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### Seasonal influences on microbiological quality of aquatic ecosystems

ALINA ROXANA BANCIU<sup>1</sup>, MONICA ALEXANDRA VAIDEANU<sup>1,2\*</sup>, DRAGOS MIHAI RADULESCU<sup>1,3</sup>, CRISTINA IFTODE<sup>1</sup>, LAURA NOVAC<sup>1</sup>, MIHAI NITA-LAZAR<sup>1</sup>

<sup>1</sup>National Research and Development Institute for Industrial Ecology – ECOIND, 57-73 Drumul Podu Dambovitei street, district 6, 060652, Bucharest, Romania

<sup>2</sup>University of Bucharest, Faculty of Biology, Splaiul Independentei Street, no. 91-95, District 5, Bucharest, secretariat@bio.unibuc.ro, Romania

<sup>3</sup>Ecological University of Bucharest, Faculty of Ecology and Environmental Protection, Bd. Vasile Milea, no. 1G, Bucharest, contact@ueb.ro, Romania

\*Correspondence author: monica.vaideanu@ecoind.ro

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#### **Abstract**

*The wide distribution of bacteria and their highly dynamic activities have a great impact on the aquatic ecosystem. Surface water bodies constitute an important drinking water source that approximatively cover half of the worldwide need. Considering the main health threats, the microbial profile represents the most important indicator on the biology quality of water environments. One of the most important criterion is fecal indicators and in consequence the aim of this study was to monitor the seasonal fecal pollution variability in the surface water of Arges and Dambovita River during 2021. These rivers are part of the Arges-Vedea basin that cover 9% of Romania's surface and they are located in the proximity of the Bucharest, the capital city of Romania.*

**Keywords:** *aquatic ecosystem, bacterial contamination, safe water*

#### **INTRODUCTION**

Bacteria are widely spread over all types of ecosystems, including aquatic ecosystems. Their wide distribution and highly dynamic activities have a great influence on the ecosystems health and stability. It has been known that bacteria can serve as principal decomposers in the ecosystem, maintaining the natural cycles of carbon, nitrogen and phosphorus. In the aquatic environment, the compositions and diversities of bacterial community may vary based on waterbodies composition and quality. Extensive studies have been carried out to match bacterial community structure and function with different aquatic systems types. There are systems that change their physical and ecological proprieties depending on the spatio-temporal and environmental conditions [1]. Over the time, the aquatic systems have been under the pressure of natural changes and, recently, they have been more and more under anthropic pollution pressure due to a rapid development of economy and population growth [2]. Water is an essential element to preserve life but at the same time it is a perfect environment to spread harmful chemical compounds and pathogens [3].

Surface water bodies constitute an important drinking water source that approximatively cover half of the worldwide need [4]. Limited drinking water resources for domestic activities force communities to use microbiologically polluted river water for these purposes presenting a risk to public health [5]. According with EU legislation, nowadays, in Romania has 3027 surface water bodies from which only 2470 are natural water bodies, 488 heavily modified water bodies and 69

artificial water bodies [6]. The assessment of water quality allows the detection of the causes of pollution and prevents possible water-borne diseases, providing information about the public health risk. The microbial community is not only a natural component of the aquatic ecosystem, but also one of the main indicators of their ecological status. Considering the main health threats, the microbial profile represents the most important biological quality parameter of the water environment. Water quality assessment takes into account physical, chemical and biological characteristics. One of the most important criteria for water quality is fecal pollution. Faecal water contamination refers to the double source of point and diffuse pollution, being evaluated by the detection and quantification of viable bacterial indicators, the so-called faecal indicator bacteria, primarily represented by *Escherichia coli* and Enterococcus species [4, 7, 8]. The qualitative evaluation process of surface waters is complex and depends on a series of criteria, methods and procedures that vary, thus imposing a relative character. Water quality does not remain constant over time, but can vary depending on the sources of natural or artificial impurities, a fact that requires a permanent control of the values of the parameters by which the quality of surface waters is defined. Also, natural waters have the function of receiving waste water loaded with waste or "losses" resulting from human activities, which alters the initial quality of the water. In the conditions of contemporary society, characterized by the accelerated pace of social and economic development, there is a tendency of a dangerous accentuation of the process of polluting water resources, which can lead to completely inappropriate situations [9].

