MICROPLASTIC CHARACTERIZATION IN ROMANIAN COASTAL WATERS, WESTERN BLACK SEA

Teodora BABOȘ[1], Costantin LAZĂR[1,2], Oana DOBRE[1], Cornel POP[1], Iulian POJAR[1]

[1]National Institute of Marine Geology and Geo-Ecology (GeoEcoMar), 23-25 Dimitrie Onciul St., 024053 Bucharest, Romania
e-mail: teodora.babos@ geoecomar.ro
[2]University of Bucharest, Doctoral School of Geology, 1 Nicolae Bălcescu Blvd., Bucharest, Romania

DOI: 10.xxxx

Abstract. The pollution carried by the Danube is evident in coastal areas, given that the river mouths at the Black Sea shore can be considered as a significant pollution hotspots. This study focuses on microplastic (MPs) abundance and distribution in the coastal waters of the western Black Sea, with considerations regarding the source of the plastic litter. An important comparison was assessed between the Danube Delta front and adjacent areas of Romanian main harbors. A total of 6 water samples were collected from various locations, revealing an average concentration of 0.63 MPs/m^3, with the highest concentration near Sf. Gheorghe (1.37 MPs/m^3). Fragments and fibers were the most prevalent forms, with foils, spherules, and fiber clumps present in smaller percentages. Spectral analysis indicated that the predominant MP types were polyethylene, polypropylene, ethylene, and polyvinyl, suggesting diverse origins such as packaging materials, textile products, and fishing gear. This research emphasizes the importance of thorough monitoring and a more comprehensive approach to the negative impact at the regional scale.

Key words: Microplastics, Black Sea, Pollution, Danube, Surface water

1. INTRODUCTION

Plastic contamination is a critical issue in every water environment throughout the world (Coleman and Wehle, 1984; Ivleva *et al.* 2017). Despite the huge reusage potential of these synthetic products, improper plastic dumping and poor collecting management is allowing plastic litter to enter and spread into the marine environment. Depending on their sizes, plastic waste can be divided into macro- (≥5 mm), micro- (<5 mm) and nano-plastics (<1000 nm). Over the past years, the scientific interest for microplastics has been increasing as microplastic contamination has become one of the most significant pollution issues of the present (Hu *et al*., 2019). Microplastics are defined as solid polymeric plastic-based materials with 2 types of origins: primary, manufactured in cosmetics, abrasion, cleaning industries, and secondary, fragments of most macro and mezo-plastics man-made (Siegfried *et al*., 2017). These particles are extremely widespread (Ahmad *et al.*, 2020), and they have harmful effects on water, sediments, and especially on animals and humans (Galloway, 2015). Recently, microplastics have been found in all oceanic zones, including remote places like the Arctic (Obbard *et al.* 2014) or Antarctica (Lusher, 2015).

According to Alexanderov *et al.* (2017) and Slobodnik *et al.* (2017), the Black Sea basin has a large drainage area of about 2.5 million km^2 divided into 107 sub-basins that cross more than 20 nations (Slobodnik *et al.*, 2017) on the European and Asian continents. The main catchment areas responsible for the plastic exported into the Black Sea are three major transboundary rivers: Danube, Don, and Dnieper (D'Hont *et al*., 2021). In addition, it was calculated that 4.2 tons per day of input from coastal industries, urbanization, agriculture, and diffusive sources enter the Black Sea *via* the Danube River (Lechner *et al.*, 2014). Besides studies addressing microplastics, there has been an increasing focus on microplastics in the Black Sea since 2014, particularly emphasizing the presence and abundance of microplastics at the water's surface. Therefore, as indicated by the article conducted by Bat and Öztekin (2020), a relatively larger number of studies

have reported the presence of microplastics at the surface compared to the water column and sediments of the Black Sea, a trend also confirmed by our results.

