HABILITATION THESIS

Bioindicators and Circular Strategies in Environmental Studies: Pollution Assessment, Apicultural Products and Waste Cooking Oil Valorization

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Contents

Abstract

Fundamental research in the field of environmental science is of major importance as it provides new, clear, and evidence-based information regarding the state of ecosystems, the level of contamination, and the impact of anthropogenic activities on environmental factors. These studies contribute essentially to the understanding of environmental degradation processes and to the identification of sustainable directions for the protection and valorization of natural resources.

The habilitation thesis, entitled *"**Biondicators and Circular Strategies in Environmental Studies: Pollution Assessment, Apicultural Products and Waste Cooking Oil Valorization**"*, summarizes the main results of my scientific activity conducted after obtaining the PhD title, as well as the professional path developed in the field of environmental sciences, with an emphasis on pollution assessment and the development of solutions based on circular economy principles.

There are three major research directions addressed in the habilitation thesis. The first focuses on the assessment and monitoring of environmental pollution, with particular emphasis on water quality in pollution-affected areas (Alba County), including the use of diatoms as bioindicators for assessing surface water pollution, as well as research on soil quality in polluted areas.

The second direction concerns apicultural products as bioindicators of environmental pollution and microbial bioinhibitory agents, highlighting the role of honey and propolis in reflecting environmental contamination and their potential antimicrobial properties.

The third direction explores the circular valorization of waste cooking oils into antimicrobial soaps, integrated with environmental risk assessment under the ISO 14001 framework, by incorporating apicultural products and essential oils into sustainable formulations and valorization strategies.

The research directions addressed primarily focused on the assessment of environmental quality factors, especially surface and groundwater in areas subjected to anthropogenic pressure. The studies aimed to identify spatial and temporal variations of quality indicators (physicochemical and microbiological parameters), as well as to highlight pollution sources. In this context, bioindicator organisms—diatoms—were used as efficient tools for assessing the ecological status of aquatic ecosystems, due to their high sensitivity to environmental changes, evidenced also by the occurrence of teratological (abnormal) forms, frequently associated with ecological stress and pollution.

Another important direction was the analysis of soil contamination, particularly in areas affected by industrial and mining activities, through the determination of heavy metal concentrations and the assessment of associated ecological risks. These studies contributed to the understanding of pollutant accumulation and transfer mechanisms in the environment and to highlighting their long-term impact on ecosystems.

In parallel, I investigated apicultural products (honey and propolis) as bioindicators of environmental quality, focusing on how pollution influences their composition, safety, and biological activity. The results highlighted correlations between the degree of environmental contamination and the characteristics of these natural products, confirming their usefulness in integrated environmental monitoring.

A complementary research direction focused on the valorization of organic waste, particularly used cooking oils, through the development of circular strategies for their conversion into value-added products, such as soaps with functional properties. These studies included the use of bee products and essential oils as ingredients, the optimization of conversion processes, and the assessment of environmental impacts within a production facility, in accordance with the principles of sustainability and environmental management.

The conducted research contributes to an integrated understanding of environmental issues by combining pollution monitoring methods with innovative resource valorization approaches, supporting the transition towards sustainable development models and a circular economy.

Rezumat

Cercetările fundamentale în domeniul mediului au o importanță majoră deoarece aduc informații noi, clare și bazate pe dovezi privind starea ecosistemelor, nivelul de contaminare și impactul activităților antropice asupra factorilor de mediu. Acestea contribuie esențial la înțelegerea proceselor de degradare ambientală și la identificarea unor direcții sustenabile de protecție și valorificare a resurselor naturale.

Teza de abilitare, intitulată „***Bioindicatori și strategii circulare în studiile de mediu: evaluarea poluării, produse apicole și valorificarea uleiurilor alimentare uzate***”, sintetizează principalele rezultate ale activității mele științifice desfășurate după obținerea titlului de doctor, precum și parcursul profesional construit în domeniul științelor mediului, cu accent pe evaluarea poluării și pe dezvoltarea unor soluții bazate pe principii de economie circulară.

Există trei direcții majore de cercetare abordate în teza de abilitare. Prima se concentrează pe evaluarea și monitorizarea poluării mediului, cu accent pe calitatea apei în zone afectate de poluare (din județul Alba), incluzând utilizarea diatomeelor ca bioindicatori pentru evaluarea poluării apelor de suprafață, precum și cercetări privind calitatea solului în zone poluate.

A doua direcție vizează produsele apicole ca bioindicatori ai poluării mediului și agenți bioinhibitori microbieni, evidențiind rolul mierii și propolisului în reflectarea contaminării mediului și potențialul lor antimicrobian.

A treia direcție explorează valorificarea circulară a uleiurilor alimentare uzate prin transformarea acestora în săpunuri antimicrobiene, prin încorporarea produselor apicole și a uleiurilor esențiale în formulări și strategii sustenabile de valorificare, respective integrate într-o evaluare a riscului de mediu conform standardului ISO 14001.

Direcțiile de cercetare abordate au vizat în primul rând evaluarea calității factorilor de mediu, în special a apelor de suprafață și subterane din zone supuse presiunii antropice. Studiile au urmărit identificarea variațiilor spațiale și temporale ale indicatorilor de calitate (parametri fizico-chimici si microbiologici), precum și evidențierea surselor de poluare. În acest context, au fost utilizate și organisme bioindicatoare – diatomeele, ca instrumente eficiente pentru aprecierea stării ecologice a ecosistemelor acvatice, datorită sensibilității lor ridicate la modificările de mediu, evidențiate inclusiv prin apariția formelor teratologice (anormale), asociate frecvent cu condiții de stres ecologic și poluare.

O altă direcție importantă a fost analiza contaminării solului, în special în zone afectate de activități industriale și miniere, prin determinarea concentrațiilor de metale grele și evaluarea riscurilor ecologice asociate. Aceste cercetări au contribuit la înțelegerea mecanismelor de acumulare și transfer al poluanților în mediu și la evidențierea impactului pe termen lung asupra ecosistemelor.

În paralel, am investigat produsele apicole (miere și propolis) ca bioindicatori ai calității mediului, urmărind modul în care poluarea influențează compoziția, siguranța și activitatea biologică a mierii și propolisului. Rezultatele au evidențiat corelații între gradul de contaminare ambientală și caracteristicile acestor produse naturale, confirmând utilitatea lor în monitorizarea integrată a mediului.

O direcție complementară de cercetare a vizat valorificarea deșeurilor organice, în special a uleiurilor alimentare uzate, prin dezvoltarea unor strategii circulare de transformare în produse cu valoare adăugată, precum săpunuri cu proprietăți funcționale. Aceste studii au inclus atât utilizarea ca ingrediente a produselor apicole sau a uleiurilor esențiale, optimizarea proceselor de conversie, cât și evaluarea impactului asupra mediului într-o unitate de producție, în concordanță cu principiile sustenabilității și ale managementului de mediu.

Cercetările realizate contribuie la o înțelegere integrată a problemelor de mediu, prin combinarea metodelor de monitorizare a poluării cu abordări inovative de valorificare a resurselor, susținând tranziția către modele de dezvoltare sustenabilă și economie circulară.

Part I. PROFESSIONAL AND ACADEMIC ACHIEVEMENTS

1.1. Introduction

This thesis adopts a holistic approach, developing a multidisciplinary framework within the field of environmental engineering, with direct applications in environmental quality management, food safety, and waste recovery and valorization. By integrating the elements required by national and European regulations, it synthesizes the research and insights accumulated throughout the academic career, resulting in an interdisciplinary contribution to environmental protection and engineering.

Within this framework, the development potential of the proposed topics is emphasized, with sustainability underpinned by the practical applicability of the findings. The focus extends beyond theoretical conceptualization, highlighting the relevance and usability of the results in real-world contexts.

The research conducted to date has aimed to enhance scientific contribution through publications in high-impact, peer-reviewed journals, participation in various scientific events, and the integration of teaching with research activities in environmental engineering. Particular emphasis is placed on quality management applications, with a focus on ensuring the safety and integrity of water resources and naturally derived products intended for human consumption, including apicultural products, while promoting the sustainable reuse of waste materials within a circular economy framework.

The thesis is organized into three main sections:

1. Professional and academic achievements.
2. Research and scientific achievements.
3. Development plan of scientific, academic and professional career.

The first part of the habilitation thesis presents the candidate's professional and academic achievements, outlining the motivational framework that underpins the research interests and the adopted conceptual approach, together with an overview of the academic and professional activities carried out. It further highlights the professional development trajectory and the competencies acquired and demonstrated throughout the candidate's career. Particular attention is given to continuous professional development, the integration of teaching with research and innovation activities, the modernization of teaching and learning methods, and the active involvement of students in research projects and academic initiatives.

The interdisciplinary nature of the subject enabled the integration, within the habilitation thesis, of the research results obtained over the years and their alignment with the field of ***environmental engineering***, with applications grounded in environmental quality management and in the sustainable management of the quality and safety of ecosystem-based food products..

The second part, *"Research and scientific achievements",* provides an overview of the research activity, scientific contributions, and results achieved. It is structured into three sections, namely: *" Interdisciplinary Research on the Assessment and Monitoring of Pollution Impacts on the Environment and Human Health"*, *"Apicultural Products as Bioindicators of Environmental Pollution and Microbial Bioinhibitory Agents."* and "*Circular Valorization of Waste Cooking Oils into Antimicrobial Soaps Integrated with Environmental Risk Assessment under the ISO 14001 Framework".*

The contributions were synthesized along the three main directions pursued throughout the entire career, namely: the purpose and objectives of the research, and the results obtained, with particular emphasis placed on identifying needs (problems) in order to correctly establish the measures required for remediation or, ideally, for the prevention of undesirable situations. Naturally, all these results were grounded in the requirements stipulated by European legislation and standardization, thereby contributing to the continuous improvement of techniques and technological operations through knowledge and control of environmental impacts, as well as of potential issues that may affect the population.

Each subchapter presents the activities carried out, as well as the results obtained through the implementation of projects and research activities, which are reflected in the publication record presented separately in each chapter, in accordance with the addressed topics. The entire research activity aimed to increase scientific contribution through articles published in prestigious journals, participation in various national and international scientific events, and, at the same time, through the harmonization of teaching activities with research activities.

The final part of the thesis addresses the directions and perspectives for the development of the academic career, through direct actions that will support the practical implementation of the proposed objectives and future development directions. This section is based on the idea that education represents one of the fundamental pillars of society, and that an academic career has autonomous characteristics arising from the integration of teaching and research activities, as well as from the immediate and long-term needs of the socio-economic reality for which most students are being prepared by completing the three cycles of higher education: bachelor's, master's, and doctoral studies.

1.2. The Conceptual Approach to the Research Activity

The preparation of the habilitation thesis is founded on competencies demonstrated through previous achievements, professional development, scientific contributions and results obtained, as well as on the prospective projection of future career development. These elements define a cumulative professional trajectory in which research experience, academic practice, and methodological development converge toward a coherent scientific profile oriented toward environmental engineering and applied environmental analysis.

The thesis adopts an interdisciplinary approach, primarily within the field of environmental engineering and, implicitly, within the field of environmental quality control, with applications aimed at reducing pollution and the effects of environmental pollution. The conceptual focus is placed on understanding environmental systems as interconnected structures in which technical processes and natural dynamics interact, generating measurable outcomes that require structured analysis and interpretation

The subject is addressed in a holistic manner, being connected to the "quality–environment" concept. Within this perspective, emphasis is placed on the continuity of environmental processes and on the need to interpret system behaviour through integrated analytical models capable of capturing both direct and indirect influences on environmental components.

Within this socio-economic context, and under the conditions of ensuring a clean, pollution-free environment, Government Emergency Ordinance no. 92/2021 [1], which transposes Directive 2008/98/EC [2], places particular emphasis on the integrated management of waste. In this framework, the conceptual relevance lies in the necessity of aligning scientific investigation with structured environmental governance mechanisms that support decision-making, operational control, and long-term resource efficiency.

On the other hand, the thesis discusses and presents research results related to the management of quality and safety of biological products resulting from ecological interactions, aiming to strengthen knowledge regarding the use of apicultural products both as natural therapeutic resources and as tools for environmental biomonitoring. From a conceptual standpoint, these aspects illustrate the interface between environmental conditions and biological responses, highlighting the value of natural matrices as indicators of system integrity. The obtained results have applicability in organic agriculture, the food industry, and public health. These domains are addressed from a systems perspective, in which environmental inputs, biological processes, and final product quality are interdependent, requiring integrated analytical and methodological approaches.

The quality system must ensure the fulfillment of established objectives while simultaneously integrating environmental protection principles. Conceptually, this reflects the need for structured process control mechanisms that allow the identification of deviations, the analysis of their causes, and the implementation of corrective pathways within complex operational systems. In this context, in order to comprehensively address the concept of quality, it is essential to consider all factors contributing to the achievement of the proposed objectives, with a focus on maintaining and continuously improving performance in both qualitative and ecological terms. This requires the interpretation of quality not as a static attribute, but as a dynamic property emerging from the interaction between processes, resources, and environmental constraints.

At the same time, the environmental quality management system, ISO 14001 [3] must be organized so as to allow appropriate and continuous control over all activities and processes that influence quality [4]. From a conceptual perspective, this highlights the importance of system coherence, feedback loops, and iterative improvement mechanisms in maintaining environmental performance at operational level.

The focus must be on both corrective actions for nonconformities and their prevention. This dual perspective reinforces the preventive dimension of environmental management, emphasizing anticipation of system deviations rather than solely reactive intervention.

A process-based approach to any system should always be grounded in the analysis of inputs and the evaluation of outputs, regardless of the context in which it is applied. This analytical structure supports the understanding of transformation mechanisms within environmental systems and enables the identification of key control points influencing system efficiency. Whether addressing primary processes (PP) or support processes (SP), they must meet requirements not only in terms of efficiency and quality but also through the integration of principles of environmental responsibility. This ensures that operational structures are evaluated not only by performance metrics but also by their systemic impact on environmental integrity.

The results obtained, feedback received, and commitment to properly conducted activities must simultaneously reflect a positive impact on natural resources and the ecosystems involved. This reflects the conceptual assumption that environmental performance is inherently linked to resource stewardship and long-term ecological balance.

Within the scope of this thesis, environmental quality—or, more specifically, the quality of bio-natural resources provided by ecosystems—can be assessed through physical, chemical, and microbiological characteristics. This multidimensional assessment approach supports the interpretation of environmental status through complementary analytical dimensions, ensuring a comprehensive understanding of system behaviour.

At the same time, attention is given to identifying the factors that contribute to pollution, alteration, or modification of these properties, thereby considering both consumer satisfaction and environmental impact. This introduces a diagnostic dimension to the conceptual framework, oriented toward understanding causality and system vulnerability.

This integrated approach to quality management, ISO 9001:2015 [5], food safety (ISO 22000:2018 [6], alongside environmental protection [3] and its impact on product quality (ISO 14001:2015), reflects a commitment to responsible and sustainable resource management. From a conceptual standpoint, this illustrates the convergence of multiple regulatory and methodological frameworks into a unified systems-based governance model.

The thesis consolidates research conducted over the course of an academic career into a complex, interdisciplinary endeavor dedicated to integrated quality management within the context of contemporary environmental challenges. This consolidation reflects the transition from individual research outputs toward an integrated scientific perspective.

Over 22 years of academic activity, the research topics pursued and the results obtained—closely aligned with teaching responsibilities—form the motivational foundation of this habilitation thesis. This experience underpins the development of a coherent academic identity shaped by the continuous interaction between teaching practice, research activity, and applied environmental investigation.

The thesis is structured into three main sections: scientific, professional, and academic achievements; research and scientific contributions; and the perspectives for future academic and professional development.

The first section presents the main stages of the academic and professional trajectory, highlighting the development of competencies and the consolidation of teaching and research activities within the field of environmental engineering. It reflects the progressive integration of academic practice with applied research, contributing to the formation of a coherent scientific profile.

The second section, "Scientific Contributions and Results Achieved," synthesizes the main research directions and outcomes, emphasizing their continuity, relevance, and scientific impact. The presented studies are organized around key thematic areas of environmental research, focusing on the analysis of environmental processes and the development of applied, interdisciplinary approaches supported by research activities, publications, and scientific dissemination.

The third section outlines the main directions for future academic and professional development, focusing on strengthening research capacity, enhancing the integration of teaching and research activities, and aligning academic work with emerging scientific and societal challenges. This section reflects the dynamic and continuous nature of academic evolution within the broader context of environmental engineering and higher education.

1.3. Professional Career Development and Domain-Specific Experience of the Candidate

1.3.1. Academic Background and Summary of Professional Activities

The candidate's professional career spans 22 years, predominantly within the academic sector. The initial three years (2004–2007) were served at the Politehnica University of Timișoara (UPT), Faculty of Industrial Chemistry and Environmental Engineering, followed by the period 2009–present at "1 Decembrie 1918" University of Alba Iulia (UAB), and most recently (2025–present) at Politehnica University of Timișoara (UPT), Faculty of Engineering in Hunedoara. Concurrently, the candidate held a specialist position at the Alba Sanitary Veterinary and for Food Safety Directorate, acting as an advisor responsible for inspection and control of food industry facilities.

The following section provides a synthesis of the candidate's professional activity, emphasizing the key milestones and achievements that have shaped and developed the professional career to date.

In 2004, I graduated from the Faculty of Industrial Chemistry and Environmental Engineering at Politehnica University of Timișoara, specializing in Engineering of Natural Product Processing, and defended my bachelor's thesis entitled: *"Design and Automation of a Natural Concentrated Apple Juice Production Facility"*, under the supervision of Prof. Dr. Eng. Delia Perju. At this level, I also completed courses at Mihai Eminescu University of Timișoara, majoring in Economics of Trade, Tourism, and Services.

In order to consolidate my theoretical and practical knowledge in chemical engineering and organic compounds, I engaged in teaching activities, pursued intensive individual study, and attended master's level courses at Politehnica University of Timișoara (2004–2005), specializing in Fine Organic Synthesis, Semi-Synthesis, and Natural Products.

In 2007, following three years of intensive research under the guidance of Prof. Dr. Delia Perju, I was awarded the Doctorate in Chemical Engineering. My doctoral thesis was entitled: *"Contributions to the Improvement of Juice Quality through Mathematical Modeling Techniques, with a View to Extending Shelf Life and Its Impact on Food Safety."* The research conducted during my doctoral studies focused on food quality and safety control, emphasizing the application of methods and techniques from chemical engineering to improve product quality and ensure consumer safety.

During my doctoral studies, I was admitted and employed at Politehnica University of Timișoara as a full-time doctoral student, starting in 2004, within the Faculty of Industrial Chemistry and Environmental Engineering. My doctoral research was carried out at the Department of Chemical Engineering (currently the Department of Applied Chemistry and Engineering of Organic and Natural Compounds – CAICON), within the Automation in the Chemical Industry research group. This stage represented a crucial step in my professional and scientific development, providing me the opportunity to actively engage in interdisciplinary research projects and to consolidate my competencies in applied chemical engineering.

I was involved in laboratory activities for the course "Automation of Chemical Processes I and II", contributing to the development of students' practical skills through supervision of experimental work, explanation of theoretical principles, and assistance in the use of equipment specific to industrial process automation.

Additionally, I participated in the CNCSIS Type A grant, No. A1/GR181/19.05.2006, Project No. 27, CNCSIS code 625, entitled "Simulation and Optimal Operation of Research and Laboratory Installations in the Chemical Industry Using Advanced Control Systems" (2006), as well as the CEEX contract No. 057/03.10.2005, subcontract No. 15/05.10.2005, P.II, titled "Soil Rehabilitation through Intensive Methods in Areas of Exploitation and Processing of Radioactive Ores under Natural or Anthropogenic Disaster Conditions" – acronym RESOLRAD (2005). Participation in these projects allowed me to deepen my knowledge of advanced technologies in industrial process control and to gain experience in monitoring and remediating soil pollution in ecologically high-risk areas. Moreover, these projects enabled me to contribute to the development of practical solutions in environmental engineering and chemical process management.

Since the 2008–2009 academic year, I have been employed as an associate faculty member at the Faculty of Informatics and Engineering, “1 Decembrie 1918” University of Alba Iulia. During this period, I have been responsible for teaching specialized courses such as “Environmental Protection Technologies and Biotechnologies” (lecture) and “Environmental Chemistry” (lecture and laboratory), contributing to students’ professional development by delivering theoretical knowledge and supervising practical activities specific to environmental protection.

I have also been a member of the research team for the project PN-III-P2-2.1-PED-2019-3739, titled “Development of Innovative Products from Advanced Materials (Mortars) for the Rehabilitation of Heritage Buildings” – acronym DIPAM. This project aimed to develop modern and sustainable solutions for the conservation of built heritage. Participation in this project provided the opportunity to contribute to the implementation of innovative technologies in construction materials, directly impacting the preservation of architectural and cultural values.

In the 2024–2025 academic year, I began working as an associate lector at Politehnica University of Timișoara, Faculty of Engineering in Hunedoara, where I am responsible for delivering lectures and coordinating projects for two courses “Chemistry”, “Chemistry II” and “Inorganic Chemical Technology”. This collaboration reflects my ongoing commitment to the academic development of students and to integrating fundamental chemistry knowledge into applied engineering contexts.

A summary of my university studies and major academic milestones is presented below:

- Engineer (Bachelor’s degree) – Faculty of Industrial Chemistry and Environmental Engineering, Politehnica University of Timișoara, 2004
- Master’s degree – Fine Organic Synthesis, Semi-Synthesis, and Natural Products, Politehnica University of Timișoara, 2005
- Economist (Bachelor’s degree) – Faculty of Tourism, Hotel, and Commercial Management, Mihai Eminescu University of Timișoara, 2006
- Doctor of Chemical Engineering – “Contributions to the Improvement of Juice Quality Using Mathematical Modeling Techniques, to Extend Shelf Life and Assess Its Impact on Food Safety”, Faculty of Industrial Chemistry and Environmental Engineering, Politehnica University of Timișoara, 2007

My professional training and 22 years of teaching and research experience are based on systematic and sustained work, as well as practical teaching experience. These elements have contributed to the development of pedagogical, didactic, and creative competencies. All courses I deliver are supported with teaching materials, lithographed lecture notes, or published books, available in the university library or bookstore.

The activities carried out during this period highlight qualitative aspects, demonstrating the professional and teaching competencies acquired. They ensure a direct transfer of knowledge within the university education process, as well as a transfer of research results to the national and international academic community, industry, institutions, and local authorities.

My professional trajectory has allowed me to acquire extensive experience, valuable for an academic career in teaching and scientific research.

1.3.2. Academic and Professional Activities

Teaching and professional activity is demonstrated through my personal contributions to the university environment, including the publication of specialized books, as well as active involvement in the student-teacher interaction process to enhance and invigorate the teaching-learning experience.

Particular emphasis has been placed on motivating students to actively participate in various practical activities (laboratory research, market studies, etc.) and events (conferences, scientific meetings, etc.) by fostering their curiosity and encouraging the exploration of processes and phenomena specific to this field—both the critically important field of environmental engineering and the domains of food safety and quality control throughout all stages of a product’s lifecycle.

Due to the interdisciplinary nature of the field, even at the level of bachelor's and master's theses, I have established interdisciplinary student teams from different specializations, including economics and environmental engineering.

In parallel with my teaching activities, I have participated in numerous professional training programs and international certifications, which have supported both my own competencies and the instruction of students in the fields of food safety and product quality management:

- Participation in BTSF – Better Training for Safer Food programs: Food Additives, Enzymes and Flavourings (2017); Food Hygiene and Flexibility (2022); Food Improvement Agents (2023)
- Postgraduate Certificate – Training of Food Safety Inspectors, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 2008
- Professional Certificate – Certified Associate in Project Management – Fundamentals of Project Management, Delaware Technical & Community College, 2008
- Competency Certificate – Food Safety & Applied HACCP Principles, organized by WHO and ANSVSA, 2007
- Certificate – Strategy for Initiating and Managing a Business, Politehnica University of Timișoara, 2006

The specialized books and publications I have authored primarily address the crucial domain of environmental quality control and product quality management, covering both fundamental theoretical concepts and practical aspects, such as sampling methods, detection of counterfeit products, adulteration of honey, and agro-food commodities. I have authored ten books or chapters in specialized volumes dedicated to environmental and product quality, the most representative of which are:

- **Glevitzky Mirel**, Popa Maria**,** *Tehnologii curate de protecția mediului***,** *229 p. ISBN 978-606-613-049-3,* Aeternitas, Alba Iulia, 2012;

- Glevitzky Mirel, Popa Maria, *Calitatea apelor freatice - Principii teoretice şi studii de caz,* p. 270*; Editura* Aeternitas, ISBN 978-606-613-074-5, Alba Iulia, 2012;

- Popa M., **Glevitzky M.**, *Contaminarea mărfurilor agroalimentare – Metode și tehnici experimentale de cercetare* **-** Editura Casa Cartii de Ştiinţa, ISBN 978- 973-133- 678-7, Cluj Napoca, 2009 (190 pg);

- Popa Maria**, Glevitzky Mirel**, *Bazele Merceologiei – Teorie si aplicaţii*, 269 P. Casa Cărții de Stiința, Cluj - Napoca, ISBN 978-606-17-0276-3, 2012;

- Popa Maria, **Glevitzky Mirel**, *Chimia mediului - Noțiuni de baza și studii de caz,* Casa Carții de Știinta, Cluj Napoca, 2014;

- Popa, M., **Glevitzky M.,** Calitatea și securitatea mărfurilor - Teorie și aplicații, Seria Didactica, Alba Iulia 2018;

- Popa, M., **Glevitzky M.**, Standarde europene în domeniul siguranței alimentare, Note de curs, Alba Iulia 2018

-Popa M., Glevitzky I., **Glevitzky M.**, Oprean C., *Managementul riscului în industria alimentară, în contextul combaterii efectelor infecției virale cu Covid-19- chapter in the volume***:** **Studii de caz relevante privind managementul organizațiilor din Romania, SAMRO**, Editura Pro Universitaria, ISBN 978-606-26-1244-3, 2020;

- **Glevitzky M.**,*. si colab, Modern instrument for nonconformities` management within quality management systems*, **chapter in the volume:** "Romanian management theory and practice", pp. 99–120, SAMRO, Editura Springer Nature, 2024 (ISBN 978-3-031-60342-6 ISBN 978-3-031-60343-3 (eBook).

My contribution to the development of students' competencies and the enhancement of the educational process has also been reflected through active involvement in international conferences and editorial boards:

- Member of the organizing committee of the *International U.A.B.-B.EN.A. CONFERENCE "Environmental Engineering and Sustainable Development"*, Alba Iulia, editions 2011, 2013, 2015, 2017, 2019
- Member of the editorial board of:

- *Acta Technica Corviniensis – Bulletin of Engineering*, e-ISSN 2067-3809, Politehnica University of Timișoara – Faculty of Engineering Hunedoara
- *Annals of Faculty Engineering Hunedoara – International Journal of Engineering*, ISSN: 2601-2332, Politehnica University of Timișoara – Faculty of Engineering Hunedoara

The contribution to enhancing the effectiveness of the educational process, the development of teaching competencies, and the ability to mentor students and young researchers is demonstrated through initiatives undertaken in the following areas:

- Continuous improvement of academic activities;
- Integration of the teaching process with research, development, and production activities;
- Modernization of teaching and learning methods for the courses delivered;
- Engagement of students in research activities.

1.3.2.1. Continuous Improvement of Academic Activities

Throughout my career, I have been committed to continuously improving the scientific content of the courses I teach, updating informational content, and enhancing the knowledge imparted to students.

University courses published in the Didactic Series of the University provide new clarifications and strengthen the foundational concepts taught, starting from theoretical aspects and progressing to applications specific to the field of integrated quality management.

Attention is also given to improving operations related to the technological process flow for producing a product, by applying the requirements of European standards in quality, food safety, and environmental factors. Particular emphasis is placed on developing competencies in identifying pollution sources and correctly addressing environmental issues.

Moreover, the published materials highlight the identification of non-compliant products and the application of corrective and preventive actions, in accordance with system or operational procedures required by European standardization.

All these initiatives contribute to the development of competencies related to understanding technologies and technological operations underlying product manufacturing and their impact on the environment. Specific elements of environmental protection, ecological risk identification, pollution control, and food fraud are addressed.

These concepts support the transfer and enhancement of knowledge for students preparing for careers in engineering and economics, providing them with a solid foundation to understand the relationship between technological processes, product quality, and environmental protection.

I am a member of the following professional associations: the National Society of Environmental Science and Engineering and the Balkan Environmental Association (B.EN.A) (2007–2020).

1.3.2.2. Integration of Teaching with Research, Development, and Production Activities

Given the importance of research and development activities in highlighting novel aspects of economic phenomena and processes, teaching should stimulate and cultivate students' interest in a scientific approach to various socio-economic challenges.

Based on the premise that every industry—including the food industry—must meet societal expectations not only by producing safe and legally compliant products but also by minimizing environmental impact, I have integrated modern elements of integrated quality management, environmental protection, and pollution prevention into the courses I teach.

Three main systems contribute to this objective:

- Good Manufacturing Practices (GMP): These define the conditions and procedures for processing—based on extensive experience—not only to ensure consistent product quality and safety but also to reduce pollutant emissions, optimize resource consumption, and manage industrial waste properly.

- Risk analysis and assessment (Hazard Analysis and Critical Control Points): This proactive method identifies potential risks not only for food safety but also for environmental factors, preventing contamination of air, water, and soil from the earliest stages of production.
- Quality Assurance Standards: Compliance with ISO standards (ISO 9000) and European norms (including ISO 14001 for environmental management) ensures that industrial processes follow procedures that limit pollution, optimize resource use, and promote sustainable development.

These integrated management systems, applied by economic operators, also govern relationships with suppliers, transporters, and distributors, ensuring that the supply chain complies with environmental protection requirements at every stage—from raw material production to processing, transport, and commercialization.

1.3.2.3. Modernization of Teaching and Learning Methods

To ensure effective transfer of knowledge to students, I employ teaching techniques and methods specific to each discipline. Lectures are structured according to the course syllabus, presenting theoretical aspects followed by analytical models applied to the topics covered.

Modern teaching methods, such as case studies and examples, are integrated into lectures to enhance teaching efficiency. By applying the full range of didactic principles, I aim to create teaching-learning situations in which students are actively involved, developing interest, motivation, and the skills necessary for their future professional careers.

1.3.2.4. Student Engagement in Research Activities

Initial contact with research activities takes place in laboratories for food product quality control or environmental quality monitoring, where students, using modern methods and equipment specific to experimental research, have the opportunity to define research objectives and identify the techniques required to carry out their investigations.

The results of these preliminary "research activities" are further developed by students in bachelor's, master's, or doctoral theses.

Research projects and studies, materialized in specialized articles under my coordination, have involved students at bachelor's, master's, and doctoral levels, who actively participated in data collection and processing.

Additionally, I mention several student-led research projects conducted within student research clubs, where I served on the coordination team: Presence of Food Additives in Meat Products, Contamination and Adulteration of Dairy Products and Honey, Sustainable Recycling of Used Oils with Emphasis on Environmental Protection and Soap Production etc. These projects culminated in presentations at Student Scientific Communication Sessions, academic events for faculty and researchers, and participation in national competitions. Some of these results were also incorporated into bachelor's and master's theses.

Part II. RESEARCH AND SCIENTIFIC ACHIEVEMENTS

2.1. Interdisciplinary Research on the Assessment and Monitoring of Pollution Impacts on the Environment and Human Health

Purpose and Objectives

The research carried out to date has focused on increasing scientific contributions through publications in prestigious journals and participation in various scientific events. At the same time, it has aimed to integrate teaching activities with research in the field of environmental engineering. This research is closely related to environmental pollution and food contamination, with practical applications in environmental quality management and food safety management.

The purpose of this section, entitled "*Interdisciplinary Research on the Assessment and Monitoring of Pollution Impact on the Environment and the Human Health*," is to provide a systemic approach to the phenomenon of heavy metal pollution in industrially contaminated areas, such as the Zlatna or Roșia Montană area, while also extending the analysis to other regions. This approach includes the assessment of pollution impacts on agricultural areas in the Ampoi Valley Basin, as well as on agri-food products.

The study focused on the investigation of pollution phenomena, starting from the pollution source and continuing with the assessment of impacts on agricultural areas, based on original analytical determinations.

The specific objectives are:

- To investigate the quality of groundwater and surface water in areas affected by pollution;
- To assess the impact of industrial pollution on soil;
- To conduct experimental research on the contamination of goods in polluted areas;
- To perform qualitative and quantitative analyses using statistical and mathematical models.

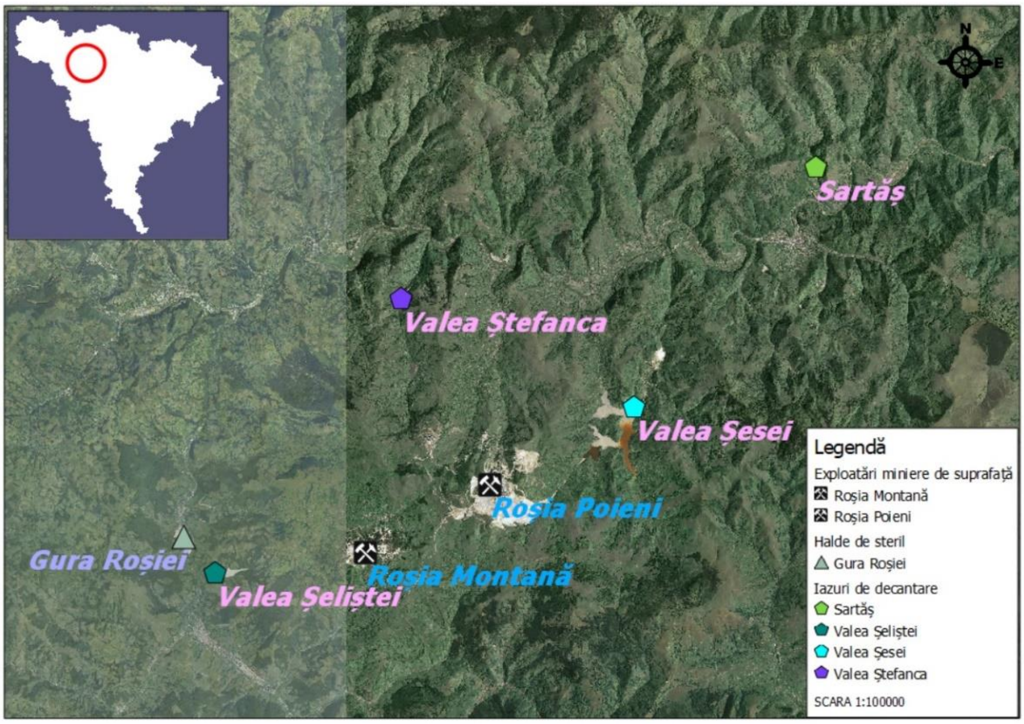
2.1.1 Water Quality in Pollution-Affected Areas: The Use of Diatoms as Bioindicators for Assessing Surface Water Pollution

Pollution in Alba County represents a complex environmental problem, largely caused by long-term industrial and mining activities. The Apuseni Mountains region, especially areas such as Roșia Montană and Roșia Poieni, is characterized by significant historical pollution generated by ore extraction and processing. These activities have led to the accumulation of heavy metals in soil and water, as well as the formation of waste rock dumps and tailings ponds, which continue to affect the environment. Thus, Alba County stands out through the presence of areas with a high level of pollution, where the impact on ecosystems and human health is persistent and long-lasting [7].

Maps no. 1, 2, and 3 highlight the existence of areas with a high degree of pollution within and near Alba County, caused by long-term mining and industrial activities. Locations such as Zlatna, as well as mining areas in the Apuseni Mountains, reflect intense historical pollution generated both by resource extraction and processing. The environmental impact is significant and persistent, affecting soil, water, and air, and the effects extend beyond administrative borders, negatively influencing neighboring regions as well. Thus, these areas are considered major pollution hotspots at a regional level, with long-term consequences for ecosystems and human health [8].

Map no. 1 highlights the main polluted areas concentrated around the Roșia Montană – Roșia Poieni mining region, territories marked by historical pollution generated by mining activities carried out over a very long period of time. Mining activity in the Roșia Montană area dates back to the Dacian period (1st century BC – 1st century AD), being significantly developed during the Roman occupation after 106 AD, when the settlement was known as Alburnus Maior. Mining continued throughout the Middle Ages (13th–18th centuries), later being expanded and modernized during the Habsburg period, starting in the 18th century. In the 19th and 20th centuries, mining activity experienced strong industrial development, reaching a peak during the communist period (1948–1989), when resource extraction was intensified on a large scale [9].

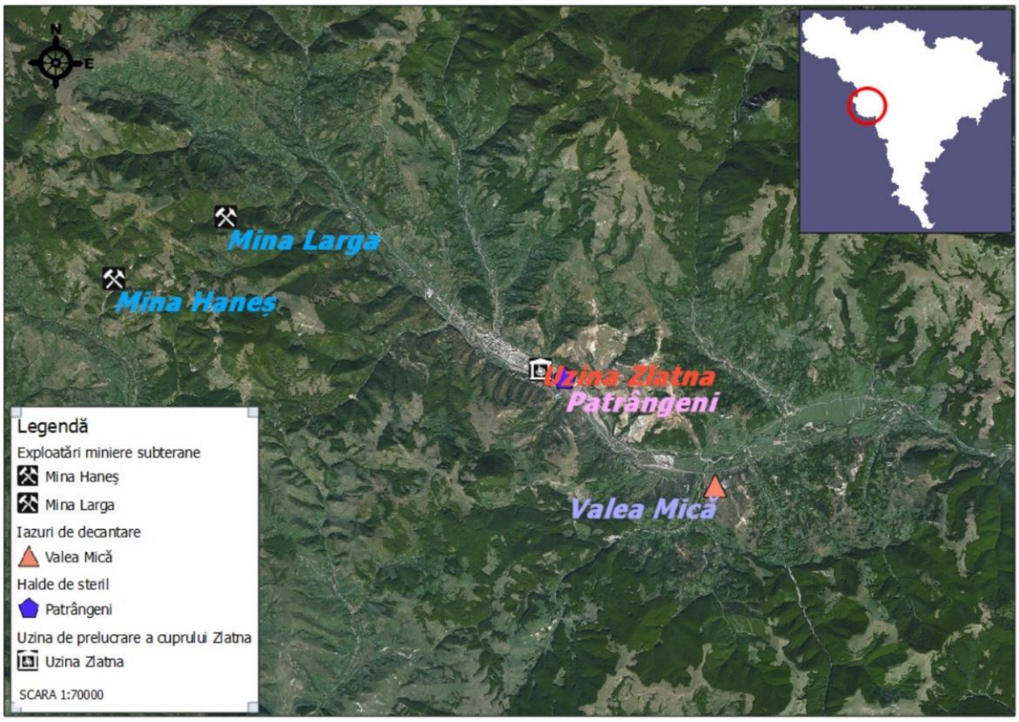
In this context, the area is recognized as an important mining basin with a long tradition in the extraction of metal resources, especially gold, silver, copper, zinc, lead, and tellurium. Extraction and processing activities have generated large quantities of waste and toxic residues, progressively accumulated over centuries. After 1990, many operations were reduced or closed; however, the effects of historical pollution remain evident, manifesting through soil and water contamination. Thus, the Roșia Montană – Roșia Poieni area stands out as a region with a high level of pollution, where the impact of historical mining activities is profound and long-lasting on the environment.



Map 1 – Pollution areas in the Roșia Montană – Roșia Poieni mining region

Areas such as Valea Șesei, Valea Șeliștei, Valea Ștefanca, and Sartaș are occupied by tailings ponds and waste rock dumps, indicating a high degree of soil and water pollution. These deposits contain hazardous substances resulting from ore processing, and their expansion in the mountainous area contributes to the degradation of local ecosystems. The presence of these structures shows a clear concentration of pollution around mining activities, making the area one of the most affected in the county.

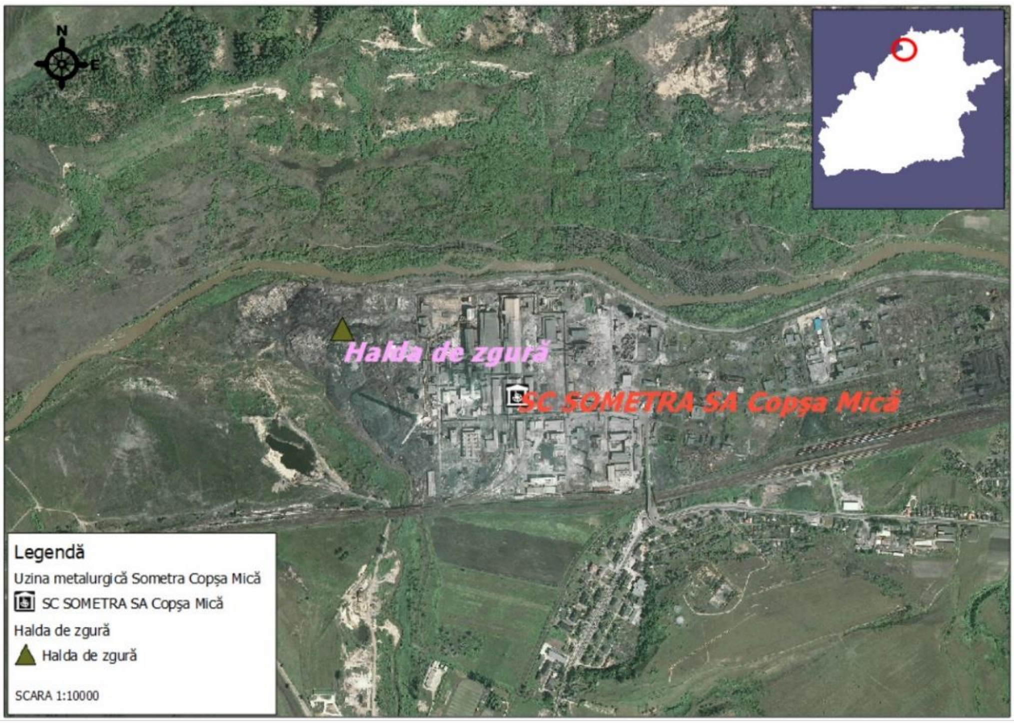
Map no. 2 highlights a heavily polluted area in Alba County, concentrated around the town of Zlatna, a region with a long tradition of metal ore exploitation in the Apuseni Mountains. Mining activity in this area dates back to the Dacian period, being intensified starting in the 18th century, while mines such as Haneș and Larga played an essential role in the extraction of polymetallic ores, especially copper, but also gold, silver, lead, and zinc. Throughout the 19th and 20th centuries, these operations were expanded and modernized, reaching their peak during the communist period, when Zlatna became an important metallurgical center. However, this development was accompanied by the massive accumulation of waste and environmental degradation. The closure of mining operations, officially completed in 2003, did not eliminate the negative effects, as the region remains affected by significant historical pollution caused by heavy metals and mining waste.



Map 2 – Zlatna pollution area and mining regions in the Apuseni Mountains

A major role in the intensification of pollution was also played by the industrial activity carried out within the copper processing plant in Zlatna, which generated emissions of toxic substances into the atmosphere and contributed to the contamination of soil and water in the surrounding areas. In addition, waste rock dumps such as the one at Pătrângeni and the tailings pond at Valea Mică represent additional sources of pollution, indicating areas where mining waste was extensively deposited. Thus, the entire Zlatna region is characterized as an area with a high level of pollution, where the cumulative impact of mining exploitation and industrial activities has generated long-lasting effects on the environment and on the quality of life.

Map no. 3 highlights an important pollution area located near Alba County, namely the town of Copșa Mică.



Map 3 – Industrial pollution area of Copșa Mică (Sometra SA)

This locality is known at the national level for its intense historical pollution, generated by industrial activities, especially ore smelting, refining, and processing (mainly non-ferrous metals such as zinc and lead were processed). The presence of the metallurgical plant indicates the main source of contamination, as it was responsible for massive emissions of toxic substances into the air over time.

Near the plant, a slag heap can be observed, an industrial waste deposit resulting from metal processing activities. This contributes to the pollution of soil and water, and fine particles can be carried by the wind, also affecting nearby areas. Due to these factors, the level of pollution in Copșa Mică has long been considered extremely high, with visible effects on the environment and the health of the population.\

To further investigate the environmental quality of the identified polluted areas, a detailed assessment was carried out, focusing on the impact of contamination on well and spring water resources.

2.1.2.1. Impact of Pollution on Well and Spring Waters in Alba County

The initial study aimed to identify and apply specific methods for assessing the degree of pollution and contamination of drinking water, with a focus on sources from public wells and springs. The study was carried out in plain, hilly, and mountainous areas located in Alba County, according to Table 2.1.1. The county was divided into five zones, as shown in Figure 2.1.1, centered around the main cities of the county.

Table 2.1.1. Study areas in Alba County

Area no.	**Urban Areas**
I	Alba Iulia - Teiuș
II	Sebeș - Cugir
III	Cîmpeni – Abrud - Zlatna
IV	Blaj
V	Aiud - Ocna Mureș

Figure 2.1.1. The map of Alba County and the investigated areas

<u>*Area no. I - Alba Iulia - Teiuș*</u>

Research on the quality of drinking water was carried out in Alba Iulia (urban area), while groundwater contamination was investigated across different sources and locations within the study area.

This investigation was structured on two levels:

1) A study based on the analysis of <u>spring and well water samples collected within the Alba Iulia area (urban)</u>. A total of 4 public spring water samples and 4 well water samples from different areas of Alba Iulia were collected. The sampling locations were randomly selected to ensure the broadest possible coverage of the municipality.

2) A study of <u>the variation in time of physico-chemical and microbiological parameters</u> was carried out for a single urban water source located in Pâclișa – Alba Iulia, belonging to a company involved in the production of soft drinks and bottled water. As part of the research, quarterly water samples were collected and analyzed before physico-chemical treatment, in order to evaluate the evolution of water quality over time [10]. This aimed to monitor the physico-chemical and microbiological parameters of the water over time.

1) The results obtained from the analyses of the physico-chemical parameters for spring and well water samples collected from Alba Iulia (Area no. I) are presented in Table 2.1.2.

Table 2.1.2. Results of analyses for the physico-chemical parameters of spring and well water from the urban area

Parameter **Source**	**pH**	**Hardness, °dH**	**NO_2^-, mg/L**	**NO_3^-, mg/L**	**NH_4^+, mg/L**	**Acidity, mg/L**	**Alkalinity, mg/L $CaCO_3$**	**Ca^{2+}, mg/L**	**Mg^{2+}, mg/L**	**Cd^{2+}, mg/L**	**Cu^{2+}, mg/L**	**Pb^{2+}, mg/L**
Bărăbanț Spring	7.71	22.44	ND	22.0	ND	0.8	176.2	99.39	32.10	0.003	ND	ND
Şanţurile Cetăţii Spring - Alba Iulia	7.54	21.31	0.05	21.0	ND	0.9	124.0	121.84	42.80	$6.55\cdot10^{-4}$	0.046	0.007
Tolstoi area Spring - Alba Iulia	7.44	26.92	ND	9.0	ND	0.7	185.5	97.79	37.93	$1.91\cdot10^{-4}$	ND	ND
Brândușei street Spring - Alba Iulia	7.67	37.02	0.03	0.5	0.2	1.1	180.1	149.09	68.09	$3.12\cdot10^{-4}$	ND	ND
Alba Iulia Well – Moţilor Street	8.29	27.37	0.08	20.5	0.3	0.8	196.9	134.66	36.96	ND	0.014	0.003
Alba Iulia Well – Regina Maria Street	7.85	27.15	0.05	28.9	0.3	1	187.8	129.85	38.91	0.004	ND	ND
Alba Iulia Well – Lalelelor Street	7.23	36.75	ND	**55.7**	ND	1.3	256.7	216.27	27.23	$5.74\cdot10^{-4}$	0.059	0.009
Alba Iulia Well – Ipotești Street	7.83	30.74	0.03	0.2	0.1	0.6	166.2	153.90	39.88	$3.08\cdot10^{-4}$	0.052	0.002
Normal values*	***≥ 6.5; ≤ 9.5***	*> 5*	***0.50***	***50***	***0.50***	***-***	***-***	***-***	***-***	***0.005***	***2***	***0.01***

*Note: ND - Not detected; * - according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2184.*

A single exceedance of the maximum admissible limits was identified: the well water sample collected from Lalelelor Street (Alba Iulia), showed a nitrate [11] concentration exceeding the legal limit of 50 mg/L for drinking water Trace metals were detected at low concentrations in the water samples, and their elemental levels were measured using ICP-MS with an Agilent 7700x instrument (Agilent Technologies, Inc., Tokyo, Japan).

Table 2.1.3 summarizes the experimental results for the microbiological parameters of the analyzed waters – public spring water and well water from Alba Iulia.

Table 2.1.3. Results of analyses for water samples from Alba Iulia.

Parameter **Source**	**TVC, CFU/mL**		**Total coliforms /100 mL**	***E.coli*/ 100 mL**	**Enterococci /100 mL**	***Pseudomonas aeruginosa* /250 mL**
	22^{0}C	37^{0}C				
Bărăbanț Spring	1	ND	ND	ND	ND	ND
Şanţurile Cetăţii Spring - Alba Iulia	37	**76**	**42**	**34**	**17**	ND
Tolstoi area Spring - Alba Iulia	**190**	**432**	**401**	**348**	**23**	ND
Brânduşei street Spring - Alba Iulia	3	**80**	**22**	**15**	ND	ND
Alba Iulia Well – Moţilor Street	5	**29**	**13**	**13**	ND	ND
Alba Iulia Well – Regina Maria Street	20	**95**	**31**	**23**	**49**	ND
Alba Iulia Well – Lalelelor Street	**140**	**162**	**20**	ND	**130**	ND
Alba Iulia Well – Ipotești Street	42	**109**	**102**	**79**	ND	ND
Normal values*	***≤100***	***≤25***	***0****	***0***	***0***	***0***

*Note: ND - Not detected; * - according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2184; ** - according to Law 458/2002 on the quality of drinking water*

Most of the public well and spring water samples from Alba Iulia show total coliforms, *E. coli*, and enterococci levels above normal limits, indicating fecal contamination and potential human health risks. Additionally, high total viable counts (TVC) in some sources suggest abundant microbial flora, possibly linked to local pollution or insufficient source maintenance. In contrast, *Pseudomonas aeruginosa* [12] was not detected, indicating the absence of this opportunistic bacterium.

Analysis of water samples collected from public wells in the rural areas of the Ampoi River basin revealed widespread microbiological contamination exceeding the permissible limits established for drinking water, indicating significant environmental pollution, with only a few isolated cases meeting the required standards. [13,14].

Table 2.1.4. Results of analyses for public well water samples from the Ampoi River basin.

Parameter / **Source**	**pH**	**Hardness, °dH**	**Alkalinity, g/L $CaCO_3$**	**Acidity, mg/L**	**TVC, CFU/mL**		**Total coliforms/ 100 mL**	***E.coli*/ 100 mL**	**Enterococci /100 mL**
					22°C	37°C			
Feneş	6.9	17.2	150.4	1.0	ND	8	ND	ND	**2**
Presaca Ampoiului	7.25	14.3	113.3	4.0	25	6	ND	ND	ND
Poiana Ampoiului	7.4	27.3	181.0	3.5	22	ND	ND	ND	ND
Meteş	7.7	39.2	283.6	7.0	**$2.0\cdot10^{2}$**	**$1.7\cdot10^{2}$**	120	94	**9**
Tăuţ	7.2	45.0	320.8	5.5	**$1.2\cdot10^{2}$**	17	ND	ND	**16**
Şard	7.5	23.1	163.2	6.0	**$1.2\cdot10^{2}$**	18	ND	ND	**4**
Miceşti	7.5	26.4	195.1	3.5	**$1.1\cdot10^{2}$**	4	ND	ND	ND
Normal values*	***6.5 – 9.5***	**-**	**-**	**-**	***≤100***	***≤25***	***0****	***0***	***0***

*Note: ND - Not detected; * - according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2184; ** - according to Law 458/2002 on the quality of drinking water.*

The pH [15] of water from public wells in the Ampoi River basin falls within normal limits, indicating a good chemical balance. The high hardness [16] and alkalinity [17] values reflect increased mineralization, while variations in acidity [18] may indicate local pollution influences.

All public well water samples studied from the rural areas of Presaca Ampoiului and Poiana Ampoiului were negative for the presence of microorganisms. Coliform bacteria and *E. coli* [19] were detected only in the well water sampled from Meteș. Enterococci [20] were identified in well water collected from Feneș, Meteș, Tăuț, and Șard. The total number of microorganisms at 22°C and 37°C [21] exceeded the limits established by Law no. 96/2024 (approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption), which transposes European Parliament and Council Directive (EU) 2020/2184, in the well water from Meteș, Tăuț, Șard, and Micești, when the results are compared with the standards for bottled water.

2) Figures 2.1.2–5.1.8 illustrate the quarterly variations over time of the main physico-chemical and microbiological parameters for the analyzed groundwater source located in Alba Iulia (Pâclişa) – Area No. I. This dataset is particularly important considering the intended use of the water as an ingredient or raw material in the production of soft drinks, as well as for bottling as drinking water.

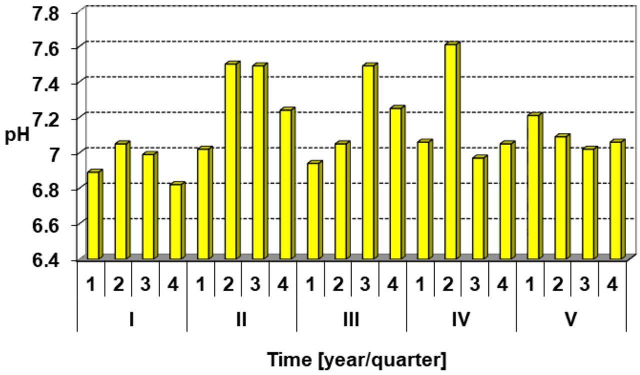


Figure 2.1.2. Variation of pH in groundwater from the Pâclișa (Alba Iulia) site

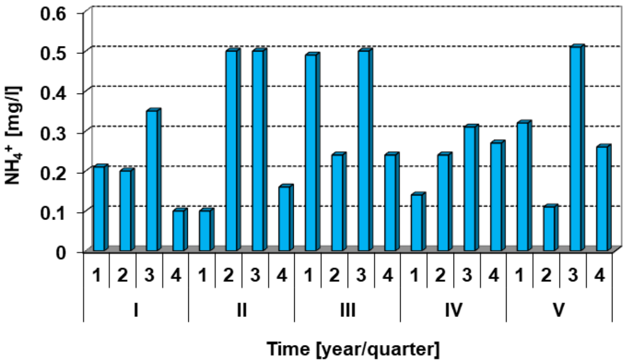


Figure 2.1.3. Variation of ammonium ions in groundwater from the Pâclișa (Alba Iulia) site

The concentration of hydrogen ions in water plays an important role in the selection of organisms and in the structural organization of biocenoses. The pH of the samples collected from the analyzed water source in Alba Iulia falls within the limits permitted by current legislation, with values ranging between 6.82 and 7.61. Ammonia in groundwater and surface waters originates mainly from the decomposition of organic matter or from animal and human waste. The presence of ammonia indicates recent contamination with microorganisms.

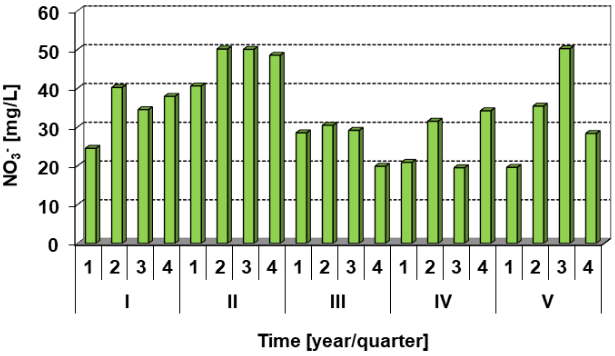


Figure 2.1.4. Variation of nitrates in groundwater from the Pâclișa (Alba Iulia) site

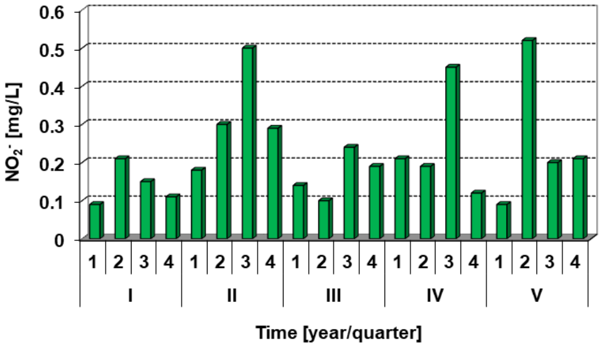


Figure 2.1.5. Variation of nitrites in groundwater from the Pâclișa (Alba Iulia) site

Nitrates may originate from soil leaching or from contamination of water by nitrogen-based fertilizers, wastewater, or domestic and industrial effluents. No significant changes in nitrate concentrations were observed over time in the analyzed water sources from Alba Iulia. Nitrites result from the incomplete oxidation of organic substances, and their presence in water usually indicates older or ongoing contamination.

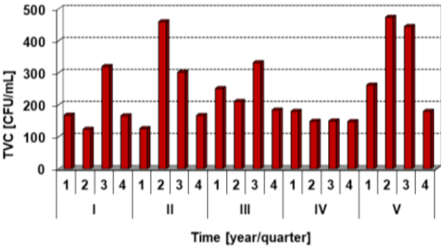


Figure 2.16. Variation of total viable count in groundwater from the Pâclișa (Alba Iulia) site

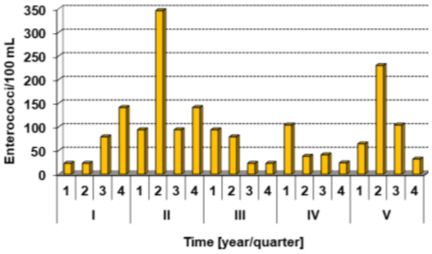


Figure 2.1.7. Variation of enterococci in groundwater from the Pâclișa (Alba Iulia) site

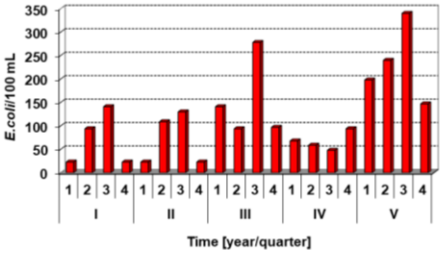


Figure 2.1.8. Variation in *E. coli* in groundwater from the Pâclișa (Alba Iulia) monitoring site

The seasonal variations of physico-chemical and microbiological parameters in the analyzed groundwater source over the five-year monitoring period reflect typical patterns for such water bodies.

Microbiological indicators (TVC, *E. coli*, and enterococci) generally peaked in spring and summer (quarters II and III), likely due to higher temperatures, increased biological activity, and enhanced surface runoff or infiltration. The lowest values were observed during winter (quarters I and IV), when microbial activity is reduced.

Area no. II - Sebeş - Cugir

The analysis of the microbiological parameters (total viable count expressed as CFU/ml at 37°C, *E. coli*, and enterococci/100 ml) in water samples collected from the localities of Boz, Doștat, Pianu de Jos, Sebeş, and Vințu de Jos reveals a variable level of contamination, but one that is present at all investigated sites, with significant differences between locations and sampling periods.

Table 2.1.5 presents the results of the total viable count (CFU/ml at 37°C), reflecting the microbial load identified in the collected water samples.

Table 2.1.5. Results of the parameter: total viable count (CFU/ml at 37°C) in the collected water samples

No.	Locality	Q1 2012	Q4 2011	Q3 2011	Q2 2011	Q1 2011
1	Boz	**103**	**87**	**106**	**85**	**124**
2	Doştat	**250**	**173**	**151**	**195**	**189**
3	Pianu d Jos	**146**	**124**	**250**	**90**	**150**
4	Sebeş	**102**	**96**	**94**	**106**	**84**
5	Vinţu de Jos	**460**	**380**	**490**	**590**	**430**

The total viable count (CFU/ml) values indicate the overall bacterial load of the water and highlight significant differences between the investigated localities. Vințu de Jos records the highest values, ranging from 380 to 590 CFU/ml, indicating severe and persistent microbiological pollution, considerably higher than in the other studied areas. Doștat and Pianu de Jos also show elevated and fluctuating values, between 151 and 250 CFU/ml, suggesting unstable microbiological water quality and a continuous influence of contamination sources. In Boz, moderate values are observed, ranging from 85 to 124 CFU/ml, indicating the presence of contamination, but at lower levels compared to the previously mentioned areas. Sebeș shows the lowest values in the study area, between 84 and 106 CFU/ml; however, exceedance of permissible limits confirms that the water is not microbiologically compliant. All investigated localities exceed the accepted thresholds, indicating a generalized contamination of groundwater.

Table 2.1.6 presents the results of the *E. coli*/100 ml, highlighting the level of fecal contamination in the analyzed water samples.

Tabelul 2.1.6. Results of the parameter: *E. coli*/100 ml in the collected water samples

No.	Locality	Q1 2012	Q4 2011	Q3 2011	Q2 2011	Q1 2011
1	Boz	**56**	**35**	**24**	**ND**	**74**
2	Doştat	**4**	**24**	**52**	**15**	**24**
3	Pianu d Jos	**8**	ND	ND	ND	**7**
4	Sebeş	ND	ND	ND	ND	**5**
5	Vinţu de Jos	**34**	**48**	**59**	**69**	**56**

The presence of *E. coli* is a direct indicator of recent fecal contamination in groundwater. Vințu de Jos shows consistently elevated values, ranging between 34 and 69 CFU/100 ml, indicating persistent and active fecal contamination throughout the entire monitoring period. In Boz, significant variations are observed, from absence to 74 CFU/100 ml, suggesting intermittent sources of fecal pollution. Doștat records moderate values, ranging from 4 to 52 CFU/100 ml, indicating temporal variability in contamination levels. In Pianu de Jos, values are generally low, with occasional presence of the bacterium, confirming a microbiological risk of lower intensity. Sebeș shows absence of *E. coli* in most samples, with only one positive value (5 CFU/100 ml), suggesting the best relative microbiological quality among the investigated localities, although not fully safe from a sanitary perspective.

Table 2.1.7 presents the results of the parameter Enterococci/100 ml, indicating fecal contamination in the collected water samples.

Table 2.1.7. Results of the parameter: Enterococci/100 ml in the collected water samples

No.	**Locality**	**Q1 2012**	**Q4 2011**	**Q3 2011**	**Q2 2011**	**Q1 2011**
1	Boz	**24**	**15**	**29**	**37**	**52**
2	Doştat	**40**	**52**	**78**	**41**	**36**
3	Pianu d Jos	**56**	**85**	**128**	ND	**16**
4	Sebeş	**8**	**12**	**9**	**14**	**11**
5	Vinţu de Jos	**42**	**67**	**62**	**89**	**61**

The presence of enterococci reflects older and more persistent fecal contamination of groundwater. In Vințu de Jos, high and relatively constant values are recorded, ranging between 42 and 89 CFU/100 ml, confirming chronic and stable fecal pollution over time. Pianu de Jos shows very large variations, from absence to 128 CFU/100 ml, indicating episodes of severe contamination and instability in water quality. In Doștat and Boz, moderate values are observed, between 15–78 CFU/100 ml and 15–52 CFU/100 ml respectively, suggesting persistent but lower-intensity contamination. Sebeș records the lowest values, between 8 and 14 CFU/100 ml, indicating the lowest fecal load in the area; however, the constant presence of this indicator still reflects anthropogenic influence on water quality.

The combined analysis of the three microbiological indicators highlights the presence of variable but widespread contamination of groundwater in the Sebeș–Cugir area. Vințu de Jos stands out as the locality with the most severe and consistent microbiological pollution, while Doștat and Pianu de Jos exhibit moderate to severe contamination, characterized by significant temporal fluctuations. Boz shows moderate but persistent contamination, whereas Sebeș presents the best relative values, although not fully compliant with drinking water standards.

The results indicate diffuse fecal pollution of groundwater throughout the investigated area, likely caused by infiltration from wastewater, inadequate sewage systems, and agricultural and livestock activities. Consequently, the groundwater quality in this region is generally unsuitable for direct consumption, requiring appropriate treatment measures and continuous monitoring.

Area no. III - Cîmpeni – Abrud - Zlatna

For the study on groundwater contamination in the Abrud–Cîmpeni mountain area, Alba County, a total of 7 spring water samples were collected [22].

The results obtained from the analyses of the spring water samples collected from the Abrud–Cîmpeni area are presented in Tables 2.1.8–2.1.9.

Table 2.1.8. Analysis results for the physicochemical parameters of spring water from the mountain area.

Parameter **Water source: Spring**	**pH**	**Hardness, °dH**	**Alkalinity, mg/L $CaCO_3$**	**Acidity, mg/L**	**Ca^{2+}, mg/L**	**Mg^{2+}, mg/L**	**NO_2^-, mg/L**	**NO_3^-, mg/L**	**NH_4^+, mg/L**	**Cu^{2+}, mg/L**	**Cd^{2+}, mg/L**	**Pb^{2+}, mg/L**
Coleşeni	6.93	8.30	62.1	0.9	8.85	3.23	< 0.02	22.0	0.15	0.056	ND	ND
Dealu Mare	6.55	9.42	71.3	0.6	8.01	5.99	< 0.02	0.4	< 0.05	ND	$1.815\cdot10^{-4}$	ND
Ciuta	7.39	17.27	124.4	1.2	5.65	5.34	0.05	21.0	0.37	0.053	$2.653\cdot10^{-4}$	0.001
Gura Cornei	6.69	11.44	81.4	0.9	4.04	3.02	< 0.02	2.8	< 0.05	ND	$9.206\cdot10^{-5}$	ND
Bâdea	6.77	7.18	50.7	0.5	6.03	1.40	< 0.02	9.0	0.2	0.073	$2.34\cdot10^{-4}$	0.006
Valea Sălîştei	6.67	11.22	81.5	1	11.22	1.83	< 0.02	1.5	< 0.05	ND	$4.165\cdot10^{-4}$	ND
Lucia	7.34	7.4	50.8	1.1	5.8	0.97	< 0.02	2.04	< 0.05	ND	ND	ND
Normal values*	***6.5 – 9.5***	***>5***	***-***	***-***	***-***	***-***	***≤ 0.50***	***≤ 50***	***≤ 0.50***	***≤ 2.0***	***≤ 0.005***	***≤ 0.01***

*Note: ND - Not detected; * - according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2184.*

Table 2.1.9. Analysis results for the microbiological parameters of spring water from the mountain area.

Parameter **Water source (spring)**	**TVC, CFU/mL**		**Total coliforms /100 mL**	***E.coli*/ 100 mL**	**Enterococci /100 mL**	***Pseudomonas aeruginosa* /250 mL**
	22^0C	**37^0C**				
Coleşeni	**160**	**25**	ND	ND	ND	ND
Dealu Mare	**190**	**22**	ND	ND	ND	ND
Ciuta	**120**	**8**	ND	ND	ND	ND
Gura Cornei	**36**	4	ND	ND	2	ND
Bâdea	1	ND	ND	ND	ND	ND
Valea Sălişte	**87**	**23**	ND	ND	4	ND
Lucia	4	ND	ND	ND	ND	ND
Normal values*	***≤100***	***≤25***	***0*****	***0***	***0***	***0***
Normal values for mineral waters – at the source***	***20***	***5***	***0***	***0***	***0***	***0***

*ND-Not detected; * - according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2184; ** - according to Law 458/2002 on the quality of drinking water; ***Decision No. 1,020/2005 for the approval of the Technical Norms for the exploitation and commercialization of natural mineral waters (12 hours after bottling).*

Area no. IV - Blaj

The results obtained from laboratory analyses of the well water samples collected in the Blaj area are presented in Tables 2.1.10.

Table 2.1.10. Physico-chemical parameter values for well water samples from the Blaj area

Wells from the area	**Sample**	**pH**	**NO_3^-, mg/L**	**NO_2^-, mg/L**	**NH_4^+, mg/L**
Valea Lungă	I	6.20	103	0.093	0.017
	II	7.01	97	0.080	0.023
	III	6.83	90	0.062	0.014
	IV	7.15	77	0.050	0.010
	V	7.22	59	0.0021	0.008
Cenade	I	6.87	245	0.169	0.017
	II	6.70	200	0.197	0.023
	III	6.99	232	0.211	0.014
	IV	7.40	152	0.103	0.010
	V	7.52	97	0.092	0.008
Sâncel	I	7.04	227	0.021	0.017
	II	7.15	200	0.019	0.023
	III	7.34	157	0.010	0.014
	IV	7.22	120	0.009	0.010
	V	7.50	90	0.003	0.008
Crăciunelul de Jos	I	7.00	97	0.021	0.011
	II	7.15	60	0.011	0.002
	III	7.25	55	0.010	0.004
	IV	7.40	22	0.004	0.003
	V	7.57	18	0.003	0.001
Blaj	I	6.46	153	0.031	0.005
	II	6.62	169	0.024	0.013
	III	6.81	179	0.013	0.020
	IV	6.73	145	0.006	0.031
	V	7.09	191	0.420	0.016

The results indicate that nitrate (NO_3^-) concentrations exceed the maximum admissible limit of 50 mg/L in almost all well water samples from the Blaj area. The highest exceedances were recorded in Cenade (up to 245 mg/L) and Sâncel (up to 227 mg/L), followed by Blaj and Valea Lungă, indicating significant nitrate contamination of the groundwater.

The pH values generally fall within the acceptable range (6.5–9.5), with only one slightly acidic value recorded in Valea Lungă and Blaj. Nitrite (NO_2^-) and ammonium (NH_4^+) concentrations remain below the maximum permissible limits in all samples, suggesting that nitrate pollution is the dominant water quality issue in the study area.

At the same time, over a period of 5 years, on a quarterly basis, the variation of physico-chemical and microbiological parameters of well water/drilled wells was monitored in other localities in the Blaj area. These include: Șona, Roșia de Secaș, Cenade, Sâncel, Valea Lungă, Cergău, Sânmiclăuș, and Jidvei.

Table 2.1.11 presents the temporal variation of the physico-chemical and microbiological parameters for the analyzed water source from Șona.

Table 2.1.11. Variation of physico-chemical and microbiological parameters at the water source in Șona

Year	**Quarter**	**Microbiological**			**Physico-chemical**			
		TVC /mL	***E.coli*/ 100 mL**	**Enterococci /100 mL**	**pH, mg/L**	**NO_3^-, mg/L**	**NO_2^-, mg/L**	**NH_4^+, mg/L**
I	1	168	23	23	6.89	24.51	0.09	0.21
	2	124	94	23	7.05	40.2	0.21	0.2
	3	320	141	79	6.99	34.5	0.15	0.35
	4	166	23	141	6.82	37.9	0.11	0,1
II	1	126	23	94	7.02	40.6	0.18	0,1
	2	460	109	346	7.5	50.12	0.3	0,5
	3	302	130	94	7.49	50.04	0.5	0.5
	4	167	23	141	7.24	48.52	0.29	0.16
III	1	251	141	94	6.94	28.51	0.14	0.49
	2	211	94	79	7.05	30.45	0.1	0.24
	3	332	278	23	7.49	29.15	0.24	0.5
	4	184	97	23	7.25	19.89	0.19	0.24
IV	1	220	68	104	7.06	20.9	0.21	0.14
	2	149	59	38	7.61	31.5	0.19	0.24
	3	150	48	41	6.97	19.5	0.45	0.31
	4	148	94	24	7.05	34.2	0.12	0.27
V	1	262	198	64	7.21	19.59	0.09	0.32
	2	494	240	230	7.09	35.4	0.52	0.11
	3	475	340	104	7.02	50.22	0.2	0.51
	4	259	168	91	7.54	40.61	0.34	0.42
Normal values*		***25***	***0***	***0***	***≥ 6.5; ≤ 9.5***	***50***	***0.50***	***0.50***

*Note: * - according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2184.*

The results obtained from the quarterly monitoring of microbiological and physico-chemical parameters of well water in Șona indicate exceedances of the limits established by the current drinking water legislation, particularly with regard to bacteriological parameters, for all analyzed samples. Throughout the monitored period (Years I–V), high values of TVC, *E. coli*, and enterococci were frequently recorded, highlighting persistent microbiological contamination. The maximum value of fecal streptococci (enterococci), reaching 346 CFU/100 ml, was observed in Year II, Quarter 2. From a physico-chemical perspective, most samples generally complied with the legislative limits (according to Law no. 96/2024 (approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption), which transposes European Parliament and Council Directive (EU) 2020/2184) [23]; however, minor exceedances were occasionally recorded for nitrate, nitrite, and ammonium concentrations in certain quarters.

Table 2.1.12 presents the temporal variation of physico-chemical and microbiological parameters for the analyzed water source from Roșia de Secaș.

Table 2.1.12. Variation of physico-chemical and microbiological parameters at the water source in Roşia de Secaş

Year	Quarter	Microbiological			Physico-chemical			
		TVC /ml	***E.coli*/ 100 ml**	**Enterococi /100 ml**	**pH [mg/l]**	**NO_3^- [mg/l]**	**NO_2^- [mg/l]**	**NH_4^+ [mg/l]**
I	1	142	20	8	7.56	40.32	0.06	0.24
	2	250	23	20	7.59	50.01	0.3	0.29
	3	297	27	22	7.29	45.06	0.33	0.5
	4	96	79	10	7.02	35.6	0.16	0.17
II	1	250	79	79	7.26	25.64	0.18	0.38
	2	157	79	23	7.02	29.3	0.07	0.11
	3	206	79	12	7.5	39.5	0.15	0.4
	4	149	23	23	8.02	40.2	0.09	0.29
III	1	106	23	18	7.56	42.1	0.12	0.31
	2	286	79	36	7.22	45.03	0.3	0.5
	3	254	28	23	7.02	38.64	0.25	0.43
	4	165	36	25	7.52	44.9	0.16	0.34
IV	1	150	78	36	7.42	44.68	0.16	0.37
	2	315	96	72	8.2	50.1	0.34	0.5
	3	340	231	49	8.06	47.2	0.27	0.42
	4	310	168	68	7.59	46.5	0.23	0.34
V	1	450	369	71	7.29	49.9	0.21	0.29
	2	269	190	64	8.01	74.8	0.5	0.39
	3	520	420	43	7.02	55.02	0.5	0,5
	4	486	360	44	7.51	40.23	0.46	0.42

The quarterly monitoring of well water from Roșia de Secaș shows that several microbiological parameters frequently exceed the legal limits, particularly total aerobic mesophilic bacteria (TVC), *E. coli*, and fecal streptococci (enterococci). The simultaneous presence of *E. coli* and enterococci in multiple samples indicates persistent fecal contamination of the water source. Physico-chemical parameters generally comply with legal standards, with nitrate, nitrite, and ammonium concentrations remaining within or below the permitted limits.

Table 2.1.13 presents the variation of physico-chemical and microbiological parameters over time for the analyzed water source in Cenade.

Table 2.1.13. Variation of physico-chemical and microbiological parameters at the water source in Cenade

Year	Quarter	Microbiological			Physico-chemical			
		TVC/mL	***E.coli*/ 100 mL**	**Enterococi/ 100 mL**	**pH, mg/L**	**NO_3^-, mg/L**	**NO_2^-, mg/L**	**NH_4^+, mg/L**
I	1	89	0	0	6.93	34.2	0.09	0.34
	2	93	0	23	7.2	49.92	0.12	0.21

	3	250	0	23	6.95	45.34	0.31	0.46
	4	156	0	0	7.1	39.4	0.17	0.38
II	1	146	0	20	7.08	42.03	0.08	0.25
	2	261	0	16	7.25	54.2	0.19	0.47
	3	292	0	0	7.1	50.12	0.24	0.71
	4	298	0	0	7.6	49.24	0.25	1.8
III	1	104	0	7	7.42	43.2	0.31	0.54
	2	293	0	0	7.34	57.89	0.38	5.4
	3	204	0	0	7.8	50.55	0.29	1.6
	4	96	0	0	7.92	39.24	0.19	0.29
IV	1	146	0	0	7.02	30.45	0.32	0.35
	2	260	0	0	7.63	56.82	0.63	0.45
	3	126	0	0	7.22	39.24	0.25	0.26
	4	53	0	0	6.98	32.42	0.17	1.2
V	1	94	0	0	7.06	46.9	0.35	0.51
	2	262	0	0	7.62	50.04	0.27	3.71
	3	140	0	0	7.09	34.69	0.34	0.26
	4	89	0	2	6.95	40.2	0.42	0.34

Assuming that human activity influences water quality through contamination or pollution, the monitored well in Cenade shows relatively better hygienic quality compared to other analyzed sources, but it remains non-potable according to Law no. 96/2024 approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption, (based on limits set for drinking water). Sporadic exceedances of total aerobic mesophilic bacteria (TVC) and occasional presence of fecal streptococci were observed, while *E. coli* was not detected. From a physico-chemical perspective, nitrate (NO_3^-) and ammonium (NH_4^+) concentrations occasionally exceeded legal limits.

Table 2.1.14 presents the temporal variation of physico-chemical and microbiological parameters for the analyzed water source from Sâncel.

Table 2.1.14. Variation of physico-chemical and microbiological parameters at the water source in Sâncel

Year	**Quarter**	**Microbiological**			**Physico-chemical**			
		TVC /mL	***E.coli*/ 100 mL**	**Enterococci /100 mL**	**pH, mg/L**	**NO_3^-, mg/L**	**NO_2^-, mg/L**	**NH_4^+, mg/L**
I	1	137	94	27	7.02	33.2	0.15	0.09
	2	511	342	169	7.82	49.6	0.82	0.24
	3	296	87	141	7.25	45.8	0.53	0.38
	4	252	140	29	7.43	35.68	0.27	0.29
II	1	298	70	23	6.99	39.5	0.27	0.24
	2	249	79	26	7.02	44.9	0.29	0.5
	3	500	346	110	7.98	39.54	0.28	0.51
	4	154	98	23	7.02	44.02	0.28	0.34
III	1	305	133	91	6.89	34.52	0.09	0.28
	2	520	160	346	7.42	44.5-	0.28	0.39
	3	510	352	92	8.04	52.1	0.35	0.51
	4	286	141	27	8.2	37.91	0.21	0.42
IV	1	202	35	24	8.5	49.3	0.34	0.34
	2	417	240	90	8.95	47.6	0.51	0.45
	3	397	95	215	8.04	50.3	0.42	0.59
	4	309	142	86	8.6	44.6	0.2	0.34

V	1	495	360	62	8.5	38.2	0.19	0.29
	2	312	110	120	7.99	52.1	0.5	0.51
	3	532	430	97	7.68	42.3	0.33	0.26
	4	497	295	146	8.05	39.8	0.14	0.19

The drinking water from the individual well in Sâncel shows significant microbiological contamination throughout the entire monitoring period for all three analyzed indicators (TVC, *E. coli*, and fecal streptococci). Physico-chemical parameters generally comply with legal limits, with only minor exceedances observed for nitrate, nitrite, and ammonium concentrations. The persistent presence of *E. coli* and enterococci in many samples indicates active and ongoing fecal contamination, posing a clear health risk for consumption.

Table 2.1.15 presents the temporal variation of physico-chemical and microbiological parameters for the analyzed water source from Valea Lungă.

Table 2.1.15. Variation of physico-chemical and microbiological parameters at the water source in Valea Lungă

Year	**Quarter**	**Microbiological**			**Physico-chemical**			
		TVC /mL	***E.coli*/ 100 mL**	**Enterococi /100 mL**	**pH, mg/L**	**NO_3^-, mg/L**	**NO_2^-, mg/L**	**NH_4^+, mg/L**
I	1	249	141	79	7.02	29.6	0.09	0.43
	2	310	160	169	7.52	43.6	0.35	3.15
	3	156	131	14	7.35	28.3	0.12	1.25
	4	164	44	41	7.02	25.6	0.09	0.21
II	1	257	94	74	7.28	33.57	0.1	0.57
	2	362	109	246	6.98	45.7	0.6	1.59
	3	409	246	141	6.99	43.2	0.39	0.50
	4	146	79	23	7.08	38.5	0.25	0.44
III	1	200	146	11	7.54	45.3	0.22	0.35
	2	324	146	160	8.03	52.76	0.48	0.69
	3	310	141	79	8	45.01	0.3	0.42
	4	140	79	23	7.2	31.62	0.2	0.28
IV	1	191	101	90	7.94	38.56	0.1	0.42
	2	211	92	76	7.05	30.45	0.1	0.24
	3	242	178	20	7.49	39.21	0.23	0.42
	4	186	73	18	7.25	29.99	0.39	0.34
V	1	146	79	62	7.63	39.96	0.23	0.41
	2	264	92	83	7.81	42.87	0.13	0.37
	3	239	64	168	7.94	29.85	0.25	0.27
	4	197	43	150	7.80	32.50	0.17	0.24

All analyzed samples from Valea Lungă exceed the legal limit for total aerobic mesophilic bacteria (TVC) and are positive for *E. coli* and fecal streptococci (enterococci), indicating persistent fecal contamination. Physico-chemical parameters generally comply with legal limits, with occasional exceedances of ammonium (NH_4^+). Overall, the water is microbiologically unsafe and non-potable.

Table 2.1.16 presents the temporal variation of physico-chemical and microbiological parameters for the analyzed water source from Cergău.

Table 2.1.16. Variation of physico-chemical and microbiological parameters at the water source in Cergău

Year	Quarter	Microbiological			Physico-chemical			
		TVC /mL	*E.coli*/ 100 mL	Enterococi /100 mL	pH, mg/L	NO_3^-, mg/L	NO_2^-, mg/L	NH_4^+, mg/L
I	1	189	94	94	7.02	38.74	0.04	0.2
	2	504	346	141	7.68	54.01	0.02	0.5
	3	596	442	146	7.52	42	0.04	0.32
	4	185	140	41	7.06	38.07	0.09	0.15
II	1	161	101	60	7.02	42	0.15	0.49
	2	290	46	36	6.98	31.52	0.19	1.2
	3	143	14	94	7.02	50.02	0.02	0.3
	4	145	72	54	7.42	35.06	0.08	0.28
III	1	237	94	79	6.89	44.98	0.23	0.5
	2	398	243	110	6.87	35.27	0.3	2.17
	3	459	242	146	7.35	24.58	0.15	0.5
	4	298	141	94	7.02	45.06	0.09	0.32
IV	1	224	98	54	7.06	32.01	0.31	0.37
	2	330	178	62	7.23	44.6	0.55	1.7
	3	228	103	39	7.5	55.8	0.34	0.28
	4	267	145	84	7.1	45.36	0.38	0.34
V	1	316	161	150	7.09	10.36	0.09	0.45
	2	342	145	107	6.85	15.69	0.04	0.5
	3	298	104	140	6.92	29.3	0.1	0.54
	4	262	101	153	7.85	25.47	0.15	0.24

All analyzed samples from Cergău show a microbial load above 100 CFU/ml, with all samples positive for *E. coli* and fecal streptococci (enterococci), indicating persistent fecal contamination. Physico-chemical parameters generally comply with legal limits, with occasional exceedances of ammonium, nitrate, and nitrite concentrations. Overall, the water is microbiologically unsafe and not suitable for drinking.

Table 2.1.17 presents the temporal variation of physico-chemical and microbiological parameters for the analyzed water source from Sânmiclăuș.

Table 2.1.17. Variation of physico-chemical and microbiological parameters at the water source in Sânmiclăuș

Year	Quarter	Microbiological			Physico-chemical			
		TVC /mL	*E.coli*/ 100 mL	Enterococi /100 mL	pH, mg/L	NO_3^-, mg/L	NO_2^-, mg/L	NH_4^+, mg/L
I	1	102	0	0	6.5	36	0.19	0.46
	2	210	23	23	7.11	49	0.24	0.42
	3	208	26	20	7.06	47	0.28	0.41
	4	167	0	23	6.98	39	0.29	0.29
II	1	158	0	23	6.89	40	0.3	0.24
	2	252	23	0	7.02	18	0.29	0.43
	3	268	22	0	7.16	24	0.08	0.5
	4	242	23	0	6.5	38	0.19	0.48
III	1	196	0	0	7	42	0.24	0.34
	2	158	0	23	7.62	29	0.07	0.27
	3	244	0	23	8.02	31	0.3	0.47
	4	109	23	21	6.41	39	0.21	0.16

IV	1	204	106	49	6.59	52	0.08	0.41
	2	430	240	110	6.87	50	0.5	0.62
	3	301	51	93	7.05	43.2	0.42	0.53
	4	224	23	46	7.06	41.6	0.09	0.21
V	1	315	68	180	6.98	51	0.24	0.19
	2	440	140	210	6.8	68	0.5	0.45
	3	210	95	100	8.2	42	0.19	0.6
	4	194	43	150	7.58	40	0.31	0.23

The well water from Sânmiclăuș shows frequent microbiological exceedances, with total aerobic mesophilic bacteria (TVC) consistently above the legal limit of 20 CFU/ml. *E. coli* was detected sporadically, while fecal streptococci (enterococci) were present in many samples, indicating occasional to persistent fecal contamination. Physico-chemical parameters generally comply with legal limits, although nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) occasionally approached or slightly exceeded permissible values.

Table 2.1.18 presents the temporal variation of physico-chemical and microbiological parameters for the analyzed water source from Jidvei.

