IOSUD - "DUNĂREA DE JOS" UNIVERSITY OF GALAȚI

Doctoral School of Mechanical and Industrial Engineering

DOCTORAL THESIS

SUMMARY

Assessing the impact of industrial micropollutants on aquatic ecosystems in the Lower Danube area

Ph.D. student

Mădălina CĂLMUC

Scientific Supervisor,

Prof. Ph.D. habil. Cătălina ITICESCU

Series I4 Industrial Engineering No. 97

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Ph.D. Mădălina Călmuc

TABLE OF CONTENTS **Summary Thesis**

Keywords: industrial micropollutants; microplastics; aquatic ecosystems; Danube; negative impact; water quality.

INTRODUCTION

The development of human society is directly associated with the expansion and intensification of industry. This intensification generates an environmental impact that is considered essential for the quality of aquatic ecosystems and human life in general.

This doctoral thesis focuses on several important categories of micropollutants, such as: on the one hand heavy metals and nutrients, and on the other hand a category considered to be among pollutants for a relatively short time, namely microplastics.

Industrial activities, such as metal processing and smelting, mineral resource exploitation, chemical production, etc., are the main sources of heavy metal pollution (Yang et al., 2018), while the high level of nutrients in aquatic ecosystems is mainly due to the discharge of domestic wastewater and agricultural activities, through the excessive use of fertilizers (Grizzetti et al., 2021).

The manufacture of the first plastic materials of a synthetic origin represented a revolutionary discovery for mankind. Replacing the use of raw materials, such as wood, stone, or metal, with a new, versatile material, with applications in different industrial fields, was considered an alternative that could avoid the depletion of natural resource reserves. During World War II, the plastics industry in the United States experienced a significant expansion, with plastic production increasing by 300%. During the war, nylon was used to make parachutes, ropes, armor and helmet liners, and plexiglass for aircraft windows ("History and Future of Plastics", 2024).

Paradoxically, although over 100 years ago, plastic was considered a sustainable invention, which would have lessened the pressure on natural resources, today plastic waste represents an important source of environmental pollution. The lack of implementation of an effective plastic waste management system has determined that out of a total of 353 million tons/year of plastic waste produced worldwide, 6 million tons/year end up in rivers and coastal areas, and 1.7 million tons/year in the ocean (Ritchie et al., 2023). The COVID-19 pandemic has led to an increase in the need for plastic materials, both as medical items, such as protective equipment (goggles, masks, gloves, visors, etc.), and for single-use packaging. This increase in the consumption of plastics implicitly generated larger amounts of waste (Nugnes et al., 2022).

Once in the environment, plastic waste is exposed to different physical, chemical, biological, and mechanical factors, which favor its fragmentation. The resulting particles with sizes smaller than 5 mm (microplastics) are considered to be pollutants (Acarer Arat, 2024). Moreover, the presence of microplastics (MPs) in the environment is also influenced by other sources of industrial pollution, such as air blasting, the textile industry, the construction of buildings and vehicles, the presence of microparticles in personal care products, etc. (Kefer et al., 2021).

Because there are no legislative regulations regarding the presence of MPs in the environment, they are classified as emerging pollutants (Tang et al., 2019). Moreover, no standardized methodologies for the analysis of microplastics have been developed to date. One of the objectives of the European Commission is to reduce microplastic emissions by 30% by 2030 ("Microplastics - European Commission," 2024).

The toxic potential of MPs in aquatic ecosystems is generated by their capacity to accumulate and transfer into the upper level of the food chain. Also, due to their character as transport vectors for other contaminants, synergistic effects are created that potentiate the toxicity of microplastics (Rakib et al., 2023).

Based on the information presented previously, the need to study this class of industrial pollutants insufficiently addressed in the Lower Danube River area was identified. Thus, the present doctoral thesis entitled "Assessing the impact of industrial micropollutants on aquatic ecosystems in the Lower Danube area" aims to investigate the presence and influence of microplastics on the Romanian sector of the Danube River.

The main objectives of this doctoral thesis are as follows:

- Evaluation of the industrial activities impact carried out in the riparian areas of the Lower Danube sector, on the water quality from a physicochemical point of view;
- Estimation of microplastic concentrations present in the Danube water, at the river's entrance to the territory of Romania (Old Moldova), and before the formation of the three branches (Isaccea);
- Evaluation of microplastic presence in water and sediments collected from river, pre-deltaic, and deltaic aquatic ecosystems.
- Identification of areas with potential accumulation of microplastics in water and sediments;
- Morphological and compositional characterization of the identified microplastics to spot the main sources of MPs pollution;
- Estimation of the impact of MPs on the studied aquatic ecosystems by calculating ecological risk assessment indices (PLI, HI, and PERI);
- The use of Artificial Intelligence for the automated quantification, classification, and morphological characterization of MPs;
- Studying the behavior of MPs as a transport vector of other industrial pollutants.
- Identification and characterization of MPs in *Alosa immaculata* (Danube shad) fish species.
- The development of national and international databases regarding the occurrence of microplastics in the Danube River.

The novelty degree of this doctoral thesis is highlighted by the integrated approach of some first-of-its-kind studies, which evaluated the presence and impact of microplastics on the Lower Danube sector, among which are listed:

- Study of the spatial distribution of microplastics (MPs) in water and sediment in the predeltaic and deltaic sector of the Danube River along a length of approximately 370 km;
- Investigating the presence of MPs in the largest area of the Lower Danube River studied so far in the specialized literature;
- Development of innovative equipment for sampling MPs from freshwater aquatic ecosystems (OSIM patent No. 134991);
- Evaluation of the vertical distribution of MPs in the water column of the Lower Danube River;
- Quantification of the amount of MPs in the water at the entrance of the Danube on the Romania territory and before the division into the three main branches;
- Evaluation of the seasonal variation of MPs in the Lower Danube River water;
- Identification of MPs in migratory fish species from the Danube River (*Alosa immaculata*);
- The use of Artificial Intelligence techniques for the development of imaging models for the characterization and quantification of MP particles;

- Evaluation of the ecological risk generated by the presence of microplastics in the predeltaic and deltaic sectors of the Danube River.

This doctoral thesis is structured in 4 chapters, the introduction and the bibliography.

The introduction includes information about the motivation for choosing the research topic, the main objectives, and the originality elements of the doctoral thesis.

In **the first chapter**, information was presented regarding the current state of research, and general aspects regarding microplastics (sources, behavior, transport, analysis methods, etc.). The negative impact on aquatic ecosystems was also discussed.

Chapter 2 includes information related to the description of the study areas, the presentation of the analytical methods, and the equipment used to carry out the experimental studies of the research. In addition, the methodology based on deep-learning algorithms for automating the process of quantification, classification, and characterization of MPs is presented.

Chapter 3 is the largest, being structured into 5 subchapters that include the results and discussions of all the studies addressed in this doctoral thesis. This chapter presents the results of a preliminary study in which the quality of Danube River water was evaluated from a physical-chemical point of view, using quality indices, to track the impact of industrial activities. Also, the distribution of MPs in two areas of interest of the Danube, strategically chosen to estimate the amount of MPs in the water, at the entrance of the Danube in Romania and before the formation of the three branches, is discussed. Also in this chapter, the presence of microplastics is studied in the predeltaic and deltaic sectors of the Danube, both in the water and sediment matrix. Microplastics were compositionally and morphologically characterized to identify the main sources of pollution. Subchapter 3.4 presents the results obtained by training and applying three Artificial Intelligence models, with the help of which the quantification, morphological classification, and automated dimensional characterization of microplastics can be carried out. In sub-chapter 3.5, the research related to the evaluation of the impact of MPs on aquatic ecosystems is presented, the Pollution Load Index (PLI), the Hazard Index (HI), and the Potential Ecological Risk Index (PERI) being calculated. Also for this purpose, the presence of MPs in the fish *Alosa immaculata* (Danube shad) was studied. Moreover, the presence of other industrial pollutants on the surface of MPs was analyzed, confirming their character as a transport vector of other contaminants.

In **Chapter 4**, the final conclusions and personal contributions obtained as a result of the research carried out in this doctoral thesis are presented. Future research directions are also listed, which will be addressed to elucidate certain topics and expand the study area.

CHAPTER 1. Microplastics in aquatic ecosystems. General aspects. State of the art

Plastics are considered the most widely used and versatile materials, with global production increasing significantly to meet market demands (Andrady and Neal, 2009). In 1950, the global annual production of plastics amounted to 1.5 million tons. However, despite the decline in production during the oil crisis of 1973 as well as the economic crisis of 2007, by 2009 global plastics production had increased substantially to 250 million tons (Mt). Historically, global plastics production has increased by about 9% each year. Moreover, by 2014, the global production rate reached 311 Mt per year, representing the global annual production growth of about 25% in just 5 years (Crawford and Quinn, 2017). According to the Plastics - the Facts 2023 report, the global annual production of plastics has increased in recent years from 335 Mt in 2016 to 400.3 Mt in 2022 (Plastics Europe, 2023). According to predictions, it is anticipated that, by 2050, about 33 billion tons of plastic will still be produced globally (Cho et al., 2024).

The fragments of plastics found in the environment can be classified according to size, as follows: macroplastics (>100 mm), mesoplastics (20 mm – 5 mm), microplastics (<5 mm) and nanoplastics (< 0.1 μm). Therefore, microplastics (MPs) are considered to be plastic fragments smaller than 5 mm in size (Mai et al., 2018; Qiu et al., 2016; Rezania et al., 2018).

Microplastics (MPs) present in the environment represent a heterogeneous group of particles different in size, density, shape and chemical composition from different sources. Depending on the nature of the pollution sources, microplastics are classified into **primary and secondary MPs**.

Primary MPs They are plastic particles manufactured with microscopic dimensions to be used in household items, in the pharmaceutical industry as a vector for medicines, in personal hygiene products such as facial cleansers, toothpaste, exfoliating creams, etc. After using the products, the MPs present in their composition are frequently eliminated and can reach the environment by discharging industrial and domestic wastewater, after the purification process, into the outfall. Another important source of primary MPs is the raw materials used in the manufacture of plastic products. Accidental losses, improper handling, leaks from processing facilities, and residues from plastics manufacturing can accumulate in the environment (Rocha-Santos and Duarte, 2017). Also, the use of air blasting technology is a source of primary MPs. The process of sandblasting machines, engines, and boats to remove rust and paint involves the use of a scrubber with acrylic, melamine or polyester microplastics (Cole et al., 2011).

Secondary MPs are the result of the degradation of larger plastic particles already present in the environment, under the influence of UV radiation, microorganisms and mechanical factors. Long-term exposure to sunlight (especially UV radiation) causes oxidation of the polymer matrix, leading to bond breakage. Also, plastic debris is susceptible to fragmentation by combining mechanical forces, such as the action of waves and turbulence. The longevity of many plastics is still uncertain and many can remain in the medium for a long period of time, starting from months to centuries (Li, 2018).

CHAPTER 2. Materials and methods

This chapter describes the methods and working techniques applied that led to obtaining the data on which the studies integrated in this doctoral thesis were based. The sampling stations, selected for the spatial distribution of MPs in the Danube water, cover the longest length (370 km) studied so far in Romania in terms of microplastic assessment. The chosen area includes the Romanian sector of the Danube.

Two important sectors have been studied:

A. In order to study the quality of the aquatic ecosystem, the Lower Danube sector was chosen, starting with the city of Galati (S1 – Danube – Siret confluence) and ending with the three branches of the Danube flowing into the Black Sea. Also, the studied area includes the pre-deltaic and deltaic area of one of the most important nature reserves in the world, namely the Danube Delta Biosphere Reserve. The largest area investigated covers two of the Natura 2000 sites in Romania, namely Site of Community Importance (ROSCI0065) and Special Protection Site for Birds (ROSPA0031) (www.natura2000.eea.europa.eu). Figure 2.1 illustrates the location map of the 11 MPs sampling stations. The sampling campaigns were carried out between May 2021 and June 2022.

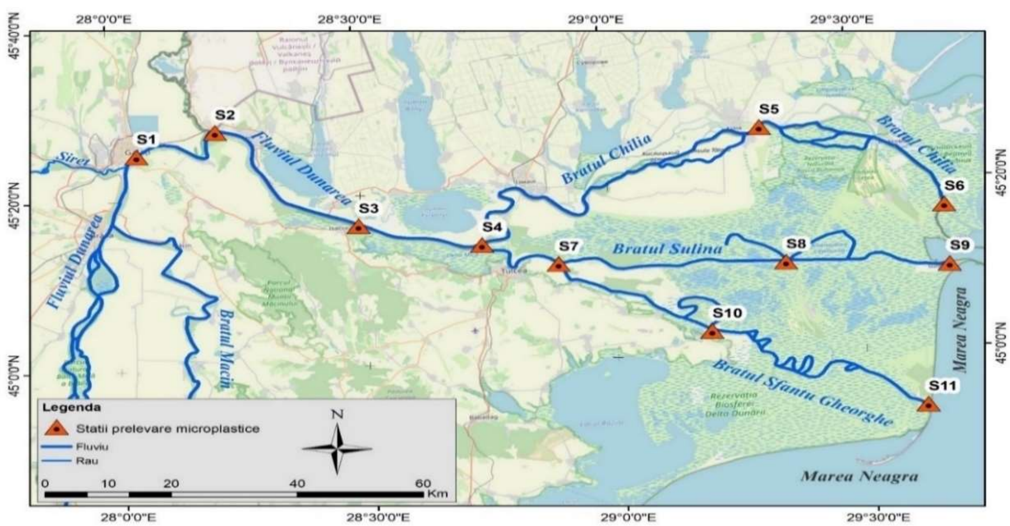

Figure 2.1 Presentation of the study area and location of sampling stations

B. To estimate the amount of MPs in the Danube water at the entrance to Romania, the first sampling station was selected near Moldova Veche (Caraș-Severin City). The second sampling station was established near Isaccea locality (Tulcea City), which is located after the last tributary of the Danube (Prut River) before the formation of the three branches (Figure 2.2). In front of each locality, the samples were taken from three points distributed in the cross-section, as follows: left bank proximity, navigable fairway proximity and right bank proximity. To assess the seasonal variation of MPs, samples were taken every season between spring and winter 2022. Table 2.2 describes the locations and periods in which the samples were taken.

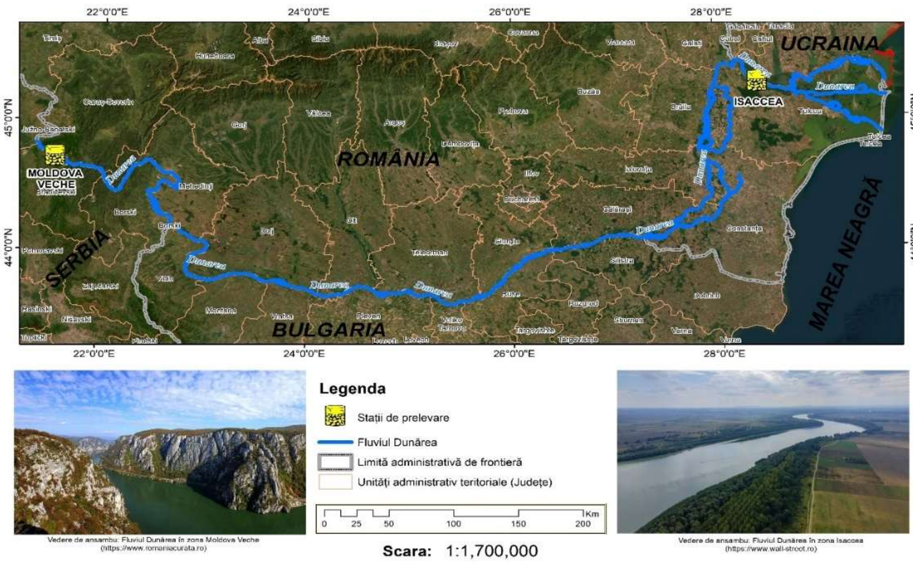

Figure 2.2 Location of sampling stations

Table 2.1. Description of sampling stations and periods

Sampling point code	Sampling point description	Sampling period
P/V/T/I M1.1	Romanian shore - *Moldova* Veche, surface	P – spring (May 2022) V – summer (July 2022) T – autumn (October 2022) I – winter (December 2022)
P/V/T/I M1.3	Romanian shore - Moldova Veche, depth	
P/V/T/I M2.1	Navigable fairway - Moldova Veche, surface	
P/V/T/I M2.3	Navigable fairway - Moldova Veche, depth	
P/V/T/I M3.1	Serbian shore - Old Moldova, surface	
P/V/T/I M3.3	Serbian shore - Old Moldova, depth	
P/V/T/I Is1.1	Romanian shore - Isaccea, surface	P – spring (May 2022) V – summer (July 2022) T – autumn (September 2022) I – winter (November 2022)
P/V/T/I Is1.3	Romanian shore - Isaccea, depth	
P/V/T/I Is2.1	Navigable fairway – Isaccea, surface	
P/V/T/I Is2.3	Navigable fairway – Isaccea, depth	
P/V/T/I Is3.1	Ukrainian shore - Moldova Veche, surface	
P/V/T/I Is3.3	Ukrainian shore - Moldova Veche, depth	

2.1 Determination of the physicochemical parameters of water

This subchapter describes the sampling and determination methods of physicochemical parameters to assess the quality of aquatic ecosystems.

