ROMANIAN ACADEMY
School of Advanced Studies of the Romanian Academy
Institute of Biology Bucharest

PhD THESIS-SUMMARY

CHARACTERIZATION OF MICROBIAL COMMUNITIES IN POLLUTED ENVIRONMENTS

PhD SUPERVISOR:
CSI Dr. Cristina Ligia PURCĂREA

PhD STUDENT:
Aurelia (VLAD) PODOSU

2024

CONTENTS

INTRODUCTION

The biological activity of ecosystems is determined by the microbial components that establish the balance between organic and inorganic substances and between living and non-living matter. Flowing waters represent a particular environment from a microbiological point of view, because their microbial composition undergoes major changes from the springs to the confluence of the river with other flowing waters or with the river-sea system. The microbial content of the waters is influenced by the hydrographic basin, the contribution of tributaries, the physico-chemical and climatic conditions and, in particular, by the anthropogenic impact.

Water quality is essential for all life on Earth, having a major impact on human communities. Throughout history, human communities have developed in the vicinity of water reserves (flowing waters, lakes, seas, oceans), using water resources for various activities, which has led to an increase in anthropogenic impact on water quality. Residue pollution resulting from industrial, agricultural, recreational or other human activities leads to significant changes in the chemical composition and physicochemical characteristics of water. They influence the community of microorganisms in the anthropized aquatic ecosystem, having direct effects on biogeochemical cycles.

There is currently no universally accepted system for classifying water as polluted or unpolluted. There are regulatory systems that classify water into several quality classes, allowing the use of water in certain types of activities. In this study, the investigated water samples were classified into the categories of polluted or unpolluted water, in relation to the values of the physicochemical and microbiological parameters established for the quality of drinking water supplied to socio-economic systems (APHA standard, 1998; Lazăr et al., 2014; Bernasconi et al., 2003; Janelidze et al., 2011) in the investigated area.

The microbiological diversity of an aquatic ecosystem is essential for its functioning in terms of the flow of matter, energy, information, and biogeochemical cycles (Azam et al., 1983). At the same time, the physicochemical changes of the ecosystem are reflected in its microbiological activity.

Taking into account the fact that water reserves are fundamental for daily human activities, and the knowledge of their (micro)biological and chemical structure is of particular importance for assessing its quality, joint studies on the chemical and microbiological composition of waters are essential for characterizing their quality.

The term or notion of pollution, with linguistic meaning as the transformation of a normal environment into a harmful one as a result of the presence of chemical or biological agents, is based on arbitrary human perceptions depending on a certain degree of tolerance of the modification. A suggestive definition of this notion can be considered the one proposed by Lynn Margulis who shows that "pollution is a mixture of ignorance and arrogance on the part of a being who has defined himself as *Homo sapiens* and superior to other beings, towards the environment". The term has entered the current language of political decision-makers, which has led to an increased interest in research in this field lately.

In this doctoral thesis, two sectors of the Pasărea River (Dimieni Bridge and Tunari Dam) located in the vicinity of economic units essential for carrying out daily activities, but also of a highly developed human community, with a significant anthropogenic impact, were investigated. The present study was mainly oriented towards the investigation of the undeveloped sector of the Pasărea River, between the Dimieni bridge and the Tunari dam, areas also established as sampling points in the area of Tunari, in Ilfov County, located in the vicinity of economic agents of national importance, but also of some residential complexes built in the last period of time.

The investigations carried out during four seasons came in continuation of a study carried out previously, at the request of the National Administration of Romanian Waters, on the high fish mortality in this sector and contributed to the scientific substantiation of the negative ecological phenomenon recorded. The studies of this research were oriented towards highlighting the chemical composition of the water sector, the physicochemical parameters that can define it and the microbial communities that develop in the water of this river, as well as some of their extracellular metabolites with potential in the biological modeling of the quality of the Pasărea River, in the context of downstream arrangements for fish farms that deliver the products to the consumer market.

This doctoral thesis is structured in two parts, the first part includes geographical and economic data about the ecosystem of the Pasărea River and the adjacent areas, the wastewater captured by the Pasărea River. The next chapter focuses on data on the main factors influencing the dynamics of aquatic ecosystems, mainly rivers/flowing waters and the role of microorganisms in the biogeochemical cycles of carbon, nitrogen and sulfur and the role of extracellular enzymes within them. The third chapter addresses the pollution of aquatic ecosystems from a microbiological point of view. The original contributions are described in the following 5 chapters, starting with the purpose and objectives of this thesis, followed by the description of the materials and methods and of the 4 studies that founded the present research, in which the characterization of the physicochemical parameters, the role of microorganisms involved in the biogeochemical cycles of the Pasărea River, isolation and morphofunctional characterization of microorganisms from the Pasărea River and the impact of pollution on groups of microorganisms from the Pasărea River. At the end, the conclusions and perspectives are presented, the paper being accompanied by an appendix with figures, the bibliography and the list of papers/articles in which the results of this paper were disseminated.

I. BIOGEOGRAPHIC AND BIOECONOMIC DATA OF THE PĂSĂREA RIVER ECOSYSTEM

I.1. The Pasărea River and its inclusion in the Bucharest – Ilfov plain hydrographic basin

The Pasărea River, a tributary of the Dâmbovița River, springs from the Otopeni Forest and crosses the localities of Tunari, Afumați, Găneasa, Brănești and Fundeni, before flowing into the Dâmbovița (Cocoș, 2006). With an average annual flow below 1 m^3/s, a dam (Tunari II) was set up to regulate the flow and prevent flooding. The river has a tributary, Șindrilița, and drains an area of 254 km^2 (Cocoș, 2006).

I.3. Demographic and economic aspects in the Pasărea river basin as potential sources of pollution

The communes crossed by the Pasărea River have populations ranging between 6,300 (Tunari) and 9,400 (Brănești) inhabitants (County Plan for Risk Analysis and Coverage, Ilfov County Committee for Emergency Situations, 2021). Tunari commune is located near Henri Coandă International Airport, and Ilfov County is industrialized with economic agents active in various fields (Cepoiu, 2007). Human activities directly or indirectly influence the course and quality of water, negatively impacting the health of the aquatic ecosystem of the Pasărea River, according to the Research Report no. 471/27/02.2019 of the Institute of Biology in Bucharest, within the Romanian Academy, carried out at the request of the National Administration "Apele Române", the Argeș Vedere Water Basin Administration and the Ilfov-Bucharest Water Management System. The Pasărea River was classified as grade IV for ammonium ion, and the Moara Domnească Lake showed high values of ammonium and nitrates, indicating severe eutrophication and the presence of pesticides (Research Report no. 471/27/02.2019, Institute of Biology in Bucharest, Romanian Academy; Sandu et al., 2023).

II. STRUCTURE AND DYNAMICS OF MICROORGANISMS IN AQUATIC ECOSYSTEMS

II.1. General aspects of aquatic environments

Aquatic environments, including fresh and salt waters, are complex and varied ecosystems, where water facilitates the circulation of nutrients and essential gases, supporting a diversity of microorganisms that contribute to biogeochemical cycles. Microorganisms play an essential role in the primary production and recycling of nutrients, influenced by factors such as temperature, pH and dissolved oxygen, which determine their adaptation and dynamics. The bacterial populations in these ecosystems are highly dynamic, responding quickly to seasonal changes and the availability of essential resources such as carbon, nitrogen, and phosphorus (Madigan et al., 2014). Microorganisms in aquatic environments play a crucial role in biogeochemical cycles, actively participating in processes such as photosynthesis, nitrogen fixation, organic matter breakdown, and nutrient recycling. (Lazăr et al., 2016).

II.2. Ecological (physico-chemical) factors influencing bacterioplankton

II.2.1 Lumina

The intensity and duration of light directly influences their productivity, while UV light can negatively affect bacteria through DNA damage. In deep areas, where light penetrates less, heterotrophic bacteria predominate, adapted to dark conditions (Pommier et al., 2007).

II.2.2 Temperature

Water temperature influences the metabolic rate and breakdown activity of planktonic bacteria, with extreme temperatures that can inhibit their activity and alter the structure of bacterial communities, from psychrophilic to mesophilic (Kirchman et al., 2008).

II.2.3 Turbidity

Turbidity, influenced by suspended sestoon, affects aquatic microorganisms by modulating sedimentation, and in oligotrophic environments such as lakes and rivers, the amount of seston is generally low (Rügner, 2013). Turbidity influences the penetration of light into the water, affecting the depth of photosynthesis for microorganisms and, through suspended organic particles, provides support for the adhesion of microorganisms, facilitating the movement and degradation of organic matter (Rusănescu, 2011; Ardelean, 2012).

II.2.4 Dissolved gases and pH value

Dissolved gases, such as CO2, O2 and N2, play a crucial role in aquatic ecosystems, supporting essential processes such as photosynthesis, respiration and biochemical cycles (David Sigee, 2005). CO2 comes from the atmosphere and the respiration of organisms, being indispensable for photosynthesis, respiration and aerobic bacteria.

pH is another crucial physicochemical factor that affects bacterioplankton. Most aquatic bacteria prefer a pH-neutral or slightly alkaline environment. pH changes can directly affect the structure of bacterial cell membranes and enzymatic functions, thus influencing the growth and survival of bacteria (Rappé and Giovannoni, 2003). In acidic aquatic environments, certain acidophilic bacterial species may become dominant, while in alkaline environments, alkalophilic species are better adapted (Zwart et al., 2002).

II.2.5 Ionic composition

Salinity and ionic composition differentiate freshwater and marine aquatic environments, determining the types of microorganisms that can populate them, although few species can survive in both (Zarnea et al., 1994; Enache et al., 2004). Oceanic waters have an average salinity of 3.5%, and extreme values, as in the Dead Sea, can reach 340 g/l of salts (Zarnea et al., 1994). Salinity variability influences the activity and diversity of bacteria, with halophilic bacteria preferring high salinity, while freshwater bacteria prefer low salinity; rapid changes can cause osmotic stress (Enache et al., 2007; Logares et al., 2009) and affects bacterial interactions, such as competition and symbiosis (Mou et al., 2008).

II.2.6 Currents and flow velocity

The currents in water basins significantly influence the microbiota, facilitating the transport of nutrients, the uniform distribution of microorganisms and the oxygenation of the deep layers, essential for ecological development. They occur in both flowing and standing waters, being generated by factors such as gravity and differences in temperature and level (Mihaescu, 2000; Zarnea, 1994).