Table 2.1.18. Variation of physico-chemical and microbiological parameters at the water source in Jidvei

Year	**Quarter**	**Microbiological**			**Physico-chemical**			
		TVC /mL	***E.coli*/ 100 mL**	**Enterococi /100 mL**	**pH, mg/L**	**NO_3^-, mg/L**	**NO_2^-, mg/L**	**NH_4^+, mg/L**
I	1	142	79	23	7.22	39.52	0.08	0.24
	2	190	109	23	7.02	42.1	0.22	0.29
	3	256	94	23	6.94	34.28	0.28	0.42
	4	136	94	0	7.24	34.67	0.24	0.36
II	1	240	79	23	7.98	45.06	0.34	0.42
	2	280	109	162	7.24	68	0.42	1.4
	3	310	146	79	8.02	44.92	0.29	0.5
	4	167	79	74	7.28	39.51	0.24	0.34
III	1	201	109	23	8	40.61	0.29	0.46
	2	295	142	74	7.68	45	1.14	2.27
	3	269	146	94	7.51	39.51	0.5	0.75
	4	201	94	23	7.24	30.42	0.22	0.37
IV	1	196	110	54	7.69	34.61	0.02	0.49
	2	410	320	34	7.28	50	0.49	1.9
	3	237	140	83	7.68	74.2	0.5	0.54
	4	259	80	72	7.02	45.2	0.28	0.42
V	1	240	106	49	7.26	35.42	0.5	0.38
	2	320	201	76	7.89	43.24	1.4	1.02
	3	157	68	80	8.1	39.4	0.42	0.35
	4	116	97	9	7.02	28.9	0.34	0.24

Microbiological analyses of the water from Jidvei show values exceeding the maximum allowable limits, with all three indicators (TVC, *E. coli*, and enterococci) frequently positive, even during winter and spring months. No sample fully met the microbiological standards, except in Quarter IV (the first year of monitoring). Physico-chemical parameters were generally within legal limits for pH. Nitrate (NO_3^-) reached a maximum of 74.2 mg/l, nitrite (NO_2^-) exceeded permissible values in several quarters (up to 1.4 mg/l), and ammonium (NH_4^+) concentrations were occasionally high (maximum 2.27 mg/l). The water is microbiologically unsafe and shows occasional physico-chemical deviations, making it non-potable according to legislation.

Area no. V - Aiud - Ocna Mureş

To assess the degree of pollution of spring waters in the Aiud–Ocna Mureş area, physico-chemical and microbiological parameters were analyzed for seven public springs located within the Aiud–Ocna Mureş localities, in order to identify possible sources of contamination [24].

Table 2.1.19 presents the values of the physico-chemical parameters and microbiological indicators for the seven spring water samples collected from the Aiud–Ocna Mureş area.

Table 2.1.19. Laboratory test results for physico-chemical parameters of spring waters

Parameter / **Spring source**	**pH**	**Cl^-, mg/L**	**Hardness, °dH**	**Alkalinity, mg/L $CaCO_3$**	**Acidity, mg/L**	**TVC, CFU/mL**		**Total coliforms /100 mL**	***E.coli* / 100 mL**	**Enterococci /100 mL**	***P. aeruginosa* /250 mL**
						22°C	**37°C**				
Războieni	7.4	114.25	32.3	26.4	2.3	4	ND	ND	ND	ND	ND
Noşlac	7.1	55.30	39.7	34.5	2.5	22	ND	ND	ND	ND	ND
Uioara	7	252.96	29.6	20.5	2.7	9	ND	ND	ND	ND	ND
Unirea	7.1	202.25	35	22.4	2.8	24	ND	ND	ND	ND	ND
Banţa	7.2	175.10	28.7	25.7	2.9	**$3.5\cdot10^2$**	**$1.5\cdot10^2$**	**12**	**3**	**4**	ND
Căprioara	6.9	53.88	37.5	21.1	2.7	25	1	ND	ND	ND	ND
Cisteiu de Mureş	7	256.20	25.13	17.8	1.8	**$1.2\cdot10^2$**	23	ND	ND	2	ND

Chloride concentrations [25] in several water sources exceeded the recommended drinking water limit of 250 mg/L, which may result in a salty taste and suggests potential anthropogenic influence.

Spring water samples collected and analyzed by the Ocna Mureş area were negative for the presence of microorganisms, except spring Banţa and Cisteiu of Mureş, where was identified a microbiological growth exceeding the maximum allowed by law. The faecal strepotococci (enterococcus) and coliforms are present only in spring water taken from source Banţa (if we compare the values obtained with the limits for bottled drinking water). The total number of germs that grow at 22°C, respectively 37°C exceeds the limits stipulated in Romanian Law no. 96/2024 (approving Government Emergency Ordinance no. 7/2023 on the quality of water intended for human consumption), which transposes European Parliament and Council Directive (EU) 2020/2184, in the waters of the two springs.

For spring Banţa was obtain a microbial growth of $3.5\cdot10^2$, respectively $1.5\cdot10^2$ colony forming units and for the spring Cisteiu of Mureş: $1.20\cdot10^2$, respectively 23 colonies.

For the study on groundwater pollution in the Blaj area, 25 well water samples were collected from different localities: Valea Lungă, Cenade, Sâncel, Crăciunelul de Jos, and Blaj, all located in the Blaj area [26]. The samples were collected in April 2012 from different sources within each location (five samples from each commune/village).

2.1.2.2. Time Assessment of Pollution in Spring Water Sources of Alba County

Between 2017 and 2019, 132 public water sources in Alba County were monitored [27]. Only 38 of these sources met the standards for potable water, while the remaining 94 sources exhibited one or more physico-chemical or microbiological parameters exceeding the legally permissible limits, indicating significant pollution. Figure 2.1.9 illustrates the water quality analysis results for these sources in 2017.

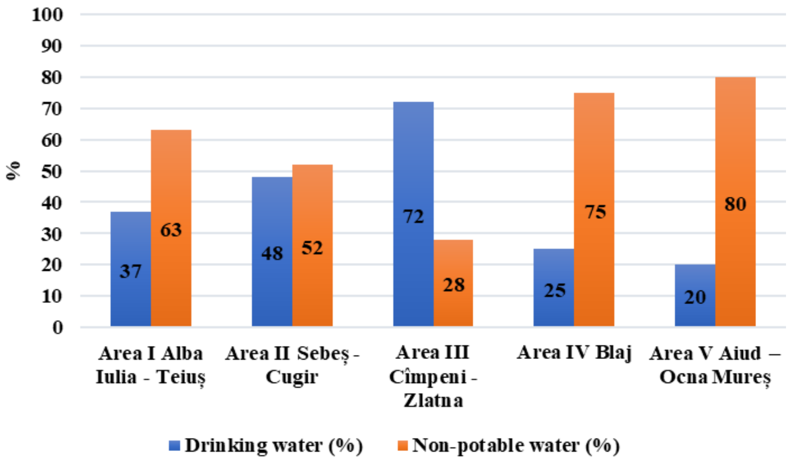


Figure 2.1.9. Quality of public spring water sources in the five areas of Alba County.

The monitoring of spring water contamination in Alba County was based on the analysis of several physico-chemical and microbiological parameters, including nitrate (NO_3^-), nitrite (NO_2^-), ammonium ions (NH_4^+), the total number of mesophilic aerobic bacteria, coliform bacteria, *Escherichia coli*, and intestinal enterococci. Monthly water samples were collected over a three-year period, from January 2017 to December 2019, from representative springs across five areas of the county. The analyses revealed that many springs exhibited levels of these parameters exceeding permissible limits, highlighting potential health risks from microbial and chemical pollution.

The analysis of the obtained results (Figure 2.1.9) indicates that the majority of the investigated spring water sources are not suitable for drinking. The highest percentage of non-potable water sources was recorded in Area V (Aiud–Ocna Mureș), where 80% of the springs exceeded the permissible limits, while the lowest percentage was observed in Area III (Câmpeni–Zlatna), with only 28% of the sources classified as non-potable. These results also highlight that the mountainous region (Area III) provides safer and higher-quality water compared to the other regions. The springs located in the Câmpeni area show the closest compliance with drinking water standards in Alba County.

Living organisms, dead organic matter, and mineral and organic compounds dissolved in aquatic ecosystems are never in an inert state. Instead, they undergo continuous transformation and circulation processes that condition the existence of living systems. As a result, complex interactions develop among ecosystem components, contributing to the dynamic stability of these systems and to the maintenance of ecological balance.

Nitrogen represents an essential nutrient in aquatic ecosystems and can enter water bodies through multiple pathways. In natural waters, nitrogen occurs in several forms, including molecular nitrogen, nitrogen oxides, ammonia, ammonium ions, nitrites, and nitrates. Within the ecosystem, nitrogen participates in the biogeochemical cycle, which is controlled by complex interactions among physical, chemical, and biological factors in the aquatic environment. Algae can utilize both dissolved molecular nitrogen and ammoniacal salts (NH_3), and once these are depleted, they can assimilate nitrate-nitrogen (NO_3^-) [28]. Bacteria also play a crucial role in the nitrogen cycle within aquatic ecosystems, as they mediate the transformation of nitrogen compounds. These bacterial processes are reversible, and the direction of transformation depends largely on the concentration of dissolved oxygen. Water samples were analyzed in time, monthly, from January 2017 to December 2019. Experimental data obtained from the analysis of physico-chemical parameters over 3 years are presented comparatively for the representative sources in the five areas in Figures 2.1.10 – 2.1.16.

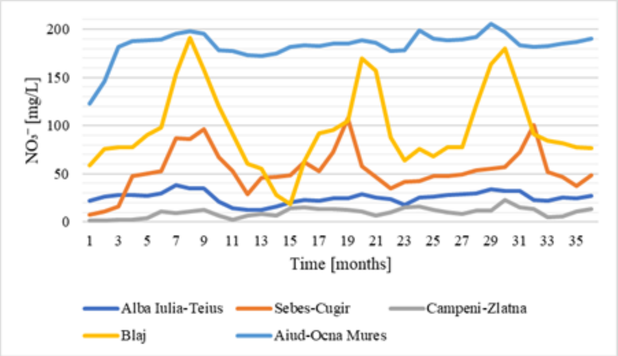


Figure 2.1.10. Variation of nitrate content in time for the 5 springs

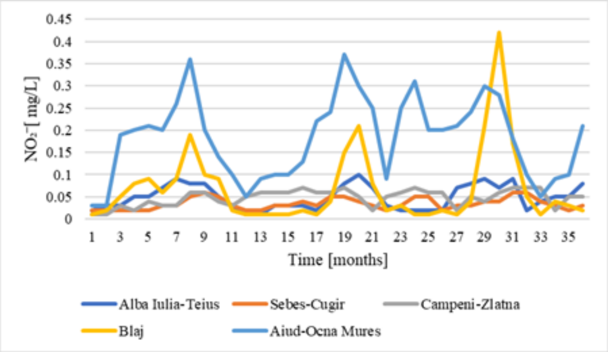


Figure 2.1.11. Variation of nitrite content in time for the 5 springs

Nitrites occur in water as a result of pollution with organic substances, either through partial oxidation of amino compounds or through the reduction of nitrates. Their presence usually indicates older pollution; however, when associated with high concentrations of ammonium ions, it may suggest continuous pollution processes [29].

Ammonium ions generally appear as a consequence of water contamination with organic substances undergoing decomposition and represent the initial stage in the degradation of nitrogenous compounds. Therefore, their presence is often considered an indicator of recent pollution.

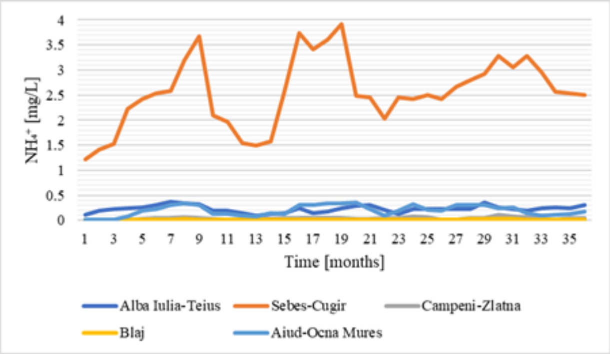


Figure 2.1.12. Variation of ammonium ion content in time for the 5 springs

The experimental data obtained from the analysis of microbiological parameters over the same three-year period are presented comparatively for the representative sources in Figures 5–8.

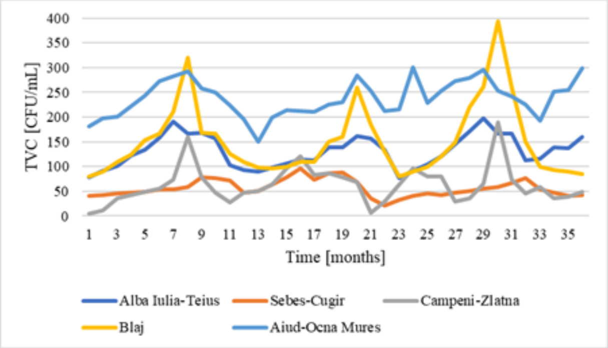


Figure 2.1.13. Variation of the total number of mesophilic aerobic bacteria in time for the 5 springs

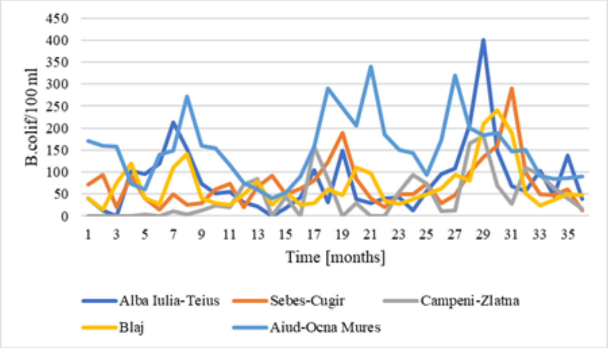


Figure 2.1.14. Variation of the number of coliform bacteria in time for the 5 springs

The results indicate that spring water from different areas presents a relatively high microbial load. The plain regions of Aiud–Ocna Mureș, Alba Iulia–Teiuș, Blaj, and Sebeș show higher microbiological contamination, with significant seasonal variations. In contrast, the mountainous and hilly regions display smaller fluctuations in microbiological parameters over time.

An increased number of mesophilic aerobic bacteria may indicate a potential risk of the presence of waterborne pathogens, including bacteria, viruses, fungi, and parasites. Exceeding the permissible limits for these microbiological indicators suggests that the springs are polluted, most likely due to infiltration from wastewater, manure deposits, or other anthropogenic sources [29].

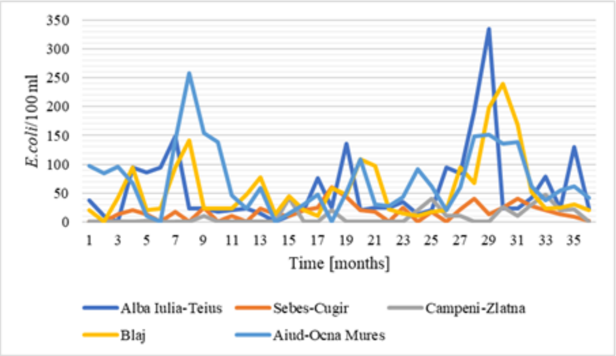


Figure 2.1.15. Incidence of *E.coli* bacteria in time for the 5 sources

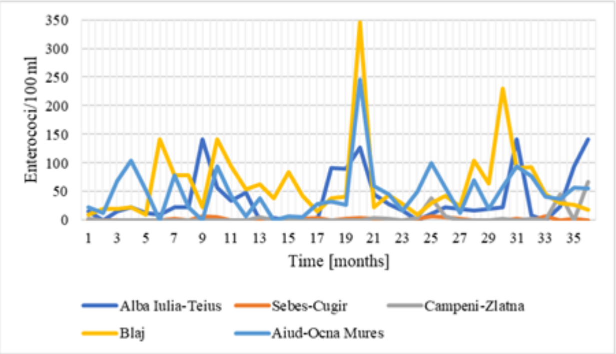


Figure 2.1.16. Incidence of intestinal enterococci in time for the 5 sources

Escherichia coli can be transmitted through the consumption of contaminated water and may cause diseases such as gastroenteritis and sepsis, particularly in children and young individuals of various animal species [30]. Studies have shown that *E. coli* can survive in drinking water from wells for up to six months and in wastewater for approximately four months [29].

The presence of diffuse microbiological pollution in several localities indicates that groundwater is affected, and consequently the natural self-purification processes are altered. Under these conditions, natural systems may no longer be able to effectively fulfil their ecological role as purifiers.

In many cases, the relationship between two or more parameters describing a particular process can be sufficiently strong that the variation of one parameter can be explained by the variation of others. Such functional relationships are known as stochastic or probabilistic links. The study of these relationships has led to the development of multiple correlation theory, which allows the quantitative analysis of interdependencies among environmental variables [31].

In order to develop statistical models describing the relationships between water parameters, the influence of several variables was evaluated through multiple correlation analysis. In this approach, the microbial load (expressed as the total number of aerobic bacteria) was considered the dependent variable, while the concentrations of nitrates, nitrites, and ammonium ions measured over time were treated as independent variables.

Considering the nitrogen cycle in aquatic systems, the dependence of the total microbial count on the presence of ammonium ions, nitrates, and nitrites over time was investigated. For this purpose, case studies were conducted using data obtained from the most polluted spring water sources identified in the five areas of Alba County. These sources were monitored monthly over a three-year period.

In order to describe as accurately as possible, the relationships between nitrate, nitrite, and ammonium concentrations and microbial load over time, a second-order polynomial model was proposed. This approach allows the identification of non-linear relationships between the analyzed parameters and provides a more accurate representation of the observed variations.

Equation (1) represents the general mathematical form of the second-order polynomial model used to describe the correlations between the investigated variables.

$$y=a_0 + a_1x_1 + a_2x_2 + a_3x_1x_2 + a_4x_1^2 + a_5x_1^2 \tag{1}$$

In equation (1), a_i denotes the coefficients of the polynomial model, whereas the variables x_1, x_2 and y represent the following parameters:

y – microbial load (total number of aerobic bacteria), log [CFU/mL];

x_1 –NO_3^-; NO_2^-; respectively NH_4^+ concentration [mg/L];

x_2 – time, [months].

The system of equations was solved using MATLAB, and the experimental data were subsequently processed and analyzed. The equations of the statistical models derived from the multiple regression analysis are presented in Table 2.1.20 and are valid within the range of the investigated values.

Table 2.1.20. Equations of the statistical models obtained in the case of the spring from Area I - Alba Iulia-Teiuș

Variable	**Equations of statistical models**
Nitrates (NO_3^-)	$y=1.891 - 0.002 \cdot x_1 + 0.004 \cdot x_2 + 1.777 \cdot 10^{-4} \cdot x_1 \cdot x_2 + 2.662 \cdot 10^{-4} \cdot x_1^2 - 1.598 \cdot 10^{-4} \cdot x_2^2$
Nitrites (NO_2^-)	$y=1.796 + 7.060 \cdot x_1 + 0.006 \cdot x_2 - 0.055 \cdot x_1 \cdot x_2 - 23.232 \cdot x_1^2 - 6.020 \cdot 10^{-5} \cdot x_2^2$
Ammonium (NH_4^+)	$y=1.750 + 1.279 \cdot x_1 + 0.006 \cdot x_2 + 0.007 \cdot x_1 \cdot x_2 - 0.06 \cdot x_1^2 - 1.522 \cdot 10^{-4} \cdot x_2^2$

The experimental data and the surfaces generated by the statistical models for Area I (Alba Iulia–Teiuș) are presented in Figures 2.1.17–2.1.19.

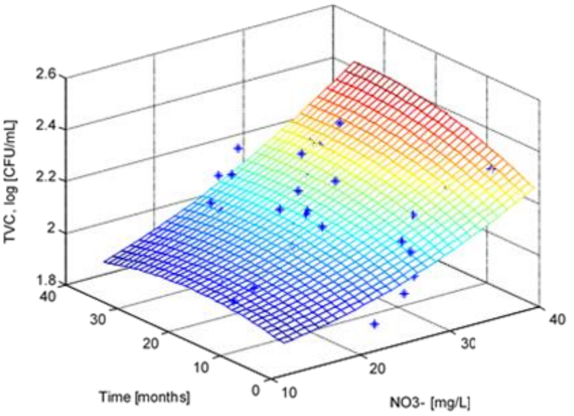


Figure 2.1.17. Variation of the microbial load depending on the nitrate content and time for the spring in Area I - Alba Iulia-Teiuș

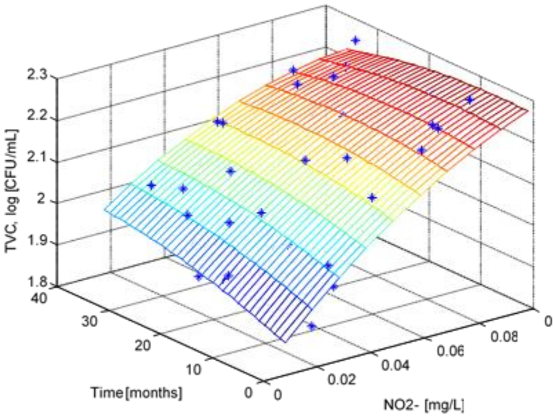


Figure 2.1.18. Variation of the microbial load depending on the nitrite content and time for the spring in Area I - Alba Iulia-Teiuș

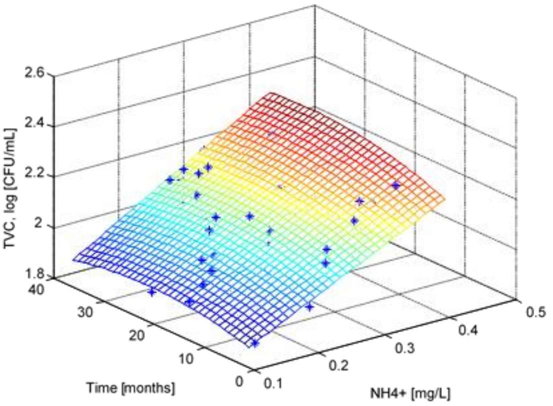


Figure 2.1.19. Variation of the microbial load depending to the ammonium ion content and time for the spring in Area I - Alba Iulia-Teiuș

After determining the model coefficients, it is necessary to compare the model predictions with the experimental data provided by the process. To evaluate the adequacy of the models, several statistical indicators were used, including theispersion σ^2, standard deviation σ, correlation coefficient R, accuracy coefficient R^2 were used as indicators of model adequacy (Table 2.2.21).

Table 2.1.21. Adequacy indicators of the determined statistical models

Variable	**σ^2**	**σ**	**R^2**	**R**
Nitrates	0.004	0.065	0.654	0.808
Nitrites	0.002	0.043	0.847	0.920
Ammonium	0.003	0.060	0.716	0.846

The values of the concordance indicators suggest a strong capacity for predicting statistical models. The experimental data, together with the surfaces generated by the statistical mathematical models for Area II – Sebeș-Cugir, are presented in Figures 2.1.20–2.1.22.

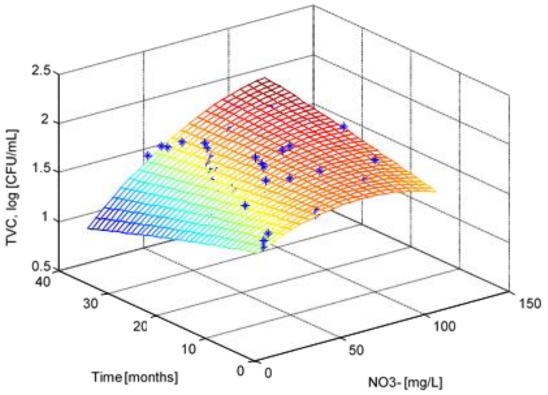


Figure 2.1.20. Variation of the microbial load depending on the nitrate content and time for the spring in Area II - Sebeș-Cugir

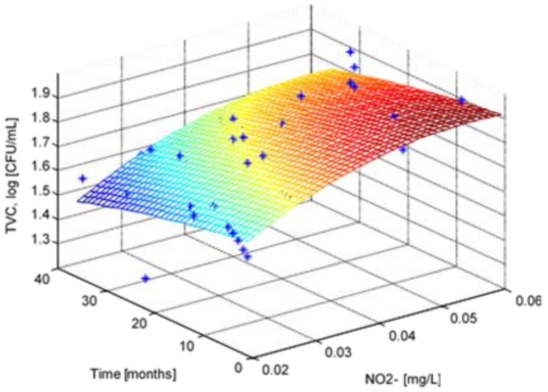


Figure 2.1.21. Variation of the microbial load depending on the nitrite content and time for the spring in Area II - Sebeș-Cugir

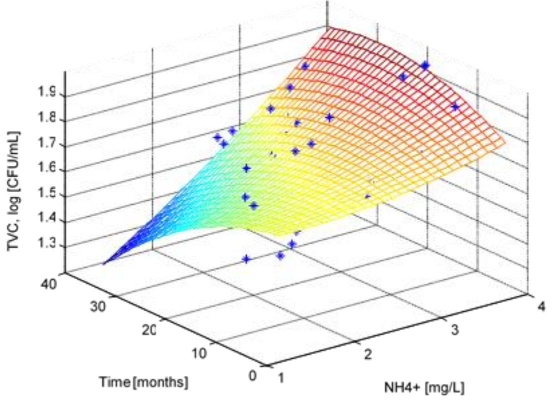


Figure 2.1.22. Variation of the microbial load depending to the ammonium ion content and time for the spring in Area II - Sebeș-Cugir

The equations of the statistical models obtained through nonlinear multiple regression are presented in Table 2.1.22. These equations are valid within the range of values studied.

Table 2.1.22. Equations of the statistical models obtained in the case of the spring from Area II – Sebeș-Cugir

Variable	**Equations of statistical models**
Nitrates (NO_3^-)	$y = 1.520 + 0.009 \cdot x_1 - 0.015 \cdot x_2 + 2.498 \cdot 10^{-4} \cdot x_1 \cdot x_2 - 7.052 \cdot 10^{-5} \cdot x_1^2 - 6.262 \cdot 10^{-5} \cdot x_2^2$
Nitrites (NO_2^-)	$y = 1.314 + 21.790 \cdot x_1 - 0.003 \cdot x_2 + 0.015 \cdot x_1 \cdot x_2 - 198.024 \cdot x_1^2 - 4.505 \cdot 10^{-5} \cdot x_2^2$
Ammonium (NH_4^+)	$y = 1.730 - 0.057 \cdot x_1 - 0.010 \cdot x_2 + 0.006 \cdot x_1 \cdot x_2 + 0.020 \cdot x_1^2 - 2.749 \cdot 10^{-4} \cdot x_2^2$

To compare the model predictions with the data obtained from the actual process, the model's suitability indicators, presented in Table 2.1.23, were calculated.

Table 2.1.23. Adequacy indicators of the determined statistical models

Variable	σ^2	σ	R^2	R
Nitrates	0.008	0.091	0.564	0.751
Nitrites	0.011	0.103	0.449	0.670
Ammonium	0.010	0.097	0.505	0.711

The values obtained for the concordance indicators associated with the statistical models substantiate the existence of statistically significant correlations among the analyzed parameters, highlighting a consistent and robust interdependence within the studied dataset.

The experimental data, together with the surfaces generated by the statistical mathematical models for Zone III – Câmpeni–Zlatna, are presented in Figures 2.1.23–2.1.25. The equations of the statistical models, obtained through second-order nonlinear multiple regression, are presented in Table 2.1.24 and are valid within the studied value range.

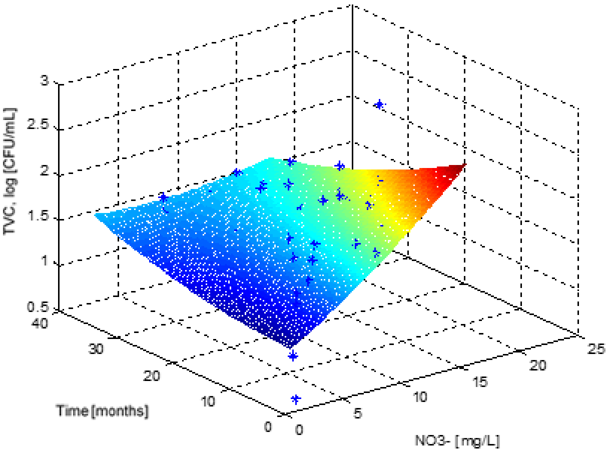


Figure 2.1.23. Variation of the microbial load depending on the nitrate content and time for the spring in Area III – Câmpeni – Zlatna

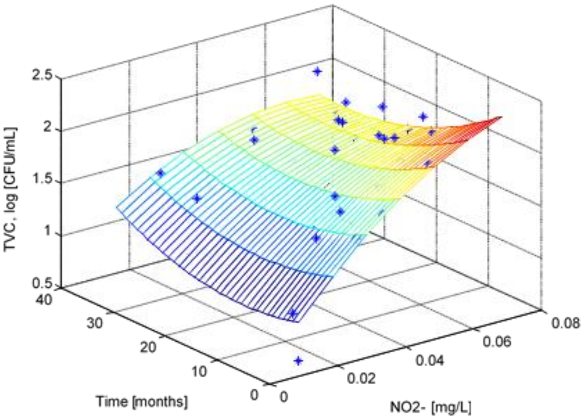


Figure 2.1.24. Variation of the microbial load depending on the nitrite content and time for the spring in Area III - Câmpeni – Zlatna

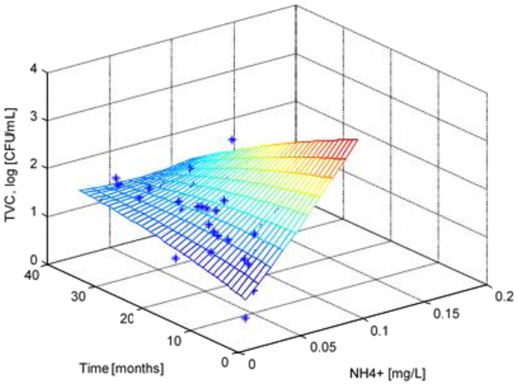


Figure 2.1.25. Variation of the microbial load depending to the ammonium ion content and time for the spring in Area III - Câmpeni – Zlatna

Table 2.1.24. Equations of the statistical models obtained in the case of the spring from Area III – Câmpeni – Zlatna

Variable	Equations of statistical models
Nitrates (NO_3^-)	$y = 1.089 + 0.078 \cdot x_1 + 0.0036 \cdot x_2 - 0.0027 \cdot x_1 \cdot x_2 + 0.0015 \cdot x_1^2 + 3.3753 \cdot 10^{-4} \cdot x_2^2$
Nitrites (NO_2^-)	$y = 0.592 + 42.040 \cdot x_1 - 0.011 \cdot x_2 - 0.424 \cdot x_1 \cdot x_2 - 219.871 \cdot x_1^2 + 6.406 \cdot 10^{-4} \cdot x_2^2$
Ammonium (NH_4^+)	$y = 0.685 + 26.003 \cdot x_1 + 0.040 \cdot x_2 - 0.856 \cdot x_1 \cdot x_2 + 44.453 \cdot x_1^2 - 3.244 \cdot 10^{-4} \cdot x_2^2$

The adequacy indicators of the statistical models, obtained by comparing the model predictions with the data from the actual process, are presented in Table 2.1.25.

Table 2.1.25. Adequacy indicators of the determined statistical models

Variable	σ^2	σ	R^2	R
Nitrates	0.049	0.221	0.574	0.757
Nitrites	0.039	0.197	0.660	0.812
Ammonium	0.048	0.219	0.583	0.763

In the case of second-order polynomial equations, the correlation coefficients indicate a functionally satisfactory relationship between the investigated variables.

The experimental data, together with the surfaces generated by the statistical mathematical models for Zone IV – Blaj, are presented in Figures 2.1.26–2.1.28.

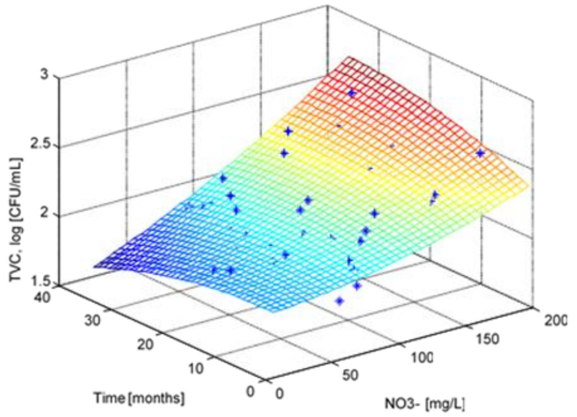


Figure 2.1.26. Variation of the microbial load depending on the nitrate content and time for the spring in Area IV – Blaj

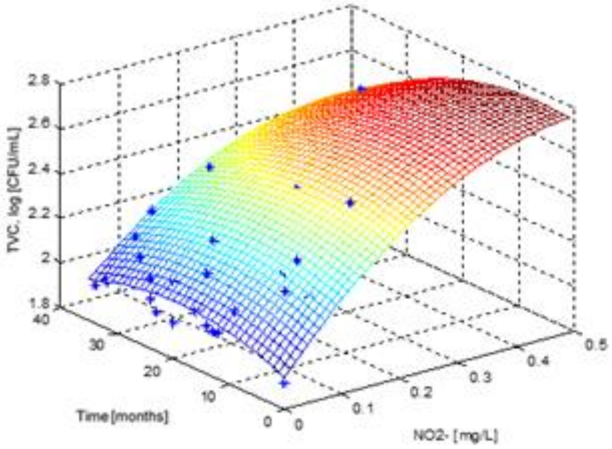


Figure 2.1.27. Variation of the microbial load depending on the nitrite content and time for the spring in Area IV – Blaj

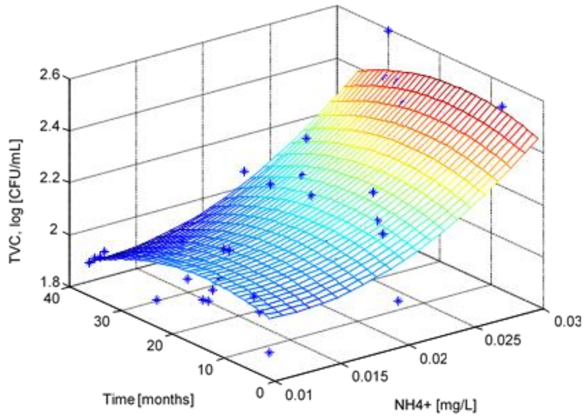


Figure 2.1.28. Variation of the microbial load depending to the ammonium ion content and time for the spring in Area IV – Blaj

The equations of the statistical models obtained from the nonlinear multiple regression are presented in Table 2.1.26 and the adequacy indicators in Table 2.1.27.

Table 2.1.26. Equations of the statistical models obtained in the case of the spring from Area IV – Blaj

Variable	Equations of statistical models
Nitrates (NO_3^-)	$y = 1.946 + 4.329\cdot10^{-4}\cdot x_1 + 8.790\cdot10^{-5}\cdot x_2 + 9.191\cdot10^{-5}\cdot x_1\cdot x_2 + 7.895\cdot10^{-6}\cdot x_1^2 - 2.396\cdot10^{-4}\cdot x_2^2$
Nitrites (NO_2^-)	$y = 1.881 + 3.217\cdot x_1 + 0.013\cdot x_2 - 0.018\cdot x_1\cdot x_2 - 2.999\cdot x_1^2 - 3.147\cdot10^{-4}\cdot x_2^2$
Ammonium (NH_4^+)	$y = 2.193 - 28.933\cdot x_1 + 0.007\cdot x_2 + 0.051\cdot x_1\cdot x_2 + 1239.3\cdot x_1^2 - 2.709\cdot10^{-4}\cdot x_2^2$

Table 2.1.27. Adequacy indicators of the determined statistical models

Variable	σ^2	σ	R^2	R
Nitrates	0.005	0.071	0.843	0.918
Nitrites	0.007	0.082	0.793	0.890
Ammonium	0.012	0.109	0.635	0.797

The values of the correlation coefficients are appreciable, which indicates a good correlation in the case of multiple 2nd order regression.The experimental data, together with the surfaces generated by the statistical mathematical models for Zone V – Aiud–Ocna Mureș, are presented in Figures 2.1.29–2.1.31.

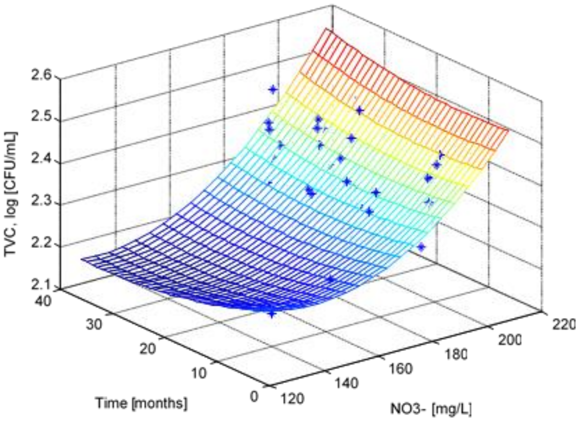


Figure 2.1.29. Variation of the microbial load depending on the nitrate content and time for the spring in Area V - Aiud-Ocna Mureș

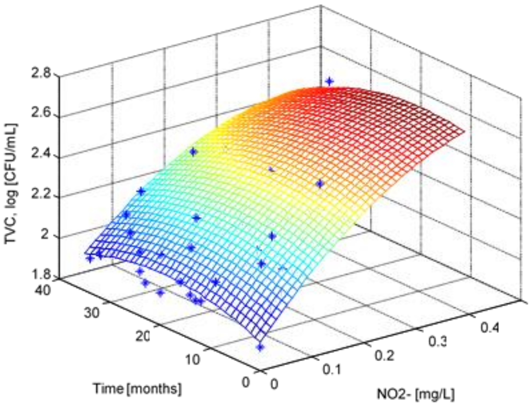


Figure 2.1.30. Variation of the microbial load depending on the nitrite content and time for the spring in Area V - Aiud-Ocna Mureș

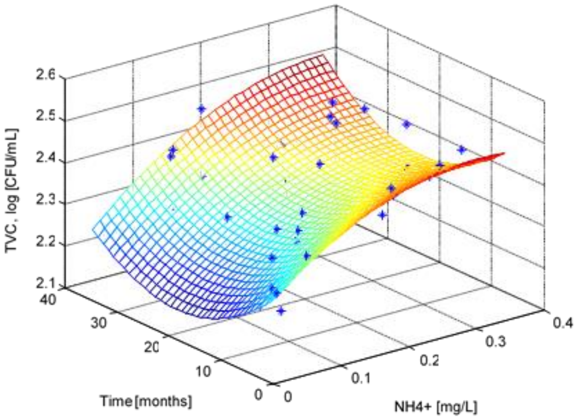


Figure 2.1.31. Variation of the microbial load depending to the ammonium ion content and time for the spring in Area V - Aiud-Ocna Mureș

The equations of the statistical models, obtained through nonlinear multiple regression, are presented in Table 2.1.28.

Table 2.1.28. Equations of the statistical models obtained in the case of the spring from Area V - Aiud-Ocna Mureș

Variable	Equations of statistical models
Nitrates (NO_3^-)	$y = 1.946 + 4.329\cdot10^{-4}\cdot x_1 + 8.790\cdot10^{-5}\cdot x_2 + 9.191\cdot10^{-5}\cdot x_1\cdot x_2 + 7.895\cdot10^{-6}\cdot x_1^2 - 2.396\cdot10^{-4}\cdot x_2^2$
Nitrites (NO_2^-)	$y = 1.881 + 3.217\cdot x_1 + 0.013\cdot x_2 - 0.018\cdot x_1\cdot x_2 - 2.999\cdot x_1^2 - 3.147\cdot10^{-4}\cdot x_2^2$
Ammonium (NH_4^+)	$y = 2.193 - 28.933\cdot x_1 + 0.007\cdot x_2 + 0.051\cdot x_1\cdot x_2 + 1239.3\cdot x_1^2 - 2.709\cdot10^{-4}\cdot x_2^2$

Table 2.1.29. Adequacy indicators of the determined statistical models

Variable	σ^2	σ	R^2	**R**
Nitrates	0.002	0.041	0.659	0.812
Nitrites	0.007	0.082	0.793	0.890
Ammonium	0.002	0.041	0.645	0.803

The calculated correlation parameters indicate a strong predictive capacity of the mathematical models. The analyses show that one or more of the determined parameters in most spring water samples exceed the maximum limits allowed by legislation.

The research provides an overview of the pollution status and contamination of spring waters in Alba County. While public drinking water supply systems rely primarily on surface water sources (such as lakes and, more often, rivers), spring water remains a largely under-investigated and underutilized resource, despite being a free, natural, and untreated source of particular importance.

A total of 132 water sources across five areas in Alba County were monitored. The most representative and highly contaminated sources were analyzed for physico-chemical and microbiological indicators over a three-year period. The results revealed that the majority of these sources were microbiologically polluted, posing potential health risks. The proportion of drinking-quality spring water sources in Alba County was relatively low, as follows: Alba Iulia–Teiuș (I) – 37%, Sebeș–Cugir (II) – 48%, Câmpeni–Zlatna (III) – 72%, Blaj (IV) – 25%, and Aiud–Ocna Mureș (V) – 20%.

The equations of the statistical models obtained can be used as predictive tools. Using these models, the microbial contamination levels in spring waters can be estimated based on the time of year when a sample is collected and its nitrate, nitrite, and ammonium ion content. The calculated correlation parameters indicate a strong predictive capacity of these statistical models, providing valuable insight into the pollution patterns of spring waters.

2.1.2.3. Time Assessment of Mining-Affected River Waters in Roșia Montană. Zeolite-Based Remediation Solution

In order to assess river water pollution in the Roșia Montană area, Alba County and to identify pollution factors along their courses, a study based on physico-chemical investigations was conducted. The study identifies possible sources of anthropogenic pollution based on the results of water contamination indicators, including water pH, oxygen content, biochemical and chemical oxygen demand, sulfates, cadmium, lead, iron, copper, manganese, arsenic, and zinc. In this context, 14 sampling points along the rivers in the area were evaluated over the course of one year.

Through experimental analyses, the impact generated by acid mine drainage in this region and possible solutions for mitigating its effects were investigated. The results contribute to ongoing discussions regarding sustainable mining practices and environmental remediation strategies. Ultimately, this research highlights the need to integrate innovative/natural materials, such as zeolites, into mine water treatment in order to ensure the protection of water resources in regions affected by mining activities. Furthermore, the potential use of a natural zeolite for the efficient removal of heavy metals from acid drainage generated at the abandoned “Gura Minei” mine was demonstrated [32].

Water samples were collected quarterly throughout 2024 from multiple points along the rivers (Figure 2.1.32) in the vicinity of the Roșia Montană area, as detailed in Table 2.1.30.

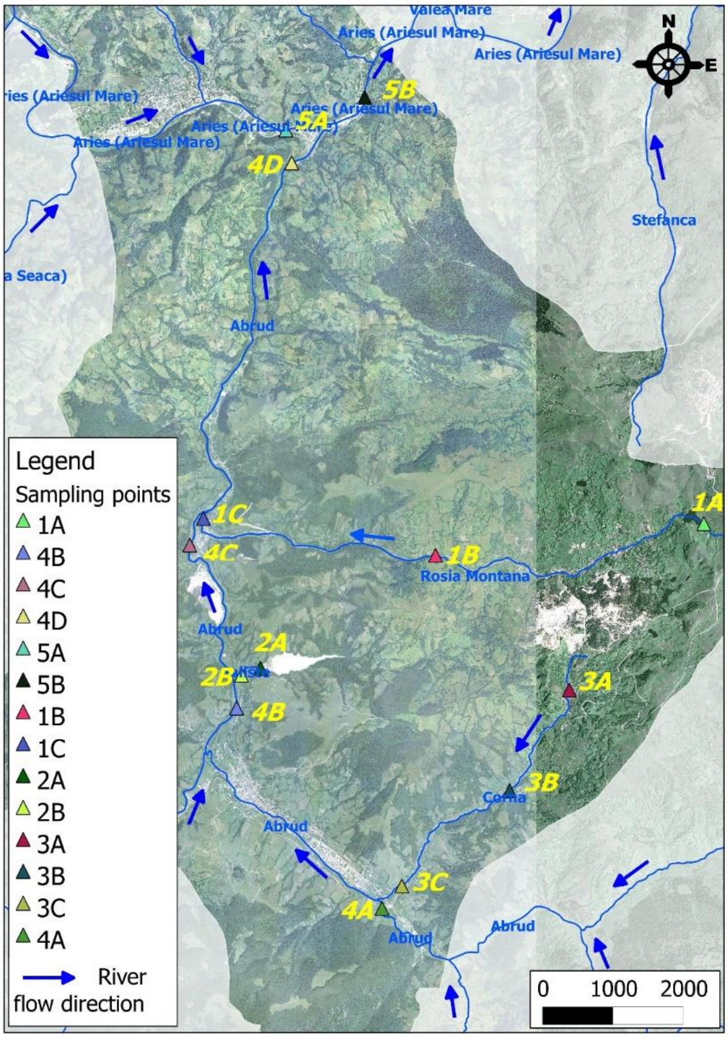


Figure 2.1.32. Map of the Roșia Montană area indicating the locations of river water sampling sites.

Table 2.1.30. Water sampling points of the analyzed rivers.

River	Samples Code	Sampling Sites	Sampling Point Details
Roșia Montană	1A	Upstream of the Tăul Mare Lake dam.	Upstream of Roșia Montană village, in an area unaffected by anthropogenic activities.
	1B	~3.8 km upstream of its confluence with the Abrud River.	Upstream of the discharge site of acid mine drainage from the Gura Minei Gallery area.
	1C	~100 m upstream of its confluence with the Abrud River.	At the confluence area of the Roșia Montană River with the Abrud River, downstream of the discharge site of acid mine drainage from the Gura Minei Gallery area.
Săliște	2A	Downstream of the Valea Seliștei tailings pond.	Downstream of the Valea Seliștei tailings pond, which is currently undergoing ecological restoration.
	2B	Upstream of its confluence with the Abrud River.	At the confluence area of the Valea Săliștei River with the Abrud River.
Corna	3A	~4.5 km upstream of its confluence with the Abrud River.	In the Corna village area, close to the site of the Roșia Montană quarry.
	3B	~2.7 km upstream of its confluence with the Abrud River.	In the Bunta village area.
	3C	~0.4 km upstream of its confluence with the Abrud River.	In the Gura Cornei village area.
Abrud	4A	~0.15 km upstream of its confluence with the Corna River.	Upstream of the Abrud town.
	4B	~0.48 km upstream of its confluence with the Valea Săliștei River.	In the Abrud Sat town area.

	4C	~0.58 km upstream of its confluence with the Roșia Montană River.	Upstream of the Gura Roșiei village.
	4D	~ 0.8 km upstream of its confluence with the Arieş River.	Upstream of the Vârşi village.
Arieș	5A	~ 0.6 km upstream of its confluence with the Abrud River.	In the Boncești village area, near Câmpeni town.
	5B	~ 0.7 km downstream of its confluence with the Abrud River.	In the town of Câmpeni, downstream of its confluence with the Abrud River.

Table 2.1.31 provides general information and estimated specific data for the selected watercourses in Alba County, Romania.

Table 2.1.31. General and specific data for the investigated watercourses.

<table>
<tr><th rowspan="3">River</th><th colspan="6">General Information About the Watercourse</th><th colspan="8">Detailed Data for the Sampling Points</th></tr>
<tr><th rowspan="2">Length, km</th><th colspan="2">Altitude, m</th><th rowspan="2">Average Slope, ‰</th><th rowspan="2">Catchment Area, km²</th><th colspan="4" rowspan="2">Width, m</th><th colspan="4" rowspan="2">Depth, m</th></tr>
<tr><th>Upstream</th><th>Downstream</th></tr>
<tr><td rowspan="2">Roșia Montană</td><td rowspan="2">8</td><td rowspan="2">1120</td><td rowspan="2">575</td><td rowspan="2">33</td><td rowspan="2">44</td><td>1A</td><td colspan="2">1B</td><td>1C</td><td>1A</td><td colspan="2">1B</td><td>1C</td></tr>
<tr><td>1</td><td colspan="2">1.5</td><td>3</td><td>0.1</td><td colspan="2">0.3</td><td>0.3</td></tr>
<tr><td rowspan="2">Săliște (unsurveyed)</td><td rowspan="2">No data</td><td rowspan="2">No data</td><td rowspan="2">No data</td><td rowspan="2">No data</td><td rowspan="2">No data</td><td colspan="2">2A</td><td colspan="2">2B</td><td colspan="2">2A</td><td colspan="2">2B</td></tr>
<tr><td colspan="2">0.5</td><td colspan="2">1</td><td colspan="2">0.1</td><td colspan="2">0.2</td></tr>
<tr><td rowspan="2">Corna</td><td rowspan="2">5</td><td rowspan="2">800</td><td rowspan="2">612</td><td rowspan="2">68</td><td rowspan="2">10</td><td>3A</td><td colspan="2">3B</td><td>3C</td><td>3A</td><td colspan="2">3B</td><td>3C</td></tr>
<tr><td>0.5</td><td colspan="2">1</td><td>2</td><td>0.1</td><td colspan="2">0.2</td><td>0.3</td></tr>
<tr><td rowspan="2">Abrud</td><td rowspan="2">24</td><td rowspan="2">1142</td><td rowspan="2">537</td><td rowspan="2">25</td><td rowspan="2">223</td><td>4A</td><td>4B</td><td>4C</td><td>4D</td><td>4A</td><td>4B</td><td>4C</td><td>4D</td></tr>
<tr><td>5</td><td>8</td><td>8</td><td>9</td><td>0.4</td><td>0.3</td><td>0.6</td><td>0.7</td></tr>
<tr><td rowspan="2">Arieș</td><td rowspan="2">166</td><td rowspan="2">1108</td><td rowspan="2">264</td><td rowspan="2">5</td><td rowspan="2">3005</td><td colspan="2">5A</td><td colspan="2">5B</td><td colspan="2">5A</td><td colspan="2">5B</td></tr>
<tr><td colspan="2">24</td><td colspan="2">30</td><td colspan="2">0.8</td><td colspan="2">0.7</td></tr>
</table>

Tables 2.1.32 – 2.1.41 present the results for each of the 5 rivers analyzed in the Roșia Montană area, which are directly or indirectly affected by acid mine drainage from the mining galleries. These results are presented from upstream to downstream of the area of interest, aiming to identify the impact of mining activities on water contamination.

Table 2.1.32. Physico-chemical data recorded at three points along the Roșia Montană River, quarterly throughout the year.

Sampling Sites	**Month of Sampling**	**pH**	**DO, mg O_2/L**	**BOD$_5$, mg/L**	**COD$_{Mn}$, mg $KMnO_4$/L**	**Sulfate, mg/L**
River 1A at the source	February	7.80 ± 0.11	9.05 ± 0.40	0.48 ± 0.10	25.06 ± 5.13	48.22 ± 2.05
	May	7,42 ± 0.08	9.47 ± 0.66	1.04 ± 0.24	17.95 ± 3.04	21.01 ± 1.18
	August	6.34 ± 0.07	9.80 ± 0.50	0.19 ± 0.08	21.25 ± 3.85	33.85 ± 3.06
	November	7.05 ± 0.09	9.53 ± 0.23	0.57 ± 0.10	17.49 ± 2.64	29.70 ± 1.93
River 1B in the middle	February	3.87 ± 0.04	9.66 ± 0.11	3.12 ± 0.32	20.11 ± 1.90	307.1 ± 6.64
	May	3,59 ± 0.08	8.40 ± 0.18	2.03 ± 0.14	18.20 ± 1.51	239.2 ± 5.27
	August	2.80 ± 0.03	10.5 ± 0.89	2.62 ± 0.25	22.74 ± 2.23	197.7 ± 8.41
	November	3.76 ± 0.05	9.32 ± 0.34	2.67 ± 0.21	16.63 ± 1.28	76.4 ± 5.23
River 1C at the river mouth	February	3.95 ± 0.07	8.61 ± 0.49	7.42 ± 0.87	20.43 ± 3.39	2185.1 ± 16.39
	May	2.69 ± 0.03	7.94 ± 0.10	9.45 ± 0.63	18.57 ± 4.17	3632.9 ± 28.20
	August	3.03 ± 0.02	7.53 ± 0.59	6.54 ± 0.70	21.79 ± 2.73	2594.0 ± 21.17
	November	3.81 ± 0.06	8.07 ± 0.78	5.16 ± 0.42	16.36 ± 3.40	1816.5 ± 15.44
Water quality classes [33]	***I***	***6.5–8.5***	***8***	***3***	***5***	***60***
	II		***7***	***5***	***10***	***120***
	III		***5***	***7***	***20***	***250***
	IV		***4***	***20***	***50***	***300***
	V		***<4***	***>20***	***>50***	***>300***

Abbreviations: DO—dissolved oxygen, BOD$_5$—biochemical oxygen demand, COD$_{Mn}$—chemical oxygen demand.

Sample 1A exhibits values for parameters such as pH, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, and sulfates that are within the normal range. However, downstream, in samples 1B and especially 1C, the results indicate significant water pollution. The higher values at point 1C can mainly be attributed to the discharge of mine water from the Gura Mine Gallery area, which is the main source of water pollution in the region. The remaining discharges are diffuse, caused by water seepage from various areas affected by mining activities in the area.

The pH values are below 6.5 (ranging from 2.69 to 3.87) at monitoring points 1B and 1C, indicating the acidic nature of the water.

The oxygen regime parameters in the river water show no significant variation; the obtained values are generally adequate for supporting aquatic ecosystems. However, the minimum value of 7.53 mg/L obtained at point 1C could be an indicator of the local stress for aquatic organisms, especially because it is accompanied by low pH and high chemical oxygen demand values. For BOD_5, the values gradually increase, suggesting an accumulation of organic matter as one moves downstream or the influence of intermittent pollution sources. The COD_{Mn} values range from 16.3 to 25.0 mgO_2/L, with an average around 20 mgO_2/L. This range is characteristic of water with significant organic and chemical load, often exceeding the recommended limits for healthy aquatic ecosystems.

Sulfate values range from low concentrations at point 1A, typical of clean water (21.01–48.22 mg/L), to high concentrations at point 1B and extremely high concentrations at point 1C (>1000 mg/L, with a maximum of 3632 mg/L), indicating severe pollution of industrial origin and uncontrolled wastewater discharges, significantly affecting water quality and ecosystems.

Table 2.1.33. The concentration of trace metals recorded at the Roșia Montană River at 3 points, at 4 time points during a year.

Sampling Sites	**Month of Sampling**	**Cu, µg/L**	**Fe, mg/L**	**Zn, µg/L**	**Pb, µg/L**	**Cd, µg/L**	**As, µg/L**	**Mn, mg/L**
River 1A at the source	February	<0.010	0.150±0.006	0.048±0.002	<0.01	0.027±0.001	<0.01	0.38±0.02
	May	<0.010	0.104±0.005	0.065±0.003	<0.01	0.020±0.001	<0.01	0.64±0.03
	August	<0.010	0.230±0.012	0.051±0.002	<0.01	0.055±0.003	<0.01	0.75±0.04
	November	<0.010	0.167±0.007	0.530±0.027	<0.01	0.041±0.002	<0.01	0.45±0.02
River 1B at the middle	February	0.374±0.017	23.79±1.10	1.070±0.051	<0.01	0.050±0.003	0.117±0.005	15.23±0.75
	May	0.820±0.041	38.90±1.93	1.632±0.080	0.010±0.001	0.020±0.001	0.134±0.007	14.78±0.74
	August	0.903±0.045	44.02±2.21	1.890±0.092	0.010±0.001	0.060±0.003	0.148±0.006	21.36±1.07
	November	0.210±0.012	29.16±1.44	1.903±0.094	<0.01	0.040±0.002	0.075±0.004	16.41±0.81
River 1C at the river mouth	February	0.366±0.018	187.55±9.38	36.440±1.820	0.031±0.001	0.074±0.004	11.230±0.560	16.37±0.82
	May	0.147±0.007	346.09±17.30	55.324±2.765	0.039±0.002	0.020±0.001	27.600±1.380	17.14±0.86
	August	0.595±0.030	207.01±10.32	51.190±2.560	0.035±0.002	0.034±0.002	13.120±0.661	22.81±1.14
	November	0.281±0.014	195.47±9.79	46.526±2.328	0.030±0.001	0.026±0.001	10.553±0.528	18.07±0.90
Water quality classes [33]	***I***	***0.005***	***0.020***	***0.0005***	***0.1***	***0.01***	***0.3***	***0.05***
	II	***0.010***	***0.030***	***0.001***	***0.2***	***0.02***	***0.5***	***0.10***
	III	***0.025***	***0.050***	***0.002***	***0.5***	***0.05***	***1.0***	***0.30***
	IV	***0.050***	***0.100***	***0.005***	***1.0***	***0.10***	***2.0***	***1***
	V	***>0.05***	***>0.1***	***>0.005***	***>1.0***	***>0.1***	***>2.0***	***>1.0***

The water quality classes used in this study were established based on the thresholds defined by the Romanian Ministry of Environment Order No. 161/2006 [33] and in accordance with the European Union Water Framework Directive [34]. These classes categorize water bodies into five quality levels—High, Good, Moderate, Poor, and Bad—based on deviations from reference or natural conditions, using physico-chemical parameters.

The water quality of the Roșia Montană river shows significant trace metal pollution at sampling points 1B and 1C, with concentrations of Fe^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and As^{3+} much higher compared to point 1A. In general, at the source (1A), the recorded values are closer to safety limits, indicating major pollution upstream of point 1B and especially in the 1C area.

Table 2.1.34. Monitoring results of the Săliște River at 2 points quarterly in a year.

Sampling Sites	**Month of Sampling**	**pH**	**DO, mg O_2/L**	**BOD_5, mg/L**	**COD_{Mn}, mg $KMnO_4$/L**	**Sulfate, mg/L**
River 2A at the source	February	8.08 ± 0.13	10.34 ± 0.49	0.74 ± 0.20	8.73 ± 2.23	57.1 ± 1.7
	May	8.10 ± 0.06	9.42 ± 0.34	0.95 ± 0.23	5.52 ± 1.14	64.4 ± 0.9
	August	7.51 ± 0.09	11.07 ± 0.53	1.48 ± 0.15	7.99 ± 1.62	71.4 ± 1.8
	November	7.87 ± 0.10	9.35 ± 0.23	1.03 ± 0.23	6.38 ± 1.35	76.8 ± 1.30
River 2B at the middle	February	6.99 ± 0.07	8.11 ± 0.19	2.66 ± 0.31	10.80 ± 2.59	80.3 ± 1.07
	May	6.68 ± 0.05	10.01 ± 0.38	4.97 ± 0.47	6.99 ± 2.04	102.2 ± 2.19
	August	6.52 ± 0.08	9.53 ± 0.21	2.10 ± 0.11	8.85 ± 1.73	79.6 ± 1.54
	November	6.80 ± 0.02	9.46 ± 0.23	3.52 ± 0.29	7.07 ± 1.95	147.0 ± 2.03

Abbreviations: DO—dissolved oxygen, BOD_5—biochemical oxygen demand, COD_{Mn}—chemical oxygen demand.

Analyzing the physico-chemical data from Table 2.1.34, it is observed that throughout the year, water quality in the Săliște River fluctuates more at monitoring point 2B, where organic pollution and sulfate concentrations are higher. In contrast, at point 2A, water quality remains relatively stable, with only moderate fluctuations in oxygen demand and sulfate levels.

Table 2.1.35. The concentration of trace metals recorded at the Săliște River at 2 points, at 4 time points during a year.

Sampling sites	**Month of sampling**	**Cu, µg/L**	**Fe, mg/L**	**Zn, µg/L**	**Pb, µg/L**	**Cd, µg/L**	**As, µg/L**	**Mn, mg/L**
River 2A at the source	February	0.597±0.030	0.206±0.010	0.905±0.045	0.036±0.002	0.035±0.002	0.015±0.001	0.372±0.019
	May	0.420±0.021	0.318±0.016	1.402±0.070	0.028±0.001	0.026±0.001	0.022±0.001	0.414±0.021
	August	0.738±0.037	0.248±0.012	1.517±0.076	0.034±0.002	0.038±0.002	0.024±0.001	0.815±0.041
	November	0.651±0.033	0.145±0.007	2.060±0.103	0.025±0.001	0.021±0.001	0.019±0.001	0.649±0.032
River 2B at the middle	February	0.455±0.023	0.188±0.009	0.875±0.044	0.034±0.002	0.029±0.001	0.011±0.001	0.420±0.021
	May	0.391±0.020	0.305±0.015	1.365±0.068	0.023±0.001	0.025±0.001	0.018±0.001	0.491±0.025
	August	0.712±0.036	0.223±0.011	1.324±0.066	0.031±0.002	0.033±0.002	0.021±0.001	0.688±0.034
	November	0.624±0.031	0.132±0.007	1.984±0.099	0.022±0.001	0.015±0.001	0.017±0.001	0.722±0.036

The concentration of trace metals in the Săliște River (Table 2.1.31) varies significantly throughout the year, with higher levels of metals such as Cu, Fe, Zn, Pb, Cd, and As observed in point 2B (middle of the river) compared to point 2A, particularly in May, while concentrations tend to decrease or stabilize by November at both sites.

Table 2.1.36. Physico-chemical data at 3 points recorded in the Corna River at 4 time points during a year.

Sampling sites	**Month of sampling**	**pH**	**DO, mg O_2/L**	**BOD_5, mg/L**	**COD_{Mn}, mg $KMnO_4$/L**	**Sulfate, mg/L**
River 3A at the source	February	4.21 ± 0.06	11.14 ± 0.34	5.56 ± 0.36	30.51 ± 4.54	106.4 ± 1.65
	May	3.46 ± 0.05	10.20 ± 0.27	7.00 ± 0.82	21.18 ± 2.19	158.6 ± 1.04
	August	4.63 ± 0.03	10.83 ± 0.26	5.81 ± 0.61	19.59 ± 3.21	176.3 ± 1.27
	November	4.70 ± 0.09	12.00 ± 0.38	6.08 ± 0.43	20.34 ± 4.83	182.8 ± 2.33
River 3B at the middle	February	6.18 ± 0.02	9.51 ± 0.41	6.37 ± 0.72	37.17 ± 5.57	631.0 ± 4.20
	May	5.90 ± 0.03	10.49 ± 0.52	7.49 ± 0.50	56.33 ± 8.16	2205.1±17.28
	August	6.29 ± 0.04	11.17 ± 0.60	7.24 ± 0.46	28.96 ± 3.76	1093.2±8.59
	November	6.47 ± 0.03	10.18 ± 0.33	8.05 ± 0.67	44.60 ± 7.00	820.5 ± 6.37
River 3C at the river mouth	February	7.51 ± 0.07	8.84 ± 0.19	4.03 ± 0.29	9.47 ± 2.41	185.9 ± 2.02
	May	7.04 ± 0.06	10.50 ± 0.24	3.95 ± 0.63	5.62 ± 1.58	265.4 ± 3.36
	August	7.09 ± 0.08	9.11 ± 0.27	3.02 ± 0.54	7.58 ± 2.05	155.7 ± 1.68
	November	7.22 ± 0.10	8.86 ± 0.18	3.69 ± 0.19	6.26 ± 2.60	154.8 ± 1.85

Abbreviations: DO—dissolved oxygen, BOD_5—biochemical oxygen demand, CODMn—chemical oxygen demand.

The Corna River shows significant variations in water quality across its three sampling points (Table 2.1.36). At the source (River 3A), the water remains slightly acidic, with moderate levels of organic and chemical pollution, and an increase in sulfate concentrations throughout the year. In the middle section (River 3B), pollution is much higher, particularly in May, with elevated levels of organic and chemical oxygen demand, as well as sulfates. The outflow point (River 3C) shows better water quality, with more neutral pH, lower oxygen demand, and significantly reduced pollution levels, especially in terms of chemical oxygen demand and sulfates.

Table 2.1.37. The concentration of trace metals recorded at 3 points at the Corna River, at 4 time points during a year.

Sampling sites	**Month of sampling**	**Cu, µg/L**	**Fe, mg/L**	**Zn, µg/L**	**Pb, µg/L**	**Cd, µg/L**	**As, µg/L**	**Mn, mg/L**
River 3A at the source	February	<0.01	58.07±2.87	0.055±0.003	<0.01	<0.01	0.020±0.001	0.31±0.010
	May	0.012±0.001	70.19±3.51	0.080±0.004	<0.01	0.02±0.001	0.028±0.003	0.43±0.021
	August	0.020±0.001	46.40±2.32	0.092±0.002	<0.01	0.02±0.001	0.029±0.002	0.69±0.034
	November	0.018±0.001	32.18±1.61	0.107±0.005	<0.01	<0.01	0.022±0.002	0.24±0.010
River 3B at the middle	February	0.015±0.001	10.94±0.53	0.273±0.010	<0.01	<0.01	0.018±0.001	0.21±0.011
	May	0.057±0.003	36.10±1.59	0.281±0.014	<0.01	<0.01	0.020±0.001	0.68±0.033
	August	0.024±0.001	17.89±0.65	0.284±0.011	<0.01	<0.01	0.024±0.001	0.94±0.037
	November	0.300±0.015	10.23±0.51	0.336±0.017	<0.01	<0.01	0.027±0.003	0.67±0.024
River 3C at the river mouth	February	<0.01	2.506±0.125	2.878±0.124	<0.01	<0.01	0.023±0.001	0.58±0.029
	May	<0.01	0.851±0.043	5.189±0.219	0.013±0.001	<0.01	0.015±0.001	0.91±0.042
	August	0.013±0.001	4.162±0.202	3.402±0.170	0.029±0.002	<0.01	0.021±0.002	1.36±0.068
	November	0.011±0.001	2.803±0.114	2.910±0.136	0.022±0.001	<0.01	0.016±0.001	0.42±0.021

The concentration of trace metals in the Corna River (Table 2.1.37) varies across three sampling points throughout the year. At River 3A (source), metal concentrations are generally low, with copper remaining below detection limits and iron decreasing from February to November. Zinc increases slightly over the year, while lead, cadmium, and arsenic concentrations remain very low. In River 3B (middle), copper and zinc concentrations rise in May and stabilize by November, while iron levels show fluctuations, and other metals remain low. At River 3C (outflow), zinc concentrations are notably higher, particularly in May, while copper is generally below detection limits, and other metals show low but detectable levels throughout the year.

Table 2.1.38. Physico-chemical data at 4 points recorded in the Abrud River in 4 time points during a year.

Sampling sites	**Month of sampling**	**pH**	**DO, mg O_2/L**	**BOD_5, mg/L**	**COD_{Mn}, mg $KMnO_4$/L**	**Sulfate, mg/L**
River 4A at the source—before the confluence with Corna River	February	6.71±0.13	11.04±0.21	3.43±0.35	0.65±0.19	3.20±0.43
	May	6.39±0.06	10.90±0.61	7.08±0.71	8.22±2.13	8.12±0.93
	August	6.52±0.09	10.32±0.23	5.16±0.48	3.74±0.90	6.26±0.79
	November	6.63±0.10	10.08±0.32	3.02±0.22	0.92±0.24	15.02±1.06
River 4B—before the confluence with Săliște River	February	6.37±0.04	8.94±0.26	4.98±0.30	1.83±0.65	42.93±1.64
	May	5.80±0.05	9.55±0.27	6.63±0.49	9.77±2.74	36.88±1.81
	August	5.65±0.08	8.51±0.14	5.11±0.72	4.80±1.49	71.44±2.59
	November	6.06±0.07	9.26±0.57	4.17±0.55	2.21±0.87	29.45±1.31
River 4C—before the confluence with Roșia Montană River	February	5.14±0.04	7.62±0.33	6.09±0.44	3.12±0.70	77.53±2.10
	May	4.78±0.03	8.71±0.36	7.15±0.83	5.01±1.35	68.15±1.14
	August	4.43±0.05	6.98±0.19	5.98±0.76	6.16±2.09	87.19±2.25
	November	4.92±0.04	7.72±0.26	6.16±0.29	5.19±1.16	184.56±3.88
	February	6.36±0.06	5.46±0.15	6.64±0.41	0.56±0.10	191.34±4.60
	May	6.21±0.07	6.76±0.23	7.52±0.70	0.95±0.34	243.93±5.86

River 4D—after the confluence with Roșia Montană River	August	6.18±0.05	4.19±0.07	6.47±0.35	3.88±0.63	142.69±3.42
	November	6.44±0.08	4.92±0.12	5.59±0.56	2.73±0.42	97.34±1.91
	February	6.71±0.13	11.04±0.21	3.43±0.35	0.65±0.19	3.20±0.43

Abbreviations: DO—dissolved oxygen; BOD$_5$—biochemical oxygen demand; COD$_{Mn}$—chemical oxygen demand.

The data reveal that water quality decreases as one moves downstream from the source to the Roșia Montană River. While point 4A (source) maintains relatively stable and better water quality, point 4B (before Săliște River) and point 4C (before Roșia Montană River) experience increasing pollution, particularly in terms of chemical and organic pollution. Point 4D (after Roșia Montană River) shows the most significant pollution, with high sulfate levels and lower oxygen content, indicating significant contamination, likely from mining and other industrial activities.

Table 2.1.39. The concentration of trace metals recorded at 4 points at the Abrud River, at 4 time points during a year.

Sampling sites	**Month of sampling**	**Cu, µg/L**	**Fe, mg/L**	**Zn, µg/L**	**Pb, µg/L**	**Cd, µg/L**	**As, µg/L**	**Mn, mg/L**
River 4A at the source—before the confluence with Corna River	February	0.016±0.001	0.49±0.03	0.18±0.01	<0.01	<0.01	<0.01	0.08±0.01
	May	0.010±0.001	0.95±0.05	0.32±0.02	<0.01	<0.01	<0.01	0.07±0.01
	August	0.027±0.002	0.42±0.02	0.12±0.01	<0.01	<0.01	<0.01	0.27±0.04
	November	0.035±0.002	0.57±0.03	0.10±0.01	<0.01	<0.01	<0.01	0.16±0.02
River 4B—before the confluence with Săliște River	February	0.452±0.019	0.18±0.02	0.09±0.00	<0.01	<0.01	<0.01	1.24±0.06
	May	0.058±0.004	0.15±0.01	0.19±0.01	<0.01	<0.01	<0.01	1.06±0.05
	August	0.054±0.003	0.17±0.01	0.27±0.03	<0.01	<0.01	<0.01	1.54±0.08
	November	0.410±0.027	0.14±0.01	0.12±0.01	<0.01	<0.01	<0.01	1.29±0.06
River 4C—before the confluence with Roșia Montană River	February	0.012±0.001	0.32±0.02	0.10±0.01	<0.01	<0.01	<0.01	1.38±0.07
	May	0.075±0.004	1.09±0.05	0.12±0.01	<0.01	<0.01	<0.01	1.65±0.08
	August	0.043±0.002	0.33±0.02	0.08±0.00	<0.01	<0.01	<0.01	1.84±0.09
	November	0.014±0.001	0.21±0.01	0.11±0.01	<0.01	<0.01	<0.01	1.42±0.07
River 4D—after the confluence with Roșia Montană River	February	0.071±0.003	1.89±0.09	0.51±0.03	<0.01	<0.01	<0.01	4.57±0.23
	May	0.085±0.004	2.35±0.12	0.63±0.03	<0.01	<0.01	<0.01	5.31±0.27
	August	0.069±0.003	1.69±0.08	0.55±0.04	<0.01	<0.01	<0.01	7.43±0.37
	November	0.078±0.004	1.68±0.07	0.46±0.02	<0.01	<0.01	<0.01	3.18±0.16
	February	0.016±0.001	0.49±0.02	0.18±0.01	<0.01	<0.01	<0.01	0.08±0.001

The trace metal data from the Abrud River suggests that copper, iron, and zinc concentrations increase as the river flows downstream, particularly in River 4D (after Roșia Montană), where mining-related pollution contributes to higher concentrations. Lead, cadmium, and arsenic levels are consistently low or undetectable across all sites and times, indicating minimal contamination from these metals. Overall, pollution appears to increase downstream, especially in terms of iron and zinc, which are associated with mining activities in the area.

The Arieș River is the main water source in the area. Its course is affected by historical mining operations through the direct discharge of the Abrud River into the Arieș. Water samples were taken from the Arieș River before and after its confluence with the Abrud River.

Table 2.1.40. Physico-chemical data recorded at the Arieș River at 2 points, at 4 time points during a year.

Sampling sites	**Month of sampling**	**pH**	**DO, mg O$_2$/L**	**BOD$_5$, mg/L**	**COD$_{Mn}$, mg KMnO$_4$/L**	**Sulfate, mg/L**
River 5A before the confluence	February	7.61 ± 0.08	12.13 ± 0.56	5.87 ± 0.41	17.9 ± 2.82	12.5 ± 1.55
	May	7.59 ± 0.06	12.44 ± 0.39	5.45 ± 0.42	18.2 ± 3.54	19.76 ± 1.79
	August	7.48 ± 0.07	11.86 ± 0.21	5.60 ± 0.65	15.6 ± 3.24	36.14 ± 1.30

with the Abrud River.	November	7.52 ± 0.09	11.76 ± 0.20	6.13 ± 0.70	19.7 ± 4.31	20.68 ± 1.85
River 5B after the confluence with the Abrud River.	February	5.90 ± 0.03	9.02 ± 0.15	6.09 ± 0.52	19.3 ± 4.42	80.49 ± 2.76
	May	6.83 ± 0.11	10,30 ± 0.22	7.02 ± 0.84	12.8 ± 2.79	93.12 ± 2.46
	August	6.04 ± 0.07	8.11 ± 0.14	6.33 ± 0.31	24.1 ± 5.64	125.6 ± 4.69
	November	5.52 ± 0.04	7.67 ± 0.17	6.71 ± 0.69	18.2 ± 3.18	74.24 ± 2.78

** Abbreviations: DO—dissolved oxygen, BOD_5—biochemical oxygen demand, COD_{Mn}—chemical oxygen demand.*

The data for the Arieș River, recorded at two points, shows distinct seasonal variations. In point 5A (before the confluence with the Abrud River), the pH remains stable, ranging from 7.4 to 7.6, reflecting slightly alkaline conditions. The oxygen demand fluctuates between 12.13 mg/L in February and 11.76 mg/L in November, with biochemical oxygen demand (BOD_5) varying from 5.45 mg/L in May to 6.13 mg/L in November. The chemical oxygen demand (COD) shows moderate variation, peaking at 19.7 mg/L in November and dropping to 15.6 mg/L in August. Sulfate concentrations rise significantly, from 12.5 mg/L in February to 36.14 mg/L in August, indicating possible pollution sources.

In point 1B (after the confluence with the Abrud River), the pH decreases slightly, ranging from 5.5 in November to 6.8 in May, indicating more acidic conditions. Oxygen demand decreases from 9.02 mg/L in February to 7.67 mg/L in November, but BOD_5 remains relatively high, ranging from 6.09 mg/L in February to 7.02 mg/L in May. The chemical oxygen demand (COD) reaches its highest level in August at 24.1 mg/L and its lowest in May at 12.8 mg/L. Sulfate concentrations significantly increase, peaking at 125.6 mg/L in August, which suggests an elevated pollution level after the confluence with the Abrud River.

Water quality seems to deteriorate downstream, especially after the confluence, as evidenced by higher sulfate levels and oxygen demand, particularly during the warmer months.

Table 2.1.41. The concentration of trace metals recorded at the Arieș River at 2 points, at 4 time points during a year.

Sampling sites	**Month of sampling**	**Cu, µg/L**	**Fe, mg/L**	**Zn, µg/L**	**Pb, µg/L**	**Cd, µg/L**	**As, µg/L**	**Mn, mg/L**
River 5A at the source	February	1.20±0.03	0.54±0.03	58.07±2.70	0.44±0.02	0.25±0.01	0.03±0.00	0.04±0.00
	May	0.90±0.05	0.96±0.05	162.60±8.03	0.49±0.03	0.44±0.02	0.06±0.00	0.08±0.00
	August	1.10±0.04	1.08±0.06	154.20±7.41	0.45±0.02	0.37±0.02	0.10±0.01	0.12±0.01
	November	1.40±0.06	0.72±0.04	71.10±3.36	0.63±0.03	0.51±0.03	0.07±0.00	0.03±0.00
River 5B at the middle	February	2.20±0.10	2.27±0.10	130.50±6.13	0.85±0.04	1.08±0.04	0.08±0.00	1.38±0.05
	May	4.40±0.21	3.09±0.15	172.10±8.21	1.04±0.05	2.20±0.10	0.05±0.00	2.06±0.10
	August	2.70±0.12	2.43±0.11	124.70±6.04	1.12±0.06	1.10±0.04	0.11±0.01	2.37±0.11
	November	3.00±0.09	1.59±0.08	143.00±6.15	0.78±0.04	0.97±0.05	0.07±0.00	1.61±0.07

Due to the high concentration of pollutants contaminating the Abrud River, the obtained values demonstrate that it has a significant impact on the Arieș River, which is also polluted.

The heavy metal concentrations recorded at the Arieș River show significant variations over the year at two points. In point 5A, Cu has the highest concentration of 1.4 µg/L in November, Fe reached 1.08 mg/L in August, while Zn was highest at 162.6 µg/L in May. The concentrations of Pb and Cd increased at 0.63 µg/L and 0.51 µg/L in November, while As attained the maximum level of 0.10 µg/L in August.

In point 5B, copper peaked at 4.4 µg/L in May, iron reached 3.099 mg/L in May, and zinc was highest at 172.1 µg/L in May. Lead peaked at 1.12 µg/L in August, cadmium reached 2.20 µg/L in May, and arsenic was highest at 0.11 µg/L in August. Overall, trace metal concentrations were higher in River 5B, particularly for copper, zinc, and cadmium. The iron concentration plays a key role in the reddish coloration of the water.

For the treatment of acidic water from the Gura Roșiei Gallery with zeolite, the study aimed to identify the potential of natural zeolite from Rupea to retain Fe, Zn, and Mn ions from wastewater. To this end, the basic physico-chemical parameters of the water were determined, as presented in Table 2.1.42.

Table 2.1.42. The obtained values for the mine water analysis from the Gura Roșiei Gallery.

Parameter	**Value**	**Maximum Allowable Limit ***	**UM**
pH	2.90 ± 0.02	6.5–8.8	-
Total Suspended Solids (TSS)	38.0 ± 1.0	35	mg/L
Filtrable Residue at 105 °C (FR)	1970.5 ± 15.4	-	mg/L
COD_{Mn}	74.0 ± 4.3	-	mgO_2/L
Fe	510.2 ± 4.8	5	mg/L
Mn	424.5 ± 6.4	1	mg/L
Zn	19.0 ± 2.0	0.5	mg/L

*Abbreviations: COD_{Mn}—chemical oxygen demand; *According to Romanian Standard NTPA 001/2002 [35].*

These parameters in Table 2.1.42 highlight the high degree of contamination with significant concentrations of heavy metals, mainly Fe, Mn, and Zn, whose concentrations exceed more than 38 to 424 times the maximum consent limits established by the Romanian legislation [35].

The XRF analysis of the natural zeolite used in this study showed that it contains significant amounts of SiO_2 (70.93%) and Al_2O_3 (16.21%), as well as various other oxides, such as: CaO (4.72%), K_2O (3.69%), Fe_2O_3 (2.82%), MgO (0.46%), Na_2O (0.45%), TiO_2 (0.25%), BaO (0.10%), and MnO (0.05%). The loss on ignition was 0.21%.

The study examines the use of zeolite for treating mine water to reduce pollution and improve environmental quality. In order to determine the influence of the natural zeolite dose and the contact time on the retention process of metals ions (Fe, Zn, and Mn) from acid water collected from the exit of the Gura Roșiei mine, the amount of zeolite was varied from 7.5 g to 20 g, using a constant volume of 100 mL of mine water with continuous stirring at 300 rpm. The variation in the concentrations of iron, zinc, and manganese ions in the acid mine drainage during its contact time with different quantities of natural zeolite is presented in Figures 2.1.33 – 2.1.35.

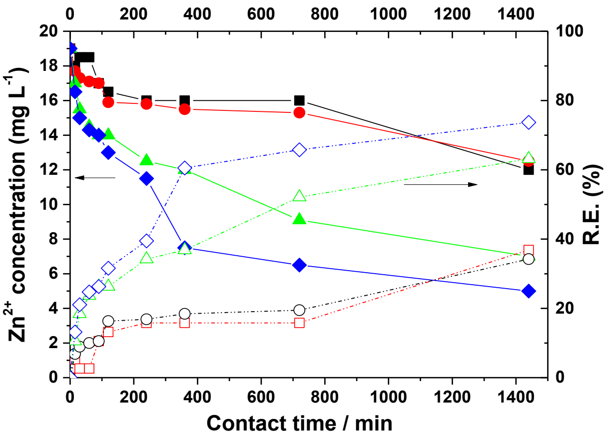


Figure 2.1.33. Influence of zeolite dosage on residual zinc concentrations in acid mine drainage and on removal efficiency after different contact times. Zeolite dosage (g): (■) 7.5; (●) 10; (▲) 15; and (♦) 20. (———) Residual concentration data. (- - - -) Removal efficiency data.

The concentration of Zn^{2+} in the acid mine drainage in contact with a 7.5 g dose of natural zeolite decreases from an initial value of 19.0 mg/L to 12.5 mg/L. As the zeolite dose increases, the removal efficiency also improves. Thus, for a 20 g dose of zeolite, after 1440 min, the concentration reaches 5.0 mg/L.

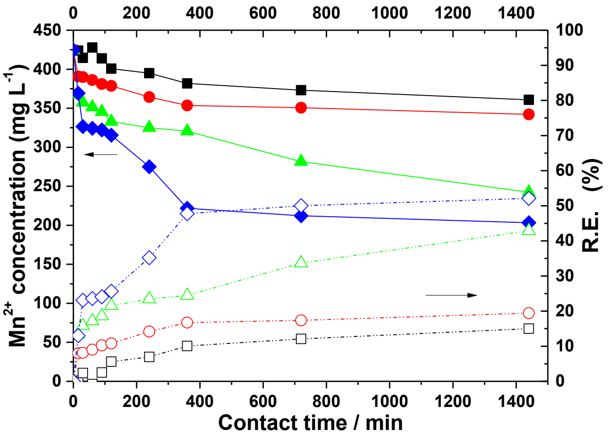


Figure 2.1.34. Influence of zeolite dosage on residual manganese concentrations in acid mine drainage and on removal efficiency after different contact times. Zeolite dosage (g): (■) 7.5; (●) 10; (▲) 15; and (♦) 20. (———) Residual concentration data. (- - - -) Removal efficiency data.

Regarding the concentration of Mn ions in the samples in contact with natural zeolite, regardless of the applied dose, a much lower removal efficiency was observed. The most notable result was for the 20 g zeolite dose, where the Mn ions were reduced by half, from 424.5 mg/L to 203.3 mg/L.

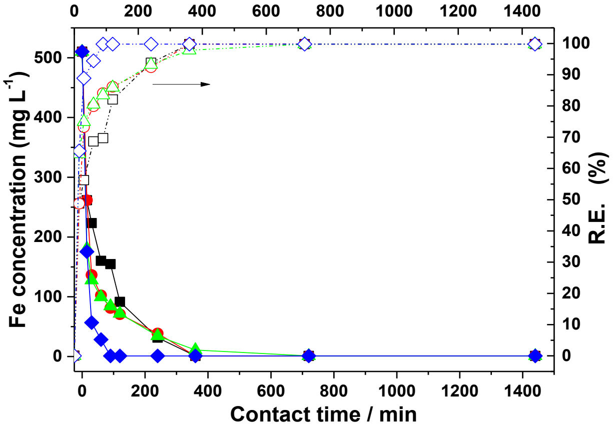


Figure 2.1.35. Influence of zeolite dosage on residual iron concentrations in acid mine drainage and on removal efficiency after different contact times. Zeolite dosage (g): (■) 7.5; (●) 10; (▲) 15; and (♦) 20. (———) Residual concentration data. (- - - -) Removal efficiency data.

The concentration of Fe ions decreased from 510 mg/L to 91.5 mg/L after a contact time of 120 min with zeolite at the lowest dose, and after 360 min, the Fe concentration reaches values below 1 mg/L, which are within the limits established by current regulations (5 mg/L).

As the zeolite dose increased from 7.5 g to 10 g, the Fe ions concentration decreased to 1 mg/L after 360 min of contact. However, the concentrations of Mn^{2+} and Zn^{2+} ions remained high. Based on the obtained data, it was concluded that increasing the zeolite dose to 10 g does not lead to a significant improvement in the retention process of Mn and Zn ions.

By treating the acidic mine water with 15 g of zeolite, a one-third reduction in the Fe concentration was observed, decreasing from the initial value to 175.5 mg/L after the first 15 min of contact. After a longer contact time, over 60 min, the concentration of Fe ions reached 1 ppm. However, the concentrations of Mn and Zn ions in the collected supernatants remained high, even at the 15 g zeolite dose. A slow decrease was observed, with Mn^{2+} reaching a concentration of 242.4 mg/L after 1440 min of contact. Regarding the Zn ion concentration, a decrease from 19.0 mg/L to 7.0 mg/L was noticed after 1440 min.

Increasing the zeolite dose to 20 g results in an improvement in the removal process of Mn and Zn metal ions.