The Danube River was identified as one of the most significant sources of microplastics in the Black Sea (Miladinova *et al.*, 2020). The influx of microplastics from land-based sources has been estimated at 4,100 tons per year (Miladinova *et al.*, 2020). Van der Wal *et al.* (2015) calculated an annual input of microplastics from the Danube to the Black Sea of about 2 trillion particles weighting 500 tons. Siegfried *et al.* (2017) determined an annual export rate of 1503 tons of microplastic from the Danube to the Black Sea, accounting for 37% of total microplastics inflow. In addition to the Danube's discharges, high inputs of microplastic were found on the southwest coast of Turkey (Istanbul), the Azov Sea, the Dnieper and in the Rioni Rivers (Siegfried *et al.*, 2017). Plastic contamination in the marine environment is very widespread, however scientific data about the presence and characterization of the microplastic in the surface waters of the Black Sea coasts are still lacking (Berov and Klayn 2020). As the Black Sea features a unique environment characterized by an anoxic zone commencing at depths of approximately 150-170 meters, which restricts aerobic life, the contamination of the oxygenated water layer poses an even greater threat. Therefore, prioritizing the monitoring of contaminants in such an ecosystem is crucial for effective risk assessments. The objective of this study is to assess the concentration of microplastics in the surface water of the Romanian Black Sea inner shelf.

2. MATERIALS AND METHODS

In April 2023, a total of six water samples were collected from diverse locations within the coastal waters of the western Black Sea. These specific sampling sites included Sulina, Sf. Gheorghe, Mamaia, Constanta and Eforie localities (Fig. 1), providing a comprehensive representation of the region's environmental conditions and enabling a thorough examination of microplastic presence and distribution patterns.

Fig. 1. Sampling sites in the Western Black Sea coastal waters. Image source: www.openstreetmap.org.

The water sampling from the surface of the Black Sea, from the mentioned locations, was carried out using a Neuston filet (Hydro-Bios, 200µm). These water samples were collected using a motorized boat, and for the sampling it was necessary to release the net and move it at least 200 m to filter at least 100 m^3 of water for a good representation of floating microplastics.

For each sample gathered during our fieldwork, precise data points including the GPS location coordinates, time and date of sampling, and specific depth at which the sample was taken were recorded, forming a comprehensive dataset as detailed in Table 1.

Table 1. Collecting details of the water samples.

Sample name	Date and hour	Depth (m)	Coordinates start		Coordinates stop		Index Flowmeter		Water volume calculated (m^3)
			Long.	Latit.	Long.	Latit.	Start	Stop	
SUL01A	19.04.2023; 17:15	2,00	29°43,661′	45°08,013′	29°43,602′	45°07,651′	26700	28801	588,28
SFG04A	22.04.2023; 14:15	3,00	29°37,549′	44°53,437′	29°37,948′	44°52,114′	28802	30081	358,12
SFG05A	22.04.2023; 14:40	5,00	29°43,661′	44°52,146′	29°37,569′	44°53,361′	30081	31354	356,44
EFO01A	24.04.2023; 10:25	5,00	28°38,617′	44°04,545′	28°39,028′	44°04,521′	31356	32671	368,20
MAM01A	24.04.2023; 10:14	9,30	28°38,646′	44°13,500′	28°38,995′	44°13,526′	32671	33994	370,44
CTA01A	24.04.2023; 11:39	5,00	28°39,631′	44°11,163′	28°39,982′	44°11,161′	33994	34439	124,60

Also, the materials used for sampling, as well as those intended for their storage, were glass, metal, textile material or wood.

2.1. Sample preparation

The analytical procedures were performed in the microplastic laboratory located in GeoEcoMar headquarter (Bucharest, Romania). The water samples were filtered through a metal sieve with the same mesh size as the filet used for collecting, in order to achieve a minimum volume. The sample processing involved organic digestion, which was carried out using a mixed solution of potassium hydroxide (KOH, 10 M) and hydrogen peroxide (H_2O_2, 30%). The digestion process typically lasts for approximately 5-7 days, during which the glass container, undergoes continuous agitation using an oscillatory shaker, as outlined in the methodology (Ehlers *et al*., 2019; Scherer *et al*., 2020). To achieve a secure filtering procedure, the samples pH was neutralized using formic acid (HCOOH, 95%) and distilled water. The resulted mixtures from each sample were filtered through 4.7 mm, 1.2 µm fiberglass membranes using a vacuum pump and a three-post stainless-steel Buchner system.