II.3 Microorganisms in rivers

The microbiota of flowing waters includes a variety of bacteria, fungi, protozoa and viruses, essential for ecological balance through nutrient cycling, the decomposition of organic matter and the elimination of pollutants. Factors such as temperature, pH, and oxygen influence their diversity, and anthropogenic activities can disrupt this balance (Ruiz-González et al., 2015; Hossain et al., 2021). Genera of bacteria such as *Pseudomonas*, *Bacillus*, *Escherichia*, *Microcystis*, *Clostridium*, *Acinetobacter* and *Vibrio* play specific roles in the processes of decomposition, bioremediation and maintenance of water quality. Monitoring these bacterial communities is crucial for protecting public health and sustainable water management (Pepper & Gerba, 2015; Towner, 2009).

II.4 The role of microorganisms in the functioning of ecosystems

Microorganisms, essential for ecological balance, support the vital processes of ecosystems through the nutrient cycle and the decomposition of organic matter (Zarnea, 1994; Lazăr et al., 2016). Flowing waters exhibit dynamic microbial diversity, influenced by physicochemical factors, the river basin, and anthropogenic impacts, such as industrial and agricultural discharges (Krâuse et al., 2022; Sinton et al., 1993). The growth of microorganisms depends on the nutrients in the water, and under conditions of organic pollution, microbial populations can accelerate ecological processes, affecting the biogeochemical balance (Lazăr et al., 2017). Metabolically adaptable and versatile bacteria use various substrates, contributing to nutrient recycling and maintaining ecosystem stability (Coyte et al., 2015; Omelon, 2016).

II.4.1 Biogeochemical Carbon Circuit (C)

Carbon, an essential element of organic and inorganic matter, is fixed from the atmosphere by autotrophic microorganisms through photosynthesis, transforming into organic compounds that support biomass and, through respiration and decomposition, is released into the atmosphere, ensuring the balance of the carbon cycle between the biosphere, atmosphere, hydrosphere and lithosphere (Zarnea, 1984; Ardelean, 2011; Lazăr et al., 2017).

II.4.2. Biogeochemical circuit of nitrogen (N)

Nitrogen, essential for the synthesis of proteins and nucleic acids, is transformed from its atmospheric form (N_2) into forms accessible to organisms through fixation processes carried out by symbiotic microorganisms, such as *Rhizobium*, followed by ammonification, nitrification and denitrification, which maintain the nitrogen balance in soil and aquatic ecosystems (Ardelean, 2012; McCabe, 2011; Krâuse et al., 2022;).

II.4.3. Biogeochemical circuit of sulphur (S)

Sulphur, essential for life, circulates in nature through microbial processes of mineralisation, oxidation and reduction, transforming between organic and inorganic forms;

bacteria break down organic compounds, oxidize sulfur to sulfates, and reduce sulfates to hydrogen sulfide, maintaining ecological balance and supporting nutrient recycling (Azam et al., 1983; Lazăr et al., 2017).

II.5. Extracellular microbial enzymes

Bacterial extracellular enzymes, regulated by environmental conditions, play an essential role in the decomposition of organic matter, mineralization of nutrients and detoxification of pollutants in aquatic ecosystems, and their activity is used as an indicator of water quality and the impact of pollution (Ruginescu et al., 2020; Cojocaru et al., 2007).

III. MICROBIOLOGICAL ASPECTS OF POLLUTION OF AQUATIC ECOSYSTEMS

Water quality monitoring, based on physical, chemical, biological and microbiological assessments, provides essential data for managing aquatic resources and assessing the impact of human activities (Fey et al., 2004; Strâub and Chandler, 2003). Sources of pollution range from agricultural runoff with pesticides and fertilizers to industrial and urban discharges, each contributing to the accumulation of heavy metals, organic pollutants, and microplastics (Liu et al., 2024). These processes are also influenced by climate change, which redistributes pollutants through flooding and increased rainfall (Thanigaivel et al., 2023). Overall, the assessment and classification of water pollution types are essential for maintaining water quality and protecting public health (Enache et al., 2017).

PURPOSE AND OBJECTIVES OF THE THESIS

The purpose of this doctoral thesis was to evaluate the degree of pollution of the Pasărea River by analyzing the spatio-temporal variability of the physicochemical characteristics, the chemical and microbiological composition monitored in two sectors of this river, namely the Tunari Dam and the Dimieni Bridge, during four seasons.

The main objectives of this doctoral thesis were:

1. In *situ determination* of the physicochemical parameters of the water samples collected from the Tunari Dam and the Dimieni Bridge during four consecutive seasons
2. Identification of seasonal variation in the chemical composition of water samples at the two sampling points by XRF (X-ray fluorescence spectrometer) analysis
3. Determination of the microbiological profile of the water samples collected at the Tunari Dam and the Dimieni Bridge over the four seasons, of the main groups of microorganisms involved in the circuits of carbon (heterotrophic microorganisms), sulfur (sulfate-reducing bacteria), nitrogen (ammoniating bacteria, nitrite-bacteria, nitrate-bacteria, denitrifying bacteria) and pollution indicator groups (total coliform bacteria, fecal coliform bacteria, fecal streptococci)
4. Isolation and taxonomic identification based on the sequence of the 16S rRNA gene of some bacterial strains from the Pasărea River
5. Functional characterization of isolated bacterial strains in order to evaluate their ability to synthesize enzymes with macromolecule degradation potential from the complex chemical composition of the polluted aquatic ecosystem
6. Evaluation of the degree of chemical and microbiological pollution of the Pasărea River

IV. MATERIALS AND METHODS

IV.1 Study areas and sampling

The analyzed water samples were taken from the Pasărea River, in the area of Tunari locality, Ilfov County, from two sampling points, namely the Dimieni Bridge (44055′24.9"N, 27014′34.4"E) and the Tunari Dam (44054′73.7"N, 26016′23.0"E).

The samples were carried out seasonally, in the summer (July), autumn (November), winter (February) in 2020 and spring (April) in 2021. Water samples were collected in 500 ml sterile clear glass containers and stored at a temperature of 4°C during transport to the laboratory for analysis.

IV.2. Determination of the physico-chemical parameters of water

The depth and transparency of the water at the sampling points were determined using the Secchi disc (Hach, USA).

The physicochemical parameters of the water were measured *in situ,* at each sampling point on the Pasărea River, using the multiparameter HI 9828 (Hanna Instruments) for the determination of turbidity values (NTU), oxidation-reducing potential - ORP (mV), oxygen saturation-OD (%), water salinity (PSU), suspended solids/total dissolved solids - TDS (ppm), conductivity (µS/cm), total dissolved oxygen (mg O_2/L), water temperature (ºC) and pH value.

The chemical composition of the water samples was determined based on a semi-quantitative method by XRF (X-ray fluorescence spectrometry) analysis using the ZSX100e Supermini XRF spectrometer (Rigaku Corporation, Japan) (Neagu et al., 2014; 2021).

IV.3. Statistical analyses

The statistical processing of the physicochemical data was carried out with the help of the student t-test. The graphical representation was made using the GraphPad Prism5 (https://www.graphpad.com/) program.

IV.4 Highlighting microorganisms involved in biogeochemical cycles

The determination of bacteria involved in the biogeochemical circuits of carbon, sulfur and nitrogen such as (aerobic heterotrophic microorganisms, sulfate-reducing bacteria, ammonifier bacteria, nitrifying bacteria, nitrifying bacteria and denitrifying bacteria) as well as pollution indicator bacteria (fecal coliform bacteria, total coliforms and fecal streptococci) was achieved by cultivation on specific selective media, solid or liquid (nutrient broth medium solid, Postgate, WanNiell, Pochon, nitrate agar medium, denitrifying medium, Levine agarized, sodium azide medium) from samples and their decimal dilutions. The substances and reagents used came from the companies Sigma-Aldrich (Germany) and Merck (Germany). (APHA standard, 1998; Lucaci et al., 2019; Neagu et al., 2021).

IV. 6. Gram staining

Gram staining (Helebian et al., 1981; Lucaci et al., 2019) of the obtained strains was performed on the resulting cultures using the kit from Sigma-Aldrich and Merck, Germany. To confirm the results of the Gram staining, the Kovacki test was additionally used, which consists of applying a solution with 3% KOH. (Lucaci et al., 2019; Azhar et al., 2014).

IV.7. Determination of enzymatic activities

IV.7.1. Catalase and oxidase

The production of catalase was evidenced using the *Catalase Assay Kit* from Merck, Germany, based on the reaction of a bacterial culture in the liquid medium to the 3% oxygen peroxide (H2O2) solution. (Steel, 1961; Azhar et al., 2014).

The oxidase test is based on the oxidation of phenylenediamine to indophenol in the presence of cytochrome - oxidase from bacterial culture, in liquid media, using the *Oxidase Bactident* kit from Merck, Germany (Steel, 1961; Shields et al., 2010).

IV.7.2. Hydrolactic activities

Qualitative and semi-quantitative determinations of hydrolactic enzymatic activities (amylases, proteases, cellulases, esterases and gelatinases) for isolated bacterial strains were performed by the method of culture in plate or liquid medium (to highlight the presence of gelatinase) (APHA standard, 1998; Lazarus et al., 2014;) using specific culture media (Table IV.2) containing the subtract corresponding to the type of hydrolase investigated, subsequently incubated at 28º C for 48 hours (APHA Standard, 1998; Lazăr et al., 2014;).

The results were expressed qualitatively (presence of activity) based on the occurrence of a substrate lysis halo in the solid culture medium, and semi-quantitatively as enzyme activity levels (LEA) based on the diameter of the hydrolysis zone (in millimeters), from which the diameter of the culture spot was subtracted. (Enache et al., 1999; Ruginescu et al., 2020).

IV.8. Extracția ADN genomic, amplificarea PCR și secvențierea genei 16S ARNr

Un număr de 20 de tulpini bacteriene au fost selectate în vederea identificării la nivel de gen/specie prin metode moleculare. Acestea au implicat: extracția ADN-ului genomic, amplificarea in vitro prin tehnica PCR a genei ce codifică pentru ARNr 16S, secvențializarea ampliconilor, secvențele ADN au fost analizate raportat la cele din bazele de date NCBI folosind algoritmul BLASTN (BLAST: Basic Local Alignment Search Tool).

Arborii filogenetici au fost construiți utilizând programul MEGA X, aplicat asupra alinierilor multiple obținute utilizând algoritmul CLUSTALW (https: // www .megasoftware .net).

