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DOCTORAL THESIS

RESEARCH ON THE QUANTIFICATION AND CHARACTERIZATION OF MICROPLASTICS PRESENT IN MILK AND DAIRY PRODUCTS - IMPACT ON HEALTH AND ENVIRONMENT

Thesis summary

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INTRODUCTION

Globally, the world is facing a triple planetary crisis, represented by climate change, biodiversity loss, and pollution. Research indicates that plastics, including micro- and nanoplastics, destabilize the biosphere and disrupt essential ecosystem processes, adversely affecting both environmental quality and human health. These initial concerns were debated at the meetings of the Scientists' Coalition for an Effective Plastics Treaty (2024), on the topic of Plastics and the Triple Planetary Crisis (Scientists' Coalition for an Effective Plastics Treaty, 2024).

Plastics significantly contribute to exacerbating crises through greenhouse gas emissions across their life cycle, as well as harming species and habitats, and contaminating the environment entirely (SAPEA 2019). In turn, climate change amplifies the spread of plastic pollution through extreme weather conditions (storms, extreme floods, tornadoes, etc.). However, plastics pose not only an environmental risk but also a potential health risk (microplastics and chemicals associated with plastics are transferred up the food chain). Microplastics, defined as small plastic particles with sizes ranging from 0.1 μm or 1 μm to 5 mm (SAPEA, 2019), are created by the abrasion and decomposition of larger plastics and are frequently identified in drinking water (Danopoulos et al., 2020) and in various food products (Da Costa Filho et al., 2021; Banica et al., 2024a, 2024b, 2024c; Conti et al., 2020; Dessi et al., 2021). On the other hand, plastics also contain other chemicals, such as harmful additives. Microplastics act as vectors for other contaminants of emerging concern, such as pesticides, polycyclic aromatic hydrocarbons, heavy metals, antibiotics, viruses, etc., which is a public health concern, as the ingestion of microplastics and their associated toxins can have harmful effects, it is only a matter of time before the health risks of microplastics become apparent, given the greater amount of plastics entering the environment and subsequently the human body. However, scientific research is lacking in information and evidence, so the extent of the effects of microplastics on human health is unclear; their effects remain unknown in terms of daily/weekly/annual exposure through various routes (ingestion, inhalation, systemic, and dermal contact). There is no data on the cumulative extent of the impact.

Given the accumulation of microplastics in the environment and their transport in the food chain through direct contamination of (natural and processed) food products, which has become a global threat to both the environment and human health, **this doctoral thesis represents a benchmark for scientific research in Romania, but also abroad,** through precise, unequivocal data regarding the significant risk to the environment and humans associated with exposure to microplastics. The interdisciplinary character of the research started from identifying the relevant societal context for a clean environment and implicitly safe food for a healthy life (without emerging contaminants) and understanding the various factors applicable to the field of environmental engineering by applying computational methods, as well as relevant experimental techniques, but also data analysis approaches.

The choice of the theme was made based on an in-depth study of the specialized literature, taking into account the fact that, at the time of starting the scientific incursion, the data were limited, the few studies being based on assumptions and presumptive hypotheses without a practical/experimental foundation and without the stability of clear correlations on a statistical basis. On the other hand, another criterion, which was the basis for choosing the theme, was related to innovation, which in the clean environment-safe food system mainly must *(i)* contribute

to reducing the plastic footprint, *(ii)* ensure the safety and sustainability of food products, *(iii)* improve the concept of safe food, and *(iv)* contribute to indeclining global objectives.

The doctoral thesis entitled **Research on the quantification and characterization of microplastics present in milk and dairy products – impact on health and the environment** represents the **first comprehensive investigation in Romania**, which systematically quantifies and characterizes microplastics (the result of the historical pollution of the planet with plastics) in a broad spectrum of commercial, packaged, high-fat dairy products. Worldwide, the demand for organic products is increasing due to greater awareness of environmental issues. This demand for organic products, a new option in the modern, civilized world, has led to profound changes in agricultural practices, especially in cultivation and production systems, from agriculture predominantly based on systemic treatments, called traditional, to organic agriculture. Historical pollution with microplastics makes organic agriculture and products unsafe. Microplastics present in the environment harm soil chemistry, acting as vectors of pathogenic bacteria and other toxic pollutants in the soil (bisphenols, phthalates, short/medium-chain chlorinated paraffins, heavy metals, and persistent organic pollutants). In addition, the presence of microplastics in the soil can affect chlorophyll, the size of the leaves of plants, crops, and even perennial grasses, and therefore "photosynthetic efficiency". Microplastics found in the soil are often present in the food consumed by herbivorous animals. These particles may accumulate in animals' bodies and can be transferred to products processed for human consumption. The potential risks associated with exposure to these pollutants remain uncertain. In conclusion, chemical contaminants such as microplastics, heavy metals, or even polycyclic aromatic hydrocarbons present in finished products from the current diet of the population can come from sources such as feed, environmental sources (air, water, and/or soil pollution), migration from habitat, work equipment materials, or cross-contamination.

The topic proposed in the doctoral thesis entitled **Research on the quantification and characterization of microplastics present in milk and dairy products – Impact on health and the environment** has an interdisciplinary character, falling within the fundamental field of Engineering Sciences, the field of Environmental Engineering, within the Doctoral School of the Faculty of Chemical Engineering and Biotechnology.

The doctoral thesis presents a **high degree of novelty**, also through the protocol developed in this thesis for isolating microplastics from dairy products, which has been patented at national and international levels.

Given the ubiquity of microplastics in the environment and the health risk associated with exposure to them, the stated aim of the doctoral thesis was to separate, quantify, and morphologically and chemically analyze, but also to highlight the health and environmental risk associated with the presence of a cumulation of contaminants, such as microplastics, polycyclic aromatic hydrocarbons, and heavy metals, taking into account the most recent and representative research in this field. Primary sorption processes that increase exposure to emerging contaminants linked to microplastics include key mechanisms such as electrostatic interactions, hydrophobic interactions, hydrogen bonding, and steric effects.

The research undertaken in the development of the doctoral thesis entitled **Search on the quantification and characterization of microplastics present in milk and dairy products – Impact on health and the environment** aimed at the following objectives:

O1. establishing sample selection criteria using a survey;
O2. selection, preparation, and processing of samples, taking into account the complex organic matrix of the samples, as well as their susceptibility to pathogens;
O3. isolation of microplastics from milk and fermented products with different fat content;

O4. complex morphological investigations on isolated microparticles through high-performance analytical techniques, such as optical microscopy, scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS), and micro-Fourier transform infrared spectroscopy (micro-FTIR);

O5. identification and chemical characterization of microplastics by micro-Fourier transform infrared spectroscopy (micro-FTIR);

O6. investigation of potential emerging contaminants, such as polycyclic aromatic hydrocarbons (by high-performance liquid chromatography with fluorescence detection, HPLC-FLD) and heavy metals (by inductively coupled plasma mass spectrometry, ICP-MS) in milk samples, to sketch a possible hypothesis regarding the property of microplastics to act as vectors for these contaminants;

O7. estimating the risk associated with exposure to emerging contaminants, in terms of the environment and human health (children and adults);

O8. studie of microplastics dimensions through complex morphological investigations, but also analytical ones regarding chemical composition, correlated and statistically interpreted, with the estimation of the risk to the environment and human health associated with exposure to emerging contaminants, an assumed objective of the doctoral thesis. Secondary factors, taking into account historical soil pollution, animal feeding methods, geographical conditions, relief, habitat, etc., and statistical analyses, contribute to the stability of fundamental correct hypotheses and interpretations to achieve the objectives assumed within this scientific research;

O9. possibilities for implementing the patented method of isolating microplastics in industry by evaluating the effectiveness of the HACCP system in controlling microplastics and other contaminants in finished products, with reference to Romania.

Novelty items:

1. development and patenting of the method for isolating microplastics from dairy products, RO 137927 B1/2025 (OSIM) and WO2025/071426 A1 (WIPO/PCT).

2. quantification and morphological, elemental, and chemical study of isolated microplastics through high-performance analytical techniques such as optical microscopy, scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS), and micro-Fourier transform infrared spectroscopy (micro-FTIR);

3. quantitative determination of potentially carcinogenic contaminants, such as polycyclic aromatic hydrocarbons and heavy metals, by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) and by inductively coupled plasma mass spectrometry (ICP-MS);

4. estimating the environmental and human health risk associated with exposure to microplastics and other emerging carcinogenic contaminants (heavy metals and polycyclic aromatic hydrocarbons);

5. alternative regarding the implementation of the patented microplastic isolation method, in terms of the effectiveness of the HACCP system, in the separation/control of microplastics in dairy products, which is based on the identification of critical points in the technological process of manufacturing dairy products and on continuous monitoring of potential contamination;

6. evaluating the effectiveness of the HACCP system in controlling microplastics and other contaminants in dairy products.

The dissemination of scientific research presented in the doctoral thesis was the subject of five articles in prestigious ISI journals (Q1), two patents, and participation in invention salons and international conferences in the field, where over 20 awards and medals were obtained.

Structure of the doctoral thesis

The doctoral thesis **Research on the quantification and characterization of microplastics present in milk and dairy products – Impact on health and the environment** is structured in two parts (I and II), totaling eight chapters:

PART I – STATE OF KNOWLEDGE, comprising **Chapter 1,** *The current state of research on the presence and associated risks of emerging contaminants in the environment and the food chain* **–** provides a critical and comprehensive assessment of the current state of research on the presence and associated risks of emerging contaminants in the environment and the food chain. Its main objective was to synthesize existing studies, identify the most significant challenges, and provide insight into innovative solutions that can overcome current limitations. The chapter begins by highlighting the main active regulations at the international level regarding the limitations of these contaminants of emerging concern, the main routes of human exposure, and the classes of contaminants relevant to the doctoral research, the motivation of the choice being based on the complexity of the risk to the environment and, implicitly, to health. The literature review addressed techniques for detecting contaminants that are adsorbed by microplastics, as well as methods for investigating interactions at the interface between microparticles and biomolecules. Understanding these factors is crucial for assessing toxicity, biological transport, and long-term health implications. A fundamental principle of toxicology and risk assessment is the stability of a clear dose-response relationship. This requires a precise quantification of the internal dose of a contaminant and a correlation to the defined results it produces. However, in the case of microplastics, achieving this has been a persistent challenge. If we include data from clinical studies on the carcinogenic effects of polycyclic aromatic hydrocarbons, microplastics, and heavy metals, the dose-response relationship becomes more complex, making it harder to quantify and understand.

PART II – ORIGINAL RESEARCH AND OWN CONTRIBUTIONS, are structured in 7 chapters as follows: **Chapter 2** entitled *Materials and methods applied in experimental research*, is systematized in the following sections: (1) Materials and methods for sampling, which include: *(i)* complete description of the samples, reagents and materials adjacent to the preparation and preparation of the samples for physico-chemical analysis, specifying the security measures adopted to avoid accidental contamination; *(ii)* the questionnaire method, the criteria that formed the basis for the sampling and selection of the samples. (2) Sample preparation methods, which included: *(i)* microplastic isolation method; *(ii)* sample preparation procedure by mineralization for determining heavy metal content; *(iii)* sample preparation procedure for determining polycyclic aromatic hydrocarbons by saponification and concentration in a nitrogen stream. (3) Analysis methods systematized as follows: *(i)* analysis by optical microscopy; *(ii)* morphological and chemical analysis method of microplastics from selected samples; *(iii)* analysis by vibrational spectroscopy, micro-FTIR; *(iv)* determination of heavy metals by inductively coupled plasma mass spectrometry (ICP-MS); *(v)* determination of polycyclic aromatic hydrocarbons by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). (4) Techniques for determining risk indices where risk/contamination/ingestion indices of contaminants in the analyzed samples are systematized. (5) Statistical analysis of data where the working technique, the program/software used, the subprograms, and the correlations used to support the reproducibility of the results are presented. **Chapter 3,** entitled *Rapid method for isolating microplastics from milk and dairy products*, presents the microplastic isolation protocol

according to the patent RO 137927 B1/2025 and the patent WO2025/071426 A1. **Chapter 4**, entitled *Research on the quantification and characterization of emerging contaminants in milk*, exhaustively presents the results obtained, following the quantification and morphological and chemical characterization of a series of contaminants of emerging concern, such as microplastics, heavy metals, and polycyclic aromatic hydrocarbons, potentially present in milk, while assessing the health risks related to human exposure. **Chapter 5**, entitled *Research on the quantification and characterization of microplastics in yogurt*, presents exhaustively the results obtained, following investigations on the quantification and analysis of microplastics in dairy products found in the current human diet. The scientific approach was naturally conducted through the stages of identification, quantification, morphological, and chemical analysis using complementary methods such as optical microscopy and micro-FTIR. **Chapter 6**, entitled *Research on the quantification and characterization of microplastics in high-fat dairy products*, presents the results obtained following the quantification and morphological and chemical characterization of microplastics in high-fat samples. **Chapter 7**, entitled *Environmental and human health risks associated with exposure to emerging contaminants*, had as its objectives *(i)* to assess the potential health risks that may arise from exposure to foods contaminated with different contaminants of emerging concern (microplastics, heavy metals, polycyclic aromatic hydrocarbons) and *(ii)* to identify the correlations that may arise between the determined risk factors. This chapter is a natural continuation of the investigations related to the quantification and characterization of microplastics and other contaminants in the carcinogenic risk class (heavy metals and polycyclic aromatic hydrocarbons), identified in a wide range of dairy products, with direct reference to the potential health risk (children and adults), due to the consumption of contaminated products. **Chapter 8**, entitled *Research on the implementation of the microplastic isolation method in industry*, focuses on presenting an alternative approach for implementing the microplastic isolation technique. It emphasizes the effectiveness of the HACCP system in controlling microplastics in dairy products. The research in this chapter assessed the effectiveness of the HACCP system in controlling microplastics and other contaminants in dairy products.

The doctoral thesis ends with **general conclusions** indicating that the assumed purpose and objectives have been met. The **Original contributions** and **Perspectives for further development are also highlighted**.

The doctoral thesis includes **Bibliographic references** and relevant **Annexes** a thorough understanding of the entire scientific research.

Chapter 1.
CURRENT STATUS OF RESEARCH ON THE PRESENCE AND ASSOCIATED RISKS OF EMERGING CONTAMINANTS IN THE ENVIRONMENT AND THE FOOD CHAIN

This chapter offers a critical and comprehensive evaluation of the current state of emerging contaminants in the environment and their associated risks in the food chain. Its main objective was to synthesize existing studies, identify the most significant challenges, and provide an insight into innovative solutions capable of overcoming current limitations. The chapter begins by highlighting the main active regulations at the international level regarding the limitations of these contaminants of emerging concern, the main human exposure pathways, and the classes of contaminants relevant to the doctoral research, the motivation for the choice being based on the complexity of the risk to the environment and, implicitly, to health.

