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INVESTIGATION OF THE PHARMACEUTICALS PRESENCE IN THE SOMOVA-PARCHEȘ AQUATIC COMPLEX

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Abstract

The Somova-Parcheș Aquatic Complex is part of the Danube Delta Biosphere Reserve and hosts a wide variety of fauna and flora species of community importance. This complex is fed with a significant volume of water from the Danube River, thus influencing the quality of the lake ecosystems. For this reason, the present study aims to investigate the presence of certain classes of pharmaceutical residues in water sampled from 6 lakes, namely Somova, Parcheș, Morun, Potica, Babele, Câșla, as well as their ability to accumulate in the tissues of fish collected from the same study area. Extraction of pharmaceutical compounds was performed using the solid phase extraction method for water samples and the QuEChERS method for fish tissues. Results obtained from analyses using high-performance liquid chromatography coupled with high-resolution mass spectrometry revealed the presence of the pharmaceutical compound caffeine in most water samples and its tendency to accumulate in some fish organs, such as the gills.

***Key words**: Somova-Parcheș Aquatic Complex, pharmaceutical residues, water, fish tissue.*

INTRODUCTION

The Danube River supplies water to the Somova-Parches Aquatic Complex, thus facilitating the entry of various contaminants into the lakes. Numerous studies have been carried out on the Danube River, which have shown that, in general, the water falls into class quality II (Good) (Mănoiu & Crăciun, 2021). However, a wide variety of chemical and physical compounds have been identified which, in certain concentrations, can have toxic effects on the aquatic environment, such as: nutrients (Malagó et al., 2017; Popa et al., 2018), gross alpha and gross beta activities (Pintilie-Nicolov et al., 2021), heavy metals (Lazăr et al., 2024; Mîndrescu et al., 2022), pharmaceutical compounds (Chitescu et al., 2015), microplastics (Procop et al., 2024), pesticides (Đurišić-Mladenović et al., 2024), etc.
To date, studies have been conducted in the Somova-Parcheș Aquatic Complex area to evaluate the quality of aquatic ecosystems, considering the common parameters regulated by Order 161/2006. Among the most frequently analyzed compounds in this study area are heavy metals, oxygen regime, and nutrients (Burada et al., 2015; Catianis et al., 2019). This study aims to investigate, for the first time in this area, a class of emerging pollutants represented by pharmaceutical residues. The main pollution sources of the Danube river with pharmaceutical residues are conventional wastewater treatment plants and animal farms (Kock et al., 2023; Radović et al., 2012).
The analysis of this type of contaminants in aquatic environments is very important, as they can have direct toxic effects on aquatic biota and can accumulate in biotic tissues depending on their concentration and the physicochemical properties of each pharmaceutical substance (Khan et al., 2020; Rojo et al., 2021; Williams et al., 2009). For this reason, the present research focuses on investigating the presence of different classes of pharmaceuticals in various organs of the *Carassius gibelio* fish species collected from the Somova-Parcheș Aquatic Complex.

MATERIALS AND METHODS

To study the presence of pharmaceutical compounds in the Somova-Parcheș Aquatic Complex, four water samples were collected from different areas (inlet, middle and outlet) of each lake, namely Somova, Parcheş, Morun, Potica, Babele and Câşla (Figure 1).

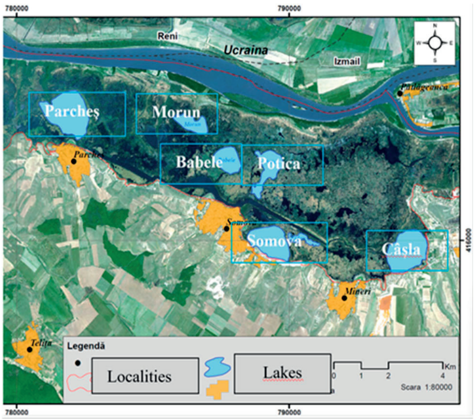

Figure 1. Map of the studied lakes in the Somova-Parcheş Aquatic Complex

Extraction of pharmaceutical residues from water samples was performed with a Dionex AutoTrace 280 Thermo Scientific automated solid phase extraction system (Figure 2).