IV.10 Assessment of water pollution

The evaluation of the degree of physico-chemical pollution was carried out based on the pH values, the concentration of dissolved oxygen (DO) and the percentage oxygen content (DO%) of the water, in accordance with the provisions of Order 161/2006 issued by the Ministry of Environment and Water Management.

The degree of **organic pollution** with heterotrophic microorganisms, ammoniatory, sulfate-reducing bacteria, denitrifying bacteria and **fecal pollution** with total coliforms and fecal coliforms, determined in this study, was evaluated according to the microbial concentration of different types of microorganisms, falling into 5 pollution classes according to the increasing ranges of their water content (Kohl, 1975; Lazăr et al., 2015; Ardelean et al., 2011; EU-Expert 2000/60/EC).

V. DETERMINATION OF THE SEASONAL VARIATION OF THE PHYSICOCHEMICAL PARAMETERS AND THE CHEMICAL COMPOSITION OF THE PĂSĂREA RIVER

V.1. Physicochemical parameters of the Pasărea River

The Pasărea River is a dynamic ecosystem characterized by physicochemical parameters that fluctuate seasonally, depending on atmospheric conditions, such as precipitation and solar radiation intensity, as well as anthropogenic impact (Ojovan, Podosu et al., 2021). These parameters were monitored over the four seasons (in two sampling points, respectively the Dimieni Bridge and the Tunari Dam).

Bridge Dimieni

The seasonal variation of the physicochemical parameters of the water, recorded at the Dimieni Bridge sampling point of the Pasărea River, in the four seasons (Table V.1), indicated an alternation of their values both dependent and independent of the anthropogenic impact.

Table V.1. The seasonal variation of the physicochemical parameters of the water recorded at the Dimieni Bridge sampling point of the Pasărea river in the four seasons (average values).

Phyzico-chemical parameters	**DIMIENI BRIDGE**				**Annual average value (± standard deviation)**
Season (month)	**2020**		**2021**		
	Summer (July)	**Autumn (November)**	**Winter (February)**	**Spring (April)**	
Temperature (°C)	28,6	11	6,7	10,4	14.2 ± 9.8
pH	8,4	8,2	6,8	7,4	7.7 ± 0.7
Solide în suspensie/ TDS (ppm)	345	510	468	396	429,75 ± 73,5
Conductivity (µS/cm)	691	1023	936	792	860,5 ± 147,7
ORP (mV)	52,8	-293	43,4	-70	-66.7 ± 160
DO (mg O2/L)	4,7	0	0	13,5	4.54 ± 6.3
OD (%)	62,2	0	0	83,5	36.42 ± 42.9
Turbidity (NTU)	16,7	36,6	29,3	22	26.1 ± 8.6
Transparency (cm)	30	30	40	40	35±5 AM
Salinity (PSU)	0,3	0,51	0,4	0,4	0.4 ± 0

In the area of the Dimieni bridge, seasonal measurements of water quality revealed notable variations, pH values ranged from 6.82 in winter to 8.44 in summer, indicating a slightly alkaline environment. The concentrations of dissolved solids were higher than in other rivers in Romania and showed an average of 429.7 ppm, reflecting the contribution of exogenous organic matter. The electrical conductivity of the water recorded high values, suggesting a significant degree of mineralization, especially in autumn (1023 µS/cm). Dissolved oxygen values were 0 mg/L in autumn and winter, indicating anoxia and organic pollution, and transparency decreased in warm seasons due to sediment and algae loading. The salinity remained low, specific to fresh waters, similar to other rivers in Romania.

Tunari Dam

In the case of water samples collected from the Tunari dam, the values of the physicochemical parameters also indicated seasonal variations (Table V.2).

Table V.2. Seasonal variation (average values) of the physicochemical parameters recorded at the Tunari Dam sampling point of the Pasărea River in the four seasons

Phyzico-chemical parameters	**TUNARI DAM**				**Average annual value (± standard deviation)**
Season (month)	**2020**		**2021**		
	Summer (July)	**Autumn (November)**	**Winter (February)**	**Spring (April)**	
Temperature (°C)	28,6	11,6	3,8	12,5	14.1 ± 10.4
pH	8,2	9,1	9,5	8,9	8.9 ± 0.5
Solide în suspensie/ TDS (ppm)	354	385	454	445	409.5 ± 48
Conductivity (µS/cm)	707	771	909	890	819.2 ± 96.5
ORP (mV)	12,8	-92	-66,7	35	-27.7 ± 61
DO (mg O2/L)	1,8	2,8	1,2	8,6	3.6 ± 3.3
OD (%)	23	25	9,2	70,6	31.9 ± 26.7
Turbidity (NTU)	6,8	21,5	16	25,5	17.4 ± 8.0
Transparency	90	30	40	40	50±23.45
Salinity (PSU)	0,3	0,3	0,4	0,4	0.4 ± 0

At the Tunari Dam, seasonal measurements of water quality indicated significant variations, the pH was slightly alkaline, slightly exceeding the limits allowed in cold seasons, with an average of 8.9.

The concentration of dissolved solids was consistently high (409.5 ppm annual average), and the electrical conductivity, indicating mineralization, ranged from 707 µS/cm in summer to 909 µS/cm in winter.

Dissolved oxygen was low, between 1.2 mg/l (winter) and 8.6 mg/l (spring), and oxygen saturation showed low values in most seasons, indicating oxygenation difficulties.

Turbidity values ranged from 6.8 NTU in summer to 25.5 NTU in spring, and high transparency in the summer season suggested relatively clear water.

The transparency of the water at the Tunari Dam varies seasonally, decreasing to 30 cm in autumn due to rainfall that increases suspended matter, and reaching 40 cm in winter and spring, when biological activity decreases and sediments stabilize. The annual average transparency is 50 cm, but with a large deviation, reflecting the influence of seasonal environmental factors.

The salinity of the water remained relatively constant between 0.3 and 0.5 PSU throughout the year, being slightly higher than the values of the Dâmbovița River, but below those of the Diyala River in Iraq (Ioniță et al., 2023; Al-Samawi et al, 2016).

V 1.2. Spatio-temporal variations of the physico-chemical parameters of the Pasărea River

The comparative representations of the physicochemical parameters of the water in the Pasărea river have highlighted significant spatio-temporal variations between the ecosystems specific to each sector, as well as seasonal. In particular, these differences concerned turbidity, conductivity and the concentration of dissolved oxygen in the water. No significant differences were observed between sampling points for salinity, temperature and resistivity.

V.2. Chemical composition of water samples from the Pasărea River

XRF analyses of water samples from the two sectors of the Pasărea River, the Dimieni Bridge and the Tunari Dam, revealed a variable seasonal composition of metallic and non-metallic ions, including some elements that are part of the lanthanide groups (Table V.4 and Table V.5).

The chemical composition of the Pasărea River showed seasonal variations and differences between the sampling sectors, with the presence of rare metal oxides such as barium, cerium, germanium and terbium, suggesting anthropogenic pollution and an exogenous contribution of pollutants. The highest concentration was recorded for BaO (73%-80% mass) in winter at the Tunari Dam, and the oxides TiO_2 and CeO_2 had high percentages in summer and spring. The toxic elements Sm_2O_3, Gd_2O_3 and TiO_2 were present in high concentrations, with ecological and health risks. At the Dimieni Bridge, SiO_2 was consistently high, and Sm and Lu oxides showed notable seasonal variations, indicating the need for continuous monitoring to protect this ecosystem.

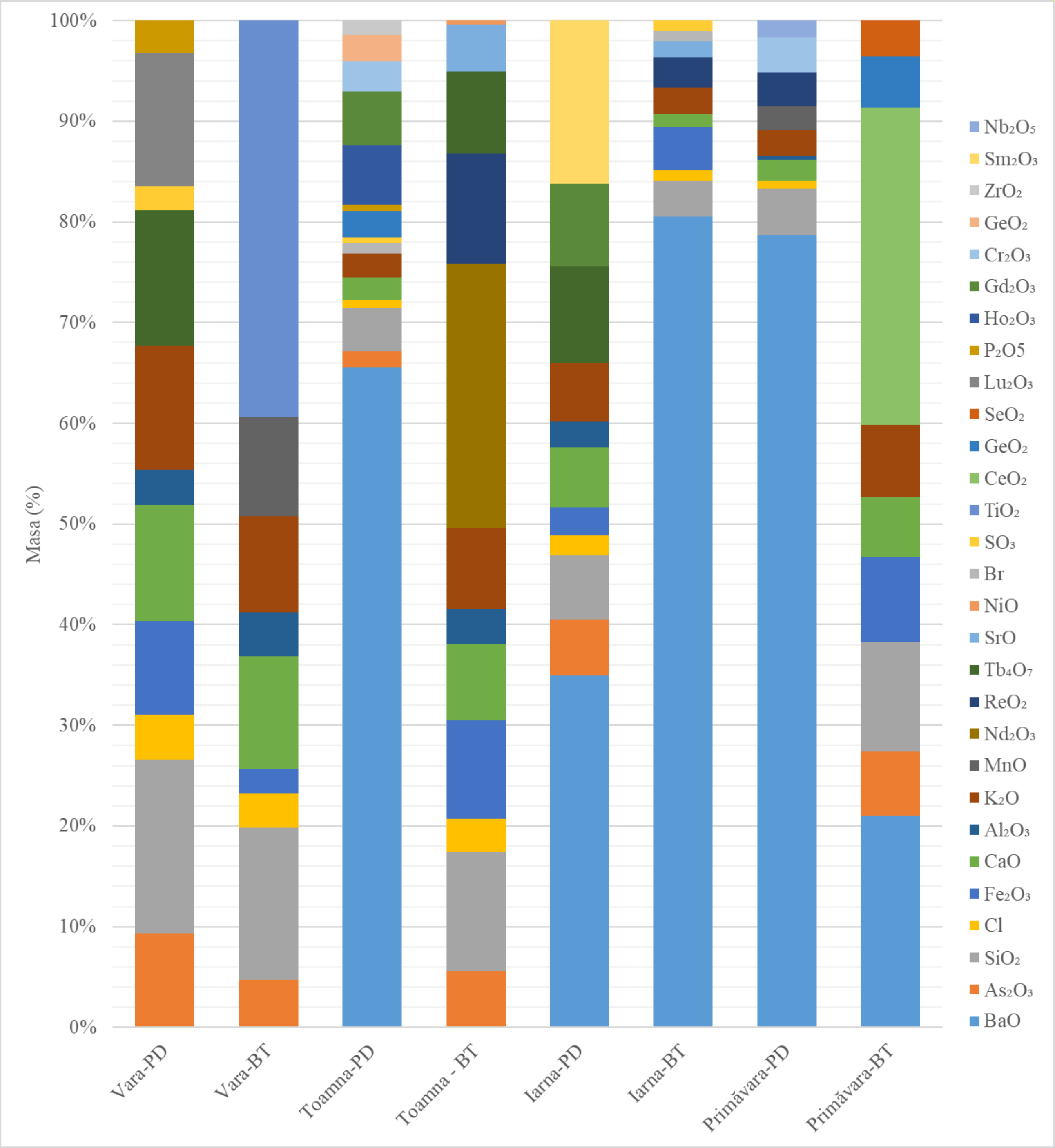

Figure V.21. Spatio-temporal distribution of chemical compounds in the water of the Pasărea River.