The global economy is driving the continuous production and release of new chemical and biological agents, including emerging contaminants, which pose a continuing challenge to global health and sustainability. Emerging contaminants, also known as contaminants of emerging concern, are either natural or synthetic chemicals and biological agents that pose potential hazards to human health and ecosystems due to risks that are not yet fully understood. Emerging contaminants include pharmaceuticals, personal care products, microplastics, nanoplastics, pesticides, per- and polyfluoroalkyl substances, polycyclic aromatic hydrocarbons, persistent organic pollutants, heavy metals, disinfection by-products, detergents, uranium radionuclides, emerging pathogens, cyanotoxins, and other natural toxins.

As of February 2024, the US Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA) chemical inventory listed 86,741 potentially hazardous chemicals, of which 42,293 are currently in active use. A study by Wang et al. (2020a) highlighted that over 350,000 chemicals and chemical mixtures have been registered for commercial use worldwide.

Sources of emerging contaminants can be classified as point discharges from wastewater treatment plants (including domestic, industrial, commercial, and medical effluents) and non-point (diffuse) sources (agricultural, livestock, and aquaculture runoff and urban areas). Emerging contaminants can also originate from landfill leachate. Traditional wastewater treatment plants were not originally designed to eliminate contaminants. Consequently, many of these contaminants have been detected in treated effluents at concentrations typically ranging from nanograms per liter (ng/L) to milligrams per liter (mg/L). Taking into account these briefly presented aspects, which have amplified global concern, regarding a series of contaminants with more or less complex structures, but which pose a significant risk to the environment and to life, through the perspective of *carrier* behavior, this chapter will represent an introspection on microplastics, classified in the category "*Other emerging contaminants*", to fully understand their behavior and degree of danger to the quality of life and the environment.

Microplastics pose risks by adsorbing and concentrating environmental pollutants, such as heavy metals and persistent organic pollutants, acting as vectors within the body. These findings highlight the urgent need for reliable diagnostic and analytical methods to detect, identify, and quantify human exposure to microparticles through ingestion, inhalation, and direct systemic exposure. The presence of these microparticles has already been confirmed in human tissues and biological fluids. Their potential impact on human health has become an essential topic of scientific investigation. The detection and quantification of microplastics in different matrices remains a challenge due to their low concentrations, diverse physicochemical properties, and

interference with organic and inorganic matter. This chapter will also critically evaluate existing methods for the separation, detection, and analysis of microplastics. Taking into account the fact that these microplastics act as vectors for other contaminants, this chapter will include data/information on a series of pollutants with a more or less carcinogenic effect, such as polycyclic aromatic hydrocarbons and heavy metals, to create a more complete picture of the complex, irreversible risk that these microplastics pose to the ecosystem and the health of the population worldwide. It is mentioned that limiting the doctoral research to the two categories of contaminants, organic (polycyclic aromatic hydrocarbons) and inorganic (heavy metals) took into account the study of the complex risk of microplastics on the quality of the environment and life, associated with the hypothesis that they act as vectors for other contaminants, the selection being made for the two classes of emerging contaminants already classified as carcinogenic. Several challenges, including the lack of standardized procedures and the difficulty in detecting microplastics, have been identified from the outset of the research as significant barriers to accurate exposure and risk assessment. A fundamental principle of toxicology and risk assessment is the establishment of a clear dose-response relationship, which requires the precise quantification of the internal dose of a contaminant and its correlation with defined health outcomes. In the case of microplastics, this has been and remains a challenge. If we include data from clinical studies regarding the carcinogenic effects of polycyclic aromatic hydrocarbons and heavy metals, the dose-response relationship becomes more complex and challenging to quantify and understand.

Over the past century, the increasingly rapid development of the globalized industrialized economy has led to a significantly polluted environment (Roskova et al., 2022), directly involving numerous problems related to human health and ecological safety. Heavy metals are included in the category of inorganic contaminants of emerging concern, due to their non-biodegradable nature, toxicity, environmental persistence and bioaccumulation potential (Santhosh et al., 2024). In particular, copper, zinc, arsenic, lead, mercury, chromium, nickel, and cadmium are classified as hazardous substances due to their documented ecotoxicological impacts (Munksgaard and Parry, 2001; Liu and Miao, 2022; Malik et al., 2022; Su et al., 2023).

As the molecular weight of polycyclic aromatic hydrocarbons (PAHs) increases, their carcinogenic potential also rises, while their acute toxicity tends to decrease. The most potent carcinogens in the PAH class have been identified to include benzo[*a*]anthracene, benzo[*a*]pyrene, and dibenzo[*a,h*]anthracene. Given the ubiquity of PAHs in the environment and the health risks associated with exposure to them, the stated purpose of this chapter was to present the risks associated with the presence of a cluster of contaminants, such as microplastics, PAHs, and heavy metals, taking into account the most recent and representative research in this field.

PART II. ORIGINAL RESEARCH AND OWN CONTRIBUTIONS

Chapter 2.
MATERIALS AND METHODS APPLIED IN EXPERIMENTAL RESEARCH

This chapter naturally leads to an understanding of the scientific approach of this research, from the objectives and assumed purpose to the results obtained, interpretations, potential applications, and conclusions. This chapter is crucial in providing credible details about the materials, strategies, and experimental protocols, including the equipment used to achieve the research objectives. The more apparent and more concisely structured the methodological framework is, the more this chapter will contribute to the understanding of the experimental protocols, as well as the results obtained, the reproducibility and validity of the working methods/results, the certification of the hypotheses, but also to the elimination of any prejudice on the way in which the study was conceived and carried out. Thus, this chapter supports the scientific approach of the research topic, allows a complete understanding of the proposed design, the experimental protocols used/adapted for this research, the complex techniques of analysis, data collection, and interpretation, as well as the correlations obtained through statistical analysis. This chapter explains and justifies the scientific approach, connecting objectives with results. The following chapters will present and interpret these results, revealed by the techniques or protocols developed here.

This chapter details the criteria by which the samples were selected/collected, the stages of sample preparation/preparation, as well as the materials used in the preparation process, environmental factors, experimental working conditions, preparation of work protocols, detailing the analytical techniques used in this scientific endeavor, the techniques for interpreting and accuracy of the results, as well as the statistical analysis.

The complete detailing of the applied methodologies contributes to the **understanding and achievement of the objectives assumed through the doctoral research**, *underscoring the importance of the topic addressed at the national and international level, knowing that such* **a topic has not yet been thoroughly researched, and has not been undertaken by any research group through the prism of complexity**.

Chapter 2 is organized into the following sections: (1) *Materials and methods for sampling*, which include: *(i)* a complete description of the samples, reagents and materials associated with the preparation and preparation of samples for physico-chemical analysis, specifying the safety measures adopted to avoid accidental contamination; *(ii)* the questionnaire method, the criteria that formed the basis for the sampling and selection of samples. (2) *Sample preparation methods*, which included: *(i)* microplastic isolation method; *(ii)* sample preparation procedure by mineralization for determining heavy metal content; *(iii)* sample preparation procedure for determining polycyclic aromatic hydrocarbons by saponification and concentration in a nitrogen stream. (3) *Analysis methods*, where the working principles and the stages of the actual analyses are presented, systematized as follows: *(i)* analysis by optical microscopy; *(ii)* method of morphological and chemical analysis of microplastics from selected samples; *(iii)* analysis by vibrational micro-spectroscopy (micro–FTIR); *(iv)* determination of heavy metals by inductively coupled plasma mass spectrometry (ICP-MS); *(v)* determination of polycyclic aromatic hydrocarbons by high-performance liquid chromatography with fluorescence detection (HPLC–FLD). (4) Determination of the risk associated with exposure to contaminants, where

risk/contamination/ingestion indices of contaminants in the analyzed samples are systematized. (5) *Statistical analysis of the data*, where the working technique, the program/software used, the subprograms, and the correlations used to support the reproducibility of the results are presented.

The accurate analysis of microplastics in selected milk and dairy samples relies on the ability to first isolate them from the complex organic matrix of the samples. This isolation step was not just a procedural formality, but represented the greatest challenge of the doctoral research. The inherent nature of milk samples presents a hostile and complex environment for detecting traces of synthetic polymers, creating a series of fundamental obstacles that must be overcome to generate reproducible data. Microplastics, as exogenous materials, are embedded in a much more abundant and chemically heterogeneous matrix, which complicates their isolation. Preliminary tests based on protocols from the literature did not yield the expected results; the isolation of these microparticles requires the complete removal of the organic matrix from dairy products. The analytical difficulty in the sample preparation steps is accentuated by the fact that microplastics are present either in low concentrations in small sample volumes (100 mL) or in high concentrations in larger sample volumes (1 L). This can lead to problems in the isolation process, such as clogging of filters and sieves, which not only slows down the workflow but can also result in the physical loss of target microparticles. This dynamic can lead to a trade-off: a smaller sample volume may be easier to process but may not contain a representative number of microparticles, whereas a larger sample volume may contain a representative number of microplastics but may be more challenging to process in the separation/isolation step.

Spectroscopic techniques for polymer identification are also highly susceptible to interference from the complex organic matrix of dairy products. In micro-Fourier transform infrared spectroscopy (micro-FTIR), strong absorption bands from residual organic matter (e.g., amide bands from proteins) can overlap and obscure the characteristic absorption peaks of specific polymers. Therefore, efficient and complete removal of the organic matrix is essential to ensure accuracy and reliability in any analytical workflow. This is why the original isolation method, first developed through doctoral research, has been internationally recognized, with microplastics identified and certified by FTIR micro-spectroscopy being correctly analyzed, without interference. The micro-FTIR technique, characterized by its chemical specificity necessary for the definitive identification of polymer structures, is one of the most valuable methods for screening microplastics. Still, it has limitations related to the quantitative assessment of exposure or regulatory decision-making. Exposure to microplastics is calculated with risk indices, and the correlations are supported by fundamental statistical analysis. All these procedures are applied successively to develop the behavior and risk associated with these microparticles.

Chapter 3.
RAPID METHOD FOR ISOLATION OF MICROPLASTICS FROM MILK AND DAIRY PRODUCTS

This chapter represents original research, unique at an international level, which, moreover, was the subject of an invention patent RO 137927 B1/2025 and an international patent WO2025/071426 A1.

The method of isolating microplastics from milk and dairy products is an integral part of this doctoral thesis, as it is a research project with a high degree of uniqueness, which is necessary for the logical approach outlined in the doctoral study plan. The isolation method was conducted within the advanced research laboratories of the Multidisciplinary Scientific and Technological Research Institute at the Valahia University of Targoviste, with financial support throughout the research.

The isolation method described and patented by RO 137927 B1/2025 and WO2025/071426 A1 includes the following steps:

A. Samples are pretreated with ultrapure reagents to facilitate digestion of the complex organic matrix. This process includes shaking at 150 rpm for 10 minutes.

B. The actual digestion takes place by ultrasonication at a temperature of 30°C for 20 minutes.

C. After digestion, the samples are filtered using filters with a porosity of 12-15 µm.

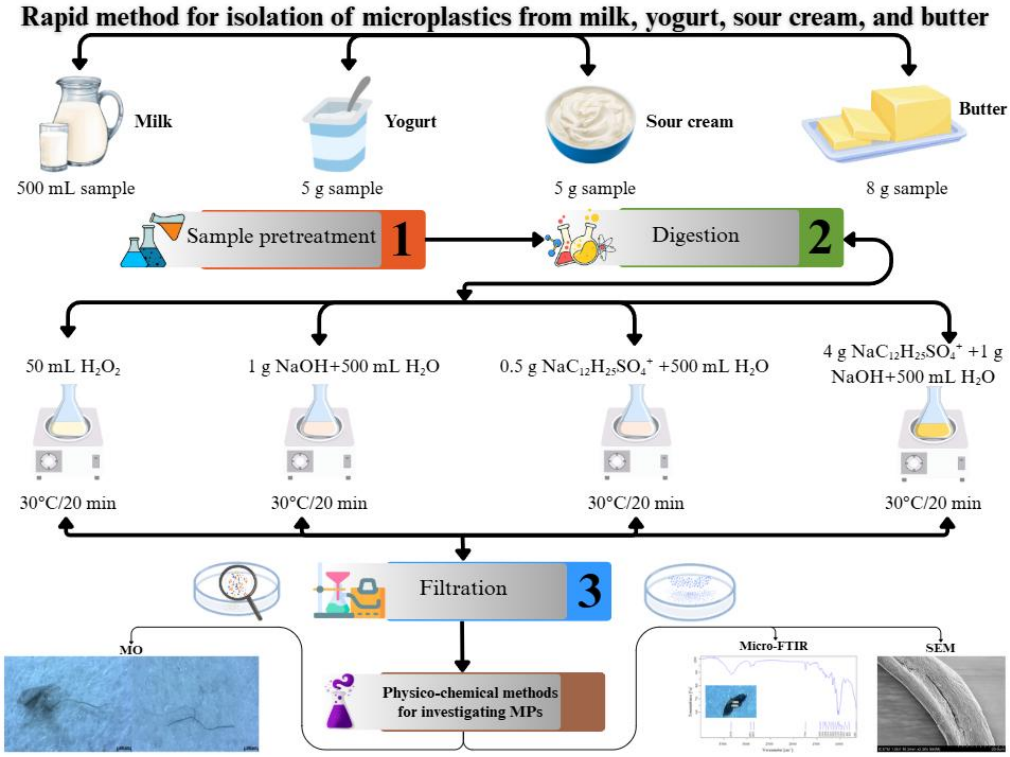


Figure 3.1. Systematization of the protocol for isolating microplastics from milk and dairy products.

Chapter 4.
RESEARCH ON THE QUANTIFICATION AND CHARACTERIZATION OF CONTAMINANTS OF EMERGING CONCERNS IN MILK

Contaminants of emerging concern are chemical compounds that are not currently regulated or have been recently added, for which concerns exist regarding their potential impact on the environment and human health, including possible interaction mechanisms, distribution, and migration pathways. Such a global concern is understandable, as some classes of emerging contaminants, presented in Chapter 1, are mainly non-biodegradable or very poorly degradable, and thus quickly reach marine, freshwater, and terrestrial ecosystems, ultimately contaminating drinking water and the food chain. Of particular interest is the ability of microplastics to transport toxic metals and other carcinogenic contaminants, such as polycyclic aromatic hydrocarbons. These hydrocarbons are generated mainly by pyrolysis and/or incomplete combustion of organic matter, and are classified as genotoxic and carcinogenic to the human body. Of these, benzo[*a*]pyrene is the most dangerous, being included in the first group of human carcinogens. Benzo[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene are compounds that are also found in food products with a high carcinogenic risk.

Of all the basic foods consumed by humans, from children to the elderly, milk is one of the most complex and widely used/consumed. The complexity of milk is given by its chemical composition (fatty substance: fats proper - glycerides, other fats - phosphatides and steroids; non-fatty substance: proteins - caseins and steric proteins, lactose, minerals - calcium, selenium, zinc, magnesium, iodine - and other substances, such as enzymes, vitamins, pigments, gases), being considered the most complete food. Starting from this brief description of the chemical composition of milk, it is understandable why research on the presence and quantification of emerging contaminants, such as microplastics, polycyclic aromatic hydrocarbons, and heavy metals, remains limited and was a focus of interest within this doctoral research.