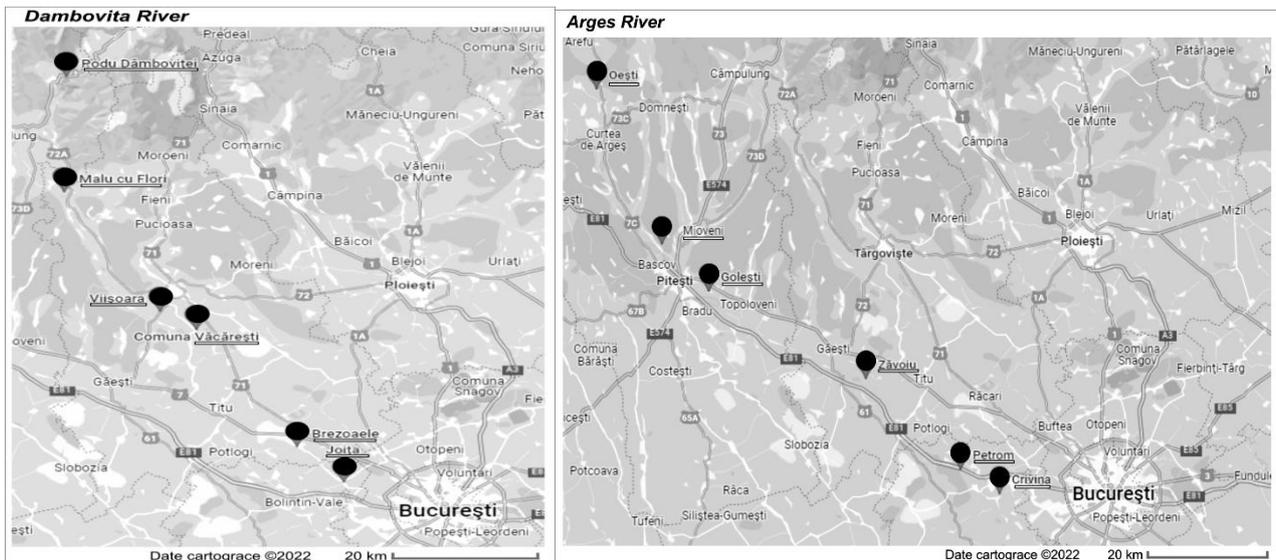
In order to protect public health, detecting the microbes sources and their changes in surface water is important for an effective water treatment [10]. Improperly treated and untreated wastewater released from wastewater treatment plants is the main source of emerging organic contaminants found in the Danube River and its tributaries, the most important sources of drinking water in Romania. In 2014, microbial fecal pollution levels were mainly linked to untreated sewage [11]. Thus, in 2014, human fecal pollution was proven to be the primary pollution source along the entire river, while animal fecal pollution was found to be the minor importance [12]. Rivers play a significant role as a major water supply, but, unfortunately, rivers are also receptors of domestic and industrial waste and pollution, which is disseminated by rivers over large and distant areas, negatively influencing those ecosystems [8]. The pathogenic characteristics of faecal bacteria make them persist in the aquatic environment for a long period of time. Pathogen densities are also influenced by weather and climate. Climate changes in temperature and precipitation influence the densities of pathogens and the structure of bacterial communities in surface water sources. High temperatures and increased precipitation favor flooding that can favor the migration of pathogens into surface waters. At the same time, increased precipitation decreases the concentration in surface water due to dilution [12].

The aim of the present study was to monitor the seasonal fecal pollution variability in the surface water on the lower reaches of Arges and Dambovită Rivers from Romania during the year of 2021.