2.1.2.4. Pollution Effects on Water and Petrophilic Diatom Communities of the Arieș River Basin

Human activities in the Apuseni Mountains region of Romania, particularly mining in the Roșia Montană area, have significantly impacted the water quality of the Arieș River and its tributaries. Historical assessments of mining operations in the Apuseni Mountains of Transylvania indicate a cumulative environmental impact, mainly associated with acid mine drainage and the mobilization of trace elements. These processes have resulted in the contamination of both surface water and groundwater, promoting bioaccumulation and ecotoxic effects, with long-term consequences for the structure and functioning of aquatic ecosystems [36].

Diatoms are key components of river ecosystems and serve as sensitive bioindicators of water quality. Their species composition, abundance, and morphological traits—particularly the occurrence of teratological forms—reflect both current and cumulative environmental stress, including heavy metal contamination [37].

In this study, water pollution in the Arieș River [38] and its main tributaries [39] near the Roșia Montană mining area was assessed by integrating physico-chemical measurements with biological analyses of diatom communities. This combined approach provides a comprehensive evaluation of organic and trace metals pollution, highlights areas most impacted by anthropogenic activities, and supplies critical information for the sustainable management of freshwater resources.

The monitored watercourses are part of the Arieș-Turda hydrotechnical system, which operates within the Arieș River Basin (3005 km²). The monitoring points are located in the vicinity of the mining operations in the Roșia Montană region, as shown in Figure 2.1.36.

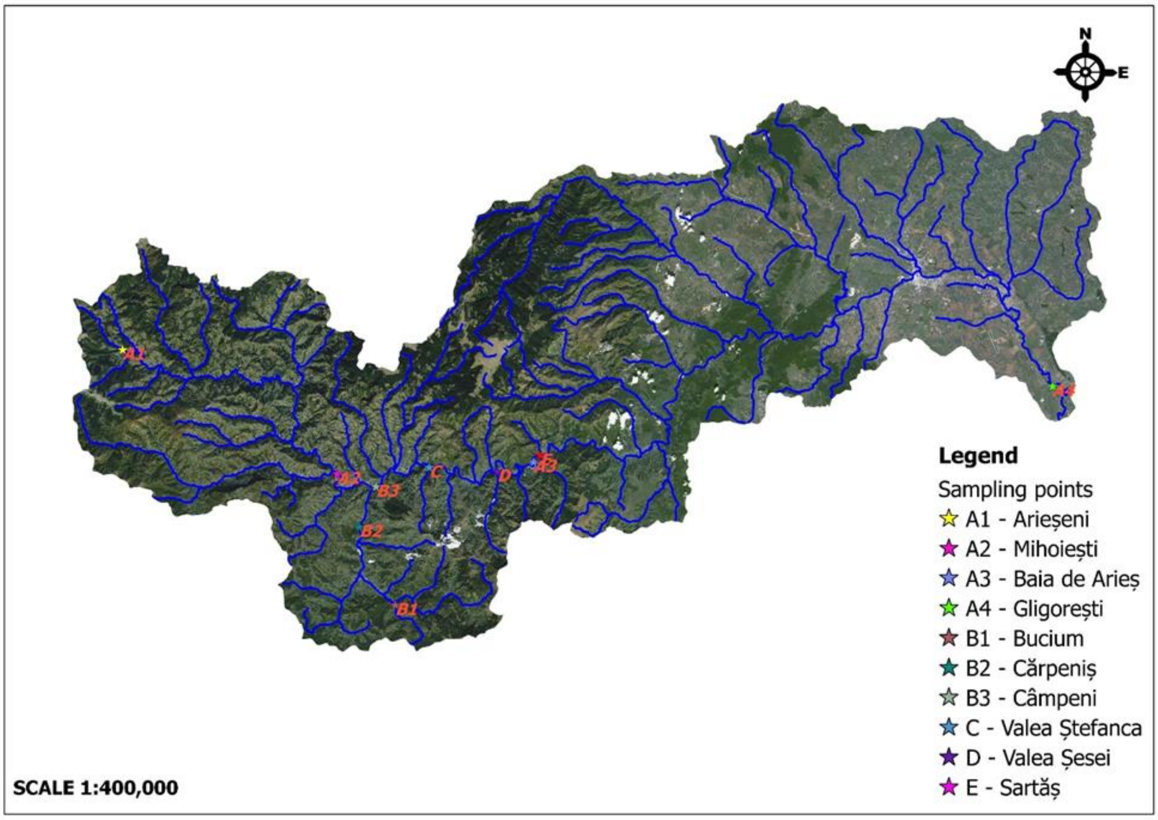


Figure 2.1.36. The sampling points with the greatest impact on the pollution of the Arieș River and its tributaries.

Water samples for physico-chemical analyses were collected during 2022, 2023, and 2024, in January, April, July, and October, to cover all seasons as well as varying climatic and river flow conditions.

Samples for periphytic biofilm and benthic diatom analyses were collected in April 2024.

Table 2.1.43. Coding and sampling points of river water samples

Code	**River**	**Sampling area**
A1	Arieș	Arieşeni
A2		Mihoiești
A3		Baia de Arieș
A4		Buru
B1	Abrud	Source in the Bucium area
B2		Downstream of the confluence with the Roşia Montană River, in the Cărpeniş area
B3		Upstream of the confluence with the Arieș River, in the town of Câmpeni
C	Ștefanca	Before the confluence with the Arieș River
D	Valea Seșii	Before the confluence with the Arieș River
E	Sartaș	Before the confluence with the Arieș River

The main mining areas near the studied region, which contribute to the pollution of the Arieș River, include: 3 mines; 4 settling ponds; 5 mine waste dumps; and 6 crushing/flotation facilities.

In Tables 2.1.44, 2.1.45, and 2.1.46, the physico-chemical parameters of the Arieș River at the source (A1) are presented, allowing the analysis of seasonal variations in water quality, as well as the identification of potential natural and anthropogenic influences on the chemical composition.

Table 2.1.44. Physico-chemical parameters of the Arieș River upstream of Arieşeni (A1).

Parameter **Time, year/month**		**pH**	**TSS, mg/L**	**DO, mgO/L**	**BOD$_5$, mgO/L**	**COD$_{Cr}$, mgO/L**	**NH$_4^+$, mgN/L**	**NO$_2^-$, mgN/L**	**NO$_3^-$, mgN/L**
2022	January	7.5	10.8	9.87	0.83	1.13	0.002	0.005	0.167
	April	7.7	13.4	10.01	0.87	1.74	0.003	0.006	0.214
	July	7.6	14.9	8.04	1.34	2.32	0.005	0.011	0.584
	October	7.5	9.8	10.81	0.81	1.81	0.004	0.007	0.298
2023	January	7.8	10.3	9.60	0.91	1.56	0.001	0.006	0.170
	April	7.6	13.4	9.91	0.91	1.71	0.002	0.008	0.199
	July	7.4	15.4	7.98	1.12	2.14	0.006	0.009	0.604
	October	7.5	9.1	10.47	0.94	1.75	0.003	0.008	0.304
2024	January	7.6	12.3	9.74	0.82	1.38	0.002	0.005	0.186
	April	7.7	14.1	10.24	0.86	1.69	0.004	0.007	0.228
	July	7.6	13.6	8.45	1.27	2.09	0.008	0.008	0.527
	October	7.5	9.8	10.57	0.89	1.94	0.001	0.005	0.356
Quality class [33]	***I***	***6.5 - 8.5***	***-***	***6.2, not less than 80% oxygen saturation***	***6***	***10***	***0.4***	***0.01***	***1***
	II					***25***	***0.8***	***0.03***	***3***
	III					***50***	***1.2***	***0.06***	***5.6***
	IV					***125***	***3.2***	***0.3***	***11.2***
	V					***>125***	***>3.2***	***>0.3***	***>11.2***

According to the physico-chemical parameters, the water quality of the Arieș River at the source is relatively stabble, with no significant fluctuations in the indicators, reflecting good water status in this area.

Table 2.1.45. Salinity parameters of the Aries River water upstream of Arieseni locality(A1).

Parameter Time, year/month		EC, µS/cm	FR, mg/L	Ca, mg/L	Mg, mg/L	Na, mg/L	Bicarbonates, mg/L	Chlorides, mg/L	Sulfates, mg/L
2022	January	104.42	71.4	13.7	1.9	5.8	52.4	0.8	32.4
	April	127.42	116.4	19.6	2.5	4.6	76.8	1.6	41.6
	July	211.79	147.4	41.5	4.7	7.1	125.7	4.1	58.0
	October	163.09	124.7	35.0	1.8	4.2	48.6	1.5	21.4
2023	January	124.93	83.6	17.4	1.4	4.7	55.2	0.1	28.2
	April	131.14	125.1	24.2	1.9	4.4	84.7	1.8	45.5
	July	198.72	138.1	34.8	3.8	6.3	113.0	3.5	51.7
	October	173.47	121.0	30.1	2.4	3.7	51.8	2.1	18.6
2024	January	142.72	79.2	13.6	1.6	5.5	64.1	1.7	31.3
	April	139.71	132.7	29.1	2.1	5.1	75.7	2.1	38.2
	July	199.67	152.4	38.3	4.1	6.6	132.4	3.8	54.8
	October	156.77	118.7	29.9	2.6	3.9	53.1	2.5	21.1
Quality class [33]	***I***	**-**	***500***	***50***	***12***	***25***	**-**	***25***	***60***
	II		***750***	***100***	***50***	***50***		***50***	***120***
	III		***1000***	***200***	***100***	***100***		***250***	***250***
	IV		***1300***	***300***	***200***	***200***		***300***	***300***
	V		***>1300***	***>300***	***>200***	***>200***		***>300***	***>300***

The salinity parameters of the Arieș River upstream of the Arieșeni village showed seasonal fluctuations, with higher values in the summer months, but without exceeding the analyzed parameter limits. These seasonal variations are typical of mountain freshwater ecosystems, where temperature and precipitation conditions influence the concentrations of salts and minerals in the water.

Table 2.1.46. The content of metal trace elements in the water of the Aries River upstream of the Arieseni locality (A1)

Parameter Time, year/month		As, µg/L	Cd, µg/L	Co, µg/L	Cr, µg/L	Cu, µg/L	Fe, mg/L	Mn, mg/L	Ni, µg/L	Zn, µg/L
2022	January	0.29	0.23	0.21	1.18	9.37	0.07	0.001	0.69	6.74
	April	0.30	0.29	0.35	2.59	10.97	0.09	0.002	0.54	14.54
	July	0.32	0.44	1.74	8.41	13.13	0.11	0.005	1.44	31.84
	October	0.28	0.20	0.13	1.93	12.08	0.08	0.004	0.41	0.11
2023	January	0.31	0.19	0.19	1.24	7.62	0.05	0.001	0.73	7.78
	April	0.29	0.23	0.33	3.08	9.07	0.06	0.002	0.47	20.14
	July	0.35	0.51	1.56	7.96	12.24	0.09	0.006	1.54	39.14
	October	0.27	0.28	0.27	2.06	8.30	0.04	0.003	0.58	0.24
2024	January	0.35	0.25	0.13	1.45	9.51	0.07	0.001	0.98	9.16
	April	0.29	0.31	0.38	2.77	11.64	0.08	0.004	3.41	18.1
	July	0.37	0.53	1.62	8.25	12.47	0.10	0.007	1.47	31.71
	October	0.33	0.22	0.19	2.17	9.14	0.03	0.006	0.69	0.19
Quality class [33]	***I***	***10***	***0.5***	***10***	***25***	***20***	***0.3***	***0.05***	***10***	***100***
	II	***20***	***1***	***20***	***50***	***30***	***0.5***	***0.1***	***25***	***200***
	III	***50***	***2***	***50***	***100***	***50***	***1.0***	***0.3***	***50***	***500***
	IV	***100***	***5***	***100***	***250***	***100***	***2***	***1***	***100***	***1000***
	V	***>100***	***>5***	***>100***	***>250***	***>100***	***>2***	***>1***	***>100***	***>1000***

The content of toxic trace elements in the water of the Arieș River, upstream of the Arieșeni village, varied significantly depending on the season, but remained within limits that do not indicate severe pollution. In most cases, the concentrations of trace elements were below the maximum admissible limits, placing the water most of the time in quality classes I and II, which are considered good or very good for aquatic ecosystems.

In July, heavy metal concentrations reached higher values (0.53 µg/L Cd), which could be associated with evaporation and the accumulation of toxic substances during the summer period.

Biofilm samples were collected by scraping with a disposable plastic toothbrush from at least five submerged stones, each with a surface area of at least 10 cm^2, selecting areas with water flow below 20 cm/s. After scraping, the toothbrush was periodically rinsed in a plastic tray containing approximately 50 mL of river water. Once sampling was complete, the resulting suspension was transferred to a polyethylene container and preserved with Lugol's solution (1 mL per 1 L of sample) to inhibit cell division and prevent decomposition of organic material [40].

Under laboratory conditions, benthic diatoms were cleaned using the hot H_2O_2 method to remove organic material, stabilizing their siliceous frustules for subsequent analysis. The sample was homogenized by agitation, and 10 mL of the sus-pension was moved to a laboratory beaker. Then, 20 mL of hydrogen peroxide was added, and the mixture was heated in a water bath at 90 °C until complete oxidation of the organic material (1–3 h). After heating, the beaker walls were rinsed with distilled water, and the suspension was allowed to settle. The washing process was repeated three times to remove any residual peroxide. The diatom suspension was then concentrated in a small volume of distilled water and transferred to a clean vial. After settling, excess supernatant was removed with a Pasteur pipette. A drop of the remaining suspension was placed on a clean microscope slide and evaporated gently using a hot plate. The prepared sample was examined under a Zeiss Axio LAB.A1 light microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) at 1000× magnification, and 300–600 individual diatom valves were identified. Data were interpreted using the saprobic index method [34]. The saprobic value for each species was obtained from Romanian Order no. 161/2006 [35]. Based on the saprobic valence, species were as-signed values as follows: Oligosaprobic–1 (o); Oligo-beta-mesosaprobic–1.5 (o–β); Beta-mesosaprobic–2 (β); Beta-alpha-mesosaprobic–2.5 (β–α); Alpha-mesosaprobic–3 (α); Alpha-meso-polisaprobic–3.5 (α–p); Polisaprobic–4 (p). The relative frequency of each species was calculated as a percentage (F. Rel%) using the formula: FRel% = (total count/species count) × 100. p is the number of individuals of a species and $\sum p$ is the to-tal number of individuals in the sample. Based on relative frequency, a weighted fre-quency (h) was assigned according to the following scale: <1%→h = 1; 1–3%→h = 2; >3–10%→h = 3; >10–20%→h = 4; >20–30%→h = 5; >30–40%→h = 7; >40–100%→h = 9. The saprobic index (S) was calculated using the formula $S = \sum(s_i \cdot h_i)/\sum h_i$, where s_i is the saprobic value of species i, and h_i is its weighted frequency. The saprobic index was interpreted as follows: 1.0–<1.5→Oligosaprobic (no pollution, Class I quality); 1.5–<1.8→Oligo-beta-mesosaprobic (slight pollution, Class I quality); 1.8–<2.3→Beta-mesosaprobic (moderate pollution, Class II quality); 2.3–<2.7→Beta-alpha-mesosaprobic (moderate to critical pollution, Class III quality); 2.7–<3.2→Alpha-mesosaprobic (strong pollution, Class IV quality); 3.2–<3.5→Alpha-meso-polisaprobic (strong to very strong pollution, Class V quality); 3.5–4→Polisaprobic (very strong pollution, Class V quality) [41].

Table 2.1.47 presents the structure of the diatom community identified at the source, sampling point A1 in the Arieșeni area of the Arieș River, including the number of individuals, corresponding saprobic zones, and saprobic values associated with each species. The data were used to calculate the saprobic index (SI), reflecting the level of organic loading in the water.

Table 2.1.47. Taxonomic structure and saprobic values of diatoms identified at the source of the Arieș River (A1).

Species name	**Number of individuals**		**Saprobic zone**	**Saprobic value**
	Normal	**Teratological**		
Planothidium rostratoholarcticum Lange-Bertalot & Bak	1	0	β	2

Achnanthidium minutissimum (Kützing) Czarnecki	188	**11**	o-β	1.5
Cymbella affinis Kützing	1	0	o-β	1.5
Cymbella ventricosa Agardh	3	0	o-β	1.5
Gomphonella olivacea (Hornemann) Rabenhorst	156	**1**	β	2
Gomphonema pumilum (Grunow) E.Reichardt & Lange-Bertalot	50	0	o	1
Total	411		-	-
Saprobic index value	1.40		-	-

The community is dominated by *A. minutissimum* and *G. olivacea*, species characteristic of waters with low to moderate organic load. The calculated SI value for this station is 1.40, which indicates Class I quality water, corresponding to an oligosaprobic–β-mesosaprobic zone, therefore clean water with reduced anthropogenic influence.

In Tables 2.1.48, 2.1.49, and 2.1.50, the physico-chemical parameters of the Arieş River downstream of the Mihoești reservoir (A2) are presented, highlighting the evolution of water quality under the influence of natural and anthropogenic factors, as well as the seasonal and spatial variations of the main pollution indicators.

Table 2.1.48. Physico-chemical parameters of the Arieş River downstream of the Mihoești reservoir(A2).

Parameter Time, year/month		**pH**	**TSS, mg/L**	**DO, mgO/L**	**BOD_5, mgO/L**	**COD-Cr, mgO/L**	**NH_4^+, mgN/L**	**NO_2^-, mgN/L**	**NO_3^-, mgN/L**
2022	January	7.6	18.4	10.53	0.97	1.21	0.004	0.003	0.327
	April	7.8	42.9	11.18	0.79	1.80	0.014	0.005	0.381
	July	7.7	21.4	8.14	1.84	3.34	0.021	0.010	0.847
	October	7.8	31.7	10.93	1.21	2.47	0.019	0.008	0.401
2023	January	7.9	12.4	10.21	1.04	1.06	0.008	0.001	0.352
	April	7.8	51.3	10.84	0.86	1.62	0.009	0.005	0.436
	July	7.6	31.1	8.59	1.89	3.21	0.014	0.009	0.773
	October	7.6	28.2	10.58	1.12	2.23	0.011	0.007	0.485
2024	January	7.7	23.8	11.23	0.93	1.17	0.003	0.004	0.318
	April	7.8	35.5	11.04	1.13	2.32	0.007	0.006	0.388
	July	7.6	12.2	8.04	1.97	3.07	0.028	0.011	0.686
	October	7.7	24.6	10.82	1.34	1.94	0.014	0.010	0.425

Between 2022 and 2024, the physico-chemical parameters of the Arieş River water downstream of the Mihoești reservoir showed seasonal fluctuations, but overall, the values remained within limits that do not severely affect water quality.

Table 2.1.49. Salinity parameters of the Aries River water downstream of the Mihoești reservoir (A2).

Parameter Time, year/month		**EC, µS/cm**	**FR, mg/L**	**Ca, mg/L**	**Mg, mg/l**	**Na, mg/l**	**Bicarbonates, mg/L**	**Chlorides, mg/L**	**Sulfates, mg/L**
2022	January	134.47	84.7	19.1	3.4	5.1	91.9	0.8	3.4
	April	145.31	123.3	19.6	6.6	5.8	101.2	2.9	15.7
	July	184.02	124.5	22.3	12.1	6.2	105.1	7.2	17.6
	October	169.33	124.7	21.5	9.8	4.4	84.8	4.6	13.7
2023	January	144.21	91.2	18.6	2.7	5.3	87.4	0.7	4.1
	April	157.09	116.3	19.0	1.9	5.5	84.7	3.8	16.3
	July	169.23	118.9	21.6	7.2	5.9	98.5	6.4	14.6

	October	171.17	121.0	20.7	10.1	4.1	91.1	4.9	18.1
2024	January	128.14	88.5	20.1	4.1	4.8	94.3	0.5	4.8
	April	145.44	105.8	21.3	8.3	5.7	75.6	4.4	13.3
	July	177.08	122.2	22.7	11.7	6.0	98.7	6.8	16.8
	October	167.49	118.7	21.8	10.5	4.5	79.4	5.1	10.8

The salinity parameters of the Arieș River water downstream of the Mihoești reservoir varied seasonally, but generally remained within acceptable limits, indicating water quality favorable to the aquatic ecosystem.

Table 2.1.50. The content of toxic metal trace elements in the water of the Arieș River downstream of the Mihoești reservoir (A2)

Parameter **Time, year/month**		**As, µg/L**	**Cd, µg/L**	**Co, µg/L**	**Cr, µg/L**	**Cu, µg/L**	**Fe, mg/L**	**Mn, mg/L**	**Ni, µg/L**	**Zn, µg/L**
2022	January	0.30	0.53	0.86	3.53	5.32	0.03	0.006	0.69	2.24
	April	0.35	0.66	1.71	6.38	11.89	0.11	0.012	1.48	11.17
	July	0.41	0.98	3.17	9.14	12.87	0.18	0.022	1.91	39.18
	October	0.39	0.41	0.62	6.14	11.42	0.06	0.018	1.09	1.31
2023	January	0.33	0.46	0.60	4.07	6.08	0.04	0.011	0.73	7.78
	April	0.36	0.57	2.07	5.17	10.43	0.09	0.013	1.37	10.53
	July	0.37	0.81	3.22	8.22	11.45	0.17	0.018	1.72	34.8
	October	0.25	0.39	0.55	4.49	9.96	0.07	0.015	1.24	1.29
2024	January	0.35	0.54	0.94	4.58	4.97	0.01	0.008	0.98	1.31
	April	0.42	0.62	1.22	6.18	9.74	0.12	0.009	1.56	5.67
	July	0.48	0.79	2.85	7.73	13.08	0.13	0.013	1.83	43.01
	October	0.31	0.44	0.51	5.16	10.22	0.05	0.011	1.22	0.91

These parameters increase slightly in summer, they remain within permissible limits, which does not indicate excessive pollution but rather naturally higher concentrations commonly found in river water. Only Cd shows a slight exception, with concentrations (0.39–0.98 µg/L) corresponding to quality classes I–II.

Table 2.1.51 presents the structure of the diatom community at station A2 (Mihoești) on the Arieș River.

Table 2.1.51. Taxonomic composition and saprobic index of diatom species identified in the Arieș River at the Mihoești sampling station (A2).

Species name	**Number of individuals**		**Saprobic zone**	**Saprobic value**
	Normal	**Teratological**		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	2	0	β	2
Achnanthidium minutissimum (Kützing) Czarnecki	339	**24**	o-β	1.5
Amphora pediculus (Kützing) Grunow	13	0	β	2
Hannaea arcus (Ehrenberg) R.M.Patrick	2	0	o	1
Cocconeis placentula Ehrenberg	1	0	β	2
Cymbella gracilis (Ehrenberg) Kützing	3	0	o	1
Cymbella ventricosa Agardh	35	0	o-β	1.5
Odontidium mesodon (Ehrenberg) Kützing	1	0	o	1
Diatoma vulgaris Bory	50	**1**	β-α	2.5
Didymosphenia geminata (Lyngbye) Mart.Schmidt	5	0	o	1

Fragilaria capucina Desmazières	9	**4**	β	2
Gomphonema pumilum (Grunow) E.Reichardt & Lange-Bertalot	5	0	o	1
Navicula cryptotenella Lange-Bertalot	4	0	β	2
Nitzschia dissipata (Kützing) Rabenhorst	2	0	o-β	1.5
Ulnaria ulna (Nitzsch) Compère	2	0	β	2
Total	502		-	-
Saprobic index value	1.39		-	-

The community is dominated by *A. minutissimum* (339 individuals) and *D. vulgaris*, species characteristic of clean waters or those slightly affected by organic matter. The SI value is 1.39, indicating Class I quality water, oligosaprobic to β-mesosaprobic. Thus, at station A2, the water maintains a low level of pollution, similar to that at the source (A1), without significant anthropogenic influences.

In Tables 2.1.52, 2.1.53, and 2.1.54, the physico-chemical parameters of the Arieș River downstream of Baia de Arieș (A3) are presented, providing relevant information for assessing the impact of mining activities and other pollution sources on water quality.

Table 2.1.52. Physico-chemical parameters of the Arieș River water downstream of Baia de Arieș (A3).

Parameter **Time, year/month**		**pH**	**TSS, mg/L**	**DO, mgO/L**	**BOD_5, mgO/L**	**COD-Cr, mgO/L**	**NH_4^+ mgN/L**	**NO_2^- mgN/L**	**NO_3^- mgN/L**
2022	January	7.6	48.7	10.53	0.97	1.46	0.017	0.002	0.157
	April	7.8	143.5	11.27	0.69	2.04	0.024	0.006	0.287
	July	5.7	75.8	8.48	1.84	4.17	0.078	0.009	0.612
	October	6.9	118.3	9.42	1.41	3.26	0.063	0.007	0.398
2023	January	7.9	33.56	10.21	1.04	1.13	0.011	0.003	0.189
	April	7.4	113.7	11.09	0.76	1.96	0.029	0.007	0.335
	July	5.9	47.3	9.14	1.89	3.97	0.081	0.008	0.585
	October	7.2	96.3	10.17	1.17	2.45	0.047	0.006	0.512
2024	January	7.7	49.7	11.23	0.93	1.21	0.008	0.004	0.201
	April	7.3	163.9	11.41	1.23	2.04	0.031	0.005	0.314
	July	6.1	78.5	8.39	1.97	3.68	0.092	0.010	0.537
	October	7.1	104.6	10.13	1.47	2.27	0.054	0.009	0.476

The water pH ranged from 5.7 to 7.9, with a significant decrease in July 2022 (5.7), which could indicate a temporary acidification of the water, possibly due to decomposition processes or the input of acidic substances during that period. In other periods, the pH remained within the normal range for river waters. Suspended solids showed significantly high values in April 2022 (143.5 mg/L) and April 2024 (163.9 mg/L), suggesting increased turbidity during these periods. These values may have been caused by heavy rainfall, leading to erosion and sediment transport into the water.

DO exhibited moderate fluctuations between 8.39 and 11.41 mg/L. BOD_5 showed an increase during the summer, which suggests a higher organic load during that period, possibly due to the input of organic waste or the decomposition of organic matter. COD-Cr presented higher values during the summer months, peaking at 1.84 mgO/L in July 2022, which may indicate a greater load of organic and inorganic substances, but without exceeding pollution limits.

Ammonium, nitrites and nitrates concentrations indicate potential temporary organic pollution, but without reaching levels dangerous to the ecosystem.

Table 2.1.53. Salinity parameters of the Arieș River water downstream of Baia de Arieș (A3)

Parameter Time, year/month		EC, µS/cm	FR, mg/L	Ca, mg/L	Mg, mg/L	Na, mg/L	Bicarbonates, mg/L	Chlorides, mg/L	Sulfates, mg/L
2022	January	169.17	122.4	53.5	4.2	4.2	41.8	6.2	118.7
	April	238.36	175.48	61.9	4.9	5.1	52.7	7.9	129.3
	July	367.12	201.7	72.7	7.5	7.2	101.5	11.2	198.4
	October	244.37	124.7	59.4	6.7	5.9	77.3	9.1	175.4
2023	January	174.20	114.3	51.7	3.1	3.9	38.4	5.8	121.0
	April	198.47	154.7	69.6	4.6	4.4	44.1	7.1	141.7
	July	417.13	196.4	81.4	11.2	6.9	94.5	10.8	201.5
	October	315.81	121.0	75.3	9.3	6.2	82.2	8.3	168.9
2024	January	168.49	128.3	48.3	2.3	5.1	54.4	6.8	116.8
	April	211.38	199.6	71.2	4.3	6.6	75.6	7.1	135.8
	July	354.25	234.7	78.0	11.7	8.1	63.8	9.8	187.2
	October	298.74	118.7	68.2	7.4	7.1	66.7	8.2	174.6

The water conductivity varied between 168.49 µS/cm in January 2024 and 417.13 µS/cm in July 2023, indicating significant fluctuations, with peaks in the summer months. The increase in conductivity during summer may be related to higher evaporation and the concentration of salts in the water under high temperatures and low precipitation conditions. Filterable residue suggests an increase in suspended materials in the water during the summer period, possibly due to rainfall and sediment influx, as well as a higher load of solid substances.

Ca^{2+} concentrations (48.3 - 81.4 mg/L) fall within quality Class II, indicating good water quality. Sulfates (116.8 - 201.5 mg/L), mainly falling within quality Class III, indicating moderate water quality typical of areas with natural or slightly anthropogenic sulfate input.

Table 2.1.54. Content of toxic metal trace elements in the water of the Arieș River downstream of Baia de Arieș (A3).

Parameter Time, year/month		As, µg/L	Cd, µg/L	Co, µg/L	Cr, µg/L	Cu, µg/L	Fe, mg/L	Mn, mg/L	Ni, µg/L	Zn, µg/L
2022	January	0.27	0.32	0.78	2.42	18.18	0.09	0.038	1.23	74.85
	April	0.31	2.14	3.26	10.04	47.33	0.36	0.096	1.84	125.39
	July	0.49	4.18	4.74	15.7	237.27	0.72	0.541	8.52	327.14
	October	0.35	1.27	2.22	8.82	101.71	0.19	0.384	3.16	165.28
2023	January	0.35	0.45	1.13	3.61	9.23	0.28	0.053	1.35	84.61
	April	0.37	3.07	2.39	9.28	39.52	0.52	0.137	2.22	120.84
	July	0.42	3.99	5.17	19.23	142.08	0.84	0.422	7.27	214.71
	October	0.40	1.52	1.56	6.74	58.32	0.43	0.226	3.69	156.71
2024	January	0.41	0.53	1.54	5.27	13.78	0.24	0.031	1.08	96.74
	April	0.42	2.25	2.34	11.29	23.27	0.41	0.127	4.26	122.6
	July	0.45	4.11	3.85	20.05	98.70	0.67	0.315	6.25	284.57
	October	0.44	1.19	1.89	7.69	44.28	0.33	0.285	5.60	165.38

Cadmium showed notable fluctuations, with values (0.32 - 4.18 µg/L) falling within classes I–IV, most of them in Class IV. This indicates moderate to poor water quality for this metal, without any extreme exceedances (Class V). Copper concentrations range from 9.23 to 237.27 µg/L, indicating possible industrial sources, and covering quality classes I–V, with three values exceeding the permissible limit (Class V). Iron values falling within quality classes I–III, indicating good to moderate water quality without exceeding upper limits. Manganese (0.031 - 0.541 mg/L) fall within quality classes I–IV, indicating water quality from very good to moderate without exceeding permissible limits. Zinc varied between 74.85 µg/L and 327.14 µg/L, falling within quality classes I–III, indicating water quality from very good to moderate and suggesting influence from industrial sources, as well as precipitation and transport in the aquatic environment.

Table 2.1.55 presents the diatom community from sampling point A3 of the Arieș River, located in the area of Baia de Arieș.

Table 2.1.55. Taxonomic composition and saprobic value of diatoms identified in the Arieş River, Baia de Arieş station (A3).

Species name	**Number of individuals**		**Saprobic zone**	**Saprobic value**
	Normal	**Teratological**		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	5	0	β	2
Planothidium rostratoholarcticum Lange-Bertalot & Bak	1	0	β	2
Achnanthidium minutissimum (Kützing) Czarnecki	193	**34**	o-β	1.5
Amphora pediculus (Kützing) Grunow	1	0	β	2
Hannaea arcus (Ehrenberg) R.M.Patrick	1	0	o	1
Discostella stelligera (Cleve & Grunow) Houk & Klee	3	0	-	-
Cymbella ventricosa Agardh	9	0	o-β	1.5
Fragilaria capucina Desmazières	13	3	β	2
Fragilaria vaucheriae (Kützing) J.B.Petersen	13	3	β	2
Gomphonema parvulum Kützing	3	0	β	2
Gomphonema pumilum (Grunow) E.Reichardt & Lange-Bertalot	1	0	o	1
Navicula cryptotenella Lange-Bertalot	4	0	β	2
Navicula gregaria Donkin	8	0	β	2
Navicula cryptocephala Kützing	2	0	α	3
Navicula viridula (Kützing) Ehrenberg	2	0	α	3
Nitzschia dissipata (Kützing) Rabenhorst	41	0	o-β	1.5
Nitzschia gracilis Hantzsch	7	0	β	2
Nitzschia palea (Kützing) W.Smith	13	0	α	3
Surirella angusta Kützing	3	0	β	2
Ulnaria ulna (Nitzsch) Compère	1	0	β	2
Total	364		-	-
Saprobic index value	1.65		-	-

The diatom community at A3 is more diverse, with the presence of species characteristic of waters with higher organic load, such as *N. cryptocephala* and *N. palea.* The dominance of *A. minutissimum* (193 individuals) remains, but the relative increase of α-mesosaprobic species indicates a greater influence of organic matter. The SI value is 1.65, corresponding to Class I–II quality water, with slight organic pollution, suggesting that at A3 the water begins to be affected by more pronounced anthropogenic influences compared to A1 and A2.

In Tables 2.1.56, 2.1.57, and 2.1.58, the physicochemical parameters of the Arieș River water are presented upstream of its confluence with the Mureș River, near the locality of Gligorești (A4), to highlight water quality before the river leaves the Arieș watershed. This represents a strategic monitoring point, located near the river's exit from the basin, allowing assessment of the cumulative impact of anthropogenic and natural activities from the entire upper and middle basin on water quality.

Table 2.1.56. Physico-chemical parameters of the Arieș River water upstream of Gligorești (A4).

Parameter / Time, year/month		pH	TSS, mg/L	DO, mgO/L	BOD$_5$, mgO/L	COD-Cr, mgO/L	NH_4^+ mgN/L	NO_2^- mgN/L	NO_3^- mgN/L
2022	January	7.9	38.5	10.69	0.83	1.27	0.021	0.003	0.164
	April	8.2	112.7	11.03	0.57	1.67	0.027	0.007	0.294
	July	6.7	68.1	8.98	1.58	3.74	0.084	0.011	0.617
	October	7.2	105.2	9.67	1.27	2.67	0.071	0.008	0.427
2023	January	8.1	30.0	10.61	0.89	1.04	0.019	0.004	0.217
	April	7.9	109.6	10.87	0.57	1.72	0.034	0.009	0.367
	July	6.4	43.8	9.63	1.36	3.30	0.091	0.011	0.597
	October	7.7	91.9	10.45	1.07	2.17	0.053	0.009	0.542
2024	January	8.1	39.3	10.87	0.84	1.07	0.015	0.007	0.231
	April	7.9	154.9	11.23	1.18	1.79	0.042	0.008	0.333
	July	6.3	69.4	8.25	1.84	3.34	0.107	0.013	0.550
	October	7.8	98.3	10.05	1.36	2.11	0.068	0.011	0.497

The Arieș River, upstream of Mihai Viteazu, shows a pH ranging between 6.3 and 8.2, indicating generally neutral water, with some fluctuations that could be influenced by climatic conditions or human activities. Suspended solids reached high values in April 2024 (154.9 mg/L), suggesting a possible increase in sedimentation, likely due to heavy rainfall or soil erosion.

Overall, this water parameters fall within quality classes I and II, but seasonal fluctuations and increased organic pollution and nutrient levels during the summer require continuous monitoring to prevent long-term deterioration of water quality.

Table 2.1.57. Salinity parameters of the Arieș River water downstream but upstream of Gligorești (A4).

Parameter / Time, year/month		EC, µS/cm	FR, mg/L	Ca, mg/L	Mg, mg/L	Na, mg/L	Bicarbonates, mg/L	Chlorides, mg/L	Sulfates, mg/L
2022	January	178.23	135.4	64.0	5.8	5.2	52.3	6.2	18.7
	April	247.37	181.2	61.3	6.7	6.4	63.1	7.9	29.3
	July	388.28	212.6	74.5	12.8	8.3	96.5	11.2	98.4
	October	269.07	135.0	48.2	9.4	7.7	78.7	9.1	75.4
2023	January	184.69	123.1	54.7	6.9	4.5	61.4	5.8	21.0
	April	217.01	161.9	55.1	8.1	5.6	69.9	7.1	41.7
	July	432.07	201.3	75.0	14.4	9.1	102.8	10.8	101.5
	October	356.61	142.5	62.1	9.9	7.0	94.4	8.3	68.9
2024	January	187.10	133.8	48.2	5.3	5.4	52.3	6.8	16.8
	April	232.24	212.4	55.3	7.6	5.6	63.8	7.1	35.8
	July	369.41	245.3	67.8	12.5	7.9	89.6	9.8	87.2
	October	301.17	137.7	74.9	8.8	7.7	78.5	8.2	74.6

The salinity parameters of the Arieș River water upstream of Gligorești show significant seasonal variations. Overall, they fall within water quality class II, due to the exceedances of the Ca, Mn, and sulfate parameters.

Table 2.1.58. The content of toxic metal trace elements in the Arieș River water upstream of Gligorești (A4).

Parameter / Time, year/month	As, µg/L	Cd, µg/L	Co, µg/L	Cr, µg/L	Cu, µg/L	Fe, mg/L	Mn, mg/L	Ni, µg/L	Zn, µg/L

2022	January	0.31	0.11	1.12	3.41	12.27	0.09	0.096	1.19	8.85
	April	0.32	1.29	2.98	8.96	35.71	0.10	0.124	1.74	42.56
	July	0.41	2.01	3.45	25.41	187.13	0.12	0.285	8.52	101.42
	October	0.39	1.43	2.01	7.62	74.36	0.11	0.181	2.97	71.38
2023	January	0.34	0.09	1.23	2.94	10.21	0.04	0.108	1.20	14.61
	April	0.37	1.42	2.11	6.52	23.84	0.06	0.143	2.74	37.62
	July	0.39	2.27	4.06	19.69	122.17	0.09	0.199	6.88	94.08
	October	0.40	1.19	1.79	9.96	63.86	0.07	0.162	4.19	75.36
2024	January	0.28	0.18	1.42	4.18	13.78	0.03	0.088	1.08	19.97
	April	0.31	1.36	1.84	12.49	28.73	0.12	0.117	1.23	32.86
	July	0.36	1.94	3.97	30.17	98.69	0.14	0.214	6.25	87.63
	October	0.37	0.85	1.49	7.12	54.28	0.08	0.249	3.48	44.20

Contamination with toxic metal trace elements in the Arieș River water upstream of Gligorești has varied considerably over the past three years, showing notable seasonal fluctuations.

Arsenic concentrations were relatively low. Cadmium showed a maximum of 2.27 µg/L in 2023, falling within quality classes I–IV, mostly in Class III, indicating moderate water quality. Chromium, peaking at 25.41 µg/L in July 2022 and 30.17 µg/L in 2023, ranged mostly within Class I, with a few values in Class II, indicating generally good water quality. Copper values ranged from 10.21 to 187.13 µg/L, covering classes I–V, with most values in Classes IV and V, indicating variable water quality with two exceedances of permissible limits. Manganese concentrations (0.088–0.285 mg/L) ranged mostly within Class III, with a few values in Class II, indicating moderate water quality.

Most of the analyzed trace metals fall within the limits of water quality class II, but during summer months, when pollution values are highest, there is an increased environmental risk. Elevated Cu, Cd, and Zn concentrations can affect aquatic organisms, such as fish, invertebrates, and algae, and may bioaccumulate, potentially impacting human health through water or food consumption.

Table 2.1.59 presents the identified diatom species, the number of individuals, and the saprobic values used to assess the degree of organic load of the water and the potential impact of anthropogenic activities.

Table 2.1.59. Taxonomic composition and saprobic value of diatoms identified in the Arieș River, Gligorești station (A4).

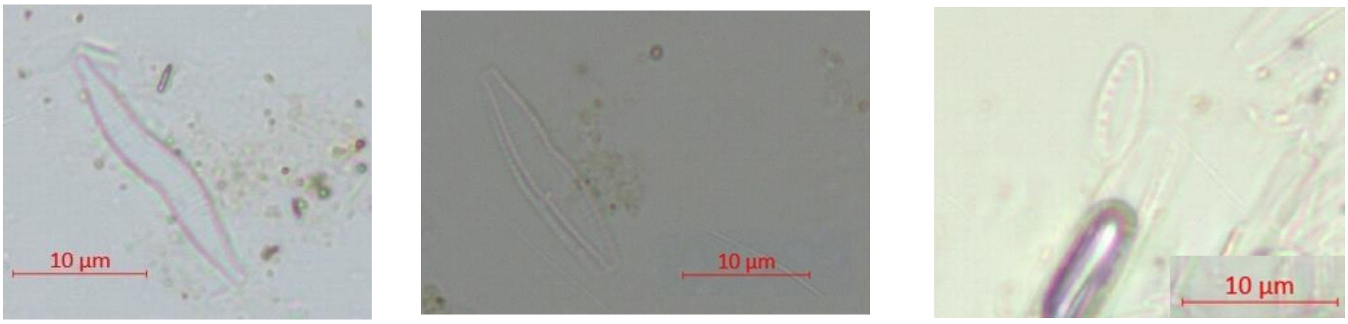
Species name	**Number of individuals**		**Saprobic zone**	**Saprobic value**
	Normal	**Teratological**		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	2	0	β	2
Amphora ovalis (Kützing) Kützing	3	0	α	3
Amphora pediculus (Kützing) Grunow	2	0	β	2
Cocconeis pediculus Ehrenberg	4	0	β	2
Cymbella affinis Kützing	19	0	o-β	1.5
Encyonema cespitosum Kützing	66	0	β	2
Cymbella tumida (Brébisson) Van Heurck	19	0	β-α	2.5
Cymbella ventricosa Agardh	3	0	o-β	1.5
Diatoma vulgaris Bory	3	0	β-α	2.5
Fragilaria capucina Desmazières	35	**5**	β	2
Gomphonella olivacea (Hornemann) Rabenhorst	33	0	β	2
Gomphonema parvulum Kützing	23	0	β	2
Luticola mutica (Kützing) D.G. Mann	7	0	α	3
Melosira varians Agardh	34	0	β	2
Navicula cincta (Ehrenberg) Ralfs	93	0	β-α	2.5

Navicula cryptocephala Kützing	9	0	α	3
Navicula cryptotenella Lange-Bertalot	15	0	β	2
Navicula recens (H.Lange-Bertalot) H.Lange-Bertalot	2	0	α	3
Nitzschia dissipata (Kützing) Rabenhorst	20	0	o-β	1.5
Nitzschia inconspicua Grunow	21	**3**	α	3
Rhoicosphenia curvata (Kützing) Grunow	1	0	β	2
Ulnaria ulna (Nitzsch) Compère	8	**10**	β	2
Total	440		-	-
Saprobic index value	1.93		-	-

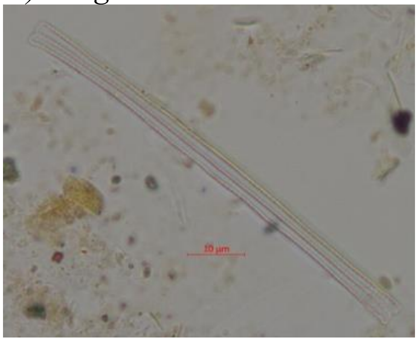
The diatom community at the sampling point in the Mureș River (A4) is diverse, with the presence of several α-mesosaprobic species, such as *A. ovalis*, *N. cryptocephala*, and *N. inconspicua*, indicating an increase in organic load and a greater influence of anthropogenic factors. The dominance of β and o-β species, such as *E. cespitosum* and *N. cincta*, suggests that the water still maintains moderate to oligosaprobic characteristics. The SI value is 1.93, which indicates Class I–II quality water, with a slight influence of organic pollution and mining activities on the diatom community.

Figures 2.1.37 a) to g) present teratological forms of diatoms, which indicate community responses to ecological stress and the presence of pollutants, reflecting alterations in frustule development.

a) Achnanthidium minutissimum *b) Gomphonella olivacea* *c) Diatoma vulgaris*



d) Fragilaria capucina *e) Fragilaria vaucheriae* *f) Nitzschia inconspicua*



g) Ulnaria ulna

Figure 2.1.37. Teratological forms (a–g) of diatoms under ecological stress.

These examples of teratological forms, observed for instance in *A. minutissimum, F. capucina, F. vaucheriae*, and *U. ulna*, reflect local ecological disturbances associated with chemical stress or the presence of pollutants. The observations were integrated into the community analysis, and the data were used to calculate the SI, providing a quantitative assessment of the organic load and the ecological status of the water.

In performing the Principal Component Analysis, all physico-chemical, salinity and metal trace element parameters were considered (figure 2.1.38).

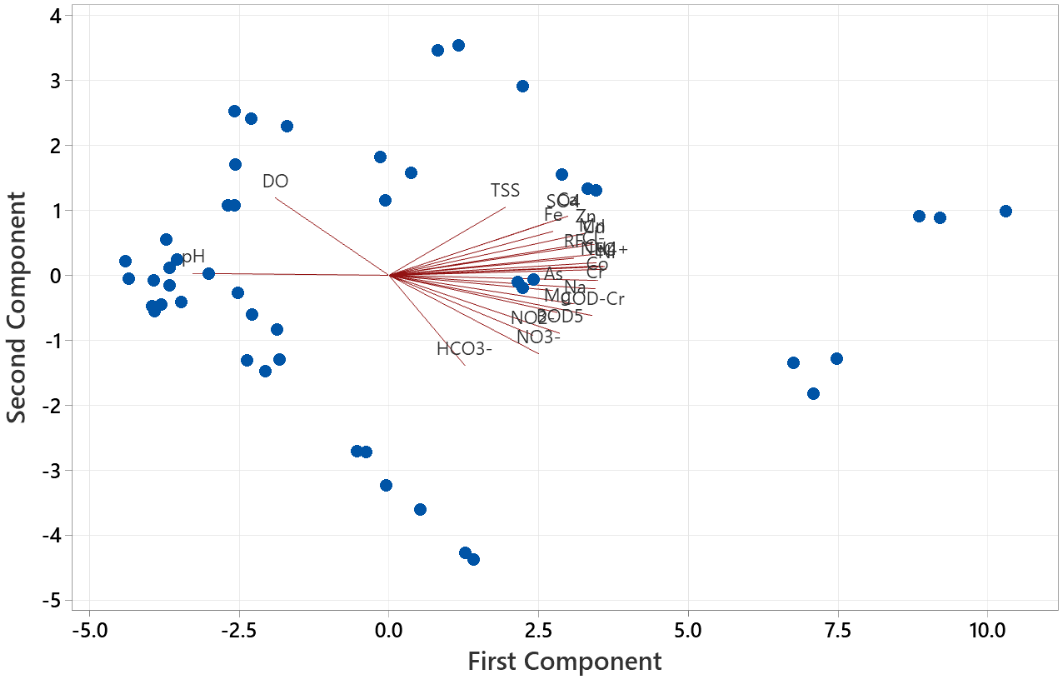


Figure 2.1.38. PCA scores plot showing sampling site distribution along gradients of organic load, nutrients, salinity, and trace metals contamination.

The Principal Component Analysis (figure 2.1.38) highlighted that the first three components explain approximately 81% of the total variance in the dataset, indicating a strong structure within the environmental dataset. PC1 accounts for 61% of the variance and clearly reflects a general pollution gradient. It is strongly positively associated with electrical conductivity, ammonium, organic matter indicators (COD-Cr, BOD_5), and several metals (Cd, Co, Cr, Cu, Mn, Ni, Zn). This axis therefore represents a clear gradient of mineralization and pollution, separating well-oxygenated, less impacted waters from those enriched in dissolved ions and trace metals.

PC2, which explains 14% of the variance, with positive correlations with dissolved oxygen and total suspended solids, and negative ones with nitrates, bicarbonates, and organic load, suggesting differences in oxygenation and nutrient balance among sites.

PC3, responsible for 6.5% of the total variance, captured additional variability related mainly to iron and zinc concentrations, reflecting localized metal inputs.

The PCA indicates that water samples are distributed along a continuum from clean, oxygen-rich conditions to more mineralized and organically enriched environments.

Figure 2.1.39 illustrates the results of a Canonical Correspondence Analysis (CCA), highlighting the relationship between environmental variables and the distribution of diatom species. The plot also shows how dominant taxa respond to variations in water quality, providing insight into the ecological effects of pollution and the potential use of these species as bioindicators.

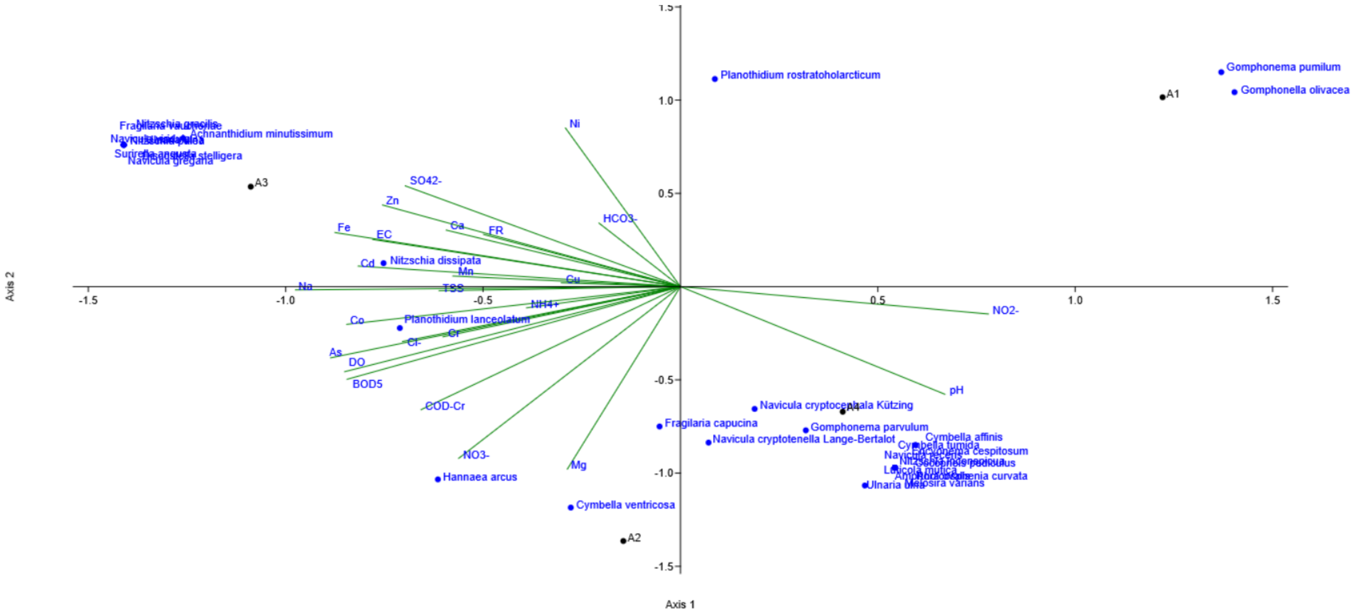


Figure 2.1.39. Canonical Correspondence Analysis (CCA) plot showing the impact of environmental variables on diatom species distribution.

The CCA ordination focusing on environmental variables (physico-chemical, salinity and trace metal parameters) and diatom abundance revealed clear ecological gradients. Axis 1 (36.8% of constrained inertia) mainly reflects organic pollution and nutrient enrichment, with variables such as BOD_5, COD-Cr, NH_4^+, and EC associated with tolerant taxa like *Nitzschia palea*, *Surirella angusta*, and *Navicula viridula*. In contrast, species such as *Achnanthidium minutissimum* and *Gomphonema pumilum* occur in cleaner, better-oxygenated waters. Axis 2 (33.4%) represents a secondary gradient related to dissolved oxygen and nutrient balance, separating taxa typical of low-oxygen, nutrient-rich sites from those of oligotrophic environments. Axis 3 (29.8% of constrained inertia) reflects secondary variation related to slight changes in salinity and metal levels (especially Fe, Cu, and Zn). Overall, the three axes show that diatom communities respond along a gradient from clean, oxygen-rich, low-nutrient waters to more mineralized and organically polluted environments. Although Monte Carlo tests showed no statistical significance ($p > 0.05$), the ordination still indicates that diatom communities respond predictably to variations in organic load, nutrients, and ionic composition, confirming their usefulness as bioindicators of water quality.

The table 2.1.60 presents the Pearson correlation coefficients between the concentrations of various trace elements (As, Cd, Cr, Cu, Fe, Ni, Zn) and the diatom species identified at the studied sites. The values highlight how dominant species respond to heavy metal contamination, providing insights into their potential as bioindicators of water quality.

Table 2.1.60. Pearson correlation coefficients between metal trace elements and diatom species distribution.

Diatoms vs. Metal Trace elements	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Ni**	**Zn**
Planothidium rostratoholarcticu	-0.083	0.194	-0.294	-0.112	**0.472**	**0.967**	**0.556**
Achnanthidium minutissimum	**0.547**	**0.840**	**0.422**	0.339	**0.985**	**0.785**	**0.976**
Cymbella affinis	-0.522	0.142	**0.602**	**0.743**	-0.301	-0.625	-0.171
Cymbella ventricosa	**0.714**	-0.247	-0.219	-0.587	-0.091	-0.358	-0.323
Gomphonella olivacea	-0.809	-0.631	-0.698	-0.347	-0.531	0.242	-0.386
Gomphonema pumilum	-0.623	-0.684	-0.848	-0.564	-0.472	0.343	-0.379
Planothidium lanceolatum	**0.742**	**0.935**	**0.719**	**0.498**	**0.939**	**0.371**	**0.874**
Amphora pediculus	**0.584**	-0.327	-0.174	-0.518	-0.256	-0.561	-0.472

Hannaea arcus	**0.898**	0.034	-0.069	-0.467	0.233	-0.111	-0.003
Cocconeis placentula	**0.573**	-0.397	-0.295	-0.627	-0.273	-0.483	-0.492
Cymbella gracilis	**0.573**	-0.397	-0.295	-0.627	-0.273	-0.483	-0.492
Odontidium mesodon	**0.573**	-0.397	-0.295	-0.627	-0.273	-0.483	-0.492
Diatoma vulgaris	**0.555**	-0.394	-0.262	-0.593	-0.294	-0.530	-0.510
Didymosphenia geminata	**0.573**	-0.397	-0.295	-0.627	-0.273	-0.483	-0.492
Fragilaria capucina	-0.074	**0.466**	**0.865**	**0.835**	0.043	-0.587	0.097
Navicula cryptotenella Lange-Be	-0.200	0.345	**0.788**	**0.797**	-0.094	-0.653	-0.024
Nitzschia dissipata	0.396	**0.994**	**0.808**	**0.749**	**0.909**	**0.451**	**0.946**
Ulnaria ulna	-0.428	0.134	0.621	0.709	-0.313	-0.710	-0.211
Discostella stelligera	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Fragilaria vaucheriae	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Gomphonema parvulum	-0.418	0.296	**0.719**	**0.833**	-0.148	-0.555	-0.023
Navicula gregaria	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Navicula cryptocephala Kützing	-0.369	0.384	**0.775**	**0.881**	-0.055	-0.491	0.071
Navicula viridula	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Nitzschia gracilis	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Nitzschia palea	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Surirella angusta	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Ulnaria ulna_1	**0.573**	**0.860**	**0.457**	**0.359**	**0.992**	**0.753**	**0.978**
Amphora ovalis	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Cocconeis pediculus	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Encyonema cespitosum	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Cymbella tumida	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Luticola mutica	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Melosira varians	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Navicula recens	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Nitzschia inconspicua	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150
Rhoicosphenia curvata	-0.478	0.174	**0.634**	**0.757**	-0.273	-0.634	-0.150

The Pearson correlation analysis between trace metal concentrations and diatom species in the Arieș River reveals differentiated responses of the communities to metal pollution (table 2.1.60). Cadmium shows strong positive correlations with species such as *N. dissipata* (r = 0.994) and *A. minutissimum* (r = 0.840). In contrast, species such as *G. olivacea* and *G. pumilum* show negative correlations. Correlations with other metals, such as As, Fe, Cu, and Zn, show similar patterns. These relationships allow for the identification of more impacted areas and provide a basis for long-term ecological monitoring, using diatoms as reliable bioindicators of aquatic ecosystem health.

The study demonstrates that water quality in the Arieș River is generally good at most monitoring points, corresponding to Class I–II physico-chemical status. Seasonal increases in physico-chemical parameters, particularly in summer, reflect combined natural and anthropogenic influences, highlighting the need for continuous monitoring. However, downstream sites (A3–A4) reflected the impact of mining and industrial activities, particularly through elevated concentrations of trace elements such as Cd and Cu.

A novel aspect of this study is the integration of diatom deformities with chemical data as indicators of sub-lethal metal stress. The occurrence of teratological forms in species such as *Amphora ovalis*, *Navicula cryptocephala*, and *Nitzschia inconspicua*, provides a sensitive biological signal of ecological stress associated with trace metal accumulation.

These findings highlight the importance of long-term biomonitoring in mining-impacted rivers, as diatom deformities can serve as early-warning indicators of ecological deterioration before water quality reaches critical levels. The results support specific management recommendations for the Arieș River, including targeted monitoring of heavy metals, regular assessment of benthic diatom communities, and implementation of restoration measures to mitigate metal and organic pollution downstream of mining areas.

The following presents the physico-chemical parameters for the Abrud River at three sampling points: at the source, downstream of the confluence with the Roșia Montană River, and upstream of the confluence with the Arieș River. This analysis allows a comparative assessment of surface water quality, highlighting the impact of pollution caused by acid waters from the Roșia Montană River, a tributary of the Abrud River.

Tables 2.1.61 – 2.1.63 show the physico-chemical parameters of the Abrud River at its source, in the locality of Bucium Sat (B1).

Table 2.1.61. Physico-chemical parameters of the Abrud River water in the locality of Bucium Sat (B1).

Parameter **Time, year/month**		**pH**	**TSS, mg/L**	**DO, mgO/L**	**BOD$_5$, mgO/L**	**COD-Cr, mgO/L**	**N-NH$_4$, mgN/L**	**N-NO$_2$, mgN/L**	**N-NO$_3$, mgN/L**
2022	Jan.	7.9	24.3	9.12	0.76	2.16	0.015	0.001	0.025
	Apr.	8.2	31.2	8.46	0.91	1.73	0.023	0.002	0.031
	Jul.	8.1	14.1	7.73	1.07	2.25	0.048	0.005	0.052
	Oct.	7.7	19.7	8.37	0.85	1.96	0.031	0.004	0.019
2023	Jan.	7.5	22.3	8.92	0.81	1.87	0.019	0.002	0.018
	Apr.	8.0	31.4	8.81	1.01	1.34	0.028	0.003	0.027
	Jul.	7.9	24.2	7.94	1.23	2.36	0.034	0.006	0.046
	Oct.	7.6	32.4	8.35	0.67	1.24	0.027	0.001	0.029
2024	Jan.	7.8	27.1	9.04	0.83	1.33	0.011	0.004	0.022
	Apr.	7.7	32.6	8.84	0.89	1.57	0.022	0.003	0.038
	Jul.	7.5	28.0	8.01	1.04	2.67	0.038	0.009	0.044
	Oct.	8.7	32.8	8.76	0.77	1.56	0.013	0.002	0.033
Quality class [33]	***I***	***6.5–8.5***	**-**	***6.2, not less than 80% oxygen saturation***	***6***	***10***	***0.4***	***0.01***	***1***
	II					***25***	***0.8***	***0.03***	***3***
	III					***50***	***1.2***	***0.06***	***5.6***
	IV					***125***	***3.2***	***0.3***	***11.2***
	V					***>125***	***>3.2***	***>0.3***	***>11.2***

The analysis of the physico-chemical parameters of the Abrud River in the village of Bucium Sat (at the source) indicates generally very good water quality, corresponding to Class I for most parameters, with a single exception in October 2024 when the pH reached 8.7, suggesting slight alkalinization possibly due to natural processes or minor anthropogenic influences.

Table 2.1.62. Salinity parameters of the Abrud River in the locality of Bucium Sat (B1).

Parameter **Time, year/month**		**EC, µS/cm**	**FR, mg/L**	**Ca^{2+}, mg/L**	**Mg^{2+}, mg/L**	**Na^{+}, mg/L**	**HCO$_3^-$, mg/L**	**Cl$^-$, mg/L**	**SO$_4^{2-}$, mg/L**
2022	Jan.	136	153	38	2.13	4.1	78.7	0.8	9.8
	Apr.	167	169	42	3.39	3.8	83.5	1.3	8.1
	Jul.	251	234	66	3.85	4.5	89,4	3.7	12.4
	Oct.	196	206	59	1.89	3.4	73.2	2.4	11.1
2023	Jan.	194	174	41	1.66	3.7	72.7	1.1	7.6
	Apr.	184	182	57	2.47	4.2	86.7	1.5	9.3
	Jul.	279	213	61	2.98	4.0	102.4	4.1	14.6
	Oct.	213	168	46	2.09	3.4	84.6	2.9	8.4
2024	Jan.	164	158	48	2.17	3.9	87.4	1.5	10.2

	Apr.	183	184	43	3.14	4.3	84.3	2.3	9.7
	Jul.	267	227	55	4.16	4.8	111.5	3.8	14.5
	Oct.	173	173	40	2.17	3.5	91.0	2.5	10.3
Quality class [33]	***I***	**-**	***500***	***50***	***12***	***25***	**-**	***25***	***60***
	II		***750***	***100***	***50***	***50***		***50***	***120***
	III		***1000***	***200***	***100***	***100***		***250***	***250***
	IV		***1300***	***300***	***200***	***200***		***300***	***300***
	V		***>1300***	***>300***	***>200***	***>200***		***>300***	***>300***

Between 2022 and 2024, the Abrud River at Bucium Sat exhibited low salinity. These results indicate weakly mineralized water with no significant influence from saline pollution sources.

Table 2.1.63. The content of toxic trace elements in the water of the Abrud River in the locality of Bucium Sat (B1).

Parameter **Time, year/month**		**As^{2+}, µg/L**	**Cd^{2+}, µg/L**	**Co_{total} (Co^{2+} + Co^{3+}), µg/L**	**Cr_{total} (Cr^{3+} + Cr^{6+}), µg/L**	**Cu^{2+}, µg/L**	**Fe_{total}, mg/L**	**Mn_{total}, mg/L**	**Ni^{2+}, µg/L**	**Zn^{2+}, µg/L**
2022	Jan.	0.32	0.31	0.91	1.47	2.54	0.02	0.054	1.64	7.69
	Apr.	0.41	0.45	1.13	2.80	3.17	0.04	0.047	2.37	9.14
	Jul.	0.77	0.63	3.47	5.78	9.76	0.09	0.124	4.46	10.54
	Oct.	0.28	0.43	2.34	2.34	3.15	0.06	0.067	2.48	8.42
2023	Jan.	0.35	0.28	0.24	2.01	3.09	0.01	0.049	1.87	6.43
	Apr.	0.46	0.51	0.69	3.17	4.17	0.03	0.061	2.27	9.84
	Jul.	0.69	0.57	2.64	6.15	10.46	0.07	0.115	3.94	13.47
	Oct.	0.25	0.39	1.86	2.36	3.18	0.04	0.055	3.04	7.62
2024	Jan.	0.39	0.41	0.36	2.17	4.64	0.01	0.049	2.07	6.64
	Apr.	0.51	0.37	0.62	2.48	5.20	0.05	0.062	2.41	7.30
	Jul.	0.59	0.58	3.42	6.37	8.12	0.08	0.097	3.97	14.52
	Oct.	0.33	0.34	1.84	3.14	4.38	0.02	0.043	1.95	11.34
Quality class [33]	***I***	***10***	***0.5***	***10***	***25***	***20***	***0.3***	***0.05***	***10***	***100***
	II	***20***	***1***	***20***	***50***	***30***	***0.5***	***0.1***	***25***	***200***
	III	***50***	***2***	***50***	***100***	***50***	***1.0***	***0.3***	***50***	***500***
	IV	***100***	***5***	***100***	***250***	***100***	***2***	***1***	***100***	***1000***
	V	***>100***	***>5***	***>100***	***>250***	***>100***	***>2***	***>1***	***>100***	***>1000***

Cadmium, one of the most toxic metals, varied between 0.28 and 0.63 µg/L, slightly exceeding the Class I limit of 0.5 µg/L in a few isolated cases (July 2022, April and July 2023, July 2024), temporarily placing the water in Class II for this parameter. These minor increases may be associated with periods of intense runoff or seasonal mobilization from soils. Dissolved metals were present at very low concentrations.

Although the biofilm samples were collected on a single date (25 April 2024), they provide a snapshot of the ecosystem's status at that time. These results are interpreted alongside long-term chemical and physical monitoring data to assess the influence of mining activities in the area, so even a single biological sample can offer valuable insight into the potential impacts of local mining on the aquatic ecosystem.

Table 2.1.64 presents the diatom species identified in the water of the Abrud River at its source, in the Bucium Sat area (A1), the number of individuals for each species, the associated saprobic zone, and the saprobic value.

Table 2.1.64. Diatom composition and saprobic index of the Abrud River at Bucium Sat (B1).

Scientific Name of a Diatom	**Number of individuals**		**Degree of Saprobity**	**Saprobic Indicator Value**
	Normal	**Teratological**		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	62	0	β	2
Achnanthidium minutissimum Kützing) Czarnecki	129	**8**	o-β	1.5
Cocconeis pediculus Ehrenberg	24	0	β	2
Cocconeis placentula Ehrenberg	9	0	β	2
Cymbella ventricosa Agardh	1	0	o-β	1.5
Odontidium mesodon (Ehrenberg) Kützing	3	0	o	1
Fragilaria vaucheriae (Kützing) J.B.Petersen	5	0	β	2
Gomphonella olivacea (Hornemann) Rabenhorst	15	0	β	2
Gomphonema parvulum Kützing	3	0	β	2
Meridion circulare (Greville) C.Agardh	3	0	o	1
Navicula cryptocephala Kützing	27	0	α	3
Navicula radiosa Kützing	59	0	β	2
Navicula tripunctata (O.F.Müller) Bory	13	0	β-α	2.5
Nitzschia dissipata (Kützing) Rabenhorst	13	0	o-β	1.5
Surirella ovalis Brébisson	40	0	α	3
Total	414			
Saprobic index value	1.91			

The diatom composition and the saprobic index (1.91) indicate water with low organic pollution, corresponding to a moderate ecological class. The dominant species in the β and o-β zones suggest a slightly impacted environment by organic matter, but with generally good conditions for maintaining the aquatic ecosystem.

Figures 2.1.40 and 2.1.41 show a comparison between normal and teratological diatom forms, representing samples collected from the tributaries of the Aries River at site A1.

Figure 2.1.40. Normal form of *A. minutissimum* at site B1.

Figure 2.1.41. Teratological form of *A. minutissimum* at site B1.

Tables 2.1.65 – 2.1.67 present the physico-chemical parameters of the Abrud River downstream of its confluence with the Roșia Montană River.

Table 2.1.65. Physico-chemical parameters of the Abrud River water downstream of the confluence with the Roșia Montană River (B2).

Parameter Time, year/month		pH	TSS, mg/L	DO, mgO/L	BOD$_5$, mgO/L	COD-Cr, mgO/L	N-NH$_4$, mgN/L	N-NO$_2$, mgN/L	N-NO$_3$, mgN/L
2022	Jan.	7.1	28.5	8.43	1.27	1.41	0.357	0.006	0.246
	Apr.	6.9	35.1	8.67	1.53	1.87	0.413	0.009	0.740
	Jul.	5.1	18.2	8.04	0.89	0.95	0.281	0.004	0.204
	Oct.	7.3	41.0	8.27	2.31	2.16	0.981	0.013	1.012
2023	Jan.	7.7	36.7	8.79	1.46	2.04	0.652	0.008	0.314
	Apr.	7.2	47.9	8.68	1.90	1.98	0.607	0.012	0.681
	Jul.	5.5	39.4	8.37	1.14	0.68	0.194	0.007	0.181
	Oct.	7.0	65.3	8.07	1.82	2.43	1.147	0.015	0.998
2024	Jan.	7.6	32.0	8.62	1.51	1.74	0.842	0.012	0.372
	Apr.	6.9	47.2	8.48	1.82	1.37	0.701	0.008	0.593
	Jul.	4.5	26.1	8.18	0.97	1.18	1.027	0.006	0.167
	Oct.	7.5	74.7	8.35	2.59	2.35	1.842	0.011	0.847

After the confluence with the Roșia Montană River, the physico-chemical parameters of the Abrud River show a noticeable increase in pollution compared to upstream. Although pH generally remains stable, pronounced decreases were observed during the summer, reaching 4.5 in 2024, indicating significant acidification, likely due to acid mine drainage in the Roșia Montană area.

Dissolved oxygen levels remain good (8–8.8 mg/L), indicating a relatively preserved self-purification capacity. BOD$_5$ and COD-Cr values reflect increased biodegradable and oxidizable organic matter. Ammonium nitrogen and nitrite concentrations are significantly elevated, frequently exceeding Class I and II limits.

Downstream of the Roșia Montană River confluence, the Abrud River shows a significant increase in water mineralization. Sulfate concentrations exceed 200 mg/L in summer, approaching Class III, suggesting infiltration from sulfate-rich mining areas. The combination of high conductivity, minerals, and sulfates points to significant anthropogenic impact, particularly from mining. These parameters highlight continuous chemical pressure on the aquatic ecosystem during the warm season.

Table 2.1.66. Salinity parameters of Abrud River at site B2 (downstream of the Roșia Montană confluence).

Parameter Time, year/month		EC, µS/cm	FR, mg/L	Ca^{2+}, mg/L	Mg^{2+}, mg/L	Na^{+}, mg/L	HCO$_3^-$, mg/L	Cl$^-$, mg/L	SO$_4^{2-}$, mg/L
2022	Jan.	294	233	43	10.3	7.6	63.4	10.4	102.1
	Apr.	374	261	86	12.7	9.1	74.3	11.6	132.5
	Jul.	514	313	102	16.3	11.3	96.0	16.1	194.3
	Oct.	263	179	43	4.7	5.5	61.2	9.2	77.2
2023	Jan.	332	231	52	7.9	7.8	68.3	12.6	116.4
	Apr.	347	246	74	17.3	9.2	89.1	11.3	126.8
	Jul.	411	374	137	22.2	10.7	109.8	15.0	214.1
	Oct.	363	262	63	8.8	6.3	55.6	8.2	51.7
2024	Jan.	384	263	74	10.0	7.7	74.1	10.9	113.0
	Apr.	473	324	94	17.8	9.8	74.3	11.7	141.2
	Jul.	562	364	93	27.4	10.1	79.7	13.5	183.4
	Oct.	318	189	46	11.1	8.4	41.0	9.8	52.3

Table 2.1.67. Metal trace element content in the Abrud River at site B2 (downstream of the Roșia Montană confluence).

Parameter **Time, year/month**		**As^{2+}, μg/L**	**Cd^{2+}, μg/L**	**Co_{total} ($Co^{2+}+Co^{3+}$), μg/L**	**Cr_{total} ($Cr^{3+}+Cr^{6+}$), μg/L**	**Cu^{2+}, μg/L**	**Fe_{total}, mg/L**	**Mn_{total}, mg/L**	**Ni^{2+}, μg/L**	**Zn^{2+}, μg/L**
2022	Jan.	0.35	3.68	6.84	23.41	127.31	1.28	10.43	21.68	412.35
	Apr.	0.42	4.87	8.14	25.12	135.07	1.67	15.17	32.17	617.22
	Jul.	0.47	8.64	9.63	28.86	187.35	3.07	18.84	48.47	896.74
	Oct.	0.36	1.37	3.61	11.58	104.28	1.08	8.67	18.09	233.17
2023	Jan.	0.38	3.02	7.45	17.61	94.36	1.69	12.20	25.34	387.19
	Apr.	0.41	3.77	8.74	22.27	125.07	2.41	14.64	39.41	697.23
	Jul.	0.37	9.14	11.08	28.94	154.08	4.44	24.04	48.30	967.74
	Oct.	0.33	2.35	5.12	15.6	112.32	1.32	12.71	17.40	214.78
2024	Jan.	0.31	3.62	7.38	17.08	89.70	1.87	11.74	21.77	386.42
	Apr.	0.38	6.42	8.42	28.17	131.22	3.24	14.32	23.48	612.37
	Jul.	0.47	7.84	12.28	61.74	166.74	4.74	21.74	42.07	814.37
	Oct.	0.39	2.47	3.18	16.37	99.71	1.39	13.62	19.42	274.62

The Abrud River exhibits a severe load of toxic trace elements, especially during the summer, indicating strong anthropogenic influences, likely from historical and ongoing mining activities.

Cadmium concentrations consistently exceeded the permissible limits, peaking above 9 μg/L in July 2023 (Class IV–V), posing a significant risk to aquatic life. Cobalt, nickel, and chromium remain elevated, with chromium reaching 61.74 μg/L in July 2024. Copper shows extreme summer values (>180 μg/L), far above the 20 μg/L limit, likely due to runoff from mining tailings. Iron and manganese reach 4.74 mg/L and 24.04 mg/L, respectively, placing the water in Classes III–V. Zinc exceeds 600 μg/L in summer, reaching over 960 μg/L in 2023, signaling significant industrial pollution with clear ecotoxic effects.

Table 2.1.68 presents the diatom species identified at the Cărpeniș station (B2), their individual counts, saprobic zones, and saprobic values, providing a basis for assessing the ecological quality and organic pollution level of the water.

Table 2.1.68. Diatom composition and saprobic index at site B2 (Abrud River, Cărpeniș).

Scientific Name of a Diatom	**Number of individuals**		**Degree of Saprobity**	**Saprobic Indicator Value**
	Normal	**Teratological**		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	1	0	β	2
*Achnanthidium minutissimum (*Kützing) Czarnecki	353	**99**	o-β	1.5
Fragilaria vaucheriae (Kützing) J.B.Petersen	2	0	β	2
Navicula cryptocephala Kützing	2	0	α	3
Nitzschia dissipata (Kützing) Rabenhorst	2	0	o-β	1.5
Total	459			
Saprobic index value	1.22			

The saprobic index (1.22) indicates very low organic pollution (Class I–II). Dominant species from the o-β and α zones suggest good environmental conditions with minimal organic impact. In the Abrud River (Cărpeniş), the saprobic index indicates a very good water status in terms of organic matter load. However, *A. minutissimum* was identified, representing 78.21% of the total counted individuals.

Additionally, 21.56% of these individuals exhibited teratological forms, which occur under heavy metal contamination. This indicates the presence of an ecological niche formed by acidic waters in the area, allowing this species, adapted to lower pH, to thrive and occur in high numbers. The result of the saprobic index does not provide a realistic assessment of the water body's condition. The strong dominance of *A. minutissimum* suggests, as supported by the high metal values shown in Table 2.1.68, that metal pollution has a significant impact, with only minor variations in concentration over time. In this case, the evaluation of the water body should be based solely on the presence of metals and pH variations, excluding the saprobic assessment.

Figures 2.1.42 and 2.1.43 compare normal and teratological diatom forms from tributary samples of the Aries River at site B2.

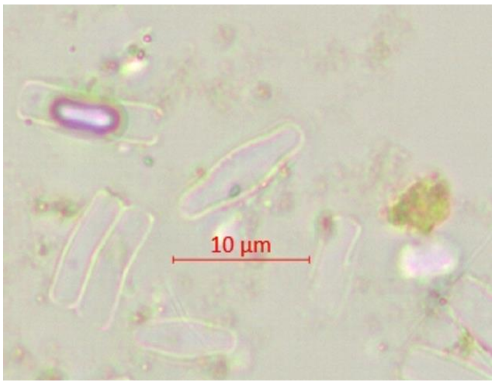


Figure 2.1.42. Normal form of *A. minutissimum* at site B2.

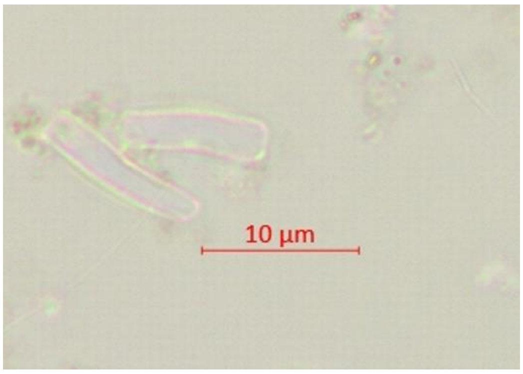


Figure 2.1.43. Teratological form of *A. minutissimum* at site B2.

Comparison of sites B1 and B2 shows a clear impact of mine waters on the diatom community. At A1, near the source, diverse species from the β and o-β saprobic zones, including *P. lanceolatum*, *A. minutissimum*, and *N. radiosa*, indicate low organic pollution and stable conditions. Downstream at B2, diversity declines, with the community dominated by metal- and pollutant-tolerant species, especially *A. minutissimum*, and a reduction in sensitive α and β species, reflecting decreased ecological quality.

Tables 2.1.69 – 2.1.71 show the physico-chemical parameters of the Abrud River upstream of its confluence with the Aries River, at Câmpeni (B3).

Table 2.1.69. Physico-chemical parameters of Abrud River at site B3 (upstream of the Aries River confluence, Câmpeni).

Parameter **Time, year/month**		**pH**	**TSS, mg/L**	**DO, mgO/L**	**BOD$_5$, mgO/L**	**COD-Cr, mgO/L**	**N-NH$_4$, mgN/L**	**N-NO$_2$, mgN/L**	**N-NO$_3$, mgN/L**
2022	Jan.	7.2	24.4	8.35	1.37	1.51	0.368	0.007	0.254
	Apr.	7.4	31.3	8.27	1.68	1.63	0.443	0.011	0.769
	Jul.	5.5	17.9	8.08	1.12	1.12	0.312	0.007	0.274
	Oct.	7.7	38.3	8.15	2.34	2.27	1.113	0.016	1.117
2023	Jan.	7.9	31.3	8.53	1.64	2.21	0.674	0.011	0.427
	Apr.	7.6	45.7	8.44	1.97	2.17	0.617	0.014	0.719
	Jul.	5.8	32.0	8.24	1.27	0.84	0.243	0.009	0.227
	Oct.	7.3	58.4	8.04	1.94	2.67	1.297	0.017	1.098
2024	Jan.	7.8	28.8	8.57	1.63	1.93	0.863	0.014	0.432
	Apr.	7.4	43.1	8.24	1.92	1.40	0.784	0.011	0.613
	Jul.	5.6	26.2	8.07	1.27	1.31	1.084	0.009	0.218
	Oct.	7.2	69.5	8.17	2.62	2.48	1.927	0.014	0.917

Ammonium nitrogen exceeded 1 mg/L in autumn, while nitrite and nitrate showed variable nitrogen forms, with a nitrate peak of 1.11 mg/L in October 2022, suggesting diffuse or agricultural/urban pollution.

Salinity parameters of the Abrud River upstream of the Arieș confluence at Câmpeni exhibited clear seasonal variability. Conductivity reached 512 µS/cm in July 2024, while sulfates peaked at 201.5 mg/L in July 2023, suggesting inputs from erosion or mining. Overall, water quality was relatively good.

Table 2.1.70. Salinity parameters of Abrud River at site B3 (upstream of the Aries River confluence, Câmpeni).

Parameter Time, year/month		EC, µS/cm	FR, mg/L	Ca^{2+}, mg/L	Mg^{2+}, mg/L	Na^{+}, mg/L	HCO_3^-, mg/L	Cl^-, mg/L	SO_4^{2-}, mg/L
2022	Jan.	279	201	38	9.3	7.6	52.1	9.9	98.7
	Apr.	356	249	74	10.2	8.7	67.9	11.2	124.4
	Jul.	487	289	98	14.1	10.2	84.4	15.4	189.1
	Oct.	237	156	34	3.4	4.7	48.3	8.7	34.1
2023	Jan.	302	197	41	7.1	6.8	56.4	10.1	101.5
	Apr.	318	212	62	14.4	7.3	74.2	10.4	117.9
	Jul.	392	328	103	18.4	9.1	96.4	13.7	201.5
	Oct.	321	187	49	5.9	5.9	41.7	7.9	42.5
2024	Jan.	342	231	59	8.6	6.9	61.2	10.1	108.7
	Apr.	408	281	81	11.4	8.1	67.1	10.7	134.9
	Jul.	512	301	87	21.5	8.7	73.8	11.8	174.8
	Oct.	298	141	28	6.7	6.4	33.4	9.3	41.4

Table 2.1.71. Metal trace element content in the Abrud River at site B3 (upstream of the Aries River confluence, Câmpeni).

Parameter Time, year/month		As^{2+}, µg/L	Cd^{2+}, µg/L	Co_{total} ($Co^{2+}+Co^{3+}$), µg/L	Cr_{total} ($Cr^{3+}+Cr^{6+}$), µg/L	Cu^{2+}, µg/L	Fe_{total}, mg/L	Mn_{total}, mg/L	Ni^{2+}, µg/L	Zn^{2+}, µg/L
2022	Jan.	0.26	3.27	6.47	17.83	94.70	1.09	9.87	19.72	384.17
	Apr.	0.31	4.46	7.68	19.71	114.61	1.47	13.40	27.81	547.70
	Jul.	0.38	8.12	9.14	24.52	157.14	2.48	16.41	42.71	847.41
	Oct.	0.26	1.07	3.24	4.56	45.78	0.87	1.24	8.41	147.79
2023	Jan.	0.29	2.79	7.04	16.37	84.87	1.27	10.37	20.17	327.18
	Apr.	0.32	3.29	8.14	18.19	104.73	1.86	11.71	32.18	647.08
	Jul.	0.27	7.14	10.24	23.74	137.37	3.07	14.62	41.83	941.73
	Oct.	0.25	1.18	4.18	5.14	65.17	0.76	2.38	7.53	178.14
2024	Jan.	0.26	3.14	6.87	15.74	79.09	1.37	8.82	17.86	357.14
	Apr.	0.28	5.07	7.63	20.16	117.49	2.34	10.08	21.18	593.41
	Jul.	0.39	6.87	11.42	21.31	146.91	3.65	13.48	39.07	796.01
	Oct.	0.33	1.32	2.78	6.37	39.70	0.39	3.14	6.07	207.78

Peaks occurred in summer, with copper up to 157.14 µg/L, iron 2.48 mg/L, zinc 847.41 µg/L, cobalt 9.14 µg/L, and cadmium 8.12 µg/L in July 2022, reflecting increased mobilization of metals due to pollution, higher temperatures, and possible erosion. In autumn and winter, metal levels decreased significantly. Although toxic metals are below hazardous limits, high summer concentrations suggest a negative influence of natural processes and potentially anthropogenic activities, such as mine water infiltration.

Table 2.1.72 lists diatom species identified at Câmpeni (B3), their individual counts, saprobic zones, and saprobic values, supporting ecological quality assessment.

Table 2.1.72. Diatom composition and saprobic index at site B3 (Abrud River, Câmpeni).

Scientific Name of a Diatom	**Number of individuals**		**Degree of Saprobity**	**Saprobic Indicator Value**
	Normal	**Teratological**		
Achnanthidium minutissimum Kützing) Czarnecki	214	**71**	o-β	1.5
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	3	**0**	β	2
Planothidium rostratoholarcticum Lange-Bertalot & Bak	1	0	β	2
Amphora pediculus (Kützing) Grunow	3	0	β	2
Cocconeis pediculus Ehrenberg	5	0	β	2
Cocconeis placentula Ehrenberg	4	0	β	2
Surirella librile (Ehrenberg) Ehrenberg	1	0	β-α	2.5
Cymbella ventricosa Agardh	17	0	o-β	1.5
Odontidium mesodon (Ehrenberg) Kützing	5	0	o	1
Diatoma vulgaris Bory	1	0	β-α	2.5
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	1	0	β	2
Fragilaria capucina Desmazières	7	0	β	2
Fragilaria vaucheriae (Kützing) J.B.Petersen	13	0	β	2
Gomphonema parvulum Kützing	15	0	β	2
Meridion circulare (Greville) C.Agardh	3	0	o	1
Navicula capitatoradiata H.Germain ex Gasse	10	0	o-β	1.5
Navicula gregaria Donkin	5	0	β	2
Navicula recens (H.Lange-Bertalot) H.Lange-Bertalot	1	0	α	3
Navicula tripunctata (O.F.Müller) Bory	1	0	β-α	2.5
Navicula veneta Kützing	3	0	α	3
Nitzschia dissipata (Kützing) Rabenhorst	3	0	o-β	1.5
Nitzschia inconspicua Grunow	2	0	α	3
Nitzschia palea (Kützing) W.Smith	6	0	α	3
Nitzschia sigmoidea (Nitzsch) W.Smith	2	0	β	2
Pinnularia subcapitata f. subconstricta A.Cleve	1	0	o	1
Reimeria sinuata (W.Gregory) Kociolek & Stoermer	2	0	β	2
Surirella angusta Kützing	9	0	β	2
Surirella brebissonii Krammer & Lange-Bertalot	19	0	β-α	2.5
Total	428			
Saprobic index value	1.79			

The saprobic index (1.79) indicates low organic pollution (Class I–II). The diatom community is dominated by β and o-β species, including *A. minutissimum* and *C. ventricosa*, with some sensitive α species, suggesting moderate ecological pressure.

Mining water impacts reduce diversity between A1 and A2, but at A3, upstream of the Aries confluence, diversity partially recovers due to dilution and less-polluted tributary inputs.

Figures 2.1.44 and 2.1.45 compare normal and teratological diatom forms from tributary samples at site B3.

Figure 2.1.44. Normal form of *A. minutissimum* at site B3.

Figure 2.1.45. Teratological form of *A. minutissimum* at site B3.

Tables 2.1.73 – 2.1.75 present the main physico-chemical parameters of the water in the Valea Ștefanca River, measured upstream of its confluence with the Arieș River.

Table 2.1.73. Physico-chemical parameters of Valea Ștefanca River water upstream of the Arieș confluence (C).