4.1.3. Cuantificarea și caracterizarea microparticulelor prin microscopie optică

The first method used to detect microplastics isolated from selected milk samples was optical microscopy. This primary analytical investigation technique does not provide complete information on the morphology and composition of the polymer in the microparticles' composition. However, this method can provide essential data on the size, number, and identity of the microplastics. In the current research, the optical microscopy technique highlighted the colors and shapes of the microparticles retained on the filter surface after isolation (Figure 4.1), which allowed a determination of the total number of microparticles (expressed as microparticles·L^{-1}).



L_3



L_6



L_8



L_9

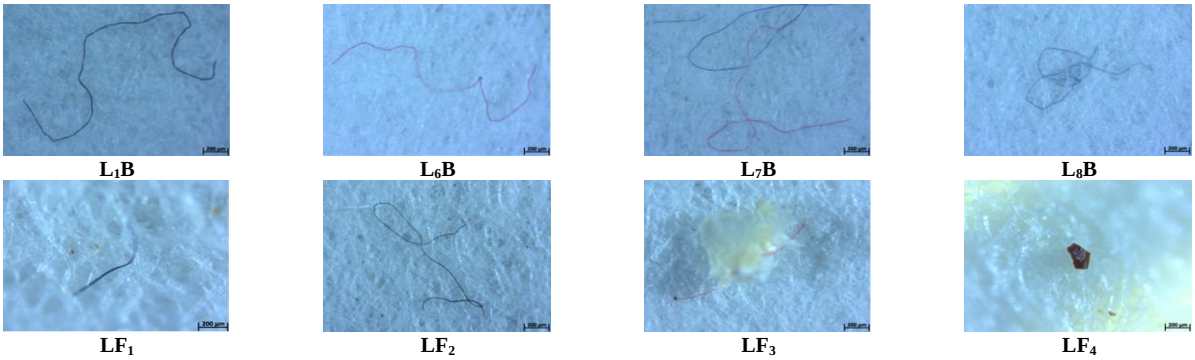


Figure 4.1. Representative optical microscopy images of microparticles isolated from the analyzed samples.

Figure 4.2 presents the results for the total number of microparticles identified in the 20 analyzed samples. It was observed that in ten conventional milk samples, 124 microparticles were identified, in six organic milk samples, 185 microparticles were found, and in four raw milk samples, 115 microparticles were detected. After all 60 filters (20 samples analyzed in triplicate, the volume of each sample being 1 L) were analyzed and the results were quantified (mean value), resulting in a total number of 424 microparticles.

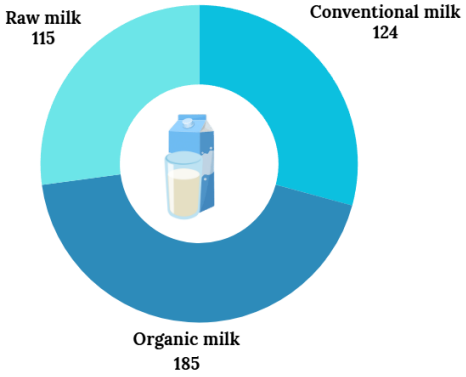


Figure 4.2. Average number of microparticles identified in the analyzed samples.

4.1.4. Morphological and chemical characterization of microplastics by micro-Fourier transform infrared spectroscopy (micro-FTIR)

Vibrational spectroscopy, including Raman spectroscopy and Fourier transform infrared spectroscopy, has become more suitable for microparticle analysis, as it provides high-quality identification of polymers, even if the analysis time is extended. By using micro-FTIR images, a complete characterization (both chemical and morphological) of microplastics is achieved, yielding reliable and reproducible results. In this sense, any microplastics (dark-colored, fluorescent, fibers, fragments, etc.) can be analyzed with minimal effort (Verleye et al., 2001) (Figure 4.2). Micro-Fourier Transform Infrared Spectroscopy (micro-FTIR) plays a vital role in investigating and understanding the composition of microplastics in the food chain, including in food samples. FTIR spectra (Figure 4.3) provide valuable information on the polymeric structure of microplastics. High-resolution micro-FTIR imaging enables the visualization and mapping of microparticles at the micrometer scale, leading to a more advanced understanding of their structure.

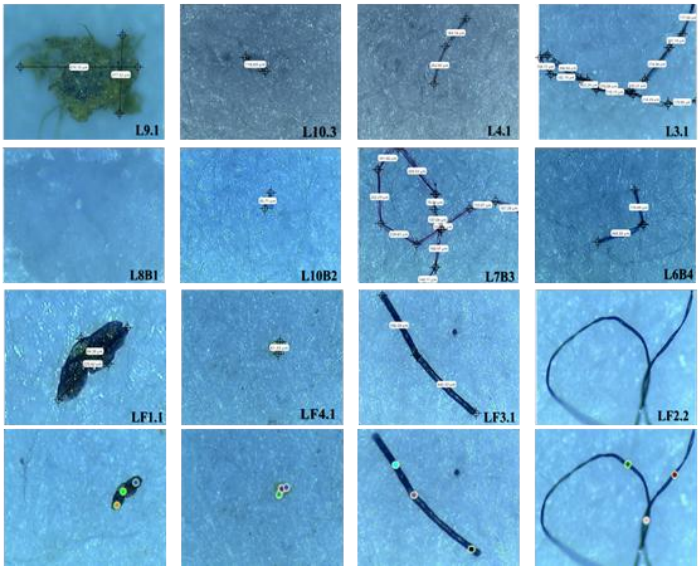


Figure 4.2. Measurement sequence and three-point mapping of microparticles identified in the analyzed samples.

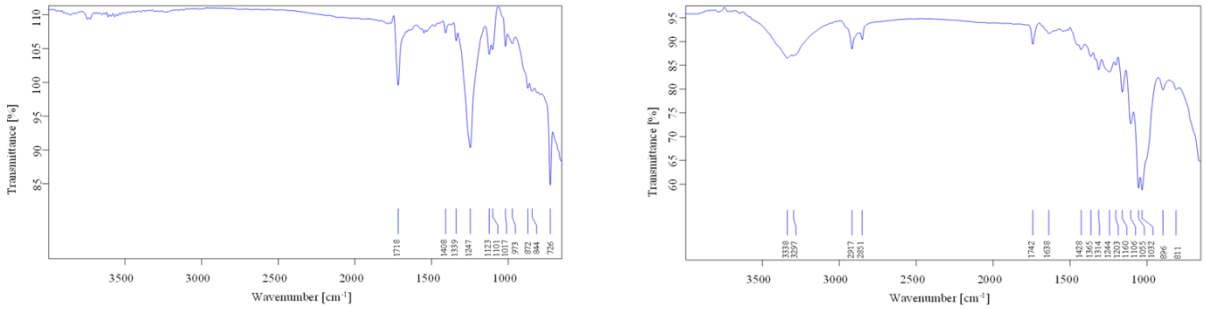


Figure 43. FTIR spectra of microplastics from milk samples (selection): a) – polyester; b) - mixture: cotton and poly(methyl methacrylate).

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) is a destructive technique. Therefore, this technique was applied after the microplastics were morphologically and chemically characterized by non-destructive techniques (optical microscopy and micro-FTIR spectroscopy). Thus, the microplastics were extracted from the surface of the filters and placed on double-sided carbon tape, which was fixed to a specific support on the equipment. Figure 4.4 presents the most representative SEM images obtained for microplastics from conventional and organic milk samples, their morphology being similar. They are characterized by finely textured surfaces, with medium roughness and longitudinal orientation.

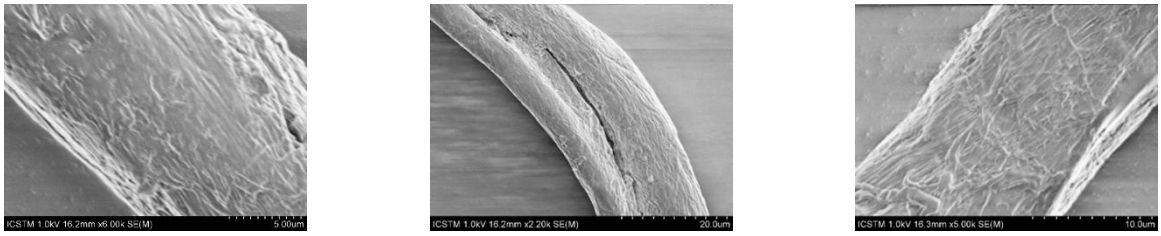


Figure 4.4. SEM images of microplastics (selection) extracted from conventional/organic milk samples.

4.2. Determination of heavy metals by inductively coupled plasma mass spectrometry

Milk, which contains essential macro- and microelements such as calcium, magnesium, manganese, potassium, phosphorus, copper, iron, zinc, sodium, and selenium, plays a crucial role in providing essential nutrients for human health. Cadmium (Cd), for example, is widely found in nature and often coexists with lead-zinc ores and cadmium sulfide ores. Industrial activities, such as mining and metal smelting, lead to soil contamination with cadmium, which enters dairy products through the "*soil-pasture-animal*" pathway.

European legislation, as outlined in the Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed, STAN 193-1995, and Regulation EC 1881/2006, regulates the maximum level of lead in milk at 0.020 mg/L. Compared to these European standards, all the samples analyzed (except sample L_3) recorded lead values higher than the maximum permitted limit. From the perspective of national legislation, Order 975/1998 establishes and approves the sanitary hygiene norms for food, including the maximum levels of copper, zinc, cesium, and lead in milk. Compared to these values imposed by national legislation, the analyzed samples recorded values below the maximum thresholds for copper and zinc, but for cadmium, in sample L_5, and for lead in samples L_6, L_7, L_8, L_9, and L_{10}, values were obtained that exceeded the maximum limits allowed in national legislation. Since children are the primary consumers of milk and dairy products, the main concern related to heavy metals is the concentrations of lead and cadmium, as they are potentially carcinogenic.

The study showed that the level of heavy metals (Figure 4.5) was within acceptable limits, and out of the 20 milk samples analyzed, only six conventional milk samples (L_5, L_6, L_7, L_8, L_9, and L_{10}) can be considered potentially emerging in terms of carcinogenic risk (cadmium and lead).

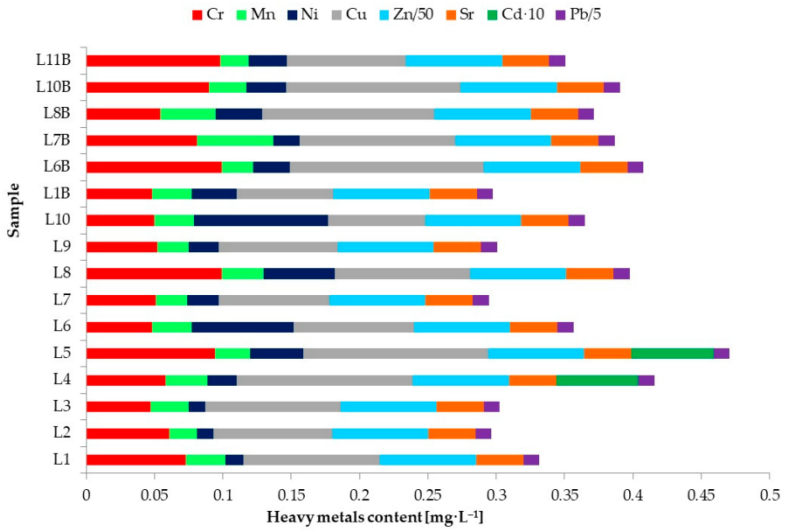


Figure 4.5. Heavy metal content in analyzed samples determined by ICP–MS.

4.3. Determination of polycyclic aromatic hydrocarbons by high-performance liquid chromatography with fluorescence detection

The term "polycyclic aromatic hydrocarbons" refers to a group of chemicals formed during the partial combustion of organic compounds through both natural and anthropogenic processes. The "16 polycyclic aromatic hydrocarbons" or "16PAHs" for short have been identified by the USEPA as the most essential persistent organic pollutants due to their high

mutagenicity, teratogenicity, and carcinogenic properties. In particular, 88-98% of exposure to polycyclic aromatic hydrocarbons can occur through the consumption of contaminated food, including milk.

Following a comprehensive review of the literature, which indicated the presence of polycyclic aromatic hydrocarbons in milk samples collected from various parts of the world, the research objectives were expanded to include further investigations of the collected milk samples. In this regard, a method for determining 16 polycyclic aromatic hydrocarbons in milk samples was validated, utilizing the saponification and concentration in a nitrogen stream method for sample preparation. From the data provided by the internal standard, 13 polycyclic aromatic hydrocarbons were identified in the 16 conventional and organic milk samples analyzed (Figure 4.6). Acenaphthylene, benzo[*g*,*b*,*i*]perylene, and indeno[1,2,3-*c*,*d*]pyrene were not identified in the analyzed samples.

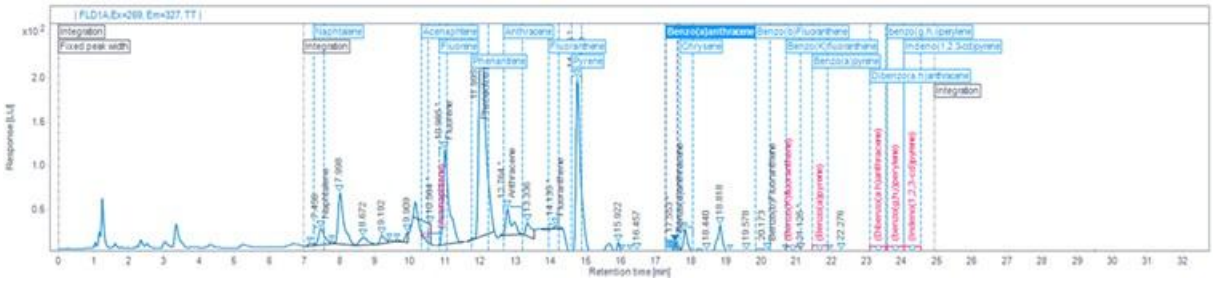


Figure 4.6. Chromatogram of polycyclic aromatic hydrocarbons identified in the analyzed samples.

The polycyclic aromatic hydrocarbons identified in the 16 milk samples are presented grouped in the form of box-plot graphs (Figure 4.7). The grouping of the compounds is related to the number of samples in which these hydrocarbons were identified and their concentration in the samples.

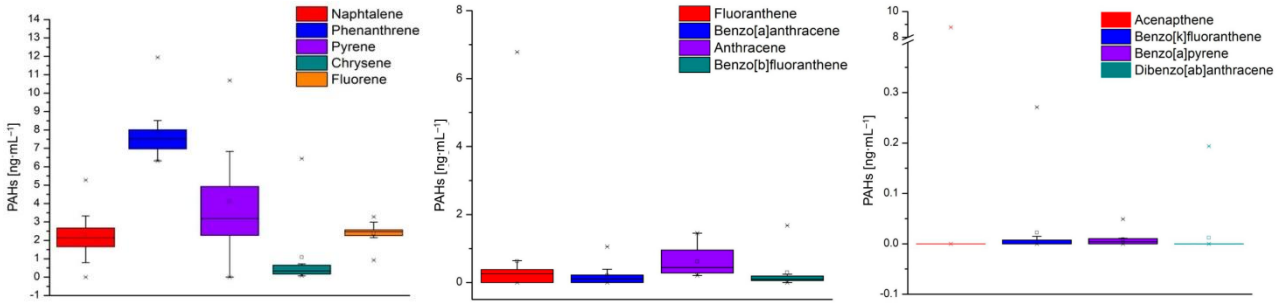


Figure 4.7. Concentration of polycyclic aromatic hydrocarbons identified in the analyzed samples.