The water samples were taken from the upper layer of the water column (the first 10 cm) using a water sampler with a telescopic rod. The samples were transferred to polyethylene (PE) containers with a volume of 500 mL, previously decontaminated in the laboratory with ultrapure water, and later, in the field, with the sample. In the next stage, the samples were transferred, preserved at 4 ºC and analyzed within 24 hours of sampling, within the European Center of Excellence for the Environment (ECEE) of the "Dunarea de Jos" University of Galati.14 parameters were analyzed, 2 in-situ (pH, DO - dissolved oxygen) and 12 ex-situ (BOD_5 - biochemical oxygen demand, COD-Cr - chemical oxygen demand, TN - total nitrogen, TP - total phosphorus , Cr-total - total chrome, $N\text{-}NH_4^+$ - ammonium nitrogen, $N\text{-}NO_2^-$ - nitrite nitrogen, N-NO_3^- - nitrate nitrogen, SO_4^{2-} - ion sulphate , Cl^- - ion chloride, Fe-total - total iron,Zn^{2+} - Zinc) using electrochemical and spectrophotometric methods.

2.2 Sampling

2.2.1 Sampling microplastics from water using pump equipment

For the quantitative determination of the MPs from the surface layer (10 cm) of the water, but also from the depth of 7 m, a pump-type sampling equipment made within the European Center of Excellence for the Environment of the "Dunărea de Jos" University of Galati was used.

- The sampling system used for this study has the following configuration:
- Gasoline water pump with a flow rate of 5 liters/second;
- Absorption hose with a length of 10 m that allows the sampling of MPs from a depth of 7 m;
- Discharge hose with a length of 5 m;
- Mesh with a diameter of 30 cm and an eye size of 125µm.

2.2.2 Sampling microplastics from water using mesh equipment

The samples were taken with the help of equipment built within the Faculty of Engineering of the "Dunărea de Jos" University of Galati. The system is composed of 3 nets, 2 located at the top and one at the bottom. Each net has a container with a diameter of 10 cm for collecting samples at the end. To quantify the volume of water, hydrometric mills mounted on each mesh were used. For this study, the meshes of the net were 250 µm, avoiding immediate clogging of the meshes, given that the goal was to filter as much water as possible.

2.2.3 Sediment sampling

The sediment sampling from the upper layer (the first 10 cm) was carried out using a Van Veen dredger. The collected sample (approximately 750 g) was stored in a container with a volume of 1 L and stored at a temperature of 4 °C.

2.3 Isolation of microplastics

2.3.1 Isolation of microplastics from water

In the present study, for the digestion of the samples, the method described by Pojar et al., 2021a, using a 1:1 mixture of 10 M KOH and H_2O_2 solution, 30%. The samples were left to shake for 5 days, and then neutralized using formic acid. During stirring, the sample containers were covered with watch bottles to prevent contamination with microplastics from the air. After the removal of the organic matter, the stage of separation of the MPs from the rest of the suspensions followed.

For the separation of MPs from denser suspensions, the most used method is the one based on the modification of the sample density, thus using a saturated salt solution favors the floating of MPs with lower density on the solution supernatant and the separation from denser particles. The saline solution used in the present study is $ZnCl_2$, 60% solution, which is considered the most effective method. The last step in separating the collected plastics consisted of transferring the samples to separation funnels with a volume of 500 mL, the collected supernatant being filtered by a vacuum pump, on a glass fiber filter paper with a pore size of 2 µm, diameter 47 mm.

2.3.2 Isolation of microplastics from sediment

The method of separating microplastics from sediment was adapted after the study published by Pojar et al., 2021c. The sediment samples taken were transported to the laboratory and dried in the oven at a temperature of 60°C for 24 h (approximately 500 g of sediment). After drying, the sample was visually analyzed and coarse materials such as vegetation, gravel, macroinvertebrates were removed. Then, 250 g of dry sediment was weighed into a 1 L beaker, to which 800 mL of 60% $ZnCl_2$ solution was added in a 1:4 ratio. The solution was mixed for 15 minutes and left to settle for another 15 minutes, until the mixture cleared so that the supernatant could be collected. 30 mL of 30% H_2O_2 was added to the collected supernatant and the sample was left to digest for 7 days. After digestion, the sample was transferred to the filter funnel (500 mL), the supernatant being filtered on a glass fiber filter paper, with a pore size of 2 µm and a diameter of 47 mm.

2.3.3 Isolation of microplastics from ichthyofauna

In the present study, 5 individuals of the *Alosa immaculata* were captured from 4 sampling stations on the Danube. The length of the analyzed specimens varied in the range 28.2 - 32.6 cm, and the weight in the range 204.26 - 313.38 g. Following dissection, the following gills and gastrointestinal system were removed from the *Alosa immaculata.* The harvested organs were transferred to Petri dishes to be subjected to digestion. The method applied in this study is alkaline digestion with 10% KOH (3x tissue volume), 12 h at room temperature and another 12 h at 60°C. After digestion, the sample was neutralized with 1M citric acid (volume equal to KOH), each sample being then filtered on quantitative filter paper (Rochman et al., 2015).

2.4 **Identification and quantification of microplastics**

2.4.1 ATR-FTIR spectroscopy

In the present doctoral thesis, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to identify the type of polymers in the composition of the sampled plastic fragments. The plastic fragments easily visible to the naked eye were analyzed individually using the Spectrum 3 Laboratory FTIR Spectrometer, manufacturer Perkin Elmer, configured with ATR, which features Diamond/ZnSe crystal. The FTIR spectra obtained were compared with the reference spectra from the S.T. Japan polymer spectrum database.

2.4.2 ***FTIR Microscopy***

The Micro-FTIR equipment used in this doctoral thesis, model Spotlight 400, manufacturer Perkin Elmer, located in the REXDAN Research Infrastructure, within the "Dunarea de Jos" University of Galati, is capable of automatically analyzing both a single particle, but also a sample with multiple particles located on a filter. The micro-FTIR is also equipped with a camera in the visible range to allow the operator to see the samples they are working with and to configure their positions for performing the analysis. The equipment provides information such as: the size of MPs and the type of component polymer and, at the same time, it can map an area selected by the user, and at each point an IR spectrum is collected, thus generating an IR map of the selected sample.

2.4.3 Scanning electron microscopy (SEM) technique coupled with energy-dispersive X-ray spectroscopy

To analyze the morphology in depth (at the micron level) of the micron particles, scanning electron microscopy (SEM) was used. SEM technology combined with EDX (energy dispersive X-ray spectroscopy) was applied to perform elemental particle analysis. The model of the equipment used is *Tescan Vega*, manufacturer, *Tescan*, located in the endowment of the Center for Interdisciplinary Research in the Field of Eco-nano Technology and Innovative Materials (CC-ITI) within the Faculty of Engineering, "Dunărea de Jos" University of Galati.

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CHAPTER 3. Results and discussions

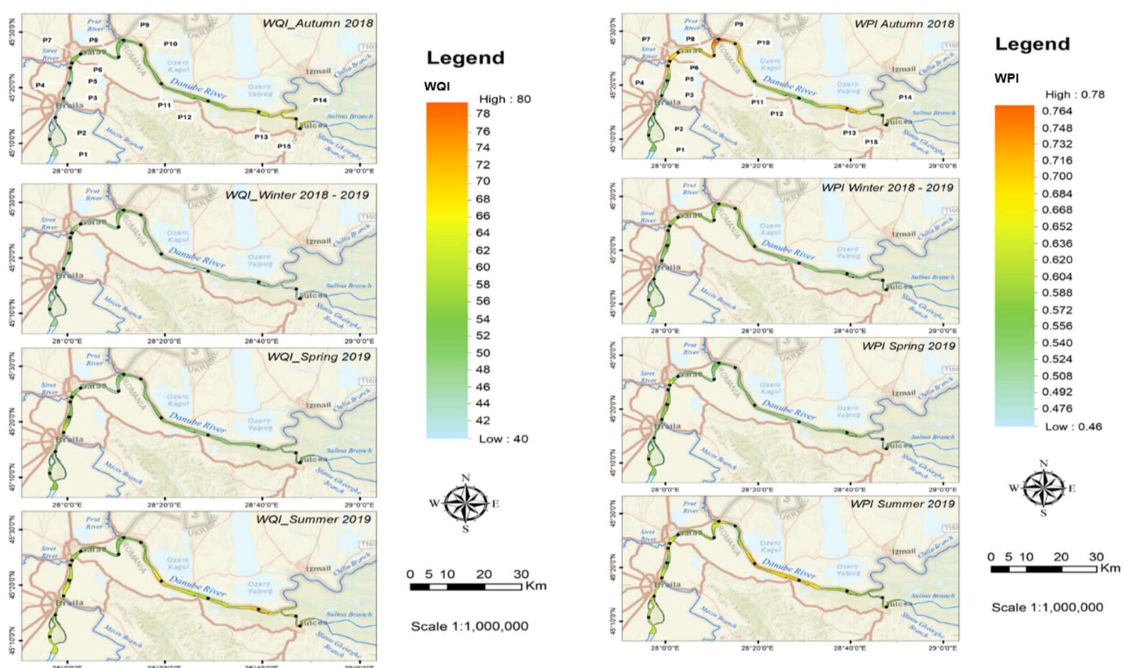
3.1 Assessing Danube water quality using quality indices

The first studies on the quality of the Danube's water were carried out on the riparian sector of the industrial cities of Brăila, Galați and Tulcea. In the study conducted by (Calmuc et al., 2020), of which I am the main author, Water quality status was assessed using the following quality indices: Water Quality Index (WQI), Water Pollution Index (WPI), Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI). Water samples were taken during the four seasons between autumn 2018 and summer 2019 from 15 stations located on a sector with a length of 120 km.The first station is located upstream from the city of Brăila (P1), and the last one downstream from Tulcea (P15). The sampling stations (Fig. 3.1) have been established to be representative, being located in areas exposed to different sources of pollution (P2, P3, P6, P9, P10, P13, P14, P15), but also in areas where the anthropogenic influence is minimal (P4, P5, P8). The main sources of pollution in the monitored sector are agricultural, industrial and domestic activities (e.g. wastewater treatment plants) (Gasparotti, 2014).

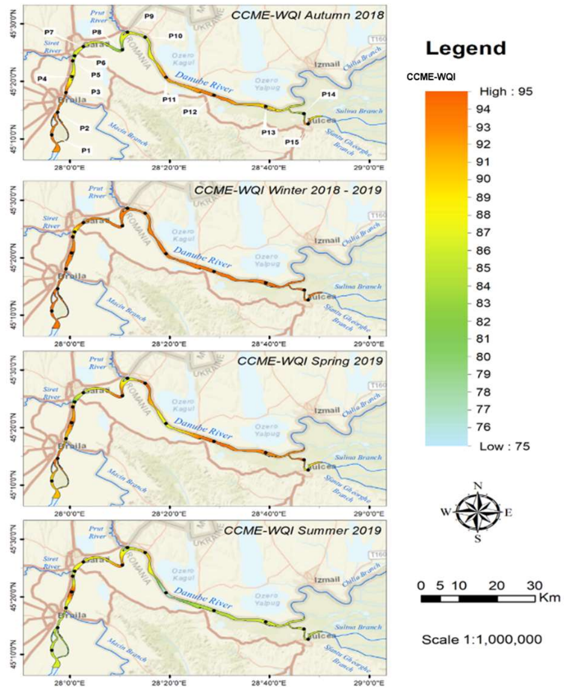
Figure 3.1. Sampling station location map (Calmuc et al., 2020)

3.1.1 WQI, WPI and CCME-WQI Index Results

The WQI index has been applied to assess the quality of the Danube water by a team of researchers from the "Dunarea de Jos" University of Galati, starting with 2013 (Iticescu et al., 2013). In this study, WQI index values ranged from 40.7 to 72.5 (Figure 3.2 - a), with these results classifying water quality into classes II and III. The maximum value of the WQI index was recorded in the P14 station (Tulcea) in the autumn season. This result may be due to the industrial activity (shipyard) carried out in the vicinity of this location. Values above 50, corresponding to quality class III, were recorded at stations P6 and P9 in all 4 seasons. These sampling stations are located at the confluence of the Danube with its main tributaries, Siret (P6) and Prut (P9), rivers with a significant contribution of pollutants, especially nutrients, which come from fertilizers used in agricultural activities. In addition, the Siret River is the emissary into which treated water from the domestic and industrial wastewater of the city of Galati is discharged. The results of the WPI index, illustrated in Fig. 3.2-b, also highlight the contribution of the two tributaries to the water quality of the Danube, with a value of 0.78 being recorded in station P9 in the autumn season. Moreover, during the spring, the highest value was obtained in the P6 station, when the N-NH_4^+ parameters and N-NO_3^- exceeded the maximum values regulated by Order 161/2006 for the second class of quality. However, according to the WPI results, the water in these stations was classified in quality class II, while the values of the index WQI have classified water quality in class III. High values were also recorded in the P10, P11, P12, and P13 locations in the summer and autumn seasons, when the BOD_5 parameters, DO, COD-Cr, N-NO_3^- and N-NO_2^- exceeded the maximum allowed concentrations (MAC). These sampling stations are located near areas with intense agricultural activity and are also the main source of pollution. Although WPI values varied both spatially and temporally (0.48 - 0.78), water was classified exclusively in quality class II ("Water clean"). Similar to the WPI index, the results of the CCME-WQI index classified the water quality in class II ("Good condition"), with one exception, namely the value recorded in the P11 station in summer (76.54), which corresponds to quality class III ("Average condition"). This particular case may be due to the 5 of the 14 parameters analyzed (BOD_5, DO, COD-Cr, N-NO_3^- and N-NO_2^-), which exceeded the maximum permissible concentrations. Although the exceedances recorded were not significant, this result was influenced by the *F1—Purpose*, which indicates the percentage of variables that have not met their targets. As in the case of the WQI and WPI indices, the spatial distribution of the CCME-WQI values (Figure 3.2-c), reinforces the claim that the two tributaries negatively influence the water quality of the Danube) (Calmuc et al., 2020).



(a) (b)



(c)

Figure 3.2 Spatial and temporal variation of the WQI(a), WPI(b) and CCME-WQI(c) results (Calmuc et al., 2020)

3.1.2 Comparative approach to the results of the WQI, WPI, CCME-WQI indices

The percentage weight of each parameter against the final value of the WQI index was established based on the value of the sub-index *Wiqi* calculation equation. The WQI index is generally influenced by parameters whose maximum permissible concentration is low (e.g. heavy metals or $N\text{-}NO_2^-$). This is also the main advantage of using the WQI index, i.e. different weighting of parameters depending on the level of toxicity and impact on the aquatic environment. Therefore, this index is appropriate to apply in the assessment of water quality, in particular from the point of view of determining the level of pollutants with toxic potential. In the case of the WPI index, it has been observed that the weight of the parameters varies from one season to another. During the summer and autumn, the BOD_5 and DO contribute the largest share to the final value of the index. This is due the two parameters significantly exceeded the maximum allowed value in the two seasons. Moreover, from the analysis of the spatial distribution of the weights, it can be seen that there are changes in the parameters that exceeded the MAC. Although this index does not show sensitivity to a specific class of pollutants, compared to the WQI, the disadvantage of using it is that the calculation formula assigns the variables the same weight. Thus, there is no differentiation of pollutants according to the ecological impact on the aquatic ecosystem. For example, the effects on ecosystems caused by the presence of nitrogen in water in high concentrations are completely different compared to those caused by the same concentration of Cr - total. Therefore, this index can rather be used to establish the pollution level of the studied watercourse, the results highlighting whether or not exceedances of the MAC were recorded) (Calmuc et al., 2020).

Compared to WQI and WPI, the algorithm used to calculate the CCME-WQI does not integrate any sub-indices. This algorithm is based on the incorporation of 3 factors (*F1, F2, F3*) that describe the purpose, frequency, and amplitude of the parameters, for all those who exceed the CMA. Unlike the other two indices calculated in the present study, the CCME-WQI measures via *F2* how many times a parameter exceeded the MAC in a season (frequency). The maximum *F2 values* (i.e. 14.29 and 17.86) can be explained by the fact that parameters such as BOD_5, DO, and $N\text{-}NO_3^-$ recorded values above the MAC in a station at least twice in the same season. Thus, this index is suitable for assessing water quality in areas where there are permanent sources of pollution.