2.2. Visual and polymeric evaluation

The visual examination of microplastics (MPs) was conducted using a stereomicroscope (Leika EZ4W), resulting in a quantitative evaluation, and providing details on the morphology, color, and dimensions of the MPs. This examination procedure is described by Hidalgo-Ruz *et al.* (2012).

For polymeric studies we performed spectrometric analysis (RAMAN Craig Apollo-M) to obtain information about the types of microplastics. The used laser beam is confocal, being an instrument that collects VIS-NIR range Raman spectra from solid samples. It is equipped with a 785 nm laser and a spectrometer system with a maximum output power of 80 nW and a bandwidth of <0.02 nm. The microscope includes three objectives: a 10X VIS-NIR BF Objective (EC-Epi), a 20X VIS-NIR BF/DF objective (EPI-Neo), and a 50X VIS-NIR BF/DF objective (Epi-Neo). For instrument control, sample spectrum acquisition, image analysis, and spectral data analysis, we used the 64-bit software (Lambdafire-R Raman Spectrometer Control & Analysis Software). After obtaining the Raman spectra, we employ the data into the database (KnowItAll Spectral Database Search Software, Wiley) that contains 25,000 searchable Raman spectra.

2.3. Quality assurance

Prior to sample preparation sessions, the working environment was been cleaned with ethanol to prevent sample contamination. For laboratory processes, glass and stainless-steel tools and materials were used and operators wore cotton lab coats throughout both laboratory work and microscopic studies. In addition, during the chemical digestion and storage, all sample beacons and recipients used for solution storage were covered with aluminum foil/ parafilm. All glassware and other utensils needed on the field and laboratory were rinsed before using with distilled water.

3. RESULTS AND DISCUSSIONS

During our analysis, we successfully identified and quantified a total of 1392 microplastic particles (MPs) across all the samples under examination. We categorized the plastic particles into two groups (smaller than 1 mm and bigger than 1 mm) to help us get a better overview of the MPs types. Therefore, the number of MPs < 1 mm is 874 while the number of MPs > 1 mm is 518. These numerical values represent the entirety of particles, unequivocally identified as being plastic-made, characterized by their distinctive color, morphology, and external features. The amount of microplastic particles smaller than 1 mm is usually greater than the number of those larger than 1 mm due to several reasons, such as environmental processes and the frequent breakdown of larger plastics into smaller ones. From a morphological perspective, it was observed that the most prevalent form was fragments, with fibers closely following as the second most dominant category. Concerning the colors observed among the MPs during our examination, blue was the most predominant color. This specific category constituted a substantial proportion, totaling nearly 50% of the identified particles (Fig. 2)

Following the thorough quantitative visual assessments of the water samples, an investigation of the pollution levels in the sea water unveiled a decrease in pollution in the areas neighboring the towns of Constanta and Mamaia. In the regions surrounding Sulina and Eforie localities, the pollution levels were found to be at an average level, whereas the area adjoining the town of Sf. Gheorghe exhibited a notably higher degree of pollution.

By processing the samples taken from the surface of the sea water, an average concentration of 0.58 particles per m^3 of water was identified. Low concentrations were recorded in the area adjacent to the municipalities of Sulina, Constanta, and Mamaia (<0.5 MPs/m^3), average concentrations have been identified in the areas adjoining the locality of Eforie (0.5 – 1 MPs/m^3). As for the areas belonging to Sf. Gheorghe (Fig. 3), one of the samples showed an average concentration (0.5 – 1 MPs/m^3) while the other one recorded high concentration (> 1 MPs / m^3) (Table 2). The discrepancy between the two samples can be attributed to various environmental and local factors, such as different sampling locations or proximity to pollution sources.

