



## **PHYSICAL CHEMISTRY 2021**

15<sup>th</sup> International Conference  
on Fundamental and Applied Aspects of  
Physical Chemistry

Proceedings  
Volume II

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*The Conference is dedicated to the*  
***30<sup>th</sup> Anniversary of the founding of the Society of Physical  
Chemists of Serbia***  
*and*  
***100<sup>th</sup> Anniversary of Bray-Liebhafsky reaction***

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**September 20-24, 2021**  
**Belgrade, Serbia**

**Title:** Physical Chemistry 2021 (Proceedings) **ISBN** 978-86-82475-40-8

**Volume II: ISBN** 978-86-82475-39-2

**Editors:** Željko Čupić and Slobodan Anić

**Published by:** Society of Physical Chemists of Serbia, Studentski Trg 12-16, 11158, Belgrade, Serbia

**Publisher:** Society of Physical Chemists of Serbia

**For Publisher:** S. Anić, President of Society of Physical Chemists of Serbia

**Printed by:** "Jovan", <Printing and Publishing Company, 200 Copies

**Number of pages:** 6+388, Format A4, printing finished in December 2021

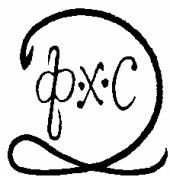
Text and Layout: "Jovan"

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*15<sup>th</sup> International Conference on  
Fundamental and Applied Aspects of  
Physical Chemistry*

*Organized by*

*The Society of Physical Chemists of  
Serbia*

*in co-operation with*

*Institute of Catalysis Bulgarian Academy of Sciences*

*and*

*Boreskov Institute of Catalysis Siberian Branch of  
Russian Academy of Sciences*

*and*

*University of Belgrade, Serbia:*

*Faculty of Physical Chemistry  
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## IMPACT OF PHARMACEUTICAL LEVELS IN UNTREATED WASTEWATER ON BELGRADE RIVER WATER QUALITY

Lj. Tolić Stojadinović <sup>1</sup>, S. Grujić <sup>2</sup>, N. Antić <sup>2</sup> and T. Đurkić <sup>2</sup>

<sup>1</sup> Innovation Centre of the Faculty of Technology and Metallurgy,  
Karnegijeva 4, 11000 Belgrade, Serbia.

<sup>2</sup>University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade,  
Serbia. (svetlana.grujic@tmf.bg.ac.rs)

### ABSTRACT

In the city of Belgrade, raw sewage is discharged directly into the Sava and the Danube rivers affecting the quality of river water. To determine the level of this impact, eight frequently used and detected pharmaceuticals were selected for the study. A previously developed method for analysis of pharmaceuticals in different water matrices was used. Sample extracts were analyzed by liquid chromatography-tandem mass spectrometry, using the electrospray ionization technique. The most commonly detected analytes in both municipal wastewater and surface water were carbamazepine, known for its high environmental stability, and diclofenac, known as having high acute ecotoxicity. The dilution effect, noted for most of the detected drugs, was not as pronounced with diclofenac, indicating a higher risk for aquatic organisms.

### INTRODUCTION

Pharmaceuticals are among the most important environmental contaminants because of their increasing production and extensive use. They are widely used in human and veterinary medicine and are continuously released into the environment through many human activities and the pharmaceutical industry. However, legal limits of pharmaceuticals in environmental waters have not been established yet, although they are commonly found in surface water at levels that may be harmful to aquatic organisms [1, 2]. Furthermore, there is still limited information on long-term effects of trace levels of pharmaceuticals in the environment. Municipal wastewater is the most significant source of these contaminants in the aquatic environment [1]. Since Belgrade does not have a wastewater treatment plant, raw sewage is discharged directly into rivers the Sava and the Danube through many sewage channels.

The aim of this work was to collect data on the concentrations of selected pharmaceuticals in Belgrade wastewater and corresponding receiving river water in order to determine the impact level of untreated sewage on the surface water quality in Belgrade. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as the most extensively used technique for the determination of trace levels of contaminants in water matrices, will be employed for this purpose.

### METHODS

Commonly used and frequently detected pharmaceuticals were selected for the study: trimethoprim, sulfamethoxazole, azithromycin, erythromycin (antibiotics); carbamazepine, lorazepam, diazepam (sedatives); and diclofenac (nonsteroidal anti-inflammatory drug, NSAID). A previously developed method for the determination of pharmaceuticals in different water matrices was applied [3]. In short, water samples were prepared using solid-phase extraction: 100 mL of the sample (pH = 6) was loaded on the Oasis HLB cartridges (Waters, USA), analytes were eluted with 10 mL of methanol-dichloromethane (1:1) mixture, and the extract was evaporated and reconstituted to the volume of 1.0 mL. As for the LC-MS/MS analysis, Dionex UltiMate 3000 HPLC system (Thermo Fisher

Scientific, Waltham, US) coupled with the linear ion trap mass spectrometer (Thermo Fisher Scientific) was used. The chromatographic separation of compounds was achieved on a reverse-phase Zorbax Eclipse® XDB-C18 column, 75 mm × 4.6 mm ID and 3.5 µm particle size. The mobile phase consisted of methanol, water, and 10% acetic acid. The mass spectrometric analysis was performed using the electrospray ionization technique in the positive mode.

