




## Article

# COVID-19 Pandemic Modulates the Environmental Contamination Level of Enteric Bacteria from WWTPs

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**Abstract:** The COVID-19 pandemic was a challenge for the whole world, and it had major secondary effects on humans and environmental health. The viral infection induced, in many situations, secondary bacterial infections, especially enteric infections, by destabilizing the balance of the gastrointestinal microbiota. The large-scale use of antibiotics and biocides for both curative and preventive purposes has resulted in an increase in bacterial resistance, and at the same time, the possibility of pathogenic microorganism multiplication and their dissemination to natural environments. Wastewater is the main vector of fecal microorganisms that favors their dissemination into natural aquatic ecosystems. The present paper aims to analyze the effect of the COVID-19 pandemic on the microbiological quality of wastewater from sewage treatment plants in Romania and its impact on receiving rivers. In order to highlight different and important areas in Romania, three cities from the east, center and west were selected for a microbiological evaluation of their WWTP influents and effluents from the pre-COVID-19 period and during the COVID-19 pandemic peak period, when the COVID-19 pandemic had a direct impact on WWTP microbial compositions. Our study shows that a higher level of contamination with fecal bacteria is linked to a higher COVID-19 incidence. The increased usage of pharmaceutical compounds, in turn, increases the number of resistant bacteria reaching the environment via WWTP effluents.

**Keywords:** pathogen; wastewater; pharmaceutical compounds; COVID-19



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## 1. Introduction

Aquatic bodies are essential for sustaining aquatic and terrestrial life; as well, they are a continuous major economic and touristic resource; therefore, they generate special attention for proper monitoring, protection and pollution issue mitigation. Most ecosystems are highly dynamic in terms of both their species compositions and abundances and their function. The abiotic and biotic elements of river and stream watersheds have been described as having a continuous pronounced ecological unpredictability from the beginning of the industrial age up to now, especially up to the development of the COVID-19 pandemic. The diversities and structures of microbial communities were inevitably impacted by environmental stress linked to natural ecosystem pollution. The microbial resilience capacity has been under the impact of a selective pressure triggered by untreated chemicals from wastewater plant effluents.

The overexploitation of water during the conduction of anthropic activities led to water depletion and/or pollution, which triggered a warning signal for proper water resource management. Furthermore, wastewaters are known sources of chemical pollutants

that mainly originate from human activities and the medical and unsupervised consumption of pharmaceuticals, which end up as parent or metabolized molecules in domestic wastewaters [1,2].

Wastewater treatment plants (WWTPs) are part of a water resource management plan to reduce chemical or microbiological pollution before the water reaches the environment [3]. Water, besides being a strategic resource for society, is an important vector in spreading infectious diseases over large areas. This was certainly the case of the COVID-19 pandemic, when water management was a major factor in SARS-CoV-2 spreading as well as its associated infections [4].

Despite the primary mode of SARS-CoV-2 transmission being the respiratory droplets that people cough, sneeze or exhale, an increasing number of articles reported the detection of this virus in the feces of COVID-19 patients. Most of the viral particles remained viable, infectious and/or able to replicate in stool under certain conditions. At the same time, during the epidemic peak, the massive use of pharmaceuticals caused a sudden increase in these drugs' concentrations in wastewaters. Unfortunately, the chemical compound increase triggered a rise in microorganisms' resistances to pharmaceutical compounds [1,5]. Changes in the microbial structures during WWTP treatment steps were correlated with viral presence in fecal matter, which eventually ended up in sewage systems.

The origin point of the COVID-19 pandemic was China, specifically Wuhan province, from where it had a very fast progression, reaching European countries in just a few months; by March 2020, all European countries confirmed active infections. The very fast worldwide spread of the virus took the medical authorities by surprise, which in response, imposed long periods of harsh social measures, such as lockdowns, to minimize the spread of the virus.

In Romania, the COVID-19 pandemic steadily spread from second part of 2020, reaching an infection peak in the middle of 2021. The COVID-19 spread rate within the population was mainly monitored by hospital units, but there were reports that it could be efficiently monitored by quantifying the virus responsible for the COVID-19 outbreak in domestic wastewater [6]. Furthermore, many studies have shown that wastewater-based epidemiology has become a practical approach for monitoring COVID-19 outbreaks. At the same time, microbiological analyses of wastewater could provide indirect data of COVID-19 progression among certain human communities. The concept of wastewater-based epidemiology indicates that wastewater is one of the main ways of disseminating antimicrobials and resistant microorganisms into the environments [3,7].