Parameter Time, year/month		pH	TSS, mg/L	DO, mgO/L	BOD$_5$, mgO/L	COD-Cr, mgO/L	N-NH$_4$, mgN/L	N-NO$_2$, mgN/L	N-NO$_3$, mgN/L
2022	Jan.	7.1	15.4	9.01	0.69	4.48	0.032	0.007	0.142
	Apr.	7.4	16.8	8.94	1.18	5.69	0.056	0.009	0.147
	Jul.	7.5	21.8	8.41	1.86	8.69	0.084	0.011	0.201
	Oct.	7.2	20.4	8.82	0.89	4.39	0.039	0.005	0.114
2023	Jan.	7.3	24.2	9.17	0.86	5.21	0.041	0.004	0.118
	Apr.	7.6	25.4	9.04	1.07	6.28	0.061	0.006	0.214
	Jul.	7.9	32.4	8.22	1.78	7.96	0.076	0.009	0.368
	Oct.	7.2	27.4	8.69	0.58	6.24	0.039	0.005	0.120
2024	Jan.	7.0	21.4	8.89	0.48	6.17	0.041	0.006	0.134
	Apr.	7.2	18.9	8.68	0.79	6.32	0.048	0.008	0.157
	Jul.	7.7	26.8	8.34	1.28	8.42	0.069	0.010	0.197
	Oct.	7.5	25.6	8.79	1.18	5.68	0.054	0.0.5	0.127

BOD$_5$ (0.48–1.86 mgO/L) and COD-Cr (4.39–8.69 mgO/L) values indicate moderate levels of biodegradable and organic matter, with higher concentrations in summer. Ammonium, nitrites, and nitrates remained low, showing balanced nitrification processes. Overall, the water quality falls within Class I–II, with seasonal fluctuations linked to increased organic load and suspended matter in summer, but without exceeding acceptable limits.

Table 2.1.74. Salinity parameters of Valea Ștefanca, upstream of the Arieș confluence (C).

Parameter Time, year/month		EC, µS/cm	FR, mg/L	Ca^{2+}, mg/L	Mg^{2+}, mg/L	Na^{+}, mg/L	HCO$_3^-$, mg/L	Cl$^-$, mg/L	SO$_4^{2-}$, mg/L
2022	Jan.	267.36	136.47	21.42	9.68	1.20	47.26	3.25	22.1
	Apr.	302.14	157.48	32.06	15.62	2.14	65.34	5.24	26.3
	Jul.	420.18	215.32	45.63	25.64	3.64	89.46	7.36	35.4

	Oct.	324.12	169.41	35.24	18.96	2.36	43.80	3.18	18.9
2023	Jan.	189.47	126.84	19.81	14.39	1.89	35.69	2.39	29.4
	Apr.	294.62	174.24	23.68	19.37	2.39	61.14	4.36	24.8
	Jul.	352.41	189.76	39.57	31.28	4.97	77.82	6.28	41.5
	Oct.	264.20	124.62	28.41	22.36	3.31	36.84	2.84	36.9
2024	Jan.	221.13	112.39	26.37	16.54	2.17	35.48	4.15	31.2
	Apr.	284.63	154.63	36.41	19.37	2.36	52.14	3.98	41.6
	Jul.	362.14	241.36	56.18	36.24	4.16	77.69	5.79	54.6
	Oct.	203.48	163.41	38.24	24.51	3.14	39.61	3.14	35.6

Electrical conductivity ranged between 189.47 and 420.18 µS/cm, with peak values in July. Filterable residue (112.39–241.36 mg/L) also peaked in July 2024, suggesting higher dissolved substances. Calcium, magnesium, and sodium showed significant increases in summer. Bicarbonates, chlorides, and sulfates indicate moderate salinity.

Table 2.1.75. Content of toxic trace elements in the water of the Valea Ștefanca River (C).

Parameter Time, year/month		**As^{2+}, µg/L**	**Cd^{2+}, µg/L**	**Co$_{total}$ (Co^{2+}+Co^{3+}), µg/L**	**Cr$_{total}$ (Cr^{3+}+Cr^{6+}), µg/L**	**Cu^{2+}, µg/L**	**Fe$_{total}$, mg/L**	**Mn$_{total}$, mg/L**	**Ni^{2+}, µg/L**	**Zn^{2+}, µg/L**
2022	Jan.	0.28	0.12	0.61	0.98	56.47	0.15	0.027	0.56	18.67
	Apr.	0.62	0.36	0.58	1.14	69.47	0.29	0.031	0.75	32.41
	Jul.	1.31	0.84	0.79	2.46	114.3	0.67	0.056	1.12	45.61
	Oct.	0.57	0.61	0.67	1.69	77.91	0.34	0.036	0.76	24.87
2023	Jan.	0.38	0.34	0.52	1.42	63.70	0.24	0.024	0.41	24.39
	Apr.	0.49	0.27	0.68	1.25	74.51	0.37	0.038	0.69	35.69
	Jul.	1.64	0.63	0.87	3.18	154.91	0.75	0.068	0.84	48.69
	Oct.	0.61	0.44	0.45	2.18	79.19	0.42	0.054	0.47	34.12
2024	Jan.	0.41	0.27	0.61	1.39	73.41	0.28	0.033	0.38	19.47
	Apr.	0.39	0.36	0.54	1.54	68.49	0.34	0.047	0.57	32.45
	Jul.	0.67	0.74	0.94	2.94	164.2	0.76	0.094	0.71	65.42
	Oct.	0.52	0.46	0.49	1.39	89.43	0.61	0.061	0.51	32.14

Copper showed significant values (56.47–164.2 µg/L), exceeding Class II–III limits, suggesting notable pollution. Iron (0.15–0.75 mg/L) and manganese (0.027–0.094 mg/L) indicated moderate levels. Water quality falls mainly into Class III–IV for trace elements, reflecting moderate to high contamination, especially from copper.

Table 2.1.76 presents the diatom species identified in the Valea Ștefanca River water, the number of individuals per species, the associated saprobic zone, and their saprobic values.

Table 2.1.76. Diatom composition and saprobic index–Valea Șefanca (C).

Scientific Name of a Diatom	**Population Size**		**Degree of Saprobity**	**Saprobic Indicator Value**
	Normal	**Teratological**		
Achnanthidium minutissimum (Kützing) Czarnecki	237	**70**	o-β	1.5
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	116	**12**	β	2
Planothidium rostratoholarcticum Lange-Bertalot & Bak	8	0	β	2
Cocconeis pediculus Ehrenberg	27	**3**	β	2
Cymbella ventricosa Agardh	9	0	o-β	1.5
Fragilaria capucina Desmazières	3	0	β	2

Gomphonema parvulum Kützing	4	0	β	2
Navicula tripunctata (O.F.Müller) Bory	2	0	β-α	2.5
Nitzschia dissipata (Kützing) Rabenhorst	30	0	o-β	1.5
Nitzschia inconspicua Grunow	41	0	α	3
Nitzschia sigmoidea (Nitzsch) W.Smith	2	0	β	2
Reimeria sinuata (W.Gregory) Kociolek & Stoermer	2	0	β	2
Surirella brebissonii Krammer & Lange-Bertalot	1	0	β-α	2.5
Total	567			
Saprobic index value	1.58			

The saprobic index of 1.58 indicates low organic pollution, corresponding to ecological quality Class I–II. The diatom community is dominated by β and o-β species, such as *P. lanceolatum* and *A. minutissimum*, while also including sensitive α species (e.g., *N. inconspicua*), suggesting good conditions for a healthy aquatic ecosystem with low to moderate anthropogenic pressure.

Figures 2.1.46 – 2.1.51 show a comparison of normal and teratological diatom forms from samples collected in the tributaries of the Arieş River in site C.

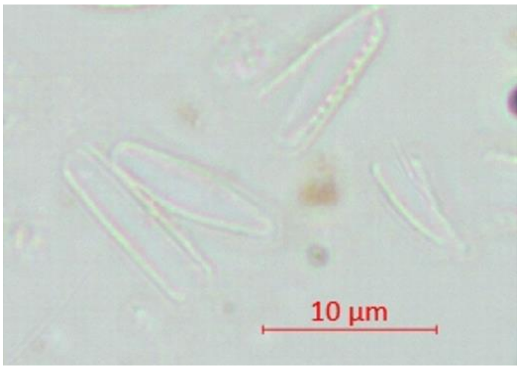


Figure 2.1.46. Normal form of *A. minutissimum* at site C.

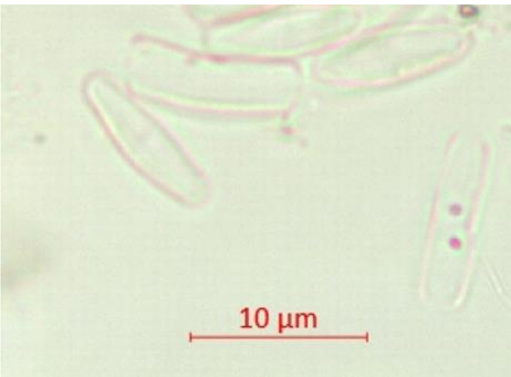


Figure 2.1.47. Teratological form of *A. minutissimum* at site C.

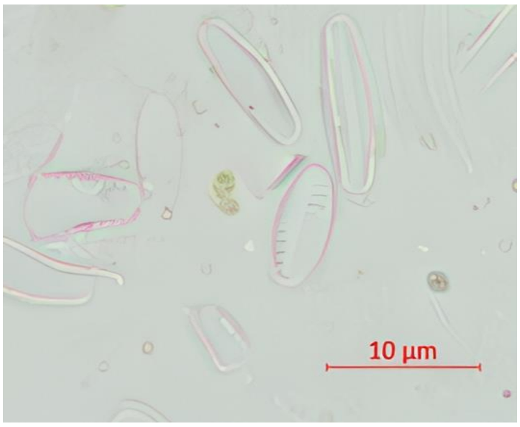


Figure 2.1.48. Normal form of *P. lanceolatum* at site C.

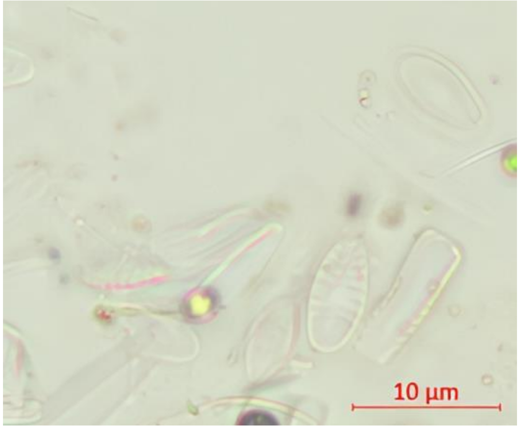


Figure 2.1.49. Teratological form of *P. lanceolatum* at site C.

Figure 2.1.50. Normal form of *C. pediculus* at site C.

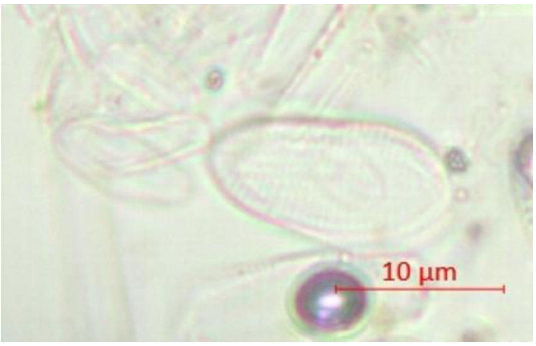


Figure 2.1.51. Teratological form of *C. pediculus Ehrenberg* at site C.

Tables 2.1.77 – 2.1.79 present the physico-chemical parameters of the Valea Șesei River water (D). Valea Șesei may have a potential impact on the water quality of the Arieș River, as it is a tributary from an area affected by mining activities, which can significantly influence the physico-chemical characteristics of the water.

Table 2.1.77. Physico-chemical parameters of the Valea Sesei River water, upstream of the Arieș confluence (D).

Parameter Time, year/month		pH	TSS, mg/L	DO, mgO/L	BOD$_5$, mgO/L	COD-Cr, mgO/L	N-NH$_4$, mgN/L	N-NO$_2$, mgN/L	N-NO$_3$, mgN/L
2022	Jan.	4.8	113	10.5	3.12	42.7	0.152	0.021	0.072
	Apr.	6.8	149	9.1	1.51	23.8	0.184	0.004	0.401
	Jul.	7.1	127	8.3	0.98	35.7	0.766	0.046	1.354
	Oct.	7.4	154	10.2	0.85	29.4	0.841	0.006	0.304
2023	Jan.	5.4	96	9.8	2.41	35.7	0.187	0.014	0.068
	Apr.	7.1	124	9.2	1.87	28.1	0.124	0.008	0.425
	Jul.	6.9	139	8.4	1.05	42.1	0.354	0.035	0.847
	Oct.	6.8	150	9.7	0.98	38.4	0.347	0.014	0.405
2024	Jan.	5.5	124	8.9	2.14	23.5	0.127	0.034	0.061
	Apr.	6.1	189	9.4	1.74	29.4	0.367	0.008	0.352
	Jul.	6.6	143	8.7	0.76	42.1	0.424	0.057	0.874
	Oct.	7.2	106	9.7	0.96	38.7	0.204	0.025	0.632

In general, the water pH ranged between 4.8 and 7.4, suggesting slightly acidic to neutral water, while concentrations of suspended solids fluctuated between 96 and 189 mg/L, indicating a moderate to high level of turbidity, except in January 2022 (113 mg/L). BOD$_5$ and COD-Cr showed variable values, suggesting moderate chemical pollution. Ammonium nitrogen concentrations were relatively low, between 0.124 and 0.841 mgN/L, and nitrite and nitrate showed fairly low values, consistent with relatively limited nitrogen pollution. Compared to water quality classes, the obtained values generally indicate a water status of Class II or III, with a moderate pollution level, suggesting acceptable water quality for aquatic ecosystems.

Table 2.1.78. Salinity parameters of the Valea Sesei River, upstream of the Arieș confluence (D).

Parameter Time, year/month	EC, µS/cm	FR, mg/L	Ca^{2+}, mg/L	Mg^{2+}, mg/L	Na^{+}, mg/L	HCO$_3^{-}$, mg/L	Cl^{-}, mg/L	SO$_4^{2-}$, mg/L

2022	Jan.	1544	569	221.4	4.11	5.62	24.0	17.32	642
	Apr.	1478	657	175.2	5.64	4.58	32.4	15.78	547
	Jul.	2541	847	552.1	5.84	8.64	42.7	22.34	614
	Oct.	1875	724	153.9	2.32	7.32	24.8	23.72	487
2023	Jan.	1237	473	178.2	3.57	4.12	27.4	24.7	514
	Apr.	1289	541	201.7	5.38	6.14	41.5	18.7	624
	Jul.	2647	947	698.3	6.23	7.48	48.1	29.4	704
	Oct.	1672	842	234.1	3.04	3.81	35.2	21.7	618
2024	Jan.	1617	652	245.1	4.01	3.39	18.7	22.3	446
	Apr.	1784	784	204.7	4.49	4.32	34.8	27.2	524
	Jul.	2631	961	618.2	6.01	5.27	47.8	31.2	716
	Oct.	1327	637	235.4	4.08	4.38	31.4	31.1	527

Electrical conductivity ranged from 1237 to 2647 µS/cm, indicating significant fluctuations in salinity depending on the period, with medium to high salinity during the summer months. Calcium concentrations varied between 153.9 and 698.3 mg/L, peaking in July 2023. Sulfates had concentrations between 446 and 716 mg/L, with higher values in the summer months. Compared to water quality classes, the results suggest that, in general, the Valea Sesei River water falls into Class V, meaning water with salinity and dissolved substance concentrations higher than the limits for Class IV, indicating significant salinity, especially during the summer months.

Table 2.1.79. The content of toxic trace elements in the water of the Valea Sesei River (D).

Parameter Time, year/month		**As^{2+}, µg/L**	**Cd^{2+}, µg/L**	**Co$_{total}$ (Co^{2+}+Co^{3+}), µg/L**	**Cr$_{total}$ (Cr^{3+}+Cr^{6+}), µg/L**	**Cu^{2+}, µg/L**	**Fe$_{total}$, mg/L**	**Mn$_{total}$, mg/L**	**Ni^{2+}, µg/L**	**Zn^{2+}, µg/L**
2022	Jan.	0.74	34.01	42.17	2.37	2147.14	3.57	1.074	24.09	1587.24
	Apr.	0.49	41.69	38.74	3.60	3241.36	4.69	1.230	26.84	2145.21
	Jul.	1.02	51.34	54.78	5.42	4162.30	6.72	2.417	42.87	3542.17
	Oct.	0.74	38.71	42.58	4.38	1897.41	4.29	1.687	36.01	2476.27
2023	Jan.	0.45	28.47	39.41	1.89	1874.35	2.89	1.339	31.74	2017.31
	Apr.	0.58	38.44	49.52	2.38	2267.09	3.67	1.876	41.58	2417.39
	Jul.	0.79	48.71	51.07	5.07	3124.21	7.61	2.472	49.37	3247.07
	Oct.	0.68	32.47	35.14	3.17	1567.41	5.14	1.961	28.63	2107.28
2024	Jan.	0.57	21.41	28.74	2.09	2146.38	3.69	0.943	24.18	1637.28
	Apr.	0.61	28.97	29.67	3.19	2643.78	4.18	1.578	29.47	1897.34
	Jul.	0.74	35.64	44.26	4.62	2894.20	6.81	3.214	37.19	2463.18
	Oct.	0.61	29.37	35.26	3.47	1897.35	5.74	2.105	24.09	1894.28

In the water of the Valea Sesei River, upstream of the Arieș confluence, cadmium ranged between 21.41 and 51.34 µg/L, suggesting relatively high pollution during certain periods of the year, exceeding the recommended limits for water quality Classes I and II. Cobalt varied between 28.74 and 54.78 µg/L, showing consistently high pollution. Copper levels were quite high, ranging from 1567.41 to 4162.30 µg/L, indicating a high level of pollution exceeding the limits. Iron concentrations ranged from 2.89 to 7.61 mg/L, with higher values in summer, indicating moderate iron pollution. Manganese values were between 0.943 and 3.214 mg/L, showing constant manganese pollution but not exceeding Class IV limits. Nickel fluctuated between 24.09 and 49.37 µg/L, peaking in July 2023. Zinc concentrations ranged from 1587.24 to 3542.17 µg/L, indicating significant pollution, especially during the summer months.

Compared to water quality classes, the results suggest that the Valea Sesei River water falls into Class V for several of these trace elements, indicating high toxic metal pollution, particularly with copper and zinc, which exceed the limits for all water quality classes.

Table 2.1.80 presents the diatom species identified in the Valea Sesei River water, the number of individuals for each species, the associated saprobic zone, and the saprobic value.

Table 2.1.80. Diatom composition and saprobic index at site D (Valea Șesei River).

Scientific Name of a Diatom	**Number of individuals**		**Degree of Saprobity**	**Saprobic Indicator Value**
	Normal	**Teratological**		
*Achnanthidium minutissimum (*Kützing) Czarnecki	236	88	o-β	1.5
Fragilaria capucina Desmazières	6	0	β	2
Nitzschia palea (Kützing) W. Smith	2	0	α	3
Total	332			
Saprobic index value	1.21			

A saprobic index of 1.21 indicates water with very low organic pollution, corresponding to ecological quality Class I. The community is dominated by species tolerant to variable conditions (e.g., *A. minutissimum*), with the presence of some sensitive α-species (*N. palea*), suggesting a healthy aquatic ecosystem with a minimal anthropogenic impact.

Figures 2.1.52 and 2.1.53 present a comparison of normal and teratological forms of diatoms, based on samples collected from the Valea Șesei River in site D.

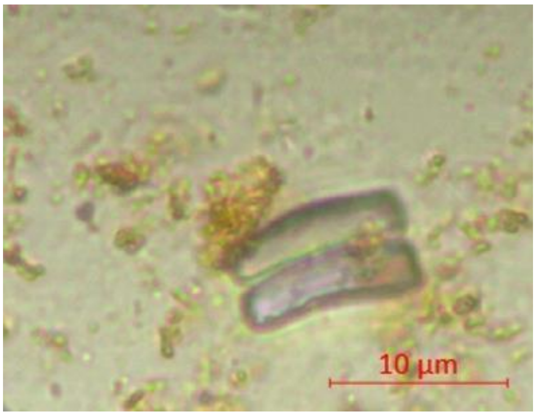


Figure 2.1.52. Normal form of *A. minutissimum* at site D.

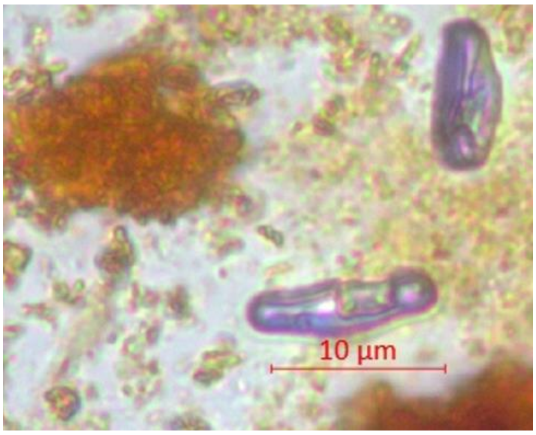


Figure 2.1.53. Teratological form of *A. minutissimum* at site D.

Tables 2.1.81 – 2.1.83 present the physico-chemical parameters of the Valea Sărtaș River water (E). The reason for choosing this surface water is that Valea Sărtaș is an important tributary of the Arieș River, and its water quality directly influences the ecological status of the Arieș in the Mihai Viteazu area. Monitoring this watercourse helps identify local sources of pollution and assess the impact of human and natural activities on water quality before the confluence with the main river.

Table 2.1.81. Physico-chemical parameters of the Valea Sărtaș River water, upstream of the Arieș confluence (E).

Parameter / **Time, year/month**		**pH**	**TSS, mg/L**	**DO, mgO/L**	**BOD$_5$, mgO/L**	**COD-Cr, mgO/L**	**N-NH$_4$, mgN/L**	**N-NO$_2$, mgN/L**	**N-NO$_3$, mgN/L**
2022	Jan.	7.4	13.3	9.64	0.87	4.64	0.003	0.001	0.093
	Apr.	7.6	19.4	9.42	0.94	6.57	0.007	0.003	0.128
	Jul.	7.1	35.6	8.94	1.08	9.82	0.016	0.009	0.232
	Oct.	7.3	21.4	9.14	0.74	6.33	0.011	0.005	0.186
2023	Jan.	7.6	16.3	9.61	0.69	5.63	0.006	0.004	0.105
	Apr.	7.5	21.2	9.23	0.81	7.76	0.009	0.002	0.134

	Jul.	7.2	41.5	9.01	1.17	10.22	0.013	0.011	0.327
	Oct.	7.5	32.1	9.34	0.84	7.51	0.008	0.006	0.214
2024	Jan.	7.5	17.3	9.77	0.54	5.10	0.004	0.002	0.131
	Apr.	7.6	21.7	9.61	0.69	6.61	0.006	0.006	0.164
	Jul.	7.0	38.9	9.04	0.97	8.64	0.019	0.014	0.245
	Oct.	7.3	31.4	9.34	0.62	5.34	0.005	0.008	0.171

The Valea Sărtaș River presents water with acceptable physico-chemical parameters; however, there is a potential risk of increased concentrations of organic matter and nutrients, especially during summer, which could affect water quality in the long term.

Table 2.1.82. Salinity parameters of the Valea Sărtaș River, upstream of the Arieș confluence (E).

Parameter **Time, year/month**		**EC, µS/cm**	**FR, mg/L**	**Ca^{2+}, mg/L**	**Mg^{2+}, mg/L**	**Na^{+}, mg/L**	**HCO$_3^-$, mg/L**	**Cl$^-$, mg/L**	**SO$_4^{2-}$, mg/L**
2022	Jan.	214.31	134.7	16.1	5.1	3.5	81.2	6.1	28.6
	Apr.	236.87	231.0	21.3	6.2	4.5	94.1	7.6	36.7
	Jul.	297.06	324.2	36.5	14.8	8.7	135.7	11.6	49.2
	Oct.	187.01	142.6	28.7	8.9	6.2	89.7	9.4	24.3
2023	Jan.	196.30	156.3	19.4	7.4	4.1	91.6	6.8	31.5
	Apr.	212.42	189.4	27.6	6.8	5.6	88.6	8.7	42.9
	Jul.	234.58	268.2	45.8	9.8	9.4	143.8	14.6	57.8
	Oct.	204.13	163.9	21.6	7.1	3.8	77.3	7.9	33.4
2024	Jan.	223.30	162.5	18.3	6.3	4.4	79.6	7.2	31.7
	Apr.	247.98	198.1	22.5	8.1	6.3	102.3	8.6	41.6
	Jul.	256.43	284.4	41.4	11.7	8.4	144.6	15.7	56.1
	Oct.	228.96	202.3	33.2	9.6	5.7	74.3	11.2	28.3

The salinity parameters of the Valea Sărtaș River indicate moderate salinity with seasonal fluctuations, particularly in summer.

Table 2.1.83. The content of toxic trace elements in the water of the Valea Sărtaș River (E).

Parameter **Time, year/month**		**As^{2+}, µg/L**	**Cd^{2+}, µg/L**	**Co$_{total}$ (Co^{2+}+Co^{3+}), µg/L**	**Cr$_{total}$ (Cr^{3+}+Cr^{6+}), µg/L**	**Cu^{2+}, µg/L**	**Fe$_{total}$, mg/L**	**Mn$_{total}$, mg/L**	**Ni^{2+}, µg/L**	**Zn^{2+}, µg/L**
2022	Jan.	0.35	0.12	0.18	0.24	12.41	0.021	0.025	0.49	21.47
	Apr.	0.41	0.24	0.36	0.31	18.63	0.034	0.037	0.68	34.65
	Jul.	0.74	0.55	0.84	0.84	35.10	0.058	0.044	1.13	54.64
	Oct.	0.44	0.41	0.52	0.42	22.42	0.025	0.032	0.74	28.30
2023	Jan.	0.43	1.18	0.23	0.33	13.47	0.019	0.019	0.56	18.94
	Apr.	0.48	0.27	0.44	0.47	21.46	0.027	0.028	0.77	24.68
	Jul.	0.57	0.66	1.12	1.13	39.42	0.044	0.033	1.34	46.37
	Oct.	0.39	0.32	0.67	0.65	31.22	0.026	0.024	0.71	28.71
2024	Jan.	0.53	0.18	0.33	0.36	18.43	0.017	0.021	0.62	23.42
	Apr.	0.46	0.27	0.42	0.58	24.69	0.028	0.033	0.86	33.44
	Jul.	0.78	0.78	0.84	1.34	38.44	0.063	0.046	1.45	61.42
	Oct.	0.47	0.41	0.50	0.61	24.81	0.030	0.022	0.60	42.13

In general, the levels of toxic trace elements in the water of the Valea Şartăş River are below the critical limits for Class I water quality. However, there are significant seasonal fluctuations, especially in the summer months.

Table 2.1.84 presents the diatom species identified in the water of the Șartăș River, the number of individuals for each species, the associated saprobic zone, and the saprobic value.

Table 2.1.84. Diatom composition and saprobic index of the Șartăș River (E).

Scientific Name of a Diatom	**Number of individuals**		**Degree of Saprobity**	**Saprobic Indicator Value**
	Normal	**Teratological**		
Achnanthidium minutissimum Kützing) Czarnecki	256	**61**	o-β	1.5
Navicula cryptocephala Kützing	26	0	α	3
Fragilaria vaucheriae (Kützing) J.B.Petersen	21	0	β	2
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	14	0	β	2
Amphora pediculus (Kützing) Grunow	11	0	β	2
Gomphonella olivacea (Hornemann) Rabenhorst	8	0	β	2
Cocconeis pediculus Ehrenberg	7	0	β	2
Nitzschia dissipata (Kützing) Rabenhorst	7	0	o-β	1.5
Nitzschia inconspicua Grunow	6	0	α	3
Navicula lanceolata Ehrenberg	5	0	α	3
Cymbella ventricosa Agardh	4	0	o-β	1.5
Navicula tripunctata (O.F.Müller) Bory	3	0	β-α	2.5
Nitzschia sigmoidea (Nitzsch) W.Smith	3	0	β	2
Rhoicosphenia curvata (Kützing) Grunow	3	0	β	2
Surirella brebissonii Krammer & Lange-Bertalot	3	0	β-α	2.5
Odontidium mesodon (Ehrenberg) Kützing	2	0	o	1
Surirella angusta Kützing	2	0	β	2
Nitzschia palea (Kützing) W.Smith	1	0	α	3
Total	443			
Saprobic index value	1.86			

Figures 2.1.54 and 2.1.55 illustrate the comparison between normal and teratological forms of diatoms, based on samples collected from the Șartăș River (E).

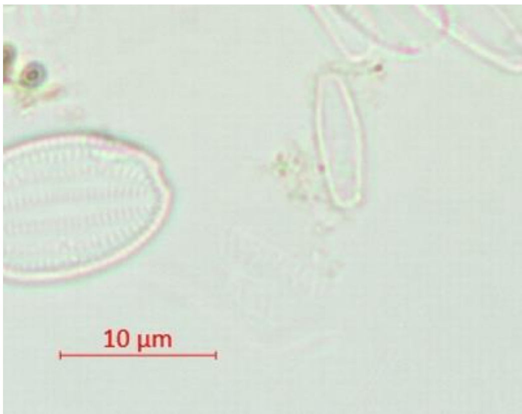


Figure 2.1.54. Normal form of *A. minutissimum* at site E.

Figure 2.1.55. Teratological form of *A. minutissimum* at site E.

The CCA (Figure 2.1.56) was thus performed to further explore the relationships between diatom species and environmental variables, including trace elements.

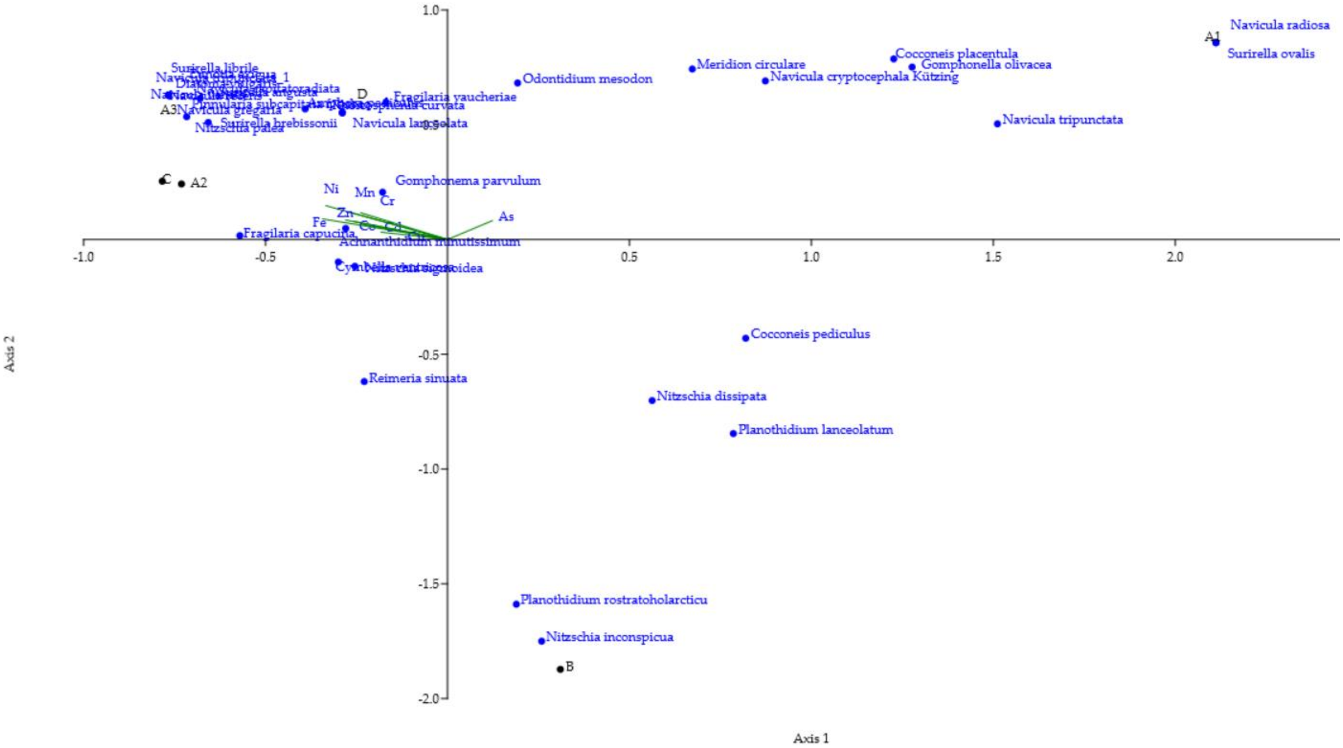


Figure 2.1.56. CCA plot illustrating the influence of trace elements on the distribution of diatom species.

The CCA shows how environmental variables influence diatom species distribution, with species position reflecting their response to environmental factors [42]. Species like *Rhoicosphenia curvata* and *Navicula lanceolata* are associated with Zn, Cu, Cd, and As, confirming tolerance to these trace elements, while *Diatoma vulgaris*, *Navicula gregaria*, and *Surirella librile* correlate with Fe, Mn, Ni, and Cr, suggesting links to natural metal loading or acidic waters.

Environmental vectors indicate the intensity and direction of influence on species. Zn, Cu, Cd, and As are associated with tolerant taxa, while Fe, Mn, Ni, and Cr define a distinct group of species. Axis 1 explains nearly 50% of the variation and represents the main ecological gradient. Axis 2 adds ~25% and differentiates metal types, together capturing ~72% of the ecological information. Axis 3 captures secondary variation, such as microhabitat differences, while remaining axes have low relevance.

Two species groups emerge: bioindicator for Zn, Cu, Cd, and As, and species linked to Fe, Mn, Ni, and Cr. The first two axes capture the essence of ecological distribution, providing a solid framework for interpreting the relationship between diatoms and metals.

Statistical tests confirm that Axes 1 and 2 are ecologically important for understanding the major variation in the data, even if they do not meet the strict significance threshold.

The physico-chemical analysis indicates that water quality in the studied rivers is strongly influenced by seasonal dynamics and by discharges from mining-related enterprises. High metal concentrations and increased salinity, particularly in the Abrud and Sartăș Rivers, are primarily associated with the Roșia Poieni mining operations and their waste deposits, while the historical Roșia Montană site contributes only minimally. The saprobic index, combined with physico-chemical parameters (especially trace metals), suggests a mild to moderate influence of organic pollution but a strong impact from acid mine drainage.

The predominant diatom species highlight the differences in water quality: oligosaprobe species, such as *N. cryptocephala* and *N. palea*, are more common in the cleaner stations, such as Valea Șesei and Șefanca, while meso-β species, such as *A. minutissimum* and *P. lanceolatum*, predominate in sectors with a more pronounced anthropogenic impact. The observation of teratological forms suggests the presence of stress factors, probably related to organic or metal pollution. By correlating the physico-chemical data with the saprobic index, it is observed that sectors with higher concentrations of nutrients and organic matter correspond to diatom communities with greater diversity of pollution-tolerant species. This relationship confirms the usefulness of the saprobic index as a tool for assessing water quality and the impact of anthropogenic activities.

The general water quality in the studied rivers is moderate, with a clear pollution gradient: Valea Şesei presents the best quality, followed by Şefanca and Sartăş, and Abrud records the highest saprobic values. To maintain ecosystem health and prevent eutrophication, continuous monitoring of water quality is recommended, particularly during periods of high temperatures and intense precipitation, to detect early signs of deterioration. In addition, reducing nutrient inputs from agricultural runoff and urban wastewater through best management practices and efficient wastewater treatment is essential. Protecting and restoring riparian buffer zones can help limit the entry of nutrients and sediments into the rivers, while promoting public awareness and local management plans can further minimize anthropogenic impacts on water quality. These combined measures can help ensure sustainable water quality and preserve the ecological integrity of the studied rivers.

2.1.2.5. Conclusions

The studies presented in this chapter provide a detailed and multifactorial overview of the groundwater and surface water quality status in Alba County, a region characterized by both historical and ongoing mining activities, as well as rural and urban anthropogenic influences. The main conclusions synthesize the results from all subsections, highlighting the degree of contamination, the role of bioindicators, and directions for remediation and management.

Regarding the quality of groundwater and surface waters, analyses performed on samples collected from public wells, springs, and boreholes in the Alba Iulia–Teiuș, Sebeș–Cugir, Câmpeni–Abrud–Zlatna, Blaj, and Aiud–Ocna Mureș areas revealed widespread microbiological contamination (coliform bacteria, *E. coli*, enterococci, total viable count) and chemical contamination (nitrates, nitrites, ammonium, increased hardness) in most sources. Only a small number of samples (e.g., some springs located in mountainous areas) comply with drinking water standards according to Law 96/2024 and EU Directive 2020/2184. Pollution is more pronounced in lowland and hilly areas (Aiud–Ocna Mureș, Blaj), while seasonal variations indicate increased microbiological indicators during spring and summer, correlated with higher temperatures and surface water infiltration. In mountainous areas (Câmpeni–Abrud), water quality is relatively better, although local exceedances are still observed.

From the perspective of the statistical assessment of pollution in public water sources, monitoring of 132 public springs between 2017 and 2019 showed that only 38 meet drinking water standards, while 94 exhibit exceedances in physico-chemical and microbiological parameters. Second-order polynomial statistical models demonstrated strong correlations between microbial load (dependent variable) and nitrate, nitrite, and ammonium concentrations (independent variables), validated by correlation coefficients (R^2 ranging from 0.45 to 0.84). These models can be used predictively to estimate microbial contamination risk based on nitrogen content and seasonal variation. Mountain areas (Zone III – Câmpeni–Zlatna) remain the cleanest (72% compliant), in contrast to lowland areas (Zone V – Aiud–Ocna Mureș), where only 20% of sources are compliant.

In the Roșia Montană basin, the waters of the Roșia, Săliște, Corna, Abrud, and Arieș rivers, affected by mining activities, exhibit, in certain areas, strong acidity (pH < 4 at some sampling points), high conductivity, extremely elevated sulfate concentrations (>3000 mg/L), and heavy metals (Fe, Zn, Mn, Cd, As, Cu) exceeding ecological quality limits. The main impact originates from acid mine drainage (AMD) from abandoned galleries (e.g., Gura Minei). Treatment using natural zeolite from Rupea demonstrated high efficiency in Fe removal (>99% reduction at optimal doses), moderate efficiency for Zn, and lower efficiency for Mn, confirming the potential of this low-cost material for AMD remediation.

The research also investigated the effects of pollution on physico-chemical parameters and periphytic diatom communities in the Arieș basin. The integration of physico-chemical analyses with the study of periphytic diatoms in the Arieș River and its tributaries (Abrud, Ștefanca, Seșii, Sărtaș) revealed a clear pollution gradient: upstream stations (Arieșeni, Mihoești) show good water quality (Class I–II, SI approx. 1.4), while downstream stations (Baia de Arieș, Gligorești, Abrud downstream) indicate strong mining influence (high heavy metals, acidic pH, SI between 1.6–1.9). The presence of teratological forms in dominant species (*Achnanthidium minutissimum*, *Fragilaria* spp., *Gomphonella olivacea*) confirms ecological stress induced by heavy metals and acidity. PCA and CCA analyses identified the main pollution gradient (mineralization and metals) and correlated tolerant species (e.g., *Nitzschia palea*, *Navicula cryptocephala*) with Zn, Cu, Cd, and As.

General Conclusions of the Section:

- Alba County exhibits persistent water resource pollution, with two major components: microbiological and nutrient (nitrogen-based) contamination in rural and urban areas, and heavy metal and acidity pollution in the Roșia Montană–Arieș mining basin.
- Periphytic diatoms proved to be sensitive and effective bioindicators, both through the saprobic index and the occurrence of teratological forms, providing integrated information on cumulative stress that complements chemical analyses.
- The developed statistical models allow prediction of microbiological risk, while natural zeolites represent a promising, sustainable, and cost-effective solution for treating acid mine drainage.
- Water quality is strongly influenced by seasonal factors (summer increases in pollutant levels) and anthropogenic pressures (historical mining, agriculture, uncontrolled discharges).

2.1.2. Research on Soil Quality in Polluted Areas

The investigations presented in the previous section provided a comprehensive assessment of water quality in pollution-affected areas, emphasizing both groundwater and surface water systems, the role of diatoms as bioindicators, and the impact of mining and anthropogenic activities on aquatic ecosystems. While these findings highlight the extent and dynamics of water contamination, environmental pollution is not limited to aquatic compartments alone.

In this context, the following section extends the analysis to the terrestrial environment, focusing on soil quality in similarly impacted areas. Given the close interaction between soil and water systems, particularly in regions affected by mining and industrial activities, the assessment of soil contamination—especially with heavy metals—offers a complementary perspective on environmental degradation. Additionally, the inclusion of bioaccumulation studies, such as those involving *Helix pomatia*, provides further insight into the transfer of contaminants through the ecosystem.

2.1.2.1. Soil Heavy Metal Contamination in the Zlatna Region, Romania

Soil contamination is a major problem not only at European but also at international level through the explosive development of the industry which led to the creation and proliferation of a wide and varied number of chemical compounds hazardous to the environment. Soil heavy metals contamination is the most dangerous pollution because those metals do not degrade like organic pollutants, their residence time in the soil being of thousands of years. The excess of heavy metals in soil can threaten the health of animals and humans, due to food chain transfer.

Zlatna is a town located in the Alba County, at 35 km from Alba Iulia city. Ampoi River connects the two cities. The main heavy metals pollution sources in the area of Zlatna were the mining and mineral processing activities. Soil pollution due to these sources was recorded both around the processing plant and along the Ampoi Valey. In accordance with those presented above, the aim of this study was to measure the levels of some heavy metals (Cu, Zn, Pb, As) in soils collected in 2004 from Zlatna and human settlements on the river Ampoi both upstream and downstream from emission sources [43].

pH and humus values of soils samples collected were measured and are reported in table 2.1.85.

Table 2.1.85. Soil properties in the Zlatna region

Sample	**Sampling place**	**Distance from the source, km**	**pH**	**Humus, %**
1	Near the Izvorul Ampoiului deposition	11.0	7.55	2.64
2	Izvorul Ampoiului deposition	10.0	7.22	1.60
3	Izvorul Ampoiului village	7.0	8.15	1.87
4	Cheneş village	5.0	8.14	5.50
5	***Zlatna town***	***0***	5.96	2.33
6	Pătrângeni village	3.0	8.11	3.19
7	Feneş village	5.8	7.67	5.88
8	Presaca Ampoiului village	10.0	6.11	8.61
9	Poiana Ampoiului village	13.0	7.60	8.99
10	Meteş village	16.5	8.26	2.28
11	Tăuţi village	19.0	8.35	0.79
12	Ampoiţa village	23.0	7.88	1.86
13	Entrance to Şard village	28.0	7.43	3.98
14	Exit from Şard village	29.0	7.34	3.27
15	Alba Iulia town	37.0	8.30	3.11

For each soil sample the following heavy metals concentrations were determined: Pb, Cu, Zn and As. The measured values are presented in figures 2.1.57 – 2.1.60 together with normal values (NV), Alert threshold – sensitive uses (ATS), Alert threshold – less sensitive uses (ATLS), Intervention threshold – sensitive uses (ITS), Intervention threshold – less sensitive uses (ITLS).

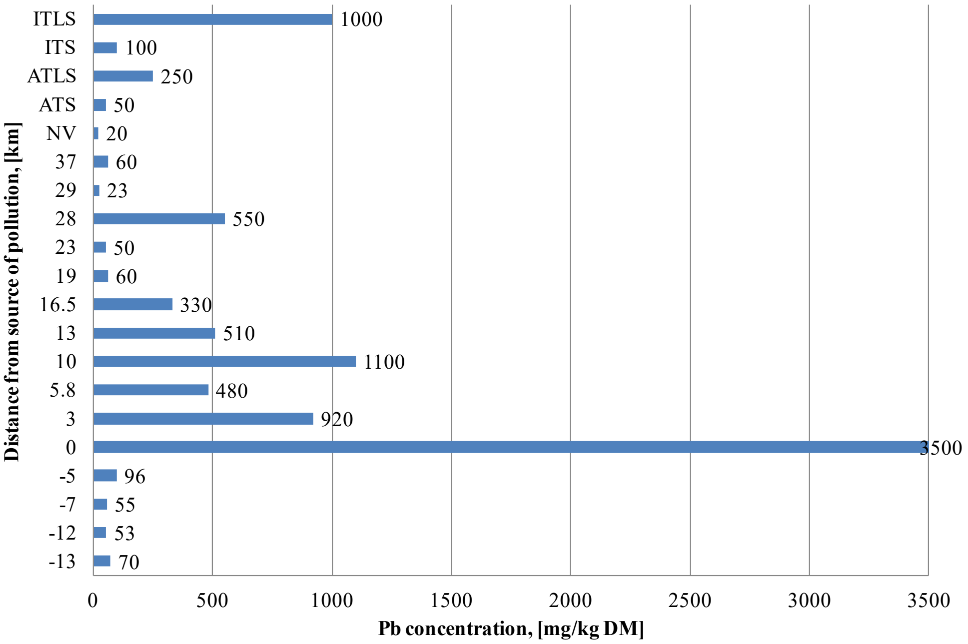


Figure 2.1.57. Lead levels recorded in the investigated area

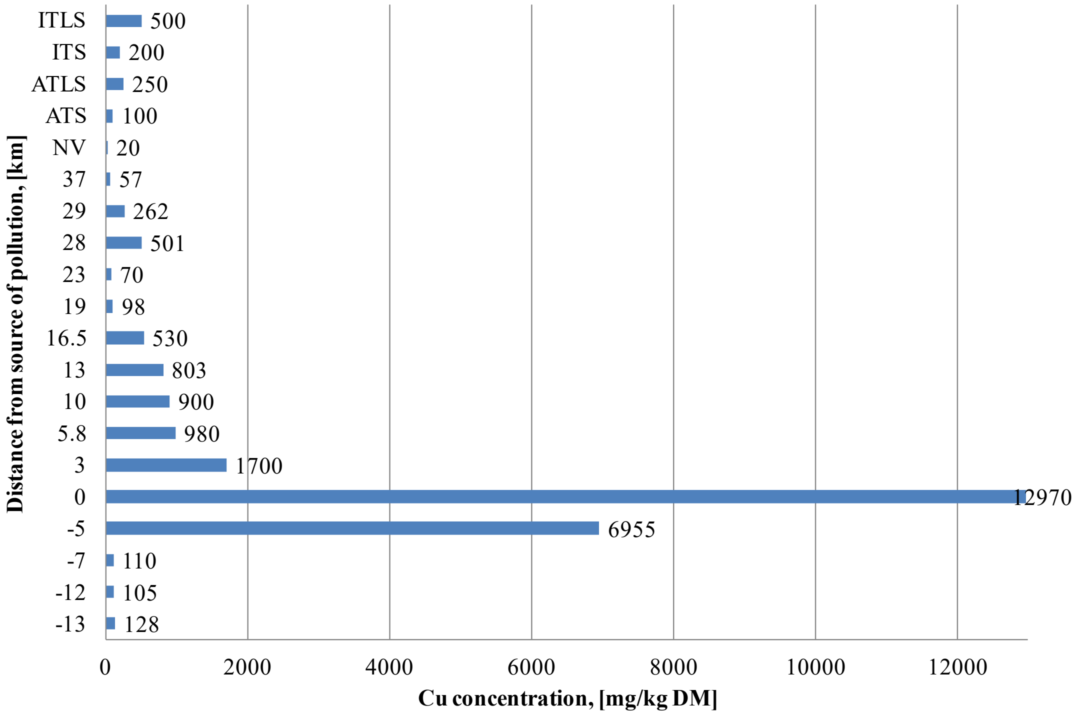


Figure 2.1.58. Copper levels recorded in the investigated area

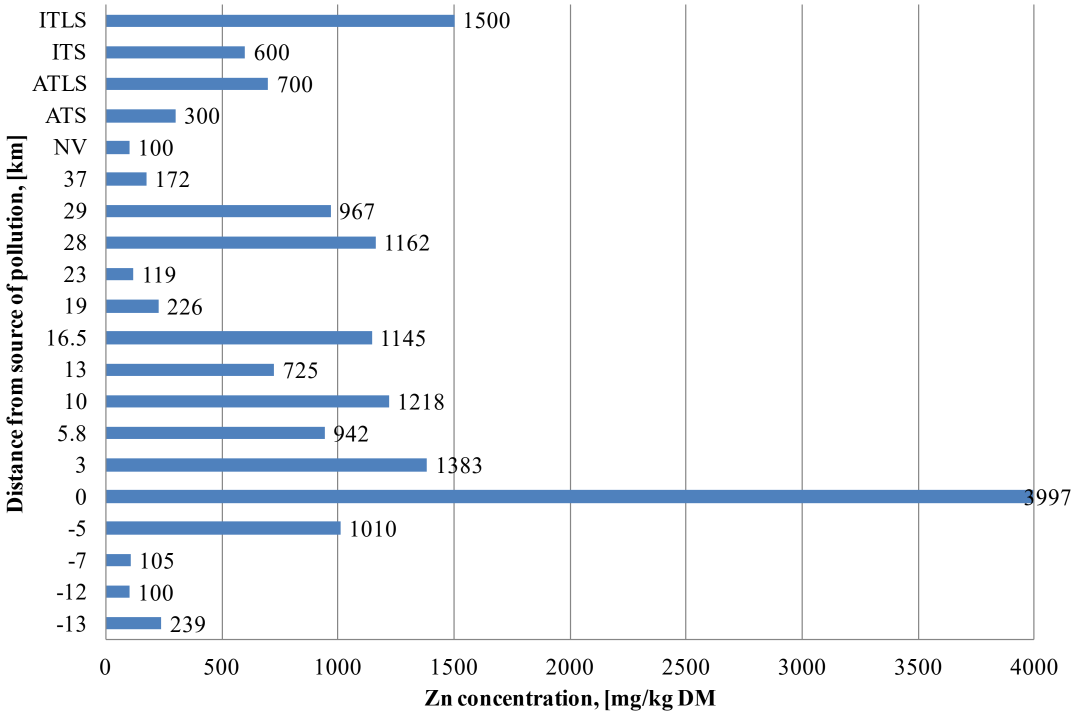


Figure 2.1.59. Zinc levels recorded in the investigated area

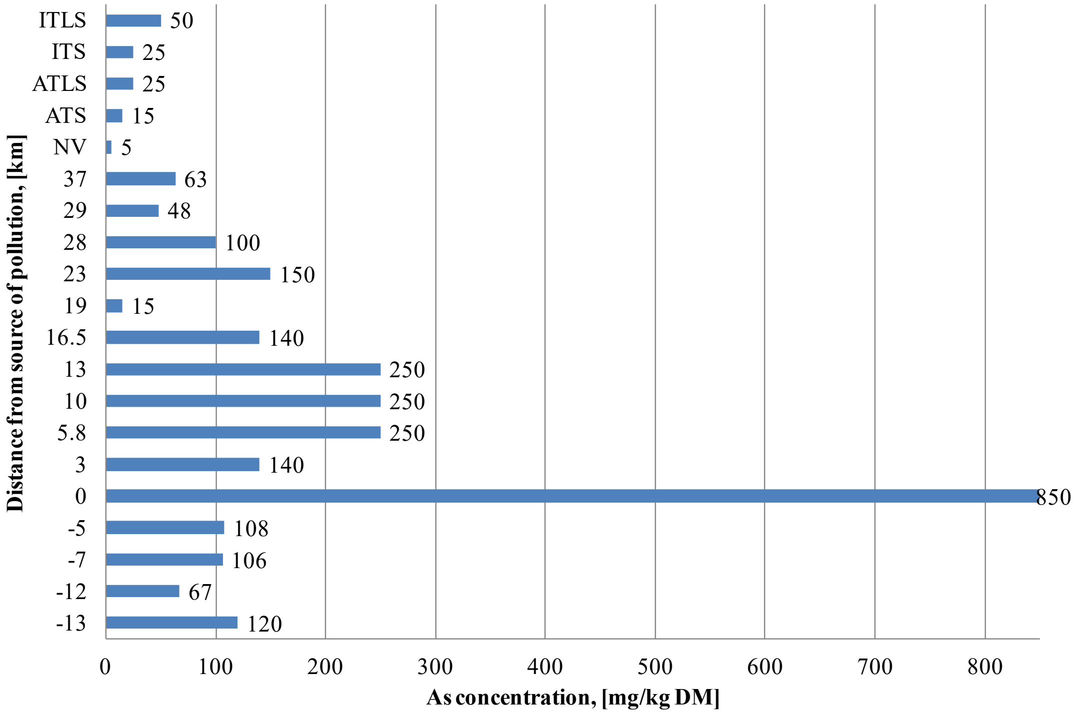


Figure 2.1.60. Arsenic levels recorded in the investigated area

In Zlatna town, the concentration of Pb, Cu, Zn and As in the soil sample was much higher that value of intervention threshold for less sensitive uses (ITLS). This is due to the action of atmospheric factors (especially wind and rain) who determined the pollution migration away from the source.

In the area Izvorul Ampoiului, Pb and Cu concentrations were higher than alert threshold for sensitive uses of soil (ATS). Zn concentration was lower than alert threshold for sensitive uses of soil (ATS) but higher than normal value (NV). As concern As, the values were higher than intervention threshold for less sensitive uses (ITLS).

In Cheneș village, situated at 5 km from Zlatna, the pollution degree was more drastic: Pb concentration was higher than the value of alert threshold for sensitive uses of soil (ATS); Zn concentration was higher than the value of intervention threshold for sensitive uses (ITS) and Cu and As concentrations were higher than the value of intervention threshold for less sensitive uses (ITLS).

Excessive pollution situation was maintained in the villages between Pătrângeni and Alba Iulia town, along the Ampoi River.

Even the soil collected at 37 km from Zlatna (at the entrance in Alba Iulia town) presented values of heavy metals higher than the normal ones (Cu and Zn). Pb concentration was higher than the value of alert threshold for sensitive uses of soil (ATS) and As concentration was higher than the value of intervention threshold for less sensitive uses (ITLS).

The investigation of soil samples from 15 urban sites in the Zlatna region revealed a clear accumulation of lead, copper, zinc, and arsenic caused by the long-term activity of the local pollution source. The concentrations of these heavy metals exceeded the intervention threshold values for less sensitive land uses (ITLS) over an area of 10 km in the direction of Alba Iulia. These levels represent a real concern for the health of the people living in the area. Under these circumstances, rapid rehabilitation of the mining area in the Zlatna region is necessary.

2.1.2.2. Soil Heavy Metal Contamination in the Town of Zlatna

The main pollutants in the Zlatna area were: Ampellum Company (copper smelter), which released pollutants into the air such as suspended particles containing zinc, lead, cadmium, arsenic, copper, as well as SO_2, SO_3, and CO; into water: Zn^{2+}, Cd^{2+}, As^{3+}, Fe^{2+}/Fe^{3+}, SO_4^{2-} (Ampoi River); and into soil: suspended particles of zinc, lead, copper, arsenic, and cadmium.

In addition, Zlatna mining activities released pollutants into water such as suspended particles, cyanide, iron, copper, and zinc (Ampoi River), and into soil through mine tailings dumps.

The sampling section was located in the Zlatna town and near the Ampoi River [44]. In July 2004 and 2012, soil samples were collected according to Figure 2.1.61 and Table 2.1.86.

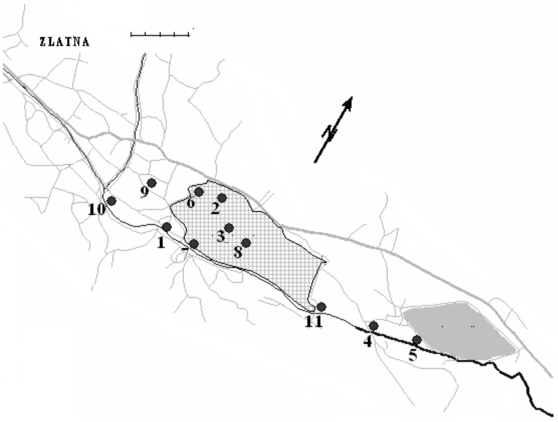


Figure 2.1.61. Study area and sampling points in Zlatna town

The hatched area represents the former location of the Ampellum Company in Zlatna, while the solid grey area represents the slag dumps located downstream of the plant specialized in copper metallurgy.

Table 2.1.86. Soil sampling points in Zlatna town

Sample	Sampling location
1	Right bank of the Ampoi River, 100 m upstream from the plant
2	Plant premises no. 1, in the vicinity of the concentrate storage area
3	Plant premises no. 1, in the vicinity of the industrial analytical laboratory
4	Waste disposal site on the right bank of the Ampoi River, 300 m downstream from Plant unit no. 1
5	Waste disposal site on the right bank of the Ampoi River, 800 m downstream from Plant unit no. 1
6	Within the premises of Ampellum SA, near the administrative building
7	Within the premises of Ampellum SA, near the transport offices
8	Within the premises of Ampellum SA, near the metallurgical building
9	In the access zone to the premises of Ampellum SA, near a grocery store
10	In the center of the town of Zlatna
11	On the right bank of the Ampoi River, 100 m downstream from the processing plants and Ampellum SA

At the beginning of the study, soil pH was measured. The values generally ranged between 4.3 and 8.1 in 2004, and between 7.4 and 7.7 in 2012, due to the soil's buffering capacity. Thus, in 2004 the soil could be classified as acidic (samples no. 5, 6, 7, and 9), slightly acidic (sample no. 8), and alkaline (samples no. 1, 2, 3, and 10).

The analysis of soils collected in 2012 showed that pH became uniform and could be considered neutral. This characteristic is important for agricultural soils. However, microbial activity is influenced by soil reaction, as nitrifying bacteria and nitrogen-fixing bacteria develop better in slightly alkaline soils. Soil reaction also affects nutrient uptake by plants and microorganisms.

The concentrations of the following heavy metals were measured for each sample: Pb, Cu, and Cd, and the results are presented in Figures 2.1.62–2.1.64. Since during the study period Romania joined the European Union and the legislation was amended, the results were reported according to the heavy metal limits established by Order no. 756/1997.

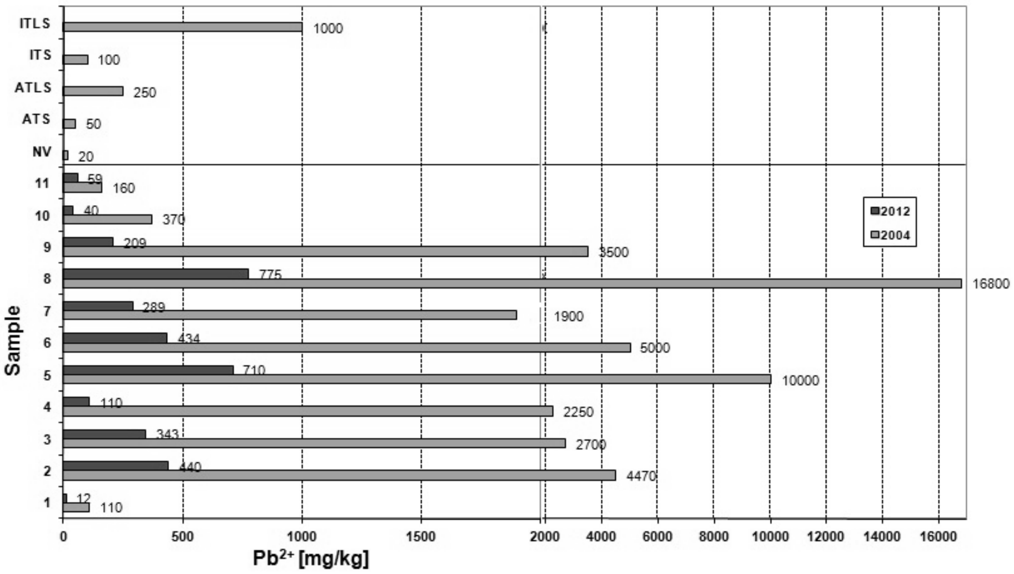


Figure 2.1.62. Lead concentration in soil samples
(NV – normal values, ATS – alert threshold for sensitive land uses, ATLS – alert threshold for less sensitive land uses, ITS – intervention threshold for sensitive land uses, ITLS – intervention threshold for less sensitive land uses)

The soil samples collected in 2004 showed Pb^{2+} concentrations that exceeded the intervention threshold at almost all sampling points (except for samples 1, 10, and 11). Eight years after the closure of the facility considered the main source of pollution (2012), the Pb^{2+} concentration remained above the normal values established by legislation at most sampling points, but was significantly lower than the initial levels.

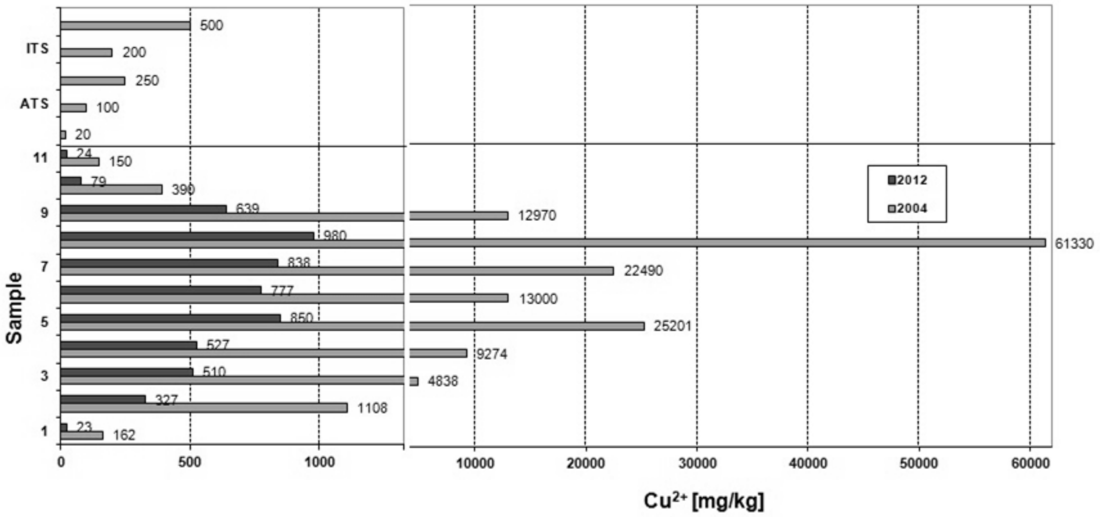


Figure 2.1.63. Copper concentration in soil samples
(NV – normal values, ATS – alert threshold for sensitive land uses, ATLS – alert threshold for less sensitive land uses, ITS – intervention threshold for sensitive land uses, ITLS – intervention threshold for less sensitive land uses)

Copper was detected at all investigated sites. The maximum soil copper level was recorded in 2004, near the metallurgical building (up to 60,000 mg/kg). After eight years, a significant decrease in Cu^{2+} content was observed at all sampling points; however, the values remain considerably above normal levels.

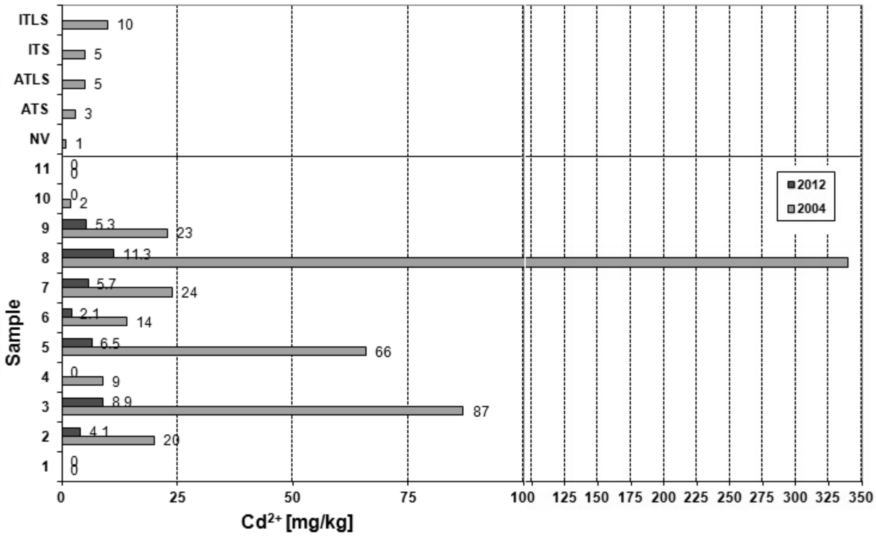


Figure 2.1.64. Cadmium concentration in soil samples

(NV – normal values, ATS – alert threshold for sensitive land uses, ATLS – alert threshold for less sensitive land uses, ITS – intervention threshold for sensitive land uses, ITLS – intervention threshold for less sensitive land uses)

The results showed that the cadmium content in the soil exceeded the normal limit values established by legislation, with the highest value recorded in the soil sample collected from inside the former Ampelum plant, near the metallurgical facility. A decrease in Cd^{2+} concentration over time was observed, but the values remained slightly above normal.

The concentrations of Pb, Cu, and Cd in the soil samples were much higher than the intervention threshold for less sensitive land uses (ITLS). Higher heavy metal concentrations were found in soil samples taken from the copper smelter yard. The elevated heavy metal levels in other areas could be attributed to atmospheric factors (particularly wind and rain) that caused the pollution to migrate away from the source. Lower heavy metal values in the soil in 2012 are due to soil remediation strategies implemented after the closure of the main pollution source.

Parameters such as lead (Pb^{2+}), copper (Cu^{2+}), and cadmium (Cd^{2+}), which describe the level of heavy metal soil pollution around Zlatna, are practically impossible to calculate directly from the experimental determinations carried out in the Zlatna area. Therefore, these parameters can be approximated based on isolated experimental measurements. Since the experimental results are not identical, it is possible to calculate a confidence interval (probability), chosen according to the desired precision.

Thus, using the eleven values of each heavy metal measured in the soil samples, the confidence interval containing the true mean content of heavy metals was calculated. The significance level was set at $\alpha = 0.050$.

As a result of the calculation, the mean values ($\bar{x}$) and standard deviations (s) were obtained (Table 2.1.87). For $\nu = 11-1 = 10$ degrees of freedom and the chosen significance level ($\alpha = 0{,}050$), $t_{0{,}05} = 2{,}228$.

Table 2.1.87. Mean values and standard deviations

Heavy metal	**Pb^{2+} (mg/kg)**		**Cu^{2+} (mg/kg)**		**Cd^{2+} (mg/kg)**	
Year	2004	2012	2004	2012	2004	2012
$\bar{x}$	4296,36	311,00	13719,36	506,73	53,18	3,99
S	5025,55	261,71	18110,47	349,97	99,09	3,95
Confidence interval	3278,46; 5314,27	257,99; 364,01	10051,17; 17387,56	435,84; 577,61	33,11; 73,25	3,19; 4,79

The average heavy metal content in the soil from the Zlatna area falls within the ranges specified in Table 2.1.87. The chosen confidence level was 95%, which practically implies certainty.

The study results demonstrated an accumulation of lead, copper, and cadmium in the Zlatna region due to the long-term activity of the local pollution source. Pollution was more pronounced in acidic soils and decreased as the distance from the pollution source increased.

The effect of soil remediation strategies applied after the closure of the main pollution source is evident, with heavy metal concentrations in the soils collected in 2012 being considerably lower than in the soils collected in 2004. The range of dispersion of heavy metal content in the soils from the investigated area decreased in all cases, with a 95% probability.

2.1.2.3. Contamination of Snail Soft Tissues (Helix pomatia) with Cadmium in Sibiu County

This research highlights the impact of industrialization in Sibiu County on soils, vegetation, and local fauna by determining the content of heavy metals, particularly cadmium, in snail meat. The presence of this heavy metal directly or indirectly affects the environment as well as human health.

The conducted studies primarily aimed to identify specific contaminants, namely cadmium, in the Sibiu County area and to determine possible sources of pollution. For this purpose, the quality of snail meat samples collected from 25 different locations within Sibiu County was investigated [45].

Within this study, the contamination level of *Helix pomatia* snail meat from the Copșa Mică area, Sibiu County, was assessed through the determination of heavy metals, with special emphasis on cadmium. In order to obtain accurate and reliable results, flame atomic absorption spectrophotometry was used as the analytical method for heavy metal determination.

The selection of the analytical technique — flame or graphite furnace — was based on the concentration of the analyzed element, with the graphite furnace method being applied for very low concentrations due to its higher sensitivity [46].

Table 2.1.88 presents the snail collection sites from which samples were taken, the corresponding localities, as well as the distances of each location from Copșa Mică, which is considered the main source of pollution in the county.

Table 2.1.88. Geographical location of snail sampling sites and their distance from the main pollution source

Sampling site	Location	Distance from the source: Copșa Mică
P1	Noiştat	59 km
P2	Apoldu de Jos	72 km
P3	Şeica Mică	14 km
P4	Micăsasa 1	18 km
P5	Micăsasa 2	18 km
P6	Cîrtişoara	89 km
P7	Sibiu	44 km
P8	Mediaş 1	19 km
P9	Laslea	40 km
P10	Şoala	13 km
P11	Saliste 1	66 km
P12	Miercurea Sibiului	80 km
P13	Ţapu	15 km
P14	Sălişte 2	66 km
P15	Şeica Mică 1	14 km
P16	Seica Mica 2	14 km
P17	Micasasa 3	18 km

P18	Seica Mica 3	14 km
P19	Mediaş 2	19 km
P20	Sibiu	44 km
P21	Seica Mica 4	14 km
P22	Seica Mica 5	14 km
P23	Mediaş 3	19 km
P24	Mediaş 4	19 km
P25	Ţapu	15 km

Sample collection was carried out in May 2014, during a period of low precipitation. Consequently, the obtained results were only slightly influenced by climatic conditions and are therefore considered relevant.

Cadmium (Cd) concentrations in snail tissue samples were determined by flame atomic absorption spectrometry (FAAS), using a PerkinElmer AA700 spectrometer (Waltham, MA, USA).

The experimental results obtained from the laboratory analyses of snail meat samples collected from Sibiu County are presented in Figure 2.1.65.

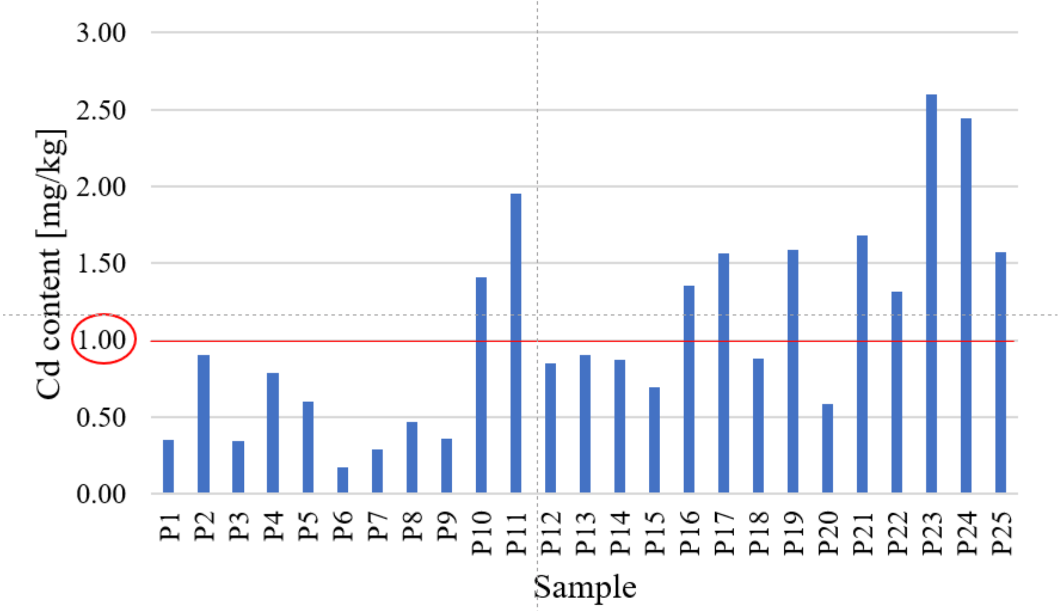


Figure 2.1.65. Cadmium concentrations in snail samples collected from the study area

The analysis of cadmium levels in snail meat from Sibiu County highlights the impact of industrialization in the Copșa Mică area on the surrounding environment. Data collected from 25 sampling sites show that locations farther from the main source of pollution, such as Cîrțișoara, Noiștat, Sibiu, and Apoldu de Jos, exhibited cadmium levels below the legal limit of 1.0 mg/kg, according to Regulation (EC) no 1881/2006 [47]. This indicates a low risk for snail consumption in these localities.

In contrast, sites closer to Copșa Mică or in areas with more pronounced industrial influence, such as Șeica Mică, Mediaș, Săliște, Șoala, and Țapu, recorded cadmium concentrations exceeding the maximum allowable level, with some values surpassing 2 mg/kg. These results suggest significant environmental contamination and underscore the need for continuous monitoring of heavy metals in local fauna.

Examining the correlation between distance from the pollution source and cadmium levels reveals a general trend: locations closer to Copșa Mică tend to have higher concentrations. However, local factors, such as soil characteristics, vegetation, and other industrial influences, may cause variations in accumulation. Therefore, the data confirm that industrial pollution has a significant effect on cadmium accumulation in snails, even at moderate distances from the main source.

These findings emphasize the importance of periodic assessment of heavy metals in edible fauna in Sibiu County and recommend caution when consuming snails from areas where cadmium levels exceed the legal limit.

2.1.2.4. Conclusions

The studies presented in this subsection highlight persistent and long-term heavy metal pollution in soils from the Zlatna (Alba County) and Copșa Mică (Sibiu County) regions, primarily generated by historical mining and metallurgical activities. The analyses demonstrate significant accumulation of Pb, Cu, Zn, As, and Cd in soils, with potential transfer into the food chain, as well as impacts on local fauna (snails).

Investigations carried out on 15 soil samples collected along the Ampoi Valley (from Zlatna to Alba Iulia, over a distance of 37 km) revealed elevated concentrations of Pb, Cu, Zn, and As, exceeding in numerous locations the alert and intervention thresholds established by legislation.

In Zlatna (0 km point), all metal concentrations exceeded the intervention threshold for less sensitive land uses (ITLS). Pollution extended significantly over a radius of approximately 10 km downstream along the Ampoi River, affecting the villages of Cheneș, Pătrângeni, Feneș, and areas near Alba Iulia. Even at 37 km distance (entrance to Alba Iulia), Cu, Zn, Pb, and As concentrations remained above natural background levels. The acidic pH in certain areas facilitated the mobility and persistence of metals in soils..

A comparison between samples collected in 2004 (during the operation of the Ampellum metallurgical plant) and in 2012 (eight years after closure) shows a significant reduction in Pb, Cu, and Cd concentrations. However, values remained well above normal limits and, in many cases, still exceeded alert and intervention thresholds. Statistically calculated average concentrations confirmed a decrease in pollution following the shutdown of the main emission source, but also highlighted the persistent nature of contamination, particularly in areas adjacent to metallurgical facilities and slag deposits. Acidic soils enhanced metal accumulation, while atmospheric factors (wind, precipitation) contributed to the long-distance dispersion of pollutants. In this context, anthropogenic soil remediation techniques were implemented in order to mitigate the environmental impact of heavy metal contamination. Although such interventions contributed to limiting further dispersion, their effectiveness remains partial, and long-term natural attenuation processes continue to play a major role in the gradual reduction of contamination levels.

Regarding the study of cadmium contamination in the soft tissues of snails (*Helix pomatia*) in Sibiu County, the analysis of 25 snail samples collected from various localities revealed a clear gradient of cadmium bioaccumulation depending on the distance from the main pollution source—Copșa Mică. Nearby localities (Șeica Mică, Mediaș, Țapu, Șoala – 13–19 km) showed Cd concentrations frequently exceeding the legal limit of 1.0 mg/kg (EC Regulation 1881/2006), with some values exceeding 2 mg/kg. In contrast, more distant locations (Cârțișoara – 89 km, Apoldu de Jos – 72 km, Sibiu – 44 km) exhibited values below the permissible limit. These results confirm the transfer of heavy metals from soil and vegetation into local fauna, representing a potential risk to human health through snail consumption.

General Conclusions of the Subsection:

- The Zlatna and Copșa Mică regions remain severely affected by historical heavy metal pollution, with long-term persistence in soils and demonstrated bioaccumulation in living organisms (snails).
- Pollution has dispersed over tens of kilometers along the Ampoi Valley, affecting both agricultural soils and natural ecosystems.
- The closure of major emission sources led to a slow but insufficient improvement; heavy metal levels remain elevated and represent a real risk to public health and food safety.
- Acidic soil pH enhances metal mobility and bioavailability, while climatic factors facilitate their long-range transport.

2.2. Apicultural Products as Bioindicators of Environmental Pollution and Microbial Bioinhibitory Agents

The previous chapter focused on the assessment of water and soil contamination in areas impacted by historical mining and metallurgical activities, highlighting the persistence and spatial distribution of heavy metals, as well as their potential transfer within terrestrial ecosystems. Building on these findings, it becomes essential to explore additional biological matrices capable of integrating environmental contamination over time and space.

In this context, the following chapter is dedicated to apicultural products, specifically honey and propolis, as bioindicators of environmental pollution and as natural agents with microbial bioinhibitory properties. These products are produced by honeybees (*Apis mellifera*), which interact continuously with the surrounding environment through foraging activities on nectar, pollen, and plant exudates, thereby facilitating the transfer of environmental contaminants into hive products. Due to their complex composition and direct connection with plant sources and atmospheric deposition, honey and propolis reflect the quality of the ecosystem. Their potential antimicrobial activity further extends their relevance beyond environmental monitoring, positioning them as valuable functional biological materials in the assessment of ecosystem health..

Research Aim and Objectives

The aim of this section is to conduct research in the field of environmental pollution, considering bee products (propolis and honey) both as bioindicators of pollution and as microbial bioinhibitors. The quality of honey and propolis is significantly influenced by the environmental conditions of their origin. Therefore, samples were collected from areas unaffected by pollution, which typically exhibit a more balanced chemical composition and higher concentrations of bioactive compounds, such as flavonoids and phenolic acids. These compounds are directly correlated with antimicrobial activity, suggesting that pollution not only affects the capacity of bee products to act as bioindicators but also diminishes their therapeutic and antioxidant properties.

The study was based on the hypothesis that propolis and honey are efficient natural bioindicators, capable of reflecting the presence and levels of trace metal contamination in industrialized or mining areas, with environmental factors potentially reducing the bioactive potential of bee products from affected regions. Furthermore, microbiological analysis demonstrated that propolis from protected areas exhibited slightly stronger antimicrobial activity against bacteria and fungi compared to samples from mining or industrially polluted regions.

Specific Objectives:

- *Analysis of quality indicators of honey;*
- *Applications and research in the field of microbial bioinhibitors;*
- *Honey and propolis as bioindicators of environmental pollution;*
- *The potential of bee products and essential oils in the development of sustainable soaps.*

Preamble and Obtained Results

Globally, the number of bee colonies has increased by approximately 85% over the past six decades, surpassing 100 million hives, according to the FAO [48]. However, this positive trend is overshadowed by the intensification of industrial and urban pollution, which exposes bees and apicultural products to increasingly high concentrations of airborne particulate matter, pesticides, fertilizers, industrial emissions, heavy road traffic, and various atmospheric deposits [49].

Apicultural products contribute to increased energy levels and the strengthening of the immune system [50] and have numerous therapeutic effects. These include antibacterial [51,52], antifungal [53], antiviral [54], hepatoprotective [55], anti- inflammatory [56], anticancer [57], immunostimulatory [58], analgesic [59] and wound-healing activities [60].

Nevertheless, bees and apicultural products such as honey, wax, and pollen can be used as effective bioindicators of environmental pollution, due to their ability to accumulate heavy metals, pesticides, and other contaminants from the air, water, and the plants they visit. [61].

Bees are increasingly exposed to environmental contaminants, especially in urban and industrial areas where air pollution is intense. One of the most significant sources of contamination is particulate matter (PM_{10} and $PM_{2.5}$), which can carry heavy metals such as lead (Pb), nickel (Ni), cadmium (Cd), and arsenic (As). These particles settle on plant leaves and flowers, from where they are then unintentionally collected by bees along with nectar and pollen [62]. In addition, emissions from industrial activities, the combustion of fossil fuels, and heavy road traffic contribute to the release of significant amounts of heavy metals into the atmosphere, which subsequently enter the apicultural ecosystem [63]. Even agricultural practices, such as the use of fertilizers and pesticides, can promote the accumulation of these toxic elements in the soil, from where they are absorbed by nectar-producing plants [64]. All these factors lead to a progressive contamination of honey and other apicultural products, endangering both the health of bee colonies and the safety of human consumers. Due to its high sensitivity to environmental changes, honey is not only a valuable food but also a useful biological matrix for monitoring pollution levels. [65]. In regions affected by mining activities, the accumulation of toxic elements such as lead, cadmium, and arsenic in honey can provide valuable information about the degree of environmental contamination and the potential risks to ecosystem and human health [66].

In various geographical regions, the contamination of soil and water resources with heavy metals has become an increasingly serious environmental concern. This phenomenon is particularly pronounced in areas characterized by a high level of anthropogenic activity, such as urbanized and industrialized zones, mining and fossil fuel extraction sites, as well as intensively managed agricultural landscapes [67]. The persistence and mobility of these toxic elements in the environment represent significant risks to natural ecosystems and to human health, making their monitoring and assessment a priority in environmental research and policies [68].

The accumulation of heavy metals in *Apis mellifera* (the honey bee) and its products is closely associated with ambient atmospheric concentrations, with particulate matter levels (PM_{10}) playing a significant role in the increased detection of lead (Pb) and nickel (Ni) within the organisms [69]. Given the risks that heavy metal contamination poses to human health, it becomes essential for such correlations with other pollution factors to be regulated through strict standards. [70], as is the case with Regulation (EC) No 1881/2006, which emphasizes the importance of food safety by establishing strict limits for contaminants, and in the case of honey, the focus is on lead (Pb), with a maximum permissible level of 0.10 mg/kg, due to its toxic effects on human health [71].

Honey is a natural food product made by bees through the enzymatic transformation of floral nectar or extrafloral secretions [72]. It is a mixture rich in simple sugars (fructose and glucose), which provides numerous nutritional benefits and contains minerals, vitamins, antioxidants, amino acids and enzymes (such as invertase and amylase), as well as hydrogen peroxide, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal and peptides derived from bees [73].

Propolis is a resinous material that bees use to seal their hives and is utilized both in traditional medicine and in homeopathic medicine, due to its numerous benefits in combating infections [74]. It contains over 300 compounds (the most important being polyphenols, sesquiterpenic quinones, coumarins, essential oils, amino acids and inorganic compounds) and represents a mixture of substances with biological activity, beeswax and bee secretions [75]. Propolis has beneficial effects on the body due to its antiviral, antibacterial, antiseptic, antifungal, antiparasitic, anti-inflammatory, antioxidant, wound-healing, and mildly anesthetic activities. It also exhibits strong immunomodulatory effects [76].

Honey bees come into contact with almost all components of the environment – soil, vegetation, water, and air – providing, through their foraging activity, numerous indicators of contamination for each season. In addition, they bring into the hive a variety of materials – nectar, pollen, honeydew, propolis, and water – which are stored according to verifiable criteria and can subsequently be analyzed in the laboratory. Honey bees are used as efficient bioindicators for detecting pesticides, heavy metals, radionuclides, polycyclic aromatic hydrocarbons (PAHs), as well as microorganisms (such as bacteria) [77].

In Table 2.2.1 are presented the average pollution levels, the diffusion of pollutants and the influence on bees.

Table 2.2.1. Sources of pollution and effects on bees depending on the exposure environment

Environment	Sources of pollution	Mode of pollutant diffusion	Areas visited by bees	Impact on bees
Air	Industrial emissions, vehicle exhaust gases, sprayed pesticides	Atmospheric dispersion, dry or wet deposition	Flowers near roads or fields	Contamination of pollen and nectar, impairment of the nervous system
Water	Industrial effluents, dissolved pesticides, fertilizers	Infiltration into soil, runoff into rivers and lakes, evaporation	Wetlands, riverbanks, aquatic melliferous plants	Pollution of melliferous plants, reduction of safe water sources
Soil	Pesticides, trace metals, toxic waste	Slow infiltration, absorption by plants	Plants with roots in the soil (flowers, grasses)	Bioaccumulation in pollen and nectar, long-term sublethal effects
Vegetation	Flowers, grasses, melliferous plants, reeds, bulrushes	Direct absorption from air, water and soil	All melliferous plants frequently visited by bees	Transfer of pollutants to pollen and nectar, impairment of bee food quality

Bees visit all three compartments of the environment through their interaction with plants, which extract water and nutrients from the soil, breathe contaminated air, and collect pollen and nectar from affected plants.

Cross-contamination is frequent: airborne pollutants can reach the soil or water through precipitation.

There are indirect risks: even if bees do not die immediately after exposure, they can transport pollutants into the hive, thereby affecting the entire colony.

2.2.1. Environmental Impact on Apicultural Products: Physico-Chemical, Microbiological, Safety and Antimicrobial Assessment

The system for ensuring an adequate supply of food products is continually evolving, with growing emphasis on how environmental factors affect the integrity, quality, safety, and nutritional value of food [78]. The concept of "food safety", based on the general principles of food hygiene and the HACCP method adopted by the Codex Alimentarius, also aims to limit biological, physical, or chemical contamination caused by environmental factors [79].