## **EXPERIMENTAL PART**

### *Sampling*

Six sampling locations were selected on the upper course of each of Arges and Dambovită rivers. (Fig. 1). These rivers are part of the Arges-Vedea river basin which cover 9% of Romania's surface. The lower course until the discharge into the Danube River takes place in the plain area where they fulfill both the role of emissaries and the role of drinking water supply. The sampling location were chosen from area where there is the capture of surface water for drinking purposes, but at the same time there is a pressure of anthropogenic pollution. The surface water sampling was done seasonally during 2021 and the samples were collected in sterile containers with a volume of 1L [13].



**Fig. 1.** Sampling sites for Dambovitza and Arges rivers

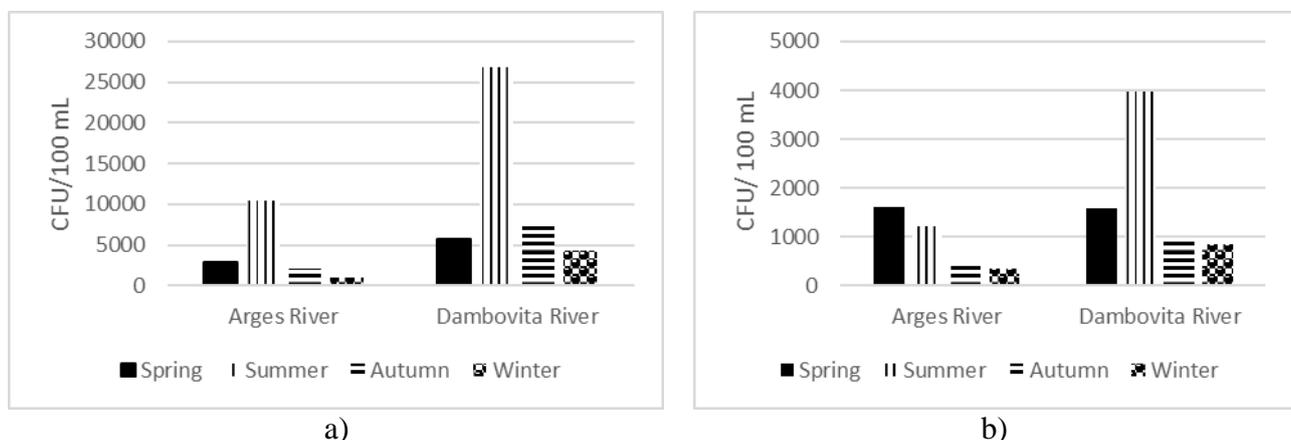
### Microbiological analysis

The densities of total coliform bacteria and *Escherichia coli* from the surface water samples were performed by Most Probable Number (MPN) method (IDEXX) [14], using Colilert-18 medium and incubate the samples at  $36\pm 2^{\circ}\text{C}$  for 18-22 hours. The positive control (*Escherichia coli* ATCC25922, *Citrobacter freundii* ATCC 8090 and *Enterobacter aerogenes* ATCC 13048) and the negative one (*Enterococcus faecalis* ATCC 29212) were tested. In the same time, a blank control with sterile distilled water was analyzed. The results were expressed in CFU/100 ml for both indicators.

The quantification of *Enterococcus sp* from surface water samples were performed by membrane filtration method using Slanetz & Bartley Agar and Bile Aesculine Azide Agar [15]. The first incubation step was for 48 hours at  $36\pm 2^{\circ}\text{C}$ , then the suspicious colonies were confirmed on Bile aesculine azide agar by incubation at  $44^{\circ}\text{C}$  for 2 hours.