Large amounts of wastewater, including those from hospital units, are generated daily into the sewage system, from where they reach, as influents, municipal WWTPs [6,8]. Modern WWTPs can process large quantities of wastewater (up to 440,000,000 m<sup>3</sup>/year) and subsequently help maintain safe urban development and a clean environment. Consequently, it is necessary for WWTPs to have efficient and stable physico-chemical and microbiological treatment steps. The microbiological treatment step relies on activated sludge, which has a unique microbial community with a high bacterial diversity and high concentration of biomass based on the wastewater pollutant composition [9]. Any impairment of the wastewater treatment steps decreases the effluent's quality, thus posing a high risk to downstream users and environments, especially to aquatic systems [10].

The presence of the virus responsible for COVID-19 and its spread rate in wastewater is clearly linked to its indirect effect on microbial populations due to an overused anti-COVID-19 medical treatment in hospitals. Unfortunately, pathogenic microorganisms and pharmaceutical compounds, which are abundantly present in hospital wastewaters, were partially biodegraded by the microbial communities from the activated sludge during a WWTP biological treatment step. In addition, the activated sludge became a hot spot for the selection and dissemination of bacterial resistance under the pressure of chemicals, most of which were used during the COVID-19 treatment.

The World Health Organization acknowledged bacterial multidrug resistance as one of humanity's greater challenges, especially for the bacterial strains linked to the ESKAPE

group, which is composed of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and species of the genus *Enterobacter* spp. A total of 50% of patients with severe SARS-CoV-2 infections were reported to develop bacterial coinfections, especially with bacterial strains from the ESKAPE group, which isolated from several extra-hospital reservoirs through various water sources, including wastewater [11,12]. The ESKAPE group may be a relevant component of the drug resistance dissemination pattern [8].

Aquatic environments under anthropic pressure are hot spots for antibiotics and antibiotic resistance genes. In addition to them, municipal WWTPs are also major incubators for stimulating bacterial resistance mechanisms due to the fact that most antibiotics used in medical systems are excreted, via urine and feces, into sewage systems [13].

The COVID-19 pandemic offered a unique opportunity to study the modulation of environmental antibiotic resistance that could be associated with changes in disinfectant and/or antibiotic usage patterns, coinfections or other behaviors. COVID-19 cases positively correlated with the disinfectant/antiseptic group of ARGs and negatively correlated with the sulfonamide and aminoglycoside resistance classes. Antibiotic residues are present in hospital wastewater, and they can subsequently adversely affect the natural biota at multiple trophic levels by exacerbating the antibiotic resistance gene contamination of aquatic systems. Unfortunately, antibiotics released from clinical units reach the municipal sewage system and from there, they can reach the aquatic environment, where they are further in contact with opportunistic pathogens, enhancing the bacterial resistance mechanism [14,15]. Municipal WWTPs reduce abiotic and biotic pollution through three main treatment steps, including a primary treatment to reduce the solid matter, secondary biological treatment to break down the organic waste matter and tertiary treatment to remove pathogenic organisms through chlorine disinfection. Recent studies showed the presence of the same *Klebsiella pneumoniae* clone in both hospital and WWTP influents and effluents after chlorination, suggesting a high adaptive potential of this clone [16]. The majority of fecal bacteria strains, such as *Klebsiella pneumoniae*, isolated from effluents, influents and clinical samples were multidrug resistant, and they were especially resistant to antibiotics (beta-lactams) [13]. Unfortunately, the microbiological and pharmaceutical compound presence in sewage systems through excreted feces from people with infections were not significantly reduced by the WWTPs; therefore, they could reach and contaminate the environment, including the human communities nearby [17]. Putative waterborne pathogens with ARGs were identified in community coinfections with COVID-19 [14].

In spite of the progress in water treatment systems, the occurrence of waterborne infections remains a hot worldwide issue [18,19]. Enteric bacteria are the most commonly used bacterial indicator groups of fecal pollution, with these being used as indicators to evaluate the quality of wastewater influents, effluents and rivers. The evaluation of fecal bacteria dynamics in sewage systems, including wastewater treatment facilities, and their resistance and resilience to pharmaceutical compounds is essential for establishing a potential impact of controlled or uncontrolled wastewater discharges on the aquatic environment. Pathogenic or potentially pathogenic fecal bacteria in aquatic ecosystems could be used as warning bioindicators of major pollution with a public health harm potential, especially when they are resistant to the pharmaceutical compounds widely used during the COVID-19 pandemic [6].