Figure 2. Automated solid phase extraction system

To extract the target compounds from fish tissues, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique was used. This method involved the extraction of contaminants from the matrix into the solvent acetonitrile in the presence of the following salt mixture: 6 g anhydrous magnesium sulfate, 5 g sodium chloride, 1.5 g disodium citrate dihydrate, and 0.75 g trisodium citrate sesquihydrate. To remove fats, lipids, and pigments from the extract, a purification step was performed using the dispersive solid-phase extraction (d-SPE) method in the presence of sorbent materials (PSA – primary secondary amine, C18, and GCB – graphitized carbon) (Figure 3).

Figure 3. QuEChERS extraction steps

The extraction step was followed by the analysis of pharmaceutical substances from the obtained extracts using high-performance liquid chromatography coupled with high-resolution mass spectrometry.
The following pharmaceutical compounds were investigated in water and fish tissue samples: metformin, carbamazepine, clindamycin, ciprofloxacin, clarithromycin, trimethoprim, sulfamethoxazole, amoxicillin, ketoprofen, diclofenac, and caffeine. To quantify these compounds, the internal standards were used depending on the class of pharmaceutical substances they belong to, as follows: enrofloxacin for antibiotics, dihydrocarbamazepine for carbamazepine, fenoprop for anti-inflammatories, phenacetin for metformin and caffeine (Figure 4).

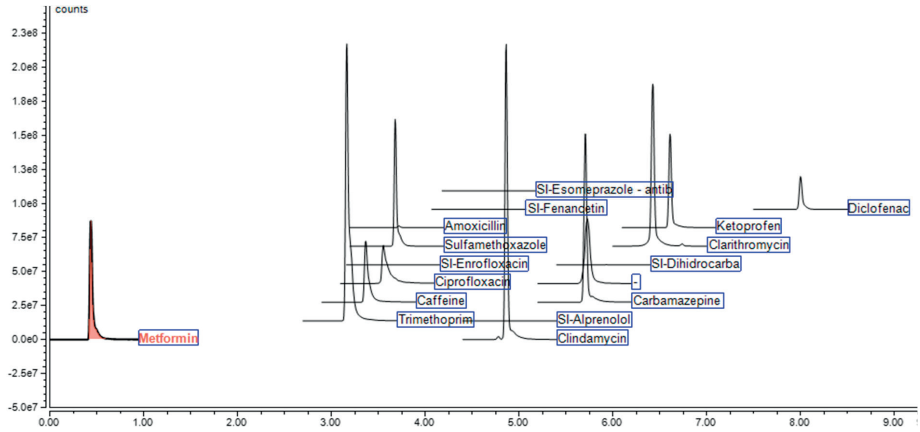

Figure 4. Chromatogram of the analyzed pharmaceutical compounds

RESULTS AND DISCUSSIONS

Spatial distribution of pharmaceutical compounds identified in water samples taken from lakes in the Somova-Parcheş Aquatic Complex

From all the 6 lakes studied in this paper, water samples were taken from 4 different stations, and the presence of different pharmaceutical substances was investigated. Of the 11 compounds monitored in water samples, only caffeine and the internal standards added to the samples for compound quantification were identified in all sampling stations (Figure 5).

Caffeine is found in beverages, foods, and medications that are consumed in significant quantities worldwide. For this reason, it is considered a significant pollutant in the class of active pharmaceutical compounds that is found with high frequency in the environment (Li et al., 2020).

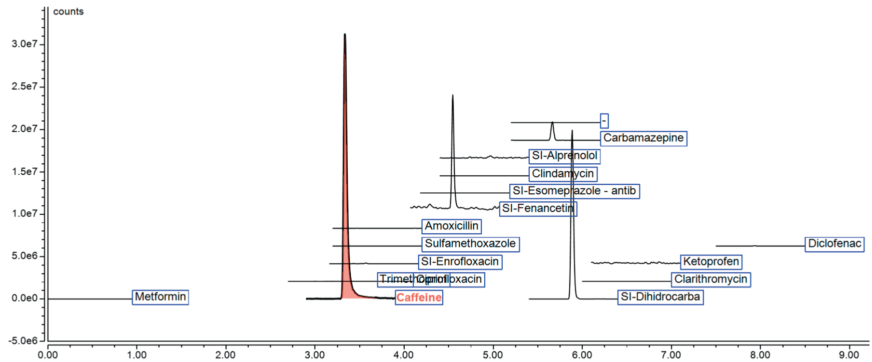

Figure 5. Chromatogram example of compounds in a water sample analyzed from lakes

