THE ACADEMY OF APPLIED
TECHNICAL STUDIES
BELGRADE

INTERNATIONAL SCIENTIFIC
AND PROFESSIONAL CONFERENCE

POLITEHNIKA 2023

CONFERENCE PROCEEDINGS

Belgrade, 15th December 2023

PUBLISHER
The Academy of Applied Technical Studies Belgrade
Katarine Ambrozić 3, Belgrade
www.atssb.edu.rs

FOR THE PUBLISHER
Marina Stamenović, PhD, Professor of Applied Studies

THEMATIC SECTION EDITORS
Olivera Jovanović, PhD
Svetozar Sofijanić, PhD
Aleksandra Nastasić, PhD
Nenad Đorđević, PhD
Ana Cvijanović, MA
Biljana Ranković Plazinić, PhD
Marko Jauković, PhD
Andrijana Đurđević, PhD
Tatjana Sekulić, PhD
Goran Zajić, PhD

TECHNICAL PREPARATION AND COVER DESIGN
The Academy of Applied Technical Studies Belgrade, Organizing Committee

DESIGN OF THE CONFERENCE LOGO
Dušan Borović

PRINT
The Academy of Applied Technical Studies Belgrade, Katarine Ambrozić 3, Belgrade

THE CIRCULATION
400

THE ACADEMY OF APPLIED
TECHNICAL STUDIES
BELGRADE

TABLE OF CONTENTS

SCOPE 1. ENVIRONMENT AND SUSTAINABLE DEVELOPMENT

INTERNATIONAL SCIENTIFIC AND PROFESSIONAL CONFERENCE
POLITEHNIKA 2023
Belgrade, 15th December 2023

ENVIRONMENT AND SUSTAINABLE DEVELOPMENT. MECHATRONICS. OCCUPATIONAL SAFETY AND HEALTH AND FIRE SAFETY. SMART MANAGEMENT SYSTEMS. GRAPHIC ENGINEERING. DESIGN. TRAFFIC ENGINEERING. BIOTECHNOLOGY AND HEALTHCARE. MECHANICAL ENGINEERING. ECOTOURISM AND RURAL DEVELOPMENT.

IMPACT OF WASTEWATER ANTIBIOTICS ON RIVER WATER QUALITY IN BELGRADE AREA

Ljiljana Tolić Stojadinović[1], Innovation Centre of the Faculty of Technology and Metallurgy
Svetlana Grujić[2], Faculty of Technology and Metallurgy, University of Belgrade
Nikolina Antić[3], Customs laboratory, Customs Administration of the Republic of Serbia
Tatjana Đurkić[4], Faculty of Technology and Metallurgy, University of Belgrade

Abstract: *Municipal wastewater in the Belgrade area is discharged into the Danube and the Sava Rivers without any treatment. This affects the quality of river water since wastewater is one of the most important sources of environmental pollutants, including pharmaceuticals. To assess the impact of this effect, the presence of three commonly used antibiotics in wastewater and receiving river water was determined. A previously developed liquid chromatography-tandem mass spectrometry method for the analysis of antibiotics in natural water matrices was validated for wastewater and applied in analysis of samples from the Belgrade area. All investigated antibiotics were detected in both wastewater and river water samples. The highest concentration of antibiotics in wastewater was found for sulfamethoxazole, while trimethoprim was detected at the highest level in surface water. The most frequently detected antibiotic in surface water was azithromycin. The environmental risk assessment was performed for surface water biota. Azithromycin was found to pose a high risk to aquatic organisms, while the risk level of sulfamethoxazole was medium or low. In conclusion, the obtained results show the detrimental impact of untreated wastewater discharge on the river water quality in the Belgrade area, emphasizing the need for urban wastewater treatment.*
Keywords: Antibiotic, Urban wastewater, River water, LC-MS/MS, Environmental Risk Assessment