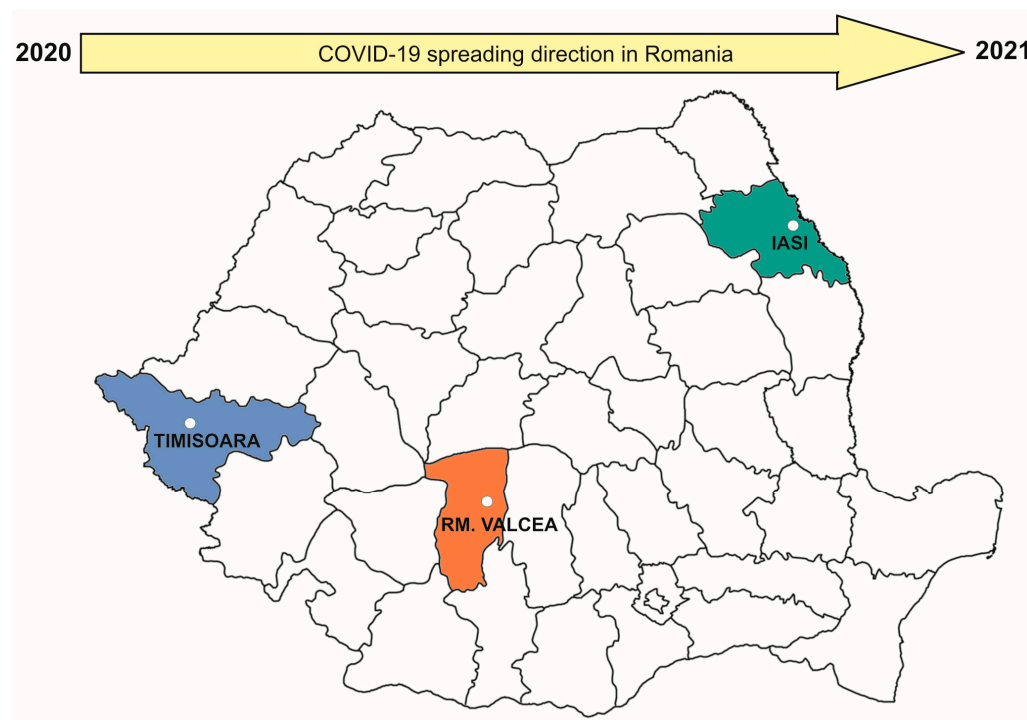
This work analyzed the microbiological modifications induced in influent and effluent wastewaters from three WWTPs in Romania pre-COVID-19 and during the peak period of the COVID-19 pandemic.

The centralized sewage systems that were studied are implemented in regions with moderate to high socio-economic capacities that have experienced a large number of cases of COVID-19. The aim of this study is to highlight the conditions that indicate an urgent need for monitoring programs and adapting the risk assessments of SARS-CoV-2 in wastewater, which can help in the early detection and limitation of future outbreaks of viral diseases.

## 2. Materials and Methods

### 2.1. Sampling

Three areas in Romania were selected (Figure 1). These are urban agglomerations with populations between 150,000 and 800,000 inhabitants: Iasi (800,000 inhabitants), Rm. Valcea (150,000 inhabitants) and Timisoara (500,000 inhabitants). The sampling period was carried out in three annual campaigns and covered the evolution of the COVID-19 pandemic in Romania (2019–2021). The sampling campaigns were grouped into two important periods: pre-COVID-19 pandemic (April 2019–September 2020) and a peak period for COVID-19 (October 2020–June 2021); these were carried out quarterly.



**Figure 1.** Sampling points selected in three areas of Romania.

Influent and effluent samples were collected from each WWTP from 24 h average samples, and surface water samples were collected from the river into which each WWTP discharges: Bahlui River (Iasi), Olt River (Rm. Valcea) and Bega River (Timisoara). Three close points were chosen at each sampling point of the rivers (upstream and downstream) for the representative sampling of a homogeneous sample of surface water.

A volume of 1 L for each water type was collected in replicates from each point of interest. The samples were collected in sterile containers in accordance with ISO 19458 [20] and then transported within a maximum of 24 h in conditions of 4–5 °C.

### 2.2. Microbiological Analysis

The Most Probable Number Method (MPN, IDEXX) was used in duplicate for the quantification of coliform bacteria densities [21]. The samples were analyzed on decimal dilutions (up to  $10^{-6}$ ), from which a homogenous suspension was prepared from 100 mL of sample with Colilert-18 medium (Idexx Laboratories, Inc., Westbrook, ME, USA).