In contrast to the MPs concentrations examined by Pojar *et al.* (2020) in three geographically similar areas, namely Sulina, Sf. Gheorghe, and Constanta localities, our study found substantially lower concentrations. In the study carried out by Pojar *et al.* (2020) the quantitative results showed an average concentration of 7 MPs/m^3, while present results showed an average concentration of 0.63 MPs/m^3.

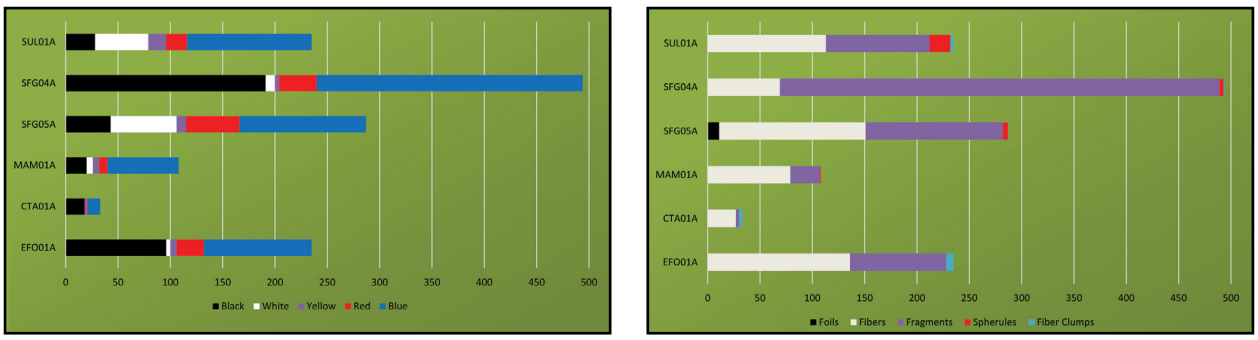

Fig. 2. Quantitative and Qualitative Assessment: colors (left) and morphology (right) of the identified MPs particles.