VI. SPATIO-TEMPORAL CHARACTERIZATION OF MICROBIAL COMMUNITIES IN THE PASĂREA RIVER

VI.1 Microorganisms involved in the biogeochemical cycles of carbon, sulfur and nitrogen

VI.1.1. Heterotrophic bacteria (carbon cycle)

In accordance with the new classification system proposed by the European Union "Bacteriological indicators for surface water quality", the number of heterotrophic bacterial colonies correlated with five quality classes, indicates organic pollution (Lazăr et al., 2015).

The abundance and morphology of microbial colonies corresponding to the summer, autumn and spring seasons, at the Dimieni Bridge and the winter season, at the Tunari Dam, illustrate variations of the microbial communities in the Pasărea River depending on the season and sampling point (Figure VI.1).

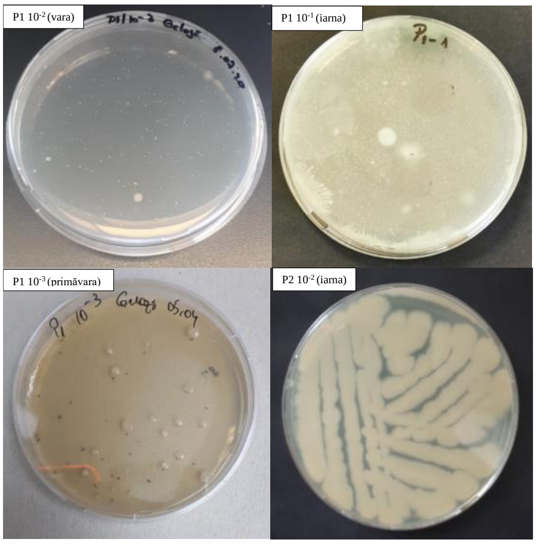

Figure VI.1. Highlighting heterotrophic microorganisms isolated from water samples, from sampling points Dimieni Bridge (P1) Tunari Dam (P2) in different seasons.

In the case of the Dimieni Bridge, the density of heterotrophic microorganisms varied between 3.2 x 105 (summer) and 4.5 x 104 (spring) (Table VI.1). For the Tunari Dam sample, the highest number of CFU/ml was highlighted in the sample taken in the winter season, 1.7 x 105 and registered a decrease in the spring season, respectively 1.4x102 CFU/ml (Table VI.1).

Table VI.1. Seasonal density of aerobic heterotrophic microorganisms from two sampling points of the Pasărea River. Dimieni Bridge (PD); Tunari Dam (BT).

Season	Sampling sector	Density microorganism heterotrophic Mean value (± standard deviation) (CFU/ml)
Summer (July 2020)	PD	(3,2 ± 0,22) x 105
	BT	5,0 ± 0,75 x 104
Autumn (November 2020)	PD	3,8 ± 0,40 x 105
	BT	1,2± 1,0 x 103
Winter (February 2021)	PD	9,9 ± 0,50 x 104
	BT	1,7 ± 0,30 x 105
Spring (April 2021)	PD	4,5± 0,30 x 104
	BT	1,4 ±0,10 x 102

VI.1.2. Bacteria involved in the biogeochemical cycle of sulfur

Sulphate-reducing bacteria

In this study on the Pasărea River, the presence of sulfate-reducing bacteria was evidenced by iron precipitation in different seasons, at both sampling points (Figure VI.4).

Figure VI.4. Highlighting sulfate-reducing bacteria in water samples collected at Dimieni Bridge (PD) and Tunari Dam (BT).

Table VI.2. Density of sulfate-reducing bacteria from the sampling points of the Pasărea River, Dimieni Bridge (PD) and Tunari Dam (BT) in the four seasons.

Season	Sampling point	Sulfate-reducing bacteria density (cells/ml)
Summer (July 2020)	PD	22,5 x 105
	BT	5,5 x 104
Autumn (November 2020)	PD	4,75 x 105
	BT	4,75 x 102
Winter (February 2021)	PD	7,5 x 106
	BT	5,5 x 107
Spring (April 2021)	PD	5,75 x 107
	BT	4,75 x 102

This group of microorganisms was highlighted during the four seasons in both sectors of the Pasărea River (Table VI.2). The high content of sulfate-reducing bacteria, recorded in the water samples from the Dimieni Bridge area, with a maximum (5.75x107 cells/ml) reached in the spring period (Table VI.2, Figure VI.5) suggests a high anaerobic activity for the degradation of organic matter.

For the sampling point at the Tunari Dam, the highest number of sulfate-reducing bacteria was recorded in the winter season (5.5x107 cells/ml), with a significant decrease during the summer (5.5x104 cells/ml) and a decreased presence in spring and autumn (4.75x102 cells/ml) (Figure VI.5, Table VI.2).

VI.1.3. Bacteria involved in the biogeochemical nitrogen cycle

In order to evaluate the microbial communities, involved in the biogeochemical cycle of nitrogen, the ammonifier, nitrifying, nitrating and denitrifying bacterial groups from the two sampling points of the Pasărea River were quantified during four consecutive seasons.

. Table VI.3. Seasonal density of groups of bacteria involved in the biogeochemical cycle of nitrogen, in the sampling points Dimieni Bridge (PD) and Dam Tunari (BT) of the Pasărea River.

Season	Sampling point	Density of ammoniating bacteria (cells/ml)	Nitrite-bacteria density (cells/ml)	Nitrate-bacteria density (cells/ml)	Density of denitrifying bacteria (cells/ml)
Summer (July 2020)	PD	0	0	0	3 x 10
	BT	0	0,8	1,18	2,2 x 105
Autumn (November 2020)	PD	0	1,4	0	2,2 x 102
	BT	0	3 x 102	0	0
Winter (February 2021)	PD	2,8 x 104	1,8 x 10	0	2.2 x 104
	BT	2,8 x 104	1,8 x 10	0	2,2 x 104
Spring (April 2021)	PD	2,8 x 105	8x10	0	0
	BT	3,0 x 103	5	0	0

The relative content of **the different groups of microorganisms involved in the biogeochemical cycle of nitrogen,** present in the Pasărea River at the sampling points Tunari Dam and Dimieni Bridge highlights the dominance of denitrifying bacteria in the summer season, in both sampling points, both at the Dimieni Bridge and at the Tunari Dam, and in the following season, in autumn, they predominated only in the water sample from the Dimieni Bridge, while in the sample at the Tunari Dam nitrifying bacteria were the majority. In the winter season, in both sampling points, ammonifying bacteria predominated (~60%), the remaining ~40% being represented by denitrifying bacteria. In the spring season, ammonifier bacteria were the majority in both samples from the two sampling points, the Dimieni Bridge and the Tunari Dam.

VI.3. Spatio-temporal distribution of potentially pathogenic microorganisms in the Pasărea River

The microbial pollution of the Pasărea River was investigated by quantitatively determining the presence of total coliforms, fecal coliforms and fecal streptococci, species frequently associated with fecal-household contamination of ecosystems (Kolarevic et al., 2011), in water samples collected from the two sectors of the river. The content of these microbial groups, measured over the four seasons, in the investigated sampling points, the Dimieni Bridge and the Tunari Dam, showed both a spatial and temporal variation in the number of pathogenic microorganisms (Table VI.5).

Table VI.5. Seasonal distribution of groups of microorganisms indicating pollution in the water samples from the Dimieni Bridge (PD) and the Tunari Dam (BT).

Season	**Sampling point**	**Patogenic microorganism group**		
		Total coliform (CFU/ml)	Fecal coliform (cell/ml)	Fecal streptococci (cells/ml)
Summer (July 2020)	PD	3,03 x 102	2,25 x 101	1,4 x 101
	BT	4,80 x 101	2,00 x 100	0
Autumn (November 2020)	PD	6,20 x 103	3,00 x 100	5,0 103
	BT	0	0	0
Winter (February 2021)	PD	1,85 x 103	0	5,0 x 103
	BT	3,65 x 102	0	1,4 x 102
Spring (April 2021)	PD	1,80 x 103	2,5 x 101	9,0 x 102
	BT	0	0	0

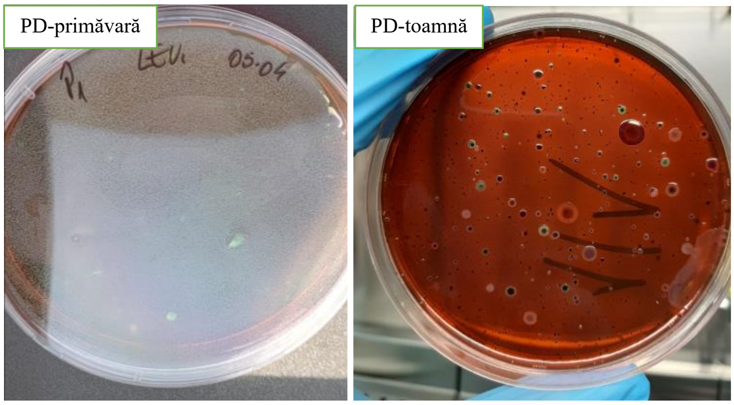

Figure VI.19. Highlighting total coliform bacteria in samples from the Dimieni Bridge (PD).

The seasonal distribution of the three categories of microorganisms, potentially pathogenic, in the Pasărea River highlighted a distinct profile of the presence of microbial pollutants in the two sampling points investigated. In this regard, a relatively important content of total coliforms and fecal streptococci was identified, accumulated in the area of the Dimieni Bridge, during the autumn and winter seasons and with the resilience of total coliforms, during the spring, while in the area of the Tunari Dam, these two groups of polluting microorganisms showed a relatively high content, only in the winter season and with substantially reduced values compared to the Dimieni Dam area.