## RESULTS AND DISCUSSION

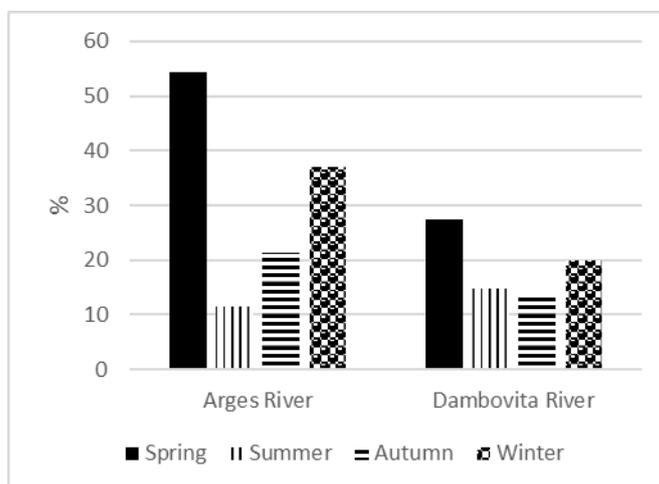
Numerous studies have shown that individual bacterial populations are highly dynamic in response to seasonal cycles and resource availability in the water body such as dissolved organic matter [16-19]. Summer is a season to match the abundance nutrients and an optimum temperature for bacterial growth. The results backed up this observation and they showed dynamic bacterial growth measured by total coliforms densities peaks in the surface water (Fig. 2.a.) during summer of 2021. Totals coliforms densities from Dambovitza River was up to 2.5 folds higher than Arges river. The same densities pattern was observed for *E. coli* (Fig. 2.b.).



**Fig. 2.** Total coliforms (a) and *E. coli* (b) seasonal densities in Dambovitza and Arges rivers

Some physical and chemical factors may trigger changes in the bacterial composition and therefore affecting in water quality [20]. Similarly, seasonal shifts in water column stability and water temperature may modulate the annual pattern of bacterial community variability.

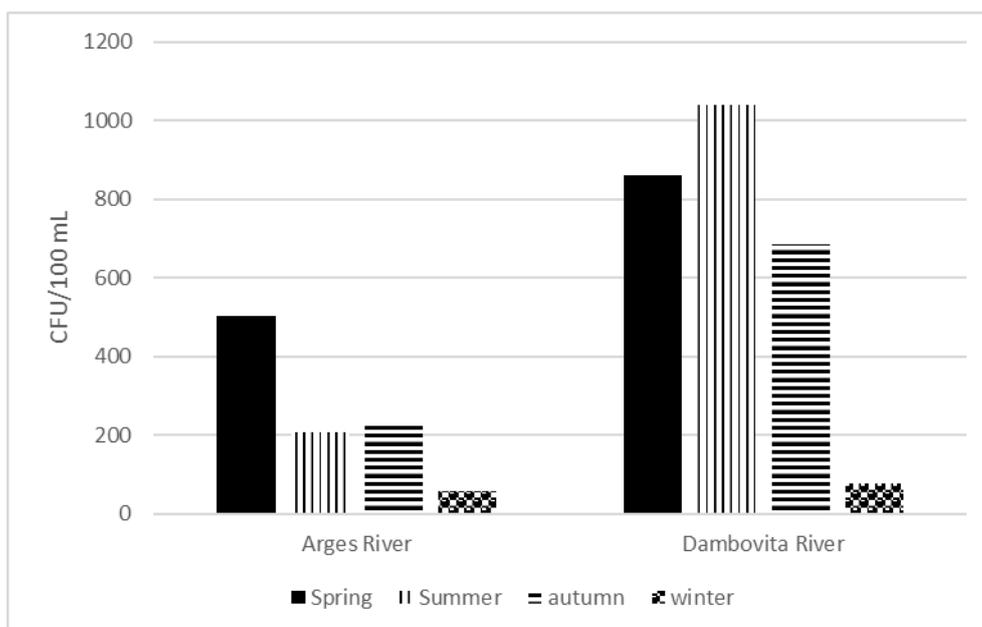
It was very interesting to observe that percentage of *E. coli* in total coliforms was higher during spring, autumn and winter in Arges river compared to Dambovită river (Fig 3). In spite of having the most differences in total coliforms densities in summer, Dambovită higher than Arges, the percentage of *E. coli* from total coliforms was higher in Arges (Fig. 3). In this respect, the *E. coli* contamination of Arges river seemed to be significant, compared to Dambovită.



**Fig. 3.** The seasonal *E. coli* percentage from total coliforms

In addition, the *Enterococcus* densities matched the total coliforms profile with high densities in Dambovită compared to Arges. Moreover, the seasonal profile showed very high densities in summer, spring and autumn for both rivers (Fig 4).