The obtained suspension was distributed in Idexx bags in 49/48 wells, with one form detecting the total number of coliform bacteria and another for the quantification of fecal coliforms. An incubation time of 18–22 h was needed for the density quantification, with specific temperatures for the total coliforms ( $36 \pm 2$  °C) and fecal coliforms (44.5 °C). All steps were controlled by using positive controls such as *Escherichia coli* (ATCC25922) (Sharlab SL, Barcelona, Spain), *Citrobacter freundii* (ATCC 8090) (Thermo Fisher Scientific, Waltham, MA, USA) and *Klebsiella aerogenes* (ATCC 13048) (Sigma-Aldrich, Saint Louis,

MO, USA) and a negative control such as *Enterococcus faecalis* (ATCC 29212) (Sigma-Aldrich, Saint Louis, MO, USA).

The membrane filtration method [22] was performed for the detection and enumeration of enterococcus. The presumption test required filtering the sample through a membrane with a 45 µm pore diameter and placing it on Slanetz and Bartley agar (Oxoid, Basinstoke, UK) plates. The membranes on which the reddish-brown colonies grew after 48 h at  $36 \pm 2$  °C were transferred to Bile Aesculine Agar (Sharlab SL, Barcelona, Spain) and were incubated for 2 h at 44 °C. *Enterococcus faecalis* (ATCC 29212) (Sigma-Aldrich, Saint Louis, MO, USA) and *Escherichia coli* (ATCC25922) (Sharlab SL, Barcelona, Spain) were used as controls for the technique efficiency.

Sample dilutions were made with sterile distilled water, which was also used for the blank control.

All final results were reported per 100 mL: MPN/100 mL for total and fecal coliforms and CFU (colony forming units)/100 mL for the enterococcus density.

### 2.3. Statistically Analysis

The statistical results were carried out using the ANOVA function of Microsoft excel (Microsoft Office Professional Plus 2019, Version 2403). This technique sought to highlight the certainty of the results regarding the significant differences recorded.

## 3. Results and Discussion

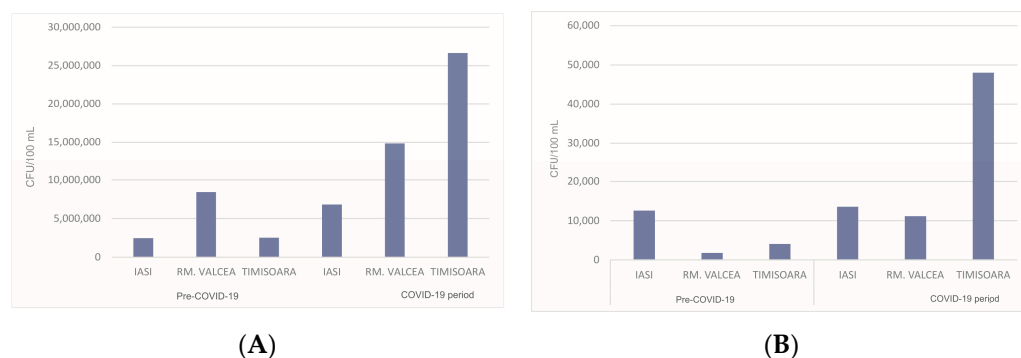
The sampling points were analyzed from west to east in Romania based on the COVID-19 progression. The sampling campaigns were correlated with the COVID-19 incidence, and were carried out at that time with the same range of incidence. The COVID-19 incidence average, measured daily in hospitals, was around five (five COVID-19 cases per one thousand inhabitants) for the city of Timisoara (west side of Romania) and three for Rm. Valcea (central part of Romania) and Iasi (eastern part of Romania) (Figure 1).

The COVID-19 pandemic was also associated with simultaneous digestive issues in humans infected with SARS-CoV-2. The binding of the virus to some proteases in the intestinal tract led to the production of inflammatory factors secondary to the viral infection that were potentially harmful to the intestinal barrier. The imbalances of the intestinal microbiota from secondary COVID-19 infections [23] caused an increase in the number of hospitalized patients with both SARS-CoV-2 infections and secondary bacterial infections. A clinical research study from Romania demonstrated that both the number of hospitalized patients and the number of bacterial isolates was lower before the emergence and installation of the COVID-19 pandemic. This phenomenon also led to the excretion and dissemination of bacteria from secondary infections to wastewater and then to the receiving aquatic environments [24]. At the same time, Amin and Co. [25] showed that a microbiological analysis on COVID-19 and non-COVID-19 hospitals from Bangladesh indicated that 97% of the wastewater samples positive for *E. coli* were positive for SARS-CoV-2 too. These results indicated that high concentrations of fecal bacteria and viral particles were discharged through the hospital wastewater into WWTPs.