Contamination of milk with polycyclic aromatic hydrocarbons is caused, on the one hand, by natural activities, and on the other hand, by anthropogenic activities (e.g., burning of forests and agricultural fields, oil spills in the oil field from exploitation areas, emissions produced by vehicles, etc.).

In addition, the high amount of polycyclic aromatic hydrocarbons in milk can be a consequence of contaminated food (natural perennials and fodder) and water. Another way milk can be contaminated is through milk processing practices. High temperatures applied to milk in the technological chain can cause the combustion of organic compounds and the production of such compounds with carcinogenic risk.

Chapter 5.
RESEARCH ON THE QUANTIFICATION AND CHARACTERIZATION OF MICROPLASTICS IN YOGURT

The main objective of this chapter is to quantify and analyze microplastics in dairy products in the current human diet. This analysis has emerged as a critical area of concern, fueled by groundbreaking research that has revealed the ubiquity of microplastics in the environment and their potential health implications. The keywords "microplastic(s)", "dairy product(s)", and “risk” outline the main objective of this chapter. Additionally, the environmental impact, health implications, and chemical aspects of microplastics remain key topics in the literature.

Microplastics have an extremely complex chemical composition and widespread prevalence in the environment, and their entry into living organisms (Dong et al., 2021), including plants (Adjama et al., 2024) and animals (Bhuyan M.S., 2022), integral components of the food chain, can be a significant risk to both the environment and human health. In addition, the presence of microplastics in food can be attributed to a diverse range of sources, including animal feed, environmental pollution, food additives (Eze et al., 2024), and packaging materials (Udovicki et al., 2022). The use of plastic in food processing and packaging has emerged as a substantial factor contributing to microplastic contamination. Furthermore, changes in food processing, transport, and storage conditions can significantly influence the microplastic content of food products (Kataria et al., 2024).

5.3. Quantification and morphological characterization of microplastics by optical microscopy

Microparticles in the yogurt samples were initially examined visually using optical microscopy. Since this technique cannot provide information on the chemical structure of microplastics, it was decided to refer to the identified contaminants as microparticles in a generic sense. The microparticles were visualized by optical microscopy, and their first characteristic is their color, which ranges from black to blue, red, brown, gray, yellow, purple, and green (Figure 5.1).

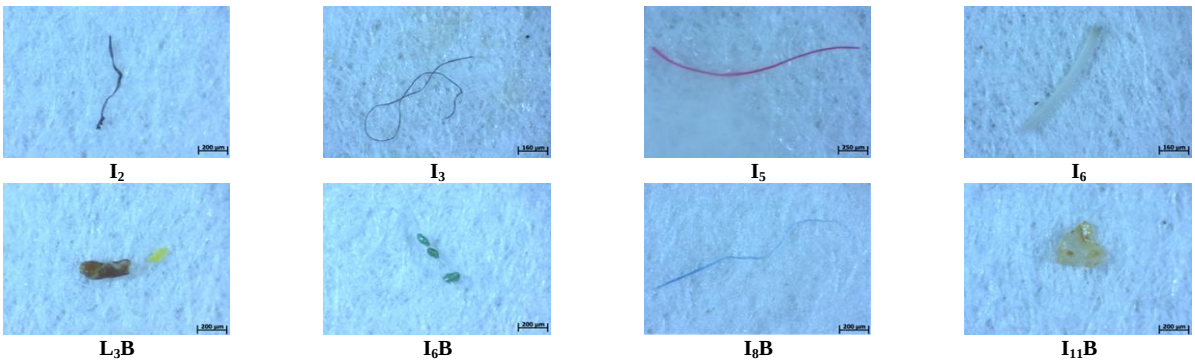


Figure 5.1. Representative optical microscopy images of isolated microparticles.

The percentage of colors identified for conventional yogurt samples was black (50%), gray (20%), red, and blue (9%), yellow (6%), purple and, brown (3%) (Figure 5.2.), and in the case of organic yogurt samples, the percentage was black (48%), gray (21%), blue (18%), yellow (7%), green (3%), red (2), and brown (1%) (Figure 5.3).

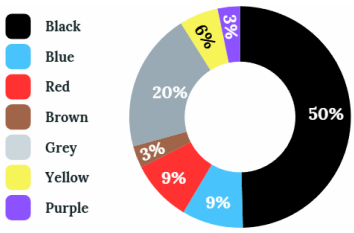


Figure 5.2. Percentage of colors identified in conventional yogurt samples.

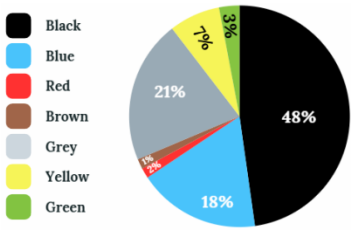


Figure 5.3. Percentage of colors identified in organic yogurt samples.

5.4. Morphological and chemical characterization of microplastics by Fourier transform infrared microspectroscopy

Micro-Fourier transform infrared spectroscopy (micro-FTIR) is one of the best techniques for morphological and compositional analysis of microplastics, based on the analysis of organic functional groups. The micro-FTIR technique used for identifying microplastics through microscopic imaging detects the FTIR absorption spectrum, reveals structural fingerprints, and reflects the optical responses of surface functional groups. With this method, the FTIR spectra (Figure 5.4) for each microplastic particle were compared with the spectra of reference polymers from the spectrum library using OPUS v.7.5 software, while determining the shape and size of the microparticles (analysis of at least three points). A selection of representative samples is presented in Figure 5.5, with the highlighted points and calculated dimensions in micrometers (µm).

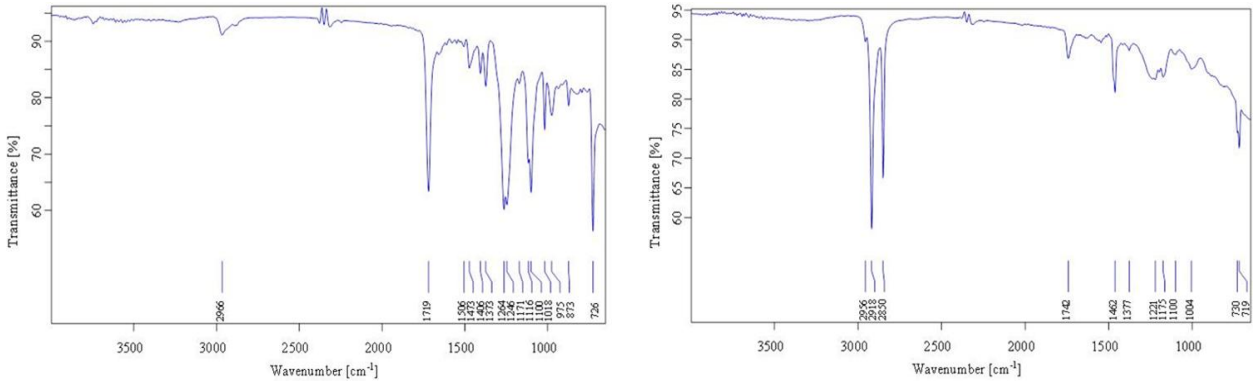


Figure 5.4. FTIR spectra of synthetic microparticles from conventional yogurt samples (selection): a) – polyester; b) – polyethylene (nylon).

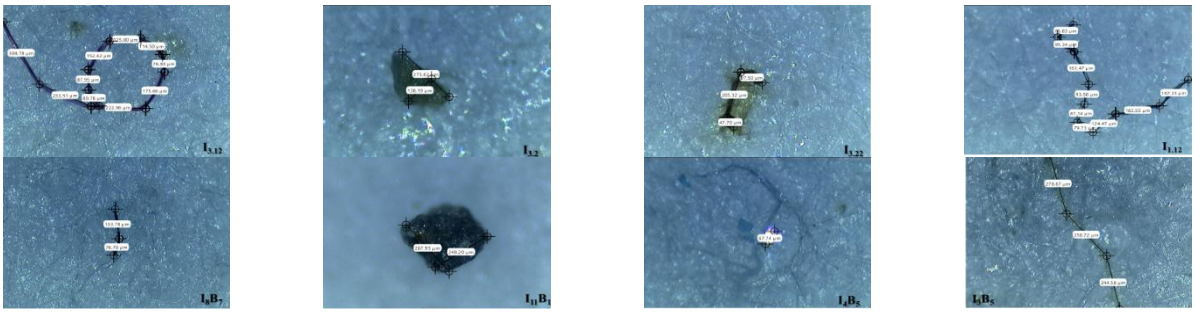


Figure 5.5. Measurement sequence of microparticles identified in the analyzed samples.

Each microparticle was assigned to a size class based on its size. In Table 5.1, based strictly on the size of the microparticles, it was considered justified to use a color code class to define those microparticles that could pose a higher risk to human health. In this sense, the color green is associated with the lowest health risk, while red is assigned to microparticles with the highest risk.

Table 5.1. Dimension classes associated with microparticle size.

Category	Size class C_x [µm]				
	[C_A] <50	[C_B] 50-100	[C_C] 100-500	[C_D] 500-1000	[C_E] >1000
Conventional yogurt	22	16	70	65	7
Organic yourt	3	4	23	24	1

Complementary to this, the discussion may include the amount and chemical structure of microparticles, which could definitely modify the health risk scale. Therefore, for the C_A class, 22 microparticles having a length under 50 µm came from conventional yogurt and three from organic yogurt; 16 microparticles from conventional yogurt and four from organic yogurt were attributed to class C_B; 70 microparticles detected in conventional yogurt, along with 23 microparticles detected in organic yogurt, are the subject of the C_C class. The last two classes (C_D and C_E) registered 65 microparticles, which were sourced from the conventional yogurt, and seven microparticles from the organic one. 24 originated from conventional yogurt, and one from the organic one.

All 236 microparticles analyzed by micro-FTIR were also characterized in terms of shape (Figure 5.6). They were assigned seven different shapes (oval, irregular, trapezoid, square, rectangle, circle, and triangle), as well as the term "fiber".

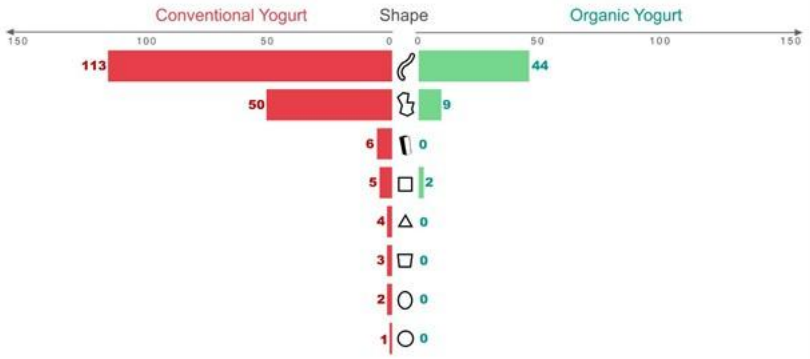


Figure 5.6. The shape distribution attributed to microplastics identified in the analyzed samples.

5.5. Statistical analysis

Statistical analysis of the data was performed using IBM SPSS Statistics v.26. The Pearson correlation coefficient, which ranges from -1 to +1 and is used as a measure of linear dependence between two or more variables, was employed to examine significant correlations (Hopkins W.G., 2000). The relationships between the variables were predicted by regression analysis. The independent variable is plotted on the x-axis, and the dependent variable is plotted on the y-axis, as shown in the methodology. Cluster analysis was performed to identify commonalities between the samples and the nature of the fibers (Figures 5.7 and 5.8).

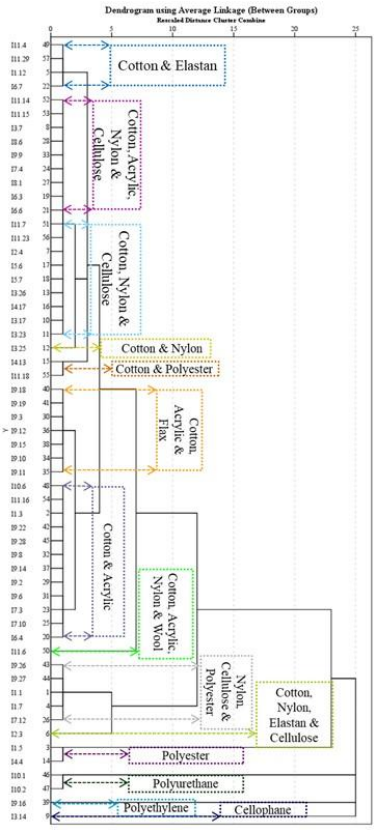


Figure 5.7. Dendrogram plot tab - synthetic microparticles identified in the conventional yogurt.

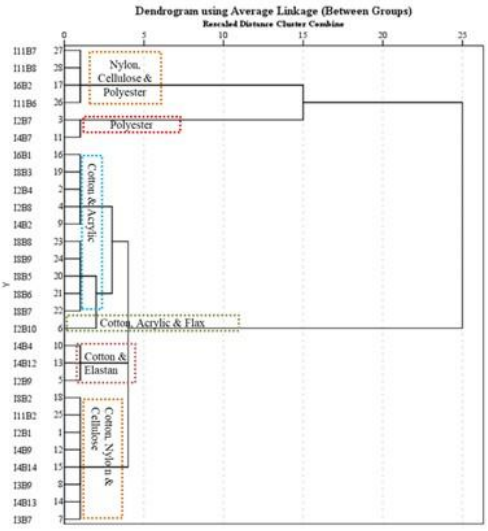


Figure 5.8. Dendrogram plot tab - synthetic microparticles identified in the organic yogurt.

This research confirmed the presence of natural and synthetic microparticles in fermented dairy products, isolated according to the method described in the patent (Radulescu et al., 2025a, 2025b). The scientific approach was naturally conducted through the stages of identification, quantification, morphological, and chemical analysis using complementary methods such as optical microscopy and micro-FTIR.

The limited information and issues regarding procedures/standards for microplastic analysis represent a challenge for the future. In this sense, this study serves as a starting point for future research on the presence of microplastics in dairy-based foods and for a more comprehensive assessment of potential human exposures and risks to human health. Furthermore, the research highlighted that the isolation method, combined with the analytical techniques used, has a low environmental impact. Efforts to optimize energy consumption could increase the environmental friendliness of the proposed methodology.

By integrating current challenges with recent innovations, this research aims to guide food industry specialists towards solving problems related to microplastic contamination of dairy products through the lens of evidence-based policies.

Chapter 6.
RESEARCH ON THE QUANTIFICATION AND CHARACTERIZATION OF MICROPLASTICS IN HIGH-FAT DAIRY PRODUCTS

This chapter aims to quantify and characterize microplastics in samples with high fat content, namely butter and cream, an objective that is, moreover, ambitious and risky to achieve. The choice of these dairy products was not accidental, as the scientific approach was led by the lack of information in the specialized literature regarding the presence and analysis of microplastics in such products.