VII. OBTAINING AND CHARACTERIZING THE BACTERIAL STRAINS FROM THE PASĂREA RIVER

VII.1. Isolation and morpho-functional characterization of microorganisms from the Pasărea River

After inoculating water samples from the Pasărea River on a solid medium at 37°C for 48 h, 11 microbial colonies were obtained from the Dimieni Bridge (P1) and 9 from the Tunari Dam (P2), with various morphologies (matte white, cream-pink, yellow, etc.) and varied structures (flat, convex, with well-defined edges), suggesting the presence of exopolysaccharide capsules.

Most strains (11) were Gram positive and catalase positive (70%), but only 40% were oxidase positive. In the summer season, all isolated bacteria were Gram positive and catalase positive, with no oxidase activity; in winter, Gram-negative bacteria with oxidase and catalase activity predominated; and in spring the isolates were divided equally, between Gram positive/negative and catalase positive/negative, all of which were oxidase negative. Seasonal variations indicate the influence of environmental conditions on bacterial composition.

VII.2. Molecular identification of bacterial strains based on the sequence of the 16S rRNA gene

The taxonomic affiliation of the strains isolated from the Pasărea River was determined based on the sequence of the 16S rRNA gene. Following the BLAST analysis (Liu et al., 2017; Sudan et al., 2018) of the sequences of the 16S rRNA gene obtained, for the 20 strains, their taxonomic identification indicated the belonging of the bacteria isolated based on the sequence identity >97% of most of them, with homologous species from the databases (Table VII.3). The taxonomy of these strains from the Pasărea River has been assigned to 3 phyla (*Bacillota, Pseudomonadota, Actinomycetota*), 3 classes (*Bacilli, Gammaproteobacteria, Actinobacteria*), 6 families (*Bacillaceae, Pseudomonadaceae, Aeromonadaceae, Enterobacteriaceae, Microbacteriaceae, Dermabacteraceae*) and 9 genera (*Bacillus, Pseudomonas, Aeromonas, Enterobacter, Exiguobacterium, Yersinia, Microbacterium, Brachybacterium* and *Lysinibacillus).*

Table VII.3. Molecular Identification of Bacterial Strains in the River Pasărea Based on 16S rRNA Gene Sequence.

No.	Isolate strain code	Collection season	Homologous species [access number]	Identity (%)	Origin of the homologous species
1	P1-2(D3)	Summer	*Bacillus sp. [KJ812450]*	99,7	Aquatic environment-sediment Pacific Ocean
2	P1-5(D3)	Winter	*Pseudomonas sp. [GCA_002234375]*	99,67	Sediment-Ganges River
3	P1a-5(D3)	Winter	*Pseudomonas sp. [1215092]*	99,7	Aquatic environment-drinking water
4	P2-5(D4)	Winter	*Bacillus sp. [536229]*	93,31	Aquatic environment
5	P2-2(D4)	Winter	*Aeromonas popoffii [AJ224308]*	99,45	Aquatic environment (drinking water)
6	P1-4N(D1)	Winter	*Exiguobacterium sp.[AY818050]*	94,52	Sediment N-E Pacific
7	P1 (D1)	Spring	*Enterobacter kobei [AJ508301.])*	98,22	Blood of a person with diabetes
8	P2-7(D4)	Spring	*Aeromonas salmonicida [LSGW00000000]*	98,44	Acvatic-somon Atlantic
9	P1-1(D3)	Spring	*Pseudomonas aeruginosa [LN681564]*	98,73	Ground
10	P1-1(D4)	Spring	*Aeromonas hydrophila [CP000462]*	97,86	Canned milk with a fishy smell
11	P2-8(D4	Spring	*Yersinia intermedia strain [EF179123]*	98,07	Human urine
12	P2-3(D4)	Spring	*Bacillus sp. [536229]*	97,31	Aquatic environment
13	P2 -1a(D4)	Spring	*Aeromonas popoffii [AJ224308]*	97,28	Aquatic environment (drinking water)
14	P1-5n (D1)	Spring	*Microbacterium marytipicum [AM181506]*	98,37	Sediment/sea sludge
15	P1-2(D4)	Spring	*Aeromonas australiensis [HE611955]*	97,04	Irrigation system
16	P1-4 (D3)	Spring	*Brachybacterium muris/zhongshanense [JX68013])*	97,93	Aquatic-aquaculture water
17	P2-4 (D4)	Spring	*Bacillus sp. [1178541]*	99,65	Aquatic environment
18	P21b(D4)	Spring	*Lysinibacillus macroides [AJ628749]*	97,73	Cow droppings
19	P1-3(D4)	Summer	*Pseudomonas psychrophila [AB041885]*	95,41	Food Cold Room
20	P2-6(D4)	Summer	*Bacillus sp. [AJ831843]*	97,04	Air

VII.3 Phylogenetic analysis of the strains isolated from the Pasărea river

The phylogenetic tree built on the basis of the 16S rDNA sequences grouped the investigated strains into two major groups (*clusters)* related to the corresponding gene, from *Halorubrum kokurii strain BG-1* used as *an outgroup* (Figure VII.11). One of these groups, containing 11 stems from the Pasărea River, is represented by members of the *families Enterobacteriaceae* and *Pseudomonadaceae* with stems belonging to the genera *Aeromonas*, *Enterobacter*, *Yersinia* and *Pseudomonas* (Figure VII.11).

The second *cluster* consists of members of the families *Bacillaceae*, *Dermbacteraceae* and *Microbacteriaceae*, grouped with species belonging to the *genera Microbacterium* (5N), *Brachybacterium* (P1-4), *Exiguobacterium* (4N), *Lysinibacillus* (P2-1b) and *Bacillus* (P2-3, P2-4, P2-5, P2-6) (Figure VII.11).

VII.4. Production of enzymes by bacterial isolates from the Pasărea River

The bacterial strains isolated from the Pasărea river were tested for the production of extracellular hydrolytic enzymes, presenting a variable profile in terms of type and level of enzymatic activity. From the category of hydrolases, esterases predominated, most of the strains hydrolyzing only one type of substrate.

Four strains (P2-8/D4, P21-b/D4, P1-3/D4 and P2-6/D4) did not record any extracellular hydrolytic activity.

Aeromonas Isolate *sp.* P1-2 (D4) was able to hydrolyze all five mediums supplemented with Tween 80, gelatin, CMC, amylase and casein, indicating a high hydrolytic potential.

The strains that showed the most enzymatic activities, with the highest hydrolysis diameter values, are *Aeromonas sp.* P1-2 (D4), *Pseudomonas sp.*, P1-1 (D3) and *Aeromonas sp*. P1-1 (D4). These strains were distinguished by their significant esterase and carboxymethylcellulase activities, showing a high application potential for obtaining new bacterial hydrolytic enzymes.

VIII. IMPACT OF POLLUTION ON THE MICROBIAL DIVERSITY OF THE PĂSĂREA RIVER

The assessment of the degree of microbial pollution of the Pasărea River was carried out according to the data obtained on the spatio-temporal variation of the microbial content of the Pasărea River, based on the classification in the water quality classes, established at European level (EU-Expert 2000/60/EC).

Table VIII.2. Classification of the water samples collected from the Dimieni Bridge and the Tunari Dam of the Pasărea River in the water quality classes for organic pollution.

Sampling sector	Summer (2020)	Autumn (2020)	Winter (2021)	Spring (2021)
Bridge Dimieni	IV	IV	III	III
Tunari Dam	III	I	IV	I

Table VIII.3. Water quality classes at the Dimieni Bridge sampling point of the Pasărea River for the faecal pollution indicator parameters.

Parameter - fecal pollution	Summer (2020)	Autumn (2020)	Winter (2021)	Spring (2021)
Total coliforms/100ml	III	IV	IV	IV
Fecal coliforms/100ml	III	II	-	III
Fecal streptococci/100ml	IV	V	V	V

Table VIII.4. Water quality classes at the Tunari Dam sampling point of the Pasărea River for the indicator parameters of fecal pollution.

Parameter - fecal pollution	Summer (2020)	Autumn (2020)	Winter (2021)	Spring (2021)
Total coliforms/100ml	II	-	III	-
Fecal coliforms/100ml	II	I/0	I/0	I/0
Fecal streptococci/100ml	-	-	V	-

The study on the Pasărea River revealed variable organic and fecal contamination, with the Dimieni Bridge sector constantly classified in pollution classes III-IV, and the Tunari Dam showing fluctuations, including a critical level of pollution in the winter season (class IV). Microbiological analyses showed an increased degree of contamination with microorganisms indicative of organic pollution, especially in winter and spring, with increased pollution at the Dimieni Bridge, where total coliforms and fecal streptococci reached high levels. At the Tunari Dam, fecal pollution was lower, but in the winter of 2021 critical levels of pollution with fecal streptococci and total coliforms were recorded.

The impact of the chemical composition on the river microbiome was observed by the analysis of metal oxides such as As_2O_3, which inhibited bacteria in cold seasons, and BaO, where an adaptation of bacterial strains belonging to the genera *Pseudomonas* and *Bacillus was noted*. Also, the presence of cerium oxide suggests the influence of industrial activities, and the isolated strains indicate the development of mechanisms of resistance to toxic pollutants, including rare metals.

CONCLUSIONS

The studies carried out within this doctoral thesis aimed at the physico-chemical, chemical and microbiological characterization of the Păsărea River in two areas (Dimieni Bridge and Tunari Dam) in order to determine the level of pollution of the river.

The originality of the study lies in determining the seasonal variations of the physicochemical and chemical profile of the investigated water sector, caused by the anthropogenic impact, which is reflected in the dynamics of microbial populations and in the spectrum of their enzymatic activities.

The *in situ* measurement of **the physicochemical parameters** of the water samples revealed spatio-temporal variations over the four seasons in the two sampling points, reflecting both natural influences and an anthropogenic pollution of an industrial nature on the water quality. At the same time, the high concentrations of some metal oxides and the presence of potentially toxic elements (PTEs) suggest significant pollution of the river that affects water quality in both water sectors investigated.

In particular, the increase in turbidity and suspended solids concentration (TDS) observed in the autumn and winter seasons at the Dimieni Bridge, as well as variations in water transparency at the Tunari Dam in the summer season, reflect seasonal changes in water composition influenced by climatic and anthropogenic factors. At the same time, the differences recorded between the two sectors of the river, for simultaneous measurements, suggest punctual episodes of local anthropogenic pollution.

