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Laboratory Assessment for Determining Microplastics in Freshwater Systems– Characterization and Identification along Somesul Mic River

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Abstract: Microplastics (MPs) pollution has become a persisting problem over the last decades and a critical issue for environmental protection and human health. In this context scientific data able to reveal the MPs presence and to improve the characterization and identification in different systems is valuable. The aim of this paper was to assess available techniques for determining MPs in real freshwater samples and subsequently to highlight the occurrence and type of MPs in the study case area (Somesul Mic River). The specific objectives of the study were: i) MPs separation and visual characterization; ii) microscopic analyses and morphological characterization of MPs; and iii) Raman and FT-IR spectroscopic identification of MPs. The MPs sampling was performed from the fresh water and sediment using planktonic nets and sieves with different mesh sizes (20 to 500µm). After digestion with hydrogen peroxide, the MPs characterization was performed using both classical microscopic techniques as well as scanning electron microscopy (SEM). For the MPs identification, Raman and FT-IR spectrometry techniques were used. Large (1-5 mm) and small (1 µm to 1 mm) MPs were observed in the shape of fibers, fragments, foam, foils and spheres in various colors (red, green, blue, purple, pink, white, black, transparent, opaque). Polymers were identified related to scientific literature and reference spectra. The presence of polyethylene (PE), polypropylene (PP) and polystyrene (PS) was registered for all sampling point. The MPs laboratory investigations have raised some issues regarding the identification of MPs particles with the size smaller than 500µm, being characterized especially under microscope. Small MPs particle dispersed on cellulose filter were identified using micro-Raman spectroscopy highlighting the same type of polymers. The results showed that both spectrometric methods Raman or FT-IR confirm the identification of the same type of polymers. No differences were registered between the sampling points due to the widespread presence of MPs. The sediments samples presented a greater abundance compared to the water samples. Overall, it is necessary to continue the optimization of the MPs separation protocol and identification according to the complexity of samples, mainly due to the limitation and lack of spectral databases.

Keywords: microplastics; polyethylene; polystyrene; polypropylene; characterization; identification; FT-IR; Raman; Somesul Mic River

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1. Introduction

Plastic pollution is considered one of the main problems of our century, being a worldwide concern. Plastics are different polymers based on synthetic compounds with very large structures that confer different abilities to be molded and shaped (such as thermoplastics: PE and PS) [1,2]. Due to the large utility of plastics and poor waste management, it finally leads to the contamination of most environment systems [3–6]. Aquatic systems are the most affected ones. Research media and also the global organization raise alarm signals concerning the accumulation of plastics in the oceans, seas and freshwater systems. According to Standard Project no. ISO/DIS 24187:2021(E), the results of plastic degradation in environment or in wastewater treatment systems are macroplastics (above 5 cm), small macroplastics (5 mm to 5 cm), large microplastics (1 mm to 5 mm), microplastics (1μm to 1 mm) and nanoplastics (less than 1 μm) [7]. The principal sources of MPs are: the breakdown of plastic products such as packaging materials, the microspheres in personal care products (detergents, exfoliants, toothpaste, sunscreens), fibers from synthetic textiles, tire wear microparticles, city dust (resulted from plastic litter fragmentation), electrical and electronics materials, building and construction sector, agriculture process. Other sources are the municipal/industrial sewage even if treated in wastewater treatment plants (WWTP) as these are not designed to properly retain microplastics [8,9].

The situation of plastic pollution tends to escalate, and even if plastic use would be stopped immediately, it will continue to persist due to the very low capacity of environmental factors and microbiome to biodegrade these polymers. The problem became even more critical with the increase of plastics use in our urge to cope with the protection requirements imposed by COVID-19 pandemic (gloves, gowns, masks, glasses, etc.). The International Solid Waste Association estimated 250-300% more consumption of single-use plastic materials during the pandemic, and the World Health Organization (WHO) estimated a consumption of 89 million medical masks per month [10]. In 2019, the global production of plastics reached almost 370 million tons. In Europe, the production of plastics has reached almost 58 million tons [11]. If current production and waste management trends continue, approximately 12 billion tons of plastic waste will end up in landfills or in the natural environment by 2050 [12].

Pollution with plastic/microplastic materials has often been reported in worldwide rivers (Rhine, Danube, Elbe, Yellow River, Amazon) [13–17] or other freshwater systems also used as drinking water sources [18–20]. The abundance of MPs in freshwater systems has been reported to vary depending on sample source in ranges from 10^{-3} to 10^{3} particles/L [21] or to over 10^{4} particles per liter with particle size distribution of 95% in the size range of 6.5 and 100 μm [22], to absolute concentrations of 0.008mg/L to 0.039mg/L [23]. The abundance of MPs in sediments, has been reported to be less than 87 particles kg^{-1} in Danube River- Iron Gate, from 87 to 165 particles kg^{-1} in Danube Delta, 630 particles kg^{-1} in marine sediment of Black Sea [24,25]. Some prospective studies showed that, in certain conditions of low flow and sandy sediment, MPs build up in concentrations of 3.6 to 10.7 particle/L in water and 360 to 1320 items/Kg in sediment, higher concentrations being registered in the samples collected from sites neighboring densely populated areas or extensive agricultural planting areas [26].

The global annual leakage of macroplastics increases from 19.4 Mt in 2019 to 38.4 Mt in 2060, while the leakage of microplastics doubles, to reach 5.8 Mt in 2060 [27].

According to PlaticsEurope statistics (2022), in 2021, the global plastics production reached 390.7 million tones, with polypropylene (PP) and polyethylene (PE) being the most demanded polymer types. Approximately 19.3% was PP, more than 20% was PE (low and high density), 5.3% was PS (polystyrene) and 6.3% was PET (polyethylene terephthalate). These plastics were predominantly used in diverse activities, especially packaging and construction activities. The demand for PP and PET increased compared to 2016, while the demand for PE and PS remained constant or slightly decreased. However, only 10% of these plastics were recycled, and only 1.5% bio-based plastics were produced [28].

Most studies have reported the occurrence of common synthetic polymers such as PE, PP and PS among others in surface waters [15,29,30]. The composition of microplastic pollution can vary in

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terms of polymer types, colors, and additives used in plastics, and of course depending on the sources and local factors. For example, in urban areas, microplastics from personal care products and synthetic textiles may be more prevalent, while in coastal regions, microplastics from degraded fishing nets and maritime debris can be significant contributors. It is important to note that microplastics are found in various including oceans, freshwater bodies, soils, and even the atmosphere [31,32]. The distribution and composition of microplastics can be influenced by local pollution sources, ocean currents, and human activities. For almost 10 years now, even in apparently non-polluted areas far from the civilization, scientific reports have revealed the occurrence of PP, PE and PS, as the most prevalent MPs in the surface water or sediment. As an example, a study of Enders in 2015 on a transect from the European Coast to the North Atlantic Subtropical Gyre using an underway intake filtration technique showed that more than half (52%) of MPs were PE (42%), PP (6%) and PS (4%) while 11% PA- polyamide, 3% PU – polyurethane, 1.8% PVC- polyvinylchloride, and 6% PES – group of polyester, 0.4% PMMA - polytetrafluoroethylene and 26.3% were unidentified polymers [33].
Moreover, 93% PE, 4% PP, 2% poly(1-Cl-1-butenylene) polychloroprene, 0.5% melamine-urea resin, and 0.5% cellulose (rope fiber) of MPs were reported in Antarctic fur seal scats., [34]. In the Danube River the most important river in Romania, PP and PE have been reported as the main MP pollutants, probably due to their various applications, especially in the packaging industry [25].

Recent studies focusing on the assessment of MPs effects/impact on living organisms, including human health showed that the main hazards with microplastics in the environment, especially aquatic ecosystems, are: physical effects, bioaccumulation, desorption and toxicity of pollutants, leaching and toxicity of additives and monomers, transport of invasive species [20]. Moreover, if we consider the absorption potential of the surface area of the MPs the concern on potential environmental and health impact increases as they would become carriers of other potential more harmful pollutants. A series of polluting chemical compounds such as metals, pesticides or pharmaceutical substances can be absorbed on their surface, thus increasing their toxic effects [11].

Some statistical studies have reported the presence of MPs in different food sources like meat, seafood, drinking bottled and tap water, beverages, and also in sugar, salt, honey and milk [8,20,35]

The small size and diversity of MPs physical and chemical properties raise problems in their characterization and detection from environmental samples and biota. Currently, the recommended technique for the morphological characterization of MPs are visual analysis, classical microscopic investigation (applied especially for large particles 1-5 mm) and also SEM-scanning electron microscopy analysis (applied for small particles <1mm). For the MPs identification are use the spectroscopy investigations applied even for 1 mm particle size. According to the literature the MPs detection methods have advantages and limitations, some are time and cost consuming. There is a lack of harmonized procedures starting to sampling and sample preparation to detection analyses. Another inconveniences are the human errors and lack of expertise. Micro-Raman or FT-IR spectroscopy, although expensive, are automated systems recognized as more reliable, precise and could contribute to reducing both the analysis time and human error, respectively [36–38].

The aim of the present study was to assess available techniques for MPs detection and to reveal the presence and type of MPs in freshwater system of Romania (study case Somesul Mic River, section Floresti-Cluj-Napoca). The specific objectives of the study were: i) the sampling of freshwater (surface water and sediment), isolation and visual characterization of MPs; ii) microscopic characterization (size, type, shape, color) and iii) Raman and FT-IR spectroscopic identification.

2. Material and methods

2.1. Experimental design

To highlight the freshwater contamination with MPs, a sampling campaign was initiated in the North-West area of Romania in September 2022. The Somesul Mic River runs through the city of Cluj-Napoca and its outskirts, including the town of Floresti. Four sampling points were selected along of river: upstream (UP) and downstream (DW) of Floresti (sampling point code: UP Floresti and DW

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Floresti), and upstream and downstream of the Cluj-Napoca Wastewater Treatment Plant (sampling point code: UP Cluj-Napoca WWTP and DW Cluj-Napoca WWTP) (Figure 1).

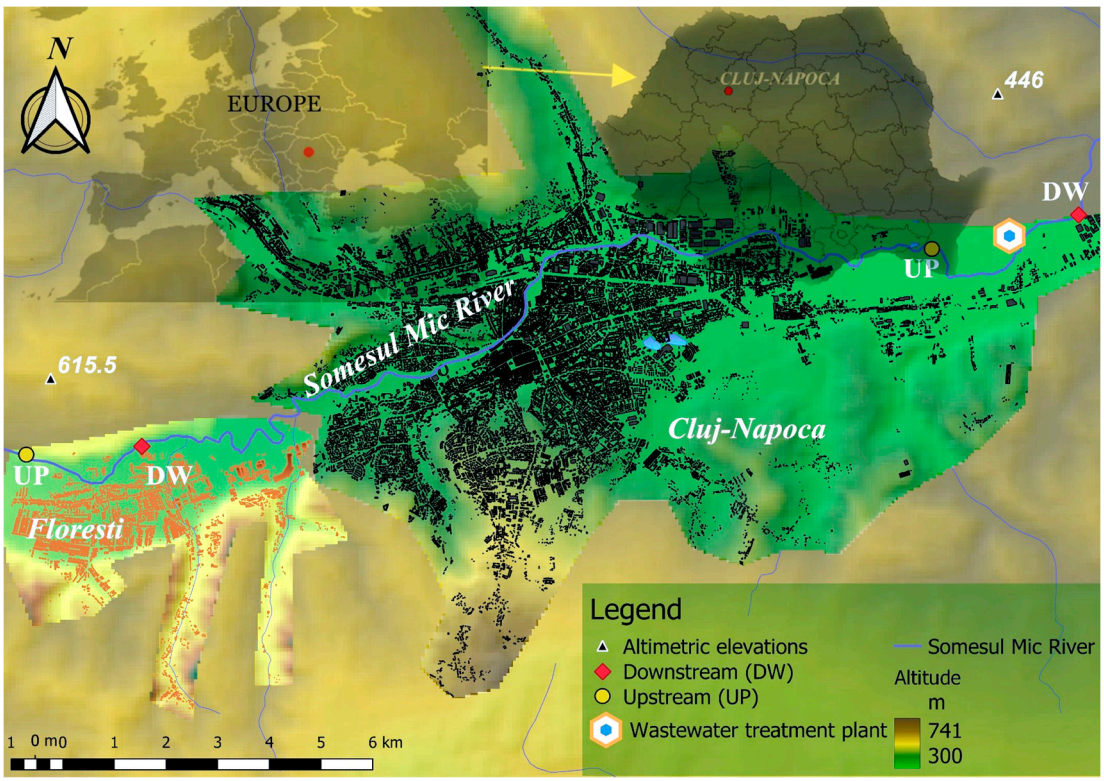

Figure 1. Map of sampling points: UP Floresti and DW Floresti and UP Cluj-Napoca WWTP and DW Cluj-Napoca WWTP (Google maps and QGis -3.26 Buenos Aires Maps Program).