1. INTRODUCTION

Antibiotics have been increasingly produced and used worldwide in human and animal medicine. As a result, significant amounts of antibiotics have been released into the environment through waste from households, hospitals, the pharmaceutical industry, and farms. Antibiotics are frequently detected in surface water samples in concentrations that could be hazardous to aquatic organisms [1, 2]. The increasing presence of antibiotic residues in the water environment is now a matter of great health concern. However, there are no regulations concerning the maximum allowed concentrations of antibiotics in the aquatic environment. The latest update of the Watch list of substances for Union-wide monitoring in the field of water policy [3] includes several antibiotics, which reflects the concern regarding the presence of these contaminants in aqueous media. Wastewater represents one of the most significant sources of pollutants, such as antibiotics, in the environment [1]. Due to the absence of wastewater treatment plants in Belgrade, municipal wastewater is directly discharged untreated into the Danube and Sava Rivers. Consequently, it is necessary to assess the presence of antibiotics in Belgrade's surface water and evaluate their adverse effects on water quality.

[1]ljtolic@tmf.bg.ac.rs
[2]svetlana.grujic@tmf.bg.ac.rs
[3]anticn@carina.rs
[4]tanjav@tmf.bg.ac.rs

The aims of the present study were (a) to validate liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of selected antibiotics in wastewater; (b) to determine the concentrations of selected antibiotics in wastewater and receiving water in the Belgrade area; and (c) to assess the environmental risks of antibiotics found in river water samples.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The pharmaceuticals selected in this study are among the most commonly used antibiotics in Serbia. Analytical standards (purity > 95%) of three chosen antibiotics (trimethoprim, sulfamethoxazole, and azithromycin) were supplied from Hemofarm (STADA Group, Vršac, Serbia). The stock standard solutions of every analyte were prepared in methanol at a concentration of 100 μg mL^{-1}. The working standard solutions were prepared by mixing the stock standard solutions of three drugs and diluting them with methanol. All solutions were preserved at –4 °C. All solvents used were HPLC grade from Sigma-Aldrich (St. Louis, US). Deionized water was obtained by passing the distilled water through a GenPure ultrapure water system (TKA, Niederelbert, Germany).

2.2 LC-MS/MS analysis

A previously developed LC-MS/MS method for the determination of pharmaceuticals was used in this study [4]. A Dionex UltiMate 3000 LC system (Thermo Fisher Scientific, Waltham, MA, US) coupled to a LTQ XL (Thermo Fisher Scientific) linear ion trap as a mass detector was used for LC-MS/MS analysis. A reverse-phase Zorbax Eclipse® XDB-C18 column, 75 mm × 4.6 mm i.d. and 3.5 μm particle size (Agilent Technologies, Santa Clara, US) was used for the separation of the analytes. A pre-column (12.5 mm × 4.6 mm i.d. and 5 μm particle size, Agilent Technologies) was installed in front of the separation column. The mobile phase consisted of methanol (A), deionized water (B), and 10% acetic acid (C). The mobile phase gradient was changing as follows: 0 min, B 33%, C 2%; 12 min, B 98%, C 2%; 15 min, B 98%, C 2%. The initial conditions were then re-established and held for 15 minutes. The flow rate of the mobile phase was 0.5 mL min^{-1}. The electrospray ionization (ESI) technique in the positive ionization mode was used. 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). An aliquot of 10 μL of the final extract was injected into the LC system.

2.3 Method validation

Using wastewater samples spiked at two concentration levels (50 and 100 ng L^{-1}), a previously developed procedure for the determination of selected pharmaceuticals in surface water and groundwater [5] was validated. Validation was performed by evaluating recovery, repeatability, sensitivity, and linearity. The repeatability of the method was expressed as the relative standard deviation (RSD). The sensitivity of the method was evaluated by determining the limits of detection (LODs) and quantification (LOQs). LODs and LOQs of the method were obtained as the minimum detectable amount of analyte with a signal-to-noise (S/N) ratio of 3 and 10, respectively. The linearity of the analytical response was studied using the matrix-matched standards prepared at five concentration levels (10–250 ng L^{-1}). The linear regression analysis was performed in order to determine the correlation coefficient (R^2).