In recent years, increasing environmental pollution has become a major concern for food safety, as contaminants originating from industrial emissions, traffic, and agricultural practices can be transferred into the food chain. In this context, apicultural products, particularly honey, are recognized as sensitive indicators of environmental quality due to their direct interaction with air, water, and vegetation.

Maintaining contaminant levels within acceptable limits—especially those originating from industrial activities, intensive agriculture, and atmospheric pollution—has become essential for ensuring the quality and safety of food products. In this context, honey is a particularly sensitive agri-food product, directly affected by the environmental conditions from which it originates. The activities of harvesting, collecting, processing, and storing honey are continually adapting not only to European legislative requirements and market demands but also to increasingly pressing ecological realities.

Honey is a semi-liquid, sweet, and aromatic food of high biological and energetic value, produced by bees from nectar, honeydew, or other sweet substances found on plants and trees. Being a natural product, honey faithfully reflects the environmental conditions in which it was produced. Consequently, its chemical composition can vary significantly depending on the pollution level of the area, potentially containing traces of heavy metals or other contaminants. These factors necessitate careful attention to the physicochemical and hygienic quality of honey, which serve as important indicators not only of its nutritional value but also of the level of environmental contamination [80]. Therefore, honey can be considered a bioindicator of environmental pollution, reflecting the presence of contaminants such as heavy metals, pesticides, and airborne particulate matter accumulated from the surrounding ecosystem.

The authentication of honey and the assessment of potential changes resulting from pollution or improper treatments are carried out by determining physicochemical parameters, and in particular by analyzing the HMF (hydroxymethylfurfural) content. Elevated HMF levels may indicate either excessive heat treatment or deterioration of the raw material quality due to unfavorable environmental factors, thereby contributing to the indirect diagnosis of the health status of the ecosystem from which the honey originates [81].

The quality of honey can be significantly influenced by environmental contamination or pollution, and these effects can be identified through a correlated analysis of various physicochemical properties of the product. In this regard, parameters such as pH, water activity, moisture content, fructose, glucose, and HMF content, among others, are evaluated and monitored to assess honey quality.

Table 2.2.2 presents the results of the physicochemical analysis of polyfloral honey samples collected from different towns in Alba County, highlighting the variations in parameters depending on the geographical area and the specific pollution level of each location.

Table 2.2.2. Physicochemical results of honey samples collected from the main towns of Alba County.

Parameter **Area**	**pH**	**a_w**	**Fructose, %**	**Glucose, %**	**Water content, %**	**HMF, mg/kg**
Alba Iulia	3.70±01	0.541±0.018	38.4±1.0	28.7±0.6	16.7±0.7	2.1±0.3
Abrud	3.66±02	0.602±0.033	40.8±1.2	29.2±0.8	15.9±0.9	7.2±1.0
Aiud	3.92±04	0.574±0.012	41.2±0.9	27.0±1.2	16.4±0.7	11.0±1.1
Baia de Arieș	3.68±03	0.559±0.027	39.9±1.5	29.8±1.6	16.5±1.1	4.7±0.4
Blaj	3.74±01	0.580±0.039	38.3±1.8	30.4±1.3	16.2±1.2	6.5±0.9
Câmpeni	3.76±02	0.567±0.034	40.5±1.3	27.9±1.1	15.3±0.7	5.2±0.6
Cugir	3.63±03	0.522±0.046	44.4±1.7	28.6±0.9	15.2±0.6	8.0±0.5
Ocna Mureș	4.08±04	0.503±0.025	41.3±2.1	29.9±2.0	17.0±1.5	12.1±1.3
Sebeș	3.81±01	0.567±0.029	38.2±0.4	27.8±1.4	15.8±0.6	7.4±0.17
Teiuș	3.79±02	0.548±0.010	39.5±0.9	27.6±1.0	15.7±0.8	4.9±0.4
Zlatna	3.95±05	0.545±0.017	39.0±0.6	29.1±0.7	16.1±1.2	6.6±0.8

The observed variations between sampling locations may be associated with differences in local environmental conditions, including proximity to industrial areas, traffic intensity, or agricultural activities. Higher HMF values and variations in moisture or acidity may indicate environmental stress factors or suboptimal storage conditions influenced by ambient pollution and temperature fluctuations. The negative impact of environmental factors, such as industrial pollution or soil and flora contamination, can be reflected in the composition of honey, and the correlation between its physicochemical properties allows for the indirect identification of the pollution level of the ecosystem from which it originates.

To investigate samples from outside Alba County and to include not only polyfloral honey but also varieties with different botanical origins, samples were collected from various regions of Transylvania. These samples, with diverse floral origins, underwent basic physicochemical analyses to determine their quality and possible contamination, particularly focusing on hydroxymethylfurfural (HMF) content. The results of these analyses are presented in Table 2.2.3. The evaluated parameters—moisture, pH, acidity, and HMF—provide essential information about the state of the honey and allow the identification of changes in its composition resulting from factors such as heat processing, improper storage, or environmental influences.

Table 2.2.3. Physicochemical analysis results of honey samples from Transylvania

Sample	Type of honey	County	Water content (%)	pH	Acidity (meq /kg)	HMF (mg/kg)
1.	Polyfloral	Alba	15.6	3.72	41	2.1±0.7
2.	Polyfloral	Sibiu	16.8	3.99	23	***52.3***±1.2
3.	Linden	Cluj	16.4	4.39	16	14.9±0.8
4.	Linden	Alba	17	4.86	11	1.0±0.4
5.	Acacia	Alba	18	3.75	12	4.3±0.5
6.	Acacia	Alba	19.4	2.89	14	8.4±0.7
7.	Honeydew	Cluj	16.2	3.66	28.9	5.7±0.3
8.	Honeydew	Sibiu	15	3.98	43.8	21.7±0.9
9.	Sunflower	Alba	16.4	3.67	22.6	22.3±0.6
10.	Sunflower	Cluj	19.8	3.59	20.9	23.8±3.0
11.	Polyfloral	Cluj	16.4	3.6	37.3	31.8±2.1
12.	Polyfloral	Alba	15.4	4.01	20.6	7.4±0.9
13.	Polyfloral	Sibiu	15.6	3.9	**55.6**	4.5±0.5
14.	Polyfloral	Cluj	16.4	3.78	28.5	2.3±0.1
15.	Polyfloral	Hunedoara	16.6	4.04	30.4	8.5±1.0
16.	Polyfloral	Alba	14.6	3.8	24.4	30.0±1.7
17.	Linden	Cluj	16.4	4.36	20.0	24.8±3.2
18.	Linden	Hunedoara	16.4	4.01	23.4	14.2±0.7
19.	Acacia	Sibiu	16.4	3.9	14.3	**64.3**±7.8
20.	Acacia	Hunedoara	16.8	3.9	14	10.7±0.9

The values of the physicochemical parameters fell within the reference ranges presented in EU or national regulations. Exceptions include samples number 2 and 19 for HMF content and sample 13 for acidity levels. The differences identified among regions suggest that environmental factors, including air quality and anthropogenic activities, may influence honey composition. Elevated HMF values in certain samples could be correlated not only with thermal processing but also with environmental stressors affecting nectar composition and stability.

Table 2.2.4 presents a comparative overview of HMF values obtained after exposing polyfloral honey to different temperatures for varying periods, highlighting how environmental factors, particularly temperature, can influence the chemical stability and quality of the product.

Table 2.2.4. HMF content values obtained at different temperatures over time.

Temp.[°C] / Time [min]	30	50	70	80	100
0	2.1±0.7				
30	5.3±0.8	10.2±0.3	11.5±0.2	11.7±0.5	15.8±0.7
60	5.4±0.5	10.8±0.8	12.2±0.7	19.6±0.4	46.3±2.8
90	5.7±0.3	11.7±0.5	14.5±0.3	20.4±0.2	50.7±4.1
120	6.6±1.1	12.3±0.9	17.8±0.15	27.3±0.9	60.2±3.7
150	9.0±1.4	15.5±1.6	19.4±2.2	36.1±1.4	74.7±2.2
180	11.4±2.3	17.2±1.0	20.3±1.8	42.9±3.1	82.3±3.8
210	15.2±4.0	22.0±3.3	44.2±4.9	50.7±3.8	84.1±4.5
240	17.1±2.8	29.6±2.4	52.1±6.7	63.9±2.5	86.5±3.9
270	19.5±3.9	32.8±1.8	60.6±2.0	74.8±4.7	88.0±2.3
300	20.1±2.7	37.5±4.1	65.0±3.3	86.6±2.9	90.4±3.7

The HMF value shows an increasing trend over time for all temperatures to which the honey was exposed, starting from 2.1 mg/kg and reaching 90.4 mg/kg for honey subjected to 100°C for 300 minutes. These results also highlight the potential impact of environmental temperature variations, including those associated with climate change, on the chemical stability of honey during storage and processing.

In chemistry, it is unusual for a process to be influenced solely by a single variable parameter. Instead, it is interesting to observe how other parameters, through their fluctuations, significantly affect the observed variations in the outcome [82].

Using MATLAB, the experimental data on the time-dependent variation of HMF content in polyfloral honey samples at different temperatures were processed and analyzed. Figure 2.2.1 presents both the experimental data and the surfaces generated by the statistical mathematical model. A correlation was established between the HMF content of honey over time (t) and the applied temperature (T), using a second-degree polynomial relationship with two independent variables.

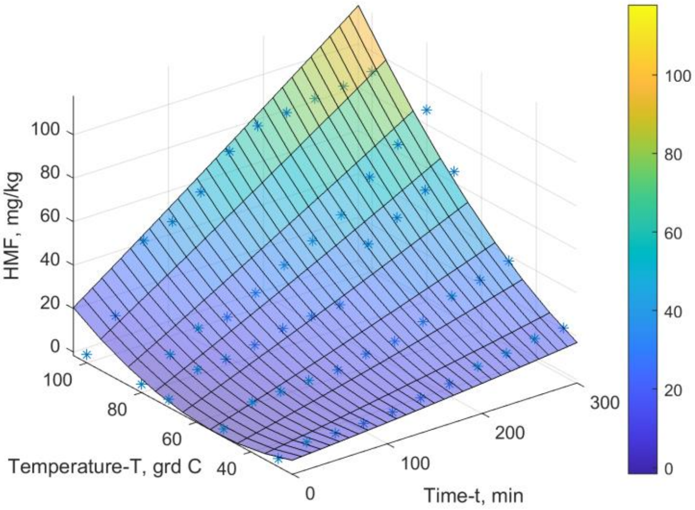


Figure 2.2.1. Variation of HMF content in polyfloral honey as a function of applied temperature and time

The second-order regression equation for the honey samples, correlating the variation of HMF content with time and temperature, has the following form:

$$\text{HMF (mg/kg)} = 21.25 - 0.06 \cdot t - 0.86 \cdot T + 0.004 \cdot t \cdot T + 3.69 \cdot 10^{-5} \cdot t^2 + 0.01 \cdot T^2$$

where: t – time, in minutes; T – temperature, in °C.

The accuracy of the correlation is confirmed by the goodness-of-fit indicators: the model's accuracy index ($r^2 = 0.944$), the correlation coefficient ($r = 0.972$), and the standard deviation ($\sigma = 6.467$). The calculated correlation parameters validate the very good predictive capability of the statistical mathematical model.

In the processing of honey within the technological process, pollution-related factors are investigated primarily to determine trace metals that may contaminate the product. Therefore, it is essential to develop a technological flow diagram that allows for the monitoring and control of these contaminants throughout the production chain.

Figure 2.2.2 presents a graphical representation of the possible stages for controlling trace metal contamination in honey.

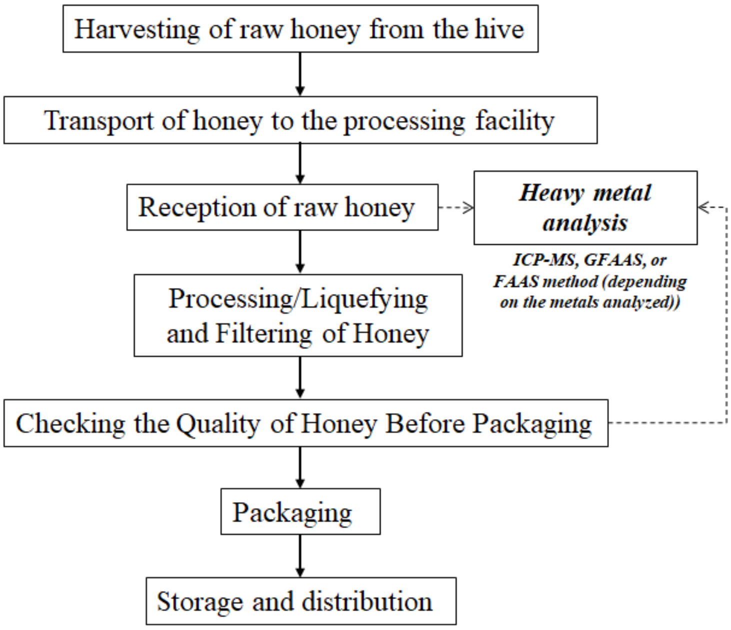


Figure 2.2.2. Technological flow diagram for trace metal control in honey

To ensure the safety and quality of honey, it is essential to identify the critical control points (CCPs) within the technological process where the risk of trace metal contamination may occur [83]. Table 2.2.5 presents the relevant stages of the honey production process, points of attention, preventive and monitoring measures, as well as the corrective actions required to maintain a safe product in compliance with food safety regulations.

Table 2.2.5. CCP Table – Control of trace metal contamination in the honey production process

Process stage	**Identified hazard**	**Critical Control Point (CCP)**	**Critical limit**	**Monitoring method**	**Corrective action**	**Responsibility**
Reception of raw honey	Contamination with trace metals	Analysis of trace metal content in honey samples	Maximum permitted concentration according to legislation (e.g. Pb ≤ 0.1 mg/kg)	Laboratory analyses (e.g. ICP-MS, AAS)	Rejection of reception and return of the contaminated batch	Laboratory responsible, production manager
Before packaging	Maintaining acceptable levels	Verification of control analyses for trace metals	Maximum permitted limit according to legislation	Review of analysis reports	Stopping packaging and conducting further investigations	Quality manager

In addition to technological control, environmental monitoring plays a crucial role in preventing contamination, as pollutants present in soil, water, or air can enter the apicultural chain even before processing begins.

The HACCP control plan for monitoring trace metal contamination in honey processing and packaging units includes the verification at the reception of raw honey, where the heavy metal content (such as lead) is analyzed through laboratory tests. If the maximum legal limit established by European legislation (Pb ≤ 0.1 mg/kg) is exceeded, the batch is rejected and returned by the laboratory responsible and the production manager.

Another production stage is the control before packaging, where control analyses are carried out to confirm compliance. In the event of exceedances, the packaging process is stopped and further investigations are initiated under the coordination of the quality assurance manager.

The presence of microorganisms in honey is influenced by both natural sources (nectar, pollen, spontaneous flora) and anthropogenic factors, particularly environmental pollution sources such as air contamination, soil quality, and water exposure, as well as hygiene conditions during harvesting, processing, and storage. Sporulating microorganisms commonly found belong to the genus *Bacillus*, while non-sporulating bacteria such as *Micrococcus*, *Pseudomonas*, and *Flavobacterium* are less numerous and originate mainly from floral organs or the digestive tract of bees. In polluted environments, these microorganisms may also reflect the microbiological load of contaminated air, dust particles, or plant surfaces. Contamination may also occur in working areas due to insufficiently sanitized equipment or dirty containers [84].

Filamentous fungi, which are widespread in nature and resistant to extreme conditions, may enter honey either through pollen carried by bees or via dust, contaminated water, or improper human handling. Their presence is often associated with environmental conditions such as high humidity, airborne particulate matter, and agricultural or industrial pollution. Although species such as *Aspergillus flavus* can be identified, honey does not provide favorable conditions for aflatoxin synthesis, thus reducing the direct risk to consumer health.

Yeasts are present in small amounts in normal honey, being naturally introduced by bees without affecting product quality. However, when originating from extraction and handling areas, yeasts can become an important microbiological risk due to their resistance and tendency to ferment honey, thereby affecting its stability and organoleptic characteristics [85]. Environmental factors such as temperature fluctuations, increased atmospheric humidity, and exposure to contaminated surfaces can further enhance yeast proliferation.

Microbiological contamination can significantly influence the hygienic quality and stability of honey. Although normal honey should be free of pathogenic microorganisms, the association of honey with *Clostridium botulinum* spores, involved in cases of infant botulism, has been frequently reported in the scientific literature. [86]. The occurrence of such spores is often linked to environmental reservoirs, including soil and dust, which may contaminate nectar or be introduced during harvesting.

In a study conducted in Portugal, honey contamination was low with regard to the presence of *Bacillus cereus* and fungi (*Mucor* spp., *Penicillium* spp., *Aspergillus flavus*, *A. candidus*, *A. fumigatus*, *A. niger*), and no aflatoxins were detected. [87]. In another study, *Clostridium perfringens* was not detected in any of the 80 analyzed samples; however, Bacillus cereus was present in 13.7% of the samples, while molds and yeasts were identified in 88.8% of them. The predominant mold genera were Aspergillus, Penicillium, and Mucor, whereas the predominant yeast genera were *Saccharomyces* and *Candida*. These findings suggest that environmental conditions and local pollution levels play a critical role in determining the microbiological profile of honey.

Furthermore, microbiological contamination during or after honey processing was confirmed by comparing samples collected directly from the hive (primary sources)—in which no microorganisms were detected—with samples obtained from local markets, where species of *Bacillus* and fungi (*Candida*, *Aspergillus*, *Geotrichum*, *Rhizopus*) were identified. These finding highlights that secondary sources of contamination, such as inadequate hygiene conditions during extraction, processing, and storage, contribute significantly to the microbial load of the final product [88]. This highlights the combined impact of environmental exposure and inadequate hygienic practices, emphasizing that honey acts as a sensitive indicator of environmental contamination.

Thus, the need for maintaining strict hygiene control throughout all stages of the production chain is highlighted, as well as the monitoring of microbiological contamination as an integral part of honey quality assurance and consumer protection, especially in the context of the increasingly pronounced influence of environmental factors. At the same time, proper processing of honey is essential for maintaining its quality and safety in the context of exposure to potentially contaminating environmental factors. In addition, monitoring environmental quality in the vicinity of apiaries is essential, as pollutants present in air, soil, and water can be directly transferred into the apicultural chain.

Technological stages such as filtration, dehydration, liquefaction, pasteurization (maximum 40–55°C for 5–6 minutes), rapid cooling, and packaging must be carried out under controlled conditions in order to prevent microbial proliferation and the possible activation of contaminants originating from polluted environments, such as industrial dust, contaminated water, or contact with unsanitized surfaces. These practices are particularly important in areas exposed to environmental pollution, where the risk of contamination with airborne particles or chemical residues is significantly higher.

In order to establish the microbiological characteristics of honey samples collected from beekeepers, the parameters presented in Table 2.2.6 were analyzed. The analyses were determined exclusively for raw honey samples, namely extracted honey that had not undergone any liquefaction or filtration processes [89]. The focus on raw (unprocessed) honey allows for a more accurate evaluation of the direct impact of environmental conditions on microbiological contamination, without the influence of technological interventions.

Table 2.2.6. Microbiological control of raw (unprocessed) honey samples

Sample	**TVC/g**	**Yeast and mould/g**
1	30	30 (*Penicillium* spp.)
2	95	15 (*Rhizopus* spp., *Aspergillus* spp., *Penicillium* spp.)
3	45	<10
4	15	15 (*Absidia* spp.)
5	20	<10
6	20	20 (*Penicillium* spp.)
7	40	40 (*Penicillium* spp., *Aspergillus* spp.)
8	45	20 (*Penicillium* spp., *Aspergillus* spp.)
9	35	<10
10	10	10 (*Fusarium* spp.)
11	30	<10
12	25	<10
13	<10	<10
14	20	10 *(Aspergillus* spp.)
15	45	10 (*Penicillium* spp.)
16	10	<10
17	20	<10
18	25	<10
19	<10	<10
20	20	<10

The antimicrobial nature of honey is supported by the results regarding TVC (total viable count), which indicated a low level of microbiological contamination, characteristic of a product with limited exposure to external environmental influences. This suggests that the sampling areas were relatively less affected by environmental pollution or that the contamination pathways from polluted air, soil, and vegetation were minimal. In all analyzed samples, the TVC was below 100 CFU/g, indicating minimal contamination with aerobic mesophilic microorganisms. This reflects both the natural antimicrobial properties of honey and the relatively low impact of environmental factors on its microbiological quality. However, TVC values may increase in areas exposed to higher levels of anthropogenic pollution, such as industrial emissions or intensive agricultural activities. The highest microbial load was observed in sample no. 2 (multifloral honey), with 95 CFU/g, a value that nevertheless remained within acceptable limits, but may indicate localized environmental influences or microclimatic conditions favoring microbial presence.

Regarding contamination with yeasts and molds—indicators that are highly sensitive to environmental and hygiene conditions—the absence of yeasts was observed in all ten analyzed samples. The mold count did not exceed 40 CFU/g in any sample, indicating a low risk of fermentation. This may reflect favorable environmental conditions, including low atmospheric humidity and reduced exposure to polluted or dust-laden air. But the identification of fungal genera such as *Penicillium* (in six samples) and *Aspergillus* (in four samples), along with the sporadic presence of fungi belonging to the genera *Absidia*

(*Mycocladus*), *Rhizopus*, and *Fusarium*, may suggest an indirect influence of the surrounding environment, particularly dust, increased humidity, or contact with contaminated materials. These fungi are commonly associated with soil, decaying vegetation, and airborne particles, indicating that environmental reservoirs play a significant role in their transfer into honey. Thus, even though the values remain low, the presence of these fungi reflects the potential for uncontrolled environmental factors, including air pollution, particulate matter, and climatic conditions, to affect the microbiological hygiene of honey.

Figure 2.2.3. *Penicillium* genus
(Samples 1, 2, 6, 7, 8, 15)

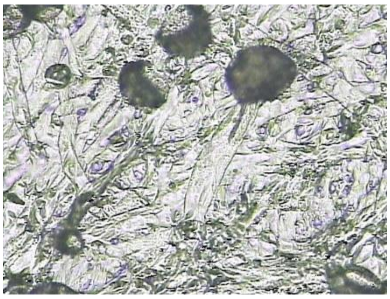


Figure 2.2.4. *Rhizopus* genus
(Sample 2)

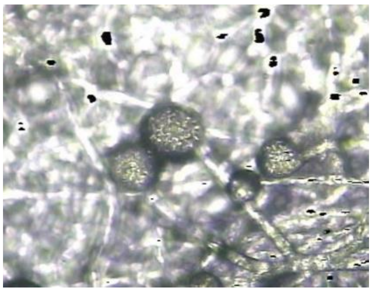


Figure 2.2.5. *Aspergillus* genus
(Samples 2,7,8,14)

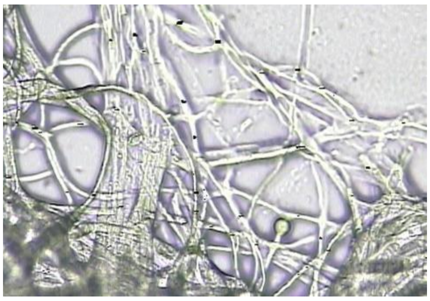


Figure 2.2.6. *Absidia* genus
(Sample 4)

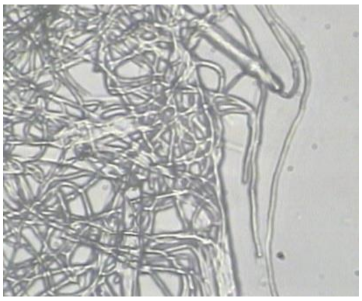


Figure 2.2.7. *Fusarium* genus
(Sample 10)

The moderate levels of total viable count (TVC) and the diversity of fungi identified in the honey samples indicate microbiological contamination resulting both from improper handling by beekeepers and from environmental influences. Factors such as atmospheric dust, air pollution, humidity, and the use of non-compliant water during the initial stages of processing may significantly affect the microbiological profile of honey. These findings highlight the combined impact of insufficient hygiene practices and uncontrolled environmental conditions, confirming that honey quality is closely linked to the state of the surrounding ecosystem.

The quality of the analyzed honey, particularly multifloral honey from Alba County and other regions of Transylvania, largely reflects compliance with the physicochemical standards imposed by current legislation. However, the indirect influence of environmental factors—such as dust contamination, air pollution, or improper storage conditions—may affect essential parameters including moisture content, acidity, and HMF levels. Non-compliant values identified in some samples may be correlated with increased exposure to environmental pollution sources or with improper processing practices, highlighting the need for rigorous monitoring throughout the entire production chain.

The correct application of HACCP system principles in honey processing is essential for preventing contamination, especially during sensitive stages such as pasteurization and packaging, which may lead to increased HMF content if not strictly controlled. Moreover, in an increasingly vulnerable ecological context, careful monitoring of potential heavy metal contamination (such as lead, cadmium, or mercury) is required, as these contaminants may be transferred into apicultural products through soil, water, or air pollution in industrialized areas. The integration of these risks into the HACCP scheme ensures not only food safety but also long-term consumer protection.

From a microbiological perspective, the obtained results demonstrated an overall low load of aerobic mesophilic microorganisms, as well as the absence of yeasts in all tested samples. Nevertheless, the presence of fungi belonging to the genera *Penicillium*, *Aspergillus*, *Fusarium*, and others highlight the risk of contamination from secondary sources related to inadequate equipment hygiene and environmental influences such as dust or humidity in processing areas. Under favorable conditions, these fungi may produce mycotoxins, emphasizing the importance of maintaining a controlled and hygienic environment throughout all stages of the technological chain.

The microbiological characterization of honey samples highlights the influence of both environmental factors and handling practices on product quality, emphasizing the strong dependence of apicultural products on ecosystem conditions. In addition to microbiological aspects, the physicochemical properties and antimicrobial activity of honey and propolis further reflect the interaction between environmental quality and biological matrices.

In order to better understand these relationships and to evaluate the influence of environmental conditions on the quality and bioactive properties of apicultural products (honey and propolis), a spatially structured sampling approach was adopted. Thus, the investigated areas were established taking into account the priority elements of the apiary, including the specific flora and flowering periods, climatic and meteorological conditions (temperature, humidity, precipitation), geomorphological features, soil composition, and demographic factors (Table 2.2.7). The selected areas are identical to those defined in Section 2.1.2.1 (Impact of Pollution on Well and Spring Waters in Alba County) – figure 2.2.8, allowing for a coherent comparative assessment of environmental quality across water, soil, and biological matrices.

Table 2.2.7. Geographical location of beekeeping products sampled in the 5 areas of Alba County.

Sample	**Apiary Location Area**	**Geographical origin**	**Landforms**
I	Alba Iulia – Teiuș	Sântimbru	Plain
II	Sebeș – Cugir	Șibot	Sub-mountainous
III	Cîmpeni – Zlatna	Abrud	Mountainous
IV	Blaj	Valea Lungă	Hilly
V	Aiud – Ocna Mureș	Ciumbrud	Hilly

Figure 2.2.8. Map of the distribution of sampling areas for beekeeping products in the five areas of Alba County

The values of the physico-chemical parameters of the polyflora honey (H) samples collected from Alba County are presented in Table 2.2.8.

Table 2.2.8. Physico-chemical parameters of honey (H) analyzed from Alba County.

Sample	**Moisture content (%)**	**Water activity, a_w**	**Ash content(%)**	**pH**	**Acidity Meq/kg**	**HMF mg/100g**	**Phenols (mg GAE/100 g)**	**Flavonoids (mg QE/100 g)**
I H	15.23±0.18	0.578±0.013	0.20±0.03	3.87±0.34	20.8±0.6	1.39±0.06	82.14±0.26	4.26±0.18
II H	18.06±0.35	0.582±0.024	0.41±0.06	4.02±0.16	24.6±0.9	1.13±0.12	70.77±0.52	2.57±0.05
III H	17.44±0.42	0.577±0.030	0.29±0.02	4.14±0.19	16.5±0.7	0.47±0.10	98.49±1.03	5.35±0.12
IV H	16.67±0.20	0.591±0.019	0.36±0.07	3.98±0.50	18.0±0.2	1.21±0.07	63.51±0.28	3.29±0.16
V H	14.51±0.11	0.569±0.027	0.45±0.04	3.85±0.22	14.3±0.5	1.08±0.09	64.20±0.47	3.40±0.31

* GAE—gallic acid equivalents; QE—quercetin equivalents.

The water content of the honey samples falls within the maximum limit of 20%, indicating not only proper maturation but also limited environmental stress factors such as excessive humidity linked to climate variability. The a_w of the polyfloral honey analyzed is lower than 0.6, a value at which microbial growth is inhibited and fermentation due to osmophilic yeasts is prevented, suggesting that environmental contamination has not significantly influenced the w_a and hygroscopic properties of the honey. According to Romanian STAS 784/3-2009 [90], it is recommended that the ash content not exceed 0.5%, as elevated ash levels may reflect environmental pollution, particularly soil and air contamination with mineral residues or industrial particulates. The pH values, crucial during honey extraction due to its impact on texture and stability, range from 3.85 to 4.14, remaining within normal limits and indicating minimal influence from environmental pollutants that could alter acidity. The pH value of honey together with its free acidity prevent the growth of various specific microorganisms, but deviations may occur in polluted environments where contaminants affect nectar composition. The values of these parameters are high only when the processing or storage of the honey is inadequate, or when bees forage in areas exposed to environmental pollution.

The results of the HMF content in the honey samples are below the recommended value of 1.5 mg/100 g of hydroxymethylfurfural, respectively the acceptable quality of a maximum of 4.0 mg/100 g in the case of honey liquefied and packaged in jars, indicating that the honey has not been significantly affected by heat stress or environmental degradation factors during storage and handling. The total phenolic content of honey samples ranged from 63.51 to 98.49 mg GAE/100 g honey, while the total flavonoid content was 2.57 to 5.35 mg QE/100 g honey, compounds known to be influenced by botanical origin but also by environmental conditions, including pollution levels that can alter plant metabolism and nectar composition..

The polyfloral honey samples analyzed have the values of the physico-chemical parameters within the reference ranges presented in the EU Directive [91] or Romanian national regulations [92], suggesting that, despite potential environmental pressures, the samples do not show significant contamination and reflect a relatively low level of environmental pollution in the harvesting areas.

The values of the physico-chemical parameters of the propolis (P) samples collected from Alba County are presented in Table 2.2.9.

Table 2.2.9. Physico-chemical parameters of propolis samples (P) analyzed from Alba County.

Sample	**Moisture content (%)**	**Water activity, aw**	**Ash content (%)**	**Phenols (mg GAE/g)**	**Flavonoids (mg QE/g)**
I P	4.38±0.1	0.62 ± 0.20	3.21 ± 0.06	180.4 ± 4.28	76.22 ± 0.17
II P	5.07±0.2	0.65 ± 0.14	2.48 ± 0.04	152.9 ± 3.71	71.53 ± 0.16
III P	6.31±0.1	0.71 ± 0.12	3.02 ± 0.07	174.3 ± 5.02	80.72 ± 0.25
IV P	4.95±0.1	0.66 ± 0.08	2.62 ± 0.08	149.2 ± 2.50	68.33 ± 0.14
V P	5.26±0.1	0.70 ± 0.11	2.79 ± 0.05	165.6 ± 2.13	70.48 ± 0.30

* GAE—gallic acid equivalents; QE—quercetin equivalents.

The moisture content in propolis ranges between 4.38 and 6.31%, values that suggest limited influence of environmental humidity and indicate relatively stable climatic conditions in the harvesting areas. The water activity of the propolis samples ranges from 0.62 to 0.71, which does not provide a suitable environment for most microorganisms, and also indicates that environmental contamination has not significantly altered its water-binding properties. The total ash content of propolis indicates that the extract obtained from the maceration process contains minerals, while also serving as a potential indicator of environmental pollution, since elevated ash levels may reflect the presence of inorganic contaminants from soil, air, or anthropogenic sources. The phenolic content of the propolis samples from Alba County, Romania varied from 149.2 to 180.4 mg GAE/g, while the flavonoid content ranged from 68.33 to 80.72 mg QE/g, these bioactive compounds being influenced not only by botanical origin but also by environmental conditions, including pollution, which can affect plant metabolism and, implicitly, the chemical profile of propolis.

Table 2.2.10 shows the diameters of the inhibition zones on the bacterial strains for polyfloral honey samples from Alba County.

Table 2.2.10. Diameters of inhibition zones of polyfloral honey on bacterial strains (mm).

Microbial Strain	**Sample**					**Ciprofloxacin 5 µg**
	I H	**II H**	**III H**	**IV H**	**V H**	
E. coli	0	11	14	8	0	29
S. typhimurium	0	12	15	9	7	29
S. enteritidis	0	7	8	7	0	27
S. anatum	0	8	7	0	0	28
S. choleraesuis	0	10	10	0	7	28
P. aeruginosa	13	18	17	14	13	25
P. fluorescens	14	16	18	16	16	24
S. aureus	19	19	20	21	21	30
S. epidermidis	17	17	17	19	18	29
B. cereus	0	8	9	0	10	30
B. subtilis	17	18	19	11	12	30
L. monocytogenes	10	8	10	8	8	24

As can be seen in Table 2.2.10, the most sensitive strains to the antibacterial action of honey were the two staphylococci, followed by *P. fluorescens*, then *B. subtilis* and *P. Aeruginosa,* a hierarchy that may also reflect differences in environmental exposure and adaptive resistance mechanisms influenced by pollution or habitat conditions. Some of the honey samples analyzed (mainly the I H sample) did not have an antibacterial effect on all strains, which could be associated not only with botanical origin but also with environmental factors, including pollution, that may alter the bioactive composition of honey. Some of the strains of *Samonella* spp. were resistant to the action of some honey samples, possibly due to adaptive responses developed in contaminated environments where microorganisms are exposed to various stressors. Samples II H and III H, from the sub-mountain and mountain areas, were the most effective regarding the antibacterial activity, suggesting that honey originating from less polluted areas may retain higher levels of bioactive compounds and, consequently, stronger antimicrobial properties.

Table 2.2.11 shows the diameters of the inhibition zones on the bacterial strains for propolis samples from Alba County.

Table 2.2.11. Diameters of inhibition zones of propolis extracts on bacterial strains (mm)

Microbial Strain	**Sample**					**Ciprofloxacin 5 µg**
	I P	**II P**	**III P**	**IV P**	**V P**	
E. coli	18	27	32	22	18	29
S. typhimurium	18	26	30	25	24	29
S. enteritidis	15	19	25	17	15	27
S. anatum	17	26	22	16	19	28
S. choleraesuis	15	24	28	17	21	28
P. aeruginosa	28	32	29	27	27	25
P. fluorescens	27	33	33	28	29	24
S. aureus	22	27	26	30	27	30
S. epidermidis	26	24	20	31	29	29
B. cereus	24	26	27	24	29	30
B. subtilis	25	29	29	23	23	30
L. monocytogenes	31	29	30	28	28	24

Regarding the antibacterial activity of propolis extracts, it can be observed that all propolis samples had an inhibition effect on all the strains used. In some cases, the diameters of the inhibition zones were even larger than those produced by the antibiotic. The most sensitive strains to the action of propolis were the two strains of Pseudomonas, L. monocytogenes, then the strains of *Staphylococci* and *Bacillus* spp. For the strains of *Pseudomonas* spp. and *L. monocytogenes*, all samples determined the diameters of the inhibition zones larger than the antibiotic control. Samples II P and III P have the most effective antibacterial effect, these samples being collected from the same regions as the II H and III H honey samples that presented the strongest antibacterial effect, supporting the idea that less polluted environments contribute to enhanced antimicrobial properties of bee products.

Table 2.2.12 shows the diameters of the inhibition zones on the fungal strains for polyfloral honey samples from Alba County.

Table 2.2.12. Diameters of inhibition zones of polyfloral honey on fungal strains (mm)

Microbial Strain	**Sample**					**Voriconazole 1 µg**
	I H	**II H**	**III H**	**IV H**	**V H**	
C. albicans	0	10	10	8	8	37
A. niger	8	9	10	10	9	45
A. flavus	9	9	10	10	10	43
P. chrysogenum	10	8	11	8	12	18
R. stolonifer	12	9	9	8	8	16
F. oxysporum	9	10	11	11	11	29
A. alternata	7	11	7	8	0	16

With two exceptions (sample I H in the case of C. albicans and sample V H in the case of A. alternata) all honey samples had an antifungal effect on the studied strains. The strongest effect was observed on the strain of *F. oxysporum*, then *P. chrysogenum* and *A. flavus,* which may also reflect differences in sensitivity shaped by environmental exposure conditions. As in the case of honey, samples II H and III H had the strongest antifungal effect.

Table 2.2.13 shows the diameters of the inhibition zones on the fungal strains for propolis samples collected from Alba County.

Table 2.2.13. Diameters of inhibition zones of propolis extracts on fungal strains (mm)

Microbial Strain	**Sample**					**Voriconazole 1 µg**
	I P	**II P**	**III P**	**IV P**	**V P**	
C. albicans	17	21	22	18	19	37
A. niger	15	23	26	25	21	45
A. flavus	17	25	27	24	23	43
P. chrysogenum	23	16	25	18	27	18
R. stolonifer	25	21	19	19	20	16
F. oxysporum	21	22	28	27	25	29
A. alternata	20	26	25	16	17	16

As can be seen from Table 2.2.13, all propolis samples inhibited the multiplication of fungi, in some cases (such as *R. stolonifer* and *A. alternata*) the diameter of the inhibition zones being greater than that produced by the antifungal substance as a control, suggesting a strong antifungal potential that may be maintained under favorable environmental conditions. As in the case of honey, the most sensitive strain was *F. oxysporum*, but it was followed by the two strains of *Aspergillus* spp. and then by *P. chrysogenum*. As in the case of honey, samples II P and III P showed the strongest inhibitory effect on the studied strains.

Regarding MIC, for the honey samples with inhibitory effects, antimicrobial activity was observed at dilutions of up to 1/16 (w/v) for one or more bacterial strains. However, sample I H did not exhibit any inhibitory effect at the 1/16 dilution against *Pseudomonas* strains and *A. alternata*. Some honey samples demonstrated antifungal activity even at a dilution of 1/32 (w/v), though not for all strains.

As for the propolis samples, with the exception of samples I P and IV P, most exhibited antimicrobial activity at concentrations as low as 6.25 mg/mL, although not against all strains. Several samples showed inhibitory effects at concentrations of 3.12 mg/mL for certain strains. Notably, samples II P, III P, and V P had an inhibitory effect at concentrations as low as 1.56 mg/mL. Specifically, sample II P was effective against strains of *P. aeruginosa* and *P. fluorescens*; sample III P inhibited *P. aeruginosa*, *B. subtilis*, *L. monocytogenes*, and *F. oxysporum*; and sample V P inhibited *P. fluorescens* and *B. cereus*.

The results obtained regarding the antimicrobial activity of honey and propolis highlight their significant inhibitory effects against a wide range of bacterial and fungal strains, confirming their well-known bioactive properties. The variability observed among samples, in terms of inhibition zones and minimum inhibitory concentrations, reflects not only differences in chemical composition but also the influence of environmental factors specific to each sampling area, including potential exposure to pollutants that may alter their bioactive profiles.

In this context, both honey and propolis can serve as valuable tools for environmental monitoring, providing indirect information about ecosystem health and pollution levels. Their composition and biological activity are closely linked to environmental conditions, including vegetation type, atmospheric quality, and potential contamination sources, making them sensitive indicators of environmental changes and anthropogenic impact. Therefore, the analysis of these apicultural products supports not only food safety assessment but also the evaluation of environmental quality in agricultural and industrial regions.

2.2.2. Assessment of Pollution Effects on the Quality Attributes and Antifungal Activity of Honey and Propolis

For the study of bee products as bioindicators of environmental pollution, the physicochemical characteristics of several honey and propolis samples collected from different locations in northwestern and central Romania were determined. The selected sampling areas included regions with varying degrees of anthropogenic impact, such as urbanized zones, areas influenced by traffic, and regions with agricultural or industrial activities, allowing for a comparative assessment of environmental pollution level At the same time, the antifungal activity of these samples against various strains was evaluated [53,93].

Bee products, particularly honey and propolis, are increasingly recognized as effective bioindicators of environmental quality, due to their capacity to accumulate pollutants such as heavy metals, pesticides, and airborne particulates from the surrounding ecosystem. Bees forage over large areas, interacting continuously with air, water, and vegetation, which facilitates the transfer of environmental contaminants into apicultural products. Their composition reflects both natural factors (flora, climate, soil) and anthropogenic influences, including industrial emissions and traffic-related pollution. Thus, bee products provide an integrative picture of environmental quality, linking soil, air, vegetation, and anthropogenic activity into a single measurable matrix.

Honey samples were used as received, without any prior treatment or dilution. The aqueous extracts of propolis were prepared from propolis powder. Briefly, aqueous suspensions of propolis powder were refluxed for 1 h, followed by two successive centrifugation steps. The resulting supernatant was then concentrated by heating until approximately 80% of the initial volume had evaporated at the boiling point of water. The obtained extracts were stored in a dark, dry environment until further use. For antimicrobial activity assays, the extracts were prepared at a final concentration of 0.1 g/mL.

Honey and propolis samples were collected from different locations in northwestern and central Romania during the period June–July 2021. Figure 2.2.9 shows Romania's position on the map of Europe and the sampling points.

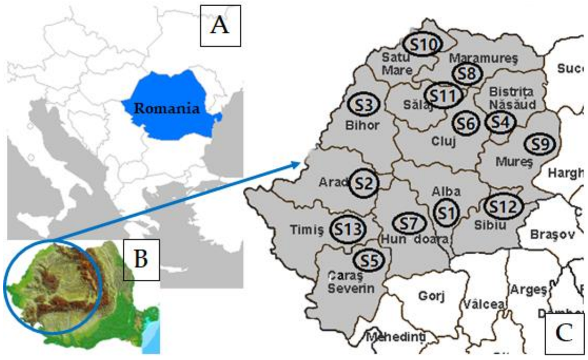


Figure 2.2.9. Romania's position on the map of Europe (A); geographical map of Romania highlighting the sampling area (B); identification of the 13 counties from which samples were collected (C).

Table 2.2.14 presents the 13 polyfloral honey and propolis samples collected from beekeepers in different counties in northwestern and central Romania, as well as 12 samples from Alba County, used for analysis. Honey and propolis samples were collected directly from the producers, with each location sampled from the same beekeeper. These bee products can reflect environmental pollution levels due to their ability to accumulate heavy metals and other contaminants from the surrounding ecosystem. The selection of sampling sites from diverse geographical areas (mountainous, hilly, and plain regions) allows for the assessment of how different environmental conditions and pollution sources influence the quality and composition of bee products. Areas with higher industrial activity or traffic density are expected to exhibit increased contaminant levels. Each sample was collected in sterile 200 g containers and stored in a dark place at a temperature between 2–8 °C until testing.

For honey, the samples were initially subjected to mild heat treatment for liquefaction/pasteurization (49.5 °C for 15 minutes) to reduce potential contamination, and microbiological purity was verified. As a control, an artificial honey sample was used. This was prepared by dissolving 40.5 g of fructose, 33.5 g of glucose, 7.5 g of maltose, and 1.5 g of sucrose in 17 mL of sterile deionized water, respecting the proportions of the four predominant sugars found in natural honey samples.

Crude propolis (50 g) was mixed with 250 mL of water, and the mixture was subjected to a reflux process. After two rounds of centrifugation and filtration, 80% of the initial volume of the mixture was evaporated, thus obtaining the aqueous propolis extract, which was stored in a cool, dark place. A concentration of 0.1 g/mL was used for analysis.

Table 2.2.14. Origin of honey and propolis samples.

Sample	**County**	**Relief form**		**Sub-sample**	**Area**
S1	Alba	Mountainous	**Alba County**		
S2	Arad	Plain		SS1	Blaj
S3	Bihor	Hill		SS2	Zlatna
S4	Bistrița-Năsăud	Mountainous		SS3	Alba Iulia
S5	Caraș-Severin	Hill		SS4	Teius
S6	Cluj	Hill		SS5	Șona
S7	Hunedoara	Submontane		SS6	Berghin
S8	Maramureș	Mountainous		SS7	Crăciunelu de Jos
S9	Mureș	Hill		SS8	Sântimbru
S10	Satu Mare	Hill		SS9	Feneș
S11	Sălaj	Submontane		SS10	Cugir
S12	Sibiu	Submontane		SS11	Abrud
S13	Timiș	Plain		SS12	Baia de Aries

The main physicochemical indicators reflecting the quality of honey and propolis were determined according to the methods proposed in the *"Harmonized Methods of the International Honey Commission"* [94], respectively the Romanian standard STAS 784/3-2009 [90]: moisture, pH, acitity, electrical conductivity, water activity (a_w), color, ash, HMF content, determination of Cu, Pb, and Cd, phenolic compounds, flavonoids, and antioxidant activity (DPPH method).

For propolis, the following were determined: moisture, ash, wax, identification of flavones and aromatic acids, quantification of flavonoid and phenolic compound content, and antioxidant activity.

These parameters were selected not only to assess product quality but also to evaluate their suitability as indicators of environmental contamination, particularly through the determination of heavy metals and antioxidant compounds, which may vary in response to environmental stressors.

Antifungal activity was determined according to CLSI-recommended procedures—agar disc diffusion method—also determining the minimum inhibitory concentration (MIC) of the honey and propolis samples. The evaluation of antifungal activity was specifically selected due to the strong relationship between environmental conditions and fungal contamination. Fungal species are highly sensitive to environmental factors such as humidity, temperature, airborne particulate matter, and soil quality, making them reliable indicators of environmental pollution and ecological imbalance. Moreover, fungi are commonly associated with environmental reservoirs (soil, dust, decaying vegetation), and their presence in honey and propolis reflects the direct influence of environmental exposure.

In contrast to bacteria, which may be more strongly influenced by handling and processing conditions, fungal contamination is more directly linked to environmental sources. Therefore, assessing antifungal activity provides relevant information not only on the bioactive properties of apicultural products but also on the environmental pressures affecting their composition.

To investigate honey quality, the physicochemical parameters were determined, as presented in Tables 2.2.15 – 2.2.18.

Table 2.2.15. Physicochemical parameters of honey samples from North-West and Central Romania.

Sample	**Moisture (%)**	**pH**	**Acidity (meq/kg)**	**Electrical conductivity (mS/cm)**	**a_w**	**Color Pfund (mm)**
S 1	15.78±0.9	4.25±0.5	22.2±4.7	0.51±0.02	0.548±0.035	52
S 2	13.05±0.6	3.88±0.3	11.2±4.6	0.38±0.02	0.536±0.022	52
S 3	14.67±0.7	3.81±0.3	12.0±3.2	0.27±0.00	0.571±0.028	57
S 4	15.04±0.9	3.93±0.2	10.8±2.4	0.34±0.01	0.588±0.034	51
S 5	14.66±0.6	3.27±0.1	10.5±3.0	0.36±0.02	0.571±0.022	53
S 6	13.48±0.8	4.08±0.3	14.5±4.2	0.40±0.01	0.532±0.010	56
S 7	14.54±0.7	4.02±0.1	17.3±3.7	0.47±0.02	0.549±0.021	57
S 8	14.82±0.8	3.74±0.3	19.7±4.4	0.58±0.02	0.545±0.025	49
S 9	13.27±0.5	3.49±0.3	12.2±4.9	0.35±0.01	0.524±0.029	55
S 10	14.45±0.8	3.55±0.2	18.2±4.5	0.61±0.03	0.562±0.028	53
S 11	14.56±0.9	3.18±0.3	16.2±3.8	0.39±0.00	0.555±0.019	50
S 12	15.63±0.8	3.92±0.3	10.5±3.2	0.32±0.02	0.579±0.023	54
S 13	14.29±0.9	3.83±0.2	19.6±3.1	0.53±0.01	0.568±0.024	49

Table 2.2.16. Physicochemical parameters of honey samples from Alba County.

Sample	**Moisture (%)**	**pH**	**Acidity (meq/kg)**	**Electrical conductivity (mS/cm)**	**a_w**	**Color Pfund (mm)**
SS 1	15.78±0.9	4.25±0.5	22.2±4.7	0.51±0.02	0.548±0.035	52
SS 2	12.64±0.6	4.06±0.2	13.8±3.2	0.23±0.03	0.552±0.024	50
SS 3	15.33±1.0	3.62±0.3	9.1±4.0	0.26±0.00	0.602±0.038	58
SS 4	13.75±0.1	3.53±0.1	14.7±3.1	0.28±0.00	0.567±0.041	54
SS 5	13.64±0.8	4.54±0.4	12.9±2.6	0.43±0.02	0.551±0.023	49
SS 6	13.07±0.7	3.35±0.3	13.3±6.5	0.42±0.01	0.584±0.019	56
SS 7	16.01±1.2	3.07±0.2	21.5±5.8	0.45±0.02	0.536±0.015	59
SS 8	13.75±0.7	3.94±0.2	18.6±3.4	0.33±0.01	0.575±0.026	48
SS 9	12.53±0.3	3.71±0.1	15.8±2.7	0.32±0.00	0.539±0.033	56
SS 10	14.96±0.6	3.82±0.3	14.0±9.1	0.54±0.01	0.593±0.025	51
SS 11	13.47±0.8	3.79±0.2	*32.7±4.2*	*0.93±0.01*	0.547±0.017	57
SS 12	16.05±1.4	4.24±0.4	*34.1±3.5*	0.77±0.02	0.536±0.012	55

The physical parameters fall within the limits of Romanian and European standards, with a slight increase in acidity for sub-samples 11 and 12, as well as an increase in electrical conductivity for sub-sample 11.

Table 2.2.17. Chemical parameters of honey samples from North-West and Central Romania

Sample	**Ash g/100g**	**HMF (mg/kg)**	**Phenols (mg GAE/100g)**	**Flavonoids (mg QE/100g)**	**Lead, mg/kg**	**Copper, mg/kg**	**Cadmium, mg/kg**	**RSA-DPPH (%)**
S 1	0.19	0.9±0.2	73.80±0.25	3.51±0.52	ND	ND	ND	27.45
S 2	0.28	1.2±0.4	53.67±0.49	2.18±0.17	ND	0.204±0.006	ND	15.12
S 3	0.31	1.1±0.3	61.38±0.52	3.04±0.26	ND	ND	ND	14.32
S 4	0.27	3.1±0.2	113.03±0.90	7.39±0.11	0.042±0.004	ND	ND	41.27
S 5	0.19	0.1±0.3	128.52±0.98	4.52±0.23	ND	0.118±0.003	0.006±0.002	40.43
S 6	0.21	4.7±0.2	39.41±0.29	2.49±0.09	0.120±0.005	ND	ND	18.16
S 7	0.25	2.5±0.4	93.09±1.12	5.19±0.15	ND	0.107±0.004	0.002±0.001	42.13
S 8	0.22	1.7±0.2	81.60±0.84	2.44±0.13	ND	ND	ND	33.65
S 9	0.19	0.4±0.2	59.45±0.87	1.92±0.04	0.051±0.003	ND	ND	13.16
S 10	0.18	1.2±0.3	72.74±0.51	2.06±0.51	ND	0.214±0.005	ND	16.13
S 11	0.24	2.3±0.3	66.07±0.44	3.23±0.08	0.106±0.008	ND	ND	37.2
S 12	0.31	5.4±0.4	49.24±0.63	3.69±0.03	ND	ND	0.004±0.001	25.84
S 13	0.23	0.2±0.1	87.49±1.21	6.43±0.16	ND	0.169±0.007	ND	48.09

RSA (%) – Free Radical Scavenging Activity; ND – not detectable (<0.05 mg/kg Cu; <0.001 mg/kg Cd; <0.01 mg/kg Pb).

Table 2.2.18. Chemical parameters of honey samples from Alba County

Sample	**Ash g/100g**	**HMF (mg/kg)**	**Phenols (mg GAE/100g)**	**Flavonoids (mg QE/100g)**	**Lead, mg/kg**	**Copper, mg/kg**	**Cadmium, mg/kg**	**RSA-DPPH (%)**
SS 1	0.19	0.9±0.2	73.80±0.25	3.51±0.52	ND	ND	ND	27.45
SS 2	0.34	4.0±0.3	100.72±1.20	3.63±0.20	0.334±0.021	ND	ND	15.32
SS 3	0.19	2.8±0.4	126.53±1.10	6.17±0.17	0.176±0.013	ND	ND	30.48
SS 4	0.22	0.4±0.1	111.68±0.92	7.06±0.03	0.471±0.043	ND	ND	26.79
SS 5	0.17	0.2±0.1	100.81±0.54	5.11±0.05	ND	0.125±0.015	ND	23.46
SS 6	0.18	0.9±0.2	99.55±0.41	5.46±0.15	ND	ND	0.002±0.001	22.39
SS 7	0.11	2.3±0.1	85.47±0.59	5.37±0.29	ND	0.138±0.011	0.003±0.001	23.04
SS 8	0.20	1.6±0.3	66.84±0.22	3.30±0.18	ND	0.100±0.008	0.007±0.001	15.5
SS 9	0.18	0.5±0.2	42.10±0.11	2.10±0.03	0.062±0.03	ND	ND	9.27
SS 10	0.17	3.1±0.2	67.52±0.63	1.84±0.11	ND	0.496±0.081	0.011±0.002	11.46
SS 11	0.21	0.8±0.3	74.06±0.48	2.68±0.04	ND	0.411±0.042	ND	20.55
SS 12	0.32	0.7±0.3	58.12±0.87	2.96±0.80	ND	ND	0.005±0.001	18.83

RSA (%) – Free Radical Scavenging Activity; ND – not detectable (<0.05 mg/kg Cu; <0.001 mg/kg Cd; <0.01 mg/kg Pb).

The total content of phenolic compounds and flavonoids in the honey samples ranged between 39.41 and 128.52 mg GAE/100g for phenols, and between 1.84 and 7.39 mg QE/100g for flavonoids. All polyfloral honey samples collected from beekeepers show chemical parameter values within the reference ranges established by legislation [91,92]. Exceptions are sub-samples SS2 and SS4, which have high lead content, nearly twice the maximum permitted limit.

The elevated lead concentrations detected in these samples highlight the direct impact of local anthropogenic activities on environmental quality. Such contamination is likely the result of long-term industrial emissions and transport-related pollution, confirming the role of honey as a sensitive bioindicator of heavy metal accumulation in the environment.

The results of the physicochemical determinations of the propolis samples are presented in Tables 2.2.19–2.2.20.

Table 2.2.19. Characterization of propolis samples from North-West and Central Romania

Sample	Moisture (%)	Ash (g/100g)	Wax (%)	Phenols (mg GAE/g)	Flavonoids (mg QE/g)	DPPH-RSA (%)
S1	8.04±0.12	3.14±0.07	25.84±0.57	189.4±5.82	84.31±0.09	16.44
S2	6.34±0.63	2.85±0.08	37.18±0.81	180.8±4.54	78.26±0.07	15.21
S3	7.64±0.27	2.96±0.04	40.56±1.06	172.9±3.25	78.55±0.08	15.08
S4	9.11±0.89	3.15±0.06	33.22±0.38	189.5±4.83	87.84±0.11	16.79
S5	7.56±0.28	3.28±0.09	46.33±1.05	193.4±7.22	88.06±0.08	17.27
S6	4.81±0.80	2.55±0.05	37.41±0.58	129.6±3.58	65.59±0.09	11.75
S7	6.52±0.46	2.73±0.03	31.19±0.71	184.3±6.04	82.27±0.25	15.04
S8	7.32±0.54	2.69±0.04	32.52±0.44	152.2±6.80	70.10±0.16	13.50
S9	5.43±0.82	3.08±0.03	28.92±0.67	157.1±5.57	74.35±0.36	14.43
S10	7.05±0.37	2.62±0.02	34.24±0.96	186.9±6.88	77.33±0.21	16.28
S11	6.27±0.91	3.10±0.05	39.47±1.05	144.2±5.51	67.41±0.14	12.66
S12	7.63±0.57	2.42±0.08	27.65±0.73	153.5±4.78	82.38±0.27	14.57
S13	8.18±0.62	2.67±0.07	38.15±0.92	144.0±2.09	81.09±0.98	13.92

GAE – gallic acid equivalents; QE – quercetin equivalents; RSA – free radical scavenging activity.

Table 2.2.20. Characterization of propolis samples from Alba County

Sample	Moisture (%)	Ash (g/100g)	Wax (%)	Phenols (mg GAE/g)	Flavonoids (mg QE/g)	DPPH-RSA (%)
SS1	8.04±0.12	3.14±0.07	25.84±0.57	189.4±5.82	84.31±0.09	16.44
SS2	7.46±0.06	3.05±0.07	34.07±1.12	172.2±6.14	70.37±0.03	14.54
SS3	7.38±0.39	2.88±0.08	32.71±0.71	158.8±5.27	86.48±0.12	15.81
SS4	8.21±0.64	3.22±0.14	30.15±0.57	203.3±7.28	90.54±0.06	17.22
SS5	6.78±0.17	3.12±0.05	28.92±0.68	181.5±6.10	82.92±0.07	18.19
SS6	8.06±0.60	2.24±0.06	31.64±0.27	134.7±4.09	71.24±0.02	13.30
SS7	7.86±0.68	3.20±0.09	29.38±0.63	190.6±5.26	80.19±0.01	14.41
SS8	7.15±0.82	2.49±0.03	30.79±1.01	169.1±8.39	69.23±0.04	12.15
SS9	6.70±0.93	2.77±0.02	27.67±0.64	135.9±7.42	79.89±0.44	13.27
SS10	7.65±0.37	2.72±0.04	31.05±0.91	133.7±6.97	61.56±0.59	10.29
SS11	6.99±0.70	3.32±0.08	31.26±0.67	131.5±3.01	73.15±0.41	11.38
SS12	7.72±0.44	2.89±0.07	33.45±0.85	140.4±5.31	73.97±0.29	14.07

GAE – gallic acid equivalents; QE – quercetin equivalents; RSA – free radical scavenging activity.

For the propolis samples from North-West and Central Romania, as well as from Alba County, the identification reactions for flavones and aromatic acids were positive. We can consider the presence of these compounds in all investigated samples, together with the phenolic and flavonoid content presented in Tables 2.2.19 and 2.2.20. The total content of phenolic compounds in the propolis samples ranged between 129.6 and 203.3 mg GAE/g, while the total flavonoid content ranged between 61.56 and 90.54 mg QE/g.

Variations in phenolic and flavonoid content among samples may also be influenced by environmental factors, including soil composition, plant diversity, and exposure to pollutants, which can induce stress responses in plants and subsequently affect nectar composition. Propolis, being collected from plant resins, may reflect environmental contamination even more directly than honey, as it incorporates substances from plant surfaces exposed to atmospheric pollutants.

Antifungal activity of honey and aqueous propolis extracts

Tables 2.2.21 – 2.2.24 present the results regarding the antifungal effect of the honey and propolis samples. As observed in Tables 2.2.21 and 2.2.22, all honey samples showed antifungal activity against all tested strains. The diameters of the inhibition zones ranged from 7 to 12 mm. These results suggest that the phenolic and flavonoid content, potentially influenced by pollution in the floral environment, can affect the bioactivity of bee products. Since the concentration of these bioactive compounds can be influenced by environmental stress conditions, including pollution, the antifungal activity of honey and propolis may indirectly reflect the ecological status of the collection area.The artificial honey sample had no effect on any of the strains.

Table 2.2.21. Diameters of inhibition zones against fungal strains for honey samples from North-West and Central Romania

No.	**Strain**	**Diameter of inhibition zone for the sample (mm)**												
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
1	*A. niger*	9	9	9	10	10	9	8	9	8	9	8	9	10
2	*A. flavus*	9	9	9	10	9	9	8	9	9	9	9	9	10
3	*C. albicans*	8	9	8	9	10	8	8	8	7	8	7	8	9
4	*P. chrysogenum*	10	12	11	12	11	10	9	10	9	11	9	11	12
5	*R. stolonifer*	9	12	11	12	10	8	9	9	9	12	9	11	11
6	*F. oxysporum*	8	10	9	11	11	9	8	10	7	10	8	9	11

Table 2.2.22. Diameters of inhibition zones against fungal strains for honey samples from Alba County.

No.	**Strain**	**Diameter of inhibition zone for the sample (mm)**											
		SS 1	**SS 2**	**SS 3**	**SS 4**	**SS 5**	**SS 6**	**SS 7**	**SS 8**	**SS 9**	**SS 10**	**SS 11**	**SS 12**
1	*A. niger*	9	8	9	9	10	8	9	10	9	11	9	10
2	*A. flavus*	9	10	9	9	11	9	9	8	10	10	9	9
3	*C. albicans*	8	9	8	8	9	9	10	9	8	8	9	10
4	*P. chrysogenum*	10	11	10	12	10	12	9	11	8	12	10	9
5	*R. stolonifer*	9	11	12	10	10	9	8	10	9	11	9	10
6	*F. oxysporum*	8	9	10	9	11	10	9	9	8	9	7	8

As observed in Tables 2.2.21 and 2.2.22, the strains most sensitive to the effect of honey were *P. chrysogenum* and *R. stolonifer*, followed by *Aspergillus* spp. Samples S4, S5, and S13 were the most effective regarding antifungal activity, showing the largest inhibition zone diameters.

Tables 2.2.25 and 2.2.26 present the diameters of inhibition zones produced by aqueous propolis extracts. All samples showed antifungal activity, with diameters ranging from 15 to 28 mm.

Table 2.2.25. Diameters of inhibition zones against fungal strains for aqueous propolis extract samples from North-West and Central Romania

No.	**Strain**	**Diameter of inhibition zone for the sample (mm)**												
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**
1	*A. niger*	24	21	19	23	26	17	18	16	22	15	19	18	16
2	*A. flavus*	26	20	17	25	27	15	18	15	20	16	17	20	18
3	*C. albicans*	22	19	18	22	22	19	21	20	19	21	18	20	19
4	*P. chrysogenum*	27	24	22	26	27	23	24	25	23	25	22	26	24
5	*R. stolonifer*	24	22	20	26	25	21	23	22	22	24	20	23	21
6	*F. oxysporum*	28	23	24	25	26	23	22	27	21	25	23	26	22

Table 2.2.26. Diameters of inhibition zones against fungal strains for aqueous propolis extract samples from Alba County.

No.	**Strain**	**Diameter of inhibition zone for the sample (mm)**											
		SS 1	**SS 2**	**SS 3**	**SS 4**	**SS 5**	**SS 6**	**SS 7**	**SS 8**	**SS 9**	**SS 10**	**SS 11**	**SS 12**
1	*A. niger*	24	20	18	24	26	19	25	17	21	16	19	20
2	*A. flavus*	26	22	16	25	27	17	20	15	21	18	18	19
3	*C. albicans*	22	18	20	21	23	17	22	21	18	20	16	20
4	*P. chrysogenum*	27	23	22	25	27	21	27	24	25	26	20	23
5	*R. stolonifer*	24	22	21	26	25	19	25	23	20	22	21	19
6	*F. oxysporum*	28	25	25	27	25	21	24	26	22	25	24	24

As observed in Tables 2.2.25 and 2.2.26, the diameters of the inhibition zones were larger for aqueous propolis extracts compared to honey, with *P. chrysogenum* and *F. oxysporum* being the most sensitive fungal strains to the effect of propolis. Aqueous propolis extracts S4 and S5 (as in the case of honey) and S1 exhibited the strongest antifungal effect [95].

The differences in inhibition zone sizes between honey and propolis can be explained by the different compositions of these products and the different mechanisms through which they induce antimicrobial effects [96]. In honey, antimicrobial activity is correlated with the amount of hydrogen peroxide present, as well as the presence of additional antibacterial compounds derived from the nectar source. The antimicrobial effect of propolis can be attributed to the presence of quercetin, which has strong antimicrobial activity, as well as the high concentration of phenols and flavonoids [97,98].

All honey samples showed an inhibitory effect at dilutions up to 1/16 (w/v) for one or more strains. The only exception was sample S11, which had no effect at the 1/16 dilution on any of the strains. Some samples exhibited antifungal inhibition even at the 1/32 (w/v) dilution, but not against all strains.

Regarding the propolis samples, with the exception of samples S13 and SS6, all showed antifungal activity at concentrations up to 6.25 mg/mL, though not for all strains. Many samples exhibited an inhibitory effect at 3.12 mg/mL on certain strains, while samples S1, S5, and SS7 had activity against *F. oxysporum* (S1) and *P. chrysogenum* (S5 and SS7) even at concentrations of 1.56 mg/mL.

Two-way analysis of variance (ANOVA) tested the simultaneous interaction of two independent variables: Romanian honey and propolis extracts, and the diameter of the inhibition zone for the studied strains.

For the 13 honey (H) and propolis (P) samples collected from North-West and Central Romania, the calculation of variances caused by each independent parameter, including residual dispersion due to random factors, produced the following results:

- $S_{1,H} = 6992$, $S_{2,H} = 4921.66$, $S_{3,H} = 6915.07$, $S_{4,H} = 6869.53$
- $S_{1,P} = 37636$, $S_{2,P} = 37090$, $S_{3,P} = 37140.77$, $S_{4,P} = 36790.21$

The results of the two-way ANOVA are presented in Table 2.2.27.

Table 2.2.27. Two-way analysis of variance for samples from North-West and Central Romania

Sum of variance of inhibition zone diameters		**Sum of squares**	**Degrees of freedom (ν)**	**Variance**	**$F_{calculated}$**	**$F_{0.05}^*$**
Between honey samples	(S_2–S_4)	52.12	m-1= 5	$s_{1.H}^2$=10.42	10.42	2.37
Between propolis extracts		299.79		$s_{1.P}^2$=18.40	18.40	
Between strains, honey (H)	(S_3-S_4)	45.53	n-1= 12	$s_{2.H}^2$=3.79	9.18	1.92
Between strains, propolis (P)		350.56		$s_{2.P}^2$=8.96	8.96	
Residual, honey (H)	Sr	24.79	(m-1)(n-1)= 60	$s_{r.H}^2$=0.41	-	-
Residual, propolis (P)		195.43		$s_{r.P}^2$=3.25	-	-

**critical F at 0.05 significance level*

Since there are 5 degrees of freedom for the honey and propolis samples (ν_1) and 12 for the tested strains (ν_2), and the calculated F values, $F_{col,H} = 10.42$ and $F_{col,P} = 18.40$, are both greater than $F_{0.05} = 2.37$, the null hypothesis that the column means are equal was rejected. It was concluded that the honey and propolis samples from North-West and Central Romania influence the diameter of the inhibition zones.

Additionally, since $F_{rand,H} = 9.18$ and $F_{rand,P} = 8.96$ are also both greater than $F_{0.05} = 1.92$, the null hypothesis that the row means are equal was rejected, concluding that the type of strain influenced the inhibition zones. The significance level was $\alpha = 0.05$.

For the 12 honey (H) and propolis (P) samples collected from Alba County, the sum of squares from ANOVA are: $S_{1,H} = 6453$, $S_{2,H} = 6381.83$, $S_{3,H} = 6386.58$, $S_{4,H} = 6365.68$, respectively $S_{1,P} = 35608$, $S_{2,P} = 35171$, $S_{3,P} = 35098.83$, $S_{4,P} = 34848$.

The results of the two-way ANOVA are presented in Table 2.2.28.

Table 2.2.28. Two-way analysis of variance for samples from Alba County.

Sum of variance of inhibition zone diameters	**-**	**Sum of squares**	**Degrees of freedom (v)**	**Variance**	**$F_{calculated}$**	**$F_{0.05}^*$**
Between honey samples	(S_2–S_4)	16.15	m-1= 5	$s_{1.H}^2$=3.23	3.53	2.38
Between propolis extracts		323		$s_{1.P}^2$=64.60	19.08	
Between strains, honey (H)	(S_3-S_4)	20.90	n-1= 11	$s_{2.H}^2$=1.90	2.07	1.97
Between strains, propolis (P)		250.83		$s_{2.P}^2$=22.80	6.73	
Residual, honey (H)	Sr	50.26	(m-1)(n-1)= 55	$s_{r.H}^2$=0.91	-	-
Residual, propolis (P)		186.16		$s_{r.P}^2$=3.38	-	-

**critical F at 0.05 significance level*

For 5 degrees of freedom in the case of honey and propolis samples (ν_1) and 11 for the tested strains (ν_2), and with calculated F values $F_{col,H} = 3.53$ and $F_{col,P} = 19.08$, both greater than $F_{0.05} = 2.38$, the null hypothesis was rejected. There is sufficient evidence to support the claim that the origin of honey and propolis samples from Alba County influenced the diameter of the inhibition zone.

Furthermore, since $F_{row,H} = 2.07$ and $F_{row,P} = 6.73$ are also greater than $F_{0.05} = 1.97$, the hypothesis that the row means are equal was rejected, leading to the conclusion that the type of strain affected the inhibition zones. The significance level was $\alpha = 0.05$.

In Table 2.2.29, the values of the Pearson correlation coefficients show the strength of the linear association between the diameter of the inhibition zone and the concentration of flavonoids and phenols in honey and propolis, respectively, for all studied microorganisms.

Table 2.2.29. Pearson correlation coefficients between the diameter of the inhibition zone and the flavonoid/phenolic content for the studied microbial strains.

Microbial strain	**Flavonoids**				**Phenols**			
	Honey		**Propolis**		**Honey**		**Propolis**	
	N-V/ Center	**Alba**	**N-V/ Center**	**Alba**	**N-V/ Center**	**Alba**	**N-V/ Center**	**Alba**
A. niger	0.545	-0.395	0.549	0.699	0.513	-0.392	0.551	*0.711*
A. flavus	0.481	-0.132	0.789	0.541	0.213	0.045	0.644	0.618
C. albicans	0.509	-0.004	0.696	0.415	0.630	-0.129	*0.710*	*0.732*
P. chrysogenum	0.388	0.279	0.697	0.250	0.216	0.461	0.559	0.592
R. stolonifer	0.266	0.073	0.728	0.465	0.172	0.42	*0.726*	*0.888*
F. oxisforumn	0.489	0.599	0.313	0.317	0.531	0.650	0.362	0.694

The correlations between the diameter of the inhibition zone and flavonoids were weak to moderate, generally indicating a positive linear relationship between the two variables. The strength of the association is lower in the case of honey samples compared to propolis extracts, which even show a strong positive correlation. The correlations between the diameter of the inhibition zone and phenols were similar, suggesting a moderate to strong positive linear relationship. This demonstrates that the surrounding environment, including local pollution, influences both the chemical composition and biological properties of honey and propolis.

Due to the variability of physicochemical parameters and composition found in bee products worldwide, each sample must be analyzed and classified according to its chemical profile. Due to the diversity of flora used by bees for the production of honey and propolis, specific to each geographical area, the number of constituents that can be quantified and identified in the samples varies from case to case. [99-101].

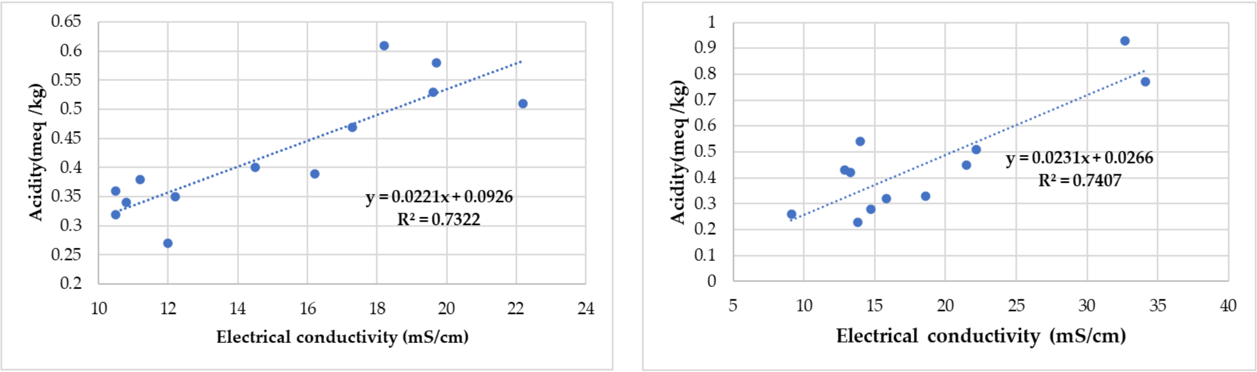
Baloš et al. reported acidity values for polyfloral honey ranging between 5.0 and 26.0 [102], while for samples from Romania, values ranged between 9.1 and 34.1 meq/kg he maximum moisture content is below 20% (according to the Codex Alimentarius), and according to Baloš et al. [103] it varies depending on the year between 15.6 and 19.6% for polyfloral honey. For the samples analyzed in the present study, the results range between 12.53 and 16.01%.

A high moisture content in honey can lead to fermentation during storage [104]. The pH value of the honey samples ranged between 3.07 and 4.54 and, together with the free acidity of honey, helps prevent the growth of various microorganisms. Parameter values were exceeded only when processing or storage was inadequate, including in cases of honey fermentation [105-107].

Water activity is an important factor that provides information about food stability by preventing or limiting microbial growth. Osmotolerant yeasts are capable of growing at a minimum water activity of 0.6 [104]. The water activity of the honey in the investigated samples ranges between 0.524 and 0.602. This range indicates an environment unsuitable for most bacteria. The results regarding the HMF content in the honey samples are generally low.

The electrical conductivity values for Romanian honey range between 0.23 and 0.93 mS/cm, while Baloš et al. [102] reported values of 0.09–0.74 mS/cm for honey from Serbia. They also established a correlation between acidity and electrical conductivity in the case of polyfloral honey.

Figures 2.2.10 a) and b) presents the correlation between acidity and electrical conductivity for the polyfloral honey samples collected for the study.



a) from north-west and central Romania b) from Alba County

Figure 2.2.10. Correlation between acidity and electrical conductivity for polyfloral honey samples

Moreover, in the case of Romanian honey, a significant correlation was established between free acidity and electrical conductivity for the polyfloral honey samples ($R^2 = 0.73$ and $R^2 = 0.74$). High conductivity values indicate a high mineral content. In polluted environments, increased electrical conductivity may also be associated with elevated levels of inorganic contaminants, including heavy metals, further supporting its relevance as an environmental indicator.

According to STAS 784/3-2009 [90], the key physicochemical parameters and recommended limits for honey marketed in Romania are as follows:

- Moisture: ≤ 20%
- Acidity: ≤ 4 ml NaOH 1N per 100 g
- Reducing sugars (expressed as invert sugar): ≥ 70%
- Easily hydrolyzable sugars (expressed as sucrose): ≤ 5%
- Diastase activity index: ≥ 6.5

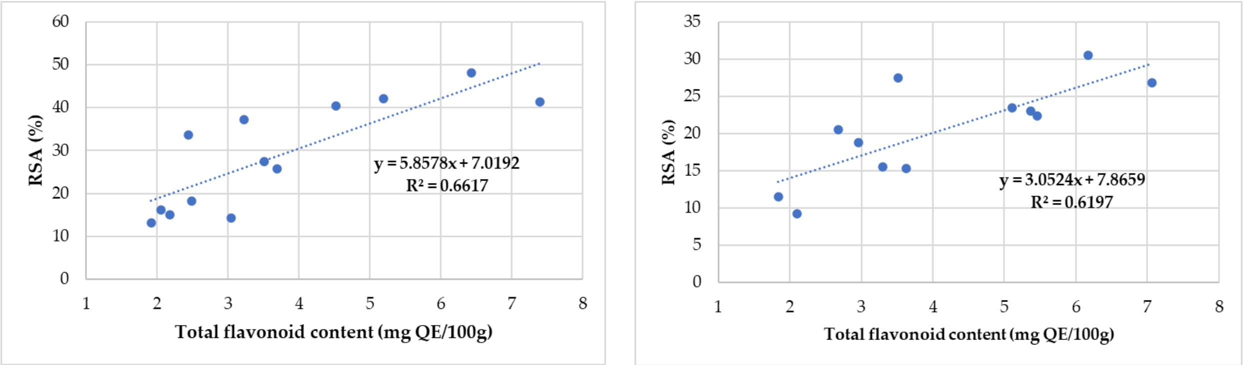
- Ash content: ≤ 0.5%
- Specific pollen grains (proportion of total pollen grains analyzed): ≥ 25%
- Hydroxymethylfurfural (HMF): ≤ 1.5 mg/100 g, although for honey packaged in jars, HMF may be up to 4 mg/100 g
- Color index (Pfund scale, mm): ≤ 18
- Insoluble matter: ≤ 0.1%
- Adulteration agents (including artificial invert sugar, industrial glucose, or substances derived from the hydrolysis of starch, gelatin, gums, cereal products, colorants, synthetic sweeteners, etc.): not permitted.

European legislation sets higher, more permissive limits [108]. For heavy metals, the values are identical at both European level (European Commission Honey Directive) and international level (Codex Alimentarius Standard of the FAO/WHO). Specifically:

- Copper (Cu): maximum 0.50 mg/kg
- Cadmium (Cd): maximum 0.02 (0.20) mg/kg
- Lead (Pb): maximum 0.20 mg/kg

In general, elevated heavy metal levels in honey may originate from industrial activities or from intense vehicular traffic, which pollute the atmosphere. Metals are deposited on plants through the air and ultimately accumulate in honey. In our study, samples SS2 and SS4, which had high lead content, were collected from the localities of Zlatna and Teiuș. Non-ferrous metals have been processed in Zlatna for a long time, while Teiuș is a major railway junction. This highlights the impact of industrial activities and traffic-related pollution on the environment and, consequently, on the quality of bee products. Heavy metals (cadmium and lead) are used as bioindicators of honey contamination [109]. In their studies, Lianda et al. [110] found that antioxidant activities and the total phenolic content are strongly correlated.

The correlation of DPPH radical scavenging activity (RSA) with total flavonoid content for honey and propolis across all samples is shown in Figures 2.2.11 a) and b).

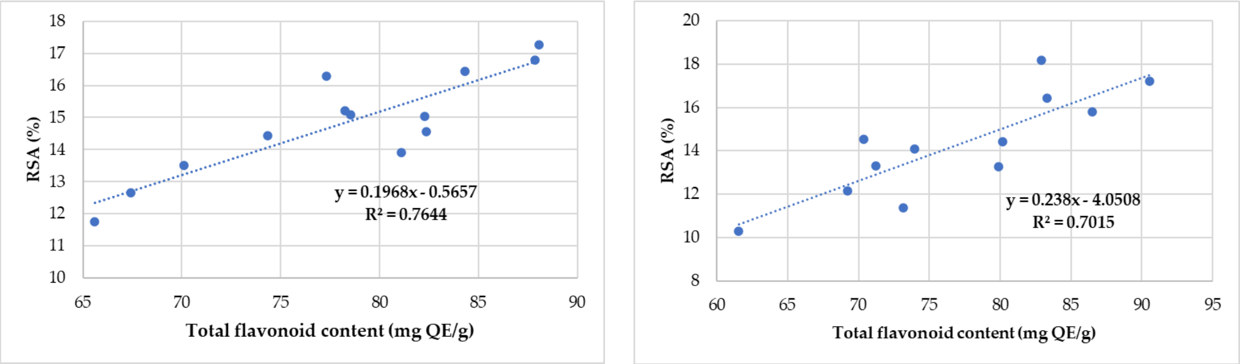


a) from north-west and central Romania b) from Alba County

Figure 2.2.11. Correlation between antioxidant activity and total flavonoid content in honey samples

The lowest radical scavenging activity (RSA) in the honey samples was observed in SS9, collected from Feneș, Alba County, while the highest was recorded in SS13 from Baia de Arieș. Sample SS10 from Cugir exhibited the lowest flavonoid content (1.84 mg EQ/100 g), whereas the highest was found in S4 from Bistrița-Năsăud (7.39 mg EQ/100 g). A moderate positive relationship was observed between antioxidant activity and total flavonoid content in honey samples, with R^2 values ranging from 0.62 to 0.66.

Figures 2.2.12 a) and b) illustrates the antioxidant activity versus flavonoid content in the propolis samples collected in this study.



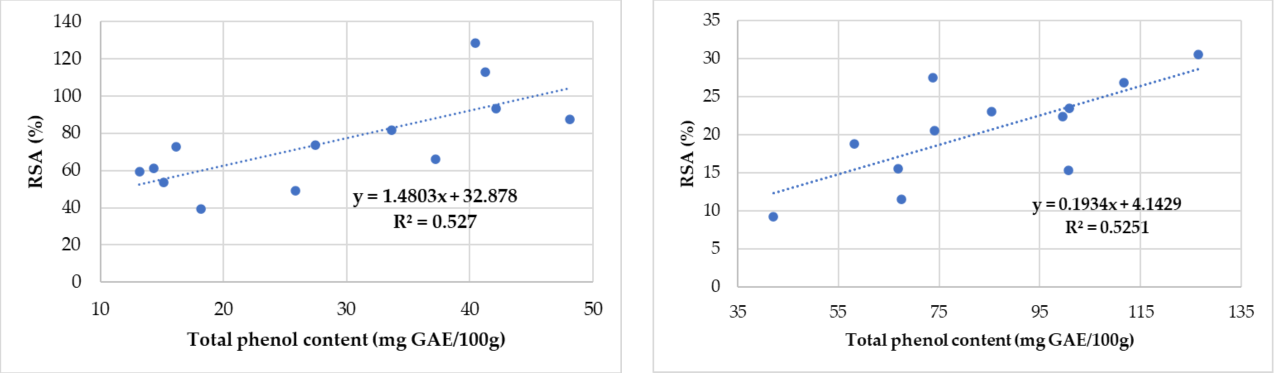
a) from north-west and central Romania b) from Alba County

Figure 2.2.12. Correlation between antioxidant activity and total flavonoid content in collected propolis samples

The antioxidant activity values ranged from 10.29% (SS10 – Cugir) to 18.19% (SS5 – Şona), while flavonoid content ranged from 61.56 mg QE/g (SS10 – Cugir) to 90.54 mg QE/g (SS4 – Teiuş). A significant positive relationship was also observed between antioxidant activity and total flavonoid content in propolis samples from north-west and central Romania, including Alba County.

Based on the correlation coefficients, it can be suggested that flavonoid compounds are largely responsible for the antioxidant activity of the selected honey samples from Romania.

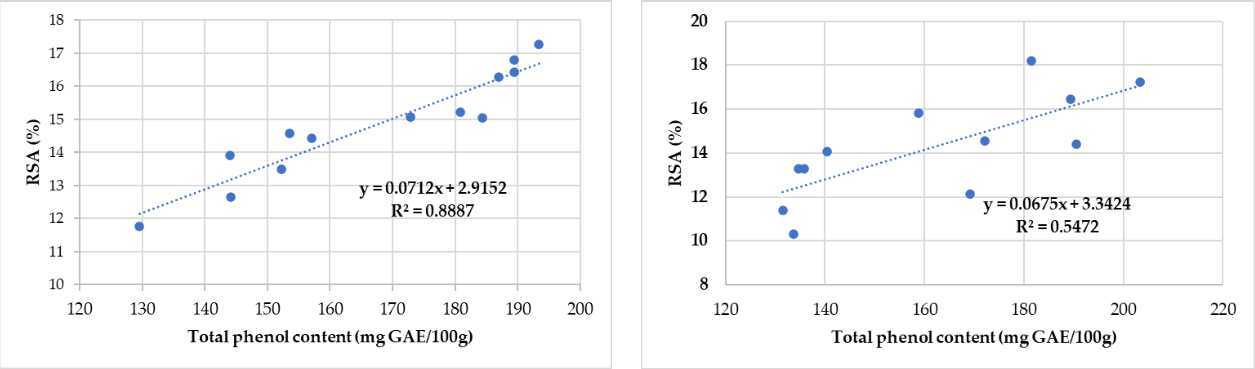
The correlation between total phenolic content and antioxidant activity for honey and propolis is shown in Figures 2.2.13 a) and b), and Figures 2.2.14 a) and b), respectively.



a) from north-west and central Romania b) from Alba County

Figure 2.2.13. Correlation between antioxidant activity and total phenolic content in collected honey samples

A weak correlation was observed between RSA and phenolic content, with values ranging from 39.14 GAE/100 g (S6 – Cluj) to 128.52 GAE/100 g (S5 – Caraş-Severin). The correlation coefficient ($R^2 = 0.53$) indicates that the variables—antioxidant activity and total phenolic content in honey samples—can be considered moderately correlated.



a) from north-west and central Romania b) from Alba County

Figure 2.2.14. Correlation between antioxidant activity and total phenolic content in collected propolis samples

Correlation coefficients ranging from 0.55 to 0.89 indicate a fairly strong positive relationship between DPPH radical scavenging activity (RSA) and total phenolic content in the examined propolis samples.

These results suggest that the antioxidant activity of different honey and propolis samples depends largely on their total phenolic and flavonoid content. Other studies have also reported a linear correlation between total phenolic and flavonoid content and the antioxidant activity of honey and honey-derived products [111-114].

The observed relationships suggest that environmental conditions, including pollution pressure, may modulate the synthesis of antioxidant compounds in plants, which are subsequently transferred into bee products.