In the lakes analyzed in the present study, caffeine concentrations varied between 15 ng/L and 65 ng/L. The highest values of the caffeine compound were identified in Somova lake, the mean concentration being 55 ng/L. The lowest concentrations were obtained in water samples collected from Morun lake, where a mean concentration of 20 ng/L was recorded. In most lakes, slight variations in caffeine concentrations were observed from one sampling station to another (Figure 6). The main contamination source of the aquatic environment with this psychoactive compound is anthropogenic, generally represented by municipal wastewater (Vieira et al., 2022).

The presence of this compound in the aquatic environment can be explained by its pseudo-persistent character, caused by its long half-life. This substance also exhibits high resistance to chemical and biological degradation in the environment, has low volatility, and its low octanol-water partition coefficient (log Kow = -0.07) explains its high solubility in water (Rodrigues et al., 2025). In the aquatic environment, this alkaloid can have direct toxic effects on biota and can even induce behavioral disorders in fish at environmentally relevant concentrations (Cerveny et al., 2022).

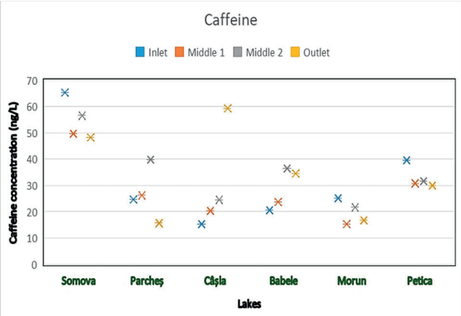

Figure 6. Spatial distribution of caffeine in the Somova-Parcheș Aquatic Complex lakes

The caffeine compound was also identified at high frequency in the Danube River in the territory of the Novi Sad, Serbia with concentrations varying from 3.10 to 621 ng/L (Milić et al., 2018).

On the territory of Romania, there is a limited number of studies that have focused on the analysis of pharmaceutical residues in the aquatic environment.

Chitescu et al., identified the following compounds in water samples taken from the Danube River: sulfamethoxazole, trimethoprim, enilconazole, carbamazepine, and tylosin. Pharmaceutical residues such as sulfamethoxazole, diclofenac, trimethoprim, carbamazepine, piroxicam and ketoprofen were quantified in the Olt, Argeș and Siret tributaries. A smaller number of pharmaceutical substances were identified in water samples taken from the Danube Delta, including sulfamethoxazole and carbamazepine (Chitescu et al., 2015).

Investigation of the pharmaceutical compounds presence in fish specimens of the species Carassius gibelio

The fish species *Carassius gibelio* is found abundantly in the Danube River basin and is considered the most consumed local freshwater fish (Raita and Georgescu, 2019). To identify the presence of pharmaceutical compounds in the species *Carassius gibelio*, 3 fish specimens were taken for analysis and extractions were made from muscle tissue, liver tissue, gills and eggs. Of all the investigated compounds, caffeine was the one that was found in all the organs analyzed from the 3 fish, with mean values ranging from 0.77 ng/g to 2.42 ng/g (Figure 7). Table 1 shows that the highest average concentration of caffeine was identified in the gills. This can be explained by the fact that the gills are in direct contact with water and can more easily accumulate pollutants found in the aquatic environment (Nikolić et al., 2020). However, it can be noted that traces of caffeine were identified even in the fish eggs analyzed in this study.

The obtained results show that the same compound identified in water samples from the Somova-Parcheș Aquatic Complex, represented by caffeine, accumulated also in the organs of fish collected from the same study area.