The Somesul Mic River has an average flow rate of 21.2 m^2/s, passes through densely populated areas and flows into the Mures River, an important cross-border water body with a route to Hungary and discharges into the Tisa River. The investigated area represents an urban environment with industrial, zoo technical, and tourist activities. The city of Cluj-Napoca (46°46′0″N 23°35′0″E) covers an area of 179.5 km^2 and has a population of 324,576 inhabitants, while the metropolitan area includes 410,766 inhabitants. Possible sources of plastic pollution in the area may include construction sites, improperly stored household waste, economic activities, and the city wastewater treatment plant. The Cluj-Napoca was chosen as the study area based on both its ranking in terms of quality of life (Cluj-Napoca ranked 43rd out of 91 European cities studied at the beginning of 2021) and the dynamics of the city's economy compared to the national level. Floresti city (46°44′52″N 23°29′27″E) covers an area of 61 km^2, with a population approx. 50,000 inhabitants growing due to expansion, being a suburb of the Cluj city with ongoing real estate development. Potential sources of plastic pollution in Floresti include construction sites, household waste, and economic activities (https://ro.wikipedia.org).

The sampling sites were chosen along the river's direction, as they are geomorphological favorable for sediment deposition. Also, the sampling points selection was based on the inspections of the left bank of the river (depending on the area accessibility) and possible sources of contamination with plastic materials. Additionally, the presence of houses in the area, waste deposits on the banks, construction sites, or proximity to the city's purification station were taken into consideration. Visual inspections of the riverbanks revealed the presence of plastic waste in the selected areas, which was mostly of household or construction origin (Figure S2).

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2.2. Sampling technique

EPA Microplastics Sampling Protocol [39] and Rocha International Sampling Guide [40] and other review studies on sampling, pretreatment and analysis of MPs [6,41,42] were consulted for the organization of the sampling campaign. The sampling protocol was adapted also based on the requirements of ISO/DIS 24187:2022 [7].

For each sampling site, three different sample units of surface water and sediment were collected.

The surface water samples were collected directly from the river and concentrated by filtration using two methods: ***1)*** by passing around 100 L of water using a 10 L bucket through the plankton nets with mesh sizes of 20 and 200 µm (Pokorny Site, Nitex, Czech Republic) and then individually collected in glass vials of 100 ml; **2)** by placing the nets against the direction of river flow for 30-40 min depending on fields accessibility (estimated passing volume being about 120 L/s considering the flowing river rate, the time of execution and the nets volume) and afterwards, collected in glass vials of 100 ml.

Sediment samples were collected from five random points along the bank from a quadrat measuring 30 x 30 cm and 5 cm deep, with the aid of a shovel, passed through granulometric sieves (40, 250 and 500 µm), washed for mud, stones and plants remove and then the material retained on the sieves was collected individually in glass containers (about 200 grams per sample). The sieved sediment was subsequently washed again in laboratory.

In addition, samples of floating and visible MPs were collected from a perimeter of 1x1 m square in order to be characterized (Table 1). The square area was established according to the accessibility and included also the bank. The floating visible plastics were collected from flowing river, from the bank as well as from the washed sediment samples. The small fragments (<30 mm) were transported to the laboratory.

Table 1. Field data - visually characterization of floating plastics and other wastes.

Sampling point	Type	Dimension	Color	No. total of particles	Classification
UP Floresti left bank, low water level, clear water, waste island on the right bank, flow speed approx. = 0.58 m/s	Fragment	>30 mm	black, green, blue	5	Macro
	Fragment - foil	≥20 mm	white, green/blue	2	Macro
	Sphere	<5 mm	red, brown	2	Large
	Fragment	~10 mm	white-gray	1	Meso
	Foam	~5 mm	yellow	1	Large
	Polystyrene pieces	>10, 20, 30 mm	white	3	Meso / Macro
	Non–plastics and other wastes				
	Surgical mask fragment / cellulose	>30 mm	green	1	-
	Fragments of aluminum packaging	>50 mm	blue	1	-
	Electronic parts	20 mm	white	1	-
	Aluminum plugs	~20 mm	gray	2	-
	Textile	>30 mm		1	-
	Plastic coated wire	~ 2, 10, 12cm	gray, white, blue	3	-
	Construction materials (brick, cement, etc.)	-	-	abundantly	-
DW Floresti	Fragment- foil	10 mm	transparent	1	Meso
	Fragment	30 mm	transparent	1	Meso

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left bank, under construction, cloudy water flow rate approx. = 0.40 m/s	Sphere	<5 mm	green	1	Large
	Polystyrene foam	5 mm	white	5	Large
	Foam / sponge	>30 mm	yellow	1	Macro
	Fragment	>30 mm	blue	1	Macro
	Plastic toy piece	> 30 mm	red	1	Macro
	Non–plastics and other wastes				
	Construction materials	-	-	abundantly	-
UP Cluj- Napoca WWTP left bank low level, flow rate approx. = 0.64 m/s	Polystyrene spheres, foam	<5 mm	white	abundantly	Large
	Pieces of polystyrene	>20 mm	white	abundantly	Meso
	Fragment	<5 mm	white	1	Large
	Fragment	~3, 5, 10 mm	white	3	Large / Meso
	Non–plastics and other wastes				
	Cigarette butts	>10 mm	brown	1	Meso
	Plastic waste from toys and packaging	>10 cm >30 cm		2	Macro
	Pet bottles	-	-	1	-
DW Cluj –Napoca WWTP left bank, there is waste on the bank, bags and foils under sediment and stones flow rate approx. = 0.62 m/s	Fragment	>30 mm	transparent	1	Macro
	Fragment	~20 mm	black	1	Meso
	Polystyrene foam spheres	~5 mm	white	1	Large
	Pieces of polystyrene	~5 mm	white	>10	Large
	Non–plastics and other wastes				
	Textile	>30 mm	-	-	-
	Construction material	-	-	abundantly	-

Note: Macro – macroplastics; Large – large microplastics; Meso – Mesoplastic; Micro - microplastic.

The same sampling methods (water and sediment) were applied in parallel for all locations. The sampling time allocated for each point was 1-1.5 hours including all sampling procedures. These activities were carried out to capture MPs less than 5 mm as best as possible.

All necessary measures were taken to prevent possible contamination from the equipment and tools used for sampling. To eliminate contamination, the use of textiles or other plastic materials was avoided as much as possible, and for the washing was used distilled water and the samples were immediately covered and transported at 4°C.

2.3. Laboratory materials and methods

The laboratory processing of the samples collected from the field, respectively the stage of MPs separation, involved the use of conventional laboratory glassware made of glass or aluminum, sterile ester cellulose filter membranes 0.45 µm (Merck KGaA, Darmsadt, Germany), separation funnels, Petri dishes, oven Binder ED 115 (Roth, Karlsruhe, Germany), vacuum/pressure pump (Millipore, Guyancourt, France), stirrer with heating, distilled water. Reagents: hydrogen peroxide H_2O_2 30%, NaCl sodium chloride and $FeSO_4 \cdot 7H_2O$ iron sulfate heptahydrate, KO_3P potassium metaphosphate, 96% sulfuric acid (all purchased from Sigma-Aldrich, USA).

Standard polymers: polyethylene (PE) CAS 9002-88-4, white color, particles size 1 mm and 40-48µm, density 0.92 g/ml; polypropylene (PP) (CAS 9003-07-0, colorless, particle size 3 mm, density 0.9 g/ml; and polystyrene (PS) CAS 9003-53-6, colorless beads, particle size 3 mm, density 1.06 g/ml, provided by Sigma-Aldrich, Saint Louse, USA.

To avoid complementary contamination with MPs, the handling of the samples was carried out in an exhaust hood, and the samples were covered and kept in glass Petri dishes. Blank sample of

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filter membrane, ambient air and distillate water were performed. The tests did not reveal background contamination suspected to be MPs, thus the field data did not take into consideration the blank.

Method of plastic particles separation

The first stage of MPs laboratory investigation was the separation of particles from the water and sediment samples by digestion and the adjustment of liquid density with NaCl. The method was adapted from the work procedure "Laboratory methods for the analysis of microplastics in the marine environment. Recommendations for the quantification of synthetic particles in water and sediment" [43].

The method involves the digestion of organic matter by subjecting the collected samples to wet peroxide oxidation (Fenton reaction) in the presence of Fe(II) catalyst. After digestion, the MPs from the mixture were separated with NaCl by flotation. Floating particles were isolated, either by using separator funnels or by vacuum filtration. According to literature reports, the method does not affect the structure of plastic particles and can be successfully used to prepare samples for further determination of polyethylene, polypropylene, polystyrene and polyvinyl chloride [44,45]. Microscopic analysis of standard PE, PS and PP treated in the same conditions with 30% H_2O_2 revealed that the particles are not affected by the oxidizing treatment (data not shown).

The water samples (200-500 ml) were subjected to evaporation 24 h, 90°C in a drying oven. After complete cooling, they were treated with 20 ml of 0.05 M $FeSO_4 \cdot 7H_2O$ (7.5 g, 278.02 g/mole in 500 ml of distilled water and 3 ml of 96% sulfuric acid).

The wet sediment samples (400 g) were again washed with distilled water and sieved to remove detritus and the mud, and the floating MPs were retained. Then, they were subjected to evaporation. The collected sediment was treated with 400 ml of KO_3P (oven-dried beforehand, 5.5 g per KO_3P per liter distilled water), stirring for 1 h and addition of 20 ml of 0.05 M $FeSO_4 \cdot 7H_2O$. Depending on sample complexity, sieving (40 and 250 μm pore size) was required once more.

In case of both matrices (water and sediment), 20 ml 30% H_2O_2 was added, followed by thermic treatment at 75 ^oC, for 5 min. If the organic matter persists, the step was repeated by adding another 20 ml of 30% H_2O_2. Supplementation was necessary only in the case of sediment samples. The resulting mixture was opaque brown in color.

After H_2O_2 treatment, it was added NaCl (6 g per 20 ml mixture, concentration 5 M) to increase the density of the aqueous solution. The resulting mixture was transparent and yellow. Each obtained mixture was transferred to separation funnels, or to a laboratory funnel or was filtered on filter membranes (0.45 μm), after settling for 24 hours. The separation was completed by taking the supernatant in glass ampoules or filtering the supernatant on filter membranes. The filters were transferred to Petri dishes at room temperature. Then, the samples were subjected to microscopic examination.

Depending on the complexity of the samples, the separation also involved additional stages as: washing the filters with plenty of distilled water to reduce the formation of salt crystals, and washing and centrifugation to concentrate the particles from the aqueous solutions, as well as collecting visible MPs.

Using the same method, two samples of waste water from the Cluj Napoca WWTP were analyzed, without concentration (because greater incidences of MPs were expected), in order to highlight the contribution of the treatment plant to the contamination with MPs.

Microscopic investigations of MPs

The second stage involved microscopic characterization, and finally, the identification of MPs.

Classical microscopic analysis (Motic optical microscope, 10X and 40X objectives) (Motic, Germany) of MPs samples highlighted particles suspected to be microplastics. In addition, Leica M205FA stereomicroscope (Germany) was used for macro/microplastics observations. The presence and physical characteristics of MPs (color, shape and size) was acquired with Leica Application Suite V4.12 image software.

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The SEM-scanning electron microscopy analysis (Thermofisher Scientific, SEM Quanta FEG 250) was used to evaluate the size and morphological characteristics of the microplastics. All samples were analyzed in LoVac (low vacuum) mode. In this type of working mode water vapors are pumped into the microscope chamber in order to preserve the samples. Also, the SEM investigations were carried out using an Everhart–Thornley Large Field Detector (LFD). This type of detector is ideal for general imaging due to the fact that this detector contains BSE (backscattered electrons) and SE (secondary electrons). All the filter membranes containing the investigated samples, were cut into small square pieces with an approximate size 1x1 cm^2. After that, the samples were mounted on stab holders which were previously covered with double sided conductive adhesive carbon tape.

Methods for MPs identification

The MPs identification was made using two complementary spectrometric techniques Raman and FT-IR, using different available equipment. Because of no availability of polymers database, for the identification of MPs spectra we used three reference polymers (that are most frequently found in surface waters: PE CAS 9002-88-4, PP CAS 9003-07-0, and PS CAS 9003-53-6) or the literature data.

1. Raman analysis - Raman-HR-TEC-785 Spectrometer

The samples were analyzed on Raman-HR-TEC-785 Spectrometer (StrellarNet Inc., Carlson Circle Tampa, Florida) equipped with the Raman Probe-785 featuring integrated optical fibers and laser excitation system with a wavelength of 785 nm. The wavelength range is 200-2700 cm^{-1}. The optical resolution is 4 cm^{-1}. The diffraction grating 1200 g/mm with gold coating. Data processing and obtaining spectra were carried out with the help of SpectraWIZ Software, SpectraWiz-ID, LabView, Spectroscopy Pro-tools.