6.3. Quantification and characterization of microplastics by optical microscopy

The first analytical technique used to identify microparticles in high-fat dairy products is optical microscopy. With the help of this analytical method, the presence of microparticles was highlighted, without establishing their nature or quantification, but instead focusing on the primary identification of their shapes and colors. Thus, the identification of representative microparticles, as well as their shape (irregular, oval, rhomboidal, film, and fibers), is presented in Figure 6.1.

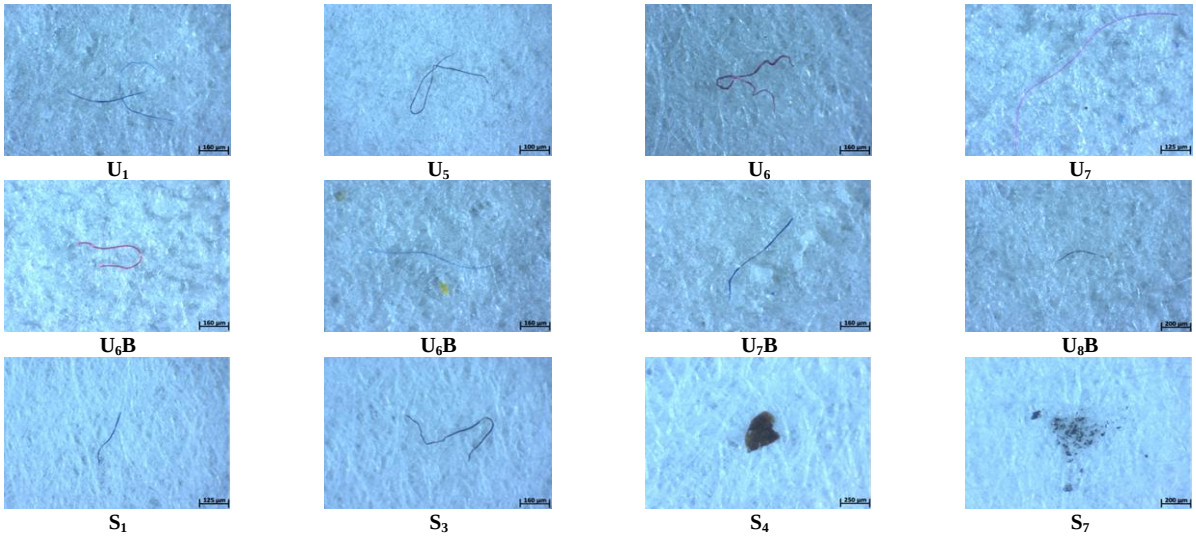


Figure 6.1. Representative optical microscopy images of microparticles isolated from high-fat dairy products.

The total number of microparticles identified in the analyzed samples is presented in Figure 6.2. The highest number of microparticles was determined in conventional butter, followed by cream and organic butter. Eight colors were identified in conventional butter samples (black, blue, red, green, brown, gray, yellow, and purple), seven colors in organic butter samples (black, blue, red, brown, gray, yellow, and purple), and six colors in cream samples (black, blue, green, gray, yellow, and purple).

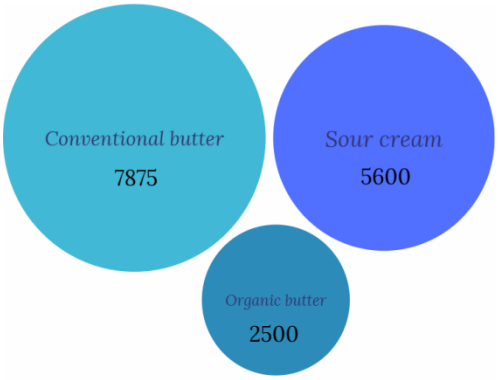


Figure 6.2. Totals microparticles identified in the analyzed samples.

6.4. Morphological and chemical characterization of microplastics by Fourier transform infrared micro-spectroscopy

A thorough chemical and morphological characterization of microparticles can be achieved by micro-FTIR imaging. Although micro-Fourier transform infrared spectroscopy (micro-FTIR) requires many investigations, at least three points, to obtain a conclusive result, it is one of the most efficient techniques for the chemical characterization of microplastics. The measurement sequence was performed for each microparticle after micro-FTIR analysis (Figure 6.3). A total of 133 microparticles were analyzed using micro-FTIR, with a focus on high-fat dairy product categories. The microparticles were classified based on their nature: 65 were natural and 68 were synthetic. Natural microparticles are those microparticles that have a 100% natural composition or a mixture of natural fibers, e.g., cotton, wool, raffia, cellulose, etc. (Silva et al., 2018). Synthetic microparticles are those microparticles that have in their composition a mixture of natural and polymeric compounds, such as poly(methyl methacrylate), polyamide (nylon), or polyurethane (elastane) (Figure 6.4, spectra selection).

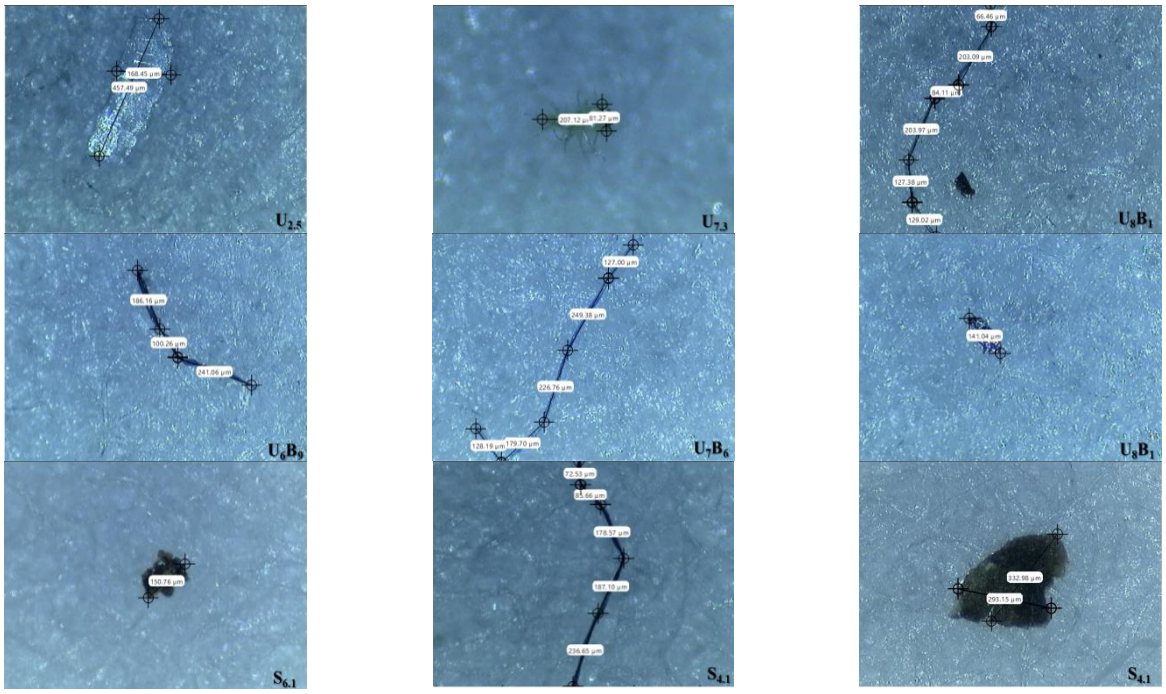


Figure 6.3. Measurement sequence of microparticles identified in the analyzed samples.

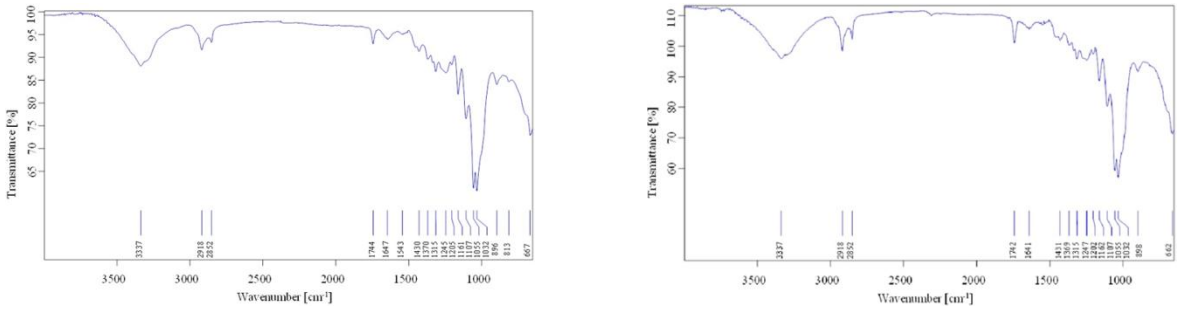


Figure 6.4. FTIR spectra of synthetic microparticles from the analyzed samples (selection): a) – cotton, poly(methyl methacrylate); b) – cotton, poly(methyl methacrylate) and flax.

According to Figures 6.5- 6.7, the highest distribution of synthetic microparticles was recorded in organic and conventional butter samples (80% in organic butter samples and 56% in conventional butter samples). In comparison, the lowest distribution was recorded in cream samples (22%). Regarding natural microparticles, the highest distribution was observed in the sour cream samples (78%), while the distribution in the conventional and organic butter samples was 44% and 20%, respectively. The microparticle content in the high-fat samples was variable (375 to 1500 microparticles/kg).

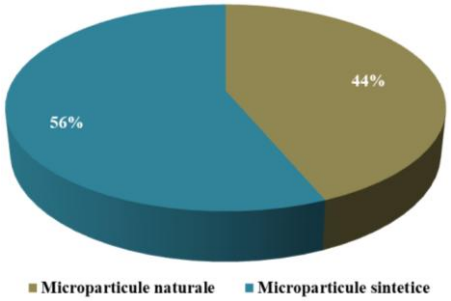


Figure 6.5. The nature of microparticles identified by the micro-FTIR technique in conventional butter samples.

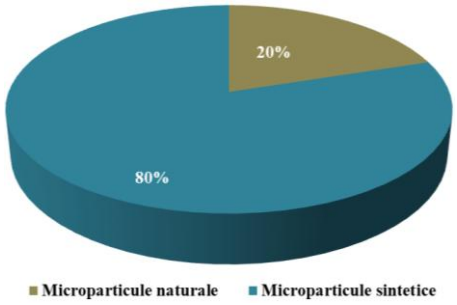


Figure 6.6. The nature of microparticles identified by the micro-FTIR technique in organic butter samples.

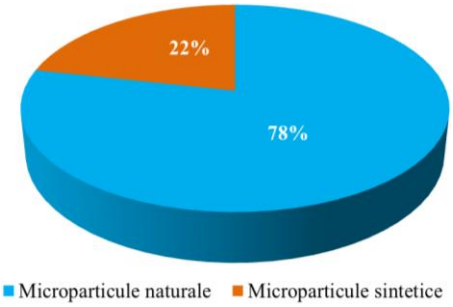


Figure 6.7. The nature of microparticles identified by the micro-FTIR technique in sour cream samples.

Chapter 7.
ENVIRONMENTAL AND HUMAN HEALTH RISKS ASSOCIATED WITH EXPOSURE TO EMERGING CONTAMINANTS

The main objectives of this chapter were: *i)* to assess the potential risks to the environment and human health that may arise from exposure to different contaminants of emerging concern (microplastics, heavy metals, polycyclic aromatic hydrocarbons) and *ii)* to identify correlations that may arise between the identified risk factors. This chapter is a natural continuation of the investigations related to the quantification and characterization of microplastics and other contaminants of carcinogenic risk (heavy metals and polycyclic aromatic hydrocarbons) identified in dairy products, with direct reference to health risks (children and adults).

The toxic effects caused by exposure to microplastics can vary depending on their physical characteristics (size, shape, and length), as well as their chemical characteristics, due to the presence of additives and polymers. These polymers can induce irritation or even toxins when assimilated by the human body. The potential risks at the gastrointestinal, hepatic, and reproductive levels, as well as neurotoxicity and synergistic toxicity caused by exposure to microplastics, are associated with oxidative stress and metabolic problems, representing a declared danger to human health (Banica et al., 2024a; Campanele et al., 2020; Prata et al., 2020). The ability of microplastics to act as vectors for heavy metals or polycyclic aromatic hydrocarbons increases the synergy between the toxicity of microplastics and that of contaminants adsorbed on their surface or in their composition, thus amplifying their harmful impact on the quality of the environment and life (Cooray et al., 2023; Cao et al., 2021; Li and Wang colab 2023).

7.2. Human health risks associated with exposure to microplastics

Microplastics can enter the human body through three main routes: inhalation, ingestion, and dermal exposure. Microplastics in the air are inhaled along with dust and other pollutants (Gaylarde et al., 2024; Mbachu et al., 2020). These tiny particles are small enough to penetrate deep into the lungs and can even enter the bloodstream (Prata et al., 2020). Both indoor and outdoor air pollution contribute to this inhalation exposure, originating from sources such as vehicle tire wear, synthetic textiles, and plastic particles found in cleaning products (Vianello et al., 2019; Liao et al., 2021; Gaston et al., 2020). On the other hand, microplastics are ingested through the consumption of contaminated food and water.

B. Specific migration limits for microplastics in dairy products

The consumption of microplastics by a person in one year was calculated for milk, yogurt, cream, and butter samples based on the formula described in this chapter of the doctoral thesis. The results obtained are presented in Table 7.1 for milk and yogurt samples and in Table 7.2 for high-fat products. The rate of microplastic ingestion over a year (365 days) increases exponentially with the amount of product consumed and/or the variety of dairy products that an adult or child consumes during a year.

Table 7.1. Average annual intake of microplastics by adults and children from consumption of low-fat products (Banica et al., 2025a).

Milk			Yogurt		
Sample code	**Adults**	**Children**	**Sample code**	**Adults**	**Children**
L_1	273.75	228.13	I_1	1442.48	1931.22
L_2	1095	912.50	I_2	776.72	1039.89
L_4	547.5	456.25	I_3	2884.96	3862.43
L_6	547.5	456.25	I_4	1886.32	2525.44
L_7	821.25	684.38	I_5	1331.52	1782.66
L_8	1916.25	1596.88	I_6	776.72	1039.89
L_9	1916.25	1596.88	I_7	1442.48	1931.22
L_{10}	1095	912.50	I_8	1442.48	1931.22
L_1B	547.5	456.25	I_9	3106.88	4159.54
L_6B	273.75	228.13	I_{10}	1553.44	2079.77
L_7B	1095	912.50	I_{11}	3439.76	4605.21
L_8B	1642.5	1368.75	I_2B	1109.6	1485.55
$L_{10}B$	547.5	456.25	I_3B	1109.6	1485.55
$L_{11}B$	273.75	228.13	I_4B	1553.44	2079.77
LF_1	821.25	684.38	I_6B	443.84	594.22
LF_2	1095	912.50	I_8B	998.64	1337.00
LF_3	1368.75	1140.63	$I_{11}B$	887.68	1188.44
LF_4	1095	912.50			

Table 7.2. Average annual intake of microplastics by adults and children from high-fat products (Banica et al., 2025a).