3.1.1 Partial conclusions

Based on the results presented in this study, the following conclusions can be drawn:

- The water quality in the study area was found to be lower in the sampling stations located near the areas where agricultural and industrial activities are carried out (metallurgical industry, shipbuilding industry, etc.).
- The spatial distribution of the values of the three indices highlighted the fact that the main tributaries of the Danube (Siret and Prut rivers) negatively influence the water quality of the river through the contribution of pollutants they carry.
- As for the seasonal variation in water quality, a lower quality was observed during summer and autumn, this being due to variations in the flow of the Danube and temperature-dependent quality parameters.
- The quality of the Danube water was evaluated differently by the three indices, the results being influenced by the weight of the parameters taken into account. According to the WQI index, 53% of sampling stations were classified in quality class III, while 47% were classified in class II. Compared to WQI, the results obtained by using WPI classified water strictly in quality class II. Roughly similar to the WPI, CCME-WQI has classified the analyzed water in 98% of all sampling stations in quality class II.
- The choice of a quality index should be made according to the complexity of the ecosystem, the type of pollution source and the purpose of the monitoring activity.

3.2 Study on the distribution of micro- and macro-plastics on the Romanian sector of the Danube

The results presented in this chapter contributed to an extensive study conducted by the "Dunărea de Jos" University of Galati in collaboration with the Global Water Partnership Association, Romania (Beneficiary) within the research contract no. 787/29.03.2022, with the title "*Qualitative and quantitative analysis of micro-plastics from solid samples (resulting from the collection of suspended solid particles) taken from flowing natural waters (Danube)"*. This study was the basis for the report entitled "The presence of plastics in the waters of the Danube on the territory of Romania" published in 2023, which had as its main objective the estimation of the annual transport of microplastics in the water of the Danube, on the territory of Romania. According to the results presented, on the Romanian sector of the Danube, between 46-51 tons/year of microplastics and between 47-49 tons/year of macroplastics are transported annually ("Studiu-microplastic_Asociatia-MaiMultVerde_2023.pdf"). Also, the results were accepted for publication in the journal Environmental Sciences Europe, SpringerOpen, the title of the paper being "*The First Spatio-Temporal Study of the Microplastics and Meso-Macroplastics Transport in the Romanian Danube".*

The personal contribution to this study was to develop the microplastics analysis procedure (microplastics separation and analysis using ATR-FTIR and micro-FTIR techniques) and to analyze 48 of the samples collected from Moldova Veche and Isaccea. Henceforth, the results regarding the spatial and seasonal variation of the concentrations of micro and macroplastics present in the 48 samples collected in the two sampling areas (Moldova Veche and Isaccea) will be presented and discussed.

3.2.1 Concentration of microplastics collected from the Danube River at Moldova Veche and Isaccea

In Fig. 3.3 (a, b, c, d) the concentrations of microplastics collected in the 4 seasons in the Moldova Veche area are graphically represented. Microplastics were collected from the surface of the water as well as from the depths of each point. Also, for each season investigated, the graphs contain information regarding the flow of the Danube recorded on the day when the sampling took place.

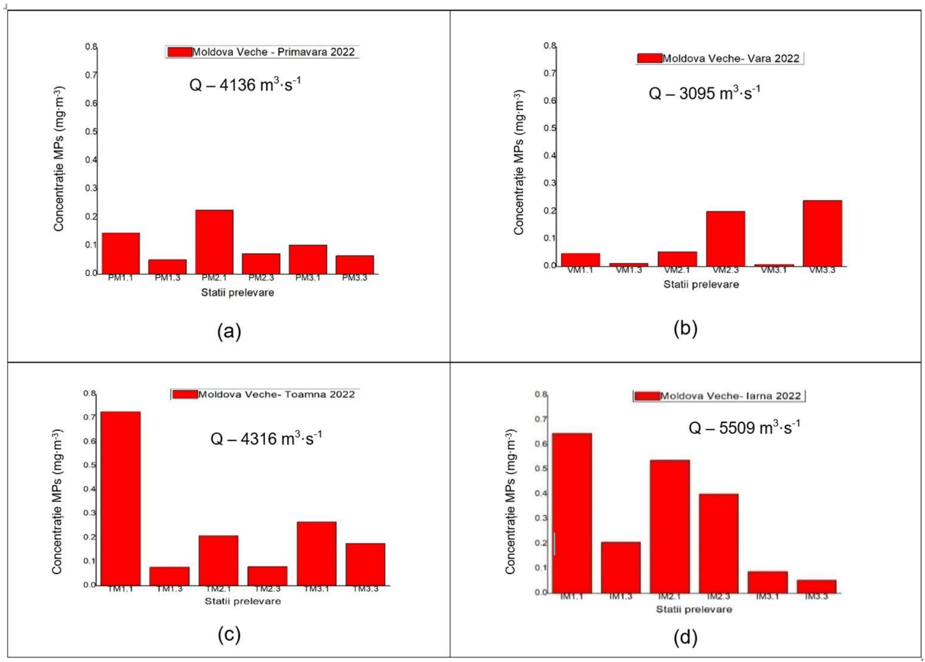


Figure 3.3 Concentration of microplastics collected from the Moldova Veche area in the 4 seasons (a – spring, b – summer, c – autumn, d – winter)

Regarding the evaluation of the seasonal variation of MPs in the Moldova Veche station, it is observed that, along with the increase in the flow of the Danube, there were also increases in the concentration of MPs. Thus, maximum values were identified in the station in M1.1 (0.726 $mg \cdot m^{-3}$ and 0.645 $mg \cdot m^{-3}$), in the autumn and winter seasons, when the flow recorded a value of 4336 $m^3 \cdot s^{-1}$, respectively 5509 $m^3 \cdot s^{-1}$. It would be expected that there would be an inversely proportional dependence of the concentration of a pollutant as a function of the flow, a higher level of the flow, favoring the dilution of the concentration. The directly proportional relationship between the flow and the concentration of MPs can be explained by the fact that, in the rainy season, when the flow of the Danube increases, the plastics (both macro and micro), accidentally located on the river bank, are carried into the water, which produces an increase in the quantity and concentration of MPs in the water.

Analyzing the distribution of MPs in the cross-section of the Danube, it can be seen that the largest quantities were reported in the vicinity of the Romanian shore. The collection of MPs on the shore is due to the flow currents, which are slower, but also to the low flow speeds. The presence of MPs in higher concentrations near the banks is also due to plastic waste that is abandoned on the shore and then ends up in the water.

Regarding the distribution of MPs in the vertical water column, it can be seen that the high values were recorded mostly in the surface layer, except for the VM3.3 station, in the summer season, when higher values were observed at depth (0.239 mg·m^{-3}) than in the surface layer (0.007 mg·m^{-3}). The entrainment of MPs vertically is generally due to currents, but also to the fixation on their surface of other materials (sediment, microorganisms, pollutants, etc.), which determine the increase in density, implicitly their transport to the depths (Zhou et al., 2021).

The concentrations of MPs recorded at Isaccea in the 4 seasons in which the samples were taken are graphically represented in Fig. 3.4 (a, b, c, d). Similar to the results obtained at Moldova Veche, the highest concentrations were associated with hydrological conditions with higher flows. Therefore, the highest concentration was observed in the PIs2.1 station (0.140 mg·m^{-3}) in the spring season, when the average flow recorded the highest value (5940 m^3·s^{-1}). Moreover, in station IIs 1.1, a higher concentration was determined in winter than in the other seasons (0.098 mg·m^{-3}), at a flow rate of 5360 mg·s^{-1}.

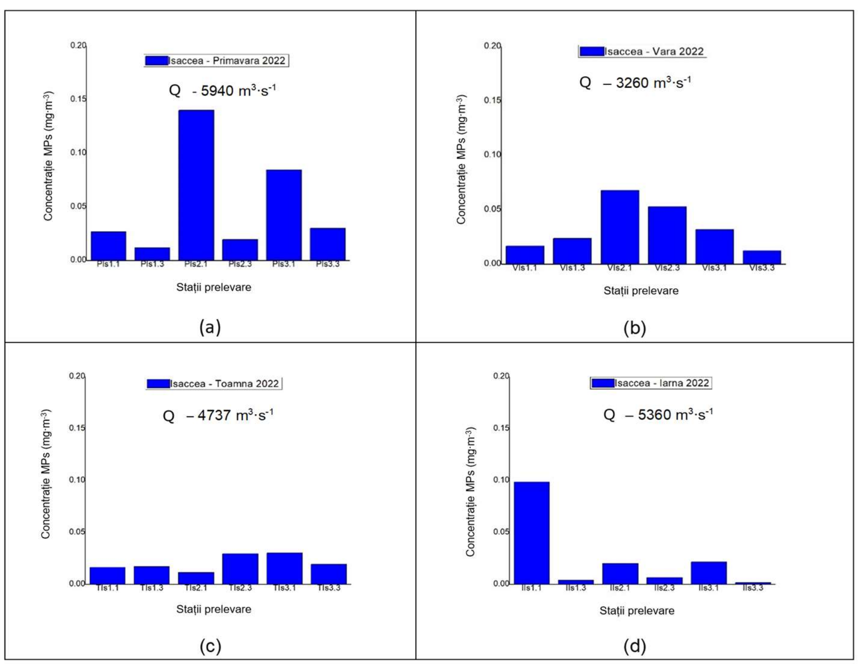


Figure 3.4 The concentration of microplastics collected from the Isaccea area in the 4 seasons (a – spring, b – summer, c – autumn, d – winter)

This time, the highest concentrations were observed near the navigable fairway (P/V Is2.1, but also near the Romanian shore of Isaccea (IIs1.1). As for the distribution of MPs in the vertical water column at Isaccea, most of the MPs were collected from the surface, except for stations TIs1.3, TIs2.3, Vis1.3 where larger quantities were quantified at a depth of 3-3.6 m. In front of Isaccea, the river traffic is much more intense, given the fact that there is a ferry crossing station that connects Romania and Ukraine, and, therefore, the hydrodynamic conditions of the water undergo changes.

Comparing the average of the concentrations of MPs recorded in the two sampling stations, it was observed that at Moldova Veche concentrations were recorded approximately 6 times higher than at Isaccea.tag. The results obtained highlight the fact that at the entrance to Romania, the Danube has a much higher concentration of MPs than in the sector near the mouths of the Danube arms into the Black Sea.

3.2.2 Concentration of macroplastics collected from the Danube River to Moldova Veche and Isaccea

Along with the sampling of microplastics, a significant amount of meso- and macroplastics was also collected. In the present doctoral thesis, meso- and macroplastics (MaPs) were quantified, considering the fact that they represent a secondary source of MPs. From Figures 3.5 (a, b, c, d) it can be noted that the highest concentration (1.92 mg·m^{-3}) of MaPs was observed from the IM1.1 station in the winter season, when the flow of the Danube registered the highest value. Analyzing the vertical distribution, most MaPs were collected from the upper layer, except for the P/V/IM3.3 and VM2.3 stations, where larger amounts were recorded at depth.

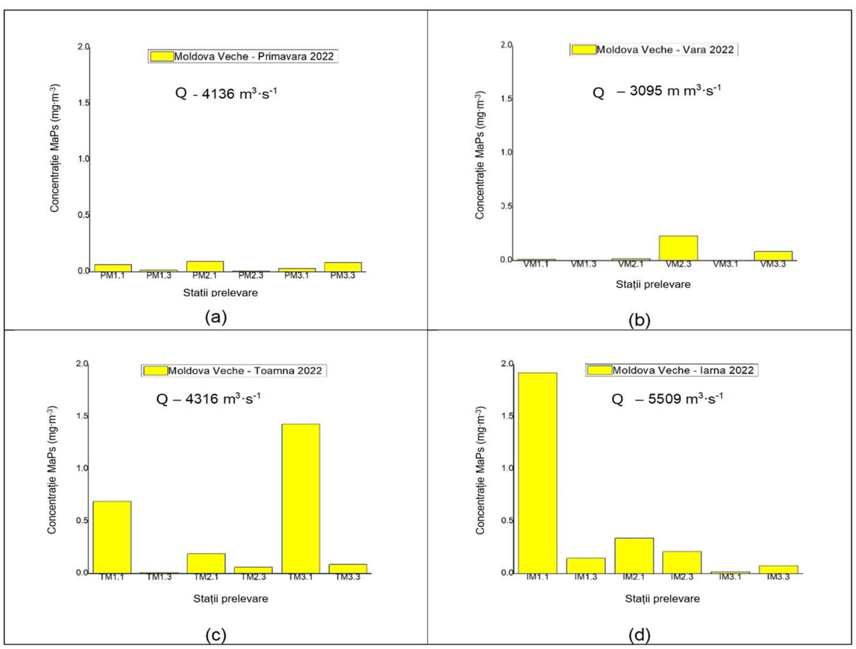


Figure 3.5 Concentration of macroplastics collected from the Moldova Veche area in the 4 seasons (a – spring, b – summer, c – autumn, d – winter)

Regarding the presence of MaPs in the Danube cross-section, it was observed that the highest concentrations were taken from the proximity of the banks. In these areas there are micro-dams due to boats, pontoons and other devices found on the banks. Also, the speed of water flow decreases towards the banks.

In Fig. 3.6 (a, b, c, d) the concentrations of MaPs collected from the Isaccea sampling station are graphically represented. The highest concentration of MaPs was observed in station VIs2.1, where the highest amount was recorded (Fig. 3.10), due to the presence of a plastic fragment with a mass of 14.36g. Except for the anomaly in station VIs2.1, the highest concentration was observed in station IIs3.1 (0.091mg·m^{-3}), when the flow recorded the value 5360 m^3·s^{-1}. Also, the highest amount of MaPs was observed in the surface layer, except for stations TIs2.3 and TIs3.3, where higher concentrations were recorded at depth.

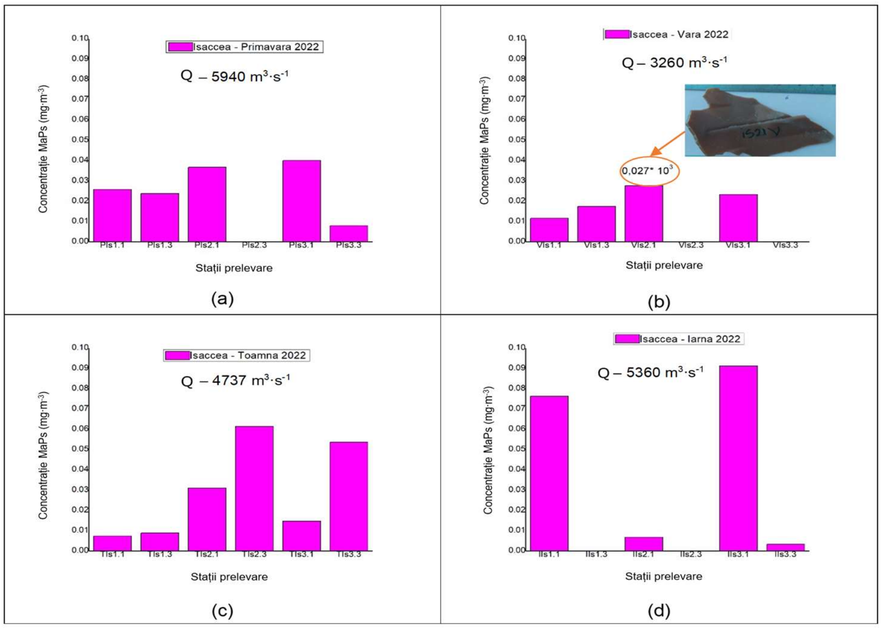


Figure 3.6 Concentration of macroplastics collected from the Isaccea area in the 4 seasons (a – spring, b – summer, c – autumn, d – winter)

Following the comparative analysis of the average concentrations of MaPs present in the two areas, it was found that in Moldova Veche concentrations were recorded approximately 10 times higher than in Isaccea.

3.2.3 Morphological and compositional analysis of micro- and macroplastic samples

In Figure 3.7 we selected 3 of the images of the MPs samples collected from the two sampling stations. From these images it can be seen that MPs with a macroscopic, mixed morphological structure were collected. MPs with a chromatic diversity were sampled, with different shades of colors such as: red, green, blue, yellow and pink. Transparent, white or black fragments were also observed. Therefore, the color variety present in the samples of MPs taken also illustrates the diversity of pollution sources.

Regarding the classification of MPs in terms of shapes, particles were collected in the form of fragments>films>lines>fibers>granules. The presence of films and fragments indicates the secondary origin of microplastics, which come from the fragmentation of larger plastics released uncontrollably into the environment. The anthropogenic source of fibers and lines is mainly represented by textiles (in the case of fibers), nets and used for fishing.