2. Raman analysis - NRS-7200 Raman Spectrometer

Polymer particles isolated on filter membranes were analyzed using the system NRS-7200 Raman Spectrometer B000461423 with microscope (JASCO INTERNATIONAL CO., LTD. Tokyo, Japan). Raman spectrometer resolution 0.7 cm-1, detector 4-Stage Peltier Cooled CCD Detector (UV-NIR range, 1024 × 255 pixel), spectral range: 5 to 8000 cm-1 General measurement information: exposure time 30 sec, accumulation 10, 20, laser wavelength 785 and 530 nm, laser power 5.6 and 56.6, objective lens 10X. The spectra were collected using JASCO Canvas Program.

3. µRaman analysis - LabRam HR800 system

The µRaman analysis were performed for the samples filtered on gridded cellulose ester membrane after the separation process. The Raman studies of MPs samples were conducted at room temperature by confocal µRaman Spectroscopy using a LabRam HR800 system (Horiba, Kyoto, Japan). All the Raman spectra were generated within 100-4000 cm^{-1} spectral range by exposing the specimens for ~200s (in total; 40s/spectral window) to a 2mW, 632nm wavelength red excitation laser using a 50x long working distance objective. The signal emitted by the samples was dispersed onto a cooled CCD detector down to -70 °C, using a 600 lines/mm grating with a spectral resolution of 0.6 cm^{-1}. All Raman spectra were collected using the LabSpec software.

4. FT-IR - Cary 630 FT-IR Spectrophotometer

The infrared spectra were recorded with a Cary 630 FT-IR Spectrophotometer (Agilent Technologies, USA) based on pellets formation with KBr (2 mg sample + 200 mg KBr, pelletizing with a hydraulic press of 7-9 tons, vacuum 2 min) in the range 400-4000 cm^{-1}. Standard spectra were obtained for polyethylene and polystyrene using MicroLab Pharma Software.

5. ATR – FTIR measurements - Perkin – Elmer Spectrum Two IR spectrometer

The ATR – FTIR measurements of samples were conducted under ambient conditions using a Perkin – Elmer Spectrum Two IR spectrometer (Spectrum TWO DTGS 102767 series). All spectra were recorded in the wavenumber range between 400 and 4000 cm^{-1} at room temperature using a DTGS detector. Each ATR – FTIR spectrum is the average over 10 scans, using air as reference, and 2 cm^{-1} as nominal spectral resolution (PerkinElmer, USA).

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3. Results and discussion

3.1. Characterization of floating plastics particles

According to specialized literature [41] we noticed that most of the plastics collected in the field, analyzed visually, are included in the group of mesoplastics < macroplastics < large microplastics (Table 1). No quantitative method was applied due to the short execution time. Table 1 – refers to field data - visually characterization of floating plastics (>5 mm) and other wastes (found in the established perimeter or in the washed sediment), in order to reveled the source of MPs.

The visual analysis of the samples filtered on filter membranes highlighted the presence of particles of various sizes, possibly MPs (Figure S3). In all the samples analyzed, fragments of different colors (red, blue, white), fibers and spheres of polystyrene type foam were highlighted. The most visible particles were highlighted in the case of sediments determined on the 250 and 500 μm sieves. MPs less than 5 mm such as fragments and spheres were cleaned with ethyl alcohol and distilled water to remove impurities for SEM and Raman analysis.

Depending on the type, the MPs exhibit regulated and unregulated shapes such as pellets, fragments, fibers, foam and spheres. Fragment and foam types were frequently observed in the collected samples. Regarding coloration, the MPs varied: white, transparent, black, red, brown, green, gray and blue. The most prevalent were the white foam spheres which are suspected to be PS.

In the wastewater influent from the local WWTP, a visually abundance of MPs particles < 5 mm was observed (Figure S4). Consequently, the urban wastewater discharges contain polymers reach in the aquatic environment. The polymer contamination depending on the retention capacity of the wastewater treatment processes. Some studies reported the presence of MPs in WWTP influents and effluents, with removal rates ranging from 20% to 90 %. The size of MPs ranging from 1μm to 2000μm, appearing on various forms (mainly fibers and fragments), and colors (predominantly white, blue, green, black, red, yellow, and transparent). Similar results were reported also by other studies [46].

These MPs were identified especially as PE, PET, PS, PVC, Polyamide and Polycarbonate [47].

3.2. Microscopic analysis of separated MPs

Using the optical microscope (10X-40X objective), particles suspected to be MPs in the form of fragments and fibers were observed. These particles were collected with the help of planktonic nets with mesh size of 20 and 200 μm. It was found that the polymers are integrated in the aquatic system, attached to organic waste or filamentous algae, and were easily confused with the carcasses/remains of organisms (especially in case of the transparent fragments) (Figure 2). Therefore, their characterization and identification require stages of removal of organic matter and the separation of the polymers.

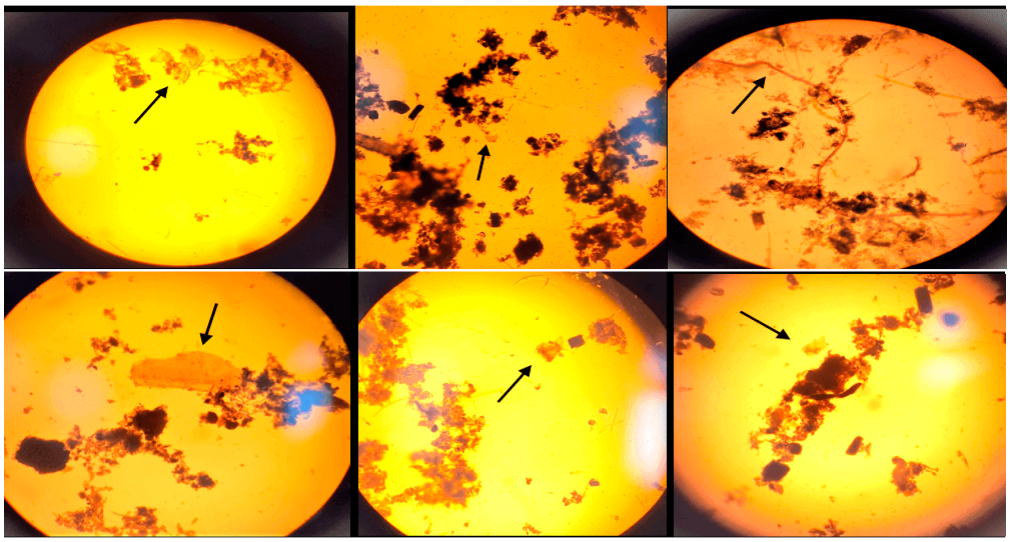

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Figure 2. Examples of microscopic visualization of particles suspected to be MPs (indicated by the black arrow) from surface waters concentrated through 20 μm nets in Floresti area. The picture was taken before the digestion of organic matter (10x-40x magnifications).

Laboratory experiments revealed that oxidation with 30% H_2O_2 completely destroyed the part of organic matter. The applied method was robust, being suitable for large MPs particles. Some tests performed on the reference MPs highlighted the fact that the particles can be separated (data not shown), but they are no longer found in the same amount at the end of the analysis, because are lost during the transfer stages, a fact also confirmed by the literature [48]. A better recovery was observed in the case of direct transfer to the filter membrane without using another intermediate glassware. Another interference is given by NaCl crystals, which can be eliminated by successive washings with distilled water. When filtering on the filter membrane, issues arise with detritus and residues of oxidized matter that make the visibility of transparent MPs particles, confusing and difficult. Through the classical microscopy method, the MPs particles less than 1 mm were separated and subsequently microscopically characterized and identified.

In all analyzed samples (sediment, surface water and wastewater) the stereomicroscopic analysis (Leica M205FA stereomicroscope) revealed the presence of MPs in different shapes, colors and sizes (Figures 3–18). The most common types of MPs were the fibers (e.g., Figure 3), appearing in a range of colors including white, blue, purple, pink, orange, black. These fibers measured between 100 μm to over 5 mm, and were more abundant in sediment samples. These fibers, with their low density, were associated with materials such as polyester, nylon, rayon, or polypropylene. The prevalence of fibers aligns with observations made by other authors [49] using the stereomicroscope, who found similar fibers in sizes less than 2 mm, from synthetic textiles as possible sources. It is supposed that the fibers are more dangerous for aquatic animals because are more difficult to be eliminate from digestive tracts compared to the fragments or spherical MPs, leading to a prolonged exposure [50].

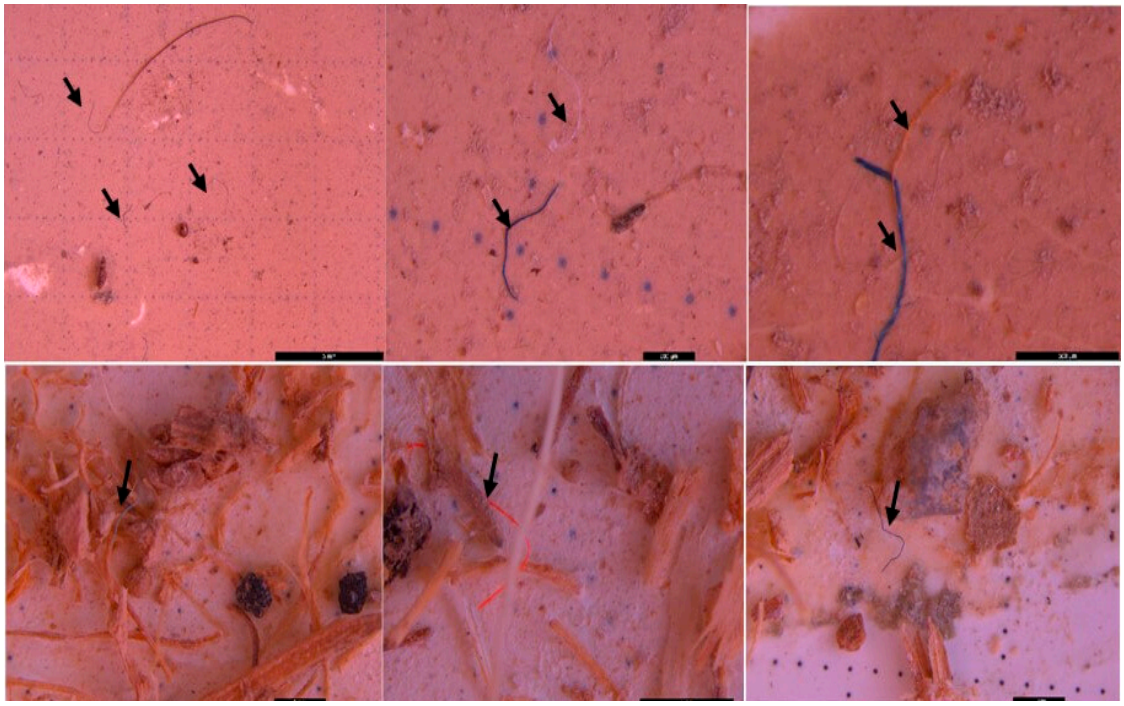

Figure 3. MPs in the form of fibers (purple, blue, black, orange) in sediment collected on a 500 μm sieve (possibly polypropylene or polyamide) - UP Floresti, scale bar = 5mm, 400 μm, 500 μm, 1mm, 1mm, 1 mm.