In addition, the COVID-19 treatment procedures relied heavily on antibiotic and biocide chemicals, which in fact disturbed the balance of intestinal bacterial communities, including the generation of antibiotic resistant bacteria. Even before the establishment of SARS-CoV-2 infections, bacterial resistance was a global problem, and after this period, the resistance rates of pathogenic microorganisms isolated from both clinical cases and the environment increased. The pathogenic microorganisms from patients were released into the sewage system and could therefore spread their resistance during the treatment steps of WWTPs. The influence of the disinfection processes and specific infection treatments with antibiotics caused the dissemination of antibiotic resistance. The antibiotic presence in wastewater can exert a selective pressure and promote the development of antibiotic resistance genes, which is an essential factor in changing the microbial community structure [26]. The fecal bacteria from the sewage systems, es-



pecially in the WWTP influents, increased during COVID-19 progression compared to the period before the COVID-19 pandemic (Figure 2A). The ratio between fecal bacteria from the COVID-19 period vs. pre-COVID-19 was similar in Iasi (2.8 ratio COVID-19/pre-COVID-19) and Rm. Valcea (2 ratio COVID-19/pre-COVID-19). Interestingly, in Timisoara, a city with a higher COVID-19 incidence, compared to Iasi and Rm. Valcea, the fecal ratio between COVID-19 and pre-COVID-19 increased to 10.8.



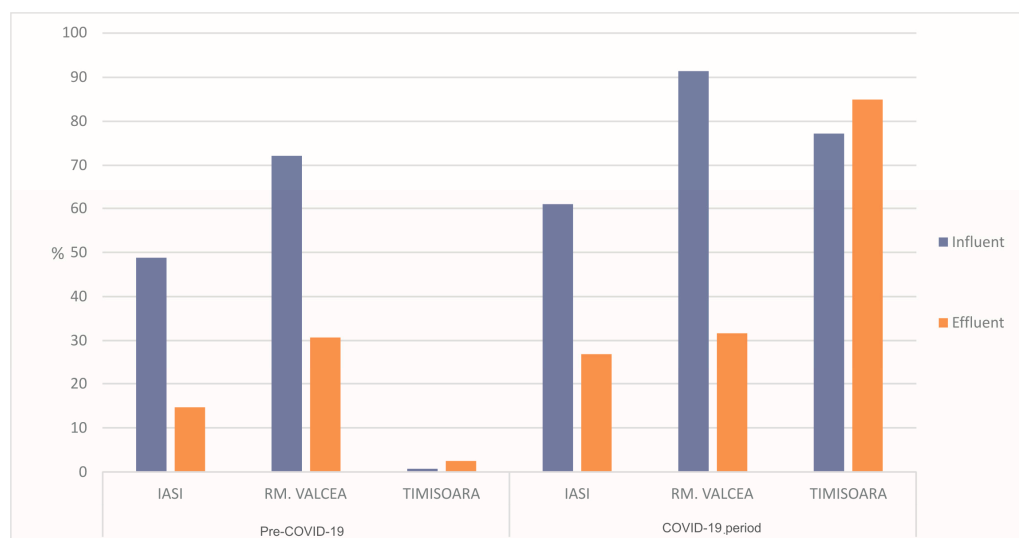
**Figure 2.** The fecal coliform loads in (A) influents and (B) effluents from Romanian WWTPs pre-COVID-19 and during the COVID-19 pandemic. The results represent the average values from at least two independent experiments, and each experiment was repeated twice ( $p$ -value < 0.05).

The WWTP efficiency in removing the bacterial load, especially fecal coliforms, from influents (around  $1.5 \times 10^7$  CFU/100 mL) to effluents (around  $1.5 \times 10^4$  CFU/100 mL) was very high and achieved by decreasing the bacterial magnitude range load with  $1 \times 10^3$  CFU/100 mL. In spite of this significant reduction in the bacterial load, the fecal presence in effluents was still substantial, and their fecal ratios between COVID-19 and pre-COVID-19 were up to 6 for Rm. Valcea and up to 13 for Timisoara but decreased to almost 1 for Iasi. (Figure 2B). The overall tendency of fecal presence was increased during the COVID-19 pandemic compared with the pre-COVID-19 progression.

The fraction of fecal bacteria detected in total coliforms from WWTP influents and effluents increased during the COVID-19 pandemic compared with the -pre-COVID-19 period (Figure 3). The statistical analysis of these results indicated a  $p$  value < 0.03 in all cases. In the city of Iasi (eastern part of Romania), the percentage rise during the COVID-19 pandemic in influents was around 10% compared with the pre-COVID-19 period. A more significant fecal percentage rise (around 15%) was observed in effluents, and this could be explained by an acquired resistance of fecal coliforms to WWTP treatment steps and chemical inputs, such as the pharmaceutical compounds used in hospital units and released into the sewage system.