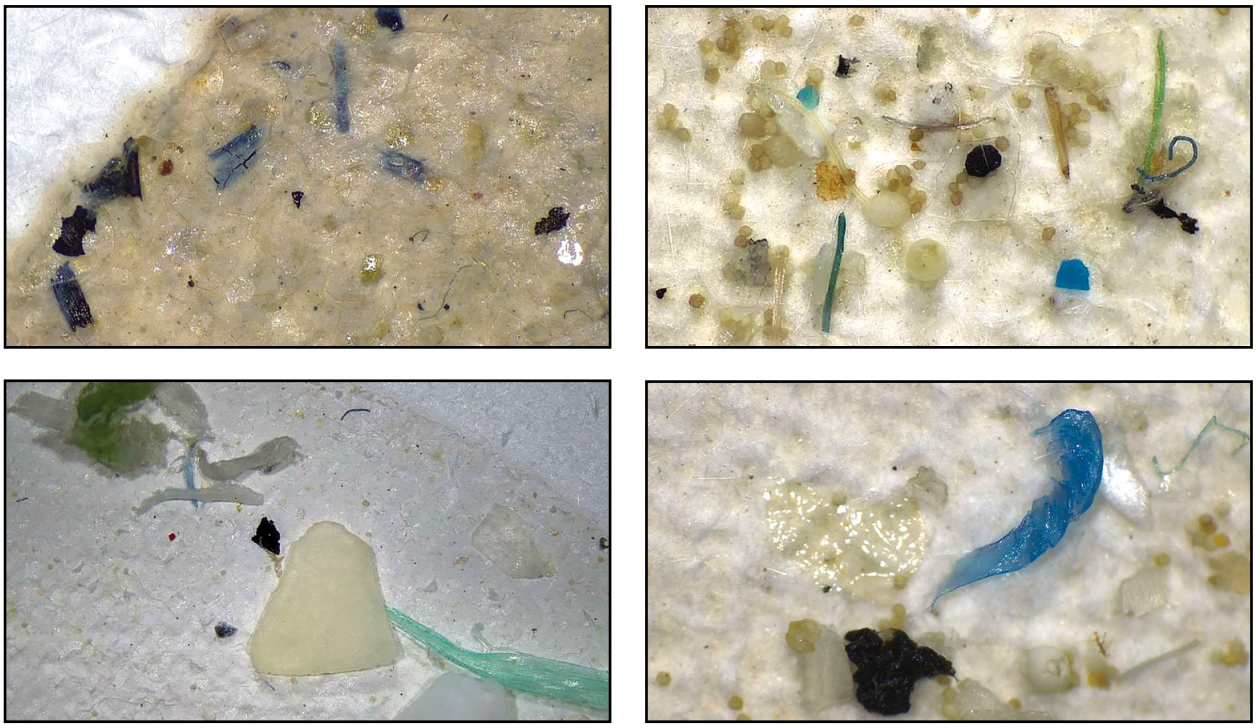

Fig. 3. Pictures taken at the microscope with microplastic particles from water samples collected in the Sf. Gheorghe area.

Table 2. Abundance and morphology of the MPs particles identified in the water samples.

Sample	Foils	Fibers	Fragments	Spherules	Fiber clumps	Total MPs	Water volume (m^3)	MPs/m^3
SUL01A	0	113	99	20	3	235	588,28	0,399
SFG04A	0	69	420	4	1	494	358,12	1,379
SFG05A	11	140	131	5	0	287	356,44	0,805
CTA01A	0	27	3	0	3	33	124,6	0,264
MAM01A	0	79	28	1	0	108	370,44	0,291
EFO01A	0	136	92	0	7	235	368,2	0,638

The major difference in the quantity of MPs identified is considered to be a result of the choice of the time period for field work. The samples from Pojar *et al.* (2020) were collected in August, while the samples from the current study were collected in April, before touristic season began.

For qualitative analysis we carefully chose various MPs from our samples, with a focus on fragments and fibers. Using the RAMAN microspectrometry, we were able to successfully identify certain polymers that were present in the surface waters we collected (Fig. 4).