Regarding the antifungal effect of honey, it was observed that all samples exhibited activity against the selected strains. The inhibition zone diameters varied depending on the strain and the honey source, ranging from 7 to 12 mm. The most sensitive strains to the antifungal activity of honey were *Penicillium chrysogenum* and *Rhizopus stolonifer*. Analysis of the minimum inhibitory concentration (MIC) showed that some honey samples retained inhibitory effects even at dilutions of 1/32. Samples S4, S5, and S13 demonstrated the strongest antifungal activity. Honey from this north-west and central region of Romania has a distinct chemical composition and physicochemical properties, as described in our previous studies [49], and exhibits a strong antimicrobial effect, consistent with other studies reporting the antibacterial activity of honey and propolis from this region [93,115].

Our results confirm that honey can exhibit antifungal activity against strains such as *Aspergillus* spp., *Candida* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp., as has been demonstrated in previous studies on honey samples from various geographic regions, including Nigeria, Pakistan, and India [116-118].

All aqueous propolis extracts exhibited antifungal activity, with inhibition zone diameters ranging from 15 to 28 mm. The most sensitive strains to propolis activity were *P. chrysogenum* and *Fusarium oxysporum*. The strongest antifungal effects were observed in samples S1, S4, and S5, with the latter two originating from the same locations as honey samples S4 and S5, which also showed the highest antifungal activity. These results confirm that geographic origin and botanical source can influence the antimicrobial properties of both honey and propolis. Similarly, our study supports previous findings on the antifungal effects of propolis samples against fungal strains [94,119].

In the present study, we evaluated the antifungal effect of aqueous propolis extracts, rather than the more commonly used ethanolic extracts, to avoid the influence of ethanol on the inhibition zone diameters. Nevertheless, the aqueous propolis extracts exhibited positive antifungal activity even at a low concentration of 1.56 mg/mL in some samples. The research demonstrated the ability of both honey and propolis samples to inhibit the growth of multiple fungal species, confirming their antifungal properties and highlighting their potential as natural antifungal agents.

Conclusions

This study enabled the analysis of honey and propolis samples collected from beekeepers in north-west and central Romania, assessing their chemical and physical characteristics as well as heavy metal content—specifically lead, copper, and cadmium—as indicators of sample quality and environmental pollution. All investigated parameters fell within the limits imposed by legislation, with the exception of lead in the localities of Zlatna and Teiuș, which are considered industrialized areas or regions with heavy traffic.

The phenolic and flavonoid content in honey samples varied according to the collection area, although no major differences were observed between samples from Alba County compared to those from the central and north-west regions of the country. For propolis, samples from north-west and central Romania showed higher bioactive compound content compared to those from Alba County.

All honey and propolis samples exhibited antifungal activity, which varied depending on the geographic origin and the type of fungal strain tested. The most sensitive strains were *Penicillium chrysogenum*, *Rhizopus stolonifer*, and *Fusarium oxysporum*. A significant correlation was observed between free acidity and electrical conductivity in polyfloral honey, as well as between antioxidant activity and total phenolic and flavonoid content.

Statistical analysis (two-way ANOVA for antifungal activity) demonstrated a link between the geographic origin of honey and propolis samples, the microbial strains used, and antifungal activity. Variance analysis indicated that both the type of strain and the geographic origin of the honey and propolis significantly influenced the inhibition zone diameters. Therefore, honey and propolis can serve as natural bioindicators for monitoring environmental pollution, providing insights relevant to human health and ecosystem conservation.

The findings support the use of honey and propolis as reliable bioindicators of environmental pollution. Their chemical composition and biological activity reflect both natural and anthropogenic influences, making them valuable tools for monitoring ecosystem health and assessing the impact of pollution on food quality and safety.

2.2.3. Honey as a Bioindicator of Environmental Pollution in Mining and Protected Areas

The study aims to monitor the trace metal content in honey and to highlight its variation depending on the environmental characteristics of the area where the apiaries are located. This is achieved by comparing regions affected by mining activities with those situated in protected natural areas. The objective is to correlate the level of contamination with the physicochemical properties and antimicrobial activity of bee products, in order to emphasize the potential of honey to act as a bioindicator of environmental pollution and quality [120].

Table 2.2.30 presents the identification of honey samples collected from areas located near former mining sites in Romania, highlighting their geographical location and the types of mineral resources associated with each area.

Table 2.2.30. Identification of Honey Samples Collected from Areas Associated with Mining-Related Environmental Pollution in Romania

Sample	County	Area	Type of Mineral Resource
S1	Alba	Zlatna – Haneș Mining Site	Gold, silver, copper, lead, and zinc
S2	Hunedoara	Certeju de Sus – Mining Site	Gold, silver, copper, lead, and zinc
S3	Bihor	Băița – Mining Site	Gold, silver, copper, lead, zinc, and uranium
S4	Harghita	Bălan – Mining Site	Copper, gold, silver, lead, and zinc

Table 2.2.31 includes the identification of honey samples collected from protected natural areas in Romania, indicating the administrative location and the Sites of Community Importance (SCI) or Special Protection Areas (SPA) in which each apiary area is situated.

Table 2.2.31. Identification of Honey Samples Collected from Protected Areas in Romania

Sample	County	Area	Protected biodiversity area
SS1	Alba	Almașu Mare Locality	ROSCI0029 Glodului, Cibului and Măzii Gorges
SS2	Hunedoara	Balșa Locality	ROSPA0132 Metaliferi Mountains
SS3	Bihor	Criștioru de Jos Locality	ROSCI0324 Bihor Mountains
SS4	Harghita	Bălan Town	ROSAC0027 Bicaz–Hășmaș Gorges

Table 2.2.32 presents the results of the physicochemical evaluation of the honey samples, highlighting key parameters such as moisture content, water activity, phenolic and flavonoid content, HMF, and sugar composition.

Table 2.2.32. Physicochemical Evaluation of Honey Samples.

Sample	**H, %**	**a_w**	**Phenols, mg GAE/100 g**	**Flavonoids, mg QE/100 g**	**Sucrose, %**	**Invert Sugars, %**	**HMF, mg/100 g**
S1	16.53 ± 0.52	0.583 ± 0.011	70.39 ± 0.47	4.11 ± 0.20	2.29 ± 0.33	72.23 ± 1.39	0.59 ± 0.10
SS1	17.18 ± 0.18	0.547 ± 0.013	82.14 ± 0.61	4.26 ± 0.18	2.84 ± 0.30	79.99 ± 2.80	0.76 ± 0.14
S2	16.42 ± 0.39	0.528 ± 0.026	63.84 ± 0.30	2.30 ± 0.11	2.88 ± 0.28	78.96 ± 2.44	1.10 ± 0.23
SS2	17.09 ± 0.31	0.569 ± 0.018	72.67 ± 0.52	2.67 ± 0.04	2.68 ± 0.36	78.44 ± 1.05	0.92 ± 0.20
S3	16.74 ± 0.20	0.571 ± 0.023	84.91 ± 0.86	4.08 ± 0.23	3.68 ± 0.41	79.11 ± 2.67	0.80 ± 0.16
SS3	16.90 ± 0.44	0.535 ± 0.009	96.49 ± 1.03	5.45 ± 0.17	3.65 ± 0.77	78.47 ± 2.53	1.03 ± 0.12
S4	18.03 ± 0.57	0.592 ± 0.014	59.73 ± 0.26	3.08 ± 0.10	1.89 ± 0.20	80.97 ± 3.31	0.68 ± 0.10
SS4	17.25 ± 0.26	0.560 ± 0.015	63.20 ± 0.44	3.31 ± 0.15	3.16 ± 0.63	79.15 ± 2.02	0.49 ± 0.15

Abbreviations: H — moisture content; aw — water activity; HMF — hydroxymethylfurfural content. Note: All values are expressed as mean ± standard deviation (n = 3).

Physicochemical analyses revealed a moisture content ranging from 16.42% to 18.03%, complying with the maximum limit of 20% established by Directive 2001/110/EC [91]. Water activity values ranged between 0.528 and 0.592. Total phenolic content varied from 59.73 to 96.49 mg GAE/100 g, and flavonoid content ranged between 2.30 and 5.45 mg QE/100 g. Sugar content was between 1.89% and 3.68%, while invert sugar ranged from 72.23% to 80.97%. HMF content was between 0.49 and 1.10 mg/kg across all analyzed samples.

Table 2.2.33 presents the concentrations of trace metals detected in the honey samples, providing an insight into potential environmental contamination.

Table 2.2.33. Trace metals identified in honey samples.

Sample	**Pb, mg/kg**	**Cd, mg/kg**	**Fe, mg/kg**	**Cu, mg/kg**	**Zn, mg/kg**
S1	0.607 ± 0.061	0.018 ± 0.001	12.131 ± 0.885	0.428 ± 0.047	5.250 ± 0.278
SS1	0.072 ± 0.005	<0.001	1.068 ± 0.070	0.073 ± 0.003	0.091 ± 0.016
S2	0.451 ± 0.043	0.020 ± 0.004	9.260 ± 0.651	0.211 ± 0.051	6.170 ± 0.235
SS2	0.058 ± 0.004	0.013 ± 0.001	0.472 ± 0.032	0.031 ± 0.002	0.114 ± 0.023
S3	0.320 ± 0.039	0.014 ± 0.002	2.210 ± 0.093	0.185 ± 0.037	0.822 ± 0.057
SS3	0.056 ± 0.003	<0.001	0.623 ± 0.081	0.060 ± 0.008	0.060 ± 0.019
S4	0.218 ± 0.025	<0.001	3.432 ± 0.126	0.545 ± 0.064	1.202 ± 0.162
SS4	0.055 ± 0.002	<0.001	0.067 ± 0.019	0.063 ± 0.005	0.075 ± 0.024

Note: All values are expressed as mean ± standard deviation (n = 3).

The values determined for trace metals in the analyzed samples (S1–S4 from mining areas and SS1–SS4 from protected areas) show significant variations depending on location. Lead concentrations were highest in sample S1 (0.607 mg/kg), suggesting a strong industrial or mining influence. Cadmium was present in small amounts in the samples, but its presence, even at low levels, indicates anthropogenic influences, such as road traffic or pesticide use. Iron appeared at high concentrations in sample S1—12.131 mg/kg—indicating a significant contribution from geological or industrial-mining sources. Copper showed moderate values, with the highest found in S4 (0.545 mg/kg), reflecting local mining activities. Zinc was present in considerable amounts in samples S2 and S1 (6.17 and 5.25 mg/kg, respectively), which, while possibly reflecting natural presence, also suggests potential environmental contamination. Overall, the lower values in samples from protected areas compared to those from mining areas may indicate a different distribution of metals between phases, influenced by solubility, pH, mining impact, or organic matter content.

Tables 2.2.34 and 2.2.35 present the antimicrobial activity (antibacterial and antifungal) of honey samples against the tested strains. Artificial honey did not inhibit any of the strains.

Table 2.2.34. Antibacterial activity of honey samples against the used strains – inhibition zone diameter (mm).

Bacterial Strain	Sample								Antibiotic
	S1	SS1	S2	SS2	S3	SS3	S4	SS4	Ciprofloxacin, 5 µg
E. coli	9	11	12	14	8	8	8	10	29
S. enteritidis	7	8	7	8	8	9	8	8	27
P. aeruginosa	14	17	13	15	11	13	15	18	25
S. aureus	18	21	19	19	18	20	18	20	30
S. epidermidis	16	18	16	18	18	19	17	18	29
B. cereus	8	8	8	9	9	10	7	8	30
L. monocytogenes	8	9	8	8	8	9	9	10	24

The results of the inhibition zone diameters (DZI) of the honey samples against different bacterial strains, compared to the standard antibiotic (Ciprofloxacin, 5 µg), indicate clear patterns. All honey samples, regardless of their collection site, exhibited antibacterial activity, with inhibition diameters ranging from 7 mm to 21 mm. The largest inhibition zones were observed for the two *Staphylococcus* strains, followed by *P. aeruginosa*.

In almost all cases, the inhibition produced by honey samples from protected areas was more pronounced compared to that of honey contaminated with heavy metals. The highest DZI (21 mm) was recorded for *S. aureus* in sample SS1 from the protected area, followed by 20 mm inhibition zones for samples SS3 and SS4, both also originating from protected areas. The smallest DZI values (7 mm) were observed for *S. enteritidis* in samples S1 and S2, as well as for *B. cereus* in sample S4, all collected from polluted areas.

Table 2.2.35. Antifungal activity of honey samples against the tested strain – inhibition zone diameter (mm).

Fungal Strain	Sample								Antibiotic
	S1	SS1	S2	SS2	S3	SS3	S4	SS4	Voriconazole, 1 µg
C. albicans	9	10	9	10	9	9	8	9	37
A. flavus	8	9	9	9	8	9	9	10	43
A. niger	9	10	9	10	7	9	8	9	45
R.stolonifer	10	11	8	10	11	12	9	9	16
F. oxysporum	8	9	9	10	9	10	9	11	29
P. chrysogenum	10	11	11	11	8	12	8	9	18
A. alternata	9	10	9	9	7	8	10	11	16

As in the case of fungal strains, Table 2.2.35 shows that all honey samples, regardless of the collection area, exhibited antifungal activity against all studied strains. The inhibition zone diameters ranged from 7 to 12 mm, with the most sensitive strains being *R. stolonifer* and *P. chrysogenum*. For *R. stolonifer*, the most effective sample was SS3, from a protected area, with a diameter of 12 mm, close to that produced by the antifungal voriconazole (16 mm). A 12 mm inhibition zone was also recorded for *P. chrysogenum* with the same sample. Similarly, for fungal strains, inhibition by honey samples from protected areas was more pronounced than that of samples from mining areas.

To understand the relationship between the chemical composition of honey from mining areas and its antimicrobial efficacy, a correlation analysis was conducted, presented in Table 2.2.36, between the inhibition zone diameter (IZD) determined for different bacterial strains and the content of bioactive compounds (phenols, flavonoids), HMF, and trace metals (Pb, Cd, Fe, Cu, and Zn).

Table 2.2.36. Pearson correlation coefficients between IZD values and chemical parameters of honey from mining areas for different bacterial strains.

Correlation of DZI versus:	**Phenols**	**Flavonoids**	**HMF**	**Pb, mg/kg**	**Cd, mg/kg**	**Fe, mg/kg**	**Cu, mg/kg**	**Zn, mg/kg**
E. coli	−0.36 *^{9}	−0.74 *^{11}	0.81 *^{3}	0.44 *^{5}	0.64 *^{4}	0.57 *^{4}	−0.45 *^{9}	0.84 *^{3}
S. enteritidis	0.27 *^{8}	0.25 *^{6}	−0.27 *^{8}	−0.89 *^{11}	−0.77 *^{11}	−0.96 *^{12}	0.15 *^{6}	−0.99 *^{12}
P. aeruginosa	−0.88 *^{11}	−0.29 *^{8}	−0.39 *^{9}	0.01 *^{6}	−0.50 *^{10}	0.32 *^{5}	0.91 *^{2}	0.19 *^{6}
S. aureus	−0.36 *^{9}	−0.84 *^{11}	0.92 *^{2}	0.21 *^{5}	0.52 *^{4}	0.35 *^{5}	−0.50 *^{10}	0.68 *^{4}
S. epidermidis	0.64 *^{4}	0.42 *^{5}	−0.15 *^{8}	−0.70 *^{10}	−0.43 *^{9}	−0.92 *^{12}	−0.22 *^{8}	−0.92 *^{12}
B. cereus	−0.60 *^{10}	−0.24 *^{8}	−0.34 *^{9}	−0.72 *^{11}	−0.96 *^{12}	−0.47 *^{9}	0.78 *^{3}	−0.53 *^{10}
L. monocytogenes	−0.60 *^{10}	−0.24 *^{8}	−0.34 *^{9}	−0.72 *^{11}	−0.96 *^{12}	−0.47 *^{9}	0.78 *^{3}	−0.53 *^{10}

*Note: *1—perfect positive correlation (r: +1); *2—very strong positive correlation (r between +0.9 and +1.0); *3—strong positive correlation (r between +0.7 and +0.9); *4—moderate positive correlation (r between +0.5 and +0.7); *5—weak positive correlation (r between +0.3 and +0.5); *6—very weak or no positive correlation (r between 0 and +0.3); *7—no linear correlation (r = 0); *8—very weak or no negative correlation (r between 0 and −0.3); *9—weak negative correlation (r between −0.3 and −0.5); *10—moderate negative correlation (r between −0.5 and −0.7); *11—strong negative correlation (r between −0.7 and −0.9); *12—very strong negative correlation (r between −0.9 and −1.0); *13—perfect negative correlation (r: −1).*

Pearson correlation values indicate both the nature (positive or negative) and the strength of these relationships, highlighting possible influences of contaminants or natural compounds on antibacterial activity. Pearson coefficients between microbial activity (DZI) and various chemical compounds show diverse relationships. For example, *E. coli* showed strong positive correlations with Cd and Fe, while *P. aeruginosa* had very strong positive correlations with Cu. *S. aureus* exhibited positive correlations with Cd and Zn. *B. cereus* and *L. monocytogenes* had strong positive correlations with Cu. The other variables showed moderate or weak correlations, suggesting complex interactions with environmental factors.

To evaluate the influence of chemical parameters on the antifungal activity of honey from mining areas, a correlation analysis was performed, presented in Table 2.2.37, between DZI values for different fungal species and the content of phenols, flavonoids, HMF, and trace metals (Pb, Cd, Fe, Cu, and Zn). The results provide insights into how contamination with metals or natural composition can affect the antifungal efficacy of apicultural products.

Table 2.2.37. Pearson correlation coefficients between DZI values and chemical parameters of honey from mining areas for different fungal strains.

Correlation of DZI versus	**Phenols**	**Flavonoids**	**HMF**	**Pb, mg/kg**	**Cd, mg/kg**	**Fe, mg/kg**	**Cu, mg/kg**	**Zn, mg/kg**
C. albicans	0.60 *^{10}	0.24 *^{6}	0.34 *^{5}	0.72 *^{3}	0.69 *^{4}	0.47 *^{5}	−0.78 *^{11}	0.53 *^{4}
A. flavus	−0.83 *^{11}	−0.93 *^{12}	0.51 *^{4}	−0.44 *^{9}	0.01 *^{6}	−0.10 *^{8}	0.24 *^{6}	0.14 *^{6}
A. niger	−0.64 *^{10}	−0.42 *^{9}	0.15 *^{6}	0.70 *^{11}	0.82 *^{3}	0.92 *^{2}	0.22 *^{6}	0.92 *^{2}
R. stolonifer	0.86 *^{3}	0.94 *^{2}	−0.57 *^{10}	−0.01 *^{8}	−0.44 *^{9}	−0.34 *^{9}	−0.15 *^{8}	−0.55 *^{10}
F. oxysporum	−0.04 *^{8}	−0.55 *^{10}	0.61 *^{4}	−0.82 *^{11}	−0.44 *^{9}	−0.76 *^{11}	−0.33 *^{9}	−0.46 *^{9}
P. chrysogenum	−0.33 *^{9}	−0.47 *^{9}	0.52 *^{4}	0.76 *^{3}	0.98 *^{2}	0.86 *^{3}	−0.28 *^{8}	0.99 *^{2}
A. alternata	−0.97 *^{12}	−0.51 *^{10}	−0.15 *^{8}	−0.04 *^{3}	0.08 *^{6}	0.32 *^{5}	0.79 *^{3}	0.28 *^{6}

Note: The same explanations as in the footnote for Table 2.2.36 apply.

Pearson correlation coefficients between fungal microorganisms (DZI) and various chemical compounds reveal complex interaction patterns. For example, *C. albicans* showed positive correlations with phenols and the metals Cd, Fe, and Zn, while *A. niger* and *P. chrysogenum* exhibited strong positive correlations with Pb, Cd, Fe, and Zn. *A. alternata* showed a strong positive correlation with Cu.

For the protected area, the correlation between DZI values for bacterial strains and the chemical composition of honey was analyzed (Table 2.2.38), highlighting possible influences of bioactive compounds and metals on antimicrobial activity.

Table 2.2.38. Pearson correlation coefficients between DZI values and chemical parameters of honey from protected areas — bacterial strains.

Correlation of DZI versus	**Phenols**	**Flavonoids**	**HMF**	**Pb, mg/kg**	**Cd, mg/kg**	**Fe, mg/kg**	**Cu, mg/kg**	**Zn, mg/kg**
E. coli	−0.53 *^{10}	−0.85 *^{11}	−0.01 *^{8}	0.19 *^{6}	0.87 *^{3}	0.01 *^{6}	−0.69 *^{10}	0.99 *^{2}
S. enteritidis	0.84 *^{3}	0.84 *^{3}	0.65 *^{4}	−0.36 *^{9}	−0.33 *^{9}	0.11 *^{6}	0.12 *^{6}	−0.72 *^{11}
P. aeruginosa	−0.79 *^{11}	−0.52 *^{10}	−0.94 *^{12}	0.31 *^{5}	−0.23 *^{8}	−0.21 *^{8}	0.37 *^{5}	0.21 *^{6}
S. aureus	0.27 *^{6}	0.54 *^{4}	−0.28 *^{8}	0.72 *^{3}	−0.82 *^{11}	0.59 *^{4}	0.95 *^{2}	−0.41 *^{9}
S. epidermidis	0.84 *^{3}	0.84 *^{3}	0.65 *^{4}	−0.36 *^{9}	−0.33 *^{9}	0.11 *^{6}	0.12 *^{6}	−0.72 *^{11}
B. cereus	−0.27 *^{8}	0.22 *^{6}	−0.75 *^{11}	−0.15 *^{8}	−0.82 *^{11}	−0.40 *^{9}	0.72 *^{3}	−0.69 *^{10}
L. monocytogenes	−0.27 *^{8}	0.22 *^{6}	−0.75 *^{11}	−0.15 *^{8}	−0.82 *^{11}	−0.40 *^{9}	0.72 *^{3}	−0.60 *^{10}

Note: The same explanations as in the footnote for Table 2.2.36 apply.

Pearson correlation coefficients for bacterial microorganisms (DZI) from the protected area reveal varied interactions with chemical compounds. *E. coli* showed strong negative correlations with flavonoids and Cu, but a strong positive correlation with Cd and Zn, while *S. enteritidis* exhibited positive correlations with phenols, flavonoids, and HMF. On the other hand, *P. aeruginosa* showed negative correlations with HMF and phenols. Among the trace metals analyzed, Pb displayed the strongest correlations with the bacterial strains studied.

For honey from protected areas, correlations were calculated and are presented in Table 2.2.39 between DZI values for different fungal strains and chemical parameters, highlighting possible links between composition and antifungal activity.

Table 2.2.39. Pearson correlations between DZI values and chemical parameters of honey from protected areas — fungal strains.

Correlation of DZI versus	**Phenols**	**Flavonoids**	**HMF**	**Pb, mg/kg**	**Cd, mg/kg**	**Fe, mg/kg**	**Cu, mg/kg**	**Zn, mg/kg**
C. albicans	−0.10 *^{8}	−0.44 *^{9}	0.20 *^{6}	0.69 *^{4}	0.58 *^{4}	0.59 *^{4}	−0.30 *^{9}	0.87 *^{3}
A. flavus	−0.72 *^{11}	−0.34 *^{9}	−0.88 *^{11}	−0.44 *^{9}	−0.33 *^{9}	−0.80 *^{11}	0.23 *^{6}	−0.29 *^{8}
A. niger	−0.10 *^{8}	−0.44 *^{9}	0.20 *^{6}	0.69 *^{4}	0.58 *^{4}	0.59 *^{4}	−0.30 *^{9}	0.87 *^{3}
R. stolonifer	0.99 *^{2}	0.86 *^{3}	0.80 *^{3}	0.28 *^{6}	−0.26 *^{8}	0.71 *^{3}	0.24 *^{6}	−0.38 *^{9}
F. oxysporum	−0.54 *^{10}	−0.32 *^{9}	−0.47 *^{9}	−0.88 *^{11}	0.00 *^{7}	−0.99 *^{12}	−0.23 *^{8}	−0.28 *^{8}
P. chrysogenum	0.91 *^{2}	0.60 *^{4}	0.96 *^{2}	0.21 *^{6}	0.13 *^{6}	0.67 *^{4}	−0.14 *^{8}	−0.06 *^{8}
A. alternata	−0.82 *^{11}	−0.52 *^{10}	−0.98 *^{12}	0.18 *^{6}	−0.26 *^{8}	−0.33 *^{9}	0.37 *^{5}	0.12 *^{6}

Note: The same explanations as in the footnote for Table 2.2.36 apply.

Pearson correlation coefficients for fungal microorganisms (DZI) from the protected area reveal distinct interaction patterns with various chemical compounds. *C. albicans* and *A. niger* showed positive correlations with Pb, Cd, Fe, and Zn, suggesting a possible stimulatory effect of these metals on their growth, while *A. flavus* and *A. alternata* exhibited strong negative correlations with HMF and flavonoids, indicating potential inhibitory effects of these compounds on their proliferation. Additionally, *P. chrysogenum* showed a strong positive correlation with phenols, flavonoids, and HMF, suggesting a possible adaptability of this species to the presence of HMF in the environment.

The values obtained for honey samples from mining and protected areas can be statistically processed using principal component analysis (PCA). This method is suitable for reducing the dimensionality of a large dataset, which includes variables such as bacteria, fungi, chemical compounds, and heavy metals, helping to identify relationships among samples and determine whether honey from mining areas can be clearly distinguished from that from protected areas.

A PCA was applied to a dataset comprising microbiological parameters (presence of bacteria and fungi), bioactive compounds (phenols, flavonoids), and trace metal content (Pb, Cd, Fe, Cu, and Zn) in honey samples. The aim of the analysis was to identify the main directions of variation in the data and highlight any differences between honey from mining areas (S) and honey from protected areas (SS).

Figure 2.2.15 presents the results of the PCA applied to honey samples to explore patterns and groupings based on chemical composition and antimicrobial activity.

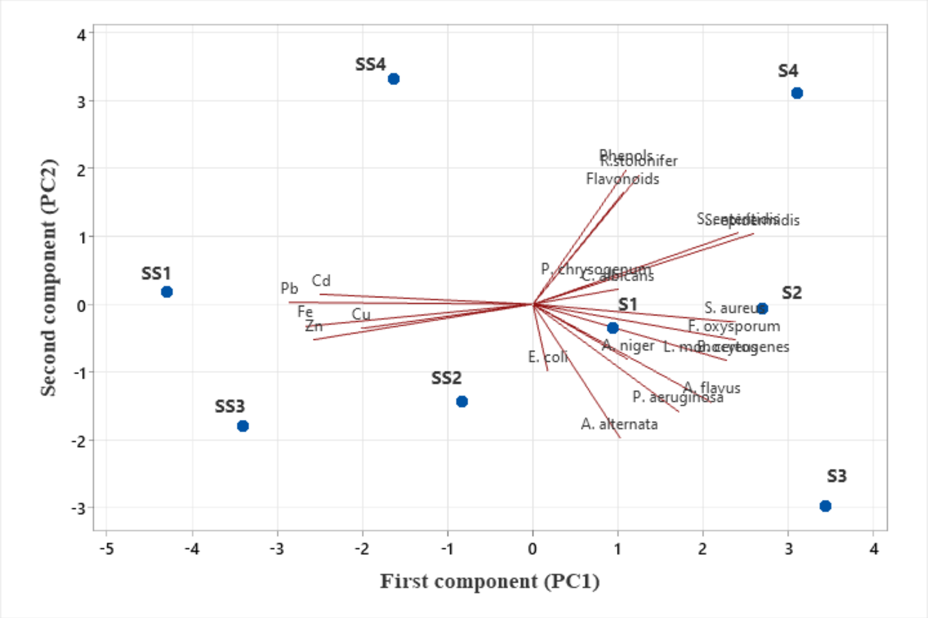


Figure 2.2.15. PCA biplot of observations for the first two principal components, generated from honey samples from mining areas and protected areas, illustrating differentiation based on chemical composition and microbiological parameters.

The resulting PCA plot highlights a clear separation between the two types of samples. Samples labeled “S” (S1–S4), corresponding to mining areas, are distinctly grouped apart from the “SS” samples (SS1–SS4), originating from protected areas.

This separation underscores the evident impact of the environment on honey quality and composition. Honey from mining areas is associated with high levels of trace metals, such as Pb, Cd, Fe, Cu, and Zn, as well as lower concentrations of bioactive compounds, such as phenols and flavonoids. In contrast, honey from protected areas contains significantly higher amounts of flavonoids and phenols, indicating superior antioxidant potential and lower heavy metal contamination.

The analysis shows that the first three principal components together explain a substantial proportion of the total variation in the dataset, namely 85.1%. The first principal component (PC1) accounts for approximately 43% of the total variation, the second (PC2) contributes 23.8%, and the third (PC3) 18.3%. Together, these three components represent a considerable portion of the total variability, suggesting that most of the relevant information is captured by just three main directions.

The PCA confirms that environmental factors, particularly exposure to heavy metals in mining areas, have a significant influence on honey characteristics. This statistical approach provides a valuable visual and analytical method for differentiating honey based on ecological origin and for assessing its potential quality and therapeutic properties.

Pollution in mining areas represents a major environmental problem, with significant impacts on ecosystems and human health. Within the studies conducted, the potential of propolis was highlighted, demonstrating that it is an effective natural bioindicator for detecting and assessing the degree of contamination in industrially polluted areas. For the propolis samples included in the study, levels of metals (Pb, Cd, As, Cr, Fe, Mn, Cu, and Zn) were determined in several mining regions in Romania, particularly in the counties of Alba, Hunedoara, Maramureș, and Caraș-Severin. The results reveal variations in anthropogenic impact, highlighting differences between affected areas, zones occupied by mine tailings, local hydrological conditions, and partial ecological rehabilitation efforts.

Moreover, the results confirm that propolis is an efficient natural bioindicator, capable of indicating the presence and concentration of heavy metal contamination in mining or industrially polluted areas. The highest concentrations of heavy metals were recorded in intensively mined areas such as Roșia Montană, Certeju de Sus, Băile Borșa, and Moldova Nouă. Comparison of samples from mining areas with those from protected areas revealed a significant impact of human activities on heavy metal contamination. The findings indicate that environmental factors can partially reduce the bioactive potential of propolis from regions affected by mining.

On the other hand, microbiological analysis showed that propolis from protected areas exhibited slightly stronger antimicrobial activity against both bacteria and fungi compared to samples from mining areas. The study confirms that propolis from protected areas has superior quality, both chemically and microbiologically. It contains higher amounts of bioactive compounds and lower levels of contamination, demonstrating stronger antioxidant and antimicrobial activity. In contrast, propolis from mining areas is affected by heavy metal contamination, which significantly reduces its therapeutic potential. These results emphasize the importance of environmental conservation and protection of natural resources for obtaining high-quality propolis with potential health benefits.

Regarding the function of honey and propolis as microbial bioinhibitors, the studies revealed a complex analysis of quality characteristics. The analyses showed that all honey and propolis samples exhibited antifungal activity, but differences were observed depending on the origin and strain type, with the most sensitive strains being *Penicillium chrysogenum*, *Rhizopus stolonifer*, and *Fusarium oxysporum*. A significant correlation was found between the acidity and electrical conductivity of polyfloral honey and its antioxidant activity with total phenol and flavonoid content. Statistical analysis (two-way ANOVA for antifungal activity) demonstrated a relationship between the geographical origin of honey and propolis samples, the microbial strains used, and antifungal activity.

For propolis, samples from the counties in northwestern and central Romania showed the highest content of bioactive compounds compared to those from Alba County.

2.2.4. Propolis as a Bioindicator of Environmental Pollution

2.2.4.1. Propolis as a Bioindicator of Environmental Pollution in Mining and Protected Areas

To highlight the role of propolis as a bioindicator, the trace metal content of propolis from four mining basins in Romania was monitored, compared with adjacent protected areas, tracking variations according to the specific characteristics of the region where the apiary is located. Additionally, the flavonoid, phenol, and metal contents of propolis samples from mining areas and protected areas were comparatively evaluated and correlated with antimicrobial activity. The scientific contribution lies in the analysis of both toxic metals (Pb, Cd, As, Cr) and essential metals (Fe, Mn, Cu, Zn) to assess their impact on the biological activity of propolis. The study evaluates the potential of propolis as a natural indicator of environmental pollution with heavy metals in mining areas, emphasizing its ability to accumulate and reflect the presence of contaminants [121].

These findings reinforce the role of propolis as a reliable bioindicator of environmental pollution, particularly in areas affected by historical mining activities. The elevated levels of trace metals detected in propolis samples from mining regions highlight the long-term environmental impact of anthropogenic contamination.

From an applied perspective, the results contribute to a better understanding of the influence of pollution on the composition and therapeutic properties of propolis.

The investigation areas were selected based on the need for a comparative analysis between mining areas, characterized by exposure to trace metal contamination, and natural protected areas, which serve as control sites with minimal anthropogenic influence. The selected regions are presented in Table 2.2.40.

Table 2.2.40. Coding and identification of propolis sampling areas in Romania.

Area Code	**County**	**Apiary Location**
AB 1	Alba	Roșia Montană
AB 2		Șugag – Natura 2000 Special Conservation Area ROSAC0085 Frumoasa
HD 1	Hunedoara	Certeju de Sus
HD 2		Vața de Jos – Natura 2000 Site of Community Importance ROSCI0325 Munții Metaliferi
MM 1	Maramureș	Băile Borșa

MM 2		Moisei – Natura 2000 Site of Community Importance ROSCI0125 Munții Rodnei
CS 1	Caraș-Severin	Moldova Nouă
CS 2		Berzasca – Natura 2000 Site of Community Importance ROSCI0206 Porțile de Fier

The investigated areas in Romania are recognized as major mining basins with a long-standing tradition in the extraction of metalliferous resources. In Roșia Montană, minerals such as gold, silver, copper, zinc, lead, and tellurium have been extensively mined [122]. In Certeju de Sus, the extraction of gold, silver, zinc, lead, and copper was also carried out [123]. Băile Borșa was the most important center for iron and gold mining in the Maramureș region, hosting one of the oldest mining operations in Romania and the deepest in Europe [124]. In Moldova Nouă, copper and zinc resources were mined, reflecting the region's long history of metalliferous exploitation [125].

These mining operations are currently closed; however, their environmental impact varies from site to site due to differences in the areas affected by mining activities, the extent of waste heaps, and local hydrological conditions. Since the mines are no longer active, there are no controlled discharges of process wastewater that could be measured. Abandoned, unreclaimed waste heaps and mines are subject to bacterial leaching, which generates acidic waters loaded with heavy metals, affecting water resources and aquatic biodiversity both within the mining areas and downstream rivers. The impact on local populations also varies depending on the environmental rehabilitation efforts undertaken by authorities, which are significant in some areas and nearly nonexistent in others [126].

Despite the cessation of mining operations, these regions remain environmentally vulnerable due to persistent sources of contamination, such as mine tailings and acid mine drainage. This ongoing pollution provides a relevant framework for evaluating propolis as a natural monitor of ecosystem health.

- The Roșia Montană mining area has a long history of gold extraction dating back to the Roman period. Human impact is significant, with large areas of land affected due to predominantly surface mining, although underground operations were also carried out. Past mining activities have led to extensive deforestation, landscape degradation, and soil deterioration, affecting local ecosystems [9].
- The Certej mining area is known for the extraction of gold and silver ores, making it one of the most important mining regions for precious metals in Romania. Intensive mining began in the 19th century and is now ceased. Extraction was carried out both through underground mines and surface mining methods, which generated significant waste. The environmental impact is similar to that of the Roșia Montană area, although of lower intensity.
- The Băile Borșa mining area dates back to the Middle Ages and experienced significant development during the 19th and 20th centuries. It is particularly known for the extraction of copper, silver, gold, and lead ores. This area, located in the Maramureș Mountains within a spectacular natural setting, was heavily affected by mining activities. Extraction was carried out mainly through underground mines, which reached considerable depths. In the 1970s, surface mining operations began, causing major visual and environmental impacts, including the creation of a crater over 200 meters deep and approximately 1 kilometer in diameter—one of the largest in Europe [127-128].
- The Moldova Nouă mining area, located in Caraș-Severin County, has a long history dating back to the Roman period, when gold and copper were extracted. Mining activities resumed in the 19th century and saw significant development in the 20th century, with intensive extraction of copper and other metals such as gold, silver, and lead. Extraction was carried out mainly through underground mines, some reaching considerable depths exceeding 1,000 meters [129].

This comparative approach aimed to evaluate the impact of mining activities on the chemical composition of propolis samples by comparing data from mining areas with those from ecologically protected regions.

The biological activity values of propolis samples collected from different areas are presented in Table 2.2.41, highlighting a comparative analysis between mining areas and natural protected areas. The mining areas include Roșia Montană (AB1), Certeju de Sus (HD1), Băile Borșa (MM1), and Moldova Nouă (CS1). These are compared with the corresponding natural protected areas.

Table 2.2.41. Evaluation of the biological activity of propolis samples.

Sample	**Phenols, mg GAE·g^{-1}**	**Flavonoids, mg QE·g^{-1}**	**IC$_{50}$, µg·mL^{-1}**
AB1	151.71±4.13	33.20±0.61	0.660±0.013
AB2	189.49±5.82	84.31±0.09	0.333±0.002
HD1	162.02±3.10	54.75±2.30	0.728±0.007
HD2	184.34±6.04	82.27±0.25	0.615±0.005
MM1	120.80±2.08	60.92±1.08	0.849±0.019
MM2	153.58±4.78	82.38±0.27	0.514±0.016
CS1	174.27±5.43	41.79±4.23	0.937±0.021
CS2	193.46±7.22	88.06±0.08	0.669±0.010

Abbreviations: GAE represents gallic acid equivalents; QE represents quercetin equivalents; IC50 is the median inhibitory concentration for DPPH radicals. Note: Values are presented as mean ± standard deviation (n = 3).

The results revealed notable differences in the biological activity of propolis between mining areas and natural protected areas. Regarding phenolic content, the lowest value was recorded in the mining area MM1 (120.80 mg GAE·g^{-1}), while the highest was found in the protected area CS2 (193.46 mg GAE·g^{-1}), indicating a richer phenolic composition in conserved regions. Similarly, flavonoid content was lowest in AB1 (33.20 mg QE·g^{-1}) and reached its maximum in CS2 (88.06 mg QE·g^{-1}), with consistently higher flavonoid concentrations in samples from protected areas.

Concerning the antioxidant activity of propolis, measured by the inhibition of 50% of free radicals (IC50), the strongest activity was observed in AB2 (0.333 µg·mL^{-1}), while the weakest was recorded in CS1 (0.937 µg·mL^{-1}). A lower IC$_{50}$ value indicates higher antioxidant activity of the propolis [130].

In general, protected areas showed lower IC50 values, demonstrating better antioxidant capacity compared to mining areas. These results suggest that environmental stressors, particularly heavy metal exposure, may interfere with the biosynthesis of bioactive compounds, ultimately diminishing the therapeutic potential of propolis.

Table 2.2.42 presents the concentrations of heavy metals (toxic) in propolis samples collected from the investigated areas — both from mining regions and protected areas.

Table 2.2.42. Evaluation of toxic metal concentrations in propolis samples.

Sample	**Pb, mg·kg^{-1}**	**Cd, mg·kg^{-1}**	**As, mg·kg^{-1}**	**Cr, mg·kg^{-1}**
AB1	9.27±1.05*	0.17±0.08*	0.87±0.14*	2.04±0.17
AB2	0.97±0.05	0.04±0.01*	0.30±0.07*	0.06±0.01
HD1	7.05±0.45*	0.14±0.05*	0.58±0.08*	7.01±0.15
HD2	0.77±0.30	0.07±0.02*	0.17±0.05*	0.06±0.02
MM1	5.62±0.22*	0.11±0.03*	0.44±0.03*	2.80±0.07
MM2	0.62±0.07*	0.03±0.01*	0.09±0.04*	0.05±0.02
CS1	5.12±0.16*	0.09±0.04*	0.71±0.06*	5.04±0.08
CS2	0.95±0.45	0.06±0.02*	0.40±0.02*	0.10±0.03

Note: Values are presented as mean ± standard deviation (n = 3), p < 0.01 (only for Pb, Cd, and As). The maximum permitted concentration in propolis is 1 mg·kg^{-1} for lead and cadmium, and 1.5 mg·kg^{-1} for arsenic, according to the Technical Regulation on Food Safety adopted within the Customs Union between Russia, Belarus, and Kazakhstan [131].

The analysis of toxic metals in propolis from the investigated regions reveals notable variations in contamination levels. Lead concentrations were highest in AB1 (9.27 mg·kg^{-1}), a mining area, indicating significant contamination. In contrast, AB2 (protected area) showed much lower levels (0.97 mg·kg^{-1}), demonstrating a cleaner environment. Other mining areas such as HD1 (7.05 mg·kg^{-1}) and CS1 (5.12 mg·kg^{-1}) also recorded high lead levels, whereas protected areas such as HD2 (0.77 mg·kg^{-1}), MM2 (0.62 mg·kg^{-1}), and CS2 (0.95 mg·kg^{-1}) had lower concentrations, consistent with the expected trend of reduced contamination in conserved regions.

Similarly, for cadmium, the highest levels were found in AB1 (0.17 mg·kg⁻¹), again reflecting the impact of mining activities. AB2 exhibited the lowest values (0.04 mg·kg⁻¹), reinforcing the idea that protected areas contain fewer contaminants. HD1 (0.14 mg·kg⁻¹), MM1 (0.11 mg·kg⁻¹), and CS1 (0.09 mg·kg⁻¹) had moderate cadmium concentrations, while the lowest levels were observed in HD2 (0.07 mg·kg⁻¹) and MM2 (0.03 mg·kg⁻¹).

Arsenic contamination was highest in the AB1 mining area (0.87 mg·kg⁻¹) and lower in AB2 (0.30 mg·kg⁻¹), a protected region. HD1 (0.58 mg·kg⁻¹) and CS1 (0.71 mg·kg⁻¹) also presented moderate arsenic levels, whereas HD2 (0.17 mg·kg⁻¹) and MM2 (0.09 mg·kg⁻¹) had lower concentrations, indicating a cleaner environment in the protected regions.

Finally, chromium levels were highest in HD1 (7.01 mg·kg⁻¹), followed by CS1 (5.04 mg·kg⁻¹), MM1 (2.80 mg·kg⁻¹), and AB1 (2.04 mg·kg⁻¹), demonstrating that mining areas contributed to elevated chromium contamination. In contrast, protected areas, including AB2 (0.06 mg·kg⁻¹) and MM2 (0.05 mg·kg⁻¹), showed much lower chromium levels.

The data clearly show that mining areas are significantly more contaminated with heavy metals such as lead, cadmium, arsenic, and chromium. In contrast, protected and natural areas exhibit substantially lower levels of these toxic metals. The clear distinction between mining and protected areas in terms of heavy metal concentrations emphasizes the strong influence of environmental pollution on propolis composition, further supporting its suitability as a sensitive indicator of localized contamination.

Table 2.2.43 presents the concentrations of essential metals in propolis samples, which are beneficial in small amounts but can become harmful if they exceed certain limits.

Table 2.2.43. Evaluation of essential metal concentrations in propolis samples.

Sample	**Fe, mg·kg⁻¹**	**Mn, mg·kg⁻¹**	**Cu, mg·kg⁻¹**	**Zn, mg·kg⁻¹**
AB1	95.73±7.08	20.11±1.01	4.99±0.18	2.50±0.20
AB2	18.67±0.92	1.30±0.04	2.20±0.03	1.56±0.3
HD1	102.26±4.05	19.0±0.90	6.00±0.29	2.67±0.14
HD2	4.77±0.56	0.28±0.06	1.39±0.05	1.03±0.25
MM1	150.43±2.01	13.20±1.01	3.68±0.45	3.28±0.8
MM2	10.62±0.60	1.69±0.02	1.97±0.08	0.65±0.04
CS1	132.23±7.48	25.43±2.07	5.40±0.11	2.90±0.21
CS2	9.96±0.33	1.5±0.03	1.19±0.02	1.65±0.09

Note: Values are presented as mean ± standard deviation (n = 3).

The analysis of essential metals in propolis reveals notable variations between mining and protected areas. Iron concentrations were highest in MM1 (150.43 mg·kg⁻¹), a mining area, followed by HD1 (102.26 mg·kg⁻¹). AB1 also showed a relatively high iron content (95.73 mg·kg⁻¹), while lower values were recorded in all protected areas. For manganese, the highest concentration was observed in CS1 (25.43 mg·kg⁻¹). In contrast, protected areas such as AB2 (1.30 mg·kg⁻¹) and MM2 (1.69 mg·kg⁻¹) exhibited much lower manganese levels.

Copper levels were highest in HD1 (6.00 mg·kg⁻¹), followed by AB1 (4.99 mg·kg⁻¹) and CS1 (5.40 mg·kg⁻¹). Protected areas such as AB2 (2.20 mg·kg⁻¹) and CS2 (1.19 mg·kg⁻¹) showed significantly lower copper concentrations. Finally, zinc concentrations were highest in MM1 (3.28 mg·kg⁻¹), whereas protected areas AB2 (1.56 mg·kg⁻¹) and CS2 (1.65 mg·kg⁻¹) had relatively lower zinc levels.

The results suggest that, although mining areas tend to have higher concentrations of essential metals, protected areas exhibit significantly lower levels. The elevated levels of essential metals in mining areas may reflect increased environmental availability due to soil and water contamination, further illustrating the influence of geochemical and anthropogenic factors on propolis composition.

Microbiological Analysis of Propolis

Table 2.2.44 presents the inhibition zone diameters for propolis samples collected from mining and protected areas of Romania, tested against selected bacterial strains.

Table 2.2.44. Antimicrobial activity of propolis samples against selected bacterial strains – inhibition zone diameter (mm).

Bacterial strain	**Sample**								**Antibiotic**
	AB1	**AB2**	**HD1**	**HD2**	**MM1**	**MM2**	**CS1**	**CS2**	**Ciprofloxacin, 5 µg**
S. aureus	26.3±0.0*	30.0±1.1	24.0±0.5*	25.0±1.7	25.0±0.0*	28.0±1.1	28.0±0.5*	30.0±0.5	30
E. coli	27.0±0.5	32.0±0.0*	28.0±1.7	30.0±0.0*	26.0±1.1	26.0±0.5*	24.5±1.7	26.0±0.0*	29
P. aeruginosa	26.0±1.1	27.3±1.7	24.3±0.0*`	30.0±0.5*	24.3±1.1	28.0±1.7	25.0±0.0*	29.3±1.1	25
E. faecalis	28.3±1.7	29.0±0.5	27.0±1.1	27.3±0.5	26.0±0.0*	26.0±0.5	25.0±1.1	27.3±1.7	27
S. mutans	23.0±1.1*	24.3±0.0*	21.3±1.7*	26.0±1.1	24.0±1.7	25.7±1.7	23.0±0.5*	26.0±0.0*	29

Note: Values are presented as mean ± standard deviation (n = 3), $p < 0.01$.

The inhibition zone diameters of propolis samples for different bacterial strains, compared with Ciprofloxacin (5 µg) as a standard antibiotic, reveal interesting trends. For *S. aureus*, inhibition zones ranged from 24 mm to 30 mm, with the highest efficacy observed in the protected areas AB2 and CS2 (both 30 mm). Propolis samples from mining areas, such as AB1 and HD1, showed slightly smaller zones, between 24 mm and 26 mm.

For *E. coli*, inhibition zones ranged from 24 mm to 32 mm. AB2 recorded the largest zone (32 mm), while CS1 and MM1 had smaller zones (24 mm and 26 mm). The inhibition zone for Ciprofloxacin was 29 mm, and in some samples from protected areas, it exceeded this value (30 mm or 32 mm).

For *P. aeruginosa*, inhibition zones ranged from 24 mm to 30 mm, with HD2 showing the largest zone (30 mm), followed by MM2 and CS2 (28 mm and 29 mm). Propolis samples from mining areas, such as AB1 and MM1, exhibited smaller zones, indicating lower antimicrobial efficiency.

For *E. faecalis*, inhibition zones varied between 25 mm and 29 mm. AB2 had the largest zones (29 mm), whereas AB1 showed a slightly smaller zone (28 mm). The inhibition zone for Ciprofloxacin was 27 mm, corresponding to the efficacy of propolis from certain mining and protected areas.

Finally, for *S. mutans*, inhibition zones ranged from 21 mm to 26 mm, with the largest zones observed in CS2 and HD2 (both 26 mm) and the smallest in HD1 (21 mm). This indicates that some propolis samples, especially from protected areas, are more effective against *S. mutans* than others.

Propolis from protected areas such as AB2, CS2, and HD2 generally showed larger inhibition zones across a broad range of microbial strains, suggesting stronger antimicrobial activity compared to samples from mining areas such as AB1 and MM1. The reduced antimicrobial efficacy observed in samples from mining areas may be associated with the negative impact of heavy metal accumulation on the chemical profile of propolis, particularly affecting phenolic and flavonoid compounds.

Table 2.2.45 presents the inhibition zone diameters for propolis samples from mining and protected areas of Romania against selected fungal strains.

Table 2.2.45. Antimicrobial activity of propolis samples against selected fungal strains – inhibition zone diameter (mm).

Fungal strain	**Sample**								**Antibiotic**
	AB1	**AB2**	**HD1**	**HD2**	**MM1**	**MM2**	**CS1**	**CS2**	**Voriconazole, 1 µg**
C. albicans	18.0±1.1*	22.0±0.0*	20.0±0.5*	21.0±0.0*	20.0±1.1*	20.3±1.7*	19.0±0.0*	22.0±0.5*	37
A. niger	16.0±0.0*	24.0±0.5*	19.0±0.0*	18.0±0.0*	17.0±0.5*	18.0±0.0*	22.0±0.5*	26.3±1.7*	45
A. flavus	20.0±0.5*	26.3±1.7*	18.3±1.1*	18.3±1.1*	19.5±1.7*	20.3±1.7*	21.0±1.1*	27.0±1.1*	43
C. neoformans	15.0±0.5*	17.0±0.0*	17.0±1.7*	21.0±0.5*	18.3±1.7*	18.0±0.0*	18.0±0.5*	19.0±0.0*	39
P. chrysogenum	25.3±1.1*	27.3±1.7*	22.0±0.5*	24.3±1.7	17.0±1.1	26.0±1.7*	24.3±1.7	27.0±0.0*	18

*Note: Values are presented as mean ± standard deviation (n = 3), * $p < 0.01$.*

The inhibition zone diameters of propolis samples for various fungal strains, compared with Voriconazole (1 µg) as a standard antifungal, provide insights into the antimicrobial properties of propolis from different regions. A summary of the results is as follows:

For *C. albicans*, inhibition zones ranged from 18 mm to 22 mm, with the largest zones observed in AB2 and CS2 (both 22 mm). The smallest zones were recorded in AB1 and CS1, with 18 mm and 19 mm, respectively. Voriconazole showed a significantly larger inhibition zone of 37 mm, indicating higher efficacy compared to the propolis samples.

For *A. niger*, inhibition zones ranged from 16 mm to 26 mm, with CS2 showing the largest zone (26 mm) and AB1 the smallest (16 mm). Voriconazole presented the largest inhibition zone at 45 mm, suggesting much higher potency than the propolis samples.

For *A. flavus*, inhibition zones ranged from 18 mm to 27 mm, with CS2 exhibiting the largest zone (27 mm), while HD1 and HD2 had the smallest (18 mm). Voriconazole had a zone of 43 mm, indicating superior antifungal activity compared to the propolis samples.

For *C. neoformans*, inhibition zones ranged from 15 mm to 21 mm, with the largest observed in HD2 (21 mm). Voriconazole demonstrated a significantly larger inhibition zone of 39 mm, exceeding those of the propolis samples.

For *P. chrysogenum*, inhibition zones ranged from 17 mm to 27 mm, with the largest in AB2 and CS2 (both 27 mm). The smallest zone was observed in MM1 (17 mm). Voriconazole had the smallest inhibition zone for this strain, 18 mm, indicating that in this case, propolis samples from nearly all regions performed similarly or even better than Voriconazole.

In general, protected areas such as AB2, CS2, and HD2 showed larger inhibition zones against fungal strains, suggesting stronger antifungal activity compared to samples from mining areas, such as AB1 and MM1.

The relationship between chemical parameters and antimicrobial activity was evaluated through statistical analysis, providing a deeper understanding of correlations and their potential significance. A Pearson correlation analysis was conducted to assess the relationship between total phenol and flavonoid content in each propolis sample from mining and protected areas and the inhibition zone diameters. The results are presented in Tables 2.2.46 and 2.2.47.

Table 2.2.46. Pearson correlation coefficients and significance for flavonoid and phenol content vs. antibacterial activity of propolis from mining and protected areas.

Bacterial strain	**Mining area**		**Protected area**	
	Flavonoids	**Phenols**	**Flavonoids**	**Phenols**
S. aureus	-0.611^{*10}	$+0.477^{*4}$	$+0.713^{*3}$	$+0.254^{*6}$
E. coli	$+0.179^{*6}$	-0.213^{*8}	-0.313^{*9}	$+0.442^{*5}$
P. aeruginosa	-0.969^{*12}	$+0.321^{*5}$	-0.021^{*8}	$+0.174^{*6}$
E. faecalis	-0.330^{*9}	-0.149^{*8}	$+0.194^{*6}$	$+0.659^{*4}$
S. mutans	-0.019^{*8}	-0.591^{*10}	$+0.227^{*6}$	$+0.155^{*6}$

*Note: *1—perfect positive correlation (r: +1); *2—very strong positive correlation (r between +0.9 and +1.0); *3—strong positive correlation (r between +0.7 and +0.9); *4—moderate positive correlation (r between +0.5 and +0.7); *5—weak positive correlation (r between +0.3 and +0.5); *6—very weak or no positive correlation (r between 0 and +0.3); *7—no linear correlation (r = 0); *8—very weak or no negative correlation (r between 0 and −0.3); *9—weak negative correlation (r between −0.3 and −0.5); *10—moderate negative correlation (r between −0.5 and −0.7); *11—strong negative correlation (r between −0.7 and −0.9); *12—very strong negative correlation (r between −0.9 and −1.0); *13—perfect negative correlation (r: −1).*

The data presented in Table 2.2.46 highlight the Pearson correlation coefficients (r – absolute value) for the association between flavonoid and phenolic content in propolis samples from mining and protected areas and their antibacterial activity against different bacterial strains. At the same time, the table indicates the direction, significance, and interpretation of the correlation coefficient (r).

For *S. aureus*, in the mining area there is a negative correlation between flavonoids and the bacterium. However, phenolic content shows a moderate positive correlation (r = 0.48). In the protected area, flavonoids exhibit a strong positive correlation with S. aureus (r = 0.71). For *E. coli*, in the protected area, flavonoids show a weak negative correlation, while phenols display a moderate positive correlation (r = 0.44), suggesting that phenols play a more significant role in inhibiting *E. coli* in protected areas. For *P. aeruginosa*, phenols show a moderate positive correlation (r = 0.32). For *E. faecalis*, in the protected area, phenols exhibit a stronger positive correlation (r = 0.66). For *S. mutans*, in the mining area, phenols show a stronger negative correlation.

In mining areas, flavonoids generally show weak or negative correlations with bacterial inhibition [132], whereas phenols tend to show weak to moderate positive correlations, particularly against *S. aureus* and *P. aeruginosa* [133]. In protected areas, flavonoids exhibit positive correlations with bacterial inhibition, especially against *S. aureus*, while phenols show stronger positive correlations, particularly against *E. faecalis* [134].

Table 2.2.47. Pearson correlation coefficients and significance for flavonoid and phenolic content versus antifungal activity in propolis from mining and protected areas.

Fungal Strain	**Mining area**		**Protected area**	
	Flavonoids	**Phenols**	**Flavonoids**	**Phenols**
C. albicans	+0.969 ^{*2}	-0.321 ^{*9}	+0.738 ^{*3}	+0.943 ^{*2}
A. niger	-0.007 ^{*8}	+0.719 ^{*3}	+0.920 ^{*2}	+0.720 ^{*3}
A. flavus	-0.687 ^{*10}	+0.382 ^{*5}	+0.862 ^{*3}	+0.581^{*4}
C. neoformans	+0.684 ^{*4}	-0.086 ^{*8}	-0.159 ^{*8}	+0.176*
P. chrysogenum	-0.909 ^{*12}	0.818 ^{*3}	+0.683^{*4}	+0.185*

Note: The same explanations as in the footnote for Table 2.2.46 apply.

Table 2.2.48 presents the Pearson correlation coefficients (r) comparing the association between flavonoid and phenolic content in propolis samples and antifungal activity in mining and protected areas.

For *C. albicans*, the mining area shows a strong positive correlation (r = 0.97) between flavonoids and fungal inhibition. In the protected area, both flavonoids and phenolics show good positive correlations (r = 0.74 and r = 0.94, respectively). For *A. niger*, in the mining area a moderate positive correlation is observed with phenolics (r = 0.72). In the protected area, flavonoids show a very strong positive correlation (r = 0.92), while phenolics show a strong positive correlation (r = 0.72), indicating that both compounds play a significant role in the inhibition of this fungus. For *A. flavus*, in the protected area flavonoids show a strong positive correlation (r = 0.86), while phenolics show a moderate correlation (r = 0.58). For *C. neoformans*, in the mining area flavonoids show a moderate positive correlation (r = 0.68). For *P. chrysogenum*, the mining area shows a strong negative correlation with flavonoids and a very strong positive correlation with phenolics (r = 0.82). In the protected area, flavonoids show a moderate positive correlation (r = 0.68), while phenolics show a weak positive correlation.

Flavonoids show stronger correlations with fungal inhibition in protected areas, especially against *C. albicans* and *A. niger*, whereas phenolics tend to have stronger effects in mining areas, particularly against *P. chrysogenum* [135].

Table 2.2.48. Pearson correlation coefficients (r) for the association between flavonoid and phenolic content and the antioxidant activity of propolis, as well as its metal and metalloid content.

Trace elements	**Mining area**			**Protected area**		
	Phenols	**Flavonoids**	**IC$_{50}$**	**Phenols**	**Flavonoids**	**IC$_{50}$**
Lead	+0.010 ^{*6}	−0.570 ^{*10}	−0.947 ^{*12}	+0.917 ^{*2}	+0.730 ^{*3}	−0.172 ^{*8}
Cadmium	−0.073 ^{*8}	−0.409 ^{*9}	−0.990 ^{*12}	+0.659 ^{*4}	+0.238 ^{*6}	+0.664 ^{*4}
Arsenic	+0.566 ^{*4}	−0.989 ^{*12}	−0.409 ^{*9}	+0.854 ^{*3}	+0.926 ^{*2}	+0.113 ^{*6}
Chromium	+0.590 ^{*4}	+0.327 ^{*5}	+0.176 ^{*6}	+0.659 ^{*4}	+0.950 ^{*2}	+0.573 ^{*4}
Iron	−0.481 ^{*9}	+0.578 ^{*4}	+0.824 ^{*3}	+0.134 ^{*6}	+0.205 ^{*6}	−0.856 ^{*11}
Manganese	+0.940 ^{*2}	−0.694 ^{*10}	+0.243 ^{*6}	−0.349 ^{*9}	+0.403 ^{*5}	−0.229 ^{*8}
Copper	+0.891 ^{*3}	−0.342 ^{*9}	−0.220 ^{*8}	−0.409 ^{*9}	−0.468 ^{*9}	−0.948 ^{*12}
Zinc	−0.604 ^{*10}	+0.726 ^{*3}	+0.716 ^{*3}	+0.909 ^{*2}	+0.814 ^{*3}	−0.069 ^{*8}

Note: The same explanations as in the footnote for Table 2.2.46 apply.

The correlation patterns indicate that heavy metal pollution not only alters the elemental composition of propolis but also affects its biological activity, highlighting the complex interactions between environmental contaminants and natural bioactive compounds.

Comparing the values for the mining area and the protected area, based on the relationship between metallic cations and the content of phenols, flavonoids, and antioxidant capacity (IC$_{50}$), significant differences are observed that reflect the influence of pollution on the synthesis of bioactive compounds. In the mining area, elevated concentrations of heavy metals negatively affect both flavonoid synthesis and antioxidant capacity, particularly in the case of cadmium and lead.

On the other hand, in the protected (unpolluted) area, metallic cations have a much more favorable impact on bioactive compounds. For lead, positive correlations were recorded with both phenols ($r = 0.92$) and flavonoids ($r = 0.73$), without significantly affecting antioxidant capacity. Similarly, arsenic and zinc show strong positive correlations with phenols and flavonoids ($r > 0.85$ in both cases).

Iron and manganese show weaker positive correlations with phenols and flavonoids, while antioxidant capacity is slightly reduced in their presence (IC$_{50}$ $r = 0.86$ for Fe and $r = -0.23$ for Mn). Thus, in the protected area, metals influence the synthesis of phenols and flavonoids in a much more balanced manner, generally contributing to the maintenance of good antioxidant activity. Unlike the mining area, where negative effects prevail, positive correlations dominate here, especially for lead, arsenic, and zinc [136].

Studies suggest that certain essential metals in propolis, such as zinc, copper, and iron, may influence its antioxidant activity. The relationship between essential and toxic metal content and the biological activity of propolis is influenced by the type and concentration of metals. These metals are involved in biochemical processes that can modulate antioxidant activity, for example through the formation of metal–phenol complexes that may enhance free radical scavenging capacity. At the same time, excessive levels of heavy metals can negatively affect antioxidant activity and may even become toxic [137].

Tables 2.2.49 and 2.2.50 present the Pearson correlation coefficients for evaluating the relationship between bacterial strains and metal content in propolis samples from mining areas and protected areas, respectively.

Table 2.2.49. Pearson correlation coefficients and significance for the relationship between inhibition zone diameter values and metal content in propolis samples from mining areas.

Bacterial strain	Lead	Cadmium	Arsenic	Chromium	Iron	Manganese	Copper	Zinc
S. aureus	-0.285 ^{*8}	-0.460 ^{*9}	+0.500 ^{*4}	-0.218 ^{*8}	+0.226 ^{*6}	+0.743 ^{*3}	+0.027 ^{*6}	+0.010 ^{*6}
E. coli	+0.668 ^{*4}	+0.795 ^{*3}	-0.043 ^{*8}	+0.152 ^{*6}	-0.641 ^{*10}	-0.474 ^{*9}	+0.233 ^{*6}	-0.461 ^{*9}
P. aeruginosa	+0.630 ^{*4}	+0.472 ^{*5}	+0.949 ^{*2}	-0.548 ^{*10}	-0.499 ^{*9}	+0.510 ^{*4}	+0.116 ^{*6}	-0.632 ^{*10}
E. faecalis	+0.964 ^{*2}	+0.996 ^{*2}	+0.436 ^{*5}	-0.275 ^{*8}	-0.792 ^{*11}	-0.262 ^{*8}	+0.143 ^{*6}	-0.692 ^{*10}
S. mutans	-0.244 ^{*8}	-0.322 ^{*9}	-0.101 ^{*8}	-0.823 ^{*11}	+0.681 ^{*4}	-0.283 ^{*8}	-0.889 ^{*11}	+0.610 ^{*4}

Note: The same explanations as in the footnote for Table 2.2.46 apply.

The correlation between the inhibition of different bacterial strains and heavy metals reflects how bacteria are influenced by metallic pollutants in mining or contaminated environments.

Bacteria from mining areas are affected differently by heavy metals, with each strain showing specific responses that suggest either adaptability or inhibition depending on the metal present and its concentration.

Table 2.2.50. Pearson correlation coefficients and significance for the relationship between inhibition zone diameter values and metal content in propolis samples from protected areas.

Bacterial strain	Lead	Cadmium	Arsenic	Chromium	Iron	Manganese	Copper	Zinc
S. aureus	+0.601 ^{*4}	-0.464 ^{*9}	+0.668 ^{*4}	+0.462 ^{*5}	+0.785 ^{*3}	+0.800 ^{*3}	+0.274 ^{*6}	+0.633 ^{*4}
E. coli	+0.421 ^{*5}	+0.122 ^{*6}	+0.065 ^{*6}	-0.376 ^{*9}	+0.408 ^{*5}	-0.531 ^{*10}	+0.440 ^{*5}	+0.297 ^{*6}
P. aeruginosa	-0.211 ^{*8}	+0.849 ^{*3}	-0.075 ^{*8}	+0.291 ^{*6}	-0.953 ^{*12}	-0.667 ^{*10}	-0.871 ^{*11}	-0.162 ^{*8}
E. faecalis	+0.791 ^{*3}	+0.005 ^{*6}	+0.521 ^{*4}	+0.030 ^{*6}	+0.725 ^{*3}	-0.119 ^{*8}	+0.414 ^{*5}	+0.704 ^{*3}
S. mutans	-0.164 ^{*8}	+0.763 ^{*3}	+0.076 ^{*6}	+0.510 ^{*4}	-0.906 ^{*12}	-0.395 ^{*9}	-0.957 ^{*12}	-0.076 ^{*8}

Note: The same explanations as in the footnote for Table 2.2.46 apply.

In a protected area, the correlation values between bacterial strain inhibition and heavy metals reflect a different dynamic compared to that observed in mining areas. In a protected environment, heavy metal concentrations are generally lower, and bacteria are not exposed to the same level of toxic stress. This is reflected in the correlation patterns, where some bacteria show increased tolerance, while others exhibit fewer negative interactions.

Tables 2.2.51 and 2.2.52 present the Pearson correlation coefficients for evaluating the relationship between fungal strains and metal content in propolis samples from mining areas and protected areas, respectively.

Table 2.2.51. Pearson correlation coefficients and significance for the relationship between inhibition zone diameter values for fungal strains and metal content in propolis samples from mining areas.

Fungal Strain	Lead	Cadmium	Arsenic	Chromium	Iron	Manganese	Copper	Zinc
C. albicans	-0.630 ^{*10}	-0.473 ^{*9}	-0.949 ^{*12}	+0.548 ^{*4}	+0.499 ^{*5}	-0.510 ^{*10}	-0.116 ^{*8}	+0.632^{*4}
A. niger	-0.690 ^{*10}	-0.738 ^{*11}	-0.041 ^{*8}	+0.662 ^{*4}	+0.240 ^{*6}	+0.714 ^{*3}	+0.500 ^{*4}	+0.118 ^{*6}
A. flavus	-0.149 ^{*8}	-0.332 ^{*9}	+0.577 ^{*4}	-0.382 ^{*9}	+0.177 ^{*6}	+0.674 ^{*4}	-0.064 ^{*8}	-0.034 ^{*8}
C.neoformans	-0.990 ^{*12}	-0.943 ^{*12}	-0.758 ^{*11}	+0.393 ^{*5}	+0.837 ^{*3}	-0.075 ^{*8}	-0.216 ^{*8}	+0.824 ^{*3}
P. chrysogenum	+0.501 ^{*4}	+0.375 ^{*5}	+0.934 ^{*2}	+0.092 ^{*6}	-0.731 ^{*11}	+0.844 ^{*3}	+0.702 ^{*3}	-0.860 ^{*11}

Note: The same explanations as in the footnote for Table 2.2.46 apply.

In the mining area, the analysis of correlations regarding the inhibition of fungal species by heavy metals shows a different response compared to bacteria. Fungi such as *C. albicans*, *A. niger*, and *P. chrysogenum* exhibit various mechanisms of adaptation and tolerance to heavy metals. These correlations may provide insights into their potential use in bioremediation of such environments.

Candida albicans shows strong negative correlations with Pb (lead), Cd (cadmium), As (arsenic), Mn (manganese), and Cu (copper), and positive correlations with Cr (chromium) and Fe (iron). *Aspergillus niger* exhibits a strong negative correlation with Pb and Cd. *Aspergillus flavus* shows a positive correlation with As ($r = 0.58$) as well as with Mn ($r = 0.67$), indicating a possible adaptation to this metal.

Cryptococcus neoformans shows positive correlations with Fe ($r = 0.84$) and Zn ($r = 0.82$), suggesting high adaptability. Unlike the other fungal species, *Penicillium chrysogenum* displays positive correlations with Pb, Cd, As, Mn, and Cu, indicating a high tolerance to these metals.

Table 2.2.52. Pearson correlation coefficients and significance for the relationship between inhibition zone diameter values for fungal strains and metal content in propolis samples from protected areas.

Fungal Strain	Lead	Cadmium	Arsenic	Chromium	Iron	Manganese	Copper	Zinc
C. albicans	+0.997 ^{*2}	+0.381 ^{*5}	+0.938 ^{*2}	+0.667 ^{*4}	+0.425 ^{*5}	-0.046 ^{*8}	-0.196 ^{*8}	+0.992 ^{*2}
A. niger	+0.899 ^{*3}	+0.089 ^{*6}	+0.965 ^{*2}	+0.784 ^{*3}	+0.530 ^{*4}	+0.399 ^{*5}	-0.154 ^{*8}	+0.938 ^{*2}
A. flavus	+0.834 ^{*3}	-0.124 ^{*8}	+0.888 ^{*3}	+0.671 ^{*4}	+0.671 ^{*4}	+0.554 ^{*4}	+0.030 ^{*6}	+0.867 ^{*3}
C.neoformans	-0.228 ^{*8}	+0.855 ^{*3}	-0.156 ^{*8}	+0.154 ^{*6}	-0.932 ^{*12}	-0.788 ^{*11}	-0.781 ^{*11}	-0.203 ^{*8}
P. chrysogenum	+0.543 ^{*4}	-0.516 ^{*10}	+0.618 ^{*4}	+0.425 ^{*5}	+0.784 ^{*3}	+0.840 ^{*3}	+0.302 ^{*5}	+0.577 ^{*4}

Note: The same explanations as in the footnote for Table 2.2.46 apply.

In protected areas, the correlation values for the inhibition of fungal species show different interactions with heavy metals compared to those observed in mining areas, highlighting more favorable environmental conditions and lower levels of pollutants. Fungi such as *C. albicans* and *A. niger* appear to be better adapted to cleaner environments, maintaining strong positive correlations with certain essential metals, whereas *C. neoformans* exhibits a different sensitivity pattern.

C. albicans shows strong positive correlations with Pb, As, Zn, Cr, and Fe, indicating a potentially favorable influence on its development at the low concentrations found in protected areas. *A. niger* shows positive correlations with Pb, As, Cr, Fe, and Mn, suggesting high adaptability to these metals. *A. flavus* also exhibits significant positive correlations with Pb, As, Zn, Cr, and Fe.

In contrast to the other species, *C. neoformans* shows negative correlations with most metals, indicating high sensitivity to their presence. *P. chrysogenum* displays moderate positive correlations with

Pb, As, Cr, Cu, and Zn, suggesting good tolerance. Additionally, Fe and Mn show strong positive correlations, indicating excellent adaptation to the presence of these metals.

Conclusions

Propolis is an effective natural bioindicator, capable of indicating the presence and level of heavy metal contamination in mining areas. The highest concentrations of heavy metals were recorded in intensively exploited mining regions such as Roșia Montană, Certeju de Sus, Băile Borșa, and Moldova Nouă. Comparing samples from the investigated mining areas with those from protected areas highlighted the significant impact of human activities on heavy metal contamination.

The comparative analysis of propolis samples from mining and protected areas revealed significant differences in chemical composition, biological activity, and levels of heavy metal contamination. Propolis from protected areas showed a higher content of phenolic compounds and flavonoids, demonstrating superior antioxidant capacity compared to samples from mining areas. The results indicate that environmental factors may reduce the bioactive potential of propolis from mining-affected regions.

Microbiological analysis demonstrated that propolis from protected areas exhibited stronger antimicrobial activity against both bacteria and fungi compared to samples from mining areas.

The study confirms that propolis from protected areas is of superior quality, both chemically and microbiologically. It contains higher levels of bioactive compounds and lower levels of contamination, resulting in enhanced antioxidant and antimicrobial activity. In contrast, propolis from mining areas is affected by heavy metal contamination, which significantly reduces its therapeutic potential. These findings emphasize the importance of environmental conservation and the protection of natural resources in obtaining high-quality propolis with potential health benefits.

2.2.4.2. Propolis as a Bioindicator of Trace Metal Contamination in the Roșia Montană Area

Metals and metalloids are naturally present in the environment, but at low concentrations. Human activities, such as mining, have led to an increase in their levels, exposing flora and fauna to potentially hazardous concentrations [138]. Industrial, agricultural, and mining activities have led to an increase in environmental pollution [139]. Heavy metals are widely studied pollutants that continuously accumulate in the environment due to their stability and resistance to degradation [140]. Mining activities can significantly impact the environment, both during extraction and long after mine closure. The main environmental issues arise from the waste generated during the extraction and processing of metals, which represent major sources of soil, water, and air pollution [141]. In mining areas, these resources may contain heavy metals or other pollutants, and the analysis of bees and their products (such as honey, wax, or pollen) can indicate the level of contamination [142]. In this context, environmental pollution is no longer a localized issue but a complex and cumulative process that affects entire ecosystems, altering biogeochemical cycles and posing long-term risks to biodiversity and human health.

In mining areas, heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) accumulate in soil, water, and air. These metals can be detected both in the bodies of bees and in their products [143]. Monitoring beekeeping products provides information about the quality of the environment from which they originate. [61]. Bees are considered bioindicators of pollution [144-151] in mining areas because they collect pollen, nectar, and water from the surrounding environment, thus reflecting the health status of the local ecosystem [152]. Mining-impacted environments are particularly vulnerable due to the persistence of contaminants, which can remain in soils and water bodies for decades, continuously influencing ecological balance even after the cessation of industrial activities.

Bees exposed to pollution may exhibit increased mortality, behavioral and health changes, as well as alterations in flight activity [153]. Bees influence the overall health of the ecosystem through pollination. If bees are affected by pollution, the entire ecological chain can be destabilized [154]. Therefore, honey bees provide an integrative and dynamic measure of environmental quality, reflecting not only localized contamination but also the cumulative exposure across landscapes.

The study aims to investigate the transfer of potentially trace elements (Cu^{2+}, Fe^{2+}, Pb^{2+}, Zn^{2+}, Sr^{2+},

As^{3+}, and Cd^{2+}) from soil and river water, to cultivated plants under the influence of the Roșia Montană mining area by determining contamination levels and assessing the properties of propolis [155]. At the same time, the research also focuses on the role of bees as vectors and practical, efficient tools for monitoring the effects of pollution on ecosystems, particularly in areas exposed to mining activities.

Figure 2.2.16 shows the map of Romania and the map of Alba County, highlighting the study area — Roșia Montană commune, located in the northwestern part of the county.

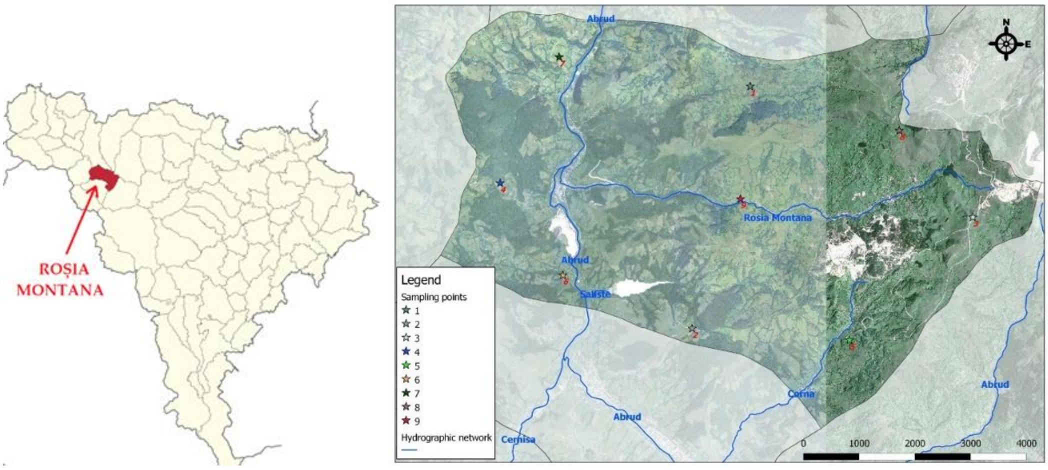


Figure 2.2.16. Map of Alba County. Location of the study area — Roșia Montană locality and sampling points

The map from Figure 2.2.16 was created using QGIS software, version 3.1.0, utilizing vector and raster data obtained from https://geo-spatial.org, as well as vectorized data prepared by the author. The points were added based on GPS coordinates collected in the field at the time of sampling, using the Android mobile application Spyglass.

Soil Analysis

Soil represents a primary reservoir for environmental contaminants, acting both as a sink and a potential secondary source of pollution through plant uptake and leaching processes.

The results obtained from the laboratory analyses of soil samples collected from the 9 points in the Roșia Montană area are presented in Table 2.2.53.

Table 2.2.53. Concentration values of metal ions (mg/kg) in soil samples from the Roșia Montană area.

Sample	**Sampling area**	**Pb^{2+}**	**Cu^{2+}**	**Cd^{2+}**	**Zn^{2+}**	**As^{3+}**
1.	Soil 1—North	91.60±7.32	15.66±2.94*	3.74±2.04*	17.41±1.56*	83.60±9.61*
2.	Soil 2—South	21.33±2.18*	8.40±1.22*	3.10±1.20*	15.80±2.71*	77.25±8.83*
3.	Soil 3—East	56.04±6.21	23.06±5.40*	2.84±1.25*	5.16±2.34*	64.15±6.50*
4.	Soil 4—West	68.75±5.04	8.19±1.63*	3.34±5.26*	12.62±2.49*	60.32±7.11*
5.	Soil 5—S-E	34.16±4.91	8.01±1.52*	2.61±1.97*	2.53±1.18*	52.50±5.72*
6.	Soil 6—S-V	21.90±3.75*	14.61±2.71*	3.09±1.74*	30.00±2.22*	81.72±7.40*
7.	Soil 7—N-V	106.43±5.96*	12.53±2.67*	3.55±2.53*	36.16±4.07*	102.17±17.0*
8.	Soil 8—N-E	108.32±4.74*	3.67±1.40*	3.47±1.05*	6.12±1.43*	95.08±12.48*
9.	Soil 9—Center	26.30±3.40*	14.68±2.08*	3.56±3.68*	27.59±3.05*	80.46±10.95*
Normal values [156]		***20***	***20***	***1***	***100***	***5***
Alert threshold [156]	***Sensitive***	***50***	***100***	***3***	***300***	***15***
	Less sensitive	***250***	***250***	***5***	***700***	***25***
Intervention threshold [156]	***Sensitive***	***100***	***200***	***5***	***600***	***25***
	Less sensitive	***1000***	***500***	***10***	***1500***	***50***

** The values obtained for soil were reported against the alert threshold – sensitive (the first signal that soil pollution could negatively impact agriculture), with a significance level defined as* $p < 0.01$.

To determine the content of metallic cations in vegetation and the degree of their translocation from soil to plants, soil samples were analyzed. From Table 2.2.53, for most metals, the normal value concentrations are exceeded in the soil from the Roșia Montană area. The maximum lead content recorded was 108.32 mg/kg in soil sample no. 8, collected in the northeast of Roșia Montană. This value slightly exceeds the established intervention threshold, which is 100 mg/kg. Zinc was present at a maximum concentration of 36.1 mg/kg, not exceeding the maximum permissible concentration, while copper was within normal limits, with a determined value of 23.0 mg/kg. The range of Cd^{2+} content in the soil varied between 2.6 and 3.7 mg/kg, values that do not exceed the intervention threshold but are above the normal value of 1 mg/kg. Regarding As^{3+} content, the maximum permissible limits were exceeded at all sampling points, with the highest value recorded in the northern area of Roșia Montană — 102.1 mg/kg of As^{3+}.These elevated concentrations highlight the ongoing environmental pressure in the area and suggest a potential risk of metal transfer through the food chain.

River Water Analysis

Aquatic systems are highly sensitive to pollution inputs, as they integrate contaminants from multiple sources, including surface runoff, atmospheric deposition, and mining effluents.

The water quality of the Roșia Montană River, which flows through the area, was investigated. The study was conducted by testing the river water for inorganic contaminants, as well as common physicochemical and microbiological parameters. The results obtained are presented in Tables 2.2.54 and 2.2.55. Samples were collected at different times of the year, depending on the season, and also across different years, in order to capture variations in the river's flow. During periods of high flow, such as during snowmelt or other periods of abundant water, pollutant concentrations are generally lower.

Table 2.2.54. Results of the analyses for monitoring chemical parameters – metalloids (µg/L) in the Roșia Montană River water.

Sampling Location	**Month of Sampling**	**Pb^{2+}**	**Cu^{2+}**	**Cd^{2+}**	**Zn^{2+}**	**As^{3+}**	**Fe^{2+}**
River 1A – at the source	February	ND	ND	27.08 ± 4.05	48.42 ± 10.03	ND	150.44 ± 40.08
	May	ND	ND	20.33 ± 0.90	65.18 ± 13.19	ND	104.03 ± 71.62
	August	ND	ND	55.72 ± 0.61	51.04 ± 17.11	ND	230.20 ± 98.34
	November	ND	ND	41.16 ± 0.82	53.22 ± 9.31	ND	167.05 ± 43.06
River 1B – midstream	February	ND	374.08 ± 11.10	50.89 ± 0.40	107.05 ± 25.00	117.20 ± 1.30	23.790.42 ± 308.26
	May	11.00 ± 0.50	820.23 ± 27.09	20.51 ± 0.22	1632.40 ± 62.83	134.44 ± 2.74	38.901.97 ± 5251.04
	August	11.00 ± 0.40	903.47 ± 41.68	60.13 ± 0.53	1890.27 ± 33.62	148.07 ± 1.95	44.024.08 ± 911.63
	November	ND	210.30 ± 18.22	40.09 ± 0.30	1903.08 ± 50.20	75.06 ± 4.22	29.162.36 ± 523.05
Water quality class [33]	***I***	***5***	***20***	***0.5***	***100***	***10***	***300***
	II	***10***	***30***	***1***	***200***	***20***	***500***
	III	***25***	***50***	***2***	***500***	***50***	***1000***
	IV	***50***	***100***	***5***	***1000***	***100***	***2000***
	V	***>50***	***>100***	***>5***	***>1000***	***>100***	***>2000***

Abbreviation: ND – Not Detectable

These dissolved parameters (Cu^{2+}, Fe^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and As^{3+}) are used in water quality monitoring to assess pollution levels and ensure compliance with environmental and health standards. At the source of the Roșia Montană River (sample 1A), only the concentrations of iron, zinc, and cadmium exceed normal levels, whereas downstream, the values indicate severe water pollution with copper, iron, zinc, cadmium, and arsenic, regardless of the season.

The Cd^{2+} concentration ranged from 27.08 µg/L in February to 55.72 µg/L in August, showing a notable increase during the warmer months. Zn^{2+} levels fluctuated between 48.42 µg/L in February and 65.18 µg/L in May, before slightly decreasing to 51.04 µg/L in August. Fe^{2+} exhibited relatively high concentrations, reaching 230.20 µg/L in August, and remained elevated throughout the year, ranging from 150.44 µg/L in February to 167.05 µg/L in November.

For river 1B, located midstream, Pb^{2+} concentrations became detectable starting in February, around 11.00 µg/L, and remained at similar levels throughout the year. Cu^{2+} concentrations were much higher compared to river 1A, peaking at 903.47 µg/L in August, with values of 820.23 µg/L recorded in

May. Cd^{2+} levels were also higher in river 1B, ranging from 50.89 µg/L in February to 60.13 µg/L in August, with the lowest value recorded in May (20.51 µg/L). Zn^{2+} concentrations were significantly elevated in river 1B, especially in May and August, reaching 1632.40 µg/L and 1890.27 µg/L, respectively. As^{3+} levels showed a steady increase, from 117.20 µg/L in February to 148.07 µg/L in August. Fe^{2+} concentrations were alarmingly high, particularly in May and August, peaking at 44,024.08 µg/L in August. Such conditions are characteristic of acid mine drainage and represent a severe threat to aquatic life, often leading to reduced biodiversity and ecosystem degradation.

When comparing these results to water quality classifications, which range from Class I (highest water quality) to Class V (lowest), data from river 1A generally indicate good water quality. Most metal concentrations are well below the limits for Class V, suggesting relatively low pollution levels. However, cadmium levels during the summer months and the elevated iron concentrations remain a concern, occasionally falling into Class III.

In contrast, river 1B shows a significant decline in water quality, as the concentrations of Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, As^{3+}, and Fe^{2+} exceed all Class III limits and approach or surpass thresholds for Class IV and Class V in some months, especially in August. This indicates that as the water flows downstream, it becomes increasingly polluted, particularly with metals such as copper, zinc, and iron.

Table 2.2.55. Values of chemical and microbiological parameters recorded in the Roșia Montană River at the source and downstream of the locality.

Sampling Location	**Month of Sampling**	**pH**	**SO_4^{2-}, mg/L**	**DO, mgO_2/L**	**BOD_5, mgO_2/L**	**COD_{Mn}, mg O_2/L**	**Identified microorganisms (genus)**
River 1A – at the source	February	7.80 ± 0.08	48.22 ± 4.11	9.05 ± 0.29	0.48 ± 0.02	25.01 ± 5.14	Developed fungi: yeasts and molds (*Mucor* spp.)
	May	7.42 ± 0.07	21.01 ± 2.00	9.47 ± 0.62	1.04 ± 0.31	17.90 ± 3.02	
	August	6.34 ± 0.08	33.16 ± 2.53	9.80 ± 0.33	0.19 ± 0.01	21.22 ± 3.81	
	November	7.05 ± 0.08	29.58 ± 1.82	9.53 ± 0.49	0.57 ± 0.03	17.43 ± 2.66	
River 1B – midstream	February	3.87 ± 0.05	1307.10 ± 60.34	9.66 ± 0.66	3.12 ± 1.00	10.10 ± 1.90	The filter residue inhibited the growth of microorganisms
	May	3.59 ± 0.06	1239.47 ± 44.71	8.40 ± 0.41	2.03 ± 0.52	8.20 ± 1.57	
	August	2.80 ± 0.04	1197.88 ± 35.40	10.50 ± 0.58	2.62 ± 0.61	12.71 ± 2.23	
	November	3.76 ± 0.05	1076.29 ± 20.22	9.32 ± 0.97	2.67 ± 0.43	6.64 ± 1.28	
Water quality class [33]	***I***	***6.5–8.5***	***60***	***8***	***3***	***5***	-
	II		***120***	***7***	***5***	***10***	
	III		***250***	***5***	***7***	***20***	
	IV		***300***	***4***	***20***	***50***	
	V		***>300***	***<4***	***>20***	***>50***	

Abbreviations: SO_4^{2-} — sulfates; DO — dissolved oxygen; BOD_5 — biochemical oxygen demand; COD_{Mn} — chemical oxygen demand.