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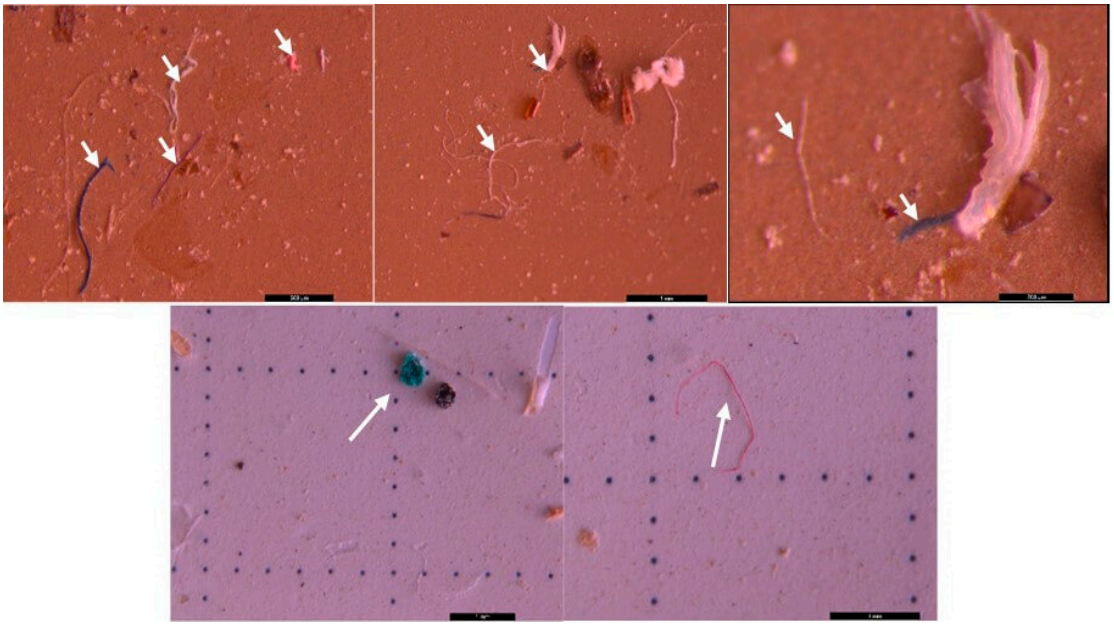

Figure 4. MPs in the form of fibers (blue, white, pink) and fragments (pink, white, blue-green) in sediment collected on a 250 µm sieve - UP Floresti, scale bar = 500 µm, 1mm, 200 µm, 1 mm, 1mm.

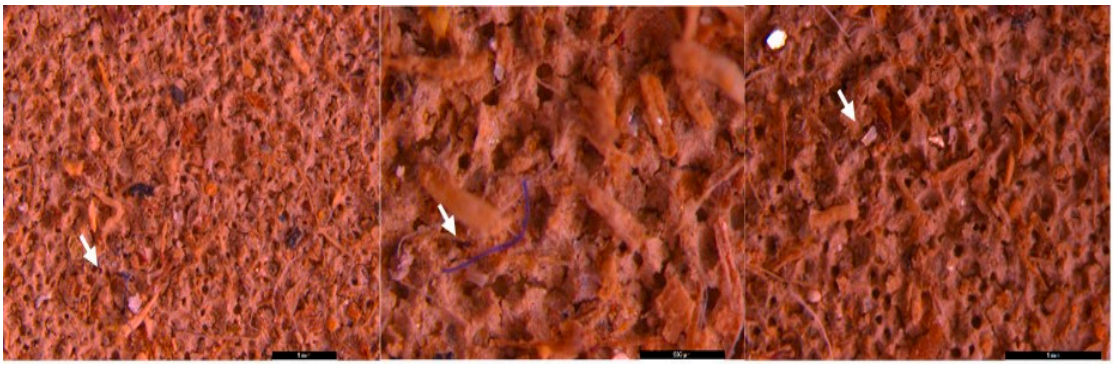

Figure 5. MPs in the form of fibers purple) and fragments (transparent) in sediment collected on a 40 µm sieve - UP Floresti, scale bar = 1mm, 500 µm, 1mm.

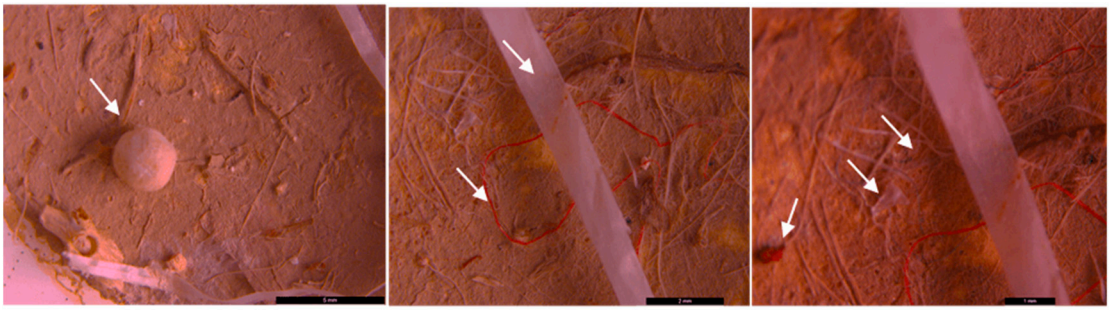

Figure 6. MPs in the form of fibers (red, blue), ribbon (transparent white), foam spheres (white) and fragments (transparent, red) in surface water collected on a 200 µm filter – UP Floresti, scale bar = 5mm, 2mm, 1mm.

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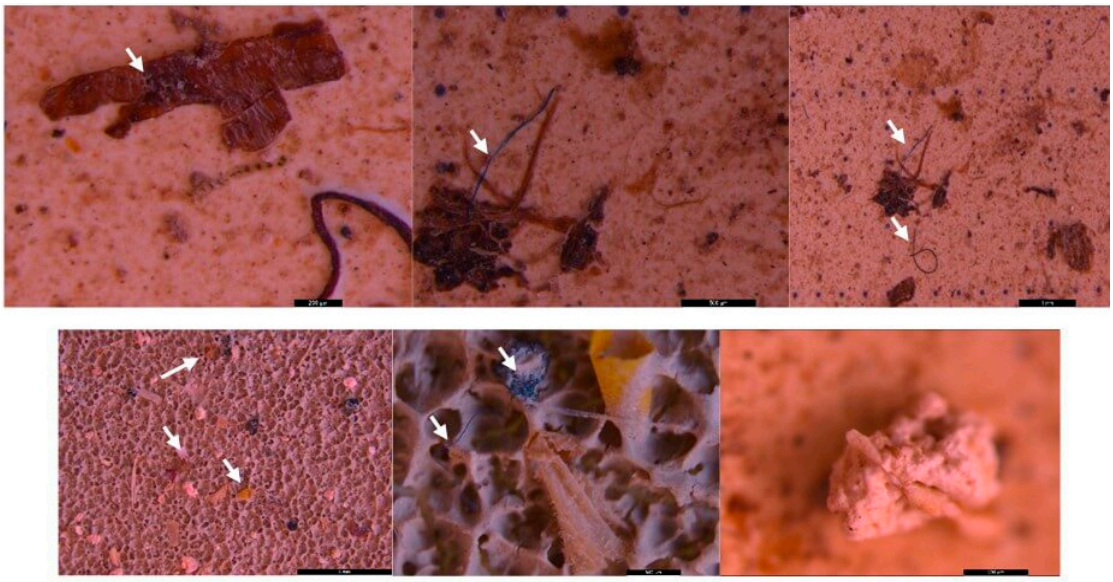

Figure 7. MPs in the form of fibers (purple and blue), fragments (brown, yellow, blue) and foam (white) in sediment collected on the 500 µm sieve - DW Floresti, scale bar = 200 µm, 500 µm, 1mm, 5mm, 500 µm, 500 µm.

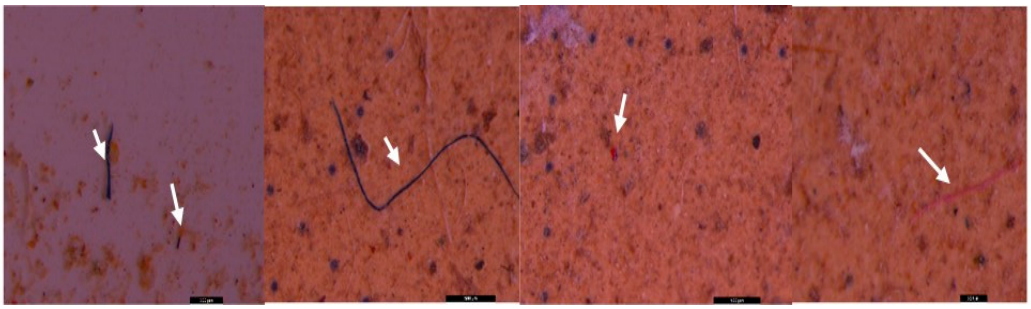

Figure 8. MPs in the form of fibers (purple and red) and fragments (red) in sediment collected on the 250 µm sieve - DW Floresti, scale bar = 500 µm, 500 µm, 1mm, 200 µm.

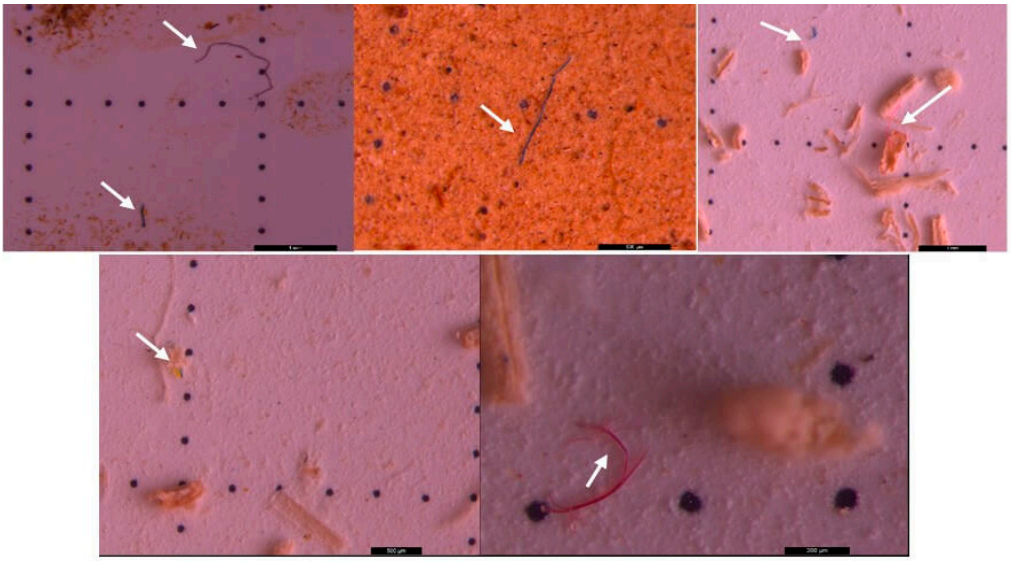

Figure 9. MPs in the form of fibers (purple, blue, pink, red) and fragments (yellow) in sediment collected on the 40 µm sieve - DW Floresti, scale bar = 1mm, 500 µm, 1mm, 500 µm, 200 µm.

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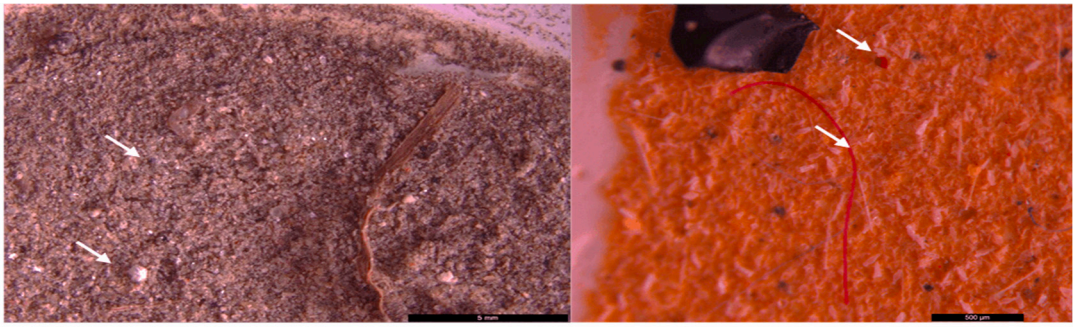

Figure 10. MPs in the form of sphere, fragments (blue and red) and fibers (red) in surface water collected a 20 µm filter - DW Floresti, scale bar = 5 mm, 500 µm.

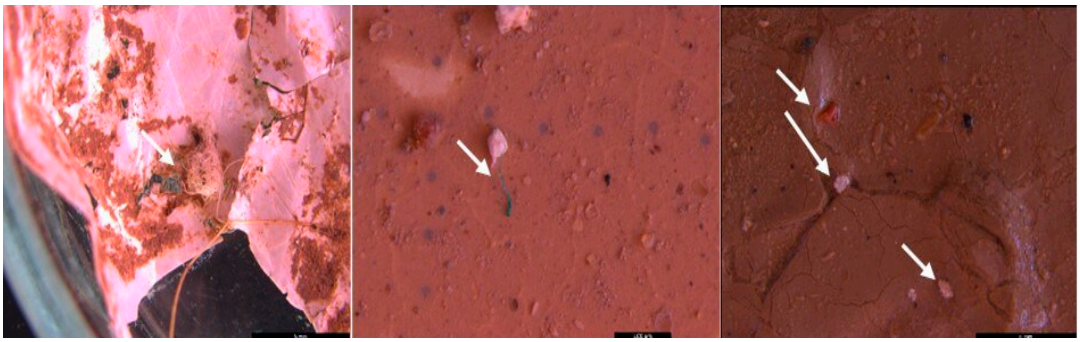

Figure 11. MPs in the form of fibers (brown, green) and fragments (red and white) in sediment collected on the 500 µm sieve – UP Cluj-Napoca WWTP, scale bar = 5 mm, 500 µm, 5 mm.