2.4 Sample collection and sample preparation

Wastewater and surface water samples were collected in the Belgrade area, around the confluence of the Danube and Sava Rivers (Figure 1). Samples of wastewater were collected at three locations of

municipal wastewater discharge. An automatic sampling device was used to collect samples continuously for 24 hours. Samples of surface water were collected at three locations downstream from wastewater discharge. 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. Samples were kept at 4 °C until analysis.

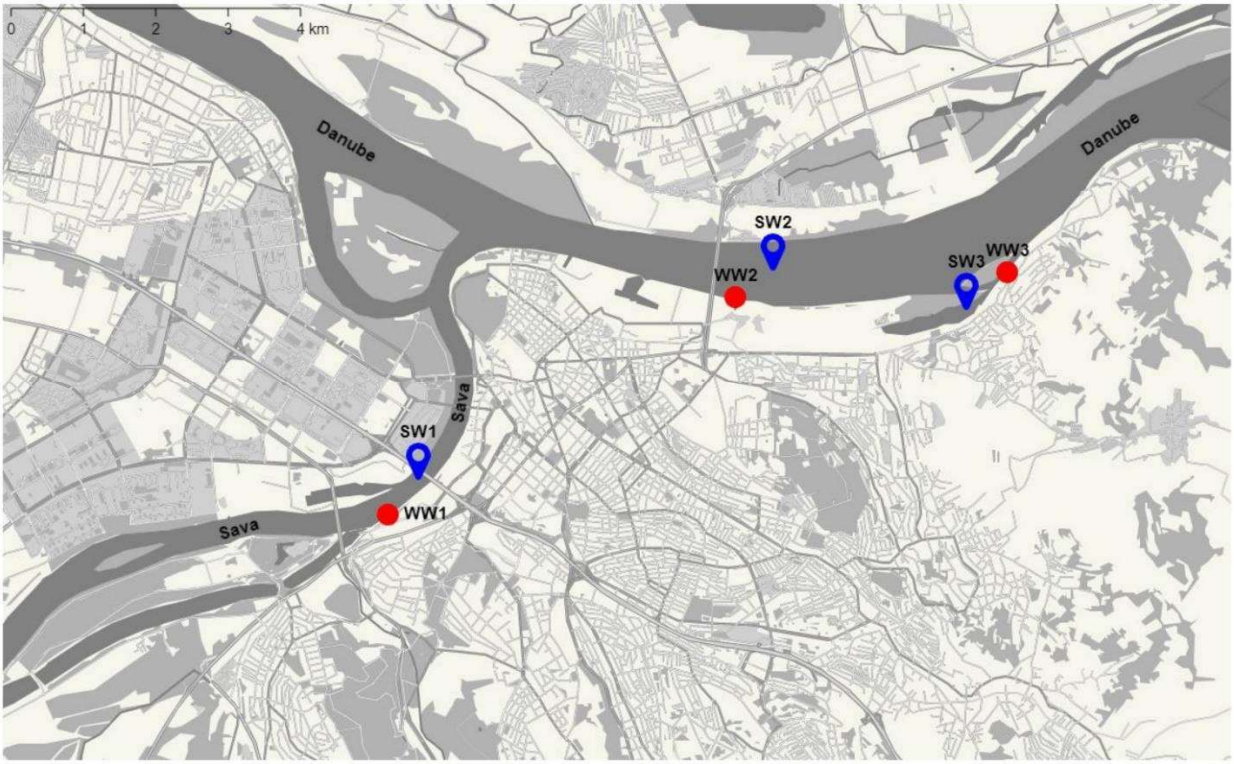

Figure 1. Map of wastewater (WW1–WW3) and river water (SW1–SW3) sampling sites
Source: https://www.stepmap.com/