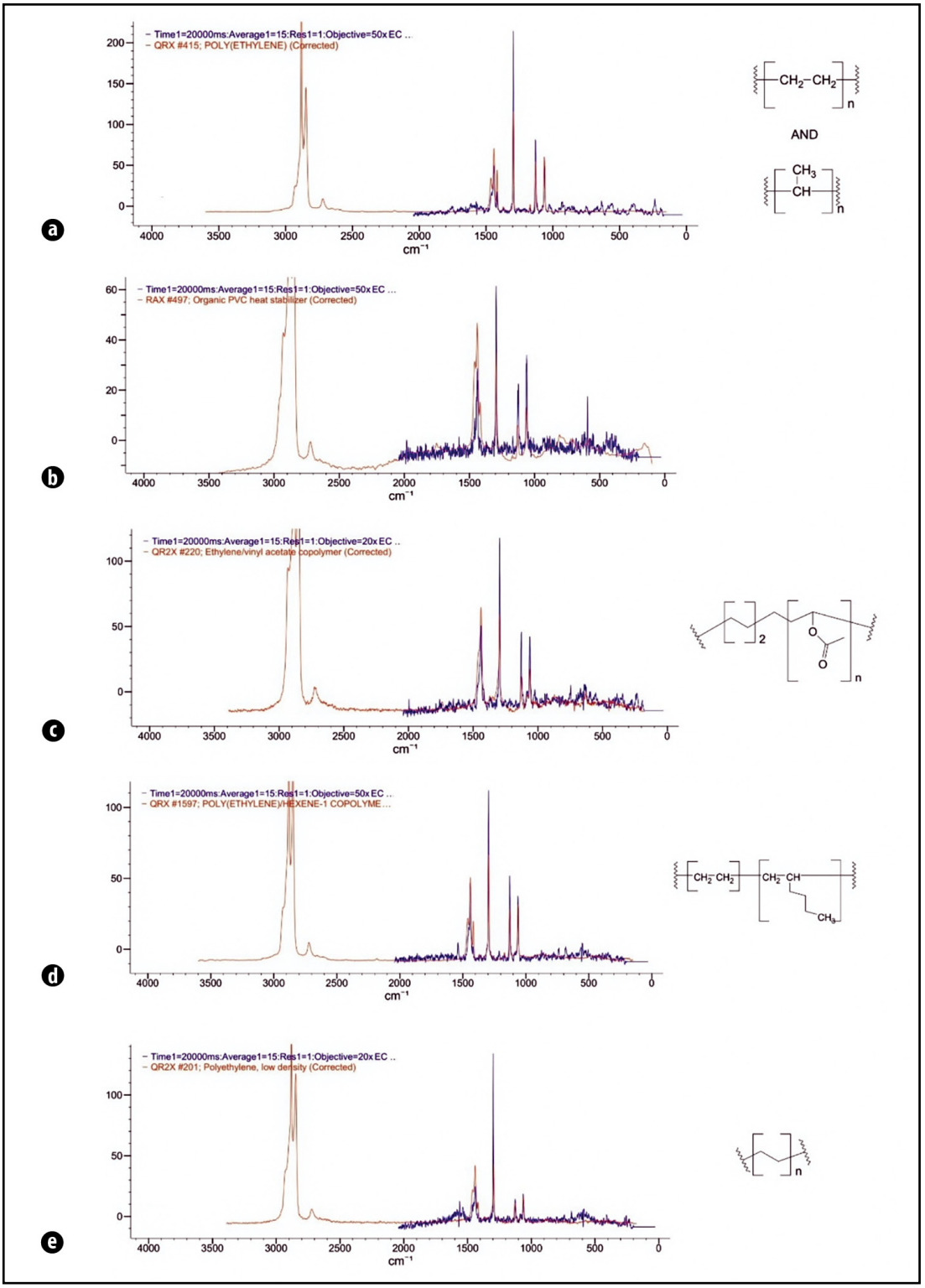

Fig. 4. Examples of Raman microspectrometry results: (**a**) Polyethylene; (**b**) Organic PVC heat stabilizer; (**c**) Ethylene; (**d**) Polyethylene; (**e**) Polyethylene; the x axis represents the wavenumber (cm^{-1}) and the y axis represents the intensity of the scattered light.

The overall results derived from our MPs analysis, reveal that most of the MPs we investigated are distinctly classified as types of Polyethylene (Fig. 4 a, d, e). However, we also observed occasional encounters of alternative polymers, including but not limited to polypropylene, ethylene, and polyvinyl, indicating a diverse composition of MPs within the samples. In addition to identifying the polymers themselves, we also successfully detected the presence of stabilizers or fillers (Fig. 4b). These substances are commonly employed during the primary processing stages to enhance the properties of the polymers while they are in their molten state (Munier and Bendell, 2018).

The MPs observed in our study exhibit potential origins from various sources, which may be one of the following: the Danube origin, local sources, or other external contributors.

The particles originating from the Danube follow a trajectory wherein they are discharged into the river and subsequently transported towards the Black Sea basin. Along this journey, spanning both river and marine environments, these particles undergo a process of accumulation, gathering significant quantities of pollutants (Issac and Kandasubramanian, 2021). This accumulation is a direct consequence of their extended exposure to the surrounding environmental conditions. Particles with local origins are typically emanated from nearby sewage systems and wastewater treatment plants. In such instances, MPs tend to absorb relatively small quantities of other pollutants compared to their counterparts from different sources. Additionally, the category of "other sources" refers to MPs that are either transported by marine currents, originate from the atmosphere or resulted from the deterioration of hull paint coatings on vessels.