Conventional butter			Organic butter		
Sample code	**Adults**	**Children**	**Sample code**	**Adults**	**Children**
U_1	427.05	328.50	U_6B	474.5	365.00
U_2	284.7	219.00	U_7B	474.5	365.00
U_3	569.4	438.00	U_8B	142.35	109.50
U_4	332.15	255.50	**Sour cream**		
U_5	284.7	219.00	**Sample code**	**Adults**	**Children**
U_6	332.15	255.50	S_2	474.5	365.00
U_7	569.4	438.00	S_6	474.5	365.00
U_8	427.05	328.50			

Corroborating the calculations made in this chapter of the doctoral thesis for dairy products with the research carried out by Kosuth et al. (2018) on salt and with the research carried out by Hernendez et al. (2019) on iced tea, an adult ends up ingesting an amount of microplastics ranging between 74.000 and 121.000 microplastic particles, according to the estimates presented by Cox et al. (2019).

The **Estimated Daily Intake** (EDI) for adults and children is presented synthetically in Figure 7.1.



a) b)

Figure 7.1. Hierarchy of milk samples analyzed based on estimated daily microplastics consumption values for: a) – children and b) – adults (selection) (Banica et al, 2025a).

The **Plastic Pollution Load Index** (PCF) was calculated for milk and processed product samples, and the results are presented in Figure 7.2. The contamination levels and risk categories for PCF were established by Ibeto et al. (2023) and adapted by Binelli et al. (2025).

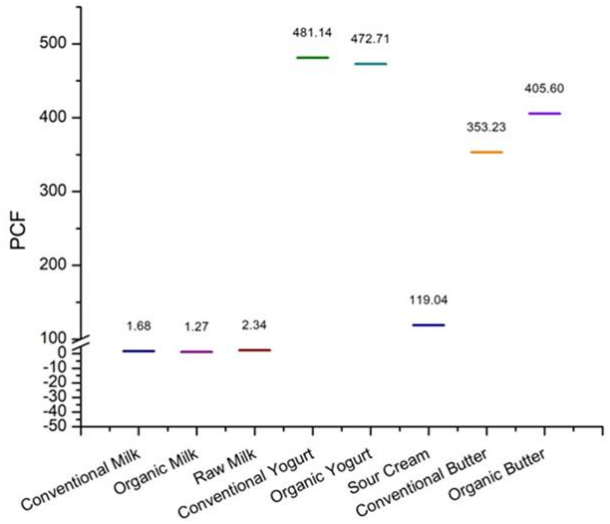


Figura 7.2. Plastic pollution load index determined in dairy product samples (Banica și colab., 2025a).

The classification of the analyzed samples was carried out based on the PCF values, and the five levels of stability risk by Ibeto et al. (2023), were: (i) very low hazard (≤1); (ii) low (≤10); (iii) medium (≤100), (iv) high (≤1000); (v) very high (≤10000). For the three milk categories, the PCF values were <10 and present a very low risk level. For the sour cream samples, the PCF was slightly higher than 100. The highest PCF values were recorded for the yogurt and butter samples, which classify these samples as being in a medium-high risk level.

In current research, the **Plastic Risk Index** (PRI) was calculated for the polymers identified in the dairy product samples analyzed. Each polymer has a risk level, which was established by Lin et al. (2023) in their study. PU has the highest risk score (13844), followed by PMMA (1021), PA (50), PS (30), and PE (10).

The PRI for the analyzed yogurt samples was calculated for the five identified polymers, using the same structure described for the milk samples; the results obtained are presented graphically in Figure 7.3. The lowest PRI value was identified in sample I_4 (PRI = 40.00), where PA and PS polymers were identified, and the highest PRI value was identified in sample I_{10} (PRI = 9569.67), where PMMA, PA, and PU polymers were identified. Using the same risk categories described by Binelli et al. (2025) for the analyzed milk samples in their study, the analyzed yogurt samples in this study fall into all five risk classes. Three analyzed samples present a low risk, with PRI values lower than 150. Four yogurt samples had PRI values between 150 and 300, placing them in the medium risk class. PRI values between 300 and 600 were identified in three yogurt samples, posing a considerable risk.

Two yogurt samples had PRI values between 600 and 1200, classifying them as high-risk, while six yogurt samples had PRI values exceeding 1200, indicating a very high risk. The average PRI value for the 17 yogurt samples is 1726.34. In the literature, a study by Ling et al. (2024) on fruit yogurt found that the risk index of plastics was reduced by a PRI value of 16. Different values were used for all the toxicity coefficients of PE and PS polymers, making it impossible to compare the results with those of other research in the literature.

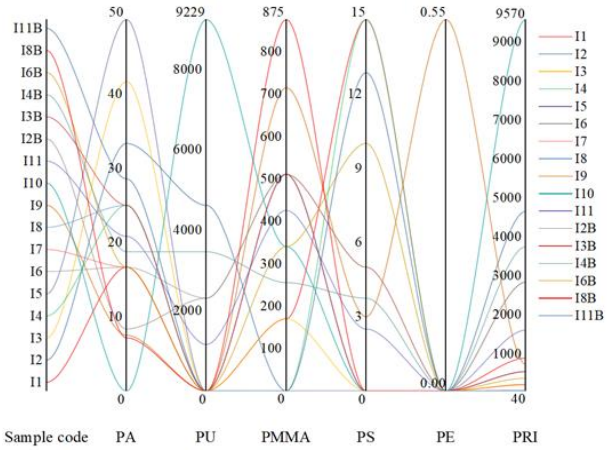


Figure 7.3. Risk index of plastic materials identified in the analyzed samples (selection) (Banica et al., 2025a).

7.5. Riscul asociat expunerii la metale grele

Carcinogenic Risk (CR) and **Total Carcinogenic Risk** (CR_t) assess the lifetime risk of developing cancer following exposure to heavy metals (Table 7.3). Exposure to heavy metals, regardless of their abundance in food products, can cause disorders in the human body, which can lead to the development of various types of cancer, neurological disorders, etc. (Badeenezhad et al., 2023).

Table 7.3. Carcinogenic risk and total carcinogenic risk for ingestion of heavy metals from the analyzed samples (Banica et al., 2025b).

Sample	CR								CR_t	
	Cr		Ni		Cd		Pb			
	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children
L_1	$2.73\cdot10^{-1}$	$1.14\cdot10^{-3}$	$9.95\cdot10^{-4}$	$4.14\cdot10^{-6}$	0.00	0.00	$1.88\cdot10^{-3}$	$1.90\cdot10^{-7}$	**$2.75\cdot10^{-1}$**	**$1.14\cdot10^{-3}$**
L_2	$2.28\cdot10^{-1}$	$9.49\cdot10^{-4}$	$9.18\cdot10^{-4}$	$3.83\cdot10^{-6}$	0.00	0.00	$1.56\cdot10^{-3}$	$1.58\cdot10^{-7}$	**$2.30\cdot10^{-1}$**	**$9.53\cdot10^{-4}$**
L_3	$1.75\cdot10^{-1}$	$7.31\cdot10^{-4}$	$9.18\cdot10^{-4}$	$3.83\cdot10^{-6}$	0.00	0.00	$5.10\cdot10^{-4}$	$5.16\cdot10^{-8}$	**$1.77\cdot10^{-1}$**	**$7.35\cdot10^{-4}$**
L_4	$2.17\cdot10^{-1}$	$9.02\cdot10^{-4}$	$1.61\cdot10^{-3}$	$6.69\cdot10^{-6}$	$2.26\cdot10^{-4}$	$8.65\cdot10^{-7}$	$2.68\cdot10^{-3}$	$2.71\cdot10^{-7}$	**$2.21\cdot10^{-1}$**	**$9.10\cdot10^{-4}$**
L_5	$3.51\cdot10^{-1}$	$1.46\cdot10^{-3}$	$2.98\cdot10^{-3}$	$1.24\cdot10^{-5}$	$4.14\cdot10^{-4}$	$1.59\cdot10^{-6}$	$2.74\cdot10^{-3}$	$2.77\cdot10^{-7}$	**$3.57\cdot10^{-1}$**	**$1.48\cdot10^{-3}$**
L_6	$1.79\cdot10^{-1}$	$7.47\cdot10^{-4}$	$5.74\cdot10^{-3}$	$2.39\cdot10^{-5}$	0.00	0.00	$1.49\cdot10^{-2}$	$1.50\cdot10^{-6}$	**$2.00\cdot10^{-1}$**	**$7.72\cdot10^{-4}$**
L_7	$1.90\cdot10^{-1}$	$7.93\cdot10^{-4}$	$1.76\cdot10^{-3}$	$7.33\cdot10^{-6}$	0.00	0.00	$6.00\cdot10^{-3}$	$6.06\cdot10^{-7}$	**$1.98\cdot10^{-1}$**	**$8.01\cdot10^{-4}$**
L_8	$3.70\cdot10^{-1}$	$1.54\cdot10^{-3}$	$3.98\cdot10^{-3}$	$1.66\cdot10^{-5}$	0.00	0.00	$6.35\cdot10^{-3}$	$6.42\cdot10^{-7}$	**$3.80\cdot10^{-1}$**	**$1.56\cdot10^{-3}$**
L_9	$1.94\cdot10^{-1}$	$8.09\cdot10^{-4}$	$1.68\cdot10^{-3}$	$7.01\cdot10^{-6}$	0.00	0.00	$5.23\cdot10^{-3}$	$5.29\cdot10^{-7}$	**$2.01\cdot10^{-1}$**	**$8.17\cdot10^{-3}$**
L_{10}	$1.87\cdot10^{-1}$	$7.78\cdot10^{-4}$	$7.50\cdot10^{-3}$	$3.12\cdot10^{-5}$	0.00	0.00	$9.92\cdot10^{-3}$	$1.00\cdot10^{-6}$	**$2.04\cdot10^{-1}$**	**$8.10\cdot10^{-4}$**
L_1B	$1.79\cdot10^{-1}$	$7.47\cdot10^{-4}$	$2.52\cdot10^{-3}$	$1.05\cdot10^{-5}$	0.00	0.00	$1.66\cdot10^{-3}$	$1.68\cdot10^{-7}$	**$1.83\cdot10^{-1}$**	**$7.57\cdot10^{-4}$**
L_6B	$3.70\cdot10^{-1}$	$1.54\cdot10^{-3}$	$2.07\cdot10^{-3}$	$8.61\cdot10^{-6}$	0.00	0.00	$1.63\cdot10^{-3}$	$1.64\cdot10^{-7}$	**$3.73\cdot10^{-1}$**	**$1.55\cdot10^{-3}$**
L_7B	$3.02\cdot10^{-1}$	$1.26\cdot10^{-3}$	$1.45\cdot10^{-3}$	$6.06\cdot10^{-6}$	0.00	0.00	$1.56\cdot10^{-3}$	$1.58\cdot10^{-7}$	**$3.05\cdot10^{-1}$**	**$1.27\cdot10^{-3}$**
L_8B	$2.02\cdot10^{-1}$	$8.40\cdot10^{-4}$	$2.60\cdot10^{-3}$	$1.08\cdot10^{-5}$	0.00	0.00	$2.26\cdot10^{-3}$	$2.29\cdot10^{-7}$	**$2.06\cdot10^{-1}$**	**$8.51\cdot10^{-4}$**
$L_{10}B$	$3.62\cdot10^{-1}$	$1.51\cdot10^{-3}$	$2.22\cdot10^{-3}$	$9.24\cdot10^{-6}$	0.00	0.00	$2.04\cdot10^{-3}$	$2.06\cdot10^{-7}$	**$3.66\cdot10^{-1}$**	**$1.52\cdot10^{-3}$**
$L_{11}B$	$3.66\cdot10^{-1}$	$1.52\cdot10^{-3}$	$2.14\cdot10^{-3}$	$8.93\cdot10^{-6}$	0.00	0.00	$1.34\cdot10^{-3}$	$1.35\cdot10^{-7}$	**$3.69\cdot10^{-1}$**	**$1.53\cdot10^{-3}$**

Farahmandkia et al. (2025) reported an acceptable limit for heavy metals ranging from 10^{-6}) to 10^{-4}, according to EPA guidelines. The calculated carcinogenic risk for adults and children had alarming values for chromium, and in the case of adults, nickel, cadmium, and lead presented values ranging from 10^{-3} to 10^{-1}, which can endanger the lives of consumers. The summation of the carcinogenic risk of the eight heavy metals led to the establishment of the total carcinogenic risk (CR_t). The determined values range from $1.98\cdot10^{-1}$ to $3.80\cdot10^{-1}$ for adults, while the calculated values for children ranged from $1.27\cdot10^{-3}$ to $9.10\cdot10^{-4}$. The analyzed milk

samples exceeded the maximum CR_t values for adults, and some samples also exceeded recommended values for children, according to the guidelines suggested by Farahmandkia et al. (2025) for CR.

Risk associated with exposure to polycyclic aromatic hydrocarbons

The **Average Daily Dose** (ADD) of polycyclic aromatic hydrocarbons was calculated for the analyzed samples, for adults and children, and the results are presented in Figures 7.4 and 7.5. The results obtained for the average daily dose indicate that children are more exposed to polycyclic aromatic hydrocarbons than adults. The highest ADD values were determined for anthracene ($kg \cdot day^{-1}$) and benzo[*a*]anthracene ($kg \cdot day^{-1}$) in conventional milk. In the case of children, benzo[*b*]fluoranthene has the highest ADD values, ranging from 0.6913 $kg \cdot day^{-1}$ to 5.7838 $kg \cdot day^{-1}$. In the study by Rawash et al. (2018), the authors reported ADD values of 0.61–1.22 mg/day for all age groups. In a study conducted in Italy by Zhao et al. (2023) on raw milk, ADD values ranged from 1.75 ng/day to 22.96 ng/day. The ADD values presented in this study are much higher than those reported by Rawash et al. (2018), but on average lower than those reported by Zhao et al. (2023).