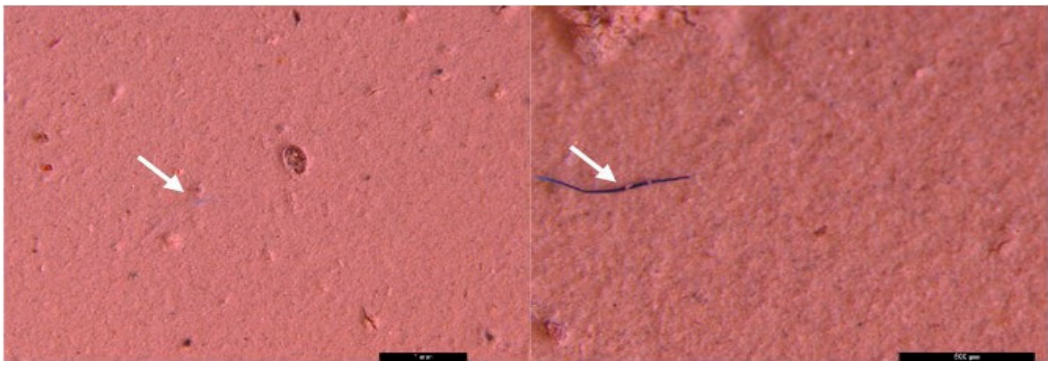

Figure 12. MPs in the form of purple fibers in sediment collected on the 40 µm sieve – UP Cluj-Napoca WWTP, scale bar = 1 mm, 500 µm.

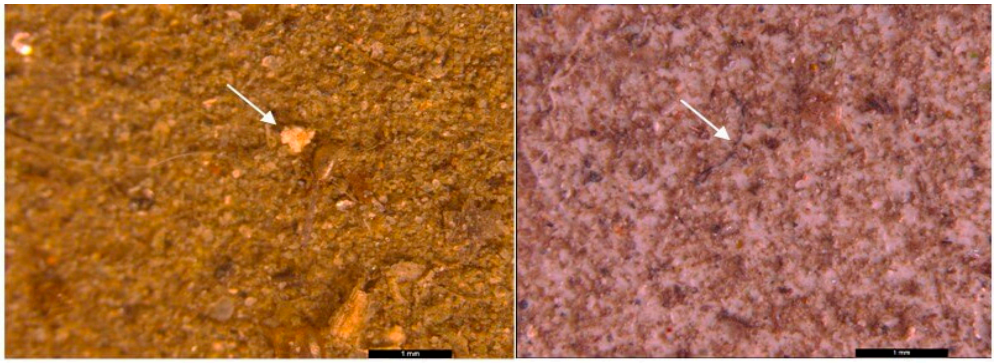

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Figure 13. MPs in the form of foam (white) and fibers (purple) in surface water collected on the 20 µm filter – UP Cluj-Napoca WWTP, scale bar = 1 mm.

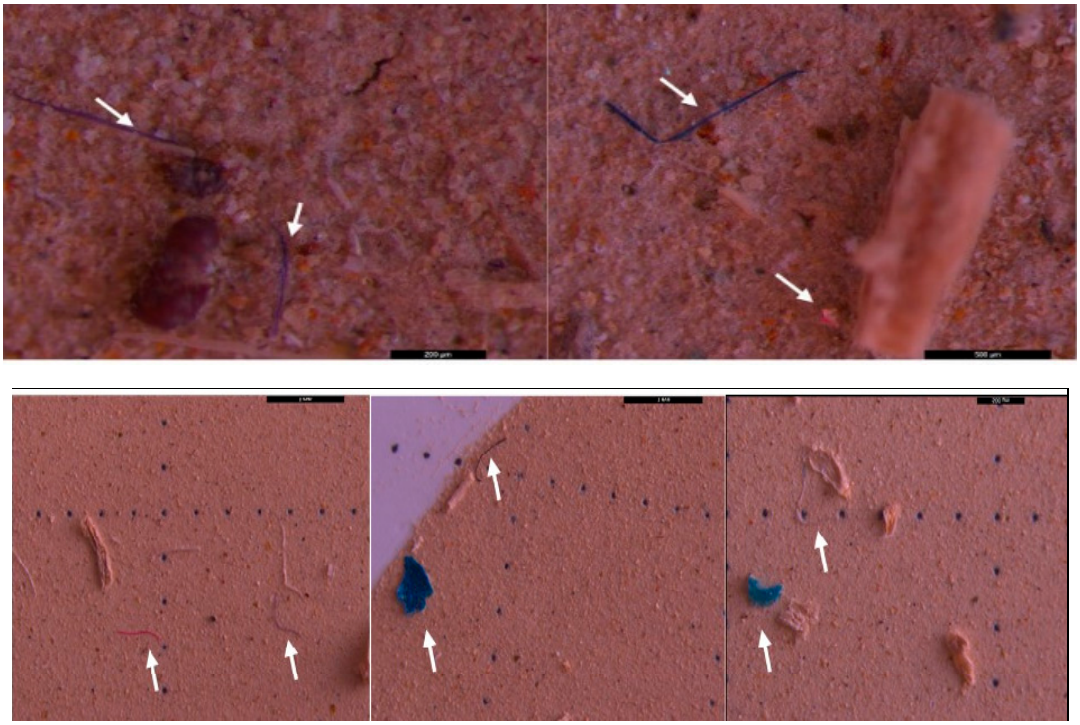

Figure 14. MPs in the form of fibers (purple, blue, pink) and fragments (blue and pink) in sediment collected on the 40 µm sieve – DW Cluj-Napoca WWTP, scale bar = 2mm, 200 µm, 200 µm, 500 µm, 1mm, 1mm, 500 µm.

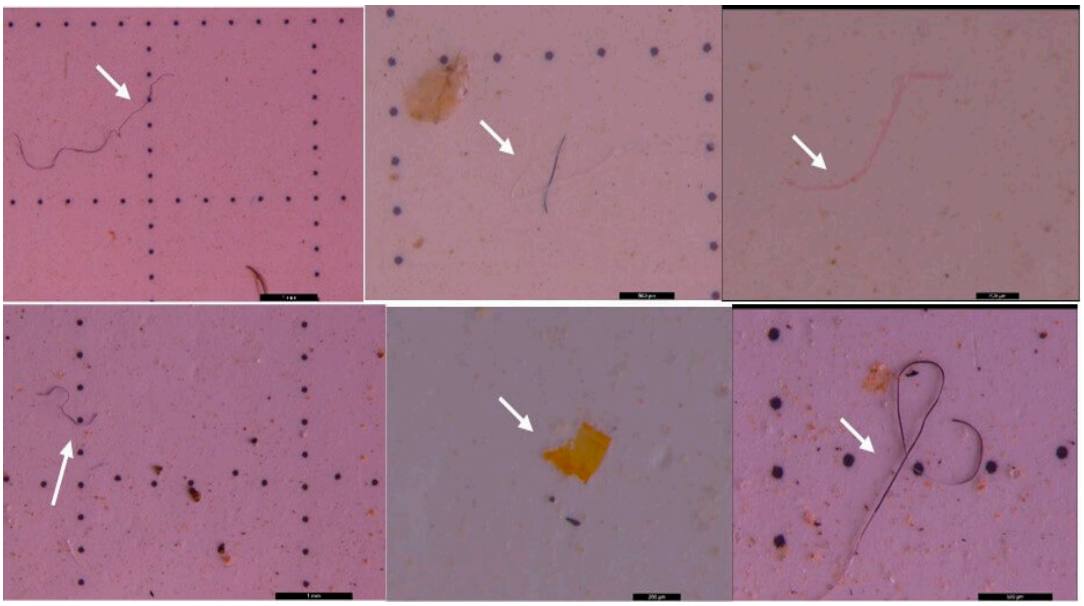

Figure 15. MPs in the form of fibers (purple, pink, transparent) and fragments (transparent yellow) in sediment collected on the 250 µm sieve – DW Cluj-Napoca WWTP, scale bar = 1mm, 500 µm, 200 µm, 1mm µm, 200 µm, 500 µm.

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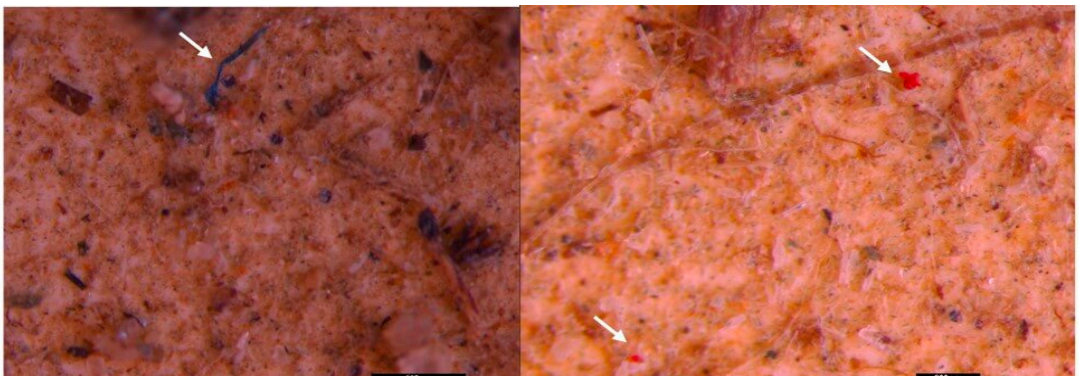

Figure 16. MPs in the form of fibers (blue) and fragments (red) in sediment collected on the 500 µm sieve – DW Cluj-Napoca WWTP, scale bar = 500 µm, 200 µm.

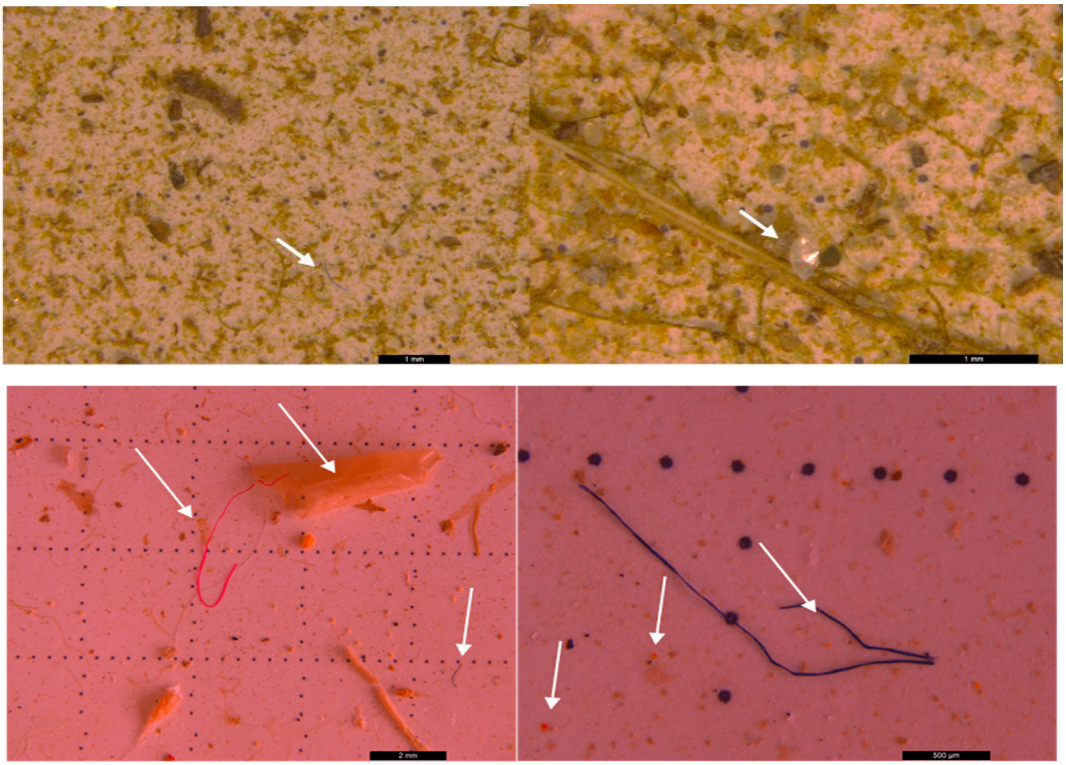

Figure 17. MPs in the form of fibers (purple, blue, pink) and fragments (transparent, orange) in surface water collected on the 20 µm filter – DW Cluj-Napoca WWTP, scale bar = 1mm, 1mm , 2mm, 500 µm.