The findings from our analysis provide evidence that the MPs encountered in the samples have multiple origins. These particles have most likely undergone weathering processes and have been shaped by a confluence of factors, including the cumulative impact of UV radiation, elevated temperatures, and mechanical forces (such as wind and waves). Heightened temperatures activated higher resistivity and cleavage formation leading to degradation, and influencing the biological activity within the aquatic environment (Shah *et al.*, 2008).

Polypropylene (PP) primarily originates from a range of sources, packaging materials, fishing gear, and agricultural films being among the key contributors to its presence in the environment. Low-density polyethylene (LDPE) finds extensive application in agriculture, where it serves multiple purposes, such as foils for protecting crops, enhancing soil temperature, and conserving irrigation water within soil layers. However, over time, these plastic materials tend to degrade and fragment into smaller pieces due to brittleness. Consequently, these fragments can make their way into soil layer and, under certain conditions like local or seasonal flooding, enter the water bodies, contributing to environmental dispersion (Lambert *et al.*, 2014; Rillig, 2012). Low density or high-density polyethylene, polyethylene and polypropylene materials can experience increased brittleness in specific locations due to elevated dissolved oxygen levels, which heighten the oxidation processes within those sites (Singh and Sharma, 2008).

4. CONCLUSIONS

To close the knowledge gap and advance our understanding of plastic pollution, this study provides a small preliminary account of the abundance and characteristics of plastic particles from surface waters of the western Black Sea and the eastern surroundings of the Danube Delta.

The results reveal a MPs concentration average of 0.63 MPs/m^3 across the 6 samples that were analyzed, with the highest concentration of MPs found in a sample from Sf. Gheorghe with 1.37 MPs/m^3. Unexpected decreased quantitative results were found in the proximity of Constanta urban area and harbor, as the MPs concentrations identified at the Danube mouths were much higher.

The most widespread forms were fragments, closely followed by fibers, while foils, spherules and fiber clumps were also found but in a smaller percentage. As our sampling was done in April, it can be assumed that a higher concentration may occur after the touristic season ends. The spectral results suggest that the MPs were mainly composed of polyethylene, polypropylene, ethylene and polyvinyl, indicating that the MPs encountered in the samples have multiple origins, such as: packaging materials, fishing gears or foils for protecting crops.

Considering the limited number of samples collected from the sites, we strongly recommend conducting a more comprehensive monitoring of the study area. Additionally, it is advisable to explore a more complex approach to sample acquisition.

ACKNOWLEDGEMENTS

The financial support was provided by the National Core Programme PN 23 30 01 02, No. 4N/30.12.2022 and the Project Research of Excellence AMBIACVA, No. 23PFE/30.12.2021 of the Romanian Ministry of Research, Innovation and Digitization (MCI).

REFERENCES

Ahmad, M., Li, J.L., Wang, P.D., Hozzein, W.N., Li, W.J. (2020). Environmental perspectives of microplastic pollution in the aquatic environment: a review. *Marine life science & technology*, **2**(*4*): 414-430.

Aleksandrov, B., Dykyi, E., Fabianova, M., Guchmanidze, A., Komorin, V., Oswald, P., Pavlovska, M., Pogojeva, M., Savenko, O. Slobodnik, J. (2017). Joint Black Sea Surveys: 12 facts about the Black Sea. UNDP, European Commission.

Aytan, Ü., Pogojeva, M., Simeonova, A. (2020). Marine Litter in the Black Sea. *Turkish Marine Research Foundation* (TUDAV), **56**.

Oztekin, A., Bat, L., Baki, O.G. (2020). Beach litter pollution in Sinop Sarikum Lagoon coast of the southern Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences,* **20**(*3*): 197-205.

Berov, D., Klayn, S. (2020). Microplastics and floating litter pollution in Bulgarian Black Sea coastal waters. *Marine Pollution Bulletin*, **156**: 111225.

Coleman, F.C., Wehle, D.H.S. (1984). Plastic Pollution: A worldwide oceanic problem. *Parks*, **9**(*1*): 9-12.