Samples code	1	3	4	5	6	7	8	9	10	11	12	13	14
	ADD Adults [ng·kg^{-1}·day^{-1}]												
L1	0.0286	0.0000	0.0276	0.0874	0.0103	0.0040	0.0586	0.0028	0.0071	0.0026	0.0000	0.0000	0.0000
L2	0.0229	0.0940	0.0173	0.0816	0.0047	0.0026	0.0223	0.0000	0.0008	0.0008	0.0000	0.0000	0.0000
L3	0.0286	0.0000	0.0099	0.0790	0.0122	0.0035	0.0336	0.0011	0.0027	0.0011	0.0000	0.0001	0.0000
L4	0.0227	0.0000	0.0282	0.0762	0.0102	0.0726	0.0000	0.0019	0.0035	0.0011	0.0000	0.0000	0.0000
L5	0.0000	0.0000	0.0270	0.1213	0.0048	0.0069	0.1145	0.0108	0.0690	0.0177	0.0029	0.0005	0.0000
L6	0.0565	0.0000	0.0254	0.1279	0.0156	0.0029	0.1112	0.0113	0.0660	0.0179	0.0000	0.0004	0.0000
L7	0.0228	0.0000	0.0274	0.0724	0.0027	0.0031	0.0219	0.0000	0.0012	0.0009	0.0002	0.0001	0.0000
L8	0.0233	0.0000	0.0320	0.0728	0.0035	0.0044	0.0238	0.0000	0.0008	0.0018	0.0000	0.0001	0.0021
L9	0.0357	0.0000	0.0248	0.0819	0.0113	0.0000	0.0382	0.0012	0.0037	0.0012	0.0000	0.0000	0.0000
L10	0.0259	0.0000	0.0267	0.0795	0.0045	0.0000	0.0311	0.0016	0.0055	0.0007	0.0000	0.0001	0.0000
	ADD Cildren [ng·kg^{-1}·day^{-1}]												
L1	0.1193	0.0000	0.1150	0.3640	0.0429	0.0165	0.2442	0.0117	5.7838	0.0109	0.0000	0.0000	0.0000
L2	0.0955	0.3916	0.0720	0.3399	0.0196	0.0107	0.0930	0.0000	0.6913	0.0032	0.0000	0.0002	0.0000
L3	0.1190	0.0000	0.0413	0.3292	0.0508	0.0146	0.1399	0.0044	2.2138	0.0047	0.0000	0.0005	0.0000
L4	0.0947	0.0000	0.1175	0.3175	0.0426	0.3026	0.0000	0.0081	2.8525	0.0046	0.0000	0.0000	0.0000
L5	0.0000	0.0000	0.1125	0.5053	0.0202	0.0286	0.4770	0.0450	5.6359	0.0738	0.0121	0.0022	0.0000
L6	0.2353	0.0000	0.1057	0.5331	0.0650	0.0120	0.4635	0.0471	5.3918	0.0748	0.0000	0.0018	0.0000
L7	0.0949	0.0000	0.1142	0.3016	0.0113	0.0128	0.0912	0.0000	0.9975	0.0038	0.0007	0.0002	0.0000
L8	0.0970	0.0000	0.1331	0.3031	0.0144	0.0184	0.0990	0.0000	0.6913	0.0077	0.0000	0.0004	0.0087
L9	0.1486	0.0000	0.1035	0.3412	0.0469	0.0000	0.1591	0.0049	3.0450	0.0051	0.0000	0.0000	0.0000
L10	0.1077	0.0000	0.1114	0.3314	0.0188	0.0000	0.1298	0.0068	4.4888	0.0030	0.0000	0.0005	0.0000

Figure 7.4. Average daily dose of polycyclic aromatic hydrocarbons ingested through the consumption of conventional milk (Banica et al., 2025b).

Samples code	1	3	4	5	6	7	8	9	10	11	12	13	14
	ADD Cildren [ng·kg^{-1}·day^{-1}]												
L1B	0.0486	0.0000	0.0351	0.0839	0.0032	0.0000	0.0277	0.0003	0.0015	0.0009	0.0000	0.0000	0.0000
L6B	0.0156	0.0000	0.0230	0.0731	0.0076	0.0009	0.0347	0.0011	0.0025	0.0000	0.0000	0.0000	0.0000
L7B	0.0205	0.0000	0.0262	0.0842	0.0026	0.0024	0.0470	0.0000	0.0069	0.0023	0.0005	0.0001	0.0000
L8B	0.0085	0.0000	0.0237	0.0794	0.0028	0.0042	0.0419	0.0000	0.0049	0.0005	0.0000	0.0000	0.0000
L10B	0.0196	0.0000	0.0251	0.0912	0.0022	0.0000	0.0732	0.0042	0.0076	0.0004	0.0002	0.0000	0.0000
L11B	0.0159	0.0000	0.0271	0.0677	0.0078	0.0000	0.0249	0.0000	0.0021	0.0000	0.0000	0.0000	0.0000
	ADD Cildren [ng·kg^{-1}·day^{-1}]												
L1B	0.2024	0.0000	0.1463	0.3496	0.0133	0.0000	0.1154	0.0014	1.2250	0.0036	0.0000	0.0000	0.0000
L6B	0.0650	0.0000	0.0960	0.3045	0.0316	0.0036	0.1445	0.0044	2.0125	0.0000	0.0000	0.0000	0.0000
L7B	0.0855	0.0000	0.1093	0.3510	0.0110	0.0101	0.1957	0.0000	5.6263	0.0096	0.0022	0.0004	0.0000
L8B	0.0356	0.0000	0.0986	0.3306	0.0116	0.0174	0.1745	0.0000	3.9813	0.0021	0.0000	0.0000	0.0000
L10B	0.0817	0.0000	0.1046	0.3800	0.0092	0.0000	0.3052	0.0175	6.2388	0.0016	0.0010	0.0002	0.0000
L11B	0.0662	0.0000	0.1128	0.2820	0.0326	0.0000	0.1036	0.0000	1.7063	0.0000	0.0000	0.0000	0.0000

Figura 7.5. Average daily dose of polycyclic aromatic hydrocarbons ingested through the consumption of organic milk (Banica et al., 2025b).

The **Toxic Equivalent Quotient** (TEQ) of polycyclic aromatic hydrocarbons was another method used to determine the risk to consumer health regarding potentially contaminated dairy products. The determining TEQ values are presented in Figure 7.6 for the analyzed samples.

Sample code	1	2	4	5	6	7	8	9	10	11	12	13	14
						[ng·L^{-1}]							
L1	0.0027	0.0000	0.0026	0.0815	0.0010	0.0004	0.5469	0.0026	0.0661	0.0245	0.0000	0.0000	0.0000
L2	0.0021	0.0088	0.0016	0.0761	0.0004	0.0002	0.2084	0.0000	0.0079	0.0071	0.0000	0.0200	0.0000
L3	0.0027	0.0000	0.0009	0.0737	0.0011	0.0003	0.3134	0.0010	0.0253	0.0105	0.0000	0.0550	0.0000
L4	0.0021	0.0000	0.0026	0.0711	0.0010	0.0068	0.0000	0.0018	0.0326	0.0104	0.0000	0.0000	0.0000
L5	0.0000	0.0000	0.0025	0.1132	0.0005	0.0006	1.0684	0.0101	0.6441	0.1653	0.2710	0.2450	0.0000
L6	0.0053	0.0000	0.0024	0.1194	0.0015	0.0003	1.0382	0.0105	0.6162	0.1675	0.0000	0.2050	0.0000
L7	0.0021	0.0000	0.0026	0.0676	0.0003	0.0003	0.2043	0.0000	0.0114	0.0086	0.0150	0.0250	0.0000
L8	0.0022	0.0000	0.0030	0.0679	0.0003	0.0004	0.2217	0.0000	0.0079	0.0172	0.0000	0.0400	0.0019
L9	0.0033	0.0000	0.0023	0.0764	0.0011	0.0000	0.3564	0.0011	0.0348	0.0114	0.0000	0.0000	0.0000
L10	0.0024	0.0000	0.0025	0.0742	0.0004	0.0000	0.2907	0.0015	0.0513	0.0068	0.0000	0.0550	0.0000
L1B	0.0045	0.0000	0.0033	0.0783	0.0003	0.0000	0.2585	0.0003	0.0140	0.0081	0.0000	0.0000	0.0000
L6B	0.0015	0.0000	0.0022	0.0682	0.0007	0.0001	0.3237	0.0010	0.0230	0.0000	0.0000	0.0000	0.0000
L7B	0.0019	0.0000	0.0024	0.0786	0.0002	0.0002	0.3384	0.0000	0.0643	0.0215	0.0490	0.0500	0.0000
L8B	0.0008	0.0000	0.0022	0.0741	0.0003	0.0004	0.3908	0.0000	0.0455	0.0048	0.0000	0.0000	0.0000
L10B	0.0018	0.0000	0.0023	0.0851	0.0002	0.0000	0.6836	0.0039	0.0713	0.0035	0.0230	0.0200	0.0000
L11B	0.0015	0.0000	0.0025	0.0632	0.0007	0.0000	0.2320	0.0000	0.0195	0.0000	0.0000	0.0000	0.0000

Figure 7.6. Equivalent toxicity coefficient of polycyclic aromatic hydrocarbons identified in conventional and organic milk (Banica et al., 2025b).

Naphthalene was absent in only one sample (L_5) out of all the samples analyzed, which means that the TEQ is 0, and the maximum value for the other samples was 0.0053 ng·L^{-1} (L_6). Acenaphthene and benzo[*g,h,i*]perylene were identified in two different samples (L_2 and L_8), and the TEQ was 0.0088 ng·L^{-1} and 0.0019 ng·L^{-1}, respectively. Fluorene, anthracene, and fluoranthene were determined in all processed milk samples analyzed, the values obtained were between 0.0009-0.0030 ng·L^{-1}, 0.0676-0.1194 ng·L^{-1}, and 0.0003-0.0015 ng·L^{-1} for conventional milk, respectively, while in organic dairy, the TEQ values determined were between 0.0022 and 0.0030 ng·L^{-1}, 0.0632-0.0851 ng·L^{-1}, and 0.0002-0.0007 ng·L^{-1}. Benzo[*a*]anthracene was identified in eight conventional milk samples and three organic milk samples, being absent in the others. The highest TEQ value for benzo[*a*]anthracene was 0.0068 ng·L^{-1}, determined in sample L_4. Chrysene was absent in sample L_4, while TEQ values were the highest compared to the other polycyclic aromatic hydrocarbons determined. The TEQ values of chrysene for the ten processed milk samples in which it was identified ranged from 0.0010 ng·L^{-1} to 0.0105 ng·L^{-1}. Samples L_5 and L_6 had values higher than 1.

Suspended particles, drinking water, fish, fruit, meat, milk, honey, vegetables, medicinal plants, and cosmetics are some examples through which microplastics can reach the human body, through ingestion, inhalation, or skin contact, and the effects on health can sometimes be extremely serious, even irreversible. What is concerning is that these particles can enter the bloodstream and lymphatic system through the gastrointestinal tract, respiratory tract, and dermis (skin lesions), and then accumulate in the body. Although the toxicity of microplastics in humans has not yet been fully demonstrated, niche studies have shown altered behavioral effects and malformations in animals used in laboratory research.

The results of this study highlight the need for targeted interventions in the dairy production chain, as well as the reduction of plastic waste in unmanaged areas and the implementation of high precision in animal farms, to mitigate environmental contamination with microplastics and minimize human health implications. The consistent association of yogurt, regardless of the production system, with high levels of PA, PU, PMMA, and PS suggests that specific processing or packaging steps may contribute disproportionately to contamination. Additionally, assessing the risk of emerging carcinogenic contaminants (heavy metals, polycyclic aromatic hydrocarbons) in milk samples, the raw material for producing other dairy products, represented another novel aspect addressed in this chapter. Establishing for the first time specific migration limits of microplastics to food products intended for human consumption, namely milk and dairy products, amplifies the novelty of this chapter and implicitly of the doctoral thesis.

GENERAL CONCLUSIONS

Microplastics are present everywhere in the environment and pose an increasing threat to ecosystems and living organisms. Concerns are growing that microplastics may contaminate water, soil, and air, potentially having adverse effects on human health through exposure. Additionally, when living organisms ingest microplastics, it may lead to unknown risks that could directly or indirectly affect the quality of life.

Microplastics, as potential vectors, can differently affect the transport and deposition of chemical contaminants, such as additives, heavy metals, polycyclic aromatic hydrocarbons, antibiotics, pesticides, and fungicides, depending on the type, concentration, and aging process of the polymeric material. The most common sorption mechanism of organic contaminants on microplastics is hydrophobic interaction, followed by electrostatic interactions, hydrogen bonds, halogen bonds, and π-π interactions. On the other hand, metal ions are adsorbed mainly through electrostatic interactions and surface complexes. The hydrophobic nature of plastics can lead to the formation of biofilms, which often carry toxic pollutants (heavy metals, polycyclic aromatic hydrocarbons) and can enter the food chain. Thus, in the case of polycyclic aromatic hydrocarbons, as the molecular weight increases, their carcinogenicity increases exponentially, accompanied by a decrease in acute toxicity. The most potent carcinogens in the class of polycyclic aromatic hydrocarbons have been identified as including benzo[*a*]anthracene, benzo[*a*]pyrene, and dibenz[*a,h*]anthracene.

Given the widespread presence of microplastics in the environment and the associated health risks of exposure, one of the main objectives of the doctoral thesis was to assess the potential risks associated with the accumulation of contaminants, including microplastics, polycyclic aromatic hydrocarbons, and heavy metals. This assessment draws on the most recent and relevant research in the field. Several mechanisms are considered primary processes by which these contaminants are absorbed, including electrostatic interactions, hydrophobic interactions, hydrogen bonding, and steric effects. These mechanisms significantly contribute to understanding the increased risk associated with exposure to the microplastic-organic/inorganic contaminant system, an emerging concern.

The demand for organic products is steadily increasing among consumers, and concerns about environmental pollution are also on the rise. The phrase "emerging contaminant" is commonly used in scientific literature, raising significant problems, particularly regarding its environmental impact. Contaminants commonly present in today's animal feed can enter the bodies of animals, potentially leading to their presence in processed products intended for human consumption. Consequently, the risks associated with exposure to these pollutants are on the rise. Emerging contaminants, such as microplastics, heavy metals, and polycyclic aromatic hydrocarbons, can be found in the food products consumed by the population. These contaminants primarily originate from animal feed (including feed and water) and environmental sources, which encompass air, water, and soil pollution, as well as work environments and protective equipment. Additionally, they can result from cross-contamination.

The doctoral thesis demonstrates a high degree of novelty through the protocol for isolating microplastics from milk, which has been proposed and patented at both national (RO 137927 B1/2025) and international (WO2025/071426 A1) levels. In addition, another assumed objective of this doctoral thesis was to investigate the presence of other emerging contaminants, such as polycyclic aromatic hydrocarbons and heavy metals, in branded milk, to support a

possible hypothesis regarding the property of microplastics to act as carriers for these contaminants.

The emergence of contaminants, including polycyclic aromatic hydrocarbons, microplastics, and heavy metals, poses a significant threat to biodiversity and the survival of living organisms. In the environment, organic compounds can decompose into toxic byproducts or break down into tiny, often invisible particles through various processes such as mechanical action, UV radiation, and thermal oxidation. Both irregularly shaped and spherical microfragments, as well as synthetic fibers, contribute significantly to the microplastic problem, primarily due to everyday human activities. These fibers pose a particular risk to humans, especially children, yet they are often overlooked in current research.

The doctoral thesis introduces a novel method for isolating microplastics from milk. It thoroughly examines three emerging contaminants–microplastics, polycyclic aromatic hydrocarbons, and heavy metals–that were previously analyzed separately. This research employs advanced analytical techniques, including optical microscopy, scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), and Fourier transform infrared micro–spectroscopy (micro-FTIR), inductively coupled plasma mass spectrometry (ICP-MS), and high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Additionally, the study incorporates statistical correlations to enhance the findings. To enhance the understanding of the research, a series of procedures was implemented to assess the risk indices related to human health for both children and adults. The composition of the microparticles was analyzed using the OPUS v.7.5 library. Microparticles made entirely from natural materials–such as cotton, cellulose, linen, and wool–were classified as natural fibers. In contrast, synthetic fibers, or microplastics, include microparticles made from materials such as polyamide, polyester, poly(methyl methacrylate), and polyurethane. This category also encompassed any microparticles that contained a mixture of natural and synthetic fibers.