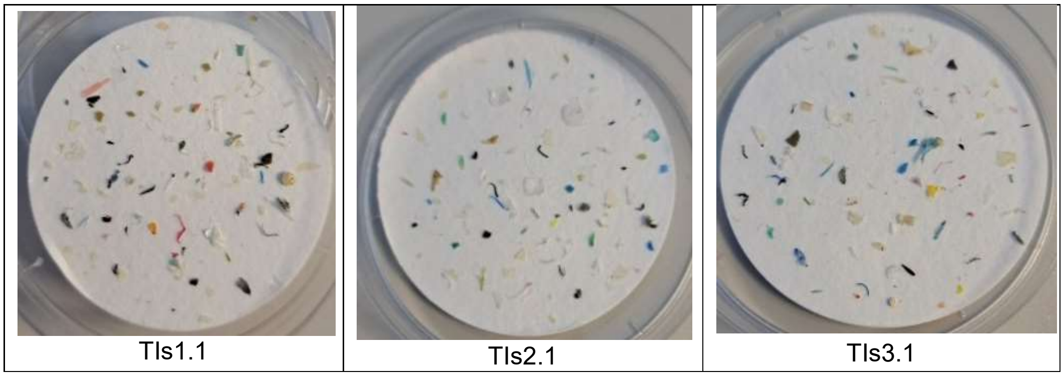


Figure 3.7. Photo images of microplastic samples

Figures 3.7 and 3.8 graphically represent the polymers identified in the samples taken from the Moldova Veche (MV) and Isaccea (Is) stations. From the two graphs it can be seen that the majority polymer identified was polyethylene (75.53% in Moldova Veche and 74.72% in Isaccea), followed by polypropylene in a percentage of 20.31% in Moldova Veche and 16.23% in Isaccea, respectively. The results obtained are due to the fact that PE is the most widely used polymer in the production of plastics. It is used for the manufacture of packaging (e.g. food bags) and household products, such as containers ("Polyethylene (PE) | Properties, Structures, Uses, & Facts | Britannica," 2024). After polyethylene, polypropylene is the second most widely used polymer in the plastics manufacturing industry (Karger-Kocsis and Bárány, 2019), it is mainly used for the manufacture of plastic packaging. It is found that the use of polypropylene in the packaging industry accounts for 16% of plastics worldwide (Sin and Tueen, 2023). Due to its property of being resistant to high temperatures, this polymer is used for the production of pipes and other items that require sterilization (jars, buckets, bottles, etc.) (Maddah, 2016). In a percentage of 1.91% at MV and 2.26% at Is, ethylene propylene dien monomer (EPDM) was identified, which due to its high resistance to heat, light and ozone, is used as an insulating material in building and car construction (Fazli and Rodrigue, 2020; Mitra et al., 2009).

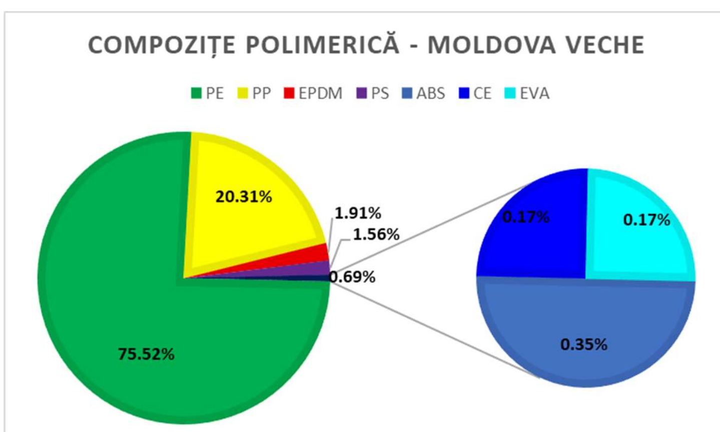


Figure 3.8 Types of polymers identified in the Moldova Veche sampling station

Polystyrene was found in a share of 1.56% at MV and 4.91% at Is, being used as thermal insulation for buildings and in the manufacture of single-use packaging. This polymer belongs to the category of thermoplastics with a high demand globally, after PE, PP and PVC (Gausepohl and Nießner, 2001). Cellulose particles were identified in a percentage of 0.17% at MV and 1.51% at Is. It has applications in the food, pharmaceutical, and packaging industries (Gupta et al., 2019). Even though, cellulose fibers decompose at a faster rate than plastics (Singh et al., 2020), they can be harmful because they can contain toxic additives (e.g. phthalates and dyes) whose release can affect biota (Queiroz et al., 2022). With a low share (below 1%) polymers such as PUR, ABS and EVA were identified. Polyurethane (PUR) is used as a thermal insulator in the construction industry, a material in the manufacture of furniture and in the production of textiles, packaging, paints and additives (Das and Mahanwar, 2020).

Ethylene-vinyl-acetate copolymer (EVA) It is present in the composition of insulating materials in the construction industry, adhesives and items such as hoses, and footwear (textile industry) (Henderson, 1993). It also has applications in the pharmaceutical industry, being a good excipient for extended-release dosage forms of drugs (Schneider et al., 2017). The presence of ABS polymer may be due to its use in the manufacture of products such as components for the interior of cars (automotive industry), medical devices, personal care products, toys, building materials (Polli et al., 2009).

The inadequate management of plastic waste containing the polymers presented above is the main source of pollution of the Danube water. Often, different areas are observed on the banks where household waste is found, which can result from anthropogenic recreational and fishing activities.

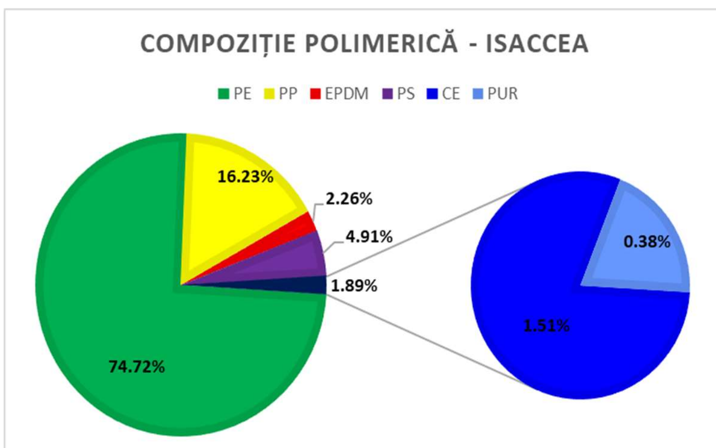


Figure 3.9 Types of polymers identified in the Isaccea sampling station

3.2.4 Partial conclusions

Based on the results presented, the following partial conclusions were drawn:

- At Moldova Veche, the maximum concentrations of MPs (0.726 mg·m^{-3}) and MaP (1.92 mg·m^{-3}) were recorded in the M1.1 station, in the surface layer of the Danube water, in the vicinity of the Romanian shore.
- At Isaccea, the highest concentration of MPs was observed in the PIs2.1 station (0.140 mg·m^{-3}), in the surface layer, near the fairway. As for the MaPs concentration, the maximum value was found in station IIs3.1 (0.091mg·m^{-3}), on the surface, in the vicinity of the Ukrainian shore.
- In Moldova Veche, concentrations of MPs were recorded about 6 times higher than in Isaccea.
- MaPs collected at Moldova Veche recorded concentrations about 10 times higher than at Isaccea.

- As for the seasonal variation, the lowest concentrations were observed in the summer season, when the lowest level of the Danube's flow was also recorded.
- From the analysis of the vertical distribution in the water column of the MPs, the highest concentrations were observed in the surface layer of the water.
- Micro- and macroplastics showed chromatic diversity, and from the point of view of particle shape, fragments>films>lines>fibers>granules were collected in the order presented. Morphological features are valuable indicators in terms of identifying sources of pollution.
- The polymers identified in the analyzed samples were: PE, PP, PS, EPDM, ABS, CE, EVA, and PUR. The largest share was held by the EP (75.53% in Moldova Veche and 74.72% in Isaccea) and the PP (20.31% in Moldova Veche, respectively 16.23% in Isaccea).
- The main sources of pollution identified are plastics manufacturing and processing industries, as well as industries where the use of plastics is secondary. Also, plastic waste (packaging, bags, containers) and domestic wastewater are important sources of pollution.

3.3 Assessment of the presence of microplastics in the pre-deltaic and deltaic sector of the Danube

The present study aims to quantify the microplastics in the Danube water, both from the surface layer (the first 10 cm) and from the depth of 7 meters. The area from which the samples of MPs were taken includes the largest area that has been studied so far on the Romanian sector of the Danube, covering approximately 370 km and transition areas such as the pre-deltaic and proxim-deltaic ones. The samples of MPs were collected from 11 locations (see map Fig. 2.1), located starting with the station located near the city of Galati, at the confluence of the Siret River with the Danube, up to the mouth of the three branches of the Danube into the Black Sea. In the following subchapters we will discuss the results obtained regarding the spatial variation of the concentration of MPs in water and sediment. Aspects related to the composition and morphology of the sampled plastic microparticles are also discussed.

3.3.1 Spatial distribution of microplastics in water

Figure 3.10 graphically shows the variation of MPs concentrations in the surface layer of the Danube water. Following laboratory analyses, variations in MPs concentrations in the range of 0.9 – 2.8 particles·m^{-3} were observed. It can be noted that the highest concentration was recorded in the S6 sampling station, located near the mouth area of the Chilia Arm into the Black Sea (2.8 particles·m^{-3}). High values were also recorded in the sampling stations S2 (2 particles·m^{-3}) and S10 (1.8 particles·m^{-3}), related to the confluence of the Prut River with the Danube (S2), respectively to the locality of Murighiol (S10). The spatial distribution highlights the critical areas where high amounts of MPs accumulate. The confluence of the Prut River with the Danube River signals the contribution of the tributary in terms of the MPs intake in the Danube. The results recorded in the sampling stations located in the vicinity of the discharge areas of the three arms of the Danube into the Black Sea illustrate the significant impact of the Danube on the amount of MPs in the Black Sea. Moreover, the maximum concentration of MPs was recorded in the confluence area of the Chilia and Black Sea arms.

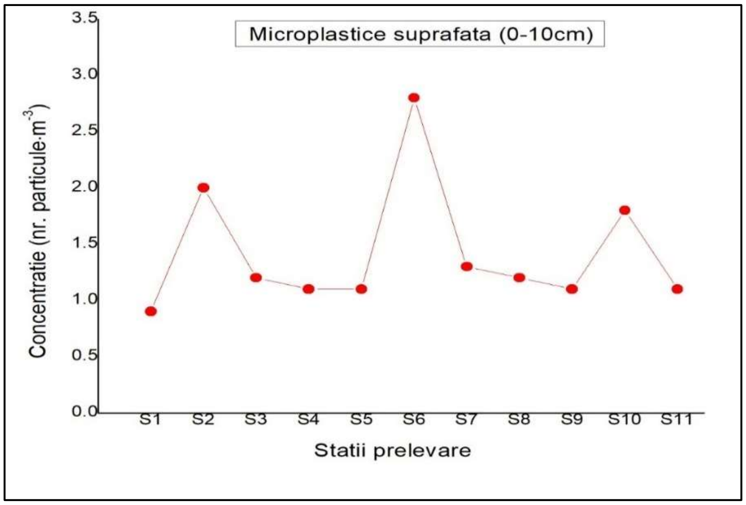


Figure 3.10. Variation in the concentration of MPs in the upper layer of water

Regarding the morphological classification of MPs, Fig. 3.11 shows the share of microplastics on each class identified in the 11 sampling stations, namely: fragment, film or fiber. It is observed that in the highest percentage fragment (16.6-88.8%) and fiber (8.3-83.3%) MPs were identified in all stations.

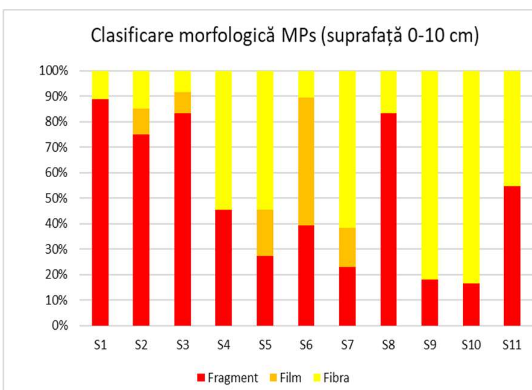


Figure 3.11. Morphological classification of MPs collected from the surface layer

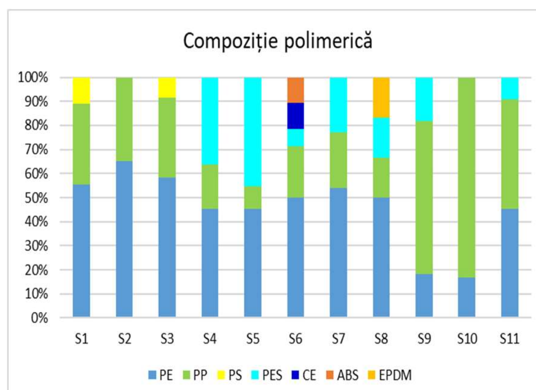


Figure 3.12. Polymers in the composition of MPs collected from the surface layer

In the samples taken from stations S2, S3, S5, S6, and S7, film-type MPs (8.3-50) were also observed. The majority presence of fragments is an important indicator for establishing the type of pollution source, the fragments being secondary MPs from the degradation of large plastic waste. Most of the collected plastic microparticles showed irregular shapes (Fig. 3.27), and the dimensions varied in 150 µm – 5 mm.

Regarding the collected MPs colors, they present a color diversity, being observed MPs, transparent, black, white, but also with different shades of yellow, green, blue and red.

Fig. 3.12 graphically shows the share of each identified polymer in the composition of microplastics collected from the surface layer of the Danube water. According to the IR spectra obtained from the analysis of the samples, the following 7 polymers were identified: polyethylene (PE), polypropylene (PP), polyester (PES), polystyrene (PS), cellulose (CE), acrylonitrile butadiene styrene (ABS), ethylene-propylene-dien-monomer (EPDM). It is worth noting that in all 11 stations, PE (16.6 – 65%) and PP (16.66 – 83.33%) polymers were mostly identified. The two polymers are in the composition of 70% of the global production of plastics, from which the majority of single-use packaging is produced (Ranjani et al., 2022), and represent an important source of MPs for aquatic ecosystems, which is also confirmed in this study, given that they were ubiquitous in all samples.

The PES polymer (9.09 – 45, 45%) was observed in 7 (S4, S5, S6, S7, S8, S9, S11) of the 11 sampling stations, the largest share being recorded in the S5 Chilia Veche station (45.45%). The main source of pollution with MPs containing PES is the fibers from textile materials, an important contribution being the wastewater resulting from washing clothes. Also, the deposition of microfibers from the atmosphere is another imported source (Schell et al., 2022). Most of the time, the polyester in the composition of these fibers is polyethylene terephthalate (PET). The greatest diversity in terms of the polymeric composition of the samples was observed in the S6 station, located near the mouth area of the Chilia arm into the Black Sea, where in addition to the polymers PE, PP, PES, were also identified ABS and CE polymers (10.7%). Isolated, in the S8 station (Crișan), the EPDM polymer (16.66%) was also observed, which is usually used as a sealing material (e.g. automotive industry), but also in building and roof construction (Mintenig et al., 2020). In stations S1 and S3, the polystyrene polymer (8.33 – 11.11%) was also observed, which is used on the scale to obtain disposable containers (Zhang et al., 2017) and materials with different applications in the manufacture of electronic gadgets and auto parts (Bouadil et al., 2024).

Considering the fact that no measurements regarding the distribution of microplastics and at depth have been made in the study area, in the present doctoral thesis, we proposed for the first time the evaluation of the presence of MPs in the water of the Danube and at a depth of 7 meters. Figure 3.13 shows the variation of the plastic microparticles at a depth of 7m in the 11 sampling stations. The concentrations of MPs varied in the range of 0.5 – 1.8 particles·m^{-3}. The maximum value was observed in the S8 station, located on the Sulina Arm, near Crisan. According to the study conducted by Born et al., 2023, the vertical transport of MPs is also influenced by inland water. Therefore, a possible explanation for the maximum concentration of MPs in the S8 station can be given by the intense naval traffic on the Sulina Arm, given the fact that it represents the only direct sea route to the Black Sea. Compared to the results recorded from the surface layer, concentrations were identified at depth lower than MPs, except for the samples taken from stations S7 and S8, located downstream from the city of Tulcea (S7) and adjacent to Crișan (S8).