D'Hont, A., Gittenberger, A., Leuven, R.S., and Hendriks, A.J. (2021). Dropping the microbead: Source and sink related microplastic distribution in the Black Sea and Caspian Sea basins. *Marine Pollution Bulletin*, **173**: 112982.

Ehlers, S.M., Manz, W., Koop, J.H. (2019). Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly Lepidostoma basale. *Aquatic Biology*, **28**: 67-77.

Galloway, T.S. (2015). Micro- and nano-plastics and human health. *In*: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing: 343-366.

Hu, D., Shen, M., Zhang, Y., Li, H., Zeng, G. (2019). Microplastics and nanoplastics: would they affect global biodiversity change? *Environmental Science and Pollution Research*, **26**: 19997-20002.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science & Technology*, **46**(*6*): 3060–3075.

Issac, M.N., Kandasubramanian, B. (2021). Effect of microplastics in water and aquatic systems. *Environmental Science and Pollution Research*, **28**(*16*): 19544-19562.

Ivleva, N.P., Wiesheu, A.C., Niessner, R. (2017). Microplastic in aquatic ecosystems. *Angewandte Chemie International Edition*, **56**(*7*): 1720-1739.

Lambert, S., Sinclair, C.J., Boxall, A.B. (2014). Occurrence, degradation and effect of polymer – based materials in the environment. *Review of Environmental Contamination Toxicology*, **227**: 1-53.

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., Schludermann, E. (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution*, **188**(*100*): 177-181.

Lusher, A. (2015). Microplastics in the marine environment: distribution, interactions and effects. In: Bergmann, M., Gutow, L., Klages, M. (Eds.). Marine Anthropogenic Litter. Springer International Publishing: 245-307.

Miladinova, S., Moy, D.M., Stips, A., Garcia-Gorriz, E. (2020). Microplastics accumulation on the Black Sea coast: Scenario analysis. In: Aytan, Ü., Pogojeva, M., Simeonova, A. (Eds.). Marine Litter in the Black Sea. *Turkish Marine Research Foundation (TUDAV)* **56**: 218-235.

Munier, B., Bendell, L.I. (2018). Macro and microplastics sob and desorb metals and act as point source of trace metals to coastal ecosystems. *PLoS One*, **13**(*2*): e0191759.

Pojar, I., Kochleus, C., Dierkes, G., Ehlers, S. M., Reifferscheid, G., Stock, F. (2021). Quantitative and qualitative evaluation of plastic particlesticles in surface waters of the Western Black Sea. *Environmental Pollution*, **268**: 115724.

Rillig, M.C. (2012). Microplastic in terrestrial ecosystems and the soil? *Environmental Science Technology* **46**: 6453-6454.

Siegfried, M., Koelmans, A.A., Besseling, E., Kroeze, C. (2017). Export of microplastics from land to sea. A modelling approach. *Water research*, **127**: 249-257.

Singh, B., Sharma, N. (2008). Mechanistic implications of plastic degradation. *Polymer degradation and stability*, **93**(*3*): 561-584.

Shah, A.A., Hasan, F., Hameed, A., Ahmed, S. (2008). Biological degradation of plastics: a comprehensive review. *Biotechnology advances*, **26**(*3*): 246-265.

Slobodnik, J., Alexandrov, B., Komorin, V., Mikaelyan, A.S., Guchmanidze, A., Arabidze, A., Korshenko, A. (2018). VII. Descriptor 10: Particlest I - Marine litter. National Pilot Monitoring Studies and Joint Open Sea Surveys in Georgia, Russian Federation and Ukraine, 2017. Final Report on EU/UNDP Project "Improving Environmental Monitoring in the Black Sea–Phase II (EMBLAS-II)": 573.

Van der Wal, M., van der Meulen, M., Tweehuijsen, G., Peterlin, M., Palatinus, A., Kovac Viršek, M. (2015). SFRA0025: C. Report for Michail Papadoyannakis. *DG Environment*, United Kingdom: 186.