This research represents the first comprehensive investigation in Romania that systematically quantifies microplastics in a wide range of commercial, packaged, high-fat dairy products. The research focused on samples of milk and fermented dairy products, such as yogurt. To enhance the novelty and complexity of the thesis, the study also analyzed microplastics in high-fat dairy products. This comprehensive approach provides a clearer understanding of the severity of the contamination issue within the dairy market.

With the increasing interest in understanding the impact of consuming contaminated food and the associated risks to ecosystems and health, this doctoral thesis presents new evidence regarding the presence of microplastics in a widely consumed food category: milk and dairy products. It also explores their potential health effects. The analyses performed highlighted PMMA, PU, PS, PE, and PA as the main polymers present in different categories of dairy products. The calculated risk indices (H, CF_i, EAI, DPI, CDI_{ng}, and PRI for microplastics, EDI, HRI, CDI_{ng}, HQ, HI, CR, CR_t, CF_i, PLI for heavy metals, and ADD, CDI_{ng}, HQ, HI, and TEQ) demonstrated that exposure levels vary substantially depending on the type of product. Yogurts, both conventional and organic, consistently showed higher levels of contamination.

These results highlight the importance of targeted monitoring and mitigation strategies for high-risk dairy products. To enhance the statistical power and better understand the relationships between products and contaminants, it is recommended to expand the dataset, include detailed polymer characterization, and evaluate seasonal and geographic variations. Implementing these approaches will improve the accuracy of risk assessments and guide policy decisions aimed at reducing dietary exposure to microplastics.

The findings from the doctoral thesis emphasize the necessity for targeted interventions in the technological processes of dairy product production. It also highlights the importance of reducing plastic waste in unmanaged areas and ensuring precision in animal farming. These measures aim to decrease environmental contamination from microplastics and mitigate the potential impacts on human health. The consistent association of yogurt, regardless of the production system, with high levels of PA, PU, PMMA, and PS suggests that specific processing or packaging steps may contribute disproportionately to contamination.

From a regulatory perspective, the observed variability in contamination across product types supports the establishment of product-specific monitoring protocols and acceptable threshold levels for microplastics in dairy products. Incorporating microplastic testing into routine food safety assessments, particularly for highly sensitive products, would allow for early detection and mitigation.

Ultimately, the doctoral thesis offers producers in the milk and dairy products market a practical approach for identifying risk points and critical stages associated with microplastic contamination. This is achieved through the use of the Hazard Analysis and Critical Control Points (HACCP) system. The implementation of the HACCP system, along with consumer education on food safety and risk analyses regarding health issues related to microplastics or other contaminants in milk, is essential for reducing or eliminating these risks. Following the hazard analysis, several critical control points were identified, described in detail in Chapter 8, to which the application of the patented isolation method can respond with control/monitoring/analysis solutions.

The lack of knowledge regarding procedures and standards for microplastic analysis presents a significant challenge for the future. The doctoral thesis was designed as a reference for those interested in reducing their plastic footprint and improving the assessment of human risks associated with exposure to emerging carcinogenic contaminants. The cumulative effects of these contaminants have yet to be fully understood.

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LIST OF PUBLICATIONS

National (Bn1, Bn2, etc.) and international (Bi1, Bi2, etc.) patents in the field of the doctoral thesis

Bn1. Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A.. Stirbescu R.M., Gorghiu L.M., *Metodă rapidă de izolare a microplasticelor din lapte, iaurt, smântână și unt*, Patent RO137927B1, 2025.

Bi1. Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A.. Stirbescu R.M., Gorghiu L.M., *Isolating microplastics from milk, yogurt, sour cream, and butter by pretreating samples with ultrapure reagents for digestion of complex organic matrix and homogenization by stirring at specific rpm, actual digestion by ultrasonically treating at specific temperature, and filtrating under vacuum,* Patent WO2025/071426 A1 (WIPO/PCT).

Articles in ISI-listed/indexed journals (S1, S2, etc.) in the field of the doctoral thesis

S1. **Banica A.L.,** Radulescu C., Buruleanu C.L., Olteanu R.L., Stirbescu R.M., Stanescu S.G., Dulama I.D., *Emerging Health Risks Associated with the Intake of Microplastics Found in Milk and Dairy Products*, Microplastics, **4**(4), 98, 2025. WOS: 001646978700001, FI = 5.1, AIS = 0.905, (Q1). https://doi.org/10.3390/microplastics4040098

S2. **Banica A.L.,** Radulescu C., Olteanu R.L., Dulama I.D., Stirbescu R.M., *Insight into the risks of milk consumption, potentially contaminated with heavy metals and polycyclic aromatic hydrocarbons*, Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry, **26**(3), 657-681, 2025. WOS: 001664805500004, FI = 0.648, AIS = 0.063, (Q4). https://doi.org/10.29081/ChIBA.2025.657

S3. **Banica A.L.,** Radulescu C., Dulama I.D., Bucurica I.A.. Stirbescu R.M., Stanescu S.G., *Microplastic Debris in Yogurt: Occurrence, Characterization, and Implications for Human Health*, Journal of Science and Arts, **24**(1), 223-248, 2024. WOS: 001241852900020, FI = 0.4, AIS = 0.069, (Q4). http://dx.doi.org/10.46939/J.Sci.Arts-24.1-b04

S4. **Banica A.L.,** Radulescu C., Stirbescu R.M., Dulama I.D., Bucurica I.A.. Stanescu S.G., Stirbescu N.M., *Microplastics Contamination of Dairy Products with High-Fat Content – Occurrence and Associated Risks*, U.P.B. Sci. Bull., Series B, **86**(4), 87-108, 2024. WOS: 001380943500007, FI = 0.3, AIS = 0.043, (Q4). https://www.scientificbulletin.upb.ro/rev_docs_arhiva/full28d_746984.pdf

Participation in international conferences (CFi1, CFi2) with papers in the field of the doctoral thesis

CFi1. **Banica A.L.**, Radulescu C., Dulama I.D., Bucurica I.A., Bucur (Popa) R.M., New extraction concept for identification and quantification of microplastics from milk and dairy products – *20th International Balkan Workshop on Applied Physics*, Constanta, July 12-15, 2022.

CFi2. **Banica A.L.**, Radulescu C., Dulama I.D., Bucurica I.A., Stirbescu R.M., Bucur (Popa) R.M., Novel method for the extraction and identification of microplastics in branded milk – *9th International Conference on Environmental Science and Technology – ICOEST*, Sarajevo, Bosnia and Herzegovina, May 17–21, 2023.

CFi3. **Banica A.L.**, Radulescu C., Dulama I.D., Bucurica I.A., Stirbescu R.M., Microplastics in Milk: Quantification and Health Risk Assessment – *2nd International Conference on Sensory Education and Food Analysis*, Targoviste, October 30th, 2023.

CFi4. **Banica A.L.**, Radulescu C., Dulama I.D., Bucurica I.A., Stirbescu R.M., Stanescu S.G., Microplastics in yogurt – Occurrence and characterization – *22nd International Balkan Workshop on Applied Physics*, Constanta, July 09-12, 2024.

CFi5. **Banica A.L.**, Radulescu C., Stirbescu R.M., Dulama I.D., Bucurica I.A., Stanescu S.G., Isolation and identification of microplastics in milk – a potential health risk for children – *XXXVII Edition National Conference of Chemistry*, Targoviste, September 25-27, 2024.

Participation in national conferences (CFn1, CFn2, etc.) with papers in the field of the doctoral thesis

CFn1. **Banica A.L.**, Radulescu C., Bucurica I.A., Dulama I.D., Bucur (Popa) R.M., Microplastics contamination in fresh milk: a rapid protocol for efficient recovery, *Conferința Națională de Chimie 2022 – CNCHIM 2022*, Calimanesti – Caciulata, October 04-07, 2022.

Participation in national conferences (CFi1, CFi2, etc.) with papers in fields related to the doctoral thesis

CFn1. Radulescu C., Dulama I.D., Bucurica I.A., Stanescu S.G., **Banica A.L.**, Assessment of tailing dumps' impact on water quality from abandoned mining areas based on health risk indices, *Conferința Națională de Chimie 2022 – CNCHIM 2022*, Calimanesti – Caciulata, October 04-07, 2022.

International awards (OPi1, OPi2, etc.) obtained, in terms of patents and/or articles published in the field of the doctoral thesis

OPi1. **Gold Medal - International Exhibition of Scientific Research, Innovation and Invention, ProInvent,** for patent application A/00528/2023/26.09.2023 - *Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter***,** Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Cluj–Napoca, Romania, October 25-27, 2023.

OPi2. **Diploma of Excellence - International Exhibition of Scientific Research, Innovation and Invention, ProInvent,** for patent application A/00528/2023/26.09.2023 - *Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Cluj–Napoca, Romania, October 25-27, 2023.

OPi3. **Diploma of Excellence awarded by the Justin Capră Association within the International Exhibition of Scientific Research, Innovation and Invention, ProInvent**, for patent application A/00528/2023/26.09.2023 - *Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Cluj Napoca, Romania, October 25–27, 2023.

OPi4. **Gold Medal - INVENTCOR International Exhibition,** for WO2025/071426 A1 (WIPO/PCT) - *Fast Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Deva, Romania, April 4-6, 2024.

OPi5. **Gold Medal - International Exhibition of Inventions and Innovative Entrepreneurship**, for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Chisinau, Moldova, May 16-17, 2024.

OPi6. **ICECHIM Award awarded by ICECHIM Bucharest during the exhibition EUROINVENT**, for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid method of isolating microplastics from milk, yogurt, sour cream, and butter*, Radulescu C., Dulama I.D., **Banica A.L.**, Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Iasi, Romania, June 6-8, 2024.

OPi7. **Certificate of Excellence awarded by Innovation & Technology Transfer Office UPT awarded during the salon EUROINVENT**, for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid Method of Isolating Microplastics from Milk, Yogurt, Sour Cream, and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Iasi, Romania, June 6-8, 2024.

OPi8. **Special Prize awarded by the Politehnica University of Timisoara during the salon EUROINVENT**, for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid Method of Isolating Microplastics from Milk, Yogurt, Sour Cream, and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Iasi, Romania, June 6-8, 2024.

OPi9. **Silver Medal - European Exhibition of Creativity and Innovation - EUROINVENT,** for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid method of isolating microplastics from milk, yogurt, sour cream, and butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Iasi, Romania, June 6-8, 2024.

OPi10. **Gold Medal - International Exhibition of Innovation and Technology Transfer Excellent IDEA 2024,** for patent application RO 138394 A0/50.05.2024 - *Rapid Screening Method of Imazalil and Thiabendazole Citrus*, Radulescu C., Olteanu R.L., Dulama I.D., Bucurica I.A., Galaon, T., Stirbescu R.M., **Banica A.L.,** Minea B.B., Chisinau, Moldova, September 20-22, 2024.

OPi11. **Gold Medal - International Exhibition of Innovation and Technology Transfer Excellent IDEA 2024,** for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Chisinau, Moldova, September 20-22, 2024.

OPi12. **Diploma of Excellence awarded by the Academy of Sciences of Moldova**, for patent application WO2025/071426 A1 (WIPO/PCT) - *Rapid Method for Isolation of Microplastics from Milk, Yogurt, Sour Cream and Butter*, Radulescu C., Dulama I.D., **Banica A.L.,** Bucurica I.A., Stirbescu R.M., Gorghiu L.M., Chisinau, Moldova, September 20-22, 2024.

OPi13. **Romanian Chemical Society Award** for the work *Isolation and identification of microplastics in milk – a potential health risk for children*, presented at the XXXVII Edition National Conference of Chemistry, Targoviste, September 25-27, 2024.

OPi14. **Special Award - International Exhibition INVENTCOR, 6th edition, offered by Politehnica University of Timisoara,** for the patent application, RO 138394 A0/50.05.2024 - *Rapid Screening Method of Imazalil and Thiabendazole from Citrus*, Radulescu C., Olteanu R.L., Dulama I.D., Bucurica I.A., Galaon, T., Stirbescu R.M., **Banica A.L.,** Minea B.B., Deva, Romania, April 3-5, 2025.

OPi15. **Special Award - International Exhibition INVENTCOR, 6th edition,** offered by Politehnica University of Timisoara, for the patent application RO 138394 A0/50.05.2024 - *Rapid Screening Method of Imazalil and Thiabendazole From Citrus*, Radulescu C., Olteanu R.L., Dulama I.D., Bucurica I.A., Galaon, T., Stirbescu R.M., **Banica A.L.,** Minea B.B., Deva, Romania, April 3-5, 2025.

OPi16. **Excellence Award - International Exhibition INVENTCOR, 6th edition,** offered by ICECHIM, for the patent application RO 138394 A0/50.05.2024 - *Rapid Screening Method of Imazalil and Thiabendazole from Citrus*, Radulescu C., Olteanu R.L., Dulama I.D., Bucurica I.A., Galaon, T., Stirbescu R.M., **Banica A.L.,** Minea B.B., April 3-5, 2025, Deva, Romania.

OPi17. **Certificate of Excellence - International Exhibition INVENTCOR, 6th edition,** offered by Research and Development Center Healthy Vibe – Holistic Treatment, for patent application RO 138394 A0/50.05.2024 - *Rapid Screening Method of Imazalil and Thiabendazole from Citrus*, Radulescu C., Olteanu R.L., Dulama I.D., Bucurica I.A., Galaon, T., Stirbescu R.M., **Banica A.L.,** Minea B.B., April 3-5, 2025, Deva, Romania.

OPi18. **Gold Medal - International Salon of Invention and Innovative Entrepreneurship,** for patent application RO 138394 A0/50.05.2024 - *Rapid Screening Method of Imazalil and Thiabendazole from Citrus*, Radulescu C., Olteanu R.L., Dulama I.D., Bucurica I.A., Galaon, T., Stirbescu R.M., **Banica A.L.,** Minea B.B., Chisinau, Republic of Moldova, June 6-7, 2025.

OPi19. **Gold Medal - International Salon of Invention and Innovative Entrepreneurship,** within the category Implementation of research results in practice, with paper *Polyphenolic Screening and the Antioxidant Activity of Grape Pomace Extracts of Romanian White and Red Grape Varieties* (Antioxidants, 13(9), 1133, 2024 IF=6.6, Q1), Chisinau, Republic of Moldova, June 6-7, 2025.

OPi20. **Gold Medal - International Salon of Invention and Innovative Entrepreneurship,** within the category Implementation of research results in practice, with paper *Heavy Metals and Associated Risks of Wild Edible Mushrooms Consumption: Transfer Factor, Carcinogenic Risk, and Health Risk Index* (Journal of Fungi, 10(12), 844, 2024, IF=4.2, Q1), Chisinau, Republic of Moldova, June 6-7, 2025.