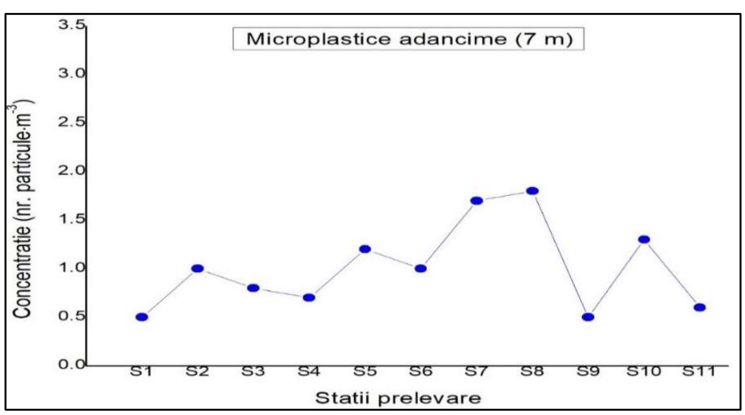


Figure 3.13 Variation in the concentration of MPs at a depth of 7 m

From a morphological point of view (Fig. 3.14), analogous to the samples taken from the upper layer, fragments (23 – 80%) and fibers (25 – 76.95%) were observed in all locations, except for the S2 location, from where no fibers were collected. Film-type MPs (20 – 25%) were observed in samples collected from stations S1, S2, S3 and S7. The mixed structure of the samples taken from these four stations is directly influenced by the anthropogenic activities carried out in the cities of Galati (S1 and S2), Isaccea (S3) and Tulcea (S7), the sampling locations being located in their proximity.

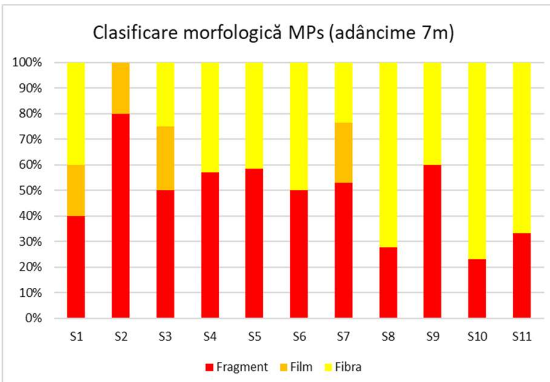


Figure 3.14. Morphological classification of MPs collected from a depth of 7 m

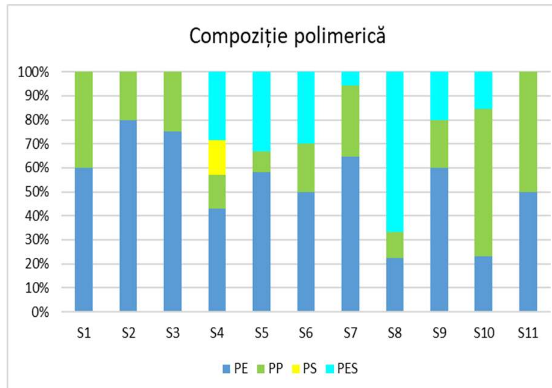


Figure 3.15. Polymers in the composition of MPs collected from a depth of 7 m

Unlike the MPs in the top layer, only 4 polymers were identified as a result of the analysis of the MPs collected at depth, namely PE, PP, PS and PES. Analogous to the results obtained from the analysis of MPs taken from the surface layer, the polymers mostly identified in the samples taken from the depth of 7 meters, in all 11 stations, were PE (22.22 – 80%) and PP (8.99 – 61.53%) (Fig. 3.15). Also, polyester (5.88 – 66.66%) was also identified in MPs collected from the depths of the S4-S10 stations

3.3.2 Spatial distribution of microplastics in surface sediment

Figure 3.16 graphically shows the variation in the concentration of microplastics in the sediment samples taken from the 11 stations. The values obtained varied in the range of 12 – 52 particles·kg^{-1}. The highest concentration was recorded in the related S2 station the area of confluence of the Prut River with the Danube. Also, high concentrations (40 particles·kg^{-1}) have been observed in the locations S1 and S11, located near the confluence of the Siret River with the Danube (S1), respectively the Sf. Gheorghe arm in the Black Sea (S11), where the low flow velocity favors the sedimentation of MPs from the water. The high concentrations recorded at the confluence of the Siret and Prut Rivers with the Danube River highlight the significant contribution of these tributaries on the amount of MPs in the Danube. Also, the Siret River is the emissary of the waters coming from SEAU Galati, the S1 sampling station being located approximately 2.55 km away from the domestic wastewater discharge area. The migration of microplastics into the sediment is favored by hydrodynamic conditions and the adhesion of other particles of biological origin (microorganisms), organic or inorganic (suspended sediments, other pollutants, etc.), which cause the density of MPs to increase and their deposition in the sediments (He et al., 2020). Moreover, the sediment is considered to be a reservoir of MPs accumulation (He et al., 2021; Yang et al., 2021).

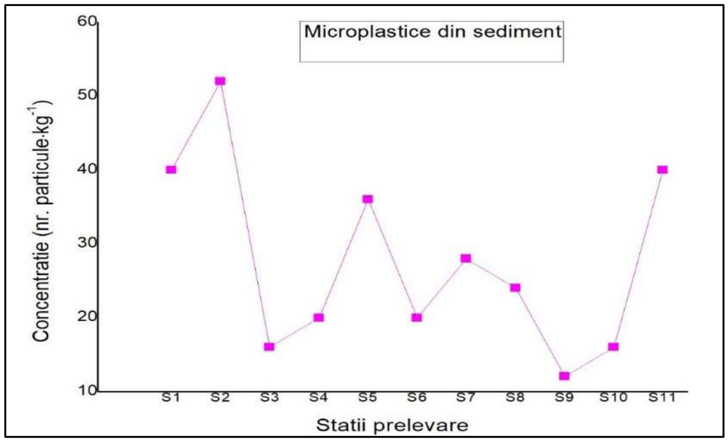


Figure 3.16. Variation in the concentration of MPs in sediment

In terms of shape (Fig. 3.17), most of the microplastics isolated from the sediment samples were fragments (66.6% - 100%), isolated fibers in stations S1, S2, S4, S5, S6 and S11 (7.6% - 40%). Film-type MPs were observed only in the S10 station. The presence of fragments in the sediment may be due to the surface that favors the adsorption of other particles that cause an increase in density.

Regarding the polymer composition of the microplastics isolated from the sediment samples, Fig . 3.18 graphically shows the share of polymers identified in the samples collected from the 11 stations. Similar to the composition of MPs taken from water, the ubiquitous and mostly identified polymers in sediment MPs were PE (40 – 75%) and PP (25 – 50%). Thus, the hypothesis is once again confirmed that, in an aquatic ecosystem, sediment is the storage reservoir of MPs. Moreover, the main sources of pollution are the same. Polyester fibers (10%) were also identified in stations S1 and S11. An explanation regarding the presence of this polymer in the S1 station is given by the fact that it is located at the confluence of the Siret with the Danube, which is the emissary of domestic wastewater from SEAU Galati. Water from SEAU is the main source of microfibers, in this case polyester fibers.

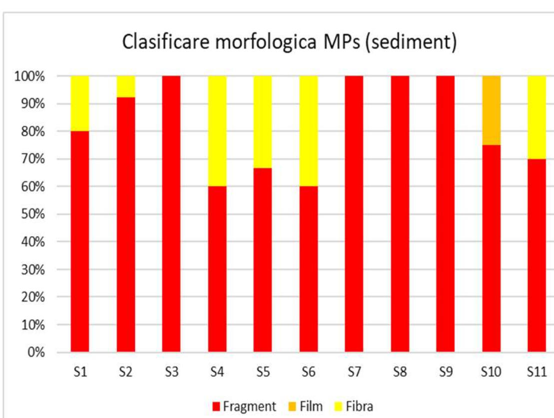


Figure 3.17. Morphological classification of MPs isolated from sediment

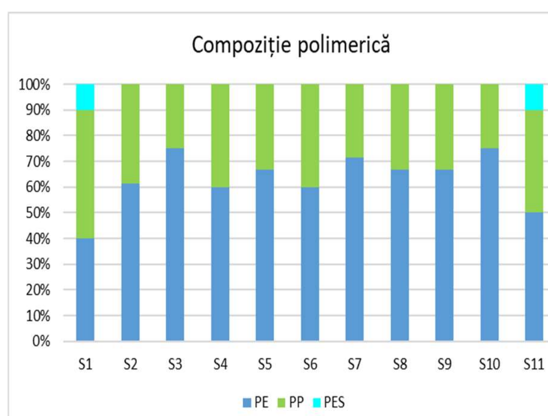


Figure 3.18. Polymers in the composition of MPs isolated from sediment

3.3.3 Partial conclusions

The results obtained allowed the elaboration of the following partial conclusions:

- The presence of microplastics in the pre-deltaic and deltaic sectors of the Danube was assessed for the first time.
- The presence of microplastics in the surface layer of the Danube water was observed in all 11 sampling stations. The highest concentrations were collected near the mouth of the Chilia Arm into the Black Sea (2.8 particles·m^{-3}) and at the confluence of the Prut River with the Danube (2 particles·m^{-3}).
- As for the vertical distribution of MPs, in most of the stations the highest concentrations were recorded in the surface layer. At a depth of 7 meters, the maximum value was identified on the Sulina arm, near Crișan locality (1.8 particles·m^{-3}).
- The polymers identified in the composition of microplastics in water were, in the following average, PE>PP>PES>PS>EPDM>ABS>EC. Ubiquitous in all 11 stations were the PE and PP.
- Most of the MPs in the water were fragments>fibers>films.
- The presence of microplastics has also been observed in the sediment of the Danube. The highest abundance was found at the confluence of the Prut River with the Danube (52 particles·kg^{-1}).
- The polymers identified in the MPs in the sediment were PE, PP and PES.
- A uniform distribution was observed in terms of the morphological characteristics of the MPs collected from the two verticals of the water and sediment column.

3.4 Using Artificial Intelligence for morphological classification and quantification of microplastics

The quantification, characterization and classification of microplastics are essential steps in monitoring their presence and assessing the ecological impact on aquatic ecosystems. However, these analytical processes are time-consuming when traditionally performed by humans. To be able to carry out these processes in an automated way, which reduces the time and effort of analysis, several Artificial Intelligence (AI) models were tested in the present study.

The problem regarding the qualitative and quantitative analysis (number of particles) of microplastics arose when, following the filtration of a large amount of water (up to 100 m^3), the abundance of MPs collected on the filter paper, in some cases, exceeded 100 particles/filter. Therefore, counting, dimensioning, and grading MPs manually becomes a difficult task, and the results can be significantly influenced by human error.

The purpose of this study is for each MP to be quantified (counted) on each morphological class and to calculate the length and width of each particle. Moreover, for each MP the area will be established. This study can be a starting point for the introduction of surface information into the definition of microplastics. Currently, in the statement that defines the concept of microplastics presented in the *ISO 24187:2023 standard - Principles for the analysis of microplastics present in the environment*, there is only information regarding the upper limit, namely 5 mm. From the point of view of the impact on aquatic biota, the toxic effects induced by the presence of a fiber with a length of 5 mm assimilable as a geometric body with a cylinder, can differ from those caused by a 5 mm fragment by having a different geometric shape, for example, that of an irregular polyhedron. The importance of establishing a maximum limit of the surface of microplastics applies, in particular, due to the fact that MPs contain other substances with toxic potential (e.g. additives), but also due to the character of transport vectors of other pollutants or microorganisms. Thus, with the ingestion of a particle with a larger surface area, a higher concentration of other toxic compounds can also enter the body.

3.4.1 Use of the YOLOv8 model for 5 morphological classes

Initially, the YOLOv8 model was tested, for the classification of microplastics into 5 morphological classes (fragment, film, fiber, line and granules). The results obtained from testing the *YOLOv8* model will be presented below. Model evaluation was performed using the line of code illustrated in Figure 3.36. Within the line of code, the metric indicator Mask P (precision) is shown, which indicates the degree of match of the predicted results with the real ones. It can be seen that the overall value of the P indicator (accuracy) was 0.629, which indicates a match of about 63%. For each class, the values of the P indicator varied in the range of 0.428 – 0.878. Values above 0.50 were observed for the classes: fiber (0.583), fragment (0.832), and granules (0.878). For the other two classes, film and lines, values below 0.5 were obtained, which indicates a match percentage below 50%. Therefore, the probability of confusing these classes of particles with the other three is quite high.

3.4.2 Application of YOLOv5, YOLOv8 and Mask R-CNN for the classification and quantification of microplastics

Following the training of the YOLOv8, YOLOv5 and Mask R-CNN models for the classification of MPs into the three classes (fragment, fibers and granules), the metric indicators P (precision) and mAP50 (*mean Average Precision 50*). P quantifies the proportion in which the classes in all predictio ns were correctly predicted. The mAP50 (mean Average Precision 50) indicator (Table 3.4), which represents the average accuracy for all objects, obtained at an overlap (intersection) of surfaces of 50%, of the predicted object mask with the real one, being essential in assessing the accuracy of object location (Ultralytics, 2024).

The overall accuracy obtained for the YOLOv5 and YOLOv8 models was 0.926 for the YOLOv5 and 0.823 for the YOLOv8. Thus, for the model with better results (YOLOv5), for each class P values of over 0.925 were obtained, which indicates a prediction accuracy of over 92%.

Table 3.1 shows the results of the mAP50 indicator obtained for the 3 trained models. It can be seen that, for all three models, general values of over 0.848 were obtained, the highest result being obtained by Mask-RCNN. As for the fragment class, the best value was obtained for the YOLOv5 model (0.971). For fibers, the YOLOv8 model indicated the highest value (0.947), and for granules, the best performance was obtained by the mAP50 Mask-RCNN model 0.863.

Table 3.1 mAP50 indicator for YOLOv5, YOLOv8 and Mask-RCNN

Class	mAP50 YOLOv5	mAP50 YOLOv8	mAP50 Mask-RCNN
General	0.901	0.848	0.870
Excerpt	0.971	0.831	0.905
Fibre	0.879	0.947	0.844
Pellet	0.852	0.764	0.863

In the following, the results obtained from the application of *YOLOv5, YOLOv8 and Mask R-CNN models* for the classification and dimensional characterization of an unknown sample with microplastics will be presented.

The number of MPs on each class, quantified and classified manually, was as follows: 50 fragments, 13 fibers and 2 granules, with a total of 65 particles. Thus, comparing with the results predicted by the three models, it was observed that:

- YOLOv8 and YOLOv5 models have estimated exactly no. of fibers with the manually counted one (13);
- YOLOv8 and Mask-RCNN models have estimated exactly no. granules with the manually detected one (2);
- No. of fragments predicted by YOLOv8 and YOLOv5 was the closest to that manually estimated (44), the rest up to 54 being mistaken for fibers (YOLOv8), granules (YOLOv5) or not detected at all;
- No. The total number of MPs was quantified as close as possible to the manual one, by the YOLOv5 model, respectively 64, instead of 65.

From the analysis of the dimensional indicators of the MPs in the non-clear sample, it is observed that the smallest widths and lengths measured 0.2 mm, and the largest 6.49 and 5.31 mm, respectively. Thus, it is noted that MPs were detected with dimensions larger than the upper limit for MPs, respectively, 5 mm (2 particles), which requires the application of an additional condition, to eliminate from detection, particles with a length or width greater than 5 mm. As for the area of MPs identified, the smallest was 0.04 mm^2, and the largest 13.14 mm^2. It is worth noting that the maximum identified surface area does not belong to the particle with the largest size (fiber), but to a microplastic (fragment), which does not exceed the maximum limit regulated for a MPs.

3.4.3. Partial conclusions

Following the training and application of the three models, YOLOv8, YOLOv5, and Mask R-CNN, the following partial conclusions were reached:

- For the classification of microplastics into the 5 morphological classes (fragments, films, lines, fibers, granules), the YOLOv8 model obtained results with an accuracy above 0.5, only for fragments, fibers, and granules.
- For the classification of the three classes (fragments, fibers, and granules), the highest accuracies were obtained with the YOLOv5 model.
- As for the mAP50 indicator, the YOLOv5 model indicated the highest accuracy for fragments, for fibers the YOLOv8 model, and for granules, the Mask-RCNN model 0.863.
- The total number of MPs particles was predicted as close as possible to the manually determined one by the YOLOv5 model, respectively 64 MPs, instead of 65 MPs.
- A method has been applied that allows the automatic establishment of the dimensional characteristics of MPs (length, width, area).
- Given the fact that particles larger than 5 mm have been detected, it is necessary to optimize the models so that particles exceeding this limit are eliminated.
- The largest surface area determined for a MPs did not belong to particles that exceeded the limit of 5 mm. Therefore, with this study, we propose the introduction of the "area" indicator for the characterization of MPs.

3.5 Assessing the impact of microplastics on aquatic ecosystems

To assess the impact of microplastics on aquatic ecosystems studied in this doctoral thesis, this chapter analyzes the presence of these industrial micropollutants in the fish species with commercial interest, namely the Danube mackarel *(Alosa immaculata),* the only migratory species. Also in this chapter, the capacity of MPs as a transport vector of other industrial pollutants, namely heavy metals, is studied. This property favors the synergy of the toxicity of MPs with that of adsorbent pollutants on their surface, or even in their composition, thus enhancing the harmful impact on biotic and abiotic components.