A previously developed sample preparation method for the determination of pharmaceuticals in different aqueous matrices [5] was used for both wastewater and river water samples. In brief, water samples were prepared using solid-phase extraction: Oasis HLB column (Waters, Milford, MA, US) was preconditioned with 5 mL of a methanol-dichloromethane (1:1) mixture and 10 mL of deionized water. A 100 mL water sample with a pH value adjusted to 6.0 was applied. Then the column was dried for 10 min, and analytes were eluted using 15 mL of a methanol-dichloromethane (1:1) mixture. The obtained extract was evaporated and reconstituted to a volume of 1 mL with methanol. The final extract was filtered through a 0.45 µm polyvinylidene difluoride (PVDF) filter (Roth, Karlsruhe, Germany) and analyzed.

2.5 Environmental risk assessment

To assess the environmental risk of antibiotics detected in surface water, risk quotients (RQ) were calculated using the equation (1):

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

where MEC is the measured environmental concentration and PNEC is the predicted no-effect concentration of the detected analyte [6]. The lowest available PNEC values (trimethoprim 120 µg L^{-1}, sulfamethoxazole 0.6 µg L^{-1}, and azithromycin 0.019 µg L^{-1}) were obtained from the NORMAN database [7]. Environmental risk was classified in three levels: RQ ≥ 1 high risk, 0.1 ≥ RQ > 1 medium risk and RQ < 0.1 low risk [8].

3. RESULTS AND DISCUSSION

3.1 Method validation

The obtained validation parameters are presented in Table 1. The recoveries were in the range of 70–92% for trimethoprim and sulfamethoxazole. For azithromycin, the obtained values of recovery were not in this range, probably due to the pH value of the sample being adjusted to 6.0 which was optimal for other pharmaceuticals. The achieved RSD values were in the range of 5–11%. Low limits of detection (0.02–1.9 ng L^{-1}) and quantification (0.07–6.3 ng L^{-1}) indicate that the method is sensitive and suitable for the determination of trace levels of selected analytes in wastewater samples. Correlation coefficients (R^2) were in the range of 0.993–0.999, which implies that the developed method is linear in the tested concentration range (10–250 ng L^{-1}). Based on the values of the obtained validation parameters, it can be concluded that the applied method is appropriate for the determination of selected antibiotics in wastewater samples.

Table 1. Validation parameters of the analytical method
Source: *original copyright*

Analyte	Recovery, % (RSD, %)		LOD, ng L^{-1}	LOQ, ng L^{-1}	R^2
	50 ng L^{-1}	100 ng L^{-1}			
Trimethoprim	88 (8)	92 (5)	1.9	6.3	0.993
Sulfamethoxazole	83 (5)	70 (8)	0.2	0.7	0.999
Azithromycin	58 (7)	55 (11)	0.02	0.07	0.999

3.2 Sample analysis

Analyzed antibiotics were detected in wastewater in the concentration range from 122 ng L^{-1} (trimethoprim and sulfamethoxazole) to 1184 ng L^{-1} (sulfamethoxazole) (Table 2). All analyzed antibiotics were detected at the sampling site WW1, unlike samples WW2 (two antibiotics detected) and WW3 (none detected), which is likely due to WW1 being the largest wastewater discharge in the Belgrade area. In surface water, antibiotics were detected in the concentration range from 2 ng L^{-1} (azithromycin) to 334 ng L^{-1} (trimethoprim). The most frequently detected antibiotic was azithromycin, which was detected in all three river water samples.