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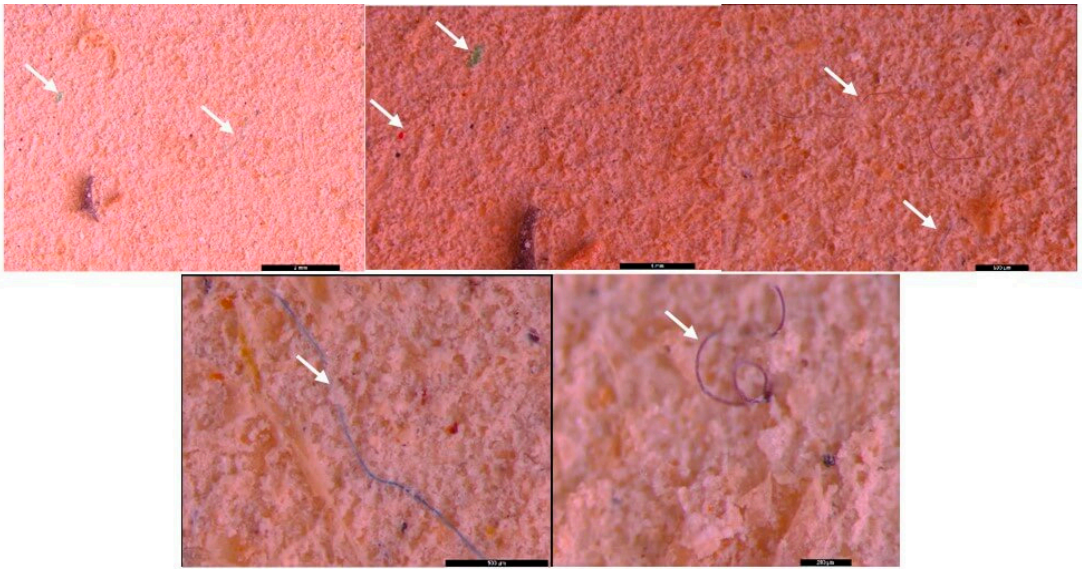

Figure 18. MPs in the form of fibers (purple, blue) and fragments (green and red) isolated from the WWTP effluent without concentration of sample, scale bar = 2 mm, 1 mm, 500 μm, 500 μm, 200 μm.

The second type of MPs was the fragments of hard polymers or foils in different colors and shapes resulting from the environmental degradation of large pieces of plastic (e.g., Figures 4, 7 and 14). Transparent or opaque, red, yellow, blue, pink or green fragments were identified. Their size varies between <100 μm and 2 mm with a higher density in the sediment samples.

Another frequently encountered category in the samples taken from the Somesul Mic River consisted of fragments and spheres of PS foam (e.g., Figures 6 and 7), generally white, gray-white, very easy to notice. PS has a low density and was frequently observed even in unconcentrated surface water and also floating on the surface of water. The prevalence of PS is likely linked to uncontrolled construction waste storage.

The size of the particles determined microscopically so as to correspond to the physical characteristics of PS were between 500 μm, 1 mm and ≥ 3 mm, but there is the suspicion of the presence of smaller fragments < 500 μm resulted under the pressure of biological processes, sunlight or purification processes. The results are comparable with the findings in national literature which indicate that PP, PS and PE are the most frequently encountered polymers in water bodies such as the Danube River and the Black Sea. Their low density allows to float being easily collectible from the surface water [25].

The microscopic analysis highlighted that most of the particles identified to be of a polymeric nature (plastic) are large MPs with size which ranged between 1 mm to ≤5 mm (fibers, fragments, foam spheres), as well as small MPs ≤1000μm such as the fragments.

Literature confirms that 90% of MPs are represented by fibers and in a smaller proportion of fragment, clumps, sphere [25]. In this case the fibers are most easily identified based on their specific form and color. Instead the fragments are more challenging to identify due to their diverse and unregulated shapes, as well as their transparency.

The types and shape of the observed MPs in sediment and surface water were also identified in the wastewater effluents, with the mention that PS foam was poorly represented. In this case the most abundant were the fragments and fibers.

We cannot make a significant difference in MPs load in terms of the sampling points, because all the samples showed the omnipresence of MPs. However, it can be appreciated that also Cluj – Napoca WWTP can be a source of small MPs (Figure 18), because the large ones are retained in the mechanical stage and in the activated sludge.

The abundance of MPs of various sizes and shapes leads us to the hypothesis that the most important source of contamination of the water body is the improperly stored waste on the banks of the river, a phenomenon also observed on the field. The washing of the banks by the river and the

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environmental conditions inevitably contribute to the destruction/degradation of macroplastics into MPs and their carrying along the body of water until they are discharged. Sediments represent storage reservoirs for MPs, as well as for numerous chemical pollutants.

Due to the fact that the MPs could be separated based on the density in NaCl, it can be appreciated that the microscopically observed MPs can be part of the class of low densities polymers such as polyethylene, polypropylenes, polystyrene, ethylene-vinyl acetates, or polyamides.

The analysis of the unused control sterile filter membranes used in the takeover of MPs for microscopic analysis did not reveal the presence of impurities or types of MPs that would contribute or interfere with their identification in the environmental samples.

The obstacle of the microscopic method was the identification of transparent fragments less than 100 µm, which are difficult to observe and characterize. The literature estimates that 20-70% of them can be identified using other more advanced methods [51]. The density of the sample sediment on the filter membrane and the presence of impurities that cannot be eliminated by digestion (such as insect carcasses, natural fibers, etc.) were impediments in the microscopic observations.

SEM microscopic analysis revealed the morphological surface structure of MPs. SEM uses an electron probe (e beam) with high energy that impacts the sample, generating a variety of signals that create an image of sample surface and composition.

Compared with classical microscopy method, the SEM technique reveled the small MPs particles ranging from less 1 µm to 500 µm (Figure 19). Also a better morphological characterization of MPs could be highlighted. The surface of fragmented polymers materials was irregular. The fibers have smooth surface and linear shape, while the foam exhibits rounder shapes. The size of MPs influences their bioavailability to organisms, the smaller they are, the more negative their effects can be [52].

Additionally, it was possible to highlight the structure of some aging particles larger than 1mm (Figure S5 and S6). Other particles were observed in the wastewater, without being subjected to oxidizing digestion treatment. They have the shape of elongated transparent films with sizes greater than 500 µm, while others are fragments of ≤50 µm (Figure S6).

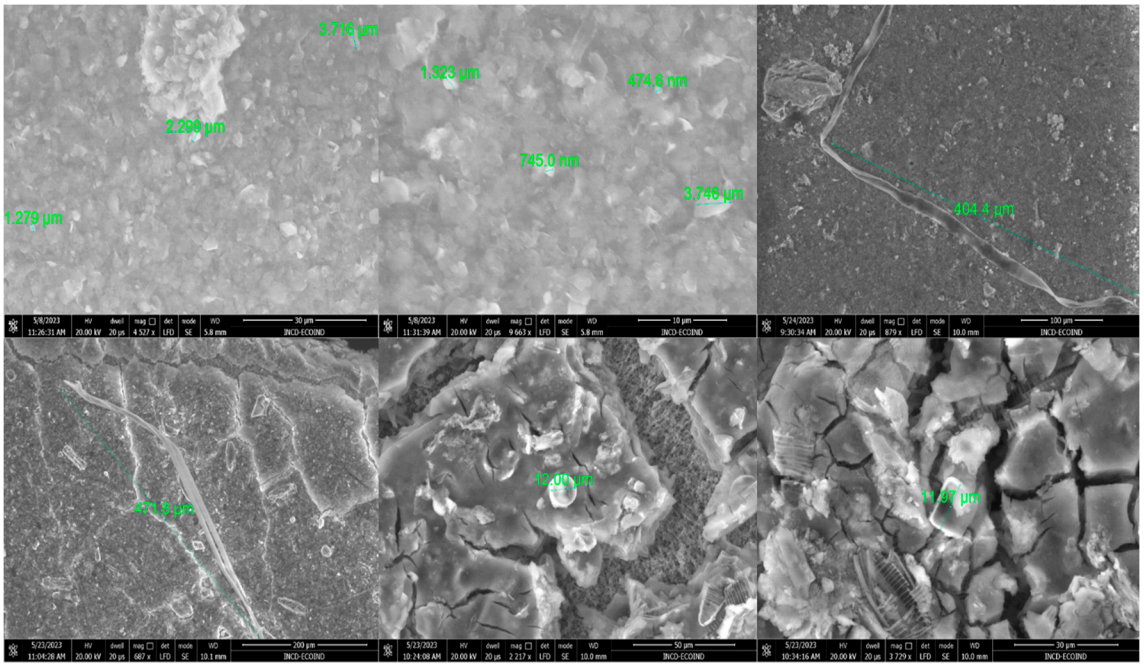

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Figure 19. SEM images of suspected MPs (sphere, fragments and fibers).

3.3. Raman investigation

Raman spectroscopy is a technique in which polymer particles were exposed to a laser light at different wavelength. The particles scatter the light in different direction according/depending to their chemical structure.

The obtained results of ***Raman-HR-TEC-785*** applied on large particles (>500 µm) and based on reference spectra of PE, PS and PP (Figure 20), showed the following estimation of identifications: PP (white and green fragments 4-5 mm) in the sediment UP Floresti (Figure 21a,b); PE (white fragment, 2 mm) in the wastewater influent of Cluj-Napoca WWTP (Figure 21c) and in the sediment of UP Cluj-Napoca WWTP (colorless fragments 1, 3 and 4 mm Figure 21e, and brown sphere 2 mm Figure 22). The obtained spectra are comparable to those obtained by [53]. Identification of fragments smaller than 500 µm was not possible (Figure 21d).

The analysis raises some limitations: i) the presence of organic or inorganic impurities that can interfere; ii) an obscure ambient environment must be ensured because light induces fluorescence; iii) the color of the MPs, additives, and their transparency can interfere with obtaining of spectra; iv) the size of the MPs – the spectra were obtained for particle sizes >1mm, the particles <1mm can be destroyed by the laser light or cannot emit sufficient signal to obtain spectra. Even so, the Raman technique using Raman-HR-TEC-785 Spectrometer allowed the identification of PE and PP polymers using particles size ≥1mm taking in to consideration the standard spectra (Figure 20a–c). Because the analyzes raise limitations and interferences, the results of identification are not clear.

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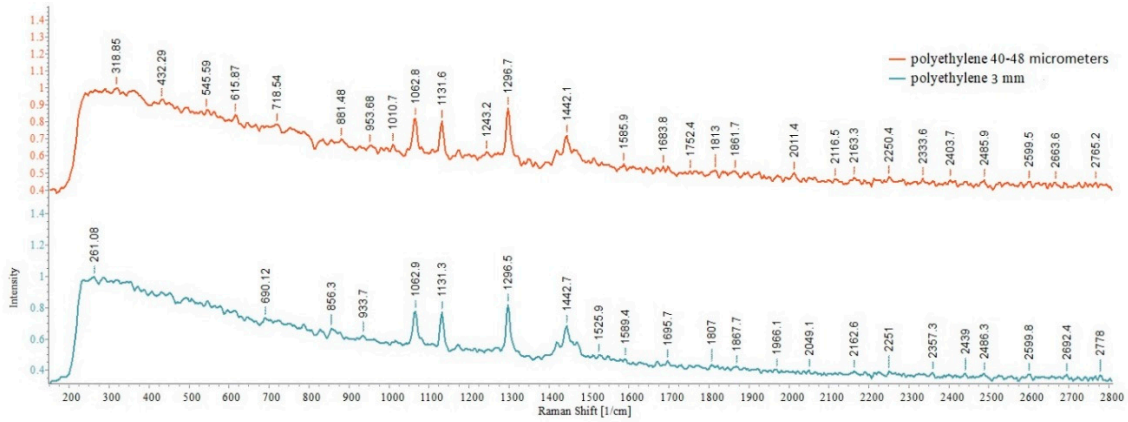
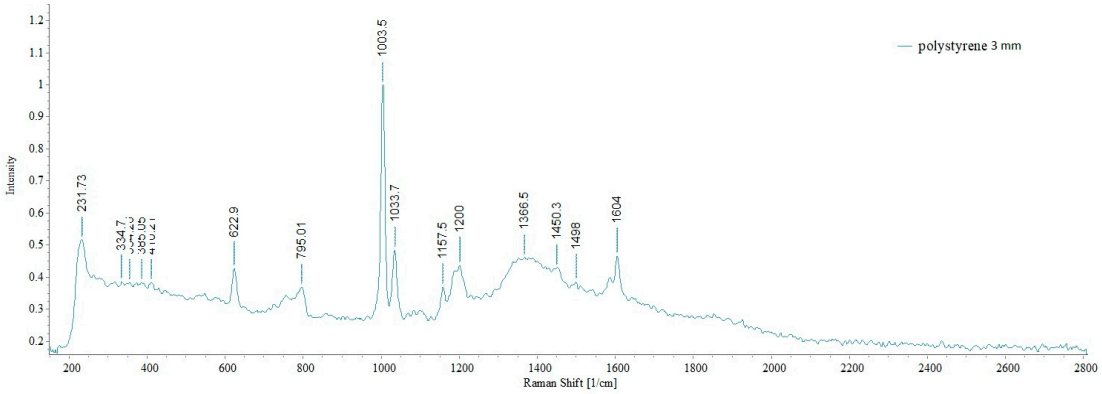
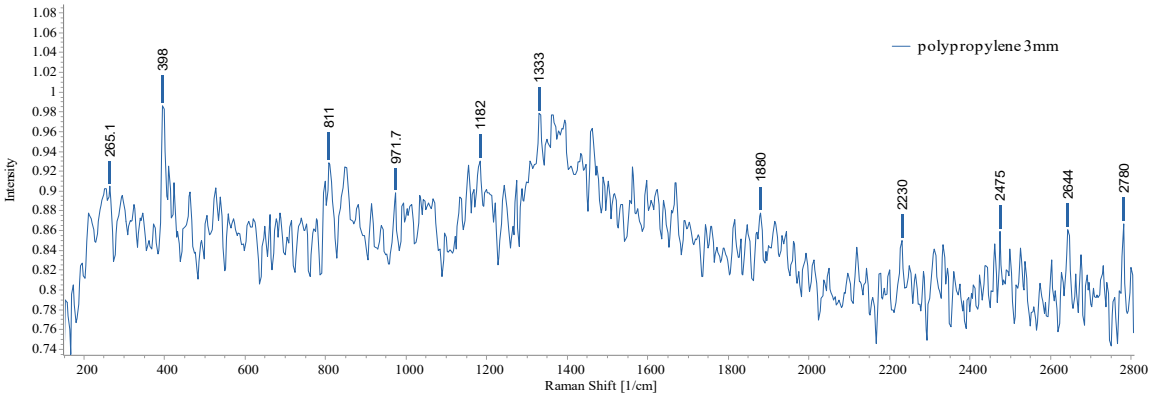