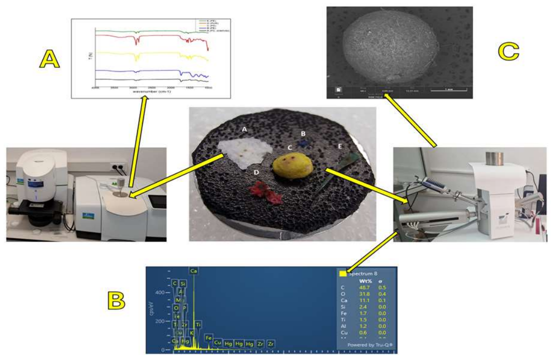


Figure 3.19 Integrated use of three non-destructive methods for microplastics analysis (A – FTIR, C and B – SEM-EDX)

The multidisciplinary character of this doctoral thesis is highlighted in this subchapter, using three non-destructive analysis techniques for the characterization of microplastics (FTIR and SEM coupled with EDX) (Fig. 3.19). Moreover, to assess the ecological risk of microplastics on aquatic ecosystems, the following three indices were calculated: the Pollution Load Index (PLI), the Hazard Index (HI), and the Potential Ecological Risk Index (PERI).

3.5.1 Study on the presence of microplastics in Alosa immaculata fish

In the present doctoral thesis, the presence of microplastics in the gastrointestinal tract and gills was collected from 20 specimens of fish of the species *Alosa immaculata* (Danube shad) caught from 4 locations on the Danube. This species of fish has an important economic value, in the Danube Delta and in the Lower Danube sector, having been caught no less than 348 tons in 2019. (Milea et al., 2023).

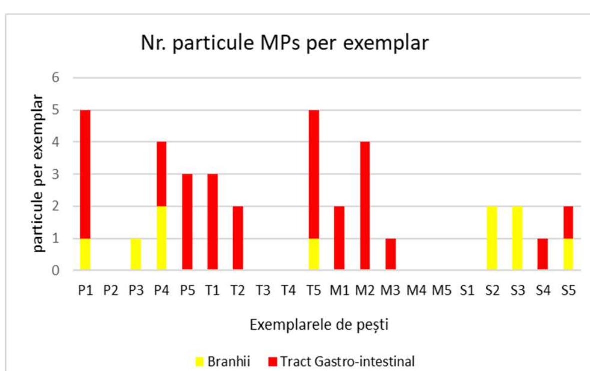


Figure 3.20. The number of MPs particles identified in the specimens of *Alosa immaculata*

Figure 3.20 graphically shows the presence of MPs microparticles isolated from the gills (B) and gastrointestinal tract (GIT) of the 20 specimens of *Alosa immaculata.*

The presence of MPs was reported in 14 of the 20 specimens investigated, in at least 3 specimens from each station from which they were captured. Most microparticles were observed in the gastrointestinal tract, with an average of 1.35 particles per specimen, with the highest abundance (4 MPs) being observed in P1, T5, and M2 specimens. The presence of MPs in GIT is due to their accidental ingestion through water and sediment or through contaminated food (Pan et al., 2021). The presence of MPs in the GIT favors the transfer of MPs to other tissues and organs, given that the small ones can cross the intestinal wall (Roch et al., 2020). At the GIT level, microplastics can cause its obstruction but also inflammatory conditions, which can affect processes such as reproduction and growth, decreased energy reserves, metabolism disorders, cell damage, etc (Atamanalp et al., 2022). A lower abundance of MPs was reported at the gill level than in GIT, the average being 0.5 particles per specimen. The largest no. of MPs (2 particles) was observed in gills collected from specimens P4, S2, and S3. The gills are one of the important pathways for MPs to enter the fish's body, their accumulation in the gills can cause physical damage and decreases in respiratory efficiency (Kılıç et al., 2022).

Figure 3.21 shows the morphological classification of MPs isolated from the analyzed biological materials. It is noted that only two classes have been identified, namely fragments and fibers. In GIT, mostly fibers were identified (60%), while in gills, fragments (51.8%).

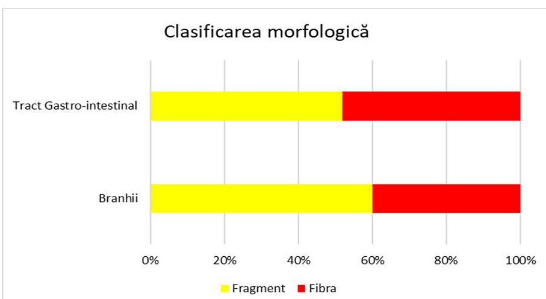


Figure 3.21. Morphological classification of MPs identified specimens of *Alosa immaculata*

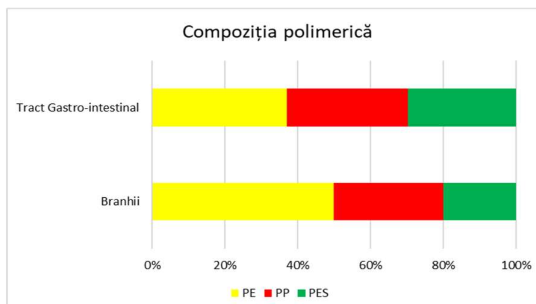


Figure 3.22. The polymeric composition of the MPs identified the specimens of *Alosa immaculata*

Regarding the type of polymers in the composition of isolated MPs (Fig.3.22), the following polymers have been identified: polyethylene (50% GIT, 34% B), polypropylene (30% GIT, 33.3% B) and polyester (20% GIT, 29.6% B). It can be seen that, in the analyzed specimens, the polymers with the highest abundance were identified in the water and sediment samples collected from the Danube. Figure 3.23 shows a fragment of MPs, approximately 150 μm in size, containing the polymer PE, isolated from the Git specimen T1.

At the GIT level in specimen P1, the PP particle illustrated in Fig. 3.24 was identified, with a size of about 700 μm. In the study conducted by Bobori et al., 2022 tests have been carried out on the evaluation of the toxicity of the PP polymer on the *Dani orerio* (zebrafish) and *Perca fluviatilis* (bibănuș). The results obtained signaled the presence of oxidative stress and the reduction of liver and gill cell functions.

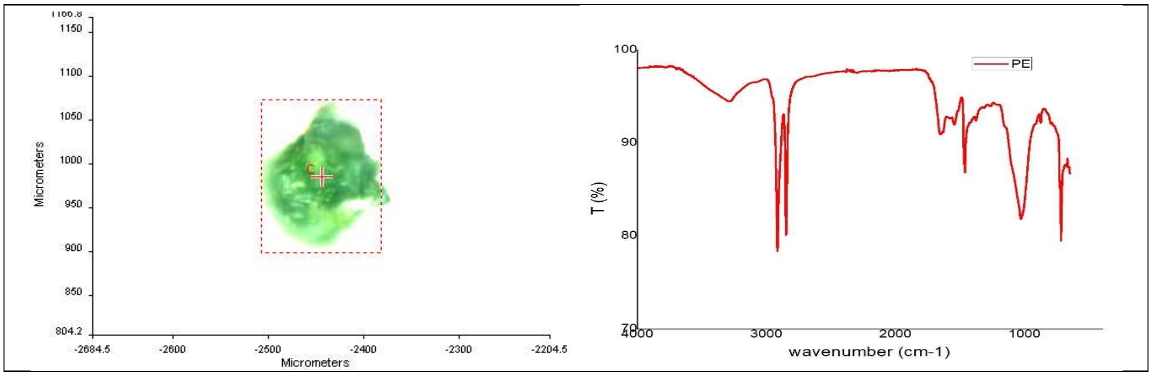


Figure 3.23 PE isolated microplastic particle in T1 specimen - GIT

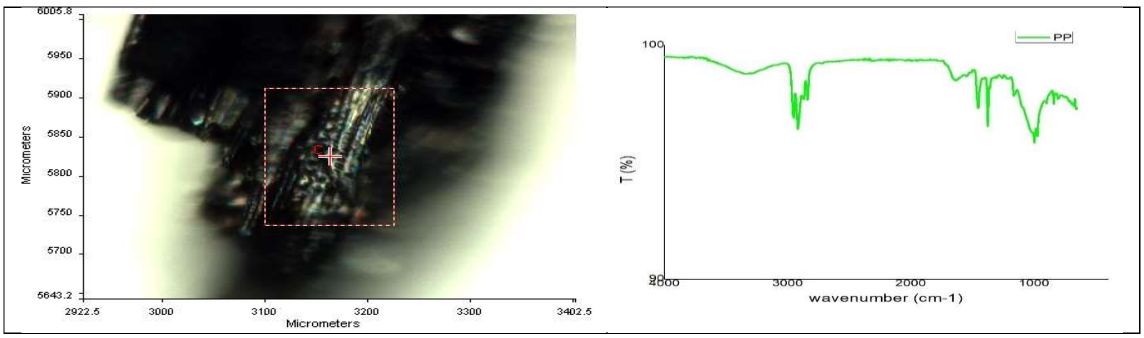


Figure 3.24 PP microplastic particle isolated in specimen P1 – GIT

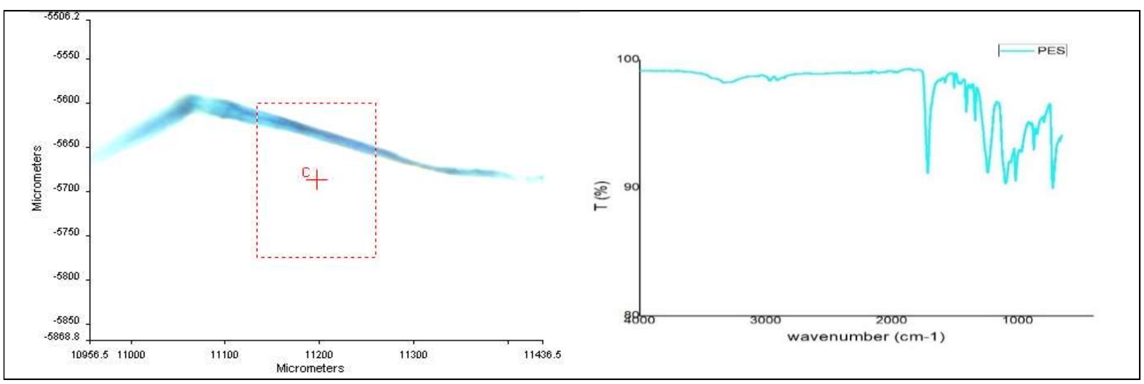


Figure 3.25 PES microplastic particle isolated in specimen S3 – gills

Figure 3.25 shows a PES fibre isolated from the gills of specimen S3, approximately 500 µm long. According to the literature, the exposure of the fish species *Oryzias latipes* (Japanese rice fish) to polyester microfibers induced the appearance of oxidative stress (Kim et al., 2023).

The existence of microplastics in the species *Alosa immaculata* from the Danube was studied for the first time in this doctoral thesis.

3.5.2 Analysis of pollutants with toxic potential on the surface of microplastics

Apart from the harmful effects that microplastics have on aquatic ecosystems and human health, there is an under-researched direction regarding the vector role that MPs (Brennecke et al., 2016; Zambrano-Pinto et al., 2024) have it in the transport of industrial pollutants and harmful microorganisms. This doctoral thesis includes experiments and interpretations related to the transport of industrial pollutants, respectively metals.

During the analyses, I combined SEM technology with EDX technology, which allowed me to formulate qualitative and quantitative conclusions on the presence of heavy metals and to confirm the role of MPs as a transport vector.

Figure 3.26 exemplifies the only sample with 5 plastic particles, easy to observe, optically and analytically, which was subjected to SEM-EDX (real size) analysis. The microplastics were collected from the water of the Danube, in the vicinity of the pontoon of the "Dunărea de Jos" University. After sampling, they were analyzed directly, without being subjected to sample preparation processes (digestion or separation), so as not to influence their composition and morphology. 5 microparticles of different colors are illustrated, fixed on the analysis support provided with a double-sided tape with a carbon conductive layer.

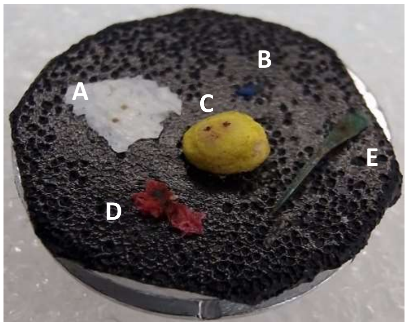


Figure 3.26. Microplastics subjected to SEM-EDX analysis

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To establish the type of polymers in the structure of the particles subjected to SEM analysis, determinations were made using the ATR-FTIR method (Fig. 3.27) from which the following conclusions emerged:

- Particle A (Fig. 3.27) is a resin-based paint particle obtained by aldehyde polycondensation (PC – aldehyde);
- Particle B contains the polymer polyurethane (PE);
- Particle C contains the polymer polystyrene (PS);
- Particle D contains the polymer polyethylene (PUR);
- Particle E in the composition of the polymer polyethylene (PE).

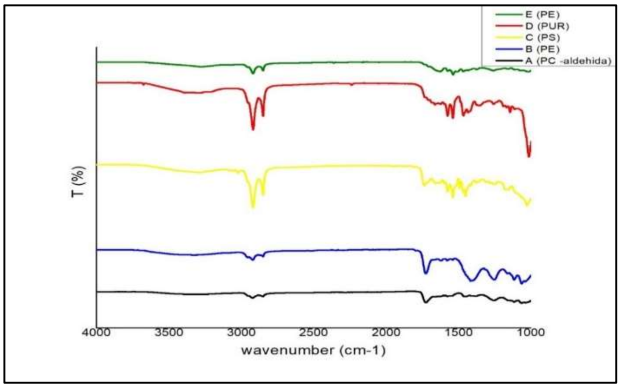


Figure 3.27. FTIR spectra of polymers identified in microparticles analyzed at SEM

From the in-depth analysis with higher magnification factors, the following characteristics of the microparticles resulted:

- uneven structure with cavernous areas that multiply the contact surface;
- the geometric shape of MPs differs depending on the provenance, the type of polymer and the disintegration conditions of the original materials.

Below we will present the qualitative and quantitative references about 2 of the 5 particles identified with SEM-EDX technology.

Particle A

In the image with a magnification of 60x (Fig. 3.28 a) there is the appearance of a smooth surface with reduced interaction areas. Taking into account the particle sizes containing industrial pollutants (heavy metals), we proceeded to increase the degree of multiplication by scanning defined areas in this image. The result can be seen in the images illustrated in Fig. 3.28 c and d, where porous/cavernous areas clearly appear suitable for the attachment of chemical species or microorganisms. It can also be considered that these fracture zones (Fig. 3.52 b) are the origin of nanoplastics by the disintegration of fragments of microplastics. In Fig. 3.28 c and d, with a higher degree of magnification (2500x and 5490x respectively), the areas where surface degradation can lead to the adsorption of chemical species with a relatively low degree of reactivity are highlighted. The surface morphology of MPs is an essential element for the adsorption of pollutants. It can be seen that we are not talking about chemical interactions per se, but about adsorption modalities based on subtle interactions between the polymer substrate and adjacent molecular media.

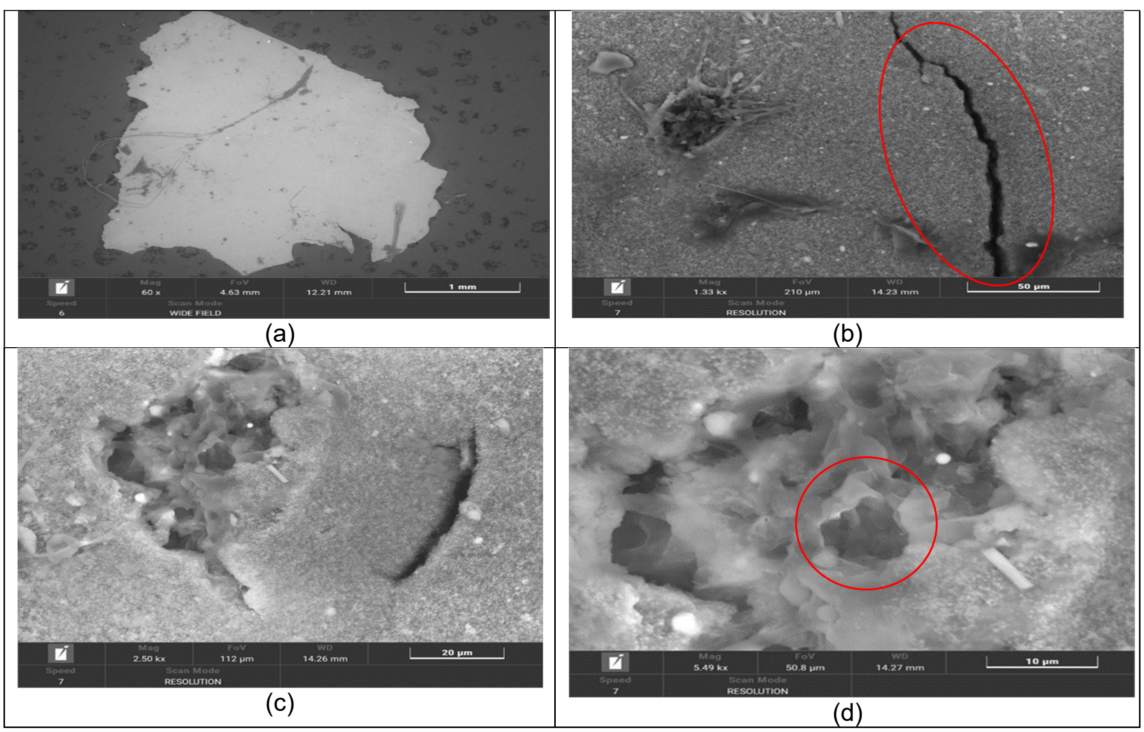


Figure 3.28. SEM images of particle A obtained at different degrees of amplification (a) 60x, (b) 1330x, (c) 2500x, (d) 5490x

The vector role analysis for MPs was highlighted by using EDX technology and determining the elemental quantitative composition of MPs particles. The EDX analysis (Table

3.2) of the particle confirms the presence of metals such as Fe, Cu, Zn, Cd, Ni, Hg, which could be adsorbed on the surface of the fragment, or even in the structure.