Regarding the parameters pH, sulfates, dissolved oxygen, biochemical oxygen demand, and chemical oxygen demand, the values obtained at the source (sample 1A) are within normal limits, but downstream (sample 1B), the results indicate significant water pollution.

The pH level is one of the most important indicators of water quality, reflecting its acidity or alkalinity. In River 1A, pH ranges from 6.34 in August to 7.80 in February, all within an acceptable range for most aquatic organisms. However, in River 1B, due to acidic mine waters, pH varies between 2.8 and 3.87, indicating extreme acidity, which can be harmful to aquatic life, disrupting the metabolic processes of fish and aquatic macrophytes specific to rivers.

SO_4^{2-} concentrations in River 1A range from 21.01 mg/L in May to 48.22 mg/L in February, well within Class II water quality limits. In contrast, downstream in the Roșia Montană River, values range from 1076.29 mg/L to 1307.1 mg/L, with the highest concentrations observed near mine drainage discharge zones. This dramatic increase indicates contamination and may lead to water acidification and the formation of toxic sulfur compounds that disrupt the aquatic ecosystem.

Assessment of the oxygen regime indicators does not show large variations. The Roșia Montană River, flowing through a mountainous area, undergoes natural aeration, enriching the water with oxygen due to turbulence and intense contact with the atmosphere.

Dissolved oxygen is essential for supporting life in aquatic ecosystems; higher values indicate better water quality. In River 1A, DO concentrations are consistently high, ranging from 9.05 mg/L in February to 9.80 mg/L in August, supporting healthy aquatic organisms. In River 1B, DO values are lower and fluctuate between 8.40 mg/L in May and 10.50 mg/L in August, still supporting life but suggesting a more stressed system, especially during summer when oxygen demand is higher.

Biochemical oxygen demand (BOD$_5$) measures the amount of oxygen consumed by microorganisms in the decomposition of organic matter. In River 1A, BOD$_5$ values range from 0.48 mg/L in February to 1.04 mg/L in May, indicating low organic pollution. However, in River 1B, BOD$_5$ levels are significantly higher, particularly in February, reaching 3.12 mg/L. This suggests a higher presence of organic contaminants in the water, which can deplete oxygen levels and disrupt aquatic life if not controlled.

Another important indicator of total pollution load is COD$_{Mn}$. In River 1A, COD$_{Mn}$ levels fluctuate between 17.43 mg/L in November and 25.01 mg/L in February, indicating the presence of pollutants requiring oxygen for decomposition. In River 1B, COD$_{Mn}$ values are slightly lower but still concerning, ranging from 6.64 mg/L in November to 12.71 mg/L in August. These elevated values suggest the presence of pollutants that may not be easily biodegradable. COD$_{Mn}$ specifically indicates the presence of oxidizable chemical substances, such as inorganic compounds.

Regarding microorganisms in the source water, fungi (*Mucor* spp.) and yeasts were identified, indicating organic pollution. Downstream, due to highly toxic acidic mine waters with low pH and high heavy metal concentrations, filter residue inhibited microorganism growth.

For this study, one sampling point in the Roșia Montană River was monitored monthly in the central area of the locality throughout 2023. Table 2.2.56 presents the values of microbial load and nutrient regime over time.

Table 2.2.56. Experimental results obtained for water sampled from the Roșia Montană River

Time [month]		**TVC/mL**	**NH_4^+-N, µg N/L**	**NO_3^--N, µg N/L**	**NO_2^--N, µg N/L**
January		278	21.40 ± 1.09	144.05 ± 23.20	1.92 ± 0.34
February		224	26.01 ± 1.22	280.43 ± 19.16	2.10 ± 0.42
March		294	35.32 ± 3.16	352.29 ± 36.08	3.74 ± 0.90
April		303	101.18 ± 2.44	761.77 ± 20.35	11.03 ± 0.51
May		422	424.29 ± 37.35	1040 ± 105.00	14.21 ± 0.74
June		450	2050.00 ± 20.91	1222.16 ± 77.14	13.80 ± 0.93
July		536	1507.17 ± 18.05	1303.52 ± 149.04	15.32 ± 1.10
August		640	1162.43 ± 15.40	781.67 ± 40.36	12.93 ± 0.22
September		505	490.20 ± 46.28	850 ± 51.27	14.60 ± 1.07
October		492	248.15 ± 44.37	513.63 ± 24.09	10.53 ± 0. 83
November		312	303.46 ± 58.06	130.55 ± 11.21	2.44 ± 0. 09
December		256	149.33 ± 5.44	242.58 ± 48.07	1.01 ± 0.05
Water [30]	***Salmonid***	***-***	***≤40***	***-***	***≤10***
	Cyprinid	***-***	***≤200***	***-***	***≤30***
Water quality class [33]	***I***	***-***	***400***	***1000***	***10***
	II	***-***	***800***	***3000***	***30***
	III	***-***	***1200***	***5600***	***60***
	IV	***-***	***3200***	***11,200***	***300***
	V	***-***	***>3200***	***>11,200***	***>300***

Abbreviations: CFU—colony-forming units; NH^{4+}*-N—ammonium nitrogen;* NO^{3-}*-N—nitrate nitrogen;* NO^{2-}*-N—nitrite nitrogen.*

Exceedances of permissible limits were observed in water samples collected from the Roșia Montană river. These exceedances occurred primarily during the summer months. The highest recorded CFU count was 640 colonies in August 2023, while the maximum concentrations of nitrates, nitrites, and ammonium ions were observed in June and July.

Vegetation Analysis

Plants serve as essential bioindicators of environmental quality, as they directly interact with contaminated soil and water, reflecting pollutant bioavailability.

Roșia Montană is an area of remarkable biodiversity, located in the Apuseni Mountains, Romania, surrounded by forests, meadows, and traditional agricultural lands. The ecosystems in this region include a wide range of plant species, many of which are specific to this mountainous environment. The vegetation consists of deciduous and coniferous forests, alpine meadows, endemic species, as well as numerous medicinal plants and vegetables traditionally cultivated in local gardens [157]. This biodiversity is supported by traditional agricultural and pastoral practices, which help maintain natural habitats and protect local species.

The analysis of the samples was was performed by flame atomic absorption spectrometry, using a Perkin Elmer AA700 atomic absorption spectrometer. To assess the degree of vegetation contamination, lead (Pb), copper (Cu), and cadmium (Cd) were selected as indicators of pollution. Table 2.2.57 presents the values obtained for the samples analyzed in the Roșia Montană area.

Table 2.2.57. Heavy metal content: lead, cadmium, and copper (mg/kg) in mixed vegetables..

Sample	**Sample Name**	**Pb^{2+}**	**Cu^{2+}**	**Cd^{2+}**
1	Mixed vegetables 1—North	0.30 ± 0.04	1.15 ± 0.09 *	0.02 ± 0.01 *
2	Mixed vegetables 2—South	0.14 ± 0.03 *	0.73 ± 0.24	0.10 ± 0.02
3	Mixed vegetables 3—East	0.13 ± 0.02 *	1.15 ± 0.13 *	0.01 ± 0.00 *
4	Mixed vegetables 4—West	0.25 ± 0.09	1.08 ± 0.06 *	0.01 ± 0.00 *
5	Mixed vegetables 5—S-E	0.56 ± 0.11	1.27 ± 0.31	0.03 ± 0.01 *
6	Mixed vegetables 6—S-V	0.42 ± 0.04	1.32 ± 0.20 *	0.12 ± 0.04 *
7	Mixed vegetables 7—N-V	0.72 ± 0.07 *	1.51 ± 0.05 *	0.15 ± 0.04
8	Mixed vegetables 8—N-E	0.38 ± 0.06	1.48 ± 0.08 *	0.11 ± 0.02 *
9	Mixed vegetables 9—Center	0.79 ± 0.13	0.80 ± 0.17	0.17 ± 0.04
Maximum levels (mg/kg fresh weight) [33]*		*0.20*	*-*	*0.05 **
Maximum permissible limits for vegetables (mg/kg) [34,35]		*0.30*	*40.0*	*0.10*

** The values obtained for vegetables were reported in accordance with the limits specified in Codex Stan 193/1995, the General Standard for Contaminants, with a significance level defined as $p < 0.01$. Note: For peeled potatoes (Solanum tuberosum), the maximum level is 0.10 mg/kg fresh weight. According to Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, established by the Codex Alimentarius Commission [47]. A total of 54 samples were analyzed (n = 6 replicates per food product).*

The water content of fresh carrots (*Daucus carota*) ranges between 88–90%, onions between 85–90%, potatoes between 75–80%, and kohlrabi approximately 90%. In their dehydrated form, the water content of onions, potatoes, and kohlrabi varies between 5–10%, while for carrots it ranges between 5–8%. To calculate the conversion factor (CF), the following formula was applied: CF = (100% − water content in fresh vegetables) / (100% − water content in processed vegetables).

Considering an average initial water content of 86% and a final value of 7%, the resulting CF was 0.15. The heavy metal concentrations in vegetables prior to dehydration were determined by multiplying the measured values by the conversion factor.

According to Table 2.2.57, lead concentrations in mixed vegetable samples collected from the Roșia Montană area exceeded the limit of 0.2 mg/kg (as specified in the 2023 updated version of Commission Regulation (EC) No 1881/2006) in samples 1 and 5 to 9, confirming that pollution represents a relevant environmental issue. The highest values (0.72 and 0.79 mg/kg) were recorded in samples collected from the north-western area and the town center.

Exceedances were also observed for cadmium in 5 out of the 9 samples, located approximately in the same areas as those with elevated lead levels, with the maximum value (0.17 mg/kg) recorded in the sample collected from the town center. This can be explained by the fact that soil samples also exceeded the maximum permissible concentrations, indicating soil contamination in the sampling areas, which is subsequently transferred to vegetation.

This transfer of contaminants from soil to edible plants raises important concerns regarding food safety and the chronic exposure of local populations. The results highlight the potential adverse health effects associated with the consumption of vegetables contaminated with heavy metals.

Copper was not found at elevated concentrations in the analyzed samples. It is an essential micronutrient for all living organisms; however, both excessive and deficient dietary intake may lead to health issues, although it is naturally present in many foods. Regarding intake levels, the European Food Safety Authority concluded that copper retention is not expected at intakes up to 5 mg/day and established an acceptable daily intake (safe level) of 0.07 mg/kg body weight for the adult population [158].

Propolis Analysis

Propolis represents a complex biological matrix that integrates environmental information from multiple sources, making it a valuable tool for monitoring environmental pollution.

The analysis of the physicochemical composition of propolis is important for determining its quality, especially when it is considered an indicator of environmental pollution in a given area. The results of the physicochemical characterization of raw propolis samples analyzed in the Roșia Montană area are presented in Tables 2.2.58 and 2.2.59.

Table 2.2.58. Determination of the physical properties of brown propolis samples from Roșia Montană.

Sample	**a_w**	**Moisture, %**	**Hygroscopicity, g H_2O/100 g Propolis**	**MP, °C**	**Density, g/cm^3**	**Dry matter, %**
S1	0.63 ± 0.08	5.33 ± 0.25	13.3 ± 0.3	65.2 ± 0.3	1.104 ± 0.003	94.67 ± 0.20
S2	0.62 ± 0.09	6.24 ± 0.19	13.8 ± 0.7	62.7 ± 0.2	0.983 ± 0.004	93.76 ± 0.23
S3	0.70 ± 0.04	5.18 ± 0.33	13.0 ± 0.5	63.4 ± 0.3	1.097 ± 0.005	94.82 ± 0.24
S4	0.71 ± 0.10	5.95 ± 0.28	13.5 ± 0.4	64.1 ± 0.2	1.055 ± 0.008	94.05 ± 0.28
S5	0.66 ± 0.07	7.67 ± 0.11	13.7 ± 0.8	63.6 ± 0.1	0.996 ± 0.007	92.33 ± 0.22
S6	0.65 ± 0.03	6.93 ± 0.24	12.9 ± 0.6	64.3 ± 0.2	1.159 ± 0.003	93.06 ± 0.19
S7	0.64 ± 0.05	5.70 ± 0.12	13.1 ± 0.5	65.0 ± 0.3	0.982 ± 0.004	94.30 ± 0.21
S8	0.66 ± 0.06	6.83 ± 0.26	13.4 ± 0.8	62.9 ± 0.2	1.080 ± 0.005	93.17 ± 0.25
S9	0.67 ± 0.09	6.12 ± 0.30	13.7 ± 0.4	63.5 ± 0.2	1.034 ± 0.006	93.88 ± 0.28

Abbreviations: a_w — water activity; MP — melting point.

Regarding water activity, the samples showed values ranging from 0.62 to 0.71. Moisture content varied between 5.33% and 7.67%. Concerning the hygroscopicity of the samples, which provides information on their capacity to absorb and retain moisture from the environment, values ranged from 12.9 to 13.8 g H_2O/100 g propolis. The melting point ranged between 62.7 and 65.2 °C. The melting point is essential for evaluating the authenticity, composition, purity, and overall quality of propolis.

Propolis density depends on the botanical source and geographic region, with recorded values ranging from 0.982 to 1.159 g/cm^3. All physical parameters are important for assessing propolis quality, including the dry matter content, which ranged between 92.33% and 94.82%.

Table 2.2.59. Determination of the chemical properties of brown propolis samples from Roșia Montană.

Sample	**Ash, g/100g**	**Wax, %**	**Water solubility, %**	**VO, %**	**OI, s**	**Reziduu insolubil în etanol, %**	**Phenols, mg GAE/g**	**Flavonoids, mg QE/g**	**IC_{50}, µg/mL**
S1	2.50±0.00	25.84±0.57	11.46±0.79	0.4±0.06	12.5±1.4	16.4±0.8	189.4±5.82	84.31±0.09	0.333±0.002
S2	2.85±0.08	37.18±0.81	10.90±0.62	0.3±0.04	12.3±2.1	17.1±0.6	180.8±4.54	78.26±0.07	0.514±0.016
S3	2.96±0.04	40.56±1.06	12.55±0.13	0.2±0.03	11.8±1.1	16.8±0.9	172.9±3.25	78.55±0.08	0.725±0.003
S4	3.15±0.06	33.22±0.38	11.16±0.45	0.3±0.08	12.5±1.9	16.5±0.2	189.5±4.83	87.84±0.11	0.669±0.010

S5	3.28±0.09	46.33±1.05	10.27±0.50	0.2±0.01	10.9±2.0	16.9±0.4	193.4±7.22	86.06±0.08	0.884±0.028
S6	2.55±0.05	37.41±0.58	10.82±0.31	0.3±0.02	11.7±1.3	16.7±0.7	129.6±3.58	68.59±0.09	0.964±0.031
S7	2.73±0.03	31.19±0.71	11.38±0.24	0.4±0.07	11.4±1.5	17.0±0.8	184.3±6.04	82.27±0.25	0.086±0.001
S8	2.69±0.04	32.52±0.44	12.04±0.33	0.2±0.03	12.6±1.7	16.6±0.3	152.2±6.80	70.10±0.16	0.517±0.04
S9	3.08±0.03	28.92±0.67	11.61±0.46	0.3±0.05	13.1±1.8	16.2±0.5	157.1±5.57	74.35±0.36	0.615±0.05

Abbreviations: VO — volatile oils; OI — oxidation index; GAE — gallic acid equivalents; QE — quercetin equivalents; IC_{50} *— half-maximal inhibitory concentration (DPPH assay).*

For the analyzed chemical parameters, no significant differences were observed among the propolis samples from the Roșia Montană area. The ash content ranged from 2.5 g/100 g (sample 1) to 3.28 g/100 g of product, reflecting the mineral and inorganic element content. Ash is an indicator of purity and mineral composition. Wax content varied depending on the sampling location, with the lowest value recorded at 25.84% and the highest at 46.33%. High wax content may indicate a lower proportion of biologically active compounds, such as phenols and flavonoids. Water solubility ranged from 10.9% to 12.55%. This parameter determines how easily active compounds can be extracted from propolis in aqueous solutions, which is important for phytotherapy and the dietary supplement industry. Volatile oils ranged from 0.2% to 0.4%, contributing to the characteristic aroma of propolis and potentially exhibiting antimicrobial and antioxidant properties. The oxidation index (10.9–13.1 s) indicates the resistance of propolis to oxidative degradation. Low values suggest increased susceptibility to oxidative degradation, which may affect the stability of active compounds. Ethanol-insoluble residue ranged from 16.2% to 17.1%, representing the amount of material insoluble in ethanol. This is an indicator of propolis purity, and high values may suggest the presence of impurities or less active bioactive components.

Phenols and flavonoids are potent antioxidants that contribute to the antimicrobial, antioxidant, and anti-inflammatory properties of propolis. The lowest phenol content was found in sample S6 (129.6 mg GAE/g), while the highest was observed in sample S5 (193.4 mg GAE/g). Flavonoid content ranged from 68.59 mg QE/g (S6) to 87.84 mg QE/g (S4). The antioxidant capacity of propolis, measured as the concentration required to inhibit 50% of free radical activity (IC_{50}), ranged from 0.086 to 0.964 µg/mL. Lower IC_{50} values indicate stronger antioxidant activity.

Water activity and moisture content of propolis are important parameters for assessing product stability, microbial growth potential (indicating whether the environment is suitable for most microorganisms), and the likelihood of chemical reactions occurring in the product [159]. Moisture content of the samples is an important indicator of propolis quality and stability.

Flavonoids exhibit a wide range of biochemical properties; however, their most extensively characterized attribute across almost all flavonoid groups is their ability to act as antioxidants. This antioxidant activity is largely determined by the specific arrangement of functional groups within their central nuclear structure [160]. The structural characteristics of flavonoids influence their activity in vegetables, particularly due to the presence of pollutants. Owing to their specific chemical structure, flavonoids can readily chelate metal ions and form complex compounds [161].

Monchanin et al. [162] demonstrated the harmful effects of metal pollutants at a historic mining site, particularly arsenic, on the behavior and cognition of honeybees, even at low exposure levels. Their results indicated neurodevelopmental issues and raised serious concerns regarding the health of bee populations in areas contaminated with potentially harmful elements.

Toxic particles adhere to the bodies of bees or are ingested. Furthermore, chemical analysis of apicultural products revealed accumulations of metal cations in regions affected by mining activities in Romania, such as the Arieș River basin, where concentrations of Cu^{2+}, Zn^{2+}, and As^{3+} increased due to mining discharges [163].

The amounts of trace metal cations (Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, As^{3+}, Sr^{2+}) obtained for all analyzed samples, as presented in Table 2.2.60, are expressed in mg/kg.

Table 2.2.60. Mineral content (mg/kg) of propolis samples.

Metal cation **Sample**	**Pb^{2+}**	**Cu^{2+}**	**Cd^{2+}**	**Zn^{2+}**	**As^{3+}**	**Sr^{2+}**
S1	8.26 ± 0.68	4.03 ± 0.22	0.16 ± 0.04	3.12 ± 0.19	0.97 ± 0.30	7.78 ± 0.86
S2	7.04 ± 0.52	4.44 ± 0.81	0.15 ± 0.08	5.71 ± 0.36	0.79 ± 0.14	9.92 ± 0.54
S3	10.14 ± 0.87	6.32 ± 0.56	0.14 ± 0.04	4.04 ± 0.60	1.04 ± 0.28	12.06 ± 0.92
S4	5.60 ± 0.42	3.21 ± 0.47	0.14 ± 0.08	3.45 ± 0.36	0.64 ± 0.18	5.48 ± 0.42
S5	8.42 ± 0.33	5.07 ± 0.75	0.15 ± 0.04	6.09 ± 0.65	0.42 ± 0.13	7.75 ± 0.75
S6	5.01 ± 0.47	2.10 ± 0.24	0.05 ± 0.09	3.36 ± 0.48	0.77 ± 0.25	7.32 ± 0.64
S7	4.65 ± 0.63	3.68 ± 0.12	0.10 ± 0.06	4.50 ± 0.22	0.18 ± 0.04	8.40 ± 0.87
S8	4.19 ± 0.30	1.29 ± 0.54	0.07 ± 0.05	3.91 ± 0.99	0.51 ± 0.10	4.16 ± 0.25
S9	5.72 ± 0.90	4.01 ± 0.55	1.15 ± 0.18	3.28 ± 0.17	0.32 ± 0.09	5.01 ± 0.35

The metal content of propolis varies significantly between samples and is not legally regulated. Nevertheless, the obtained values were correlated with those of metal cations in soil, water, and vegetables, providing insights into the degree of contamination and the geographical origin of the propolis samples, reflecting the impact of anthropogenic activities and environmental conditions on their composition.

Lead content was highest in sample S3 (10.14 mg/kg) and lowest in S8 (4.19 mg/kg). Copper was most abundant in S3 (6.32 mg/kg) and lowest in S8 (1.29 mg/kg). Cadmium reached a maximum in S9 (1.149 mg/kg) and a minimum in S6 (0.048 mg/kg). Arsenic was highest in S3 (1.037 mg/kg) and lowest in S7 (0.182 mg/kg). Strontium exhibited extreme values in S3 (12.06 mg/kg) and S8 (4.16 mg/kg). Zinc content was maximal in S5 (6.0 mg/kg) and minimal in S1 (3.1 mg/kg). These data reflect compositional differences in propolis depending on its source from various regions within the Roșia Montană mining area. The presence of heavy metals in propolis reflects not only environmental contamination but also the interaction between abiotic and biotic components of the ecosystem.

Monchanin et al. [164] demonstrated that bees are unable to detect low concentrations of metals (arsenic, lead, or zinc) present in flowers; therefore, contamination with these elements represents a major threat to pollinators.

Vegetation, as an indicator of pollution, is a useful tool for assessing the negative impact of pollutants on the environment, as it can reflect changes in air, soil, and water quality in areas affected by industrial activities such as mining [165].

The effects of pollutants from mining areas on vegetation can aid in monitoring a wide range of environmental factors and provide valuable information regarding ecosystem health. Plants are sensitive to chemical changes in their environment, and the accumulation of metal cations, such as arsenic, cadmium, or mercury, in soil and water can affect photosynthesis, growth, and overall development of vegetation [166]. In addition, atmospheric pollution with fine particles or toxic gases can lead to leaf discoloration, reduced resistance to diseases and pests, and even plant death. Therefore, analyzing the condition of vegetation in these areas can provide a rapid and effective indicator for identifying and assessing the impact of industrial pollution on the environment.

Table 2.2.61 presents the *p* values obtained using the Tukey test to evaluate the significance of differences in heavy metal content (Pb^{2+}, Cu^{2+}, Cd^{2+}) across different matrices (propolis, soil, water, and vegetables).

Table 2.2.61. *p* values from the Tukey test for heavy metal content in propolis, soil, water, and vegetables.

Treatment pair	**Tukey *p*-value**		
	Pb^{2+}	**Cu^{2+}**	**Cd^{2+}**
Propolis—Soil	0.001	0.899	0.658
Propolis—Water	0.899	0.001	0.001
Propolis—Vegetables	0.899	0.899	0.899

A significant difference was observed between the Pb^{2+} content in propolis and that in soil (p =

0.001). The observed differences are not random, indicating a relationship between the amount of Pb^{2+} in soil and that in propolis in the studied area. However, no significant differences were found between Pb^{2+} concentrations in propolis and water ($p = 0.899$) or between propolis and vegetables ($p = 0.899$). For Cu^{2+} and Cd^{2+}, a significant difference was detected between their content in propolis and in water ($p = 0.001$), highlighting a clear variation of Cu^{2+} and Cd^{2+} levels between these matrices. In contrast, no significant differences were observed in Cu^{2+} and Cd^{2+} content between propolis and soil ($p = 0.899$ and $p = 0.658$, respectively) or between propolis and vegetables ($p = 0.899$).

A satisfactory correlation was observed between the presence of metal cations in propolis and their content in soil, mixed vegetables, and water in the area, considering that the foraging range of a bee is approximately 3 km around the hive.

The findings emphasize the interconnected nature of environmental compartments, where soil, water, plants, and biological organisms collectively contribute to the transfer and accumulation of pollutants. Variations in heavy metal content in propolis are closely linked to the proximity of pollution sources. For example, lead, zinc, copper, and arsenic often accumulate in soils near mining operations due to the release of pollutants during extraction and processing. In these areas, metal content in plants—including resins collected by bees for propolis production—is elevated due to higher soil contamination and bioavailability.

Potential pathways for heavy metal accumulation in propolis involve interactions between soil, water, and plants. Metals enter soils through natural processes such as mineral erosion or anthropogenic activities including mining, agriculture, or industrial emissions. Once in the soil, metals can be absorbed by plants and translocated into tissues, including resins and exudates collected by bees. Water bodies can also act as channels for metals, mobilizing them from soils to rivers and streams, indirectly contributing to plant contamination.

These results highlight the need for continuous environmental monitoring in mining-affected areas and underscore the importance of sustainable management strategies to mitigate long-term ecological and human health risks. Bees and their products, particularly propolis, provide a practical, non-invasive tool for assessing environmental contamination over wide geographical areas. By analyzing metal cation content in propolis, the extent and type of environmental pollution can be effectively monitored.

Mining activities in the Roșia Montană area have led to significant contamination of soil, water, and vegetation with heavy metals, which is also reflected in propolis. Maximum concentrations of several trace metals in the studied matrices often exceeded health-based guideline values, with tailings, spills, and sediments from mining ponds being the primary sources.

The use of bees as bioindicators is advantageous due to their wide foraging range and their ability to integrate environmental exposure across multiple matrices. Propolis, as a product of bee-collected plant resins, effectively reflects the accumulation of metals in the surrounding ecosystem.

These findings demonstrate that propolis can serve as a valuable, natural bioindicator for monitoring ecological health and detecting anthropogenic impacts in mining regions. Continuous monitoring using bee products can inform management strategies and support the mitigation of environmental and human health risks in contaminated areas.

2.2.5. Conclusions

The research presented in this subsection demonstrates the essential role of honey and propolis both as sensitive bioindicators of environmental pollution and as natural antimicrobial agents with therapeutic potential. Analyses performed on samples collected from mining areas (Roșia Montană, Zlatna, Certeju de Sus, etc.) and protected areas in Transylvania revealed clear correlations between the degree of environmental contamination and the chemical, microbiological, and biological quality of bee products.

Regarding the quality of honey and propolis under the influence of pollution, the physicochemical parameters (moisture, pH, acidity, HMF, electrical conductivity) of honey generally fell within legal limits, with exceptions in samples from heavily polluted areas (Zlatna, Teiuș, Abrud), where increased values of HMF and acidity were recorded, correlated with thermal stress and environmental contamination. The content of phenols and flavonoids varied significantly depending on geographical origin, being lower in mining areas. All honey samples and aqueous propolis extracts showed antifungal activity against the tested strains (*Aspergillus*, *Penicillium*, *Candida*, *Fusarium*, *Rhizopus*), but samples from protected areas exhibited larger inhibition zones and superior antioxidant activity.

Using honey and propolis as bioindicators of pollution, comparison between samples from mining areas and protected regions revealed significant differences:

- Heavy metals: The content of Pb, Cd, As, Cu, Zn, and Fe was significantly higher in honey and propolis from mining areas (e.g., Roșia Montană, Zlatna), often exceeding permissible limits. Propolis from Roșia Montană accumulated Pb up to 10.14 mg/kg and As up to 1.04 mg/kg.
- Biological activity: Propolis from protected areas showed higher phenolic content (up to 193 mg GAE/g) and flavonoid content (up to 90 mg QE/g), as well as stronger antioxidant and antimicrobial activity. Pearson correlations confirmed negative relationships between heavy metal concentrations and bioactive compound content.
- Statistical analyses (PCA, ANOVA): These highlighted a clear separation between samples from mining areas and those from protected areas, confirming the impact of pollution on the quality and therapeutic properties of bee products.

General conclusions

- Honey and propolis are effective and integrative bioindicators of environmental pollution, reflecting the accumulation of heavy metals from soil, water, and vegetation within a radius of up to 3 km around the hive.
- Pollution in mining areas significantly reduces the content of bioactive compounds (phenols, flavonoids) and diminishes the antioxidant and antimicrobial properties of bee products.
- Propolis from protected areas exhibits superior quality and stronger biological activity, highlighting the importance of conserving natural ecosystems to maintain the therapeutic value of bee products.
- Antimicrobial activity is influenced by geographical origin, with samples from unpolluted areas showing higher efficiency against pathogenic bacteria and fungi.

2.3 Circular Valorization of Waste Cooking Oils into Antimicrobial Soaps Integrated with Environmental Risk Assessment under the ISO 14001 Framework

Purpose and Objectives

The main aim of this study was to develop and evaluate a sustainable approach for the valorization of waste cooking oils (WCOs) through their transformation into functional soaps with improved physicochemical and antimicrobial properties. The research focuses on reducing environmental pollution associated with improper disposal of WCOs while generating value-added products with practical applications.

The objectives of the study were to optimize the technological process for soap production from recycled oils, to characterize the obtained soaps in terms of physicochemical properties, and to assess their antimicrobial activity. Additionally, the study aimed to demonstrate that waste-derived raw materials can be effectively integrated into sustainable production systems, supporting the principles of circular economy and environmental protection.

The specific objectives are:

- To collect and pre-treat WCOs in order to obtain a suitable raw material for soap production.
- To develop and optimize the technological process for the conversion of WCOs into soaps.
- To formulate soaps with and without bioactive ingredients (honey, propolis, essential oils).
- To evaluate the physicochemical properties of the obtained soaps (pH, moisture, total solids, etc.).
- To investigate the antimicrobial activity of the formulated soaps against *E. coli* and *S. aureus*.
- To compare the performance of experimental soaps with a commercial reference product.
- To assess the environmental benefits of WCO valorization, particularly in terms of pollution reduction and waste minimization.
- To highlight the contribution of this approach to circular economy and sustainable development.

Preamble and Obtained Results

The improper disposal of WCOs represents a significant environmental issue, contributing to water and soil pollution and causing operational problems in sewage systems. In this context, their valorization into useful products such as soaps represents an efficient and sustainable alternative.

In the present study, WCOs were successfully processed into soaps through a controlled technological process involving purification, bleaching, and saponification. The obtained soaps exhibited physicochemical properties comparable to those of commercial products, indicating that recycled oils can be used without compromising product quality.

Furthermore, the incorporation of natural bioactive ingredients enhanced the antimicrobial activity of the soaps, with essential oil-based formulations showing the highest effectiveness. The results confirmed a clear dose–response relationship and highlighted differences in bacterial susceptibility, with *S. aureus* being more sensitive than *E. coli*.

The study demonstrates that the recycling of WCOs into functional soaps is not only technically feasible but also environmentally beneficial. This approach reduces the impact of waste oils, prevents pollution, and supports the transition toward a circular economy by transforming waste into high-value, eco-friendly products.

2.3.1. Sustainable Valorization of Waste Cooking Oils into Antimicrobial Soaps Enriched with Apicultural Products and Essential Oils

The use of WCOs as raw materials for soap production represents a valorization pathway that combines waste management with the generation of value-added functional products. WCOs are widely generated household and food-service residues, and their improper disposal is associated with environmental concerns, including water contamination, sewer blockages, and increased treatment costs. Converting this lipid-rich waste stream into soap contributes to waste reduction and mitigates the environmental burden associated with uncontrolled disposal.

From a resource-efficiency perspective, the valorization of WCOs aligns with circular economy principles by extending the life cycle of edible oils beyond their primary use. Instead of relying exclusively on virgin vegetable oils, which require agricultural land, water, and energy inputs, the proposed approach reintroduces a secondary raw material into the production cycle. This strategy supports more sustainable material use and reduces pressure on primary natural resources commonly employed in the oleochemical and soap industries [167].

The saponification process itself is well established, technically simple, and compatible with small- and medium-scale production, suggesting favorable prospects for scalability. From a preliminary economic standpoint, WCOs represent a low-cost and readily available feedstock compared to refined oils, potentially lowering the cost of raw materials in soap formulation. This aspect is particularly relevant for decentralized or community-based production models, where waste-derived inputs can enhance local value creation [168].

In addition to the valorization of WCOs, the incorporation of honey, propolis, and essential oils further contributes to the biobased character of the formulations. These natural additives are known for their antimicrobial properties and may reduce the need for synthetic antibacterial agents, which are increasingly scrutinized due to environmental persistence and potential health concerns. The observed antimicrobial activity of the experimental soaps indicates that functional performance can be achieved while maintaining a predominantly natural composition.

It should be noted that the present sustainability evaluation is qualitative and preliminary in nature. A comprehensive assessment, including quantitative life cycle analysis and techno-economic evaluation, would be required to fully substantiate the environmental and economic advantages of waste cooking oil-based antimicrobial soaps on an industrial scale. Nevertheless, the results support the concept that transforming WCOs into antimicrobial soaps constitutes a viable valorization strategy with promising sustainability implications.

WCOs represent one of the most abundant and complex categories of organic waste generated globally, as inevitable by-products of the modern food chain. Primarily originating from frying and cooking processes, these oils gradually lose their functional properties and, if not properly managed, become a major source of pollution. At the same time, waste cooking oils have valuable potential as a renewable raw material for the production of biofuels, polymeric materials, or "green" chemical agents [167]. WCOs, primarily produced after frying processes that exceed optimal usage time, represent a major portion of lipid residues from the food industry. Compared to virgin oils, WCOs have a more complex composition, containing degraded triglycerides, free fatty acids, oxidation and volatile compounds, metal traces, and food particles. This complexity requires thorough pretreatment and careful selection of recycling and valorization methods [169,170].

Globally, waste cooking oils are estimated to range between 41 and 67 million tons per year. Their abundance and composition highlight their potential as a valuable raw material for valorization processes within the framework of a circular economy [168]. Policies and management schemes dedicated to waste cooking oils are becoming increasingly relevant in the context of the transition toward a circular economy.

The valorization of WCOs through their transformation into soap represents a concrete example of applying the principles of sustainability and the circular economy. Instead of being improperly discarded and polluting the soil or water, these oils are reintegrated into the economic cycle as a useful and environmentally friendly product: soap [171]. This process reduces the negative environmental impact and minimizes waste and the consumption of new resources, thus contributing to a model of responsible production and consumption [172]. Furthermore, by preventing the contamination of water and soil, the initiative supports the goals related to clean water and sanitation [173]. At the same time, reusing existing resources instead of producing new raw materials reduces greenhouse gas emissions, thereby having a positive impact on climate action [174]. Thus, the transformation of WCOs into soap perfectly illustrates the transition to a circular economy, where waste becomes a resource and innovation supports sustainable development [175]. In the current context of the transition to sustainable production and consumption models, the valorization of WCOs has gained strategic importance in both environmental protection and the advancement of the circular economy. Recent studies underscore the potential of these residual streams to be reintegrated into local production cycles through the development of biodegradable soaps, thus generating added value while minimizing environmental impact [176-178].

From an environmental protection and sustainability perspective, the conversion of WCOs into biodegradable soaps simultaneously contributes to reducing the pollution risks associated with their discharge into sewage systems [179]. A recent study [180] has shown that it is technologically feasible to produce antimicrobial soaps from waste cooking oils enriched with essential oils, which exhibit good functional properties and biodegradability. From a sustainability perspective, a recent Life Cycle Assessment by Mwamba et al. [181] demonstrated that integrating WCOs with plant-based residues such as citrus peels in soap production significantly reduces environmental impacts compared to the use of virgin oils. The study also confirmed the environmental and economic advantages of this circular approach, highlighting its feasibility for small and medium-sized enterprises.

On an industrial scale, the use of NaCl for WCO purification could generate substantial saline wastewater. Implementing brine recovery, recrystallization, and appropriate wastewater treatment would be essential to ensure that the process remains environmentally sustainable [181].

From an economic and value-chain-resilience perspective, community initiatives that collect WCOs and convert them into soaps demonstrate a favorable cost–benefit ratio. These projects reduce waste management expenses while generating local micro-income. Municipal programs offering training and demonstration workshops have fostered household and small-scale adoption, enhancing community engagement and reducing uncontrolled discharges [182].

These bio-inspired approaches contribute to the simultaneous valorization of multiple waste streams—WCOs and plant-based products and residues—and lead to high-value-added products tailored for local markets and community-level ecological education initiatives [171,183].

The environmental and economic aspects of sustainability are important, as the valorization of WCOs contributes to circular economy principles by reducing waste disposal and recovering valuable resources

[177,184,185]. Recycling oils into soaps can decrease environmental pollution and lower the carbon footprint compared to conventional waste treatment methods [186,187]. Economically, WCO-based soap production can provide cost-effective alternatives to conventional raw materials, offering both social and industrial benefits, particularly in developing regions [167].

The production of soaps from WCO provides a sustainable recycling pathway, converting food waste into eco-friendly raw materials. The composition of WCO is influenced by the characteristics of the oil used as well as the conditions of the saponification reaction. In general, WCOs, previously subjected to filtration and decantation to remove solid impurities and water, reacts with a NaOH solution, thereby producing soap and glycerin as the main products. The process may include additives such as fragrances, colorants, or stabilizers, depending on the desired final application. This valorization method contributes to reducing the environmental impact of food waste and aligns with current circular economy trends.

Beyond the reduction in waste and pollution, the valorization of WCOs through soap production contributes to several Sustainable Development Goals (SDGs), particularly SDG 6 (Clean Water and Sanitation), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). The process promotes circular economy principles by reintroducing waste oils into the production chain as value-added biobased materials, thereby reducing dependence on virgin feedstocks and lowering greenhouse gas emissions associated with oil disposal or incineration [188]. Compared with energy-intensive processes such as biodiesel production, the proposed saponification route operates under milder conditions, uses fewer chemical reagents, and produces non-toxic, biodegradable end-products.

2.3.1.1. Experimental Simulation of Frying Oil Degradation and Its Environmental Implications

The peroxide value (PV) and iodine value (IV) were determined according to standardized analytical methods [189,190] to evaluate the degree of oxidative degradation and the reduction in fatty acid unsaturation in sunflower oil [191]. These parameters are important indicators of oil deterioration during thermal processing and of the potential formation of degradation products that may contribute to environmental pollution when improperly disposed of.

Sunflower oil was evaluated for oxidative stability under controlled thermal conditions simulating frying processes [180]. Laboratory frying experiments were carried out using 200 mL of oil with a mixture consisting of 20 g each of potatoes, mushrooms, and chicken breast at temperatures of 160, 170, 180, 190, and 200 °C. The temperature was maintained using a digital constant-temperature oil bath (Shanti Scientific Industries, Mazgaon, Mumbai, Maharashtra).

Food items were replaced every 5 minutes, and oil samples were collected at the same time intervals to monitor the progression of thermal degradation. The experiment lasted 30 minutes, with oil samples collected every 5 minutes. After collection, the samples were cooled to room temperature and analyzed immediately in order to assess the extent of oxidation and degradation processes that may contribute to the generation of environmentally harmful waste frying oils.

Understanding the kinetics of oil degradation under frying conditions is crucial for estimating the environmental load of waste oils [192]. Oils with high PVs and reduced unsaturation levels generate more reactive compounds, which, if released into the environment without proper treatment, can contribute to aquatic toxicity and soil pollution [193].

In Tables 2.3.1 the changes in the PV of fresh sunflower oils are presented, depending on frying temperature and duration.

Table 2.3.1**.** Peroxide value (mEq O_2/kg) of sunflower oil after frying a mix of potatoes, mushrooms, and chicken breast.

Time, Min	**Temperature, °C**				
	160	**170**	**180**	**190**	**200**
0	1.18 ± 0.07				
5	3.25 ± 0.13	7.38 ± 0.48	8.76 ± 0.55	10.25 ± 0.99	14.12 ± 1.16
10	12.72 ± 1.70	13.25 ± 0.87	13.75 ± 1.13	16.80 ± 1.51	21.48 ± 2.02

15	13.15 ± 1.50	15.68 ± 0.94	19.24 ± 1.22	27.53 ± 2.95	33.31 ± 2.41
20	15.07 ± 0.77	16.93 ± 1.01	20.18 ± 1.84	32.97 ± 2.73	42.70 ± 2.87
25	18.79 ± 1.03	21.07 ± 1.12	36.50 ± 2.46	40.44 ± 1.97	56.13 ± 3.10
30	18.38 ± 1.51	22.97 ± 1.13	39.98 ± 2.67	43.05 ± 1.97	59.61 ± 1.44

At temperatures above 180 °C, the PV increases exponentially, especially after 30 min. At 200 °C, after 30–40 min, the oil is strongly oxidized.

Frying moist foods (mushrooms) and protein-rich foods (meat) accelerates oxidation through the release of water and amino acids that interact with fats. Oxidation is influenced by temperature, time, contact with air, and the water/protein content of the food (e.g., mushrooms and meat).

There are no specific regulations in the EU establishing legal limits for the PV of used oils intended for industrial or energy reuse. However, existing standards for edible oils can serve as a reference for evaluating the degree of oxidation in used oils. Only in South Korea, according to national legislation [194], regulations require frying oils for common foods to have PV below 50 meq/kg. However, there are no specific regulations for oils used to fry animal-based foods and the corresponding fried products.

Tables 2.3.2 show the changes in the IV of fresh sunflower oils depending on frying temperature and duration.

Table 2.3.2. Iodine value (g I_2/100 g) of sunflower oil after frying a mix of potatoes, mushrooms, and chicken breast.

Time, Min	**Temperature, °C**				
	160	**170**	**180**	**190**	**200**
0	119.47 ± 0.20				
5	117.97 ± 0.23	117.48 ± 0.38	116.74 ± 0.25	116.62 ± 0.29	116.52 ± 0.26
10	116.46 ± 0.22	115.51 ± 0.37	114.42 ± 0.23	113.08 ± 0.25	113.14 ± 0.20
15	114.95 ± 0.34	113.46 ± 0.34	112.03 ± 0.22	111.35 ± 0.29	110.13 ± 0.34
20	113.07 ± 0.32	111.63 ± 0.26	109.85 ± 0.30	108.92 ± 0.37	107.27 ± 0.22
25	112.00 ± 0.38	109.17 ± 0.22	107.50 ± 0.40	105.84 ± 0.23	104.31 ± 0.33
30	110.38 ± 0.22	107.79 ± 0.31	105.87 ± 0.28	103.91 ± 0.24	102.70 ± 0.25

The obtained values for the PV and IV highlight a progressive degradation of the analyzed oils as frying temperature and duration increase. At higher temperatures, particularly above 180 °C, sunflower oil shows a pronounced increase in PV, reaching over 59 meq O_2/kg at 200 °C after 30 min, indicating severe oxidation. Such high levels of oxidation not only render the oils unsuitable for human consumption but also make them more hazardous if discarded. Oxidized triglycerides and aldehydes formed during frying can have detrimental environmental effects, including bioaccumulation in aquatic species and negative impacts on soil microbial activity.

The IV, reflecting the degree of unsaturation of fatty acids, decreased steadily for oil, confirming the degradation of the lipid structure. Sunflower oil showed a reduction from approximately 119 to 102 (at 200 °C), indicating a significant loss of unsaturated fatty acids in both cases. A decrease in the IV below 75 g I_2/100 g may indicate significant oil degradation, particularly in virgin oils, according to *Codex Alimentarius* standards [195].

Using Minitab Statistical Software version 21.4.1 (Minitab, LLC, State College, PA, USA), the experimental data were analyzed to obtain a series of statistical models describing the variation in PV and IV in the samples depending on the temperature and exposure time. An empirical second-order polynomial function was applied to model these relationships and to evaluate the effect of temperature on the oxidative stability of the oils. The experimental data and the surfaces generated by the statistical mathematical models are presented in Figure 2.3.1 for the variation in PV and Figure 2.3.2 for the variation in IV.

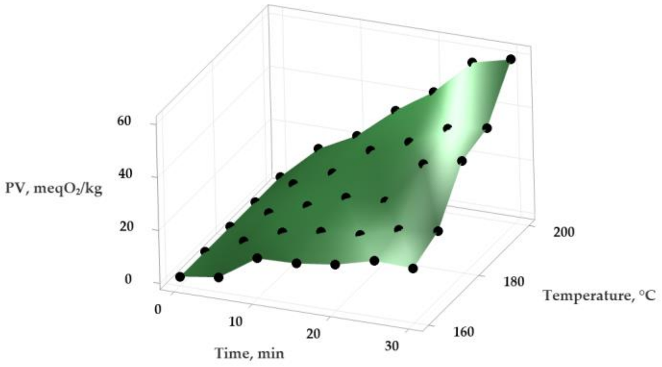


Figure 2.3.1. Variation in the PV in sunflower oil depending on temperature and time of heat treatment.

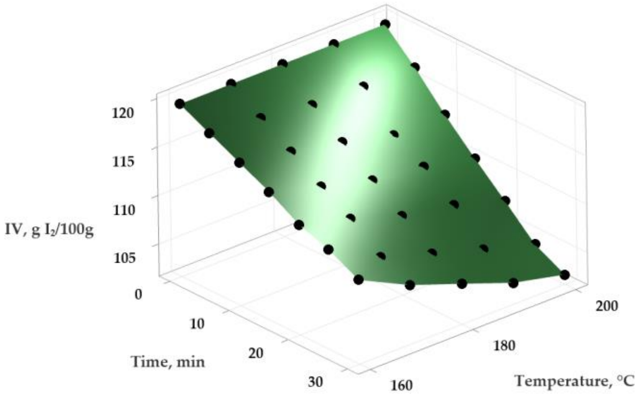


Figure 2.3.2. Variation in the IV in sunflower oil depending on temperature and time of heat treatment.

The equations of the statistical mathematical models obtained from multiple polynomial regression are presented in Table 2.3.3.

Table 2.3.3. Equations of the statistical models obtained for sunflower oil.

Parameter	**Equations of Statistical Mathematical Models**
PV	$y_{PV} = 240.6 - 2.665 \cdot x_1 - 4.887 \cdot x_2 + 0.007 \cdot x_1^2 - 0.010 \cdot x_2^2 + 0.035 \cdot x_1 \cdot x_2$
IV	$y_{IV} = 154.94 - 0.386 \cdot x_1 + 0.680 \cdot x_2 + 0.001 \cdot x_1^2 - + 0.002 \cdot x_2^2 - 0.006 \cdot x_1 \cdot x_2$

Note: PV—Peroxide Value; IV—Iodine Value. y—The value of the PV/IV of the oil; x_1—the temperature to which the oil is subjected; x_2—the time of the heat treatment applied.

After calculating the model coefficients, the predictions were compared with experimental data. The resulting concordance indicators are presented in Table 2.3.4.

Table 2.3.4. Concordance indicators of the determined statistical models.

Concordance Indicators	Peroxide Value	Iodine Value
Adequacy variance, σ^2	6.55	0.83
Standard deviation, σ	2.56	0.29
Model precision indicator, R^2	0.977	0.997
Correlation coefficient, R	0.988	0.998

The statistical models obtained for the variation in the PV and IV of sunflower oil with temperature and time demonstrated high precision, being able to explain over 95% of the variation in the indices. It should be emphasized that the PV and IV models are empirical, being valid only within the studied range of temperature and exposure time. Nevertheless, they can serve as useful tools to predict trends and provide guidance on the oxidative behavior of sunflower oil under similar conditions.

The results highlight that sunflower oil undergo significant chemical degradation at frying temperatures above 180 °C, generating reactive oxidation products. From an environmental perspective, the accumulation of such oxidized oils represents a potential pollution source if not properly managed. Sustainable practices, such as collection for biodiesel production or controlled industrial recycling, are recommended to mitigate the environmental impact of used frying oils.

2.3.1.2. Characterization of Oils and Bioactive Components: Physicochemical Properties and Environmental Considerations

The sampling of vegetable oils used was carried out from various units in Alba Iulia, Romania. Table 2.3.5 presents the types of oils tested and the specific frying parameters that correspond to each of them. The collected WCOs represent potential environmental pollutants if disposed improperly. Oils with high total polar compounds (TPCs) can contribute to soil and water contamination, as oxidized lipids are persistent and may interfere with microbial communities and aquatic ecosystems.

Table 2.3.5. Types of collected WCOs and their corresponding frying conditions.

Type of Used Oil	**Status (refined/ unrefined)**	**Field of Use**	**Type of Fried Food**	**Frying Time/Preparation, min**	**Temperature, °C**	**Number of Usage Cycles**	**Oil-to-Food Ratio**	**Type of Frying**
Sunflower	Refined	Fast food	French fries	5–10	175–190	10–15	5:1	Deep frying
Palm	Refined	Bakery/ Pastry	Donuts, Fried buns	2–5	170–180	8–12	3:1	Deep frying
Pumpkin	Unrefined	Restaurant	Vegetables/Mushrooms	2–5	120–140	3–5	4:1	Light sauté/shallow frying

Note: Collected oils were confirmed as WCOs with total polar compounds (TPCs) > 24%.

Analysis of Fresh, Used, and Purified Oils

Table 2.3.6 presents the main physicochemical parameters determined [196-200] from the analyses of the tested fresh oils. The TPCs of the oil were determined using a FOM 310 Food Oil Monitor (Ebro, Xylem Analytics Germany Sales GmbH & Co. KG, Ingolstadt, Germany). These values provide an overview of the initial quality of the products and serve as a reference point for subsequent comparisons with the WCO.

Table 2.3.6. Results of physicochemical analyses of fresh oils.

Type of Fresh Oil	**AV, mgKOH/g**	**FFA, %**	**PV, mEq O_2/kg**	**IV, g I_2/100 g**	**TPC, %**	**Moisture Content, %**	**Viscosity, mm^2/s**	**Density, g/cm^3**	**RI at 25 °C, nD**
Sunflower	0.16 ± 0.02	0.05	1.18 ± 0.07	119.47 ± 1.86	4.3 ± 0.5	0.10 ± 0.02	33.48 ± 0.04	0.88 ± 0.02	1.472 ± 0.01
Palm	0.36 ± 0.07	0.09	1.42 ± 0.16	51.44 ± 0.93	4.1 ± 0.0	0.10 ± 0.01	34.17 ± 0.03	0.89 ± 0.04	1.451 ± 0.00
Pumpkin	1.47 ± 0.35	0.74	2.80 ± 0.33	128.13 ± 1.95	1.1± 0.0	0.20 ± 0.01	34.96 ± 0.07	0.92 ± 0.05	1.474 ± 0.01

Note: AV—Acid Value; FFA—Free Fatty Acids; PV—Peroxide Value; IV—Iodine Value; TPC—Total Polar Compounds; RI—Refractive Index at 25 °C, measured at the sodium D-line (589 nm).

Table 2.3.7 presents the main physicochemical parameters determined from the analyses of WCOs. The results highlight the changes in their composition and properties during use, compared to the initial values of fresh oils. The significant increase in PVs and free fatty acids in used oils indicates a high degree of oxidation. From an environmental perspective, these chemically altered oils pose risks if released into the environment, as their reactive oxidation products can increase biochemical oxygen demand (BOD) in water bodies and impair soil quality.

Table 2.3.7. Results of physico-chemical analyses of used oils.

Sample	**AV, mg KOH/g**	**FFA, %**	**PV, mEq O_2/kg**	**IV, g I_2/100 g**	**TPC, %**	**Moisture Content, %**	**Viscosity, mm^2/s**	**Density, g/cm^3**	**RI at 25 °C, nD**
Sunflower	4.20 ± 0.20	2.11	43.27 ± 0.33	105.02 ± 2.25	43.0 ±0.01	1.29 ± 0.25	42.76 ± 0.11	0.93 ± 0.05	1.475 ± 0.001
Palm	2.56 ± 0.07	1.28	53.70 ± 0.49	44.19 ± 0.55	39.2 ± 0.1	1.02 ± 0.19	43.44 ± 0.04	0.94 ± 0.04	1.457 ± 0.004
Pumpkin	2.97 ± 0.13	1.49	31.82 ± 0.55	110.25 ± 1.18	32.2 ± 0.03	0.50 ± 0.17	41.50 ± 0.06	0.94 ± 0.07	1.479 ± 0.002

Note: AV—Acid Value; FFA—Free Fatty Acids; PV—Peroxide Value; IV—Iodine Value; TPC—Total Polar Compounds; RI—Refractive Index at 25 °C, measured at the sodium D-line (589 nm); >40.0% indicates that the TPC exceeded the upper detection limit of the FOM 310—Food Oil Monitor 215 device.

1. *Purification of WCO using saline solution*

The WCO – sunflower, palm oil and pumpkin (200 mL) was heated to 90 ± 5°C and washed with a hot brine solution (20 mL), 10% salt mine w/v, in the ratio of 10:1 (v/v) WCO: brine solution. The hot mixture (WCO and brine solution) was mixed using a stirrer at a speed of 60 rpm for 60 min. Then, the mixture was allowed to settle for 8 h to separate the two phases: the oil and the aqueous phase. The lower phase (brine solution + impurities) was separated. For the chemical method of oil purification, bulk industrial mine salt from Ocna Dej Saltmine, Cluj County, Romania was used.

The results of the physicochemical analyses of oils purified with 10% NaCl are summarized in Table 2.3.8.

Table 2.3.8. Results of the physicochemical analyses of oils purified with 10% NaCl**.**

Sample	**AV, mg KOH/g**	**FFA, %**	**PV, mEq O_2/kg**	**IV, g I_2/100 g**	**TPC, %**	**Moisture Content, %**	**Viscosity, mm^2/s**	**Density, g/cm^3**	**RI at 25 °C, nD**
Sunflower	3.95 ± 0.11	1.99	10.18 ± 0.57	103.00 ± 2.06	39.40 ± 0.13	1.30 ± 0.28	35.83 ± 0.02	0.89 ± 0.02	1.473 ± 0.03
Palm	2.56 ± 0.07	1.29	9.85 ± 0.96	45.30 ± 2.15	37.83 ± 0.15	1.14 ± 0.14	37.52 ± 0.03	0.90 ± 0.04	1.451 ± 0.01
Pumpkin	2.14 ± 0.09	1.08	14.20 ± 0.80	123.03 ± 1.97	28.2 ± 0.11	0.55 ± 0.09	36.83± 0.06	0.92 ± 0.03	1.475± 0.02

Note: AV—Acid Value; FFAs—Free Fatty Acids; PV—Peroxide Value; IV—Iodine Value; TPC—Total Polar Compounds; RI—Refractive Index at 25 °C, measured at the sodium D-line (589 nm).

The initial sunflower waste cooking oil had high acidity, peroxide value, and total polar compounds. Treatment with 10% NaCl provided only modest improvements, slightly reducing acidity and peroxide value, while total polar compounds remained largely unchanged.

Table 2.3.8 presents the main physicochemical parameters determined from the analyses of purified oils. The obtained data reflect the efficiency of the purification process and allow for the assessment of the extent to which the initial properties of fresh oils have been restored. Comparing these results with the corresponding values of used and fresh oils highlights the degree of improvement in physico-chemical characteristics following the applied treatment. Purification of WCOs not only restores physicochemical properties but also reduces their environmental hazard potential. By removing polar oxidation products and other degradation compounds, the risk of water and soil pollution from discarded oils is minimized, highlighting the importance of recovery processes in sustainable oil management. Oils with a TPC content exceeding 40% are considered highly degraded, surpassing commonly accepted food safety limits (25–27%), and therefore must be purified before reuse.

2. *Purification of sunflower-WCO using natural zeolite and egg shell.*

As natural adsorbents, clinoptinolite-type zeolite obtained from volcanic rocks (Perșani Mountain from the Eastern Carpathians), which originates from Zeolites Production Company - Rupea (Brașov County), Romania. The chemical composition of the natural zeolite estimated by X-ray fluorescence spectrometry using an ARL Quant'X spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) is presented in Table 2.3.9, together with the values of several physicochemical parameters provided by the zeolite supplier in the technical data sheet [201,202].

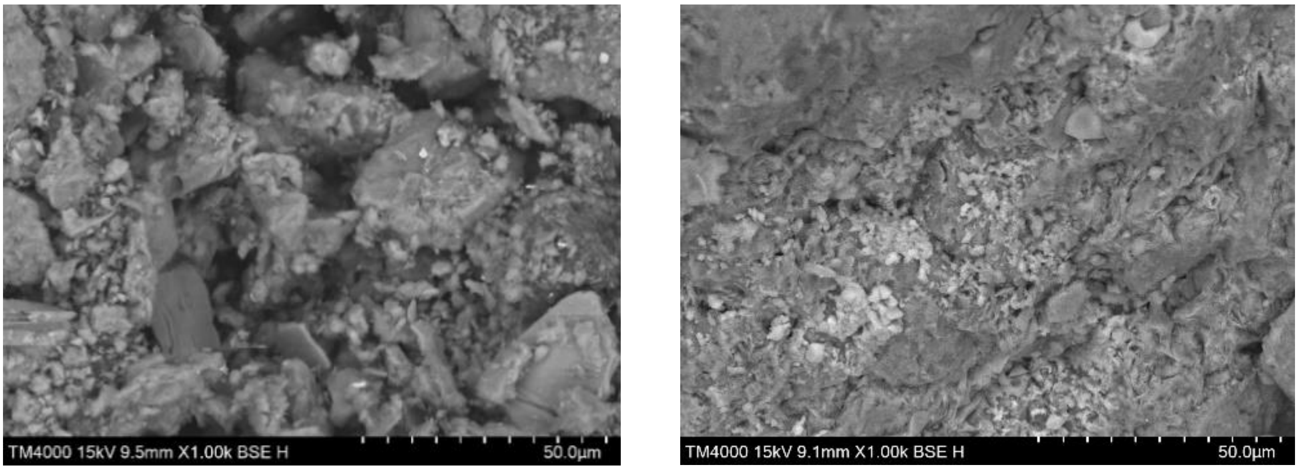
Table 2.3.9. Chemical composition and several physicochemical parameters of the zeolite.

Compound	**Formula**	**wt. %**	**Other parameters** [1]	**Value**
Silicon dioxide	SiO_2	70.93	Cation-exchange capacity (CEC)	1.51 mqv/100 g
Aluminum oxide	Al_2O_3	16.21		
Calcium oxide	CaO	4.72	Specific surface area (BET)	23.4 m^2/g
Potassium oxide	K_2O	3.69	Apparent specific gravity (AG)	1.65 – 1.75
Iron (III) oxide	Fe_2O_3	2.82	Total porosity (TP)	33.08%
Magnesium oxide	MgO	0.46	Water absorption	16.21%
Sodium oxide	Na_2O	0.45	Bulk density	0.88 kg/dm^3
Titanium dioxide	TiO_2	0.25	-	-
Barium oxide	BaO	0.10	-	-
Manganese (II) oxide	MnO	0.05	-	-
Loss on ignition	LOI	0.21	-	-

[1] Parameters from the Product Specification Sheet of the zeolite from Rupea, Romania.

The mineralogical analysis of the zeolite revealed that it mainly consists of clinoptilolite (71-83.3%), volcanic glass (4.1-9.7%), plagioclase (6.67%), SiO_2 (2.25-2.6%) and traces of other minerals.

Figures 2.3.5(a) and 2.3.5(b) show SEM micrographs of zeolite samples activated with NaOH and NaCl.



(a) (b)

Figure 2.3.5. SEM micrographs of the zeolite samples activated with NaOH (a) and NaCl (b)

The values of acidity, peroxide value, and total polar compounds, measured before and after 120 minutes of treatment (with samples taken every 15 minutes), for zeolite doses of 5, 10, 20, and 30 g per 100 mL of WCO at different contact times, are presented in Table 2.3.10. To evaluate the influence of temperature on the adsorption process, experiments were conducted at 25 °C and 80 °C.

Table 2.3.10. The values of the quality parameters of the sunflower-WCO treated with natural zeolite.

Time, min	**AV, mg KOH/g**				**PV, mEqO$_2$/kg**		**TPC, %**	
	25°C	**80°C**			**25°C**	**80°C**	**25°C**	**80°C**
	ZT[1]	**ZT[1]**	**Z_{NaOH}[2]**	**Z_{NaCl}[3]**	**ZT[1]**	**ZT[1]**	**ZT[1]**	**ZT[1]**
0	4.20 ± 0.20				43.27 ± 0.33		43.0±0.01	
5g zeolite								
15	3.00±0.13	2.89±0.11	2.74±0.10	2.61±0.14	28.37±1.85	24.84±1.01	43.0±0.02	43.0±0.03
30	2.96±0.04	2.80±0.07	2.68±0.13	2.75±0.11	25.71±1.14	20.57±0.70	43.0±0.01	42.7±0.08
45	2.79±0.18	2.74±0.02	2.42±0.09	2.50±0.06	19.96±1.62	18.31±0.96	43.0±0.02	42.5±0.10
60	2.73±0.12	2.65±0.06	2.34±0.15	2.46±0.08	17.73±0.98	15.17±0.53	43.0±0.05	42.5±0.09
90	2.73±0.06	2.52±0.13	2.34±0.11	2.42±0.09	16.52±0.49	15.05±0.32	43.0±0.07	42.5±0.15
120	2.71±0.07	2.50±0.09	2.36±0.08	2.39±0.10	16.04±1.37	14.93±0.88	42.8±0.11	42.2±0.13
10g zeolite								
15	3.06±0.15	2.79±0.08	2.61±0.10	2.34±0.09	26.73±1.80	24.26±1.36	43.0±0.01	43.0±0.02
30	2.85±0.09	2.66±0.05	2.23±0.07	2.31±0.12	19.15±1.49	20.03±1.55	43.0±0.00	42.8±0.16
45	2.79±0.10	2.69±0.09	2.51±0.05	2.47±0.10	16.67±1.04	17.78±1.21	43.0±0.02	42.4±0.08
60	2.79±0.07	2.65±0.04	2.23±0.08	2.38±0.09	13.10±0.28	16.72±0.92	42.7±0.11	42.3±0.10
90	2.76±0.05	2.63±0.04	2.20±0.09	2.35±0.03	11.55±0.17	15.84±0.54	42.6±0.07	42.2±0.14
120	2.75±0.07	2.64±0.02	2.18±0.03	2.30±0.06	11.28±0.23	12.51±0.18	42.4±0.10	42.0±0.12
20g zeolite								
15	2.65±0.11	2.47±0.12	2.23±0.05	2.33±0.17	25.24±1.70	23.46±1.48	43.0±0.02	43.0±0.01
30	2.64±0.12	2.33±0.08	2.02±0.10	2.52±0.14	18.78±1.54	17.27±1.03	43.0±0.00	42.6±0.02
45	2.74±0.07	2.33±0.09	1.92±0.08	2.41±0.10	16.17±1.81	14.39±1.27	43.0±0.01	42.4±0.07
60	2.72±0.05	2.23±0.11	1.95±0.04	2.47±0.08	11.03±0.82	12.81±0.60	42.5±0.05	42.4±0.13
90	2.70±0.10	2.20±0.07	1.91±0.07	2.40±0.09	10.35±0.77	12.23±0.35	42.3±0.17	42.3±0.08
120	2.63±0.05	2.06±0.03	1.93±0.06	2.44±0.05	9.04±0.83	10.93±0.91	42.2±0.13	41.9±0.11
30g zeolite								
15	2.79±0.17	2.49±0.12	2.01±0.11	2.53±0.08	23.35±1.39	22.14±1.08	43.0±0.03	43.0±0.02
30	2.62±0.08	2.51±0.07	1.77±0.13	2.24±0.12	15.84±1.26	17.59±0.66	43.0±0.03	42.9±0.04
45	2.62±0.12	2.63±0.04	1.80±0.10	2.21±0.10	12.47±0.84	13.45±0.52	43.0±0.02	42.9±0.07
60	2.67±0.10	2.32±0.05	1.25±0.06	2.25±0.14	10.95±0.11	10.93±0.47	43.0±0.06	42.6±0.10
90	2.68±0.04	2.18±0.04	1.20±0.05	2.13±0.08	8.38±0.37	8.04±0.73	42.7±0.11	42.3±0.04
120	2.70±0.09	2.03±0.03	1.16±0.07	2.07±0.04	7.81±0.44	7.93±0.30	42.0±0.10	41.7±0.09
15	2.79±0.17	2.49±0.12	2.01±0.11	2.53±0.08	23.35±1.39	22.14±1.08	43.0±0.16	43.0±0.12

[1] ZT - Thermally activated; [2] Z_{NaOH} - Activated with NaOH; [3] Z_{NaCl} - Activated with NaCl; All values are means ± standard deviations (n = 3).

For a zeolite dose of 5 g, slight decreases in the acidity of the WCO were observed. Thus, from an initial value of 4.2 mg KOH/g, the lowest value was recorded for the zeolite activated at 80°C with NaOH (3.36 mg KOH/g after 120 min). In the case of PV, after 120 min of adsorption using thermally activated zeolite, the results were comparatively better, with removal efficiencies of 62.9% at 25°C and 65.5% at an operating temperature of 80°C. Regarding total polar compounds, the removal efficiency was below 2% in both cases.

A similar trend was observed when a zeolite dose of 10 g was applied for all the investigated parameters; however, higher removal efficiencies were obtained. The zeolite activated at 80°C with NaOH exhibited the greatest reduction in acidity (3.18 mg KOH/g after 120 min). The highest decrease in PV was observed at 25°C, with the lowest value reaching 11.28 mEq O$_2$/kg.

In the case of 20 g dose the best values were obtained by treating the waste oil with NaOH activated zeolite. The value of the PV decreases to 9.04 mEqO$_2$/kg after 120 min of contact, the removal efficiency obtained was 73%. In general, increasing the zeolite dose provides a larger adsorbent surface, enhancing the adsorption process and reducing impurities in WCO.

At the highest zeolite dose (30 g), the acidity, PV, and TPC values were the lowest. The oil treated with NaOH-activated zeolite showed the lowest AV, and PV decreased significantly after 120 min, with a removal efficiency of approximately 80%.

For all zeolite doses, the acidity (AV), PV, and TPC of the WCO decreased progressively with increasing contact time, indicating that longer interaction enhances adsorption efficiency. Most of the reduction occurred within the first 60 minutes, reflecting the rapid adsorption when more active sites are available, while the decrease slowed after 90–120 minutes as the adsorbent approached saturation. Higher zeolite doses (20–30 g) not only achieved lower final AV and PV values but also accelerated the removal process compared to lower doses.

The results demonstrate that both dose and contact time play a critical role in the purification process, with thermally, NaOH-, and NaCl-activated zeolites showing the most effective reductions at higher doses and longer contact times.

Preparation of eggshell

Eggshells from ISA Brown hens were used as a natural adsorbent, being collected as food industry waste from a canteen. The eggshell was washed with distilled water to remove impurities and then dried in an oven at 105°C until constant mass. After drying, the eggshell was crushed in a mortar and sieved in a mesh of 42 Tyler. The eggshell was used both thermally and chemically activated. Thermal activation was achieved by calcining the eggshell at a temperature of 900°C for 4 h. Chemical activation was achieved by mixing the eggshell with a 0.1 M HCl solution (Chempur, Poland) for 48 h, followed by washing with distilled water to neutral pH.

A TM4000Plus II scanning electron microscope (Hitachi, Tokyo, Japan), coupled with a liquid-nitrogen-free energy-dispersive X-ray spectroscopy (EDS) detector (Oxford Instruments, Oxford, UK), was used for scanning electron microscopy–energy-dispersive X-ray (SEM–EDX) analysis.

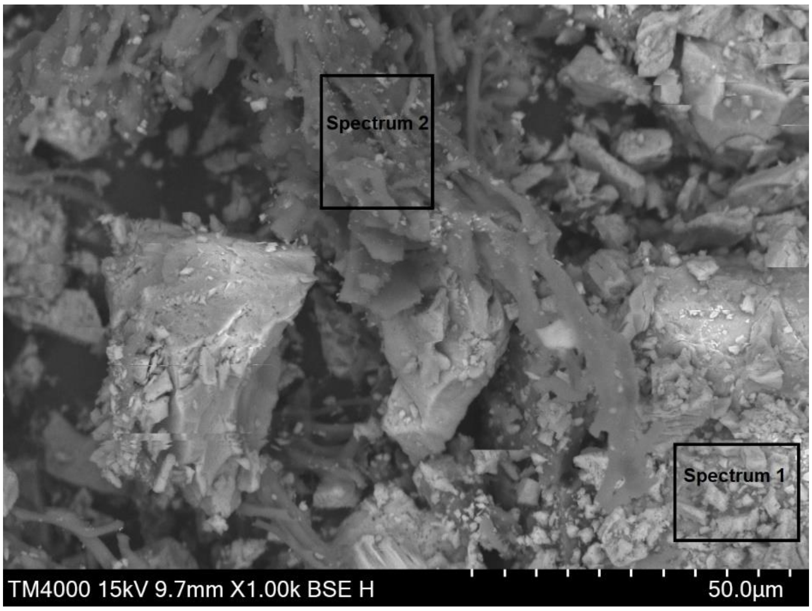


Figure 2.3.6. SEM micrographs of the eggshells.

The elemental chemical composition of eggshell samples was estimated by EDX analysis that has been performed on two regions of the surface with different morphological characteristics as shown in Figure 2.3.7.

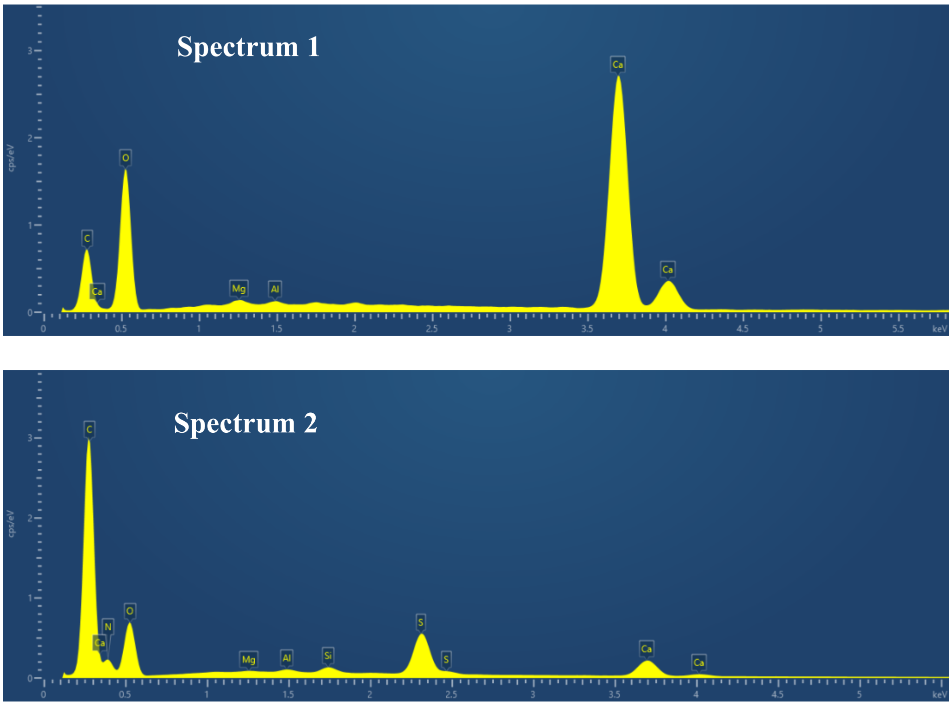


Figure 2.3.7. EDX spectra of the chicken eggshell composition corresponding to the regions indicated in Figure 2.3.6: whiter region - spectrum 1; darker region – spectrum 2.

EDX analysis of the sample shows that calcium (Ca), oxygen (O), and carbon (C) exhibited the highest concentrations. The whiter region (spectrum 1 in Figure 3) is rich in inorganic material, containing calcium (31.18 wt.%), oxygen (50.16 wt.%), and carbon (18.17 wt.%), that corresponds most probably to the calcium carbonate [203-204]. Additionally, traces of magnesium have been identified. It can be present in the form of magnesium carbonate ($MgCO_3$) [205].

The darker region corresponding to the fibrils of the eggshell membrane (spectrum 2 in Figure 3) consist mainly of organic material, namely proteins (most likely collagen), containing large amounts of carbon (57.87 wt.%), oxygen (20.13 wt.%), and nitrogen (17.26 wt.%). In addition, small amounts of S (2.56 wt.%), Ca (1.89 wt.%), Si (0.28 wt.%) and Mg (0.05 wt.%) were also detected.

This finding aligns with previous investigations using EDX mapping analysis, which indicated the predominance of calcium carbonate ($CaCO_3$), constituting the main component of the total elemental content.

To test the efficiency in the purification of WCO, different doses of thermally and chemically treated eggshell were tested. Eggshell was used in doses of 5, 10, 20 and 30 g per 100 mL of WCO - sunflower oil. The contact time was up to 120 min and the sampling was done at 15 min intervals. The experiments were carried out under continuous stirring at 300 rpm at 80°C.

Table 2.3.11 shows the variation of the quality parameters of sunflower WCO before and after 120 min from contact with the egg shell in doses between 5 and 30g / 100 mL of WCO.

Table 2.3.11. The values of the quality parameters of the sunflower WCO treated with eggshell.

Time, min	**AV, mg KOH/g**		**PV, mEqO_2/kg**		**TPC, %**	
	EST[1]	**EST$_{HCl}$** [2]	**EST**[1]	**EST$_{HCl}$** [2]	**EST**[1]	**EST$_{HCl}$** [2]
0	4.20 ± 0.20		43.27 ± 0.33		43.0±0.01	
5g egg shell						
15	1.67±0.11	3.00±0.18	38.84±1.07	37.95±1.24	43.0±0.02	43.0±0.01
30	1.66±0.17	2.50±0.15	36.41±1.27	36.06±1.27	43.0±0.03	43.0±0.00
45	1.90±0.15	2.21±0.06	37.18±0.56	36.01±0.41	43.0±0.00	43.0±0.03
60	1.82±0.20	2.20±0.11	35.15±1.03	35.14±0.87	43.0±0.05	43.0±0.02
90	1.73±0.12	2.10±0.09	34.04±0.72	33.95±0.55	43.0±0.003	43.0±0.00

120	1.51±0.26	2.04±0.17	33.61±0.44	33.84±0.23	43.0±0.04	43.0±0.02
10g egg shell						
15	2.22±0.09	2.9±0.13	38.79±1.62	36.45±1.89	43.0±0.01	43.0±0.05
30	1.75±0.06	2.1±0.05	35.27±1.08	29.42±1.30	43.0±0.03	43.0±0.02
45	1.66±0.04	1.93±0.09	34.85±1.24	27.29±1.64	43.0±0.03	43.0±0.03
60	1.79±0.08	2.07±0.07	35.16±1.76	23.83±1.04	43.0±0.06	43.0±0.02
90	1.80±0.05	2.02±0.03	33.06±1.24	24.63±0.86	43.0±0.03	43.0±0.04
120	1.77±0.05	1.95±0.06	32.62±1.43	24.52±0.81	43.0±0.02	43.0±0.08
20g egg shell						
15	2. 41±0.15	2.2±0.17	37.10±1.24	37.52±1.81	43.0±0.01	43.0±0.02
30	2.25±0.11	1.9±0.04	34.64±1.40	35.92±1.44	43.0±0.00	43.0±0.04
45	2.07±0.09	2.09±0.07	33.36±1.32	34.84±1.52	43.0±0.03	43.0±0.02
60	1.99±0.06	2.00±0.06	32.31±1.16	33.95±0.64	43.0±0.03	43.0±0.05
90	1.38±0.10	2.1±0.05	31.19±0.84	31.76±0.60	43.0±0.02	43.0±0.01
120	1.57±0.08	1.85±0.03	29.75±1.68	30.65±1.56	43.0±0.06	43.0±0.03
30g egg shell						
15	2.50±0.15	2.12±0.13	35.15±1.60	33.90±0.95	43.0±0.03	43.0±0.02
30	2.40±0.10	1.83±0.08	33.46±0.62	31.51±1.06	43.0±0.02	43.0±0.01
45	2.48±0.11	2.02±0.07	32.15±1.13	29.90±0.95	43.0±0.04	43.0±0.03
60	2.03±0.08	2.09±0.09	29.53±1.54	28.85±0.28	43.0±0.01	43.0±0.04
90	1.92±0.05	1.21±0.03	24.51±1.41	22.61±0.44	43.0±0.02	43.0±0.02
120	1.10±0.04	1.44±0.06	20.82±1.27	20.33±1.14	43.0±0.01	43.0±0.03

[1] EST - Thermally activated, [2]EG$_{HCl}$ - Activated with HCl; All values are means ± standard deviations (n = 3).

The analysis of the results shows that the values of acidity, peroxide value, and total polar compounds are influenced by both the eggshell dose and the contact time. For a low eggshell dose of 5 grams, slight decreases in acidity and peroxide value were observed, while the total polar compounds remained practically unchanged. Increasing the eggshell dose to 10 and 20 grams led to a further reduction in acidity and peroxide value, with the most pronounced effect observed at the highest dose of 30 grams. In this case, acidity decreased to 1.10 mg KOH/g for thermally activated eggshell and 1.44 mg KOH/g for HCl-activated eggshell, while the peroxide value dropped to 20.82 and 20.33 mEq O_2/kg, respectively. In general, longer contact times enhanced the adsorption process, with the most significant reductions observed after 120 minutes, indicating that both higher eggshell doses and extended contact times contribute to more efficient removal of impurities, confirming the potential of eggshell as an effective natural adsorbent for WCO purification.

Treatment of waste cooking oil with zeolite and eggshell effectively improved its chemical quality. Zeolite was particularly efficient in reducing peroxide value, achieving the lowest PV at the highest doses, while acidity decreased moderately. Eggshell, on the other hand, was more effective at lowering acidity, even at low doses, though its impact on peroxide value was slightly less pronounced. Total polar compounds remained largely unchanged for both adsorbents. The efficiency of removal increased with higher doses and longer contact times, with each adsorbent showing specific advantages depending on the targeted parameter.

The purification of sunflower waste cooking oil with 10% NaCl led to moderate improvements in chemical quality. While NaCl provides some improvement, natural adsorbents such as eggshell was more effective at lowering acidity, and zeolite excelled at reducing peroxide value. Both natural adsorbents had minimal effect on total polar compounds. Overall, natural adsorbents outperformed NaCl, each showing specific strengths depending on the targeted parameter. Although natural adsorbents showed better reductions in acidity and peroxide value, NaCl was selected for practical and economic reasons, particularly due to its suitability for subsequent soap production.

Tables 2.3.12 and 2.3.13 present the results of the physicochemical analysis of bee products collected from Alba County, Romania.

Table 2.3.12. Results of the physical and botanical parameters of the bee products.

Sample	**Bee Product**	**Moisture Content, %**	a_w	**Phund Value, mm**	**Color Category ***	**Proportion of Specific Pollen Grains, %**
H1	Poly-floral honey	15.72 ± 0.21	0.571 ± 0.016	52	Light amber	-
H2	Linden honey	17.29 ± 0.35	0.574 ± 0.031	40	Extra light amber	30.0 ± 1.4
H3	Acacia honey	18.13 ± 0.40	0.568 ± 0.020	9	Extra white	29.2 ± 1.1
H4	Manna honey	16.2 ± 0.19	0.581 ± 0.013	94	Amber	-
H5	Sunflower honey	16.4 ± 0.27	0.576 ± 0.028	39	Extra light amber	40.1 ± 2.2
P_{tot}	Propolis	5.11 ± 0.21	0.652 ± 0.19	-	-	-

*Abbreviations: a_w—water activity. Note: * United States Standards for Grades of Extracted Honey [59]. Data are presented as mean ± standard deviation (n = 3).*

Table 2.3.13. Chemical parameters of bee products.

Sample	**pH**	**Acidity, meq/kg**	**Ash, %**	**EC, mS/cm**	**HMF, mg/kg**	**EHS, %**	**Inverted Sugar, %**	**Phenols H: mg GAE/100 g; P: GAE/g**	**Flavonoids H: mgQE/100 g; P: mgQE/g**
H1	3.66 ± 0.18	21.7 ± 0.9	0.40 ± 0.05	0.591 ± 0.020	1.21 ± 0.07	2.30 ± 0.17	73.26 ± 1.58	83.52 ± 0.26	4.68± 0.15
H2	4.20 ± 0.30	11.9 ± 0.2	0.32 ± 0.03	0.542 ± 0.013	1.10 ± 0.10	2.73 ± 0.48	74.36 ± 1.23	71.03 ± 0.52	2.79 ± 0.11
H3	3.71 ± 0.26	12.3 ± 0.5	0.21 ± 0.02	0.159 ± 0.027	0.68 ± 0.12	2.90 ± 0.30	79.87 ± 1.93	90.11 ± 1.17	5.54 ± 0.16
H4	3.94 ± 0.32	28.8 ± 0.8	0.35 ± 0.07	1.025 ± 0.036	1.09 ± 0.09	2.71 ± 0.50	79.50 ± 1.3	64.90 ± 0.28	3.66 ± 0.19
H5	3.85 ± 0.29	22.5 ± 0.4	0.37 ± 0.04	0.501 ± 0.025	1.00 ± 0.13	3.59 ± 0.29	79.26 ± 1.83	66.33 ± 0.84	3.32± 0.20
P_{tot}	-	84.6 ± 0.9	2.55 ± 0.08	0.411 ± 0.020 *	-	-	-	159.83 ± 2.05	74.68 ± 0.59

*Abbreviations: H—honey; P—propolis; EC—electrical conductivity; HMF—hydroxymethylfurfural; EHSs—easily hydrolysable sugars; GAE—gallic acid equivalents; QE—quercetin equivalents. Note: * to the extract; Data are presented as mean ± standard deviation (n = 3).*

Honey samples from Alba County showed moisture content between 15.2% and 18.1%, values within the accepted quality standards. The pH ranged from 3.66 to 4.20 and free acidity from 11.9 to 28.8 meq/kg, with manna honey displaying the highest acidity. The water activity of the investigated honey samples (0.568–0.581) confirms their microbiological stability, as these values are below the threshold required for microbial proliferation. Propolis exhibited a slightly higher value (0.652), yet its rich content of bioactive compounds contributes to maintaining its stability. Electrical conductivity distinguished floral honeys (0.15–0.59 mS/cm) from honeydew honey (1.02 mS/cm). All samples presented very low HMF levels (<2 mg/kg), confirming freshness. Easily hydrolysable sugars ranged from 2.30% to 3.59% and inverted sugar from 73.26% to 79.87%, with the highest values in acacia and sunflower honeys. Melissopalynological analysis validated the monofloral origin of linden, acacia, and sunflower honeys, which contained 30–40% specific pollen. The content of bioactive compounds varied among samples:

phenolics ranged from 64.9 to 90.1 mg GAE/100 g, and flavonoids from 2.7 to 5.5 mg QE/100 g, with acacia honey showing the highest values. Propolis was significantly richer, with ~160 mg GAE/g phenolics and 75 mg QE/g flavonoids, confirming its superior antioxidant potential compared to honey.

Table 2.3.14 presents the results of the physico-chemical analysis conducted on commercially available EOs.

Table 2.3.14. The results of the physical and chemical analysis for EOs.

Sample	**Essential Oil**	**Density at 20 °C, g/cm^3**	**Refractive Index at 20 °C**	**Optical Rotation (°) at 20 °C**	**Phenols, mg GAE/g**	**Flavonoids, mg QE/g**
EO1	Clove	1.0490 ± 0.0005	1.5315 ± 0.0013	(+)0.2	0.800 ± 0.10	0.074 ± 0.010
EO2	Rosemary	0.9070 ± 0.0003	1.4701 ± 0.0017	(+)8.7	nd	0.096 ± 0.025
EO3	Mace	0.8952 ± 0.0002	1.4790 ± 0.0015	(+)18.2	nd	0.080 ± 0.017
EO4	Nutmeg	0.8850 ± 0.0001	1.4925 ± 0.0012	(+)22.5	nd	0.065 ± 0.011
EO5	White pepper	0.8875 ± 0.0002	1.4772 ± 0.0019	(+)4.1	nd	nd
EO6	Juniper	0.8602 ± 0.0001	1.4739 ± 0.0016	(+)6.0	nd	nd

Note: The total phenolic content and flavonoids were determined using different colorimetric assays with different sensitivities and selectivities. Total phenols were quantified by the Folin–Ciocalteu (limit of quantification 0.05 mg GAE/g), while flavonoids were determined using the $AlCl_3$ method (limit of quantification: 0.01 mg QE/g). Values reported as "nd" indicate concentrations below the respective quantification limits of each method. Data are presented as mean ± standard deviation (n = 3).

The physico-chemical and bioactive profiling of commercial EOs revealed species-specific differences. Density at 20 °C ranged from 0.8602 g/cm^3 (juniper) to 1.0490 g/cm^3 (clove), with higher values associated with oils rich in phenolic compounds. Refractive indices varied between 1.4701 and 1.5315, the highest observed in clove oil, reflecting the greater content of aromatic compounds with higher molecular weight. Optical rotation was positive for all samples (+0.2° in clove to +22.5° in nutmeg seeds), indicating differences in the composition of optically active isomers.