Significant seasonal variations in pH, oxidation-reducing potential (ORP), turbidity and implicitly water transparency together with electrical conductivity, indicated drastic changes in the habitat of aquatic organisms during the four seasons, with possible contribution due to chemical pollution. Also, major variations in dissolved oxygen (DO) concentration and percentage oxygen concentration (DO) were observed, worrying decreases (0mg/L and 0%/L respectively) in the autumn and winter seasons at Dimieni Bridge.

Notable variations in these physicochemical parameters affect water quality and reflect seasonal climate and hydrological changes.

For the salinity parameter, the values did not show major spatio-temporal variations over the four seasons, remaining relatively constant, also the variations of water temperature were in accordance with the seasonal influence, no major differences being identified between the two sampling points.

The chemical analysis carried out by XRF revealed significant variations in **the chemical composition** of the water samples depending on the season and the sampling point.

A total number of 22 chemical elements/compounds were identified at the Dimieni Bridge and 20 chemical elements/compounds in the form of oxides, at the Tunari Dam in the four sampling seasons.

The spatial distribution of the identified oxides varied seasonally, with a punctual presence and, in some cases, in important quantities (titanium oxide TiO2 with 39% mass in the summer season sample at the Tunari Dam, cerium oxide CeO2 30% mass in the spring season, in the same water sector, together with neodymium oxide Nd2O3 with 26.18% mass identified in the autumn season at the Tunari Dam), over 10% mass (samarium oxide Sm_2O_3, lutetium oxide Lu_2O_3,) over 5% mass (manganese oxide MnO, holmium oxide Ho_2O_3, germanium oxide GeO_2) so that a regional influence of water compounds has been identified.

Also, a series of chemical elements/compounds in the form of oxides were identified either in at least two seasons, or in both sectors of the Pasărea River, in important concentrations (gadolinium oxide 5.48% mass to 8.19% mass at the Dimieni Bridge, terbium oxide up to 8.12% mass at the Tunari Dam, the value being far exceeded at the Dimieni Bridge.

Very high concentrations (73%-80%) of barium oxides (BaO) were identified in both sampling points of the Pasărea River, indicating an exogenous contribution of chemical pollutants. The extreme seasonal variation of this compound in water samples collected in spring and summer, between maximum values and its absence, confirms the anthropogenic origin of this compound in the Pasarea River, suggesting major industrial pollution.

Some of the identified oxides such as titanium oxide (TiO_2), niobium oxide (NbO_2), strontium oxide (SrO), cerium oxide (CeO_2), neodymium oxide (Nd_2O_3), samarium oxide (Sm_2O_3), gadolinium oxide (Gd_2O_3), terbium oxide (Tb_4O_7), lutetium oxide (Lu_2O_3) and holmium oxide (Ho_2O_3) are considered toxic to aquatic ecosystems and human health, found on the European Commission's List of Critical Raw Materials (https :/ /rmis .jrc. ec . europa.eu/), their presence indicating a source of industrial pollution or uncontrolled discharges.

The microbiological analyses of the water samples from the Pasărea River, which targeted the groups of bacteria involved in the biogeochemical cycles of carbon, sulfur and nitrogen, highlighted variations in the composition of the aquatic microbiome depending on the season and the sampling point, as well as the presence of some groups of pathogens.

The heterotrophic microorganisms recorded the lowest number, of the order of 10^2, in the spring season, at the Tunari Dam, the maximum number of order 10^5 being recorded in the autumn season, at the Dimieni Bridge and in the winter season at the Tunari Dam, having a constant presence in both sampling points, in accordance with the temperature of the habitat.

The largest number of **sulfate-reducing bacteria**, of the order of 10^7, was present in the winter season at the Tunari Dam and in the spring at the Dimieni Bridge, their number remaining high in the rest of the seasons in both sectors of the Pasărea River, except for the Tunari Dam for which a minimum density of 10^2 was recorded in the spring and autumn seasons.

Ammonifier bacteria were absent in the summer and autumn seasons from both sampling points, reaching the highest density at the Dimieni Bridge, of order 10^5 in the spring season.

Both nitrite-bacteria and nitrate-bacteria were very poorly represented in the two investigated sectors, the latter being present in an extremely small number, of the order 10^1, only in the summer season at the Tunari Dam.

The denitrifying bacteria varied both according to the season and the sampling sector, being absent at the Tunari Dam, in the autumn and spring seasons and reaching high densities in the summer season, of order 10^5, while at the Dimieni Bridge they reached a maximum in the winter season, of order 10^4 but were absent in the spring season.

These quantitative variations in the communities of ammonifier, nitrite- and nitrate-bacteria and denitrifying bacteria recorded, suggest the achievement of an incomplete biogeochemical cycle of nitrogen in the two sectors of the Pasărea River.

The physicochemical parameters of the water samples, determined from these investigated sites, suggested the presence of polluting factors with an inhibitory effect on the specific enzymatic activities involved in the biogeochemical transformations of nitrogen.

In the water sample from the Dimieni Bridge, the microbiological analyses showed the presence **of fecal streptococci**, of order 10^3 in the cold seasons, of autumn, winter and of **total coliforms** with the maximum number, of order 10^3, in the autumn season, persisting during the four seasons and of fecal coliforms, in a lower content, of order 10^1, during the summer season, autumn and spring. For the Tunari Dam, the microbial pollution was due to the increased number of total coliforms, of order 10^3, recorded in the winter season and fecal coliforms during summer and spring (10^1) and fecal streptococci, in winter, of order 10^2.

The values obtained indicate a persistent pollution, both organic and chemical and microbiological, of the river during the four seasons in the two investigated sectors. In most seasons, the water of the Pasărea River was classified as **class IV** for organic pollution and **class V** for the group of fecal streptococci, persisting for three seasons at the Dimieni Bridge and in the winter season, at the Tunari Dam. The water in the Tunari Dam sector was not as affected by fecal-household pollution, being classified in most seasons as class I pollution.

From the water samples taken from the Pasărea River, **20 strains were isolated** and taxonomically identified by sequencing the 16S rRNA gene, belonging to 3 phyla, 3 classes, 6 families and 9 genera, with a high identity (>97%) with homologues from aquatic environments, in most cases.

Testing of isolates for the production of extracellular hydrolytic enzymes revealed a predominance of esterase-producing strains, most of which hydrolyzed a single type of substrate.

Four strains (P2-8/D4, P21-b/D4, P1-3/D4 and P2-6/D4) showed no extracellular hydrolytic activity.

Aeromonas isolate *sp.* P1-2 (D4) was able to hydrolyze all supplemented media with the tested substrates (Tween 80, gelatin, CMC, amylase and casein), demonstrating a high hydrolytic potential.

Aeromonas sp. P1-2 (D4), *Pseudomonas sp*., P1-1 (D3) and *Aeromonas sp*. P1-1 (D4) showed the most enzymatic activities with the highest hydrolysis diameter values. These strains were distinguished by their significant esterase and carboxymethylcellulase activities, indicating a high application potential for obtaining new bacterial hydrolytic enzymes.

The corroborated analysis of the data obtained indicates possible correlations between the chemical and microbiological composition of the Pasărea River. Of these, the presence of high concentrations of As_2O_3 had a potential inhibitory effect on some groups of bacteria (heterotrophic, sulfate-reducing, ammonifier and total coliform bacteria), while the very high concentrations of BaO and the high content of lanthanides in the form of oxides of Tb, Ho, Gd and Nd did not have an apparent direct impact on the microbial density of the groups of bacteria studied, suggesting polluting, punctual discharges, the effect of which can be observed through continuous monitoring.

PERSPECTIVES

The research of this doctoral thesis highlighted the pollution of the Pasărea River, both at a chemical and microbiological level. In this sense, exposure to various chemical compounds present in high concentrations, such as barium, various heavy metals and elements belonging to CRM, of microorganisms in river water, could favor the development of resistance mechanisms to these pollutants. An in-depth investigation of the structural and functional diversity of microbial communities in the Pasărea River, using both molecular methods (Illumina sequencing of the 16S rRNA gene and metagenomics of the microbiome) and cultivation methods (isolation from new microbial strains) and gene expression analysis by RT-PCR in the presence of various pollutants, can contribute to deciphering the adaptation mechanisms of aquatic microorganisms to polluted environments.

The widespread presence of different types of plastics (polyethylene, polypropylene, polyethylene terephthalate) in the aquatic environment also raises a major current issue on the adaptation of the microbiome in river water and sediments to high concentrations of these pollutants (Tim et al., 2022). The corroborated study of the degree of plastic pollution of the Pasărea River, as well as seasonal and spatial variations and the impact on the aquatic microbiome, represents an important perspective of research on the water quality of this river. In addition, *the screening* and functional characterization of the strains isolated from this river can lead to the identification of new microbial strains effective in the biodegradation of macro and microplastics.

Also, the highlighting of the extracellular hydrolytic activities of the strains P1-2, P2-1a, P2-7, P2-2 belonging to the genus *Aeromonas,* isolated, in this doctoral thesis, constitute important preliminary data for the further investigation of their application potential in various technologies. Regarding water treatment, the ability of the extracellular hydrolase mixture (Alokpa et al., 2022) to transform small amounts of CRM under environmental ecosystem conditions could be exploited for the further development of different systems for monitoring polluted areas and for the treatment and recovery of some materials (Lulea et al., 2022).