Table 2. Antibiotics detected in Belgrade urban wastewater (WW1–WW3) and corresponding river water (SW1–SW3) samples (n = 2)
Source: *original copyright*

Analyte	Concentration (SD), ng L^{-1}					
	WW1	SW1	WW2	SW2	WW3	SW3
Trimethoprim	122 (12)	–	482 (72)	–	–	334 (54)
Sulfamethoxazole	122 (19)	–	1184 (152)	11 (1)	–	127 (7)
Azithromycin	318 (48)	9 (0)	–	2 (0)	–	58 (1)

In river water samples SW1 and SW2, collected downstream from the corresponding wastewater discharges, antibiotic concentrations were significantly lower than those detected in the wastewater, or analytes could not be detected, probably due to dilution. However, in the case of sampling site SW3, all analytes were detected at relatively high concentrations. SW3 was located in a small bay, away from the river mainstream, with limited water flow, consequently accumulation of contaminants occurs at this sampling site.

3.3 Environmental risk assessment

In order to assess the intensity of the environmental impact of wastewater discharge on aquatic biota, RQs were determined for analytes detected in surface water. It was determined that the presence of trimethoprim was related to a low environmental risk (RQ = 0.003, Figure 2). The risk level for sulfamethoxazole was low (RQ = 0.018) at the sampling site SW2 and medium (RQ = 0.212) at the sampling site SW3. It was determined that azithromycin presents medium environmental risk at sampling sites SW1 and SW2 (RQ = 0.474 and RQ = 0.105, respectively). However, azithromycin posed a high risk to the aquatic organisms at the sampling site SW3 (RQ = 3.053). A high environmental risk for azithromycin was previously reported, such as in the Bay of Biscay in Spain [9]. In addition to the risk to aquatic organisms, the presence of azithromycin in water is highly harmful as it induces microbial resistance [10]. Obtained results indicate that wastewater in the Belgrade area discharged into rivers without treatment negatively impacts the quality of the receiving water.

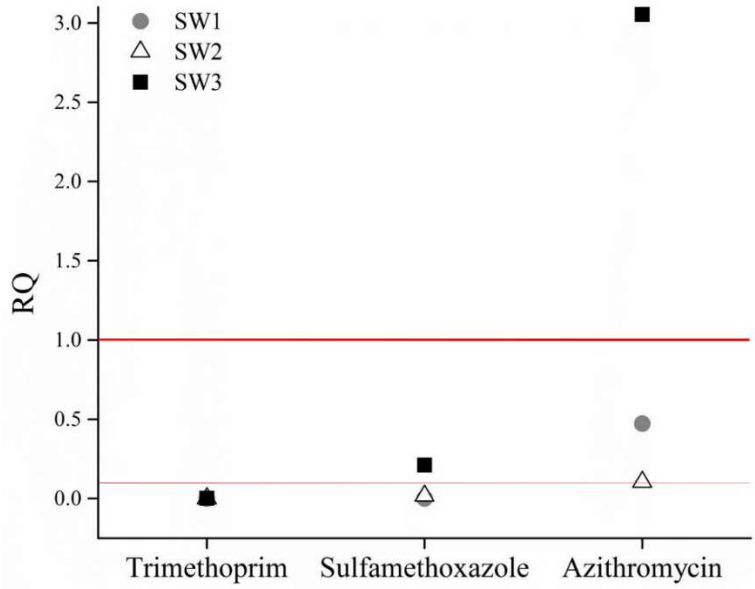

Figure 2. Risk quotients (RQs) calculated for antibiotics detected in river water
Source: original copyright

4. CONCLUSION

In this paper, a previously developed method for the determination of pharmaceuticals in various water matrices was successfully validated for wastewater. The content of selected antibiotics in municipal wastewater and corresponding surface water in the area of Belgrade was determined. Samples were collected at three locations of municipal wastewater discharges and at three locations in the Danube and Sava Rivers downstream from the wastewater discharges. Analyzed antibiotics were detected in both wastewater and surface water samples. Based on the determined RQ values, it was revealed that azithromycin posed a high risk to the aquatic biota. The obtained results demonstrate that the discharge of untreated municipal wastewater has a negative impact on the quality of river water in the area of Belgrade.