In Rm. Valcea (central part of Romania), the fecal coliform percentage rise for total coliforms was observed only for the WWTP influent, which was up to 20% during COVID-19 compared to the pre-COVID-19 period. No significant changes occurred in the fecal percentage from total coliforms in the effluent. In Timisoara (western part of Romania), we observed a significant rise in the fecal percentage from total coliforms during the COVID-19 pandemic, regardless of the sample being the influent or effluent. This could be explained by the fact that Timisoara was the SARS-CoV-2 entrance gate in Romania, and therefore, the COVID-19 incidence was higher than in the central and eastern parts of Romania; as well, the medical treatment was implemented earlier, which could have induced an early acquired resistance of fecal bacteria to pharmaceutical compounds.

Some studies showed that Gram-negative bacterial antibiotic resistance, especially fecal bacteria such as *E. coli*, increased during the COVID-19 progression compared with Gram-positive bacteria [24].

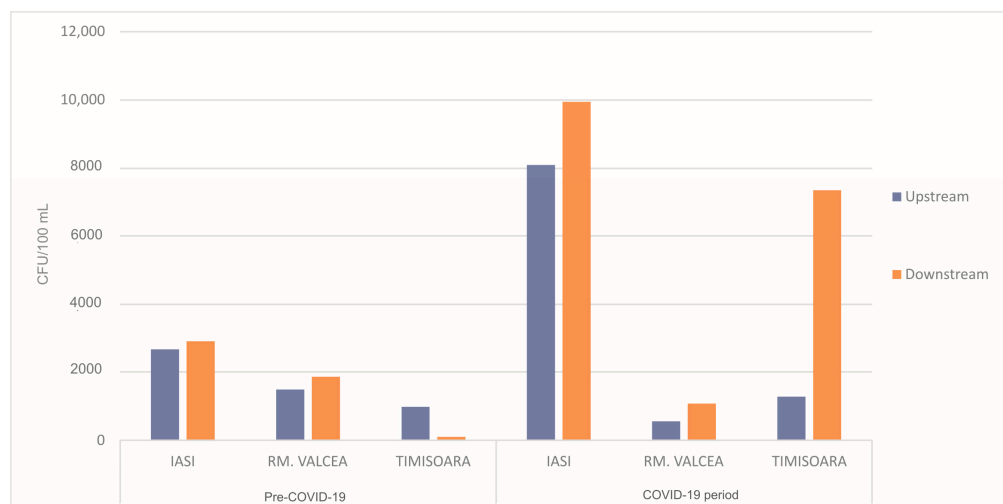


**Figure 3.** The proportion of fecal bacteria from total coliforms detected in WWTP influents and effluents during the COVID-19 pandemic or pre-COVID-19 pandemic ( $p$ -value < 0.05).

In this respect, during SARS-CoV-2 infection, Gram-negative bacteria such as total coliforms and *E. coli* were in contact with a large number of pharmaceutical compounds compared to pre-COVID-19 pandemic, increasing the resistance mechanism and subsequently, the resistant Gram-negative bacterial population. This case scenario was analyzed for Romanian WWTPs before the COVID-19 pandemic and during infection progression.

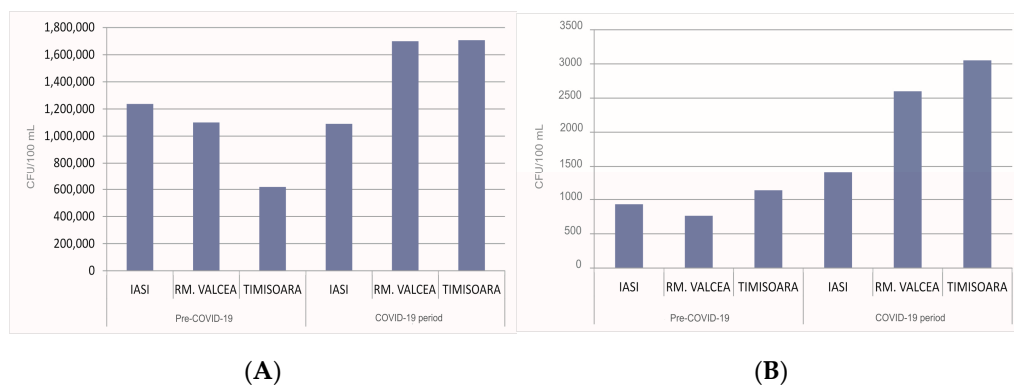
The fecal bacterial load from the streams, upstream the WWTP discharge site, was in the same range during the COVID-19 pandemic and pre-COVID-19 pandemic (Figure 4) with the exception of Iasi, where the fecal bacterial load was massively increased during the peak SARS-CoV-2 infection. Also, in this situation, the statistical results recorded a  $p$  value around 0.03. Overall, the fecal bacterial load discharged into the environment had an effect of increasing the fecal contamination downstream of the WWTP discharge site. The downstream fecal coliform loads from Iasi and Rm. Valcea had a comparable increase in the bacterial contamination level for the pre-COVID-19 period and during the COVID-19 pandemic (Figure 4). Timisoara, with a much higher SARS-CoV-2 incidence, showed a huge fecal contamination of WWTPs downstream during the COVID-19 pandemic compared to the pre-COVID-19 period, in spite of having a comparable fecal load into the environment upstream the WWTP discharge site.