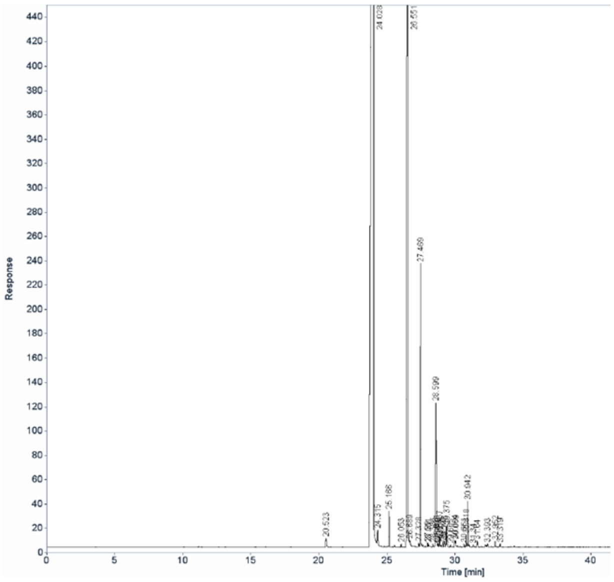
Phenolic and flavonoid contents were generally low, with clove oil showing the highest phenolic content (0.800 mg GAE/g). Flavonoids were detected at low concentrations (0.065–0.096 mg QE/g), while some oils, such as white pepper and juniper, contained undetectable levels.

Volatile phenols differed considerably among species: clove was rich in thymol, carvacrol, and eugenol; rosemary contained fewer volatile phenols and mainly partially non-volatile compounds such as carnosol and rosmarinic acid; while mace, nutmeg, white pepper, and juniper exhibited very low levels, dominated by terpenes. The remaining non-volatile phenols remained in the aqueous residue or degraded during distillation, so they were not present in the EOs but only in hydroalcoholic or aqueous extracts.

These results highlight the significant phytochemical variation among EOs, directly reflecting their botanical origin, chemical composition, and the volatility of phenolic compounds. Clove oil was characterized by high levels of bioactive volatile phenols, whereas other species contained mostly non-volatile phenols or terpenes, which limited their representation in the EO fraction.

It should be noted that the Folin–Ciocalteu method is nonspecific and may have reacted with other reducing compounds in the extracts, potentially leading to an overestimation of total phenolic content.

The GC-FID chromatograms of the investigated EOs (Figure 2.3.8 a–f and Table 2.3.15) provide a detailed qualitative and quantitative profile of their volatile constituents, enabling the identification and comparison of the main compounds across different botanical species.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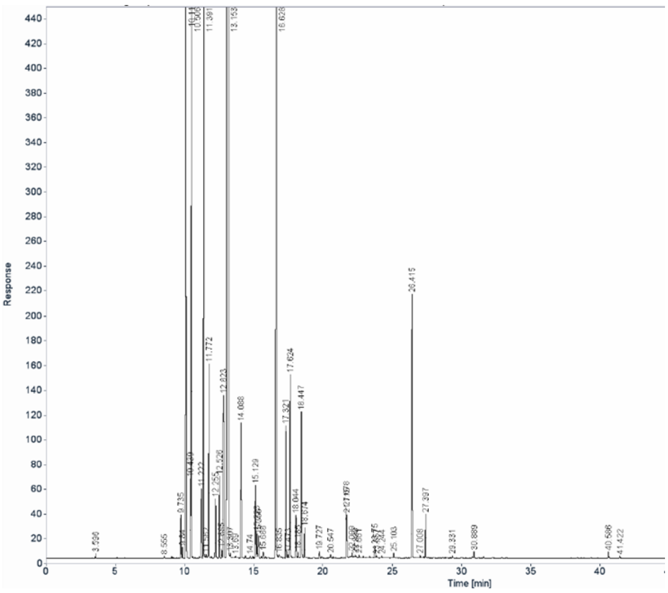
26.551
27.469
28.599
30.942
25.168
24.315
20.523
26.053
26.889
27.328
32.303
32.952
33.319

(**a**)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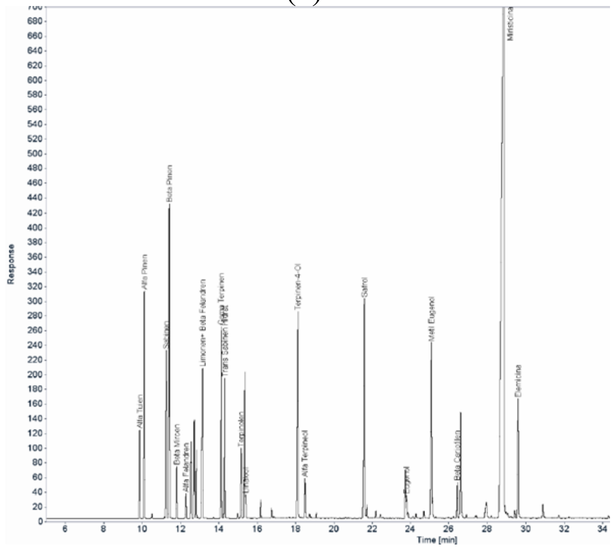
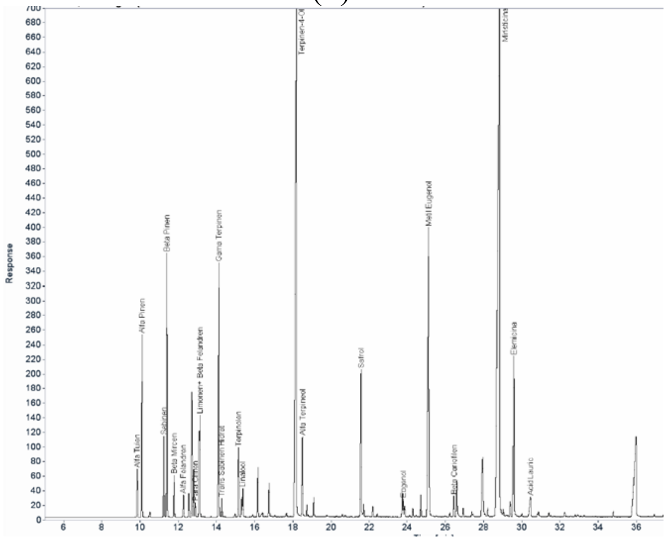
13.153
16.628
26.415
11.772
17.624
12.823
18.447
17.321
14.088
10.489
11.222
12.526
12.255
15.129
9.735
18.044
18.674
27.397
3.596
8.555
14.74
15.688
16.835
19.727
20.547
25.103
27.008
29.331
30.889
40.586
41.422

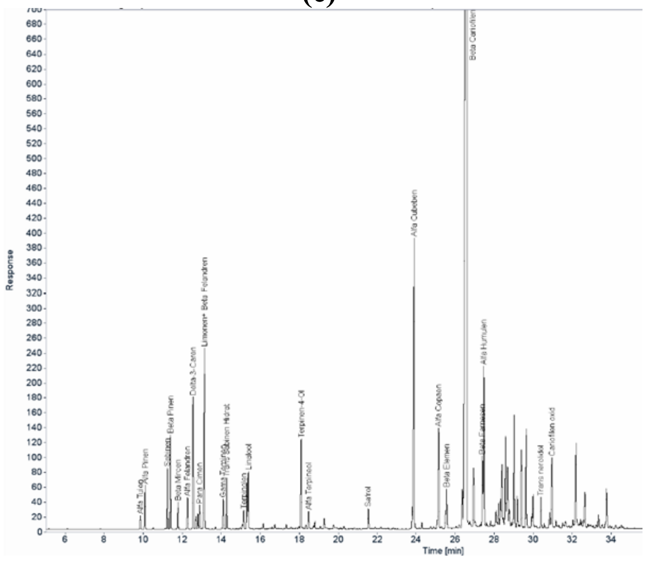
(**b**)


Response
Time [min]
Miristicina
Beta Pinen
Alfa Pinen
Sabinen
Alfa Tuien
Beta Mircen
Alfa Felandren
Limonen+ Beta Felandren
Gama Terpinen
Trans Sabinen Hidrat
Terpinolen
Linalool
Terpinen-4-Ol
Alfa Terpineol
Safrol
Eugenol
Metil Eugenol
Beta Cariofilen
Elemicina

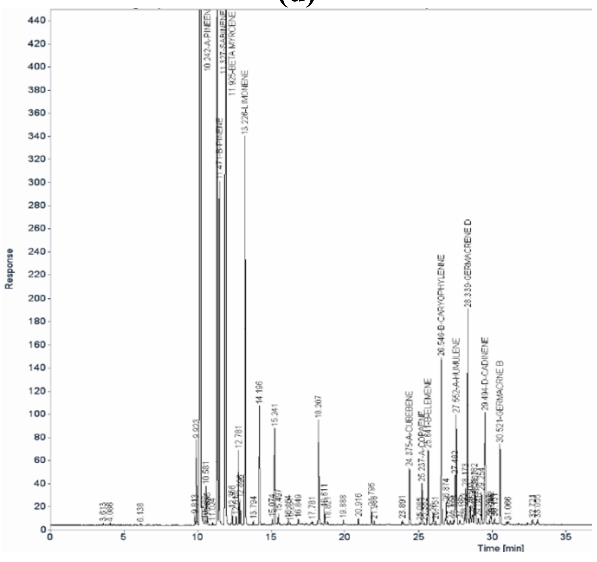
(**b**)


Response
Terpinen-4-Ol
Miristicina
Alfa Tuien
Alfa Pinen
Sabinen
Beta Pinen
Beta Mircen
Alfa Felandren
Para Cimen
Limonen+ Beta Felandren
Gama Terpinen
Trans Sabinen Hidrat
Terpinolen
Linalool
Alfa Terpineol
Safrol
Eugenol
Metil Eugenol
Beta Cariofilen
Elemicina
Acid Lauric

(**d**)


Response
Time [min]
Beta Cariofilen
Alfa Cubeben
Alfa Tuien
Alfa Pinen
Sabinen
Beta Pinen
Beta Mircen
Alfa Felandren
Delta 3-Caren
Para Cimen
Limonen+ Beta Felandren
Gama Terpinen
Trans Sabinen Hidrat
Terpinolen
Linalool
Terpinen-4-Ol
Alfa Terpineol
Safrol
Alfa Copaen
Beta Elemen
Beta Farnesen
Alfa Humulen
Trans nerolidol
Cariofilen oxid

(**e**)


Response
Time [min]
10.242-A-PINEEN
11.327-SABINENE
11.925-BETA MYRCENE
11.471-B-PINENE
13.226-LIMONENE
9.923
10.581
12.781
14.196
15.241
18.207
24.375-A-CUBEBENE
25.237-A-COPAENE
25.641-B-ELEMENE
26.546-B-CARYOPHYLENNE
27.552-A-HUMULENE
28.339-GERMACRENE D
29.494-D-CADINENE
30.521-GERMACRNE B
3.613
4.066
6.138
9.813
11.024
13.794
15.074
16.849
17.781
18.611
19.888
20.916
21.796
21.988
23.891
27.403
31.066
32.721
33.033

(**f**)

Figure 2.3.8. GC-FID chromatogram of essential oils: (**a**) EO1 - clove; (**b**) EO2 - rosemary; (**c**) EO3 - mace (aril of *Myristica fragrans*); (**d**) EO4 - nutmeg (seeds of *Myristica fragrans*); (**e**) EO5 - white pepper (*Piper nigrum*) (**f**) EO6 - juniper (*Juniperus communis*).

Table 2.3.15. Major compounds and their percentages in essential oils: EO1—clove; EO2—rosemary; EO3—mace (aril of Myristica fragrans); EO4—nutmeg (seeds of Myristica fragrans); EO5—white pepper (Piper nigrum); EO6—juniper (Juniperus communis).

EO1—Clove		**EO2—Rosemary**		**EO3—Mace (Aril)**		**EO4—Nutmeg (Seeds)**		**EO5—White Pepper**		**EO6—Juniper**	
Compound	**%**	**Compound**	**%**	**Compound**	**%**	**Compound**	**%**	**Compound**	**%**	**Compound**	**%**
Eugenol	69.15	1,8-Cineole	42.31	Myristicin	43.08	Myristicin	27.31	β-Caryophyllene	49.74	α-Pinene	46.96
β-Caryophyllene	23.61	α-Pinene	14.12	Safrole	5.16	Terpinen-4-ol	17.76	α-Cubebene	5.48	β-Myrcene	18.44
Humulene	3.08	Camphor	11.05	β-Pinene	5.11	Methyl eugenol	8.38	Limonene + β-Phellandrene	3.25	Sabinene	5.51
Eugenyl acetate	1.63	β-Pinene	7.83	Terpinen-4-ol	4.43	γ-Terpinene	4.56	α-Humulene	2.90	Limonene	4.45
-	-	Camphene	5.01	Methyl eugenol	3.93	β-Pinene	4.30	δ-3-Caren	1.99	Germacrene D	2.58
-	-	Limonene	2.76	Limonene + β-Phellandrene	3.66	α-Pinene	2.80	α-Copaene	1.98	β-Caryophyllene	1.99
-	-	β-Caryophyllene	2.67	α-Pinene	3.47	Limonene + β-Phellandrene	1.64	Terpinen-4-ol	1.56	α-Humulene	1.34
-	-	Borneol	0.95	γ-Terpinene	3.29	α-Terpineol	1.51	β-Pinene	1.37	δ-Cadinene	1.33
-	-	Linalool	0.83	Sabinene	2.81	Terpinolen	1.18	Linalool	0.91	Germacrene B	1.00
-	-	α-Terpineol	0.72	Elemicin	2.54	Linalool	0.50	α-Terpineol	0.29	β-Elemene	0.92
-	-	-	-	Eugenol	0.95	β-Caryophyllene	0.41	trans-Nerolidol	0.69	α-Cubebene	0.60
-	-	-	-	α-Terpineol	0.72	Safrole	3.15	Caryophyllene oxide	1.27	α-Copaene	0.44
-	-	-	-	β-Caryophyllene	0.68	Elemicin	3.72	β-Farnesene	1.25	-	-
-	-	-	-	-	-	Sabinene	1.34	β-Elemen	1.04	-	-
-	-	-	-	-	-	trans-Sabinen hydrate	0.32	-	-	-	-
-	-	-	-	-	-	Para-cymene	0.23	-	-	-	-
-	-	-	-	-	-	α-Thujene	0.72	-	-	-	-
-	-	-	-	-	-	α-Felandrene	0.37	-	-	-	-

GC-FID analysis of clove (Syzygium aromaticum) EO revealed a profile dominated by eugenol, which is the main bioactive constituent responsible for its potent antimicrobial, analgesic, and antioxidant activities. The oil also contained significant amounts of β-caryophyllene (23.61%) and smaller proportions of humulene and eugenyl acetate.

The chemical profile of rosemary EO is dominated by 1,8-cineole (42.31%), followed by α-pinene (14.12%) and camphor (11.05%). Other notable constituents included β-pinene, camphene, limonene, and β-caryophyllene, while minor compounds such as borneol, linalool, and α-terpineol were also detected in lower proportions.

GC-FID analysis of aril from mace EO revealed a profile dominated by myristicin (43.08%), accompanied by safrole, β-pinene, terpinen-4-ol, and methyl eugenol. Other relevant constituents included limonene with β-phellandrene, α-pinene, γ-terpinene, sabinene, and elemicin, while several compounds such as eugenol, α-terpineol, and β-caryophyllene were detected in minor proportions.

Nutmeg EO revealed a profile dominated by myristicin (27.31%), which is the main phenylpropanoid responsible for the characteristic aroma and bioactivity of the oil. Other major constituents included terpinen-4-ol (17.76%) and methyl eugenol (8.38%), both contributing to antimicrobial and aromatic properties. Monoterpenes such as γ-terpinene (4.56%), β-pinene (4.30%), and α-pinene (2.80%), along with limonene and β-phellandrene (1.64%), form the hydrocarbon fraction that shapes the volatile profile of the oil. Minor oxygenated terpenes, including α-terpineol, terpinolen, and linalool, together with sesquiterpenes such as β-caryophyllene, safrole, and elemicin, contribute to both aroma complexity and bioactive potential. Other minor monoterpenes identified were sabinene, trans-sabinene hydrate, para-cymene, α-thujene, and α-felandrene.

GC-FID analysis of the white pepper revealed a chemical profile dominated by β-caryophyllene (49.74%), which is the main sesquiterpene contributing to the oil's anti-inflammatory and antimicrobial activities. Other major constituents included α-cubeben (5.48%), limonene + β-phellandrene (3.25%), and α-humulene (2.90%), which collectively contribute to the characteristic aroma and synergistic bioactivity. Monoterpenes such as δ-3-caren (1.99%), α-copaene (1.98%), terpinen-4-ol (1.56%), and β-pinene (1.37%) form the hydrocarbon fraction, enhancing volatility and aroma complexity. Minor oxygenated terpenes, including linalool, α-terpineol, trans-nerolidol, as well as sesquiterpene derivatives like caryophyllene oxide, β-farnesene, and β-elemen, further contribute to the therapeutic potential of the oil.

GC-FID analysis of the juniper EO revealed a profile dominated by α-pinene (46.96%), which defines the main monoterpene fraction and contributes strongly to the characteristic aroma and potential anti-inflammatory properties. Other major constituents included β-myrcene (18.44%), sabinene (5.51%), and limonene (4.45%), forming the hydrocarbon monoterpene fraction that enhances volatility and aroma complexity. Sesquiterpenes such as β-caryophyllene (1.99%), germacrene D (2.58%), α-humulene (1.34%), δ-cadinene (1.33%), and germacrene B (1.00%) were also detected, contributing to the bioactive potential of the oil, particularly its antimicrobial and anti-inflammatory properties. Minor sesquiterpenes, including β-elemene, α-cubebene, and α-copaene, further enrich the chemical complexity.

FT-IR Analysis of Used and Purified Oils

Figure 2.3.6 shows the FT-IR spectra of sunflower oil, used (green trace) and purified (sky blue trace); palm oil, used (red trace) and purified (purple trace); and pumpkin oil, used (blue trace) and purified (mustard yellow trace).

The FTIR spectra of used and purified oils (Figure 2.3.9) show characteristic absorption bands typical of triglyceride structures, with minor differences related to oil degradation and purification. In the spectrum of WCO, a broader absorption band around 3400 cm^{-1} was observed, which can be attributed to O–H stretching vibrations associated with moisture and oxidation products. For all samples, the band at approximately 3006 cm^{-1} corresponded to =C–H stretching and indicated the presence of unsaturated fatty acids, while the bands at 2922 and 2852 cm^{-1} were attributed to asymmetric and symmetric CH_2 stretching vibrations of aliphatic chains. The intense band at 1745 cm^{-1} reflected the C=O stretching of triglyceride esters. After purification, slight changes in band intensity were observed, suggesting a possible removal of polar degradation compounds. Bands at 1465 and 1377 cm^{-1} corresponded to CH_2 and CH_3 bending vibrations. The region 1235–1160 cm^{-1} and the band at 1098 cm^{-1} were assigned to C–O–C stretching vibrations of ester linkages. The band near 720 cm^{-1} was assigned to CH_2 rocking, typical of long-chain fatty acids.

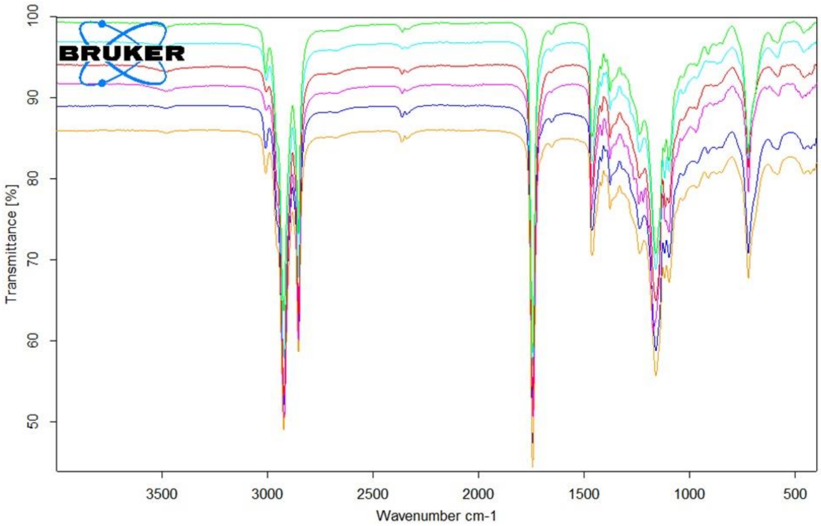


Figure 2.3.9. FTIR spectra of used and purified oils: used oils are represented by green (sunflower), red (palm), and blue (pumpkin) traces, while purified oils are shown in sky blue (sunflower), purple (palm), and mustard yellow (pumpkin) traces.

The similarity of the FTIR spectra before and after purification indicates that the treatment did not modify the main chemical structure of the oils, while the removal of impurities and polar compounds occurred without generating new IR-active functional groups.

FTIR analysis confirms the removal of oxidation products, which are considered environmentally harmful. Oxidized compounds such as aldehydes and ketones can persist in ecosystems if WCOs are discarded without treatment, contributing to aquatic toxicity and soil contamination. Proper purification therefore serves both quality recovery and environmental protection purposes.

Compared to purified oils, the used oils exhibited a reduced intensity of the unsaturation band at ~3006 cm^{-1} and an increase in the carbonyl band at ~1745 cm^{-1}, indicating the oxidation of unsaturated fatty acids and formation of secondary oxidation products such as aldehydes/ketones. These spectral changes were more pronounced in pumpkin oil, likely due to its high content of polyunsaturated fatty acids and increased susceptibility to thermal degradation. The preservation of the main ester and methylene bands after purification confirms that the purification process effectively removed oxidation products while maintaining the fundamental lipid structure of the oils.

2.3.1.3. Technological Process for Soap Production from Waste Cooking Oils

In general, WCO, previously subjected to filtration and decantation to remove solid impurities and water, is reacted with a NaOH solution, thereby producing soap and glycerol as the main products. The process may include additives such as fragrances, colorants, or stabilizers, depending on the desired final application. This valorization method contributes to reducing the environmental impact of food waste and aligns with trends in the circular economy. Moreover, recycling WCO into soap prevents their uncontrolled disposal into drains or landfills, which can cause soil contamination, water pollution, and adverse effects on aquatic ecosystems. By diverting waste oils from sewage systems, this process mitigates the formation of persistent fat, oil, and grease (FOG) deposits that block wastewater infrastructure.

Figure 2.3.10 presents the technological process for the production of soaps from WCO, along with the equipment corresponding to each stage, detailing the sequence of physical and chemical processes through which residual oils are transformed into finished soap products [206]. The figure highlights both the necessary technological operations—such as filtration, purification, bleaching, saponification, and molding—and the corresponding industrial equipment for each stage:

1. Collection and storage of WCOs. The process begins with the collection of WCOs from sources such as restaurants, households, or food processing units. Proper collection and storage of WCOs not only ensures process efficiency but also prevents environmental contamination by reducing accidental spills, seepage into soil, or mixing with other hazardous wastes. These oils are transferred to collection and storage tanks made from corrosion-resistant materials such as stainless steel or food-grade plastic. Storage is carried out safely, avoiding fur-ther contamination and separating the oils from other wastes.

2. Mechanical filtration (removal of solid impurities). Collected oils contain food residues, solid particles, and water, which must be removed before processing. Filtration is performed using a mesh filter (nylon), a filter press, a fine metal sieve, or by gravitational decantation. This step is essential to prevent contamination of subse-quent chemical processes and to protect the equipment.

3. Physico-chemical purification with brine solution (sodium chloride—NaCl). After filtration, WCOs may undergo purification with a concentrated NaCl solution. This treatment aims to separate polar compounds, mucilages, proteins, and other solu-ble impurities that dissolve or precipitate under the action of salt. The oil is mixed with 10% (w/w) NaCl solution at a 1:1 v/v ratio in a stirred reaction vessel and left in contact for 30–60 min at room temperature or slightly heated (~30–40 °C). The saline solution aids impurity separation through differences in solubility and density.

4. Decantation and phase separation. After the reaction, the mixture is allowed to decant in the mixer, where phase separation occurs: the upper layer contains the oils, and the lower layer contains water and heavy impurities. For higher efficiency, a centrifuge may be used to accelerate separation. This step removes most of the water and other residues. Mechanical filtration and decantation remove impurities, which otherwise, if released into the environment, could contribute to eutrophication or microbial contamination of water bodies. The separation of solids and water also reduces the chemical load entering

wastewater streams.

5. Bleaching with hydrogen peroxide. To improve the visual appearance of the soap, the purified oil undergoes an oxidative bleaching step with 30% H_2O_2 solution, at 1–2% v/v relative to the oil. This chemical treatment removes natural colorants or those formed through oxidation. Because H_2O_2 is a strong oxidizing agent, the pH (7–8) and temperature (40–60 °C) must be rigorously controlled during the 30 min reaction to avoid excessive oxidation of fatty acids and degradation of the raw material. The treatment is carried out with a 30% H_2O_2 solution at 1–2% v/v relative to the oil.

6. Post-bleaching separation. After bleaching, the mixture undergoes a decanta-tion/separation process in the mixer, either in a static decanter or by centrifugation, allowing phase separation. The bleached, purified oil rises to the surface, while the aqueous phase, containing impurities and excess adsorbed substances, settles at the bottom and is subsequently removed. Residual peroxide is checked using potassium iodide-starch paper.

7. Treatment with caustic soda (saponification). After bleaching, the oils are transferred into a mixer, where NaOH is added in a calculated excess based on the tri-glyceride content to ensure complete conversion of the remaining triglycerides into soap. This stage guarantees a complete reaction and stability of the final product. The saponification vessel is equipped with a constant stirring system, and the process is carried out at approximately 40 °C.

8a. Glycerin separation. After saponification, the soap paste contains glycerin as a by-product, which can affect stability and texture. Glycerin in excess is separated from the soap paste by decantation (or centrifugation). The recovered glycerin can be col-lected for further use. This step ensures that the soap has a consistent quality and re-duces irritation.

Note: If a superfat content of 5% is maintained, a portion of glycerin may remain in the soap together with the superfat, contributing to increased moisturization and a softer texture, without compromising product safety.

8b. Washing and neutralization of soap. To ensure safety, stability, and quality of the final product, the soap is washed with warm demineralized water or a 5–10% (w/v) saline solution. The mixture is stirred during washing and then allowed to settle. The washed soap is subsequently drained, and the process may be repeated. The pH of the final soap is carefully monitored and adjusted if necessary to ensure dermal safety. After washing and neutralization, the soap is allowed to cool and solidify, and the final product is formed into solid soap.

The production of soap from WCO thus converts a potential environmental pollutant into a safe, solid product, promoting circular economy principles and minimizing the ecological footprint of food industry waste. In contrast to uncontrolled disposal, this valorization reduces the risk of hydrocarbon pollution in soil and waterways.

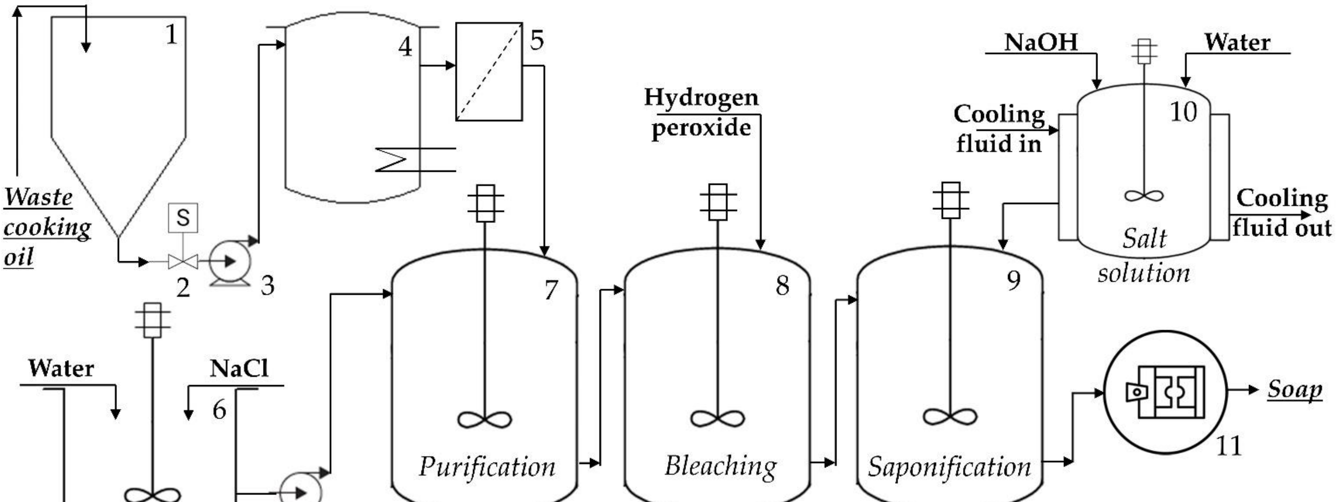


Figure 2.3.10. Process flow diagram for soap production from WCO, including the main stages and

corresponding equipment. Description: 1—Waste oil storage tank; 2—Solenoid valve; 3—Electric pump; 4—Preheating tank; 5—Filtration; 6—Brine preparation tank (with agitator); 7—Mixing reactor for oil purification by decantation; 8—Mixing reactor for oil bleaching by decantation; 9—saponification reactor; 10—Caustic soda dissolving tank; 11—Soap molding machine.

Soap Formulation

For soap production, the amounts of NaOH and distilled water were calculated considering a 5% superfat (SF), as follows:

- Total mass of oils, g:

$$\mathrm{M_{oils}} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{m_i} \tag{1}$$

- Theoretical mass of NaOH (SF = 0%), g:

$$\mathrm{m_{NaOH,0\%}} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{m_i} \cdot \mathrm{SV_i} \tag{2}$$

- Superfat correction (SF), g:

$$\mathrm{m_{NaOH,SF}} = \left(1 - \frac{\mathrm{SF}}{100}\right) \cdot \mathrm{m_{NaOH,0\%}} \tag{3}$$

- Water (distilled water) as a percentage of the mass of oils:

$$\mathrm{m_{H_2O}} = \left(\frac{\mathrm{W}}{100}\right) \cdot \mathrm{M_{oils}} \tag{4}$$

where $\mathrm{M_{oils}} = \sum \mathrm{m_i}$—total mass of oils (g); $\mathrm{m_i}$—mass of oil i (g), n—total number of types of oils; $\mathrm{SV_i}$—saponification value of oil i (g NaOH/g oil); SF—superfat percentage (0–10%, usually); $\mathrm{m_{H2O}}$—mass of distilled water required—usually 30% of the total oil weight (mL).

All soap formulations were prepared using the same base composition and saponification parameters, resulting in comparable alkalinity. The pH of the plain soap base after curing was 9.87 ± 0.04, and the incorporation of bioactive ingredients at low concentrations (1–3% w/w) did not significantly modify the final pH. Therefore, pH was considered constant across all tested samples.

Table 2.3.16 presents the composition of the formulation for functional soaps.

Table 2.3.16. Composition of the soap formulations obtained.

No.	**Ingredient**		**Quantity, g**	**SV, g NaOH/g Oil**
1	Purified sunflower oil		60	0.134
2	Purified palm oil		170	0.141
3	Purified pumpkin oil		170	0.136
4	Sodium hydroxide		52.37	-
5	Distilled water		125.5	-
6	Bioactive ingredient	Essential oil	8	-
		Honey	12	-
		Propolis extract	4	-

Note: SV—saponification value.

The proportions of oils used in the soap formulation were 15% sunflower oil, 42.5% palm oil, and 42.5% pumpkin seed oil, calculated relative to the total oil mass (400 g). At the final stage of soap preparation, different soap formulations were obtained by incorporating a single bioactive ingredient per batch, based on the total oil content. Specifically, honey was added at 3% (12 g), EOs at 2% (8 g), or propolis extract at 1% (4 g), depending on the formulation.

The amount of NaOH and distilled water was calculated (Equations (4)–(7)) for a superfat (SF) of 5% as follows:

$$\mathrm{m_{NaOH}} = (\text{amount of sunflower oil} \times 0.134 + \text{amount of palm oil} \times 0.141 + \text{amount of pumpkin oil} \times 0.136), 0\%\ \mathrm{SF} \quad (5)$$

$$\mathrm{m_{NaOH}} = \text{amount of NaOH, g SF } 0\% - \frac{5}{100} \times \text{amount of NaOH, g } 0\%\ \mathrm{SF} = \text{amount of NaOH, g } 5\%\ \mathrm{SF} \quad (6)$$

$$\mathrm{m_{NaOH}} = (60 \times 0.134 + 170 \times 0.141 + 170 \times 0.136) = 55.13\ \mathrm{g\ NaOH}, 0\%\ \mathrm{SF} \quad (7)$$

$$\mathrm{m_{NaOH}} = 55.13 - \frac{5}{100} \cdot 55.13 = 52.37\ \mathrm{g\ NaOH}, 5\%\ \mathrm{SF} \quad (8)$$

A plain soap, prepared from the same oil base without bioactive ingredients, was also prepared and used as a control for all physico-chemical analyses to assess the baseline properties of the soap matrix. For the antimicrobial activity assays, a commercial soap -Safeguard (Procter & Gamble (Pvt.) Ltd., Karachi, Pakistan) was used as a reference, since plain soap typically exhibits only modest antibacterial effects and does not provide a relevant biological comparison.

2.3.1.4. Physico-chemical Analysis and Antimicrobial Activity of Recycled Soaps

The increasing environmental concerns related to the disposal of used cooking oils (WCOs) have driven the search for sustainable valorization methods. Transforming WCOs into soap represents a practical approach to reducing waste, preventing soil and water pollution, and promoting circular economy principles. Besides minimizing environmental contamination, this process generates value-added products with both cosmetic and antimicrobial properties. The present chapter evaluates the physico-chemical characteristics of soaps derived from recycled oils and investigates the antimicrobial potential of formulations enriched with natural bioactive ingredients, highlighting their ecological and functional advantages.

Table 2.3.17 presents the main parameters used for the characterization of plain soap (soap without bioactive ingredients), compared with the analyzed commercial soap.

Table 2.3.17. Physico-chemical parameters for the quality assessment of plain soap.

Parameter	**Experimental Soap Without Bioactive Ingredients**	**Commercial Soap - Safeguard**
pH	9.87 ± 0.04	10.30 ± 0.07
Moisture content, %	15.30 ± 0.08 *	8.20 ± 0.09
Total Dissolved Solids, TDSs	0.46 ± 0.02	0.61 ± 0.05
Total Solids, TSs	1.25 ± 0.09	1.15 ± 0.06
Free caustic alkalinity, %	0.01 ± 0.00	0.00 ± 0.00
Foam height, cm	10.50 ± 1.00	12.40 ± 2.00

*Note: * Measured 4 weeks after soap preparation. Values are reported as mean ± standard deviation (n = 3).*

Table 2.3.17 presents the main parameters used for the characterization of plain soap (soap without bioactive ingredients) compared with the analyzed commercial Safeguard soap. The pH and free caustic alkalinity values indicate good skin compatibility and safety for both products. The experimental soap shows a higher moisture content, which may affect hardness and shelf life. Total dissolved solids and total solids are similar for both soaps, suggesting comparable concentration and solid content. The foam height is slightly higher in commercial soap, reflecting a better foaming capacity. Overall, the experimental soap demonstrates satisfactory physicochemical properties, close to those of the commercial product, despite the absence of bioactive additives.

The physico-chemical properties of the experimental soap indicate that recycling WCOs does not compromise the quality of the final product. Additionally, this approach prevents the potential release of oils into wastewater systems, reducing environmental contamination and promoting sustainable waste management.

Antimicrobial Properties of Soaps

The antimicrobial activity of the formulated soaps was evaluated using the diameter of the inhibition zone (mm) against *E. coli* and *S. aureus*. By incorporating bioactive natural ingredients such as honey, propolis, and essential oils, the recycled soaps not only achieve enhanced antibacterial properties but also demonstrate that value-added products can be derived from waste streams. This dual functionality aligns with sustainability goals by combining environmental protection, health benefits, and circular economy principles, converting a potential pollutant into a safe, functional product.

Tables 2.3.18 and 2.319 summarize the results for soaps enriched with various types of honey and propolis, while Tables 2.3.20 and 2.3.21 present the data for soaps containing different EOs. Each sample was tested at three concentrations (500, 250, and 125 mg/mL), and the inhibition zones were measured to assess the efficacy of the bioactive ingredients in limiting bacterial growth. For comparison, a commercial soap (Safeguard) was included as a reference. The results highlight the influence of the type and concentration of bioactive ingredients on the antibacterial properties of the soaps.

Table 2.3.18. Diameter of the zone of inhibition (mm) against *E. coli* for soap samples containing honey or propolis.

Concentration	**Sample Inhibition Diameter Area (mm)**						
	Soap with Polyfloral Honey—BPS1	**Soap with Linden Honey—BPS2**	**Soap with Acacia Honey—BPS3**	**Soap with Manna Honey—BPS4**	**Soap with Sunflower Honey—BPS5**	**Soap with Propolis—BPS6**	**Safeguard—Commercial Soap—SGS**
500 mg/mL	6.00 ± 0.50 ^{cde}	6.50 ± 0.50 ^{bc}	3.83 ± 0.29 ^{f}	4.83 ± 0.29 ^{ef}	5.17 ± 0.29 ^{de}	7.00 ± 0.87 ^{bc}	9.83 ± 0.29 ^{a}
250 mg/mL	4.83 ± 0.29 ^{de}	6.50 ± 0.00 ^{b}	3.50 ± 0.50 ^{f}	4.33 ± 0.29 ^{ef}	4.17 ± 0.29 ^{ef}	5.83 ± 0.29 ^{bcd}	8.17 ± 0.29 ^{a}
125 mg/mL	4.00 ± 0.00 ^{ef}	6.50 ± 0.00 ^{a}	4.17 ± 0.29 ^{def}	3.50 ± 0.50 ^{f}	3.67 ± 0.29 ^{ef}	5.00 ± 0.50 ^{bcd}	7.17 ± 0.29 ^{a}

Note: BPS—Bee Product Soap. Means followed by the same letter are not significantly different by the Tukey test at 5% level. Values are reported as mean ± standard deviation (n = 3).

Table 2.3.19. Diameter of the zone of inhibition (mm) against *S. aureus* for soap samples containing honey or propolis.

Concentration	**Sample Inhibition Diameter Area (mm)**						
	Soap with Polyfloral Honey—BPS1	**Soap with Linden Honey—BPS2**	**Soap with Acacia Honey—BPS3**	**Soap with Manna Honey—BPS4**	**Soap with Sunflower Honey—BPS5**	**Soap with Propolis—BPS6**	**Safeguard—Commercial Soap—SGS**
500 mg/mL	7.83 ± 0.29 ^{cde}	8.33 ± 0.29 ^{cd}	6.00 ± 0.50 ^{g}	7.17 ± 0.29 ^{ef}	6.67 ± 0.29 ^{fg}	8.67 ± 0.29 ^{bc}	19.00 ± 0.00 ^{a}
250 mg/mL	7.00 ± 0.50 ^{cde}	6.83 ± 0.58 ^{cde}	5.17 ± 0.29 ^{g}	6.00 ± 0.00 ^{efg}	5.67 ± 0.29 ^{fg}	7.83 ± 0.29 ^{bc}	17.17 ± 0.29 ^{a}
125 mg/mL	6.50 ± 0.50 ^{bc}	6.00 ± 0.00 ^{bcd}	4.17 ± 0.29 ^{ef}	5.17 ± 0.0.29 ^{def}	5.67 ± 0.76 ^{bcd}	6.67 ± 0.58 ^{b}	14.00 ± 0.00 ^{a}

Note: BPS—Bee Product Soap. Means followed by the same letter are not significantly different by the Tukey test at 5% level; Values are reported as mean ± standard deviation (n = 3).

Table 2.3.20. Diameter of the zone of inhibition (mm) against *E. coli* for soap samples containing EOs.

Concentration	**Sample Inhibition Diameter Area (mm)**						
	Soap with Clove EO—EOS1	**Soap with Rosemary EO—EOS2**	**Soap with Aril of Mace EO—EOS3**	**Soap with Nutmeg EO—EOS4**	**Soap with White Pepper EO—EOS5**	**Soap with Juniper EO—EOS6**	**Safeguard—Commercial Soap—SGS**
500 mg/mL	7.00 ± 0.50^{bc}	7.50 ± 0.00^{b}	7.17 ± 0.29^{bc}	6.00 ± 0.50^{cde}	6.17 ± 0.29^{cd}	6.83 ± 0.29^{bc}	9.83 ± 0.29^{a}
250 mg/mL	5.67 ± 0.76^{bcd}	6.17 ± 0.29^{bc}	6.00 ± 0.00^{bc}	4.83 ± 0.29^{de}	5.17 ± 0.29^{cde}	6.17 ± 0.29^{bc}	8.17 ± 0.29^{a}
125 mg/mL	5.17 ± 0.29^{bc}	5.33 ± 0.29^{b}	5.00 ± 0.00^{bcd}	4.33 ± 0.29^{cdef}	4.17 ± 0.29^{def}	4.50 ± 0.50^{bcde}	7.17 ± 0.29^{a}

Note: EOS—Essential Oil Soap. Means followed by the same letter are not significantly different by the Tukey's test at 5% level. Values are reported as mean ± standard deviation (n = 3).

Table 2.3.21. Diameter of the zone of inhibition (mm) against *S. aureus* for soap samples containing EOs.

Concentration	**Sample Inhibition Diameter Area (mm)**						
	Soap with Clove EO—EOS1	**Soap with Rosemary EO—EOS2**	**Soap with Aril of Mace EO—EOS3**	**Soap with Nutmeg EO—EOS4**	**Soap with White Pepper EO—EOS5**	**Soap with Juniper EO—EOS6**	**Safeguard—Commercial Soap—SGS**
500 mg/mL	8.50 ± 0.50^{bc}	9.50 ± 0.00^{b}	7.33 ± 0.29^{def}	6.50 ± 0.50^{fg}	6.50 ± 0.00^{fg}	7.83 ± 0.76^{cde}	19.00 ± 0.00^{a}
250 mg/mL	7.17 ± 0.29^{bcd}	8.17 ± 0.29^{b}	6.67 ± 0.29^{def}	5.00 ± 0.00^{g}	5.50 ± 0.50^{g}	7.17 ± 0.29^{bcd}	17.17 ± 0.29^{a}
125 mg/mL	6.50 ± 0.50^{bc}	6.17 ± 0.29^{bcd}	5.00 ± 0.50^{def}	4.00 ± 0.00^{f}	4.17 ± 0.29^{ef}	5.33 ± 0.29^{cde}	14.00 ± 0.00^{a}

Note: EOS—Essential Oil Soap. Means followed by the same letter are not significantly different by the Tukey test at 5% level. Values are reported as mean ± standard deviation (n = 3).

The results indicate that all soaps containing honey and propolis demonstrated measurable antimicrobial activity against S. aureus and E. coli, although the effectiveness varied according to both the soap concentration and the type of ingredient incorporated in the formulation. Overall, the inhibition zones decreased as the concentration was reduced, reflecting a clear dose–response relationship. The inhibition zones were larger in the case of the *S. aureus* strain than in the case of the *E. coli* strain. Among the formulations, the soap prepared with propolis produced the largest inhibition zones, suggesting a stronger antibacterial potential. When compared with the experimental soaps, the commercial Safeguard sample consistently produced larger inhibition zones across all concentrations, confirming its superior antimicrobial performance, likely due to the inclusion of specific antibacterial agents in its formulation.

The results revealed that all tested soaps containing EOs displayed moderate antimicrobial activity against both bacterial strains, with inhibition zones generally larger than those recorded for soaps containing honey and propolis. A concentration-dependent trend was observed, as the antimicrobial activity decreased with lower bacterial concentrations. Among the formulations, the rosemary EO-based soap consistently produced larger inhibition zones, suggesting greater antibacterial effectiveness. In contrast, the soaps formulated with nutmeg EO and white pepper EO showed comparatively weaker activity. Like the results observed for soaps containing honey and propolis, the commercial Safeguard soap exhibited the largest inhibition zones across all concentrations, confirming its superior performance, likely attributed to the presence of dedicated antibacterial agents in its industrial formulation.

The antimicrobial performance of the formulated soaps was mainly governed by several key parameters. The most influential factor was the type and chemical composition of the incorporated bioactive ingredient, with phenolic-rich components such as propolis and clove EO showing stronger antibacterial effects. The concentration of the soap solution also played a critical role, as a clear dose–response relationship was observed for all formulations. In addition, bacterial susceptibility significantly influenced the results, with Gram-positive *S. aureus* being more sensitive than Gram-negative *E. coli*.

In contrast, formulation-related parameters such as the soap base composition and pH were kept constant across all samples and therefore did not contribute to the observed differences in antimicrobial activity.

The differences in antimicrobial activity between the soap formulations can be mechanistically linked to the phenolic and flavonoid content of the incorporated bioactive ingredients. Phenolic compounds are known to disrupt bacterial cell membranes, alter permeability, and induce protein denaturation, leading to cell lysis. In the present study, soaps containing propolis, which exhibited the highest total phenolic and flavonoid content, consistently produced larger inhibition zones against both bacterial strains compared to honey-based formulations. Similarly, clove EO, characterized by a high phenolic content, demonstrated enhanced antibacterial performance relative to EOs with lower phenolic levels.

GC-FID analysis further supports these observations by revealing clear differences in the dominant chemical constituents of the EOs. Clove oil was dominated by eugenol, a phenylpropanoid with well-documented antimicrobial activity attributed to its ability to disrupt cytoplasmic membranes and interfere with enzyme systems. Rosemary oil, rich in 1,8-cineole and camphor, exhibited moderate antibacterial activity consistent with the membrane-disrupting effects of oxygenated monoterpenes. In contrast, EOs dominated by hydrocarbon monoterpenes or sesquiterpenes, such as juniper and white pepper oils, displayed weaker antimicrobial effects, reflecting the lower reactivity of these compounds toward bacterial membranes.

The higher susceptibility of S. aureus compared to *E. coli* can be explained by differences in the structure of the cell. The outer membrane of Gram-negative bacteria acts as an additional permeability barrier, limiting the diffusion of hydrophobic phenolic and terpenoid compounds, whereas Gram-positive bacteria are more readily affected by membrane-active agents. This structural difference accounts for the consistently larger inhibition zones observed against *S. aureus* across all formulations.

Although the overall inhibition zones are relatively modest, the differences among formulations correlate with the phenolic and flavonoid content of the bioactive ingredients, indicating that the observed antimicrobial activity is mainly attributable to the added compounds rather than the alkaline soap matrix. A one-way ANOVA was conducted separately for each soap concentration (125, 250, and 500 µg/mL) to evaluate the effect of formulation type on E. coli growth inhibition. The results indicated highly significant differences among the 14 formulations at all concentrations: 125 µg/mL, $F_{value} = 33.43$, $p < 0.0001$, $R^2 = 0.940$; 250 µg/mL, $F_{value} = 35.97$, $p < 0.0001$, $R^2 = 0.943$; 500 µg/mL, $F_{value} = 35.39$, $p < 0.0001$, $R^2 = 0.942$.

Levene's tests for homogeneity of variances were nonsignificant (all $p > 0.05$), which confirms that the assumption of equal variances was satisfied. Tukey's HSD post-hoc analysis revealed that at 125 µg/mL, SGS (7.17 mm) and BPS2 (6.50 mm) formed the top efficacy group and did not differ significantly from each other ($p > 0.05$), whereas all other soaps showed significantly smaller inhibition zones ($p < 0.05$). At 250 µg/mL, SGS (8.17 mm) remained the most active formulation, followed closely by BPS2 (6.50 mm; $p > 0.05$ vs. SGS), while BPS3–BPS5 exhibited the weakest activity ($p < 0.001$ vs. SGS). At the highest concentration, 500 µg/mL, SGS (9.83 mm) continued to display the greatest inhibition, but EOS2 (7.5 mm), EOS3 (7.17 mm), EOS1 (7.00 mm), and BPS6 (7.00 mm) achieved statistically comparable effects ($p > 0.05$ vs. SGS).

Antibacterial efficacy increased significantly with concentration, indicating a clear dose-dependent response. SGS consistently showed the strongest inhibitory activity, while several essential-oil-based soaps (particularly EOS2 and EOS3) approached similar performance at higher doses. Conversely, BPS3 remained significantly less effective at all concentrations ($p < 0.0001$).

Regarding the effect of soap formulation on *S. aureus* growth inhibition, one-way ANOVA showed significant differences among the 13 formulations at all concentrations: 125 µg/mL: $F_{value} = 121.38$, $p < 0.0001$, $R^2 = 0.982$; 250 µg/mL: $F_{value} = 252.54$, $p < 0.0001$, $R^2 = 0.991$; 500 µg/mL; $F_{value} = 235.61$, $p < 0.0001$, $R^2 = 0.991$.

Levene's test indicated unequal variances for the 125 µg/mL and 500 µg/mL data sets ($p < 0.05$), but not for the 250 µg/mL set ($p > 0.05$), so results at 125 and 500 µg/mL should be interpreted with caution. Tukey's HSD post-hoc comparisons showed that none of the tested soap formulations matched SGS in mean inhibition-zone diameter at any concentration—SGS formed a unique top group (SGS: 14 mm at 125 µg/mL; 17.17 mm at 250 µg/mL; 19.00 mm at 500 µg/mL), and all other formulations were significantly lower (Tukey grouping letters: SGS alone in group A at each concentration). The next-best formulations across concentrations were BPS6/EOS1/EOS2 (means ≈ 6.7–9.5 mm depending on concentration), but these remained significantly lower than SGS.

Antibacterial activity increased with concentration for most formulations (dose-dependent response), yet SGS maintained a clear superiority against S. aureus across the tested range.

Because Levene's test indicated significant heterogeneity of variances for the S. aureus data at 125 and 500 μg/mL, Welch's ANOVA followed by the Games–Howell post-hoc test was applied to provide variance-robust statistical comparisons. Welch ANOVA revealed significant differences among the groups for all three datasets (125: $F_{(13,14.28)} = 19.60$, $p < 0.001$; 250: $F_{(13,12.88)} = 24.49$, $p < 0.001$; 500: $F_{(13,11.72)} = 43.61$, $p < 0.001$). Games–Howell post-hoc tests indicated that the SGS category consistently showed significantly higher values compared to most BPS and EOS categories, with the magnitude of differences increasing with the dataset level.

2.3.1.5. Conclusions

The research presented in this subchapter demonstrates a sustainable and innovative approach to the valorization of WCO by transforming them into functional soaps with enhanced antimicrobial properties. The study integrates the principles of circular economy, pollution reduction, and the generation of value-added products, while meeting quality and safety requirements.

This study demonstrates a sustainable method for recycling WCOs into soaps with satisfactory physico-chemical and antimicrobial properties. Soaps enriched with honey, propolis, and essential oils exhibited dose-dependent antibacterial activity, with essential oils showing the strongest effects. The enhanced antimicrobial activity is attributed to the combined effects of the alkaline nature of the soaps and the bioactive compounds present in the natural additives. Among essential oils, rosemary and clove showed the strongest antibacterial potential, correlated with their higher phenolic and volatile bioactive contents, particularly eugenol and 1,8-cineole, which disrupt bacterial membranes. *S. aureus*, being Gram-positive, is more susceptible than Gram-negative *E. coli*.

For the valorization of WCO into antimicrobial soaps enriched with apicultural products and essential oils, the used oils were pretreated through filtration, purification using saline solution, natural zeolite or eggshell, and bleaching with hydrogen peroxide. The optimized technological process (saponification with NaOH at 5% superfat) enabled the production of soaps with physicochemical properties comparable to commercial products. FTIR analyses confirmed the preservation of the main lipid structure after purification, along with a reduction in oxidation products.

Frying simulations highlighted a rapid increase in the peroxide value (PV) and a decrease in the iodine value (IV) at temperatures above 180°C, confirming intense oxidative degradation. Second-order polynomial statistical models accurately predicted ($R^2 > 0.95$) the evolution of these parameters depending on time and temperature. Purification with zeolite and eggshell proved effective in reducing acidity and PV, while FT-IR analysis indicated the removal of polar oxidation compounds without altering the basic triglyceride structure. These treatments reduce the environmental risks associated with the uncontrolled disposal of used oils.

The enriched soaps demonstrate both cosmetic and antimicrobial potential while promoting the sustainable reuse of WCOs. This approach supports circular economy principles by transforming food industry waste into value-added, eco-friendly products. Future studies could focus on optimizing bioactive concentrations, expanding antimicrobial testing to additional microbial strains, and evaluating long-term stability and consumer acceptability of formulations.

The valorization of used cooking oils into functional soaps provides a sustainable solution for waste management, combining environmental protection with practical and economic benefits, and demonstrating how waste materials can be transformed into high-value consumer products.

General Conclusions:

- The transformation of waste cooking oils into soaps represents a viable technological, economic, and environmental solution, reducing hydrocarbon pollution and preventing the clogging of sewage systems.
- The obtained soaps show satisfactory physicochemical properties (pH, moisture, hardness), comparable to commercial products, while the incorporation of apicultural products and essential oils provides significant antimicrobial activity, with higher efficiency against Gram-positive bacteria.
- Purification using natural adsorbents (zeolite, eggshell) and saline solution enables the recovery of oil quality, reducing the content of polar compounds and oxidation products, with direct environmental benefits.

- The approach supports circular economy principles by converting a problematic waste into a value-added product, contributing to sustainable development goals (SDG 6, 12, and 13) and reducing the carbon footprint compared to conventional disposal methods.
- Statistical analyses (ANOVA, polynomial models) confirmed significant correlations between the concentration of bioactive ingredients, phenolic content, and antimicrobial efficiency.

2.3.2. Integrated Environmental Risk Assessment of Soap Manufacturing Processes under the ISO 14001 Framework

In the context of growing global concerns regarding environmental protection and the reduction of industrial pollution, soap production, even at a small scale, can have significant impacts on natural resources and ecosystems. The use of recycled raw materials, such as waste cooking oil (WCO), offers important opportunities for waste valorization and for reducing pressure on virgin resources; however, it also introduces operational variability and additional environmental risks related to atmospheric emissions, wastewater, solid waste, and the handling of chemical substances.

Integrated environmental risk assessment is essential for identifying, quantifying, and managing these aspects, in accordance with the principles of ISO 14001 environmental management systems [3] and ISO 31000 risk management [207]. The methodology proposed in this study employs a semi-quantitative Environmental Impact Score (EIS) to prioritize critical environmental aspects [208], supporting both operational decisions and the planning of preventive measures within WCO-based soap production processes.

By applying this approach, the aim is both to maximize the ecological benefits generated by waste valorization and to minimize the risks associated with pollution and chemical use, thereby contributing to the development of a sustainable and environmentally responsible production process..

2.3.2.1. Environmental Impact Score Assessment Methodology

The method facilitates the systematic identification, assessment, and classification of environmental aspects associated with cold-process soap production using recycled raw materials, particularly waste cooking oil (WCO). The assessment considers both normal operating conditions (N) and abnormal conditions (A), including accidental spills, equipment failures, fires, and process loss.

Environmental aspects were systematically identified for each unit operation, encompassing the collection and transport of waste cooking oils, storage of raw materials, cold-process soap production, laboratory testing, equipment maintenance, utility use, and administrative operations. The evaluation considered resource use (energy, water, and chemicals), atmospheric emissions, wastewater and solid waste generation, accidental spills, and noise. Particular focus was placed on operations involving recycled oils, given their potential to elevate emissions, material losses, and environmental contamination risks.

Each identified environmental aspect was evaluated using three criteria: legal requirements (L), pollutant characteristics (P), and control measures (C). Numerical values were assigned according to predefined scales.

The legal requirements (L) criterion reflects the degree of regulatory applicability, compliance obligations, and the potential risk of legal non-compliance associated with each environmental aspect. The scoring scale ranges from 1 (negligible legal relevance) to 10 (strict regulatory control with high non-compliance risk), as detailed in Table 2.

Table 2. Scoring framework for legal and regulatory requirements (L).

L Score	Description
1	No specific legal requirements apply; aspect is not regulated
2	General environmental principles apply, no explicit limits
3	Minor regulatory references, no monitoring required
4	Legal requirements apply indirectly
5	General environmental legislation applies
6	Specific legal requirements with basic compliance obligations
7	Mandatory monitoring or reporting obligations apply
8	Strict legal limits apply (e.g., emission thresholds)
9	High regulatory scrutiny, frequent inspections required
10	Critical legal relevance; high risk of non-compliance or penalties

The pollutant characteristics (P) criterion evaluates the intrinsic hazard, persistence, and potential environmental impact of emissions, effluents, or waste streams generated by the assessed activity. The scale ranges from low-impact, non-hazardous pollutants to highly hazardous substances with long-term environmental effects. Table 3 outlines the scoring framework applied to pollutant characteristics (P) for the comparative environmental risk assessment.

Table 3. Scoring framework for pollutant characteristics (P).

P Score	Description
1	Negligible impact; non-polluting or inert substances
2	Very low environmental impact
3	Low impact; biodegradable or easily managed
4	Slight environmental impact
5	Moderate environmental impact
6	Persistent pollutant with limited dispersion
7	Significant environmental impact
8	High-impact pollutant with potential accumulation
9	Very high environmental impact; long-term effects
10	Hazardous pollutant; severe and irreversible impact

The control measures (C) criterion assesses the effectiveness, reliability, and adequacy of existing technical, organizational, and procedural controls implemented to prevent or mitigate environmental impacts. Higher scores indicate lower control effectiveness. Table 4 outlines the scoring framework for control measures (C) within the overall environmental risk assessment methodology.

Table 4. Scoring framework for control measures (C).

C Score	Description
1	Fully effective controls, continuously monitored
2	Highly effective controls with redundancy
3	Effective controls, minor gaps
4	Controls generally effective, limited monitoring
5	Partially effective controls
6	Controls exist but are inconsistently applied
7	Limited control effectiveness
8	Controls insufficient for risk level
9	Minimal control measures in place
10	No control measures implemented

The Environmental Impact Score (EIS) for each aspect was calculated as the sum of the three evaluation criteria:

$$EIS_i = L_i + P_i + C_i \tag{9}$$

where $L_i \in [1,10]$, $P_i \in [1,10]$, $C_i \in [1,10]$. The EIS can vary between a minimum value of 3 and a maximum value of 30.

Based on the calculated EIS, environmental aspects were classified into two categories: non-significant (NS), if $EIS_i < 20$ and significant (S), if $EIS_i \geq 20$.

The EIS threshold defining significant environmental aspects ($EIS \geq 20$) was selected as a conservative screening criterion, corresponding to approximately two-thirds of the maximum possible score. This threshold is consistent with ISO 14001-based environmental management practices, where significance levels are defined to support prioritization rather than absolute risk quantification.

The semi-quantitative scoring approach involves expert judgment in assigning L, P, and C scores, which introduces inherent subjectivity. While this approach supports relative prioritization of environmental aspects and ensures transparency, it does not provide an absolute quantification of environmental risk. To ensure objectivity and consistency in scoring, the L, P, and C values (1–10) were assigned by three domain experts, each with over 10 years of experience in asphalt production and environmental assessment. The scoring was guided by predefined criteria for each aspect, as described in Tables 2–4, to minimize subjective interpretation. Inter-rater reliability was evaluated using Cronbach's alpha, resulting in a value of 0.87, indicating high agreement among the experts. This approach ensures that semi-quantitative scoring provides consistent and reproducible results while reflecting expert judgment.

For processes involving recovered waste materials (e.g., used cooking oils), an additional evaluation step was applied to account for increased uncertainty and variability. The EIS was recalculated under abnormal operating conditions by adjusting the pollutant and control parameters as follows:

$P_A = P + \Delta P$, where $\Delta P \in \{0, 2.5, 5\}$ reflects additional emissions or material losses; (10)

$C_A = C + \Delta C$, where $\Delta C \in \{0, 2.5, 5\}$ reflects reduced control effectiveness during abnormal conditions. (11)

Abnormal operating conditions were evaluated separately, considering accidental spills, fires, and equipment failures. In such cases, environmental aspects were assumed to have higher impact severity and reduced control efficiency, leading to increased EIS values. Environmental aspects identified as significant under abnormal conditions were prioritized for emergency planning, preventive maintenance, and staff training.

The stepwise increments ΔP and ΔC were deliberately defined as discrete values (0, 2.5, and 5) to represent increasing levels of deviation from normal operating conditions. A value of 0 corresponds to minor disturbances with negligible additional emissions or loss of control, while intermediate (2.5) and maximum (5) increments represent moderate and severe abnormal scenarios, such as partial equipment malfunction or major accidental events. This semi-quantitative approach allows the methodology to capture the increased uncertainty, variability, and reduced process control typically associated with the use of recovered waste materials, without resorting to overly conservative worst-case assumptions.

Uncertainties arise from the semi-quantitative nature of L, P, C scores and $\Delta P/\Delta C$ increments, as well as from process variability. Consequently, the EIS values should be interpreted as relative indicators of environmental significance rather than precise measures of impact severity.

Aspects classified as significant were further analyzed and addressed through mitigation measures, environmental objectives, and monitoring programs.

The EIS was calculated using a simple additive approach to ensure transparency, reproducibility, and alignment with semi-quantitative environmental aspect assessment practices under ISO 14001. At this screening level, legal requirements, pollutant characteristics, and control measures were considered equally relevant, as the EIS is intended to support the relative ranking and prioritization of environmental aspects under normal and abnormal operating conditions rather than to quantify absolute environmental risk.

Future applications of the methodology may incorporate weighted scoring approaches to reflect sector- or site-specific priorities. In such cases, the EIS may be expressed as (4):

$$EIS_i = w_L \cdot L_i + w_P \cdot P_i + w_C \cdot C_i \quad (12)$$

where the weighting coefficients (w_L, w_P, w_C) reflect the relative importance of legal, environmental, and control-related factors. However, these weights are inherently context- and industry-dependent and may introduce subjectivity if not supported by extensive expert elicitation or regulatory guidance. An example could include weighting factors such as $w_L = 0.35$, $w_P = 0.40$, and $w_C = 0.25$; however, no universal weighting scheme can be considered valid across different industrial sectors.

2.3.2.2. Application of the Environmental Impact Score Methodology to Soap Production from Waste Cooking Oil

The proposed methodology was applied to a representative cold-process soap production unit using waste cooking oil (WCO) as a secondary raw material. The analysis covered key operational processes and activities with potential environmental relevance, including raw material collection, purification, chemical treatment, saponification, separation, and finishing stages.

Tables 5–10 summarize the environmental aspects and associated impacts of soap production using WCO, with significant and non-significant risks identified for air, soil, water, resources, and waste management.

Table 5. Environmental impacts of logistics and transport operations.

Activities	**Environmental Aspect**	**Conditions**	**Environmental Impact**	**L**	**P**	**C**	**EIS**	**Classification**
Operation of vans, trucks	Fuel consumption	N	Resource depletion	5	3	1	9	NS
	CO_2, NOx, particulate emissions	N	Air pollution	10	5	5	20	S
	Traffic noise	N	Noise pollution	1	3	1	5	NS
Accidental oil spills	Environmental contamination	A	Soil pollution	10	5	5	20	S

Abbreviations: N—normal operating conditions; A—abnormal operating conditions; L—legal requirements; P—pollutant characteristics; C—control measures; EIS—Environmental Impact Score; NS—non-significant; S—significant.

Table 6. Environmental impacts of raw material and auxiliary substance storage.

Activities	**Environmental Aspect**	**Conditions**	**Environmental Impact**	**L**	**P**	**C**	**EIS**	**Classification**
WCO storage	Leakage/spill risk	A	Soil and water pollution	10	6	5	21	S
Chemical storage (NaOH, H_2O_2)	Chemical hazard	A	Soil/water pollution	10	8	5	23	S
Storage tanks	Odor emissions	N	Air pollution	5	3	3	11	NS
Packaging	Packaging waste	N	Waste management	5	3	3	11	NS

Table 7. Environmental impacts of soap production process.

Activities	Environmental Aspect	Conditions	Environmental Impact	L	P	C	EIS	Classification
Filtration	Solid residues	N	Waste generation	5	3	3	11	NS
Purification (NaCl)	Saline wastewater	N	Water pollution	10	6	5	21	S
Bleaching (H_2O_2)	Oxidative chemicals	N	Chemical pollution	10	7	5	22	S
	Accidental release	A	Soil/water pollution	10	8	6	24	S
Saponification (NaOH)	Corrosive substances	N	Soil/water pollution	10	8	5	23	S
	Chemical spills	A	Soil pollution	10	7	6	23	S
Glycerin separation	By-product generation	N	Waste/resource	4	2	2	8	NS
Washing/neutralization	Alkaline wastewater	N	Water pollution	10	7	6	23	S

Table 8. Environmental impacts of laboratory and quality control activities.

Activities	Environmental Aspect	Conditions	Environmental Impact	L	P	C	EIS	Classification
Soap testing	Chemical waste	N	Waste management	10	8	5	23	S
	Wastewater	N	Water pollution	10	5	5	20	S
	Vapor emissions	N	Air pollution	5	3	3	11	NS

Table 9. Environmental impacts of technical and maintenance activities.

Activities	Environmental Aspect	Conditions	Environmental Impact	L	P	C	EIS	Classification
Equipment maintenance	Used oil	N	Waste management	10	5	3	18	NS
	Accidental spills	A	Soil pollution	10	5	5	20	S
	Solid waste	N	Waste management	5	3	3	11	NS

Table 10. Environmental impacts of administrative and utilities activities.

Activities	Environmental Aspect	Conditions	Environmental Impact	L	P	C	EIS	Classification
Office activities	Paper waste	N	Waste management	5	1	1	7	NS
	Energy consumption	N	Resource depletion	1	1	1	3	NS
Cleaning	Wastewater with detergents	N	Water pollution	10	3	3	16	NS

Note: Aspects classified as significant are managed through environmental objectives, monitoring, and operational measures in accordance with ISO 14001.

The analysis highlights that processes with significant risk (S) are mainly those involving chemical handling, wastewater generation, accidental spills, and hazardous substances. These aspects require operational measures and continuous monitoring, while non-significant risks can be managed through standard environmental procedures.

Table 11. Key environmental risk factors identified for soap production processes.

Process	**Identified Environmental Aspect**	**Legal Requirements (L)**	**Pollutant Characteristics (P)**	**Control Measures (C)**
Bleaching process	Use of hydrogen peroxide	Strict handling and storage regulations	Strong oxidizing agent with environmental impact	Controlled dosing, temperature and pH monitoring
Saponification	Use of sodium hydroxide	Chemical safety and waste regulations	Highly corrosive substance with high environmental risk	Automated dosing and PPE use
Purification stage	Saline wastewater	Discharge limits apply	High salinity affecting ecosystems	Basic separation, limited treatment
Washing/neutralization	Alkaline wastewater	Water protection regulations	High pH and organic load	Neutralization and discharge control systems

The results indicate that processes involving chemical reactions and wastewater generation represent the most significant environmental risks in soap production from WCO. While the use of waste oil contributes to circular economy objectives and reduces environmental burdens associated with waste disposal, it also introduces additional risks related to raw material variability and increased processing steps.

Wastewater management was identified as the highest-ranked environmental risk, emphasizing the need for improved treatment systems and stricter operational control.

A comparative risk analysis was established between conventional soap production (using fresh oils/fats) and WCO-based soap production. To quantify the influence of waste-derived materials, a comparative risk index was calculated:

$$\Delta EIS = EIS_{RAP} - EIS_{Conventional} \tag{13}$$

Table 12. Comparative EIS for conventional and WCO-based soap production.

Scenario	**L**	**P**	**C**	**EIS**
Conventional oils	7	5	4	16
WCO-based soap	8	7	5	20
ΔEIS = 20 − 16 = +4	+1	+2	+1	+4

The observed ΔEIS increase of +4 for WCO-based soap production indicates a moderately higher environmental risk, primarily due to additional purification steps, chemical usage, and wastewater generation. However, this increase must be balanced against environmental benefits such as waste reduction and resource recovery.

The results confirm that soap production using WCO presents higher operational environmental risks compared to conventional processes. However, these risks are controllable and can be significantly reduced through appropriate technological and organizational measures.

Table 13. Environmental impact assessment after implementation of control measures.

Activities	**Control Measures**	**L_f**	**P_f**	**C_f**	**EIS_f**	**Reclassification**
Transport operations	Process optimization, monitoring	10	5	3	18	NS
Oil storage	Leak prevention systems	10	3	3	16	NS
Chemical handling	Controlled dosing, PPE	10	4	3	17	NS
Purification process	Wastewater treatment	10	4	3	17	NS
Bleaching	Process monitoring and control	10	4	3	17	NS
Saponification	Automation and training	10	4	3	17	NS
Washing/neutralization	Neutralization and treatment	9	4	4	17	NS
Laboratory activities	Controlled waste disposal	10	4	3	17	NS
Maintenance	Preventive maintenance	10	3	3	16	NS

Abbreviations: L_f—Final Legal Requirements; P_f—Final Pollutant Characteristics; C_f—Final Control Measures; EIS_f—Final Environmental Impact Score; NS—non-significant.

Processes involving chemical handling and wastewater generation represent the highest environmental risks in soap production from WCO. The use of waste-derived raw materials increases operational complexity but supports circular economy objectives. The methodology provides a clear framework for prioritizing environmental management actions.

It should be noted that the present application of the EIS methodology is specific to the cold-process soap production described above. Its applicability to other soap production techniques, such as hot-process or industrial continuous processes, has not been evaluated and represents a limitation of this study.

The reviewed studies and the present assessment demonstrate that the use of waste cooking oil (WCO) as a raw material in soap production represents a technically feasible and environmentally beneficial alternative to conventional formulations based on virgin oils. The valorization of WCO contributes to waste reduction and supports circular economy principles, while enabling the production of soaps with satisfactory physicochemical properties and functional performance.

However, the quality and performance of the final product are strongly dependent on the proper pre-treatment and purification of the waste oil. WCO is characterized by variability in composition, the presence of degradation products, free fatty acids, water, and impurities, which may negatively affect the saponification process and the quality of the resulting soap. Consequently, adequate filtration, separation, and purification steps are essential to ensure process stability and product consistency.

The analysis highlights that the use of recycled raw materials introduces additional technological complexity compared to conventional soap production. The incorporation of stages such as saline purification and oxidative bleaching increases the number of process steps, the consumption of auxiliary chemicals, and the generation of secondary waste streams, particularly wastewater. Moreover, the handling of reactive substances such as NaOH and H_2O_2 requires strict control measures due to their corrosive and oxidizing properties.

From an environmental perspective, the use of WCO significantly reduces the need for virgin raw materials and prevents improper disposal of used oils, which are known to cause severe environmental pollution if discharged into water systems. At the same time, the process generates environmental risks associated with alkaline and saline wastewater, chemical handling, and potential accidental releases. These findings confirm that while waste valorization offers clear environmental advantages, it must be accompanied by effective risk management and process control.

An important observation is that the integration of multiple treatment stages can improve the overall quality of the recycled raw material, but may also increase environmental burdens if not properly optimized. Therefore, process efficiency must be balanced with environmental performance through careful selection of operating conditions, reagent dosages, and treatment technologies.

The variability of WCO properties represents a key challenge for industrial implementation. Differences in source, composition, and degradation level require continuous monitoring, standardized characterization, and strict quality control to ensure reproducibility and product safety. Without such controls, the performance of the final soap product and the stability of the process may be compromised.

Finally, the lack of harmonized technical standards and regulatory guidelines for the use of WCO in cosmetic and detergent applications remains a significant barrier to large-scale implementation. The development of clear specifications, quality criteria, and environmental guidelines would facilitate the safe and efficient integration of waste-derived raw materials into soap production processes.

In the long term, maintaining conventional soap production processes based exclusively on fresh/virgin oils may lead to increased pressure on natural resources, higher production costs, and greater environmental impacts associated with agricultural production, processing, and transportation of raw materials. Additionally, the improper disposal of waste cooking oil continues to represent a significant environmental issue, contributing to water pollution and infrastructure damage.

By contrast, the controlled integration of WCO into soap production processes offers substantial long-term environmental and economic benefits. The reuse of waste oils reduces the demand for virgin raw materials, decreases waste generation, and contributes to resource efficiency. This approach aligns with circular economy principles and supports the transition toward more sustainable production systems.

To mitigate the environmental risks associated with WCO-based soap production, the implementation of integrated technical and organizational measures is essential. These include the optimization of purification and saponification processes, strict control of chemical dosing, proper handling and storage of hazardous substances, wastewater neutralization and treatment, periodic equipment maintenance, and staff training. In addition, the development of preventive measures for accidental spills and process deviations plays a critical role in minimizing environmental impacts.

The long-term environmental impact of WCO valorization is particularly significant in terms of waste reduction and pollution prevention. By diverting used oils from uncontrolled disposal pathways, the process contributes to the protection of aquatic ecosystems and reduces the burden on wastewater treatment systems.

From an economic perspective, the use of WCO can lead to cost savings by replacing more expensive virgin oils, although these benefits may be partially offset by the additional costs associated with purification, chemical treatment, and environmental protection measures. Nevertheless, over the long term, the increased efficiency in resource utilization and waste management can improve the overall sustainability and resilience of the production system.

The findings indicate that the integration of WCO into soap production represents a viable and sustainable strategy, provided that environmental risks are properly managed. The balance between environmental benefits and operational challenges is essential for the successful implementation of such processes at an industrial scale.

2.3.2.3. Conclusions

This study presents a structured framework for environmental risk assessment in soap production processes using waste cooking oil, based on the Environmental Impact Score (EIS) methodology. The approach enables the systematic identification, evaluation, and prioritization of environmental aspects associated with each stage of the technological process.

The results indicate that the use of WCO introduces additional environmental and operational risks compared to conventional soap production, primarily due to the need for purification stages, the use of reactive chemical agents, and the generation of wastewater. The most significant environmental impacts are associated with chemical handling and wastewater management.

Despite these challenges, the analysis confirms that the environmental risks can be effectively controlled through appropriate technical and organizational measures, including process optimization, monitoring systems, and staff training. The methodology demonstrates that sustainable production practices based on waste valorization can be successfully implemented within an environmental management framework aligned with ISO 14001.

The findings highlight that the use of WCO represents a sustainable alternative to conventional raw materials, contributing to waste reduction, resource efficiency, and environmental protection. At the same time, the study emphasizes the importance of integrating environmental risk assessment with process management to ensure safe and efficient operation.

From a practical perspective, the results provide guidance for producers interested in implementing WCO-based soap production, indicating the need for enhanced control measures, particularly in stages involving chemical reactions and wastewater generation. For decision-makers and regulators, the study supports the development of policies and standards that encourage the safe use of recycled materials in small- and medium-scale production systems.

Future research should focus on the optimization of purification and treatment processes, the development of standardized quality criteria for WCO, and the integration of advanced environmental monitoring techniques. Such efforts would further enhance the applicability and robustness of the proposed framework and support the transition toward sustainable production practices based on circular economy principles.

General conclusions:

- The conversion of waste cooking oils into enriched antimicrobial soaps proves to be a practical and sustainable solution, reducing pollution, limiting the use of virgin resources, and generating products with real functional and economic value.
- The EIS methodology provides a clear and easy-to-apply tool for environmental risk assessment. It allows risks to be identified and prioritized in a structured way and can be smoothly integrated into environmental management systems such as those based on ISO 14001.
- From an environmental perspective, the benefits are evident: reduced waste, more efficient resource use, and prevention of pollution. The potential operational risks are generally manageable, provided that the technological process is properly optimized and controlled.
- This approach supports sustainable development goals and demonstrates that even small- and medium-scale production can be carried out responsibly, in line with the principles of the circular economy.

Part III. DEVELOPMENT PLAN OF SCIENTIFIC, ACADEMIC AND PROFESSIONAL CAREER

The overall objective of my academic activity is to respond effectively to future demands from both the institution in which I am active and from the business environment and the local community. Achieving this objective requires the continuous improvement of teaching activities as well as the sustained advancement of scientific research. The derived objectives and the courses of action through which I intend to implement these goals in practice have been structured along two main dimensions: *the enhancement of teaching activities* and *the development of research activities*.

3.1. Continuous Improvement of Teaching Activities

In a higher education institution, teaching activity is the primary means through which discipline-specific knowledge is transferred, while simultaneously developing the practical skills and competencies required by the labor market. At the doctoral school level, the focus is on promoting and achieving excellence in both fundamental and applied research, aiming to enhance the quality of university training and to establish partnerships for interdisciplinary research at both national and international levels.

The *teaching objectives* set for my future activity aim to translate intellectual achievements, knowledge, and professional competencies in my field into the students' professional profile. These objectives include the development and enhancement of knowledge regarding concepts, methods, procedures, and techniques for analyzing operational and investment activities relevant to the profession, as well as the formation and improvement of skills and abilities related to specific aspects of integrated quality management. These goals will be achieved through targeted teaching activities, such as delivering high-quality courses and seminars aligned with current scientific knowledge and industry realities, participating in national and international scientific events, and modernizing teaching technologies, methods, and assessment approaches.

The following outlines several key directions on which I will focus in my future teaching activities:

3.1.1. Value-Oriented and Quality-Focused Approach to the Educational Process

To achieve educational objectives with high performance, I intend to expand the use of active-participatory teaching and learning methods (such as problematization, case studies, heuristic dialogue, etc.) as well as audio-visual techniques within the structure of courses and seminars. The use of these didactic tools not only increases the efficiency of the educational process and the accuracy of specialized knowledge transmitted, but also fosters deeper student engagement in specific learning situations. In such conducive learning contexts, there is a more effective transfer of core values, concepts, theories, and analytical methods, shifting the focus from mere knowledge transmission to the development of the formative and educational character of the course content.

In this regard, particular attention will be given to expanding the use of the problematization method, which is currently frequently applied in seminars and laboratories, especially for master's and doctoral students. The correct and effective application of this method involves several stages, characterized by interactive collaboration between the instructor and students: thorough theoretical preparation by the students on the course topic, presentation of key aspects through heuristic dialogue, integration of students into the problem-based context, exploration by students within their own cognitive frameworks to address unknown aspects, formulation of solutions, validation of these solutions, and synthesis of the knowledge acquired in the new educational context. Through these approaches, not only is a value-oriented direction established in teaching activities, but the quality of instruction is also enhanced by centering the educational process on the students.

3.1.2. Anticipation of Learning Outcomes

Learning outcomes are demonstrated through the knowledge acquired, as well as the general and specialized skills and competencies developed during courses and seminars. These outcomes can be anticipated at various points throughout the semester based on student assessments. The results obtained indicate the level of information assimilation by students and their ability to apply the acquired knowledge. Grades provide informative value and can serve as a predictor of success or failure in the final examination session.

In this context, to better guide students, encourage their preparation throughout the semester, and reduce academic failure, I propose two main courses of action: conducting more in-semester assessments based on tests for undergraduate and master's students, and holding more frequent meetings with doctoral students. Additionally, I plan to revise or update laboratory manuals and potentially develop new guides to support student learning in a structured and effective manner.

3.1.3. Evaluation of the Effectiveness of Conducted Activities

Appreciating the quality and effectiveness of the activities carried out requires continuous feedback, that is, the establishment of a reverse connection through which the instructor verifies the achievement of the proposed objectives. The quality of learning outcomes ultimately reflects both the level of attainment of the educational objectives and the overall effectiveness of the teaching process. To improve the completeness, accuracy, and depth of the knowledge acquired by students, I plan to conduct careful monitoring of academic results, individualized by specialization and doctoral level, to establish a database tracking student performance over several years, analyze these results, and adapt the content of courses in accordance with changes in the economic and legislative environment, emerging trends and requirements of the labor market, as well as the students' actual capacity to understand and assimilate the material.

3.1.4. Continuous Improvement of the Teaching-Learning Process

To ensure the transmission of deep, lasting, and thorough knowledge, as well as the development of specific skills and competencies, I intend to maintain a continuous adjustment of the teaching-learning process, taking into account the actual capabilities of participants in each instructional activity. The interactive nature of these activities will allow me to identify students' cognitive achievements, detect possible gaps in knowledge, or recognize incorrectly formed connections between the categories and concepts specific to the disciplines taught. Based on this approach, and in close correlation with other teaching resources (such as the number of hours and course scheduling), I will undertake measures to enhance the accessibility and attractiveness of the courses offered. To achieve this goal, I will adopt various teaching strategies, including the restructuring of information, presenting it in accessible and integrated formats, providing illustrative examples, and initially demonstrating the application of analytical methodologies.

3.2. Development of Research Activities

It is well known that scientific research activities are closely intertwined with educational and instructional activities. The advancement of science is based on the accumulation of knowledge, which is primarily achieved through the educational and instructional processes organized within the academic system. At the same time, science addresses society, and individuals must be capable of utilizing the results obtained from scientific research.

Being aware of the role of universities as the main pillars of scientific research, my primary objective in research activities is to develop fundamental and applied studies that make meaningful contributions to the advancement of knowledge and practice in the field of integrated quality management, with applications in food quality and safety, as well as in the monitoring of environmental quality components. The activities I will undertake to achieve this objective include: thorough documentation using appropriate and relevant sources in line with the research topic, preparation of scientific studies and articles reflecting research results, dissemination of findings through publication in reputable journals or in the proceedings of national and international conferences in which I participate, development of research grants, initiation and implementation of national and international research projects within my field of expertise, establishment of partnerships with specialized research teams, and engagement of students in research activities.

In the long term, my research activity will focus on several directions aimed at advancing knowledge and applying European standards regarding environmental management (ISO 14001) and food safety (e.g., ISO 22000, HACCP). Additionally, I intend to initiate and support research and development projects, including grants or research contracts with local economic agents. I will prioritize the valorization of research results through the publication of scientific papers. The research will culminate in a series of studies and articles that I plan to publish in prestigious, peer-reviewed journals indexed in ISI Thomson Reuters and other recognized databases such as CNCSIS.

These studies will include results from the proposed research projects as well as outcomes from scientific research aligned with my competencies and areas of scholarly interest..

3.2.1. Enhancing Scientific Reputation

An important focus of my future research activity will be to enhance the scientific reputation of the institution where I work. To achieve this goal, I intend to actively participate in the initiatives of my academic team, attend national and international conferences, and establish collaborations and partnerships with the business community, local stakeholders, and other institutions, as well as collaborate within research teams at my university and other academic centers.

Internationalization and Academic Collaboration:

- Participation in international grants and projects, such as Horizon Europe and Erasmus+, to promote scientific excellence.
- Organizing and co-organizing international conferences to disseminate research findings and increase the institution’s visibility.
- Publishing scientific articles in collaboration with international partners, thereby strengthening academic networks and research impact.
- Establishing partnerships with foreign institutes and universities to facilitate the exchange of expertise and joint research projects.

Applied and Fundamental Research:

- Expanding modeling and simulations related to the degradation and oxidation of used oils to better understand chemical mechanisms and optimize recycling processes for conversion into valuable products.
- Identifying and testing new substrates and residual materials, including used oils from various food sources, to develop soaps with antimicrobial and sustainable properties.
- Utilizing digital simulations and artificial intelligence tools to optimize the processes of transforming used oils, reduce environmental impact, and increase the technological efficiency of soap production.
- Developing pilot soap prototypes from used oils and conducting additional experimental tests to evaluate their physicochemical properties, antimicrobial efficacy, and safety, thus facilitating practical implementation.

Through these directions, my research activity will contribute both to the advancement of scientific knowledge and to increasing the visibility and prestige of the institution at national and international levels..

3.3.2. Integrating Teaching Activities with Research

The academic environment, through its foundational objectives, requires the integration of its two core components: teaching activities and research. The educational process is fundamentally based on knowledge, which logically derives from research activities. I aim to build a career grounded in axiological principles, ensuring professional satisfaction and fulfillment while simultaneously contributing to the development of the institutions where I work and enhancing their prestige within academic, economic, and social contexts.

Publications and Dissemination

Regarding the dissemination of teaching and research outcomes, I intend to develop updated manuals and guides for students across all study cycles, create digital materials, simulations, and interactive tutorials, and actively participate in editorial boards and peer-review processes in prestigious journals. Additionally, I plan to publish ISI-indexed articles and review papers, thereby contributing to academic visibility and reinforcing the institution's reputation.

Social and Community Impact

My research activities will also focus on practical applications, through implementation in industry and environmental projects, including pilot installations. I will foster collaborations with local authorities, non-governmental organizations, and the business sector, while actively involving students to prepare them for practical integration into the community and industry. In this way, teaching and research activities will mutually support each other, contributing both to students' professional development and to the institution's social and economic impact.

The development plan for my academic career aims to establish a strong synergy between teaching and research, so that each component reinforces the other and contributes to professional, scientific, and social progress. Continuous improvement of teaching activities will enable the formation of students capable of meeting the complex demands of the labor market, developing both theoretical knowledge and practical competencies in integrated quality management and food safety.

Simultaneously, research activities, structured around fundamental and applied directions, will generate new knowledge, promote innovation, and enhance the visibility and reputation of the institution, both nationally and internationally. Integrating teaching with research will also facilitate knowledge dissemination, student involvement in real projects, and the application of results in industry and environmental contexts, generating a positive impact on the community and the academic environment.

Through this plan, I commit to developing a career based on solid axiological principles, ensuring excellence in education and research, and contributing to the continuous development of the institution, its prestige, and the strengthening of links between the university, the economic sector, and the community. The medium- and long-term perspectives aim not only to achieve the established objectives but also to continuously identify opportunities for innovation, collaboration, and valorization of scientific and educational outcomes.

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