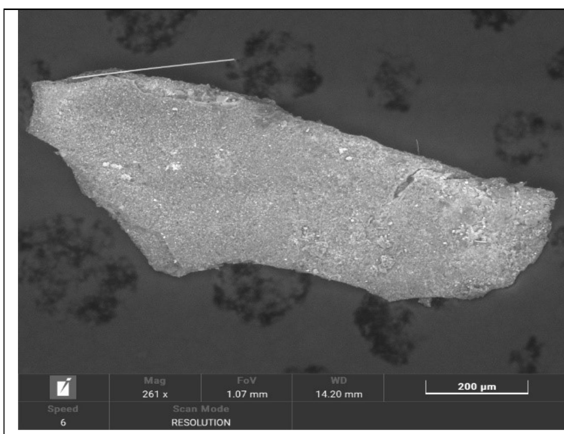
Table 3.2. EDX elemental composition of particle A

Element	C	A	Na	Mg	Of	And	K	Lik e	Your	Fe	Ni	Wi th	Zr	CD	Hg	TOTA L
Recent Mass %	36. 57	31.8 9	0. 1	0.1 1	0.9 5	1.1 4	0.0 6	1.5 1	26.3 4	0.1 4	0.0 6	0. 2	0.8 2	0.0 5	0.0 7	100

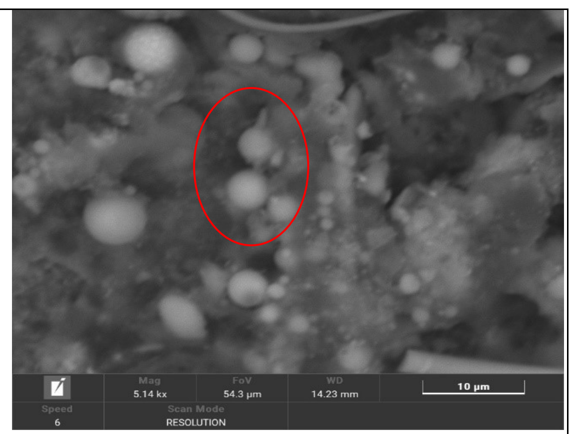
A high concentration of titanium (26.34%) is found, much more important than in the other samples, an observation that can be explained by the presence of the TiO_2-based dye in the composition of the paint, given the fact that the analyzed microparticle has the specific color of this oxide, respectively, white. The FTIR analysis confirmed the origin of the microparticle (paint), given the fact that a resin obtained by aldehyde polycondensation (generally between urea, or its derivatives, and formaldehyde) was identified. According to (Tagg et al., 2024), paints are considered to be secondary sources of MPs. Most likely, this microparticle comes from shipping, respectively from the paint on the surface of boats. According to the literature, paints used for ships are an important source of metal pollution, especially heavy metals (Cu, Zn, Pb), given the fact that they contain various metal-based compounds with an anti-corrosion or antifouling.

Particle B

Fig. 3.29 (a and b) illustrates the SEM images of particle B, with a degree of magnification 261x and a detail obtained with an amplification of up to 5140x.



(a)



(b)

Figure 3.29. SEM images of the B particle obtained at different degrees of amplification (a) 261x, (b) 5140x

From Fig. 3.29 a a relatively uniform structure can be observed, which when viewed in depth (Fig. 3.29 b) presents various cavities and the presence of spherical particles can be observed, which could be organic, inorganic or amorphous. Also, forms of degradation of the particle are observed. The irregular shape of the plastic particle, implicitly the sharp tips, can pose a danger to aquatic biota, causing damage and inflammation in the intestine, when ingested by fish (Choi et al., 2018). As regards the results obtained from the EDX analysis (Table 3.7), carbon and oxygen are found in the largest share, being the main components of plastics (Jeyasanta et al., 2023). Also, the presence of metallic elements Fe, Ti and Zr, as well as heavy metals, such as Cu and Hg, is observed, highlighting once again the role of microplastics as a transport vector for metals.

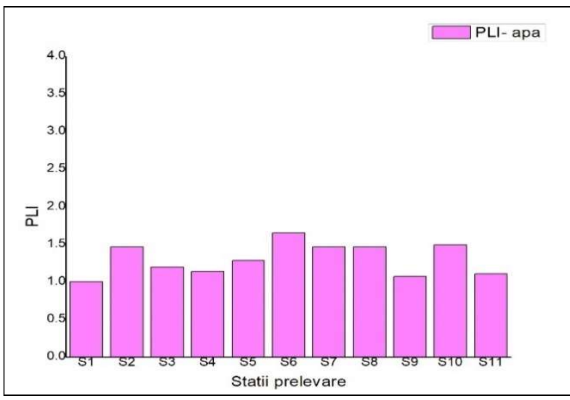
Table 3.3. EDX elemental composition of the B particle

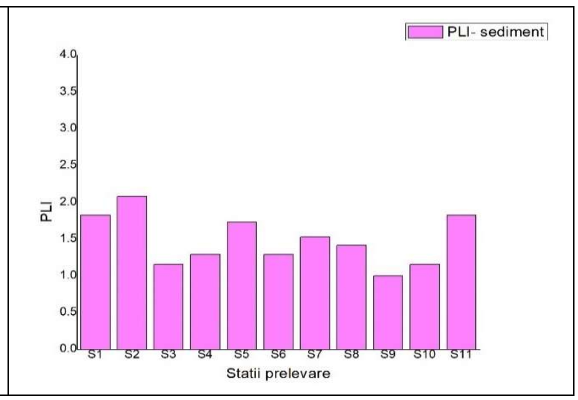
Element	C	A	Mg	Of	And	P	K	Like	Your	Fe	With	Zr	Hg	Total
Recent Mass %	48.66	31.83	0.36	1.18	2.37	0.2	0.24	11.06	1.51	1.67	0.58	0.22	0.1	100

3.5.3 Ecological risk assessment of microplastics

In order to assess the ecological risk regarding the presence of microplastics in the studied aquatic ecosystems, the following three indices were applied in the present doctoral thesis: Pollution Load Index (PLI), Hazard Index (HI) and Potential Ecological Risk Index (PERI) (Lithner et al., 2011). These indices were calculated for the first time based on the results obtained from the evaluation of the presence of microplastics in the water and sediment collected from the 11 sampling stations (S1-S11), located on the Lower Danube sector, the pre-deltaic and deltaic area.

The Pollution Load Index (PLI) was applied in this study to assess the level of pollution with MPs of the water and sediment of the Danube. Figures 3.30 a and b plot the results obtained from the calculation of the PLI index using the concentrations of microplastics in water (Fig. 3.30 a) and sediment (Fig. 3.30 b). For the Danube water, the values varied in the range 1 - 1.63, the highest value being recorded in the S6 station located near the mouth area of the Chilia Arm into the Black Sea, where the highest concentration of MPs was observed.



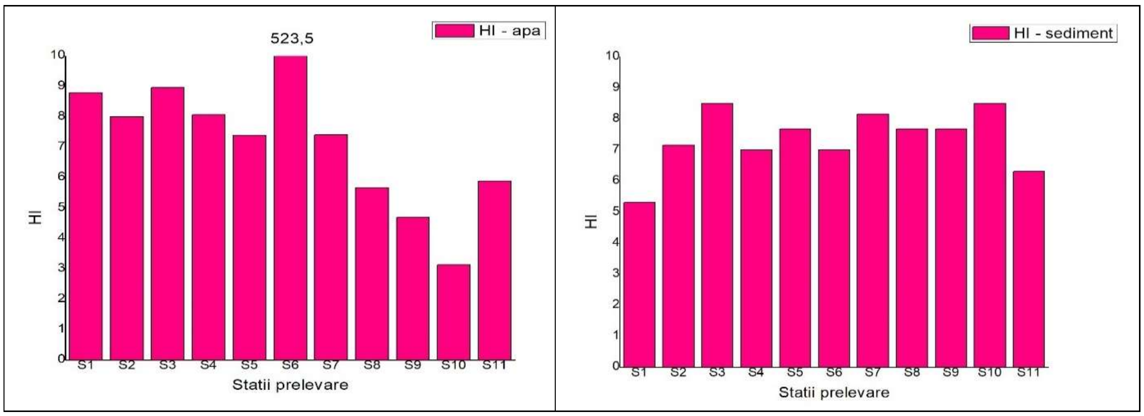


(a) (b)

Figure 3.30. PLI index values for water (a) and sediment (b)

According to the results obtained, the degree of pollution with MPs is low (level I), with values below 10 being obtained (see Table 2.10). As for the PLI results obtained for sediment, the values were higher than for water, being in the range of 1 - 2.08, the maximum value being recorded in the S2 station, located at the confluence of the Prut River with the Danube. However, even in the case of sediment, the level of pollution with MPs is low.

To assess the toxic potential of MPs in aquatic ecosystems, it is necessary to determine both the concentration and the chemical composition, respectively the type of polymers. The Hazard Index (HI) or the Polymer Hazard Index, as it is also known in the literature (PHI), is calculated based on the toxic potential of each polymer on the environment (Xu et al., 2018). Fig. 3.31 a and b plot the values of the HI index for water (Fig. 3.57 a) and sediment of the Danube (Fig. 3.31 b). The HI index recorded results in the range of 3.1 – 8.7, except for the value obtained in the S6 station (523.5). This particular case is due to the high toxicity score (Sn) of the ABS polymer found in the S6 station, which has the value 6552 (Table 2.11), compared to the other polymers (PE-11, PP-1, etc.). The results obtained corresponded to a medium level of toxicity (level II), except for the value obtained in the S6 station, which classified water with a high level of toxicity (level IV). In the sediment, the values of the HI index varied in the range 5.3 – 8.5, corresponding to the level II of hazard, due to the presence of the majority of polymers with a toxicity score of S_n lower (e.g. PE and PP).

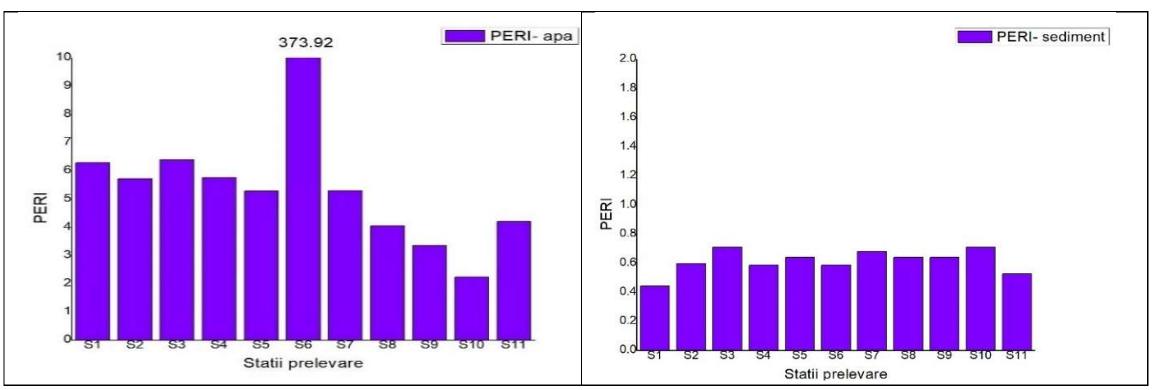


(a) (b)

Figure 3.31 HI index values for water (a) and sediment (b)

Unlike the other two indices used (PLI and HI), the Environmental Risk Assessment Index (PERI) takes into account both the effects produced by the abundance of potential microplastics in the environment and the toxicity of the polymers in their composition (Yin, 2023).

In Fig. 3.32 (a and b) the values of the PERI index for the water (Fig. 3.52 a) and sediment (Fig. 3.32 b) of the Danube are graphically represented. For water samples, the values of the PERI index varied in the range of 2.2 – 6.3, except for the value 373.92 recorded in the S6 station.



(a) (b)

Figure 3.32. Values PERI index for water (a) and sediment (b)

Analogous to the HI index, this result is due to the presence of the ABS polymer, in the calculation formula of this index being taken into account the Sn toxicity score, which for this polymer is high (6552). The results obtained correspond to a minor ecological risk in all stations, except for the S6 station, where the value exceeded the threshold of 300, the risk level being high (III). The values obtained for sediment were in the range of 0.4 – 0.7, the risk level according to these results being minor.

3.5.4 Partial conclusions

The results allowed the following partial conclusions to be drawn:

- The presence of MPs in the gills and gastrointestinal tract in the fish species *Alosa immaculata* from the Danube River was evaluated;
- The highest abundance of MPs was identified at the GIT level (4 particles per example) in 3 individuals. The average concentration of MPs in GIT was 1.35 particles per specimen;
- At the level of the gills, an abundance of MPs was reported lower than in the GIT, the average being 0.5 particles per specimen, the highest abundance being 2 particles per specimen;
- In the analyzed fish specimens, fragments and fibers with PE, PP and PES polymers were identified;
- The SEM-EDX technique was applied for morphological and elemental analysis of microplastics;
- The EDX results highlighted the presence of traces of toxic metals in the composition of microplastics, such as: Fe, Cu, Zn, Cd, Ni, Hg;
- The combined SEM-EDX analysis led to the confirmation of the hypothesis that microplastics affect the environment and human health, both directly and as transport vectors for polluting chemical species. Uneven structures affect the mechanical strength of these microparticles and create the conditions for partial decays and transformation into nanoplastics;
- The ecological risk of MPs in the water and sediment of the Danube was assessed by calculating the following indices: Pollution Load Index (PLI), Hazard Index (HI) and Potential Ecological Risk Index (PERI);
- According to the PLI index, the degree of pollution with MPs of the Danube water and sediment in the monitored sector is low (level I);
- The results of the HI index applied for microplastics in water indicated a medium level of toxicity (level II), except for the value obtained in the S6 station, which classified water with a high level of toxicity (level IV);
- In the sediment, the values of the HI index indicated a medium level of toxicity (II);
- The PERI index highlighted the presence of a minor ecological risk in all stations, except for the values obtained in station S6, the risk level being high (III);
- The PERI index values obtained for the sediment were corresponding to a minor risk level.