Wastewater samples were collected from three sewage discharges in Belgrade, while the corresponding receiving river water samples were collected downstream (Figure 1).



**Figure 1.** Sampling sites of wastewater (W1–W3) and surface water (S1–S3).

## RESULTS AND DISCUSSION

All investigated pharmaceuticals were detected in Belgrade municipal wastewater, at concentration levels ranging from 54 ng L<sup>-1</sup> (for azithromycin, Table 1) to as high as 1184 ng L<sup>-1</sup> (for sulfamethoxazole). Wastewater sample W1, collected at the discharge with the highest sewage inflow, showed trace levels of all selected analytes. The most frequently detected pharmaceuticals, found in all river water samples, as well as in all wastewater samples, were diclofenac and carbamazepine. In ecotoxicity studies of diclofenac, it was determined that it has high acute toxicity for aquatic organisms, the highest within the NSAID group [2]. As for carbamazepine, studies have shown that it is among the most persistent pharmaceuticals in the environment [4].

**Table 1.** Pharmaceuticals detected in Belgrade municipal wastewater (W1–W3) and corresponding surface water (S1–S3) samples.

Pharmaceuticals	Concentration (SD), ng L <sup>-1</sup>					
	W1	S1	W2	S2	W3	S3
Trimethoprim	122(12)	– <sup>a</sup>	–	–	482(72)	–
Sulfamethoxazole	122(19)	–	–	–	1184(152)	12(1)
Azithromycin	318(48)	8(1)	54(4)	3(0)	–	–
Erythromycin	133(22)	–	266(34)	–	–	–
Carbamazepine	274(44)	27(4)	313(30)	24(3)	127(25)	56(3)
Lorazepam	171(24)	–	84(3)	–	77(10)	–
Diazepam	163(22)	–	–	–	–	–
Diclofenac	442(73)	263(10)	579(64)	370(9)	782(156)	252(12)

<sup>a</sup>–: not detected

Our results have shown that there is evident contamination of the aquatic environment in Belgrade as a consequence of untreated wastewater discharge. In river water samples collected downstream from the corresponding sewage discharges, significantly lower levels of detected pharmaceuticals were found or drug traces could not be detected, indicating an intense dilution effect (Table 1). However, in the case of diclofenac, the dilution effect was not as pronounced, leading to a higher risk for river organisms. In addition, when aquatic species are continuously exposed to trace levels of contaminants over a long period of time, negative effects slowly accumulate and major changes occur when the cumulative level of these effects is reached [1]. Moreover, the mixtures of drug traces occurring in the environment may have an even greater impact than can be expected from the cumulative effects of individual substances.

## CONCLUSION

The study has shown that the discharge of untreated municipal wastewater significantly affects the quality of river water in Belgrade. All investigated pharmaceuticals were detected in wastewater samples, and 50% of the drugs were found in corresponding surface water samples. Although the dilution effect was generally very pronounced, the exception in the case of diclofenac led to a higher risk for aquatic organisms because this drug has high acute toxicity.

## Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract no. 451-03-9/2021-14/200287 and 451-03-9/2021-14/200135).

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CIP - Каталогизација у публикацији  
Народна библиотека Србије, Београд

544(082)  
66.017/.018(082)  
502/504(082)  
343.98(082)

**INTERNATIONAL Conference on Fundamental and Applied Aspects of Physical Chemistry  
(15; 2021; Beograd)**

Physical Chemistry 2021: proceedings: the Conference is dedicated to the 30th Anniversary of the founding of the Society of Physical Chemists of Serbia and 100th Anniversary of Bray-Liebhafsky reaction. Vol. 2 / 15th International Conference on Fundamental and Applied Aspects of Physical Chemistry, September 20-24, 2021, Belgrade, Serbia; [organized by The Society of Physical Chemists of Serbia in co-operation with Institute of Catalysis Bulgarian Academy of Sciences ... [et al.]]; [editors Željko Čupić and Slobodan Anić]. - Belgrade: Society of Physical Chemists of Serbia, 2021 (Belgrade: Jovan). - VI str., str. 347-732: ilustr.; 30 cm

Tiraž 200. - Bibliografija uz svaki rad. - Registar.

ISBN 978-86-82475-39-2  
ISBN 978-86-82475-40-8 (niz)

а) Физичка хемија -- Зборници б) Наука о материјалима -- Зборници в) Животна средина -- Зборници  
г) Форензика -- Зборници

COBISS.SR-ID 53325065