(a)



(b)



(c)

Figure 20. Raman spectra of the standard MPs: (**a**) PE 40-48 μm and 3 mm; (**b**) PS 3 mm; (**c**) PP 3 mm.

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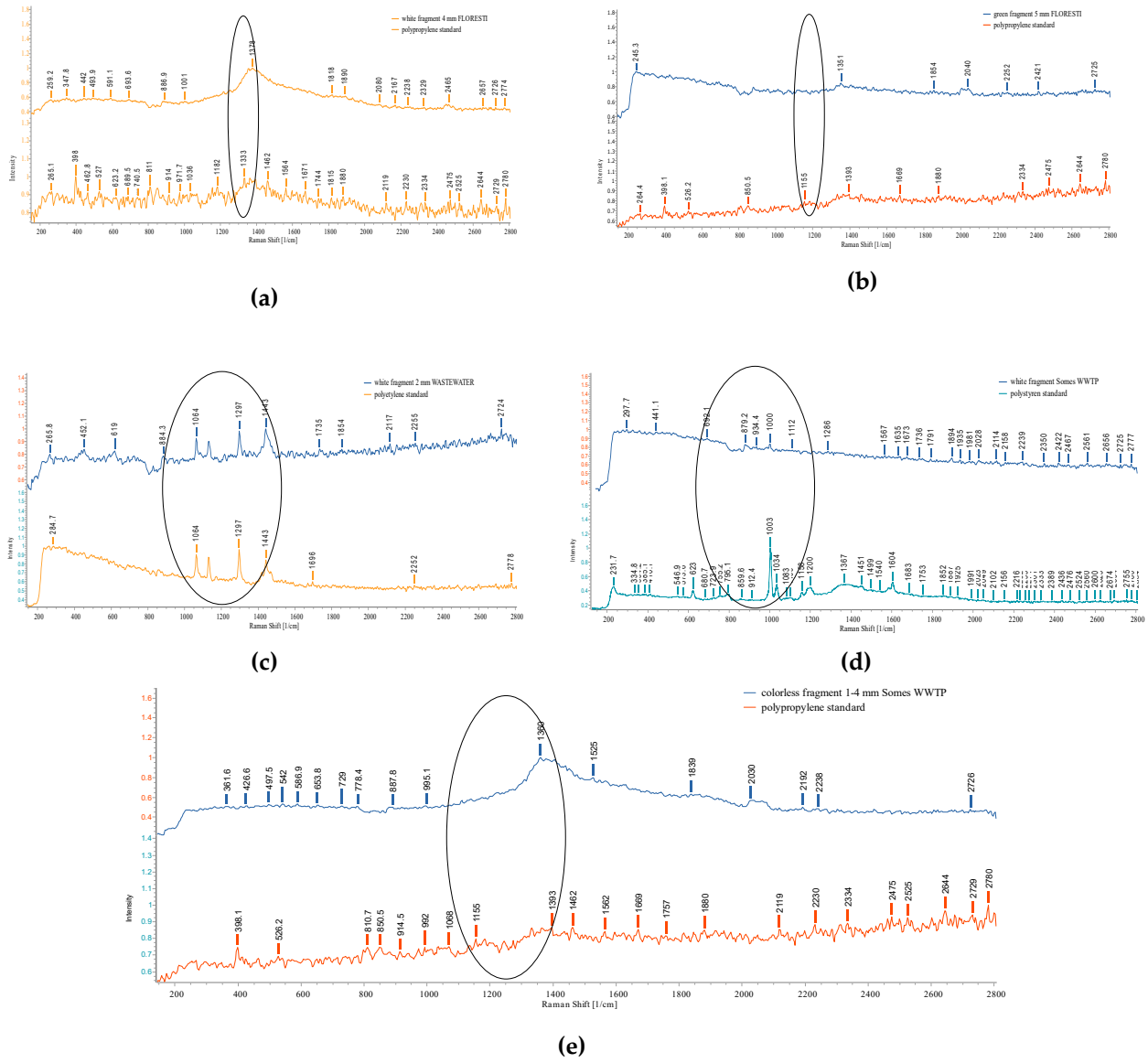

Figure 21. Raman spectra: (a) sediment UP Floresti – PP (white fragment) 4 mm, spectra compared to reference spectra PP (shown in dark orange)**; (b)** sediment UP Floresti - PP (green fragment) 5 mm, spectra compared to reference spectra PP (shown in orange)**; (c)** Wastewater influent – PE (white fragment) 2 mm, spectra compared to reference spectra PE (shown in orange)**; (d)** sediment UP WWTP - fragment 500µm- the identification was unclear, spectra compared to reference spectra PE (shown in light blue); **(e)** sediment UP Cluj-Napoca WWTP - fragments - PE, 1mm, 4 mm, spectra compared to reference spectra PP (shown in orange).

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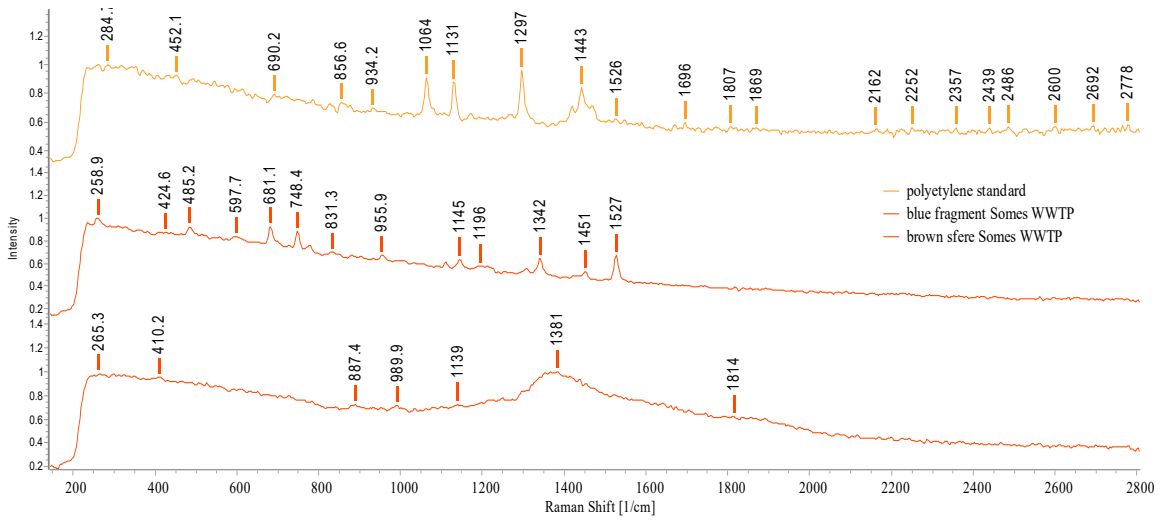
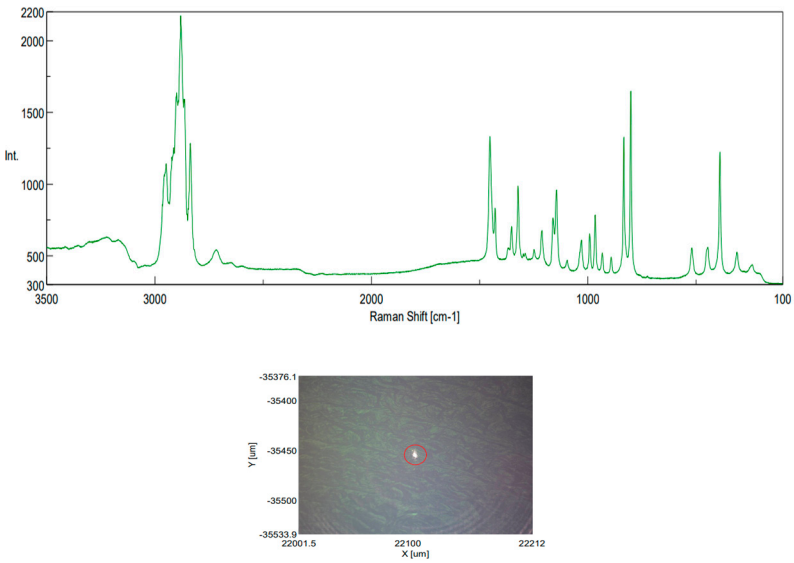


Figure 22. Raman spectra – sediment UP Cluj-Napoca WWTP – fragments 3 mm and brown sphere 2 mm – suspected to be PE.

The ***NRS-7200 Raman Spectrometer*** obtained good results that confirmed the presence of PP, PE and PS and in addition, polycarbonate (PC) in sediment samples (Figure 23). Even so, the method encountered challenges in identifying MPs trapped in the sediments or organic matter residues in filter membranes.

The size of MPs and the absence of databases for polymer spectra also contribute to difficulties in analysis and interpretation. According to the specialized literature, the ranges of Raman vibration frequencies for the main common plastic polymers are as follows: PE in the range 1062-2883 cm^{-1}, PS 621-3054 cm^{-1}, PP 809-1458 cm^{-1}, 2883-2952 cm^{-1} [1]. The obtained spectra reveled peaks –CH starting with more than 3000 cm^{-1} to 1500 cm^{-1} characteristic of PP, 1000 cm^{-1} for PS, 500 cm^{-1} to 1500 cm^{-1} for polycarbonate and 1000 cm^{-1} to 1500 cm^{-1} for PE, similar to [53].