SELECTED BIBLIOGRAPHY

1. Abatenh, E., Gizaw, B., Tsegaye, Z., & Wassie, M., 2017. The role of microorganisms in bioremediation-A review. Open Journal of Environmental Biology, 2(1), 038-046, https://doi.org/10.17352/ojeb.000007.
2. Abbott, B. W., Moatar, F., Gauthier, O., Fovet, O., Antoine, V., Ragueneau, O., 2018. Trends and seasonality of river nutrients in agricultural catchments: 18 years of weekly citizen science in France. The Science of the Total Environment, 624, 845–858, DOI: 10.1016/j.scitotenv.2017.12.176.
3. Abdullah A. U., Faisal, S., Almostafa, M. M., Younis, N. S., & Yahya, G., 2023. Multifunctional *spirogyra-hyalina*-mediated barium oxide nanoparticles (BaONPs): synthesis and applications.Molecules,28(17), 6364, https://doi .org /10. 3390 / molecules28176364.
4. Achahbar, Nouha, 2020. “Physicochemical and bacteriological quality assessment of spring waters in the Tetouan region (Morocco).” https :// doi .org /10 .48421 /IMIST.PRSM/ewash-ti-v4i3.21459
5. Alexander, T. C., Gulledge, E., & Han, F., 2016. Arsenic occurrence, ecotoxicity and its potențial remediation.Journal of Bioremediation and Biodegradation,7, e174 doi:10.4172/2155-6199.1000e174.
6. Alfaro, M. A., Jarvis, S. C., & Gregory, P. J., 2004. Factors affecting potassium leaching in different soils. Soil use and management, 20(2), 182-189 https://doi.org /10.1111/j.1475-2743.2004.tb00355.x.
7. Almeida R. C., M., Botero, W.G. & de Oliveira, L.C., 2022. Natural and anthropogenic sources of potențially toxic elements to aquatic environment: a systematic literature review. Environ Sci Pollut Res 29, 51318–51338. https://doi.org /10.1007/s11356-022-20980-x.
8. Alokpa, K., Lafortune, P., Cabana, H., 2022. Application of laccase and hydrolases for trace organic contaminants removal from contaminated water, Environmental Advances, 100243, https://doi.org/10.1016/j.envadv.2022.100243.
9. Al-Samawi , A. A. A. and Al-Hussaini , S. N. H., 2016. The oxidation reduction potențial distribution along Diyala river within Baghdad city. Mesop. environ. j., Vol. 2, No.4, pp. 54-66 .
10. Alves, P.D.D.; Siqueira, F.d.F.; Facchin, S.; Horta, C.C.R.; Victória, J.M.N.; Kalapothakis, 2014. E. Survey of Microbial Enzymes in Soil, Water, and Plant Microenvironments. TOMICROJ 8, 25–31, doi: 10.2174/1874285801408010025.
11. APHA.,1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, Washington, DC.
12. Ardelean, I.I., 2012. Microbiologie generală vol. 2, Bucharest, Ro: Ars Docendi Publishing House.
13. Arnosti C., 2003, Microbial Extracellular Enzymes and their Role in Dissolved Organic Matter Cycling, Academic Press, 315-342, https://doi.org/10.1016/b978-012256371-3/50014-7.
14. Aronsson, K., & Rönner, U., 2001. Influence of pH, water activity and temperature on the inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* by pulsed electric fields.Innovative Food Science & Emerging Technologies, 2(2), 105-112, https://doi.org/10.1016/j.ifset.2023.103460.
15. Ashbolt, N. J., Grabow, W. O. K., & Snozzi, M., 2001. Indicators of microbial water quality. In L. Fewtrell & J. Bartram (Eds.), Water quality: Guidelines, standards and health, World Health Organization (pp. 289-316)

16. Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., Thingstad, F., 1983. The ecological role of water - column microbes in the sea. Marine ecology progress series, 257-263, http://dx.doi.org/10.3354/meps010257.
17. Azhar M., Uniyal V., Chauhan N., Rawat S.D., 2014. Isolation and biochemical characterization of Halophiles from Sahastradhara region, Dehradun, India. International Journal of Current Microbiology and Applied Sciences, 3, 12, 753-760
18. Azizian, M., Boano, F., Cook, P. L. M., Detwiler, R. L., Rippy, M. A., Grant, S. B., 2017. Ambient groundwater flow diminishes nitrate processing in the hyporheic zone of streams: Ambient groundwater and stream N-cycling. Water Resources Research, 53, 3941–3967, https://doi.org/10.1002/2016WR020048.
19. Bareum, Kwon., Na, Young, Ha., Joeun, Jung., Pangyi, Kim., Younglim, Kho., Kyungho, Choi., Kyunghee, Ji., 2016. Effects of Barium Chloride Exposure on Hormones and Genes of the Hypothalamic-Pituitary-Gonad Axis, and Reproduction of Zebrafish (*Danio rerio*).Bulletin of Environmental Contamination and Toxicology, 96(3):341-346. doi: 10.1007/S00128-016-1731-9
20. Batrinescu-Moteau, C., Neagu, S., Lucaci, A.I., Ruginescu, R., Maria, G., Cojoc, R., Purcărea, C., Podosu, A., Enache, M., 2022. Preliminary data concerning communities of microorganisms in a volcanic tuff endolytic habitat. Oltenia.Studii și Comunicări. Științele Naturii, 38 (1): 168 – 173.
21. Bazeera, A. Z. and M. Irfana Amrin., 2017 "Synthesis and Characterization of Barium Oxide Nanoparticles."IOSR Journal of Applied Physics01 : 69-72.
22. Benciu, F., Enciu, M., Bujor, L., Bogan, E., Puia, O.-A., & Gabor, S., 2014. Evaluation of the Physico-Chemical Water Quality Parameters of Lake Branesti, România. International Journal of Academic Research in Enviornment & Geography, 4(1), 24-36, DOI: 10.46886/IJAREG/v4-i1/2568.
23. Bolea, V., Gavrilescu, G., Mihalache, L., & Alexandru, C., 2020. Păsările–componentă vitală a ecosistemelor pădurilor urbane. Revista de Silvicultură și Cinegetică, 24(46).
24. Bowes, M. J., Neal, C., Jarvie, H. P., Smith, J. T., & Davies, H. N., 2010. Predicting phosphorus concentrations in British rivers resulting from the introduction of improved phosphorus removal from sewage effluent. Science of the Total Environment, 408(19), 4239-4250, https://doi.org/10.1016/j.scitotenv.2017.02.100.
25. Brâun, A., Spona-Friedl, M., Avramov, M., Elsner, M., Baltar, F., Reinthaler, T., & Griebler, C., 2021. Reviews and syntheses: Heterotrophic fixation of inorganic carbon–significant but invisible flux in environmental carbon cycling. Biogeosciences, 18(12), 3689-3700, https://doi.org/10.5194/bg-18-3689-2021.
26. Burescu, P., 2003. Studiu fitocenologic cuprinzând vegetaţia acvatică şi palustră din Nord–Vestul României. Complexul Muzeal de Ştiinţele Naturii Ion Borcea, Bacău, Studii şi Comunicări. Edit. Ion Borcea, Bacău, 18, 96-102.
27. Burian, S.J., Nix, S.J., Pitt, R.E., Rocky, D.S., 2000. Urban wastewater management in the United States: past, present, and future. J Urban Technol. 7, 33–62, https://doi.org/10.1080/713684134.
28. Burns, R.G.; Dick, R.P. 2002.(Eds.) Enzymes in the Environment: Activity, Ecology, and Applications; Books in soils, plants, and the environment; Marcel Dekker: New York, NY, USA,; ISBN 978-0-8247-0614-2.

29. Cabral, J. P. S., 2010. Water microbiology. Bacterial pathogens and water. International Journal of Environmental Research and Public Health, 7(10), 3657-3703, http://dx.doi.org/10.3390/ijerph7103657.
30. Carmichael, W. W., 1994. The toxins of *cyanobacteria*. Scientific American, 270(1), 78-86.
31. Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H.,1998. "Nonpoint pollution of surface waters with phosphorus and nitrogen." Ecological Applications, 8(3), 559-568, https://doi.org/10.2307/2641247.
32. Catana, D.R., Podosu A., Florescu I.L., Mihai, A.A., Enache, M., Cojoc, R., Moldoveanu, M., 2023. Quantitative Analyses of Chemical Elements in *Phragmites australis* as Bioindication of Anthropization in Urban Lakes, Sustinability, 15, 553, DOI: 10.3390/su15010553.
33. Chen, Haiyang & Teng, Yanguo & Li, Jiao & Wu, Jin & Wang, J., 2016. Source apportionment of trace metals in river sediments: A comparison of three methods. Environmental Pollution. 211. 28-37. 10.1016/j.envpol.2015.12.037.
34. Chifiriuc M.C., Mihăescu G., Lazăr V., 2011. Microbiologie și virologie medicală. Editura Universităţii din Bucureşti;
35. Chrost, J. R., Siuda, W. 2002. Ecology of Microbial Enzymes in Lake Ecosystems. In Enzymes in the environment p. 52.
36. Cirtina, D., Mihut, M.N., 2020, Study on the Assessment of the Oxygen Regime and the Nutrients Content of Some Water Streams in Gorj County, Rev. Chim., 71(2), , 315-323.
37. Civitello, D. J., Hite, J. L., & Hall, S. R., 2014. Potassium enrichment stimulates the growth and reproduction of a clone of Daphnia dentifera. Oecologia, 175, 773-780.
38. Cocoş, O., 2006. Managementul apei în municipiul Bucureşti, Sistemele hidrogrofice Bucureşti. Bucharest, Ro: Ars Docendi Publishing House, 68 – 103.
39. Cojocaru D.C., Olteanu Z., Ciornea E., Oprică L., Cojocaru S., 2007. Enzimologie generală, Editura Tehnopress, Iaşi
40. Cole, J., 1999. Aquatic Microbiology for Ecosystem Scientists: New and Recycled Paradigms in Ecological Microbiology, Ecosystems, 2, 215–225
41. Colwell, R. R., Kaper, J., & Joseph, S. W., 1981. *Vibrio cholerae, Vibrio parahaemolyticus*, and other vibrios: Occurrence and distribution in Chesapeake Bay. Science, 212(4492), 984-986.
42. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.
43. Coyte, KZ, Schluter, J, Foster, KR, 2015. The ecology of the microbiome: networks, competition, and stability, Science, 350, pp. 663–666.
44. Da Silva, M. R. F., Souza, K. S., Motteran, F., de Araújo, L. C. A., Singh, R., Bhadouria, R., & de Oliveira, M. B. M. , 2024. Exploring biodegradative efficiency: a systematic review on the main microplastic-degrading bacteria.Frontiers in Microbiology,15, 1360844, https://doi.org/10.3389/fmicb.2024.1360844.
45. David, I,. 2022. Analysis of Some Physical-Chemical Indicators in Two Sections of the Cricovul Dulce River. Annals of" Valahia" University of Târgovişte. Agriculture, 14(2), 9-13, DOI:https://doi.org/10.2478/agr-2022-0013.