In Iasi, *Salmonella* spp., a pathogenic bacterium belonging to the fecal group, was detected in influent, effluent, downstream and upstream sites regardless of the sampling campaign period being before the COVID-19 pandemic or during the COVID-19 pandemic. The same pattern of *Salmonella* spp. presence regardless of the sampling campaign was identified for the WWTP influent from Rm. Valcea. A very interesting case was observed for Timisoara, where *Salmonella* spp. was detected only upstream samples during the COVID-19 pandemic compared to pre-COVID-19, where *Salmonella* spp. was detected in influent, effluent and upstream samples. This could be explained by *Salmonella* spp.'s sensitivity for pharmaceutical compounds that are present in the sewage system and discharged into the environment by the WWTP. The COVID-19 pandemic in Timisoara was characterized only by *Salmonella* spp. presence in upstream samples, which could be correlated with a high SARS-CoV-2 incidence, and subsequently, with a high rate of usage of pharmaceutical compounds during medical treatments for a large population. As an observation, Iasi (800,000 people) and Timisoara (500,000 people) have a comparable population, but a high COVID-19 incidence in Timisoara indirectly decreased the *Salmonella* spp. load due to the increased pharmaceutical treatment of COVID-19.



**Figure 4.** The fecal coliform loads upstream and downstream of each receiver river pre-COVID-19 or during the COVID-19 pandemic. The results represent the average values from at least two independent experiments, and each experiment was repeated twice ( $p$ -value < 0.05).

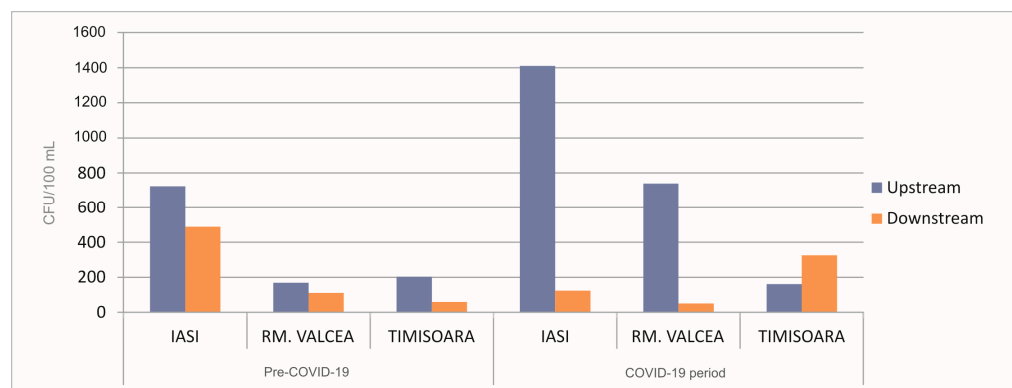
The enterococcal load pattern presence matched the fecal quantification in influents and effluents the pre-COVID-19 period and after the pandemic started. During COVID-19 progression, the enterococcal load increased, regardless of the sample being the influent of effluent, compared with the period before the pandemic (Figure 5). In addition, the maximum increase was in Timisoara, which correlated with a high SARS-CoV-2 incidence compared to Rm. Valcea and Iasi.



**Figure 5.** Densities of enterococci in (A) influent and (B) effluent of each WWTP. The results represent the average values from at least two independent experiments, and each experiment was repeated twice ( $p$ -value < 0.05).