The density of Enterococci resulting from the analyzes indicates that there are sources of pollution from rural discharges. And in this case, the influence of the external temperature factor can be observed.

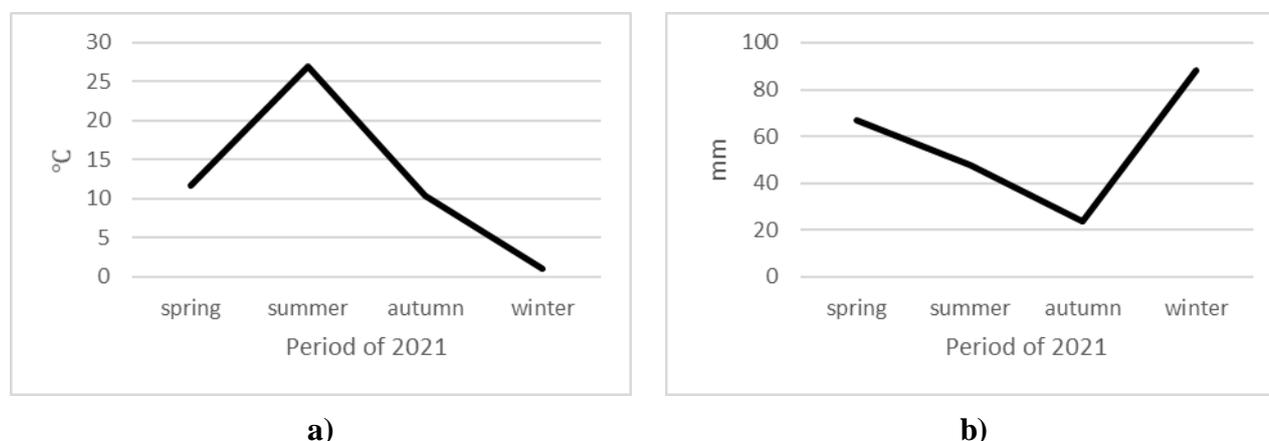


**Fig. 4.** Enterococcus sp. seasonal densities in Dambovită and Arges rivers

Bacterial densities variation was clearly linked to temperature and precipitation. The optimum temperature was associated with summer, where the temperature peak was up to 30°C (Fig. 5a).

The relationship between bacterial growth and precipitation was linked to nutrient availability and dilution factor (Fig. 5b) [16].

The difference in density can be observed depending on the external factors conditioned by the season, namely temperature and precipitation, extremely important factors for the growth of bacteria.



**Fig. 5.** Variation of the regional temperature (a) and precipitations (b) gradient during 2021 [16]

Other studies performed on Galda river from Alba County, Romania showed that potentially pathogenic bacterial contamination was correlated with increased values of temperature and physical-chemical parameters [21]. The seasonal variability temperature influence on fecal coliforms was also reported on four different sites in the USA and Korea with a peak in summer and low in winter [22].

An argument that supports this aspect is the degree of surface water pollution that highlights the role of human activities where there is no sewage system.

Also, a major influence in decreasing the bacterial density during the winter can be the lower intensity of anthropogenic activities.

## CONCLUSIONS

The aspects reported in this paper support the idea that rivers are the main source of bacterial contamination over large areas. According to the analyzed microbiological indicators, it was found that the degree of surface water contamination increased, especially in summer and spring.

It was also identified by the presence of enterococci and the source of human and/or animal sewage discharges.

At the same time, meteorological conditions (precipitation and temperature) affect the levels of potentially pathogenic bacteria such as total coliforms, *E. coli* and enterococcus. High temperatures and low precipitation favor the multiplication of microorganisms and increase their density in the surface water. The decrease in temperatures and dilution of the environmental matrix caused by precipitation determines the decrease of bacterial populations.

The variation of the bacterial population structure is dependent on the annual seasons with a peak in summer and a low level in winter.

## ACKNOWLEDGEMENTS

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