46. Dinu, C., Zaharia, S.E., Pietreanu, C.V., 2019. Considerations on Aircraft On-Ground De-Icing and Sustainable Airport Development, Rev. Chim., 70(2), 560-564.
47. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). In: Official Journal of the European Union L164: 19-40.
48. Enache, M., Dumitru, L., Faghi, A.M. 1999. Occurrence of halocins in mixed archaebacteria culture, Proc. Inst. Biol., II, 151-154.
49. Enache, M., Faghi, A.M., Dumitru, L., Teodosiu, G. Zarnea, G., 2004. Halocin Hf1 a bacteriocin produced by *Haloferax sp.* GR1, Proc. Rom. Acad. Series B, 6, 27-32.
50. European Commission, 2020. Study on the EU's List of Critical Raw Materials—Final Report; European Commission: Brussels, Belgium,.
51. Fujimura R., 2012. Analysis of Early Bacterial Communities on Volcanic Deposits on the Island of Miyake (Miyake-jima), Japan: a 6-year Study at a Fixed Site doi: 10.1264/jsme2.ME11207;
52. Henriksen, S. D., & Hansen, F., 2001. *Acinetobacter*. In M. Dworkin (Ed.), The Prokaryotes, pp. 363-379.
53. Huys G, 1997. *Aeromonas popoffii sp.* nov., a mesophilic bacterium isolated from drinking water production plants and reservoirs. Int. J. Syst. Bacteriol. 47: 1165-1171. PubMed: 9336924.
54. Ibekwe, A.M., et al., 2016. Bacterial community composition and structure in an Urban River impacted by different pollutant sources, Sci Total Environ, http://dx.doi.org/10. 1016/j.s citotenv.2016.05.168.
55. Javor B. J., 1989. Hypersaline environments. Microbiology and Biogeochemistry, Springer-Verlag KG, Berlin, Germany, 77-97.
56. Jin, Y., & Gao, X., 2019. "Review of pollution and risk of plastics in marine environment." Science of the Total Environment, 676, 742-754.
57. Jones, S. E., Chiu, C. Y., Kratz, T. K., Wu, J. T., Shade, A., și McMahon, K. D. 2020. Typhoons reshape bacterial communities in large subtropical reservoirs. Environmental Microbiology, 22(3), 967-979.
58. Joshi, D.M. & Kumar, A. & Agrawal, N., 2009. Assessment of the irrigation water quality of river Ganga in Haridwar district. 2. 285-292.
59. Kaper, J. B., Morris, J. G., & Levine, M. M., 1995. Cholera. Clinical Microbiology Reviews, 8(1), 48-86.
60. Kazamia, E., Czesnick, H., Nguyen, T. T. V., Croft, M. T., Sherwood, E., Sasso, S., Hodson, S. J., Warren, M. J. and Smith, A. G., 2012. Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. Environmental Microbiology, 14: 1466– 1476;
61. Khorshid, M.S.H.; Thiele-Bruhn, S. 2016. Contamination status and assessment of urban and non-urban soils in the region of Sulaimani City, Kurdistan, Iraq. Environ Earth Sci 75.16, 1-15.
62. Kunz, J. M., 2024. Surveillance of Waterborne Disease Outbreaks Associated with Drinking Water—United States, 2015–2020. MMWR. Surveillance Summaries, 73.
63. Kuzníková, L.; Dědková, K.; Kupková, J.; Váňa, R.; Kukutschová, J. Synthesis, characterization and acute aquatic toxicity of samarium oxide nanoparticles to freshwater green algae. NANOCON 2017, Oct 18th - 20th 2017, Brno, Czech Republic, EU.

64. Laist, D. W., 1997. "Impacts of marine debris: Entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records." Marine Pollution Bulletin, 32(3), 201-212.
65. Lazăr, V., Măruțescu, L.G., Chifiriuc, M.C., 2016. Microbiologie generală și aplicată. Bucharest, Ro: Bucharest University Press.
66. Lucaci, A. I., Moldoveanu, M., Florescu, L., Cojoc, R., Neagu S., Ruginescu, R., Enache, M., 2019. The seasonal dynamics of the cultivable microbial communities in Letea saline lake. AgroliLife Scientific Journal, 8 (1): 160-166.
67. Lulea A.C., Ruginescu R., Banciu R.M., Pantazi C., Brinduse E., Ion M., Quintela S., Elejalde E., Fernandez-de-Castro L., Villaran M.C., Ruiz-de-Vergara Z., Ruiz C., Epure P., Purcărea C., Vasilescu A., 2022. Fast Electrochemical Measurement of Laccase Activity for Monitoring Grapes' Infection with Botrytis cinerea. Processes, 10. 3.
68. Madigan M., Martinko J.M., Bender S., Buckley D., Stahl D., Brock T., 2015, Brock Biology of Microorganisms, 14th Edition;
69. McCabe, D. J., 2011. Rivers and Streams: Life in Flowing Water. Nature Education Knowledge 3(10) 19.
70. Mihaela, T.; Popa, P.; Murariu, G.; Georgescu, L.; Iticescu, C.; Barbu, M., 2016. Complementary approach for numerical modelling of physicochemical parameters of the Prut river aquatic system. J. Environ. Prot. Ecol., 17, 53–63.
71. National Center for Biotechnology Information. PubChem Compound Summary for CID 73963, 2021. Cerium Dioxide; National Center for Biotechnology Information: Bethesda, MD, USA,.
72. Neagu, S., Enache, M., Cojoc, R., Ruginescu, R., Moldoveanu, M., Florescu, L., Lucaci, I., 2021. Seasonal variation of the water color from the IOR lake – Bucharest. Oltenia. Studii şi comunicări. Ştiinţele Naturii Tom., 37(1), 205 – 210.
73. Ojovan, B., Catana, R., Neagu, S., Cojoc, R., Lucaci, A.I., Marutescu, L., Florescu, L., Ruginescu, R., Enache, M., Moldoveanu, M., 2021. Metabolic Potențial of Some Functional Groups of Bacteria in Aquatic Urban Systems. Fermentation, 7, 242, https://doi.org/10.3390/fermentation7040242.
74. Podosu (Vlad) A., Neagu S., Lucaci I., Ruginescu R., Cojoc R., Bătrînescu-Moteau C. și Enache M., 2023. Anthropogenic impact on the chemical and microbiological profile of Pasarea river, România. Oltenia. Studii si Comunicari, Seria Stiintele Naturii, Tom. 39, 2, 176 – 184.
75. Podosu (Vlad) A., Neagu S., Lucaci I., Ruginescu R., Cojoc R., Bătrînescu-Moteau, Purcărea C., Enache M., Ruginescu R., 2023. Extracellular hydrolases produced by microorganisms isolated from the polluted river Pasarea, România. Românian Journal of Biology, Plant Biology, 68, 1-2, 29-40.
76. https://isubif.ro/local/wp-content/uploads/2015/06/Planul-de-Analiz/ (accesat în ianuarie 2020)
77. https://www.google.com/maps/place/ (accesat în ianuarie 2020)
78. https://blast.ncbi.nlm.nih.gov/Blast.cgi. (accesat în iunie 2022)
79. https://www.graphpad.com/ (accesat în aprilie 2021)

DISSEMINATION OF RESULTS

Articles published on the subject of the doctoral thesis in ISI (Web of Science with Impact Factor) journals

1. **Catană R.D$^{\#}$., Podosu A$^{\#}$.**,Florescu L.I., Mihai R.A., Enache M., Cojoc R., Moldoveanu M.**,** 2023. Quantitative Analyses of Chemical Elements in *Phragmites australis* as Bioindication of Anthropization in Urban Lakes. ***Sustainability*, 15**(1): Art.No. 553. DOI: 10.3390/su15010553 (# = autori cu contribuţie egală). IF = 3,3 (Web of Science).

Articles published on the subject of the doctoral thesis in ISI (web of Science) indexed journals

1. **Aurelia Podosu (Vlad)**, Simona Neagu, Anca Ioana Lucaci, Robert Ruginescu, Roxana Cojoc, Costin Bătrînescu-Moteau, Cristina Purcărea, Mădălin Enache, 2023, Anthropogenic impact on the chemical and microbiological profile of Pasărea river, România. *Oltenia. Studii și Comunicări, Seria Științele Naturii*, Tom. 39, 2, 176 – 184.

Articles published on the subject of the doctoral thesis in BDI indexed journals

1. **Aurelia Podosu (Vlad)**, Simona Neagu, Anca Ioana Lucaci, Roxana Cojoc, Costin Bătrînescu-Moteau, Cristina Purcărea, Mădălin Enache, Robert Ruginescu, 2023, Extracellular hydrolases produced by microorganisms isolated from the polluted river Pasărea, România. *Românian Journal of Biology, Plant Biology*, 68, 1-2, 29-40.

Articles published on topics related to the doctoral thesis in ISI (web of Science) indexed journals

1. Bătrînescu-Moteau Costin, Neagu Simona, Lucaci Anca-Ioana, Ruginescu Robert, Maria Gabriel, Cojoc Roxana, Purcărea Cristina, **Podosu Aurelia**, Enache Mădălin, 2022, Preliminary data concerning communities of microorganisms in a volcanic tuff endolytic habitat. *Oltenia. Studii şi Comunicări. Ştiinţele Naturii*. Tom. 38, No. 1, 168 – 173.
2. Bătrînescu–Moteau Costin, Neagu Simona, Lucaci Anca-Ioana, Ruginescu Robert, Cojoc Roxana, **Podosu (Vlad) Aurelia**, Purcărea Cristina, Negru Mircea, Enache Mădălin, 2022, New data on microorganisms isolated from ceramic materials of the Romula archaeological site, România. *Oltenia. Studii şi Comunicări. Ştiinţele Naturii*. Tom. 38, No. 2, 147 – 153.

3. Bătrînescu-Moteau Costin, Lucaci Anca Ioana, Neagu Simona, Cojoc Roxana, Purcărea Cristina, **Podosu (Vlad) Aurelia**, Enache Mădălin, Ruginescu Robert, 2023, Superoxide dismutase activity in microorganisms inhabiting volcanic tuff rock. *Oltenia. Studii și Comunicări, Seria Științele Naturii*, Tom 39, 1, 201-209.

Articles published on topics related to the doctoral thesis in BDI indexed journals

1. Costin Bătrînescu-Moteau, Simona Neagu, Oana Cătălina Mocioiu, Anca Ioana Lucaci, Roxana Cojoc, Cristina Purcărea, **Aurelia Podosu (Vlad)**, Maria Zaharescu, Mădălin Enache, Robert Ruginescu, 2023, Evidence of microbial antagonism in volcanic tuff rock. *Romanian Journal of Biology, Plant Biology*, 68, 1-2, 9 – 20.