The enterococcus load ratios between the upstream and downstream samples increased during the COVID-19 pandemic, pointing to a possible increased sensitivity of upstream bacterial loads to pharmaceutical WWTP discharges for all cities (Figure 6). An interesting situation arose for Timisoara, where the upstream vs. downstream enterococcus ratio was inverted, perhaps due to antibiotic resistance of the enterococci discharged from Timisoara's WWTP. Previous results showed an increased antibiotic resistance due to a high pharmaceutical compound usage [27,28]. Timisoara had a higher COVID-19 incidence than Iasi and Rm. Valcea, and therefore, Timisoara had a higher usage of pharmaceutical compounds, which triggered the presence of more antibiotic resistant bacteria.





**Figure 6.** Densities of enterococci upstream and downstream of receiver river. The results represent the average values from at least two independent experiments, and each experiment was repeated twice ( $p$ -value < 0.05).

In Romanian clinics, the COVID-19 pandemic marked an increased number of Gram-negative pathogenic bacilli compared to the previous period, where Gram-positive cocci were more common [28,29]. This observation corroborated with a higher COVID-19 incidence in Timisoara, where the bacterial load had more interaction with larger amounts of pharmaceuticals, which generated resistant bacteria that ended up in natural streams (downstream) compared to the bacterial load upstream.

According to recent studies [4,6,30–32], the degree of pollution in the aquatic systems in Romania decreased with the decrease in the incidence of COVID-19 cases. Post-pandemic effects can be seen in the horizontal transmission of acquired antibiotic resistance and the perpetuation of resistant bacteria in aquatic environments, with impacts on population health. Although the pandemic was significantly reduced, bacterial infections with resistant microorganisms continued to persist, especially in hospital units. Thus, various pharmaceutical compounds, including the antimicrobial substances used in various clinical treatments, end up in WWTPs, where they spread the ARGs to the environment and human communities, subsequently affecting medical treatments. Recently, many solutions to improve the WWTP efficiency were proposed, focusing on the microbial treatment step, from increasing the energy efficiency by using bacterial–algal granules [33–35] to resilient chemical removal by specific bacterial strains. Also, phosphate recovery by struvite crystallization and using a CO<sub>2</sub> degasification reactor can significantly reduce the level of contamination of wastewater that is subsequently discharged into natural ecosystems [36–38].

Given that ecosystem stability is about temporal continuity, bacteria can continuously respond and adapt to secondary perturbations by changing their patterns of assembly and function. Antimicrobial resistance remains a serious and emerging health system problem after COVID-19 that is exacerbated and widespread globally. This continues to generate unprecedented stimuli for doctors and researchers looking for the solution, prevention and management of this problem, both at the medical level and at the environmental impact level.

#### 4. Conclusions

The COVID-19 pandemic had an indirect impact on microbial populations due the medical treatments against COVID-19 that involved using large amounts of pharmaceutical compounds, especially antibiotics. The COVID-19 progression in Romania could be monitored by the rise of fecal bacterial loads from WWTP influent wastewaters. The same fecal pattern could be observed in enterococcus enteric bacteria. Enterococcus was linked to most gastrointestinal infections and inflammatory bowel diseases reported during the COVID-19 peak period of the pandemic. Salmonella, another pathogenic bacterium, showed no significant changes (monitored only by presence or absence) in influents and

effluents, regardless of whether the sample was taken during the COVID-19 pandemic or not.

Unfortunately, we observed clear fecal contamination of the natural aquatic bodies (downstream) by the WWTP effluents, and this contamination seemed to increase in areas with a high COVID-19 incidence, such as Timisoara (five: incidence number) compared to Iasi and Valcea (three: incidence number). *Enterococcus*, a Gram-positive bacterium, had a greater resistance rate when in contact with larger number of pharmaceutical compounds, and it could be observed in the receiving river from Timisoara. In Valcea and Iasi, which both had lower COVID-19 incidence rates, the *Enterococcus* presence downstream compared to upstream was similar, regardless of during which COVID-19 period of time the sample was taken. It was clear that a higher COVID-19 incidence increased the usage of pharmaceutical compounds, which in turn, increased the number of resistant bacteria reaching the environment via WWTP effluents.

Fecal coliform bacteria in the final effluents play the role of quality indicators and indicate the presence of pathogens in wastewater, with a high risk of transfer to natural water/environmental resources. In addition to information about the microbiological quality of wastewater or surface water, in the present case, coliforms also provide valuable subsequent information, such as the progression of COVID-19 and the increase in pharmaceutical use: phenomena with which they are correlated.

**Author Contributions:** Conceptualization, A.R.B. and M.N.-L.; Formal analysis A.R.B., C.S., S.G. and I.L.; Writing—original draft, A.R.B., L.F.P., L.F. and M.N.-L.; Writing—review and editing, A.R.B. and L.F.P. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ongoing research in this field.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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