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [grant numbers 451-03-47/2023-01/200135 and 451-03-47/2023-01/200287].

LITERATURE

[1] Daughton, C., Ternes, T.: Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?, Environmental Health Perspectives, Vol. 107 (1999), No. 6, pp.907–938, Online ISSN 1552-9924.

[2] Fent, K., Weston, A., Caminada, D.: Ecotoxicology of human pharmaceuticals, Aquatic Toxicology, Vol. 76 (2006), pp. 122–159, ISSN 0166-445X.

[3] Commission Implementing Decision 2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council, Official Journal of the European Union, Vol. L197 (2022), pp. 117–121.

[4] Grujić, S., Vasiljević, T., Laušević, M.: Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography–ion trap–tandem mass spectrometry, Journal of Chromatography A, Vol. 1216 (2009), pp. 4989–5000, ISSN 0021-9673.

[5] Radović, T., Grujić, S., Petković, A., Dimkić, M., Laušević, M.: Determination of pharmaceuticals and pesticides in river sediments and corresponding surface and ground water in the Danube River and tributaries in Serbia, Environmental Monitoring and Assessment, Vol. 187 (2015), No. 4092, pp. 1–17, ISSN 0167-6369.

[6] European Commission, 2003. Technical guidance document on risk assessment, Part II. [Internet] Available on: https://urlis.net/5fpch948 (Accessed: 25.08. 2023.)

[7] NORMAN PNEC, Ecotoxicology Database — Lowest PNECs. [Internet] Available on: https://urlis.net/45vae33o (Assessed: 25.08.2023.)

[8] Hernando, M.D., Mezcua, M., Fernández-Alba, A.R., Balceló, D.: Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments, Talanta, Vol. 69 (2006), pp. 334–342, ISSN 0039-9140.

[9] Solaun, O., Rodríguez, J.G., Menchaca, I., López-García, E., Martínez, E., Zonja, B., Postigo, C., López de Alda, M., Barceló, D., Borja, A., Manzanos, A., Larreta, J.: Contaminants of emerging concern in the Basque coast (N Spain): Occurrence and risk assessment for a better monitoring and management decisions, Science of the Total Environment, Vol. 765 (2021), pp. 142765, ISSN 0048-9697.

[10] Milaković, M., Vestergaard, G., González-Plaza, J., Petrić, I., Šimatović, A., Senta, I., Kublik, S., Schloter, M., Smalla, K., Udiković-Kolić, N.: Pollution from azithromycin-manufacturing promotes macrolide-resistance gene propagation and induces spatial and seasonal bacterial community shifts in receiving river sediments, *Environment International*, Vol. 123 (2019) pp. 501-511, ISSN 0160-4120.

==========
CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

6(082)(0.034.2)
5(082)(0.034.2)
331.45/.46(082)(0.034.2)
005(082)(0.034.2)

INTERNATIONAL Scientific and Professional Conference Politehnika (2023 ; Beograd)

Conference Proceedings [Електронски извор] / International Scientific and Professional Conference Politehnika 2023, Belgrade, 15th December 2023 ; [organizer] The Academy of Applied Technical Studies "Belgrade", Belgrade. - Belgrade : The Academy of Applied Technical Studies "Belgrade", 2023. - 1 USB fleš memorija ; 1 x 1 x 5 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 400. - Bibliografija uz svaki rad.

ISBN 978-86-7498-110-8

а) Техника -- Зборници б) Примењене науке -- Зборници в) Заштита на раду -- Зборници г) Менаџмент -- Зборници

COBISS.SR-ID 132801289

==========

THE ACADEMY OF APPLIED
TECHNICAL STUDIES
BELGRADE

skup-politehnika.atssb.edu.rs
atssb.edu.rs

ISBN-978-86-7498-110-8