CHAPTER 4. Final conclusions, future research directions and personal contributions

The results obtained from the research carried out in this doctoral thesis led to the formulation of the following final conclusions:

- The industrial activities carried out in the cities bordering the Danube, namely Brăila, Galați and Tulcea, influence the quality of the Danube's water, with a lower level being observed in the sampling stations located near the areas with direct impact. Of course, the contribution coming from upstream of the direct observation area must also be taken into account;
- According to the WQI index, 53% of sampling stations were classified in quality class III (poor), while 47% were classified in quality class II (good). Compared to WQI, the results obtained by using WPI classified water strictly in quality class II. The CCME-WQI has classified the water collected from 98% of the locations in quality class II. The value of the WQI index, according to the algorithm applied and supported in publications by the team of researchers to which I belong, in the Lower Danube sector, has, most of the time, average values (categories II and III). These results are corroborated by the evaluations made by the three indices, respectively, PLI, HI and PERI, which in most cases support an average degree of pollution on the Danube with microplastics. Thus, the prospect of introducing MPs as integrated parameters in the WQI index is opened;
- The experiments developed in the present doctoral thesis managed to clarify some aspects related to the contribution of the riparian countries to the pollution of the Danube, resulting in the fact that there is, at least in the field of MPs, a significantly higher quantitative contribution,the concentrations of MPs observed in Moldova Veche were approximately 6 times higher than in Isaccea, and those of MaPs collected in Moldova Veche recorded concentrations approximately 10 times higher than in Isaccea. These results confirm the problem of the contribution of the riparian countries because they are much higher than the ratios between the flows recorded between the two observation points;
- MPs and MaPs are distributed in the cross-sections of the Danube according to parameters, such as the topography of these sections and the velocities in their different areas, thus it is found that the presence of MPs and MaPs was observed in all three cross-sections of the Danube. At Moldova Veche, the maximum concentrations of MPs and MaP were collected from the surface, from the proximity of the Romanian shore. In the Isaccea location, the highest concentration of MPs was observed near the fairway, and that of MaPs, on the surface, in the vicinity of the Ukrainian shore;
- The seasonal variation highlighted the dependence of plastic particle concentrations on the level of the Danube flow, the lowest concentrations being observed in the summer season, when the flow recorded the lowest values;
- From the analysis of the vertical distribution in the water column of the MPs, in most cases, the highest concentrations were observed in the surface layer of the water;

- Micro- and macroplastics showed chromatic diversity, and from the point of view of particle shape, fragments>films>lines>fibers>granules were collected in the order presented;
- The main sources of pollution identified are plastics manufacturing and processing industries, as well as industries where the use of plastics is secondary. Also, plastic waste (packaging, bags, containers) and domestic wastewater are important sources of pollution.
- The polymers identified in the samples collected from Moldova Veche and Isaccea were in the following order: PE>PP>PS>EPDM>ABS>CE>PUR>EVA;
- The presence of microplastics in the pre-deltaic and deltaic sectors of the Danube was assessed for the first time. The highest concentrations were collected near the mouth area of the Chilia Arm into the Black Sea (2.8 particles·m^{-3}) and at the confluence of the Prut River with the Danube River (2 particles·m^{-3});
- In the water column, in most stations, the highest concentrations were recorded on the surface. At a depth of 7 meters, the maximum value was identified on the Sulina arm, near Crișan locality (1.8 particles·m^{-3});
- Polymers identified in the composition of microplastics from the pre-deltaic and deltaic zone, in the following order: PE>PP>PES>PS>EPDM>ABS>CE. Also, from a morphological point of view, most of the MPs in the water were fragments>fibers>films;
- The presence of microplastics has also been observed in the sediment of the Danube. The highest abundance was found at the confluence of the Prut River with the Danube (52 particles·kg^{-1});
- The polymers identified in the MPs from the sediment samples were PE, PP, and PES;
- In general, a uniform distribution was observed in terms of the morphological characteristics of the MPs collected from the two verticals of the water and sediment column;
- The presence of MPs in the gills and gastrointestinal tract in the fish species Alosa *immaculata* from the Danube River was evaluated;
- The highest abundance of MPs was identified at the GIT level (4 particles per example) in 3 individuals. The average concentration of MPs in GIT was 1.35 particles per specimen;
- At the level of the gills, an abundance of MPs was reported lower than in the GIT, the average being 0.5 particles per specimen, the highest abundance being 2 particles per specimen;
- The SEM-EDX technique was applied for morphological and elemental analysis of microplastics. The EDX results highlighted the presence of traces of toxic metals in the composition of microplastics, such as: Fe, Cu, Zn, Cd, Ni, Hg;
- The combined SEM-EDX analysis led to the confirmation of the hypothesis that microplastics affect the environment and human health, both directly and as transport vectors for polluting chemical species. Uneven structures affect the mechanical strength of these microparticles and create the conditions for partial decays and transformation into nanoplastics;
- The impact of microplastics on the water and sediment of the Lower Danube was assessed, calculating the following indices: Pollution Load Index (PLI), Hazard Index (HI) and Potential Ecological Risk Index (PERI);

- According to the PLI index, the degree of pollution with MPs of the Danube water and sediment in the monitored sector is low (level I);
- The results of the HI index applied for microplastics in water indicated a medium level of toxicity (level II), except for the value obtained in the S6 station, which classified water with a high level of toxicity (level IV). In the sediment, the values of the HI index indicated a medium level of toxicity (II);
- The PERI index highlighted the presence of a minor ecological risk in all stations, except for the S6 station, the risk level being high (III). The PERI index values obtained for the sediment corresponded to a minor risk level;
- In the present study, three models (YOLOv8, YOLOv5 and Mask R-CNN) of Artificial Intelligence were used to automate the processes of classification and morphological characterization of MPs in the three classes (fragments, fibers and granules). The highest accuracies were obtained with the YOLOv5 model;
- We propose the introduction of the "area" indicator for the characterization of MPs.

Future research directions

- Extension of the study area to other aquatic ecosystems to obtain an overview of the presence of microplastics in the Danube River Basin on the territory of Romania;
- Analysis of the presence of microplastics in other fish species from the Danube and the Black Sea, both in organs and muscles;
- Carrying out tests to evaluate the toxicity of microplastics on the behavior of Zebrafish (*Danio rerio*), having the physiological parameters closest to those of humans;
- Analysis of the presence of microplastics in domestic wastewater and quantification of the contribution of the SEAU riparian to the Lower Danube area on the concentration of MPs in the river water.
- Evaluation of the interaction between microplastics and oxidants used in water disinfection processes in wastewater treatment plants (chlorination, UV exposure, etc.);
- Analysis of the presence of other organic pollutants (e.g. pharmaceutical compounds, pesticides, PAH) and pathogens on the surface of microplastics;
- Analysis of the concentration of nano- and microplastics in bottled drinking water, using gas chromatography with thermodesorption mode;
- Apparently, microplastics come from the interaction of different materials with water, thus, at the level of the research team there is a study initiated through which we have carried out sampling with systems for filtering and pumping particles from the air, to determine the weight by chemical species, of particles in the atmosphere. So far, the observations have been made by separating particles with a metallic component and ICP-MS determinations. In the next period, we will determine the presence of chemical structures of organic particles, including MPs;
- To evaluate in a controlled manner the accumulation of MPs in ichthyofauna, in the next period at the level of our team we will set up a natural laboratory (pond) supplied with water from the Danube, which will be populated with fish (broodstock) from areas independent of the Danube.

Personal contributions

Web of Science Indexed Scientific Articles

1. **Calmuc, M**., Calmuc, V., Arseni, M., Topa, C., Timofti, M., Georgescu, L.P., Iticescu, C., 2020. A *Comparative Approach to a Series of Physico-Chemical Quality Indices Used in Assessing Water Quality in the Lower Danube.* Water 12, 3239. https://doi.org/10.3390/w12113239; **IF 2.544**
2. **Madalina Calmuc**, Valentina Calmuc, Maxim Arseni, Ira-Adeline Simionov, Alina Antache, Constantin Apetrei, Puiu-Lucian Georgescu, Catalina Iticescu, *Identification and characterization of plastic particles found in the lower Danube river*, Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering 2022, Vol. XI, Print ISSN 2285-6064, 320-325
3. **Calmuc, M**., Calmuc, V.-A., Lazar, N.N., Arseni, M., Simionov, I.-A., Mihaela, T., GEORGESCU, P.-L., Iticescu, C., Study on microplastics occurrence in the lower Danube river water, Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XII, 2023 Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064 **IF=0.4**.
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5. Lazăr, N.-N., Călmuc, M., Milea, Ștefania-A., Georgescu, P.-L., Iticescu, C., 2024. Micro and nano plastics in fruits and vegetables: A review. Heliyon 10, e28291. https://doi.org/10.1016/j.heliyon.2024.e28291, **IF-3.4.**
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7. Calmuc, V.-A**., Calmuc, M.,** Arseni, M., Simionov, I.-A., Antache, A., Milea, Ștefania-A., Iticescu, C., GEORGESCU, P.-L., 2023. Spatial distribution of pharmaceuticals in the lower danube river water, 2023, Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XII, Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-513 **IF=0.4**
8. Simionov, I.-A., **Călmuc, M**., Iticescu, C., Călmuc, V., Georgescu, P.-L., Faggio, C., Petrea, Ş.-M., 2023. Human health risk assessment of potentially toxic elements and microplastics accumulation in products from the Danube River Basin fish market. Approximately. Toxicol. Pharmacol. 104, 104307. https://doi.org/10.1016/j.etap.2023.104307 **IF=4.3**
9. Milea, Ștefania-A., Lazăr, N.-N., Simionov, I.-A., Petrea, Ștefan-M., **Călmuc, M**., Călmuc, V., Georgescu, P.-L., Iticescu, C., 2023. Effects of cooking methods and co-ingested foods on mercury bioaccessibility in pontic shad (Alosa immaculata). Curr. Res. Food Sci. 7, 100599. https://doi.org/10.1016/j.crfs.2023.100599 **IF=6.3**
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11. Ira-Adeline SIMIONOV, **Mădălina CĂLMUC**, Alina ANTACHE, Valentina CĂLMUC, Ștefan-Mihai PETREA, Aurelia NICA, Victor CRISTEA, Mihaela NECULIȚĂ, The Use Of *Pectinatella Magnifica As Bioindicator Forbheavy Metals Pollution In Danube Delta*, Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering 2022, Vol. XI, Print ISSN 2285-6064, 320-325.
12. Alina ANTACHE, Valentina CALMUC, Stefan-Mihai PETREA, Ira-Adeline SIMIONOV, **Madalina CALMUC,** Aurelia NICA, Dragos CRISTEA, Mihaela NECULITA, *The influence of pharmaceutical residues from surface waters on fish oxidative stress: A review*, Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering 2022, Vol. XI, Print ISSN 2285-6064, 320-325.
13. Arseni, M., Rosu, A., Petrea, SM., **Calmuc, M**., Rosu, B., Constantin, DE., ; Iticescu, C., Georgescu, PL, *The positive effects of channels restoration in the Danube Delta Biosphere Reserve,* Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering 2022, Vol. XI, Print ISSN 2285-6064, p. 314-319.

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3. Calmuc, V.A**., Călmuc, M**., Arseni, M., Iticescu, C., Georgescu, L.P., 2020. *Preliminary statistical relationship between heavy metals in Lower Danube sediments* Annals of the "Dunarea de Jos" University of Galati. Fascicle II, Mathematics, Physics, Theoretical Mechanics 43, 62–67;
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6. Valentina Andreea Calmuc, **Madalina Călmuc**, Maxim Arseni, Catalina Iticescu, Puiu-Lucian Georgescu, Identification and quantification of pharmaceuticals in the Lower Danube River using UHPLC - Orbitrap Mass Spectrometer, The Tenth Edition SCDS-UDJG 2022,
7. Valentina CALMUC, **Madalina CALMUC**, Maxim ARSENI, Ira-Adeline SIMIONOV, Alina ANTACHE, Catalina ITICESCU, Puiu-Lucian GEORGESCU Assessment Of Water And Sediment Quality In Dunavăț- Dranov, Razim-Sinoie Aquatic Complexes, The International Conference Agriculture For Life, Life For Agriculture, June 2022.
8. Ira-Adeline SIMIONOV, **Madalina CĂLMUC**, Alina ANTACHE, Valentina CĂLMUC, Ștefan-Mihai PETREA, Aurelia NICA, Victor CRISTEA, Mihaela NECULIȚĂ The Use Of Pectinatella Magnifica As Bioindicator Forbheavy Metals Pollution In Danube Delta, The International Conference Agriculture For Life, Life For Agriculture, June 2022;
9. **Madalina CALMUC,** Valentina CALMUC, Maxim ARSENI, Ira-Adeline SIMIONOV, Alina ANTACHE, Constantin APETREI, Puiu-Lucian GEORGESCU, Catalina ITICESCU Identification and characterization of plastic particles found in the lower Danube river, The International Conference Agriculture For Life, Life For Agriculture, June 2022;
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12. **M. Călmuc**, V.A. Calmuc, M. Arseni, C. Apetrei, Lucian P. Georgescu, C. Iticescu, Application of ATR-FTIR spectroscopy for plastic debris identification in the Lower Danube water, The Ninth Edition of SCDS-UDJG, Galați, 10-11 June, 2021, poster presentation (3rd prize for poster presentation).
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15. **Madalina Călmuc**, Valentina Andreea Calmuc, Maxim Arseni, Lucian P. Georgescu, Catalina Iticescu, *Assessment of nutrients and oxygen regime in the Lower Danube water*,(poster presentation), Doctoral Schools Conference: 8th Edition of SCDS-UDJG, Galați, 18-19 June, 2020.
16. Valentina Andreea Calmuc, **Madalina Călmuc**, Maxim Arseni, Catalina Iticescu, Lucian P. Georgescu, *Preliminary statistical relationship between heavy metals in Lower Danube sediment,* (poster presentation)*,* Doctoral Schools Conference: 8th Edition of SCDS-UDJG, Galați, 18-19 June, 2020.

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18. Adrian Roșu, Maxim Arseni, Daniel Eduard Constantin, Mirela Voiculescu, Puiu Georgescu Lucian, Bogdan Roșu, **Madalina Calmuc**, Valentina Calmuc, Comparison of NO2 pollution level in Galați city before COV-19 and during the quarantine, (poster presentation), Conferința Escolalor Doctorale: 8th Edition of SCDS-UDJG, Galați, 18-19 June, 2020.
19. **Călmuc Mădălina**, Călmuc Valentina-Andreea, Iticescu Catalina, Georgescu P. Lucian, Timofti Mihaela, Arseni Maxim, Țopa Maria Cătălina, Roșu Adrian. *Assessing the lower Danube water quality using the Water Pollution Index,* The 20th Edition of the International Conference TEHNOMUS NEW TECHNOLOGIES AND PRODUCTS IN MACHINE MANUFACTURING TECHNOLOGIES, Section 3 Environmental engineering and sustainable development, Oral presentation no. 4, 2019. http://www.tehnomus.usv.ro/
20. Călmuc Valentina-Andreea, **Călmuc Mădălina**, Georgescu P. Lucian, Iticescu Catalina, Timofti Mihaela, Arseni Maxim, Țopa Cătălina, Roșu Adrian. *Spatial distribution of heavy metals in the Danube surface sediment near the Galati City.* The 20th Edition of the International Conference TEHNOMUS NEW TECHNOLOGIES AND PRODUCTS IN MACHINE MANUFACTURING TECHNOLOGIES, Section 3 Environmental engineering and sustainable development, Oral presentation no. 3, 2019, http://www.tehnomus.usv.ro/
21. Arseni Maxim, Rosu Adrian, Georgescu Puiu Lucian, Iticescu Catalina, Calmuc Valentina, **Calmuc Madalina**. *Impact of expansion and contraction cocoefficients on water surface profile,* The 20th Edition of the International Conference TEHNOMUS NEW TECHNOLOGIES AND PRODUCTS IN MACHINE MANUFACTURING TECHNOLOGIES, Section 3 Environmental engineering and sustainable development, Oral presentation no. 2, 2019, http://www.tehnomus.usv.ro/

Published Patents

1. I contributed to the elaboration of the invention patent **no. 134991 - Method and apparatus for collecting microplastics from rivers and lakes.**

List of projects implemented during the doctoral period

1. POC project "Integrated system for complex environmental research and monitoring in the area of the Danube River REXDAN", SMIS code 127065;
2. POIM project Improvement of hydrological conditions in the natural aquatic habitats of the Danube Delta Biosphere Reserve for the conservation of biodiversity and fishery resources - Gorgova-Uzlina, Roșu-Puiu lake complexes", POIM 120890;
3. POIM project "Improvement of hydrological conditions in the natural aquatic habitats of the RBDD for the conservation of biodiversity and fishery resources - Dunăvăț-Dranov, Razim-Sinoie lake complexes, Sinoie-Istria-Nuntași area";
4. POIM project, "Improvement of hydrological conditions in the natural aquatic habitats of the RBDD for the conservation of biodiversity and fishery resources - Șontea-Furtună, Matița-Merhei, Somova Parcheș lake complexes" SMIS Code 2014+ 120889
5. Project "HORIZON-MISS-2021-OCEAN-02, DANUBE REGION WATER LIGHTHOUSE ACTION", Project: 101094070 — DALIA

6. PNRR project "Integrated research and sustainable solutions to protect and restore Lower Danube Basin and coastal Black Sea ecosystems" ResPonSE 760010/30.12.2022.
7. HORIZON project "Restoration of wetland complexes as life supporting systems in the Danube Basin (Restore4Life)" financed from non-reimbursable European funds, financing contract no. 101112736/01.06.2023.
8. Qualitative and quantitative analysis of micro-plastics from solid samples (resulting from the collection of suspended solid particles) taken from flowing natural waters (Danube) – Beneficiary - Global Water Partnership Association in Romania, financing contract no. 787/29.03.2022 (April 2022-March 2023).
9. Contract with third parties, Analysis of total petroleum hydrocarbons (TPH) from soil samples no. 803/12.04.2023 concluded with S.C. X SERV S.R.L.
10. Project: 101156533 "Innovative sediment management framework for a SUstainNable DANube black SEa system" (SUNDANSE)— HORIZON-MISS-2023-OCEAN-01
11. Participation in training internship – In May 2022 I participated in a training internship at the INCDO-INOE 2000 institute, ICIA Analytical Instrumentation Research Institute Branch.

CHAPTER 5. Bibliography

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