Table 1. Concentration levels of pharmaceuticals in fish specimens of the species *Carassius gibelio* – the values are expressed as mean ± standard deviation

Pharmaceutical compound identified	Fish organs analyzed	Mean concentration ± std (ng/g)
Caffeine	Muscle tissue	1.04 ± 0.19
	Liver tissue	1.60 ± 0.23
	Gills	2.42 ± 1.10
	Eggs	0.77 ± 0.30

In the literature, there are laboratory studies that monitored the behaviour and physiology of fish exposed to caffeine and it was highlighted that, even in relatively low concentrations, this compound can cause liver enlargement and anxiety of fish (Bikker et al., 2024). There are also studies reporting accumulations of the caffeine compound in the organs of different fish species. For example, Wang and Gardinali reported the presence of caffeine in the fish species *Gambusia holbrooki* at a mean concentration of 1.3 ng/g (Wang & Gardinali, 2012).

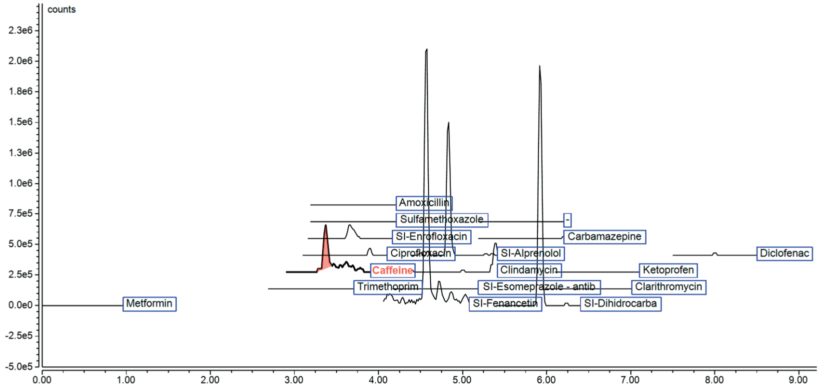

Figure 7. Chromatogram example of compounds identified in fish tissue from the species *Carassius gibelio*

In the panga fish (*Pterogymnus laniarus*) caffeine was detected in the intestine, liver and gills with a concentration of 2.03 ng/g (Ojemaye and Petrik, 2019). Caffeine was also identified in the fish species Common Silver biddy (*Gerres oyena*), Golden snapper (*Lutjanus johni*), Emperor fish (*Lethrinus, nebulosus*), Nile Tilapia (*Oreochromis niloticus*), and Milk fish (*Chanos chanos*) (Ali et al., 2018).
No results have been reported in the specialized literature regarding the presence of pharmaceutical residues in the fish species *Carassius gibelio*.

CONCLUSIONS

Due to their high toxicity, it is important to monitor the emerging contaminant class represented by pharmaceutical compounds in all water bodies.
The present study emphasizes that a wide variety of pharmaceutical substances were not found in the lakes of the Somova-Parcheș Aquatic Complex. This may be due to the fact that there are no direct sources of pollution in the area. This may also be influenced by the low concentrations of these compounds in the Danube water, which may undergo various processes of accumulation, degradation, dilution until they reach the lakes of the Somova-Parcheș Aquatic Complex.
However, the psychoactive substance caffeine was identified in all water samples analyzed. A possible explanation could be that it is generally found in higher concentrations in the aquatic environment compared to the other pharmaceutical compounds analyzed and can therefore reach even areas far from direct sources of pollution.
This study also highlights the ability of the caffeine compound to accumulate in aquatic biota. Low concentrations of caffeine were identified in the organs of the fish analyzed from the species *Carassius gibelio*.

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REFERENCES

Ali, A. M., Rønning, H. T., Sydnes, L. K., Alarif, W. M., Kallenborn, R., & Al-Lihaibi, S. S. (2018). Detection of PPCPs in marine organisms from contaminated coastal waters of the Saudi Red Sea. *Science of The Total Environment, 621,* 654–662. https://doi.org/10.1016/j.scitotenv.2017.11.298
Bikker, J., MacDougall-Shackleton, H., Bragg, L. M., Servos, M. R., Wong, B. B. M., & Balshine, S. (2024). Impacts of caffeine on fathead minnow behaviour and

physiology. *Aquatic Toxicology, 273,* 106982. https://doi.org/10.1016/j.aquatox.2024.106982

Burada, A., Topa, C. M., Georgescu, L. P., Teodorof, L., Năstase, C., Seceleanu-Odor, D., & Iticescu, C. (2015). Heavy metals environment accumulation in Somova-Parches aquatic complex from the Danube Delta area. *Revista de Chimie, 66,* 48–54.

