP and subsequent episodic release. Another possible explanation is enzymatic deconjugation of conjugated metabolites, leading to the regeneration of parent compounds during the treatment.[40] Despite their similar configurations, the two WWTPs exhibited significant differences in pharmaceutical removal efficiency, that may be explained by differences in process parameters, such as hydraulic retention time and sludge retention time.[41] WWTP2 generally exhibited higher efficiency, particularly in the removal of diclofenac and lorazepam (95 and 100%, respectively).

Overall, these results suggest that the wastewater treatment processes in the two WWTPs were insufficient to fully eliminate the studied contaminants,

highlighting the need for advanced treatment strategies to reduce pharmaceutical contamination in surface waters.

CONCLUSION

This study assessed the presence and impact of pharmaceuticals in Belgrade's wastewater on surface waters, revealing considerable pollution and associated environmental risks. A sensitive and reliable LC-MS/MS method was developed, validated, and successfully utilized for pharmaceutical analysis. The most frequently detected pharmaceuticals in wastewater and surface water were metoprolol, carbamazepine, and diclofenac, with diclofenac reaching concentrations up to 1.1 μg L^{-1} in surface water. Risk assessment revealed that diclofenac and azithromycin pose a high environmental risk in surface waters, while the cumulative risk of pharmaceutical mixtures exceeded safety thresholds at all sampling sites. The results from the two conventional wastewater treatment plants (WWTPs) demonstrated variable removal efficiencies, with some pharmaceuticals exhibiting negative removal rates, suggesting potential issues in the treatment processes. Overall, the study underscores the need for advanced wastewater treatment technologies and regulatory measures to mitigate pharmaceutical contamination and its ecological risks.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13395, or from the corresponding author on request.

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И З В О Д

ЛЕКОВИ У БЕОГРАДСКОЈ ОТПАДНОЈ ВОДИ: УТИЦАЈ НА ПОВРШИНСКУ ВОДУ И ПРОЦЕНА ЕКОЛОШКОГ РИЗИКА

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На подручју Београда, испуштање отпадних вода директно у реке Саву и Дунав има штетан утицај на квалитет вода. Циљ ове студије је процена овог утицаја испитивањем присуства лекова у Београдским отпадним водама и одговарајућим површинским водама, уз процену еколошког ризика. Развијена је и валидирана високо-осетљива и селективана LC-MS/MS метода и примењена на узорке отпадних и површинских вода. Од седамнаест испитиваних лекова, тринаест је детектовано у отпадној води, док је девет пронађено у површинској води, при чему је диклофенак детектован у највишој концентрацији (1,1 μg L^{-}

[1]). Метопролол, карбамазепин и диклофенак су најчешће детектовани у узорцима и отпадне и површинске воде. Процена ризика је показала да диклофенак и азитромицин представљају висок ризик по животну средину (RQ > 1), док је процена кумулативног ризика указала да на свим местима узорковања постоји значајан ризик услед присуства мешавине лекова. Додатно, у раду је процењена ефикасност уклањања лекова у два постројења за пречишћавање отпадних вода у Србији, која је указала на променљиву ефикасност, чак и појаву концентрисања појединих лекова, што указује на недостатке конвенционалних постројења у ефикасном уклањању ових супстанци. Ови закључци наглашавају потребу за регулаторним мерама и адекватним техникама третмана у циљу смањења загађења водене средине лековима.

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