Catianis, I., Pojar, I., Grosu, D., & Pavel, A. B. (2019). Investigation of the surface water quality parameters in the predeltaic area of the Danube Delta in 2017. Study case: Somova-Parcheș aquatic complex lakes. *Scientific Annals of the Danube Delta Institute,* 139–146. https://doi.org/10.7427/DDI.24.15

Cerveny, D., Cisar, P., Brodin, T., McCallum, E. S., & Fick, J. (2022). Environmentally relevant concentration of caffeine - effect on activity and circadian rhythm in wild perch. *Environmental Science and Pollution Research, 29,* 54264–54272. https://doi.org/10.1007/s11356-022-19583-3

Chitescu, C. L., Kaklamanos, G., Nicolau, A. I., & Stolker, A. A. M. (Linda). (2015). High sensitive multiresidue analysis of pharmaceuticals and antifungals in surface water using U-HPLC-Q-Exactive Orbitrap HRMS. Application to the Danube river basin on the Romanian territory. *Science of The Total Environment, 532,* 501–511. https://doi.org/10.1016/j.scitotenv.2015.06.010

Đurišić-Mladenović, N., Živančev, J., Antić, I., Rakić, D., Buljovčić, M., Pajin, B., Llorca, M., Farre, M., 2024. Occurrence of contaminants of emerging concern in different water samples from the lower part of the Danube River Middle Basin – A review. *Environmental Pollution 363*, 125128. https://doi.org/10.1016/j.envpol.2024.125128

Khan, H. K., Rehman, M. Y. A., & Malik, R. N. (2020). Fate and toxicity of pharmaceuticals in water environment: An insight on their occurrence in South Asia. *Journal of Environmental Management, 271,* 111030. https://doi.org/10.1016/j.jenvman.2020.111030

Kock, A., Glanville, H.C., Law, A.C., Stanton, T., Carter, L.J., Taylor, J.C., 2023. Emerging challenges of the impacts of pharmaceuticals on aquatic ecosystems: A diatom perspective. *Science of The Total Environment 878,162939.*
https://doi.org/10.1016/j.s env.2023.162939

Lazăr, N.-N., Simionov, I.-A., Petrea, Ș.-M., Iticescu, C., Georgescu, P.-L., Dima, F., & Antache, A. (2024). The influence of climate changes on heavy metals accumulation in *Alosa immaculata* from the Danube River Basin. *Marine Pollution Bulletin, 200,* 116145. https://doi.org/10.1016/j.marpolbul.2024.116145

Li, S., Wen, J., He, B., Wang, J., Hu, X., & Liu, J. (2020). Occurrence of caffeine in the freshwater environment: Implications for ecopharmacovigilance. *Environmental Pollution, 263,* 114371. https://doi.org/10.1016/j.envpol.2020.114371

Malagó, A., Bouraoui, F., Vigiak, O., Grizzetti, B., Pastori, M., 2017. Modelling water and nutrient fluxes in the Danube River Basin with SWAT. *Science of The Total Environment, 603–604*, 196–218. https://doi.org/10.1016/j.scitotenv.2017.05.242

Mănoiu, V.-M., Crăciun, A.-I., 2021. Danube river water quality trends: A qualitative review based on the open access web of science database. *Ecohydrology & Hydrobiology 21,* 613–628. https://doi.org/10.1016/j.ecohyd.2021.08.002

Mîndrescu, M., Haliuc, A., Zhang, W., Carozza, L., Carozza, J.-M., Groparu, T., Valette, P., Sun, Q., Nian, X., Gradinaru, I., 2022. A 600 years sediment record of heavy metal pollution history in the Danube Delta. *Science of The Total Environment, 823*, 153702. https://doi.org/10.1016/j.scitotenv.2022.153702

Milić, N., Milanović, M., Radonić, J., Turk Sekulić, M., Mandić, A., Orčić, D., Mišan, A., Milovanović, I., Grujić Letić, N., & Vojinović Miloradov, M. (2018). The occurrence of selected xenobiotics in the Danube River via LC-MS/MS. *Environmental Science and Pollution Research, 25,* 11074–11083. https://doi.org/10.1007/s11356-018-1401-z

Nikolić, D., Skorić, S., Rašković, B., Lenhardt, M., & Krpo-Ćetković, J. (2020). Impact of reservoir properties on elemental accumulation and histopathology of European perch (*Perca fluviatilis*). *Chemosphere, 244,* 125503. https://doi.org/10.1016/j.chemosphere.2019.1

Order 161/2006 of Romanian Ministry of Environment and Water Management regarding norms for surface water classification in order to establish ecological state of water bodies. (2006). Romanian Official Monitor: Bucharest, Romania. Available online: http://www.legex.ro/Ordin-161-2006-71706.aspx (accessed on May 15, 2025).

Ojemaye, C. Y., & Petrik, L. (2019). Occurrences, levels and risk assessment studies of emerging pollutants (pharmaceuticals, perfluoroalkyl and endocrine disrupting compounds) in fish samples from Kalk Bay harbour, South Africa. *Environmental Pollution, 252,* 562–572. https://doi.org/10.1016/j.envpol.2019.05.091

Pintilie-Nicolov, V., Georgescu, P.-L., Iticescu, C., Moraru, D. I., & Pintilie, A. G. (2021). The assessment of the annual effective dose due to ingestion of radionuclides from drinking water consumption: Calculation methods. *Journal of Radioanalytical and Nuclear Chemistry, 327,* 49–58. https://doi.org/10.1007/s10967-020-07438-5

Popa, P., Murariu, G., Timofti, M., & Georgescu, L. P. (2018). Multivariate statistical analyses of Danube River water quality at Galati, Romania. *Environmental Engineering and Management Journal, 17,* 1249–1266.

Procop, I., Calmuc, M., Pessenlehner, S., Trifu, C., Ceoromila, A.C., Calmuc, V.A., Fetecău, C., Iticescu, C., Musat, V., Liedermann, M., 2024. The first spatio-temporal study of the microplastics and meso–macroplastics transport in the Romanian Danube. *Environmental Sciences Europe, 36*, 154. https://doi.org/10.1186/s12302-024-00969-8

Radović, T., Grujić, S., Dujaković, N., Radišić, M., Vasiljević, T., Petković, A., Boreli-Zdravković, D., Dimkić, M., & Laušević, M. (2012). Pharmaceutical residues in the Danube River Basin in Serbia—a two-year survey. *Water Science and Technology, 66,* 659–665. https://doi.org/10.2166/wst.2012.225

Raita, S. M., & Georgescu, M. (2019). Monitoring *Carassius gibelio* muscle tissue quality under black cumin oil fortification and typical cold storage. *Scientific Works. Series C. Veterinary Medicine, LXV,* 21–28.

Rodrigues, S., Alves, R. S., & Antunes, S. C. (2025). Impact of caffeine on aquatic ecosystems: Assessing trophic-level biological responses. *Journal of Xenobiotics, 15,* 86. https://doi.org/10.3390/jox15030086

Rojo, M., Cristos, D., González, P., López-Aca, V., Dománico, A., & Carriquiriborde, P. (2021). Accumulation of human pharmaceuticals and activity of biotransformation enzymes in fish from two areas of the lower Rio de la Plata Basin. *Chemosphere, 266,* 129012. https://doi.org/10.1016/j.chemosphere.2020.129012

Vieira, L. R., Soares, A. M. V. M., & Freitas, R. (2022). Caffeine as a contaminant of concern: A review on concentrations and impacts in marine coastal systems. *Chemosphere, 286,* 131675. https://doi.org/10.1016/j.chemosphere.2021.131675

Wang, J., & Gardinali, P. R. (2012). Analysis of selected pharmaceuticals in fish and the fresh water bodies directly affected by reclaimed water using liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 404,* 2711–2720. https://doi.org/10.1007/s00216-012-6139-8

Williams, M., Ong, P. L., Williams, D. B., & Kookana, R. S. (2009). Estimating the sorption of pharmaceuticals based on their pharmacological distribution. *Environmental Toxicology and Chemistry, 28,* 2572–2579. https://doi.org/10.1897/08-587.1