(a)

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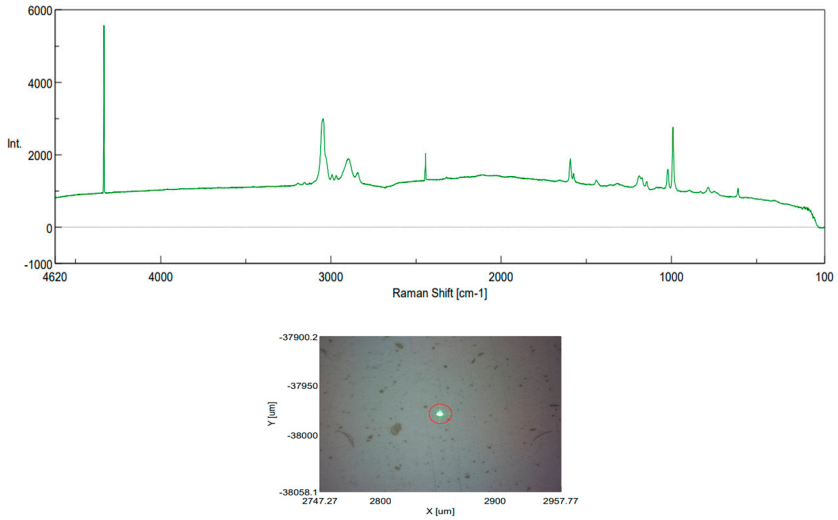
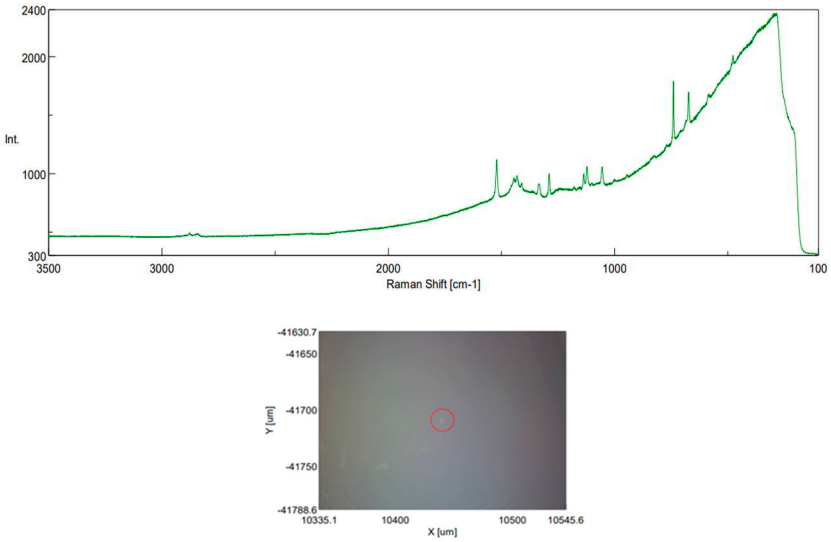
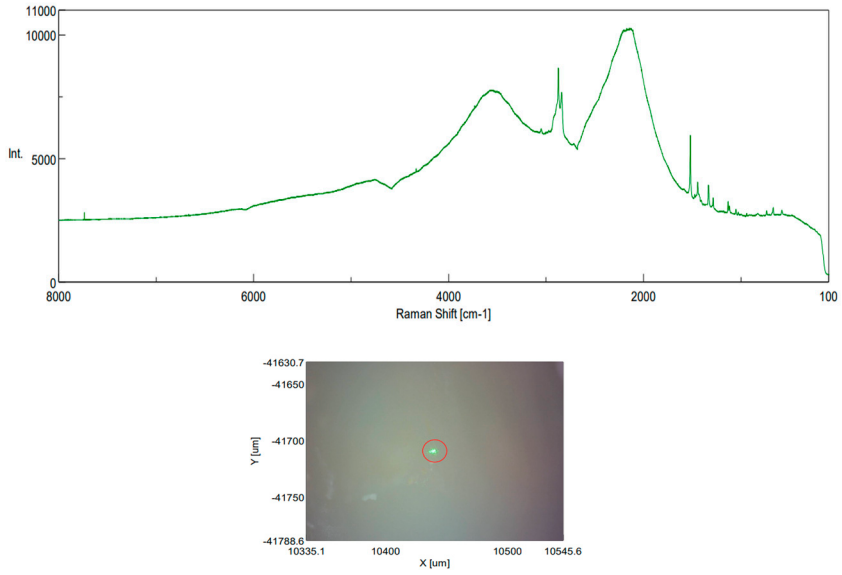


(b)



(c)



(d)

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Figure 23. Raman spectra recorded at the highlighted locations - sediment samples, identifications on filter paper*:* **(a)** PP identification (UP Floresti)**:** exposure time 30 sec, accumulation 10, center wavenumber 740.11 cm^{-1}, Z position 16061.1 µm, binning interval 240-374, valid channel 1-2048, laser wavelength 784.74 nm, resolution 9.02 cm^{-1}, 0.61 cm^{-1}/pixel, objective lens LMPLFLN 10x, slit 200x1000µm, aperture d-4000µm, laser power 56.6 mW, CCD temperature -70°C; **(b)** PS identification (DW Cluj Napoca WWTP)**:** exposure time 30 sec, accumulation 20, center wavenumber 1520.21 cm^{-1}, Z position 17003.6 µm, binning interval 240-374, valid channel 1-2048, laser wavelength 531.94 nm, resolution 4.79 cm^{-1}, 1.30 cm^{-1}/pixel, objective lens LMPLFLN 10x, slit 50x1000µm, aperture d-4000µm, laser power 5.5 mW, CCD temperature -70°C; **(c)** PC identification (DW Cluj Napoca WWTP)**:** exposure time 30 sec, accumulation 10, center wavenumber 740.11 cm^{-1}, Z position 17053.7 µm, binning interval 240-374, valid channel 1-2048, laser wavelength 784.74 nm, resolution 9.02 cm^{-1}, 0.61 cm^{-1}/pixel, objective lens LMPLFLN 10x, slit 200x1000µm, aperture d-4000µm, laser power 56.4 mW, CCD temperature -70°C; **(d)** PE or PP unclear identification - green fragment (influent of Cluj Napoca WWTP): exposure time 20 sec, accumulation 10, center wavenumber 1520.21 cm^{-1}, Z position 17213.7 µm, binning interval 240-374, valid channel 1-2048, laser wavelength 531.94 nm, resolution 4.79 cm^{-1}, 1.30 cm^{-1}/pixel, objective lens LMPLFLN 10x, slit 50x1000µm, aperture d-4000µm, laser power 5.6 mW, CCD temperature -70°C.

The µRaman study using ***LabRam HR800 system*** was performed in several areas of the MPs samples, identified using the integrated optical microscope. Some representative spectra for the investigated samples (filtered surface water) are shown in Figure 24b–o. Only a few investigated regions showed clear Raman bands; the others only showed significant fluorescence, and a series of broad bands of low intensity that can be attributed to potential organic degradation that could have been induced also by laser exposure. Figure 24a presents the Raman signature exhibited by the filter paper used in sample preparation.

As can be seen, the Raman signature of the DW Cluj-Napoca WWTP 200µm sample (Figure 24l,m) consists of the following Raman bands 2882 cm^{-1}, 2849 cm^{-1}, 1441 cm^{-1}, 1294 cm^{-1}, which can clearly be assigned to PE. These are the only Raman spectra from the Horiba LabRam based experiments that were clearly attributed to the detection of MPs.

There are regions where multi-peak Raman profiles were detected with clear/sharp bands, but extremely difficult, unclear assignment, such as the ones shown in Figure 24b (UP Cluj-Napoca WWTP 20µm) and Figure 24h–i (DW Cluj-Napoca WWTP 20µm). In all these cases, some of the bands, e.g., 1351 cm^{-1}, 1462 cm^{-1}, can be attributed to PP and thus the presence of this polymer can be confirmed, even if these bands are not the most intense/main for this plastic. It should be noted that additives and/or dyes/pigments are usually used in these plastics, and their presence can cover the main bands or even the entire specific set of bands of the polymer. However, the other bands, except those assigned to PP, could not be identified. On the other hand, for some regions of UP Cluj-Napoca WWTP 20µm sample (Figure 24d,e), the detected bands were attributed to pigment residues, such as TiO_2 (anatase phase), white pigment, and α-Fe_2O_3/$FeO(OH)_nH_2O$, red pigment, respectively, which were probably used to color some polymers (not detected in these cases).

The Raman spectra shown in Figure 24c,j and o reveal the presence of D and G bands at approximately 1137 cm^{-1} and 1578 cm^{-1}, respectively, specific for carbonaceous materials. This may either confirm the presence of C-forms in the wastewater samples or may be the result of real-time degradation of the organic components in the samples as a result of their exposure to the laser beam.

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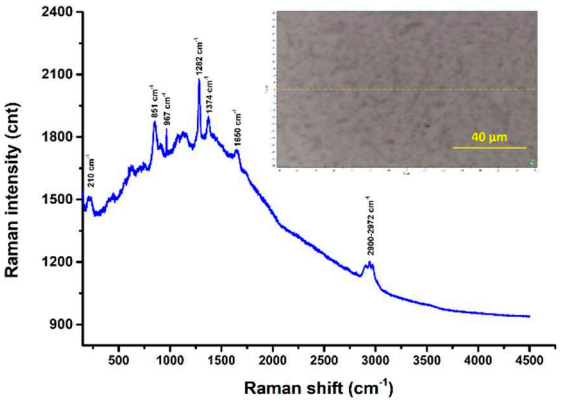
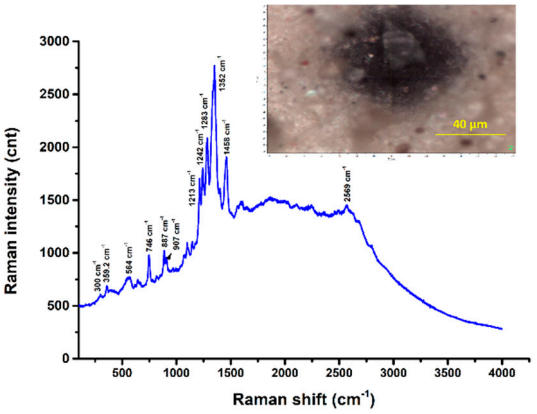


(a)



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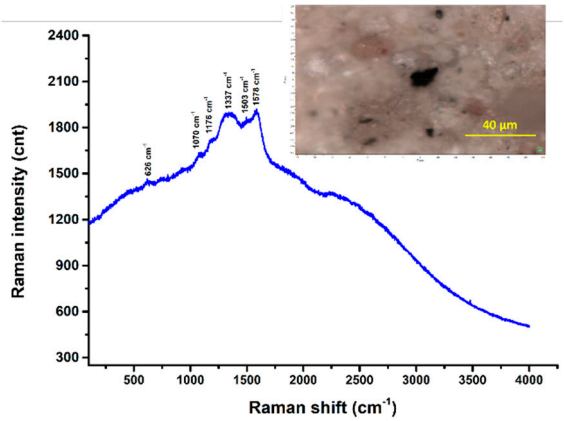
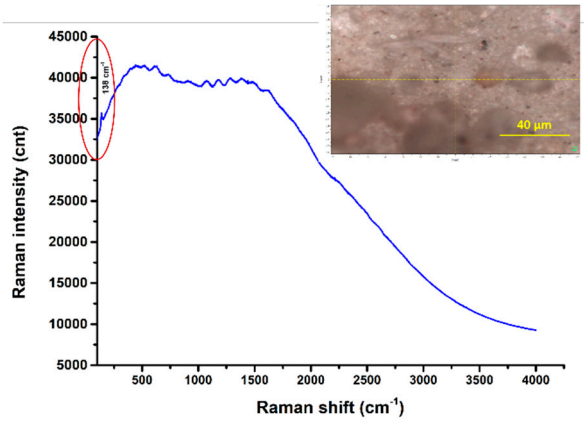
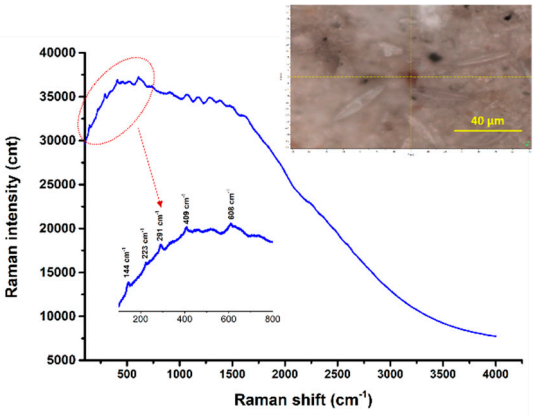


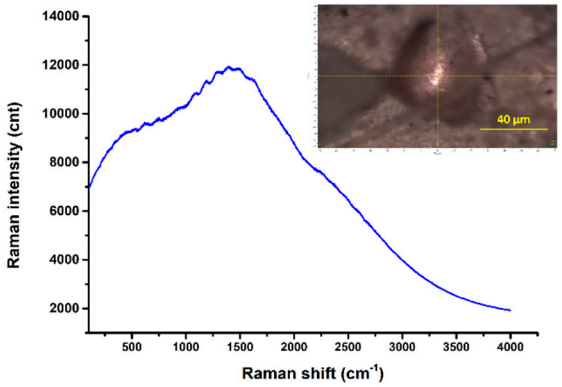
(c)



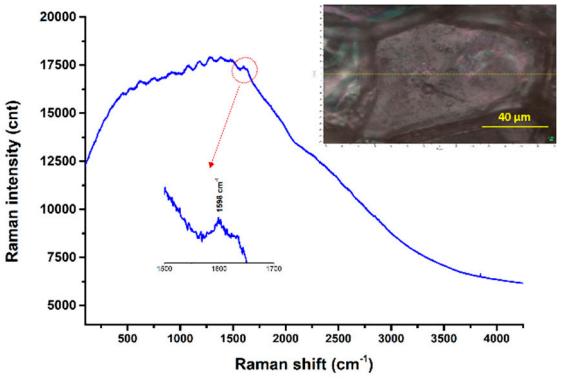
(d)



(e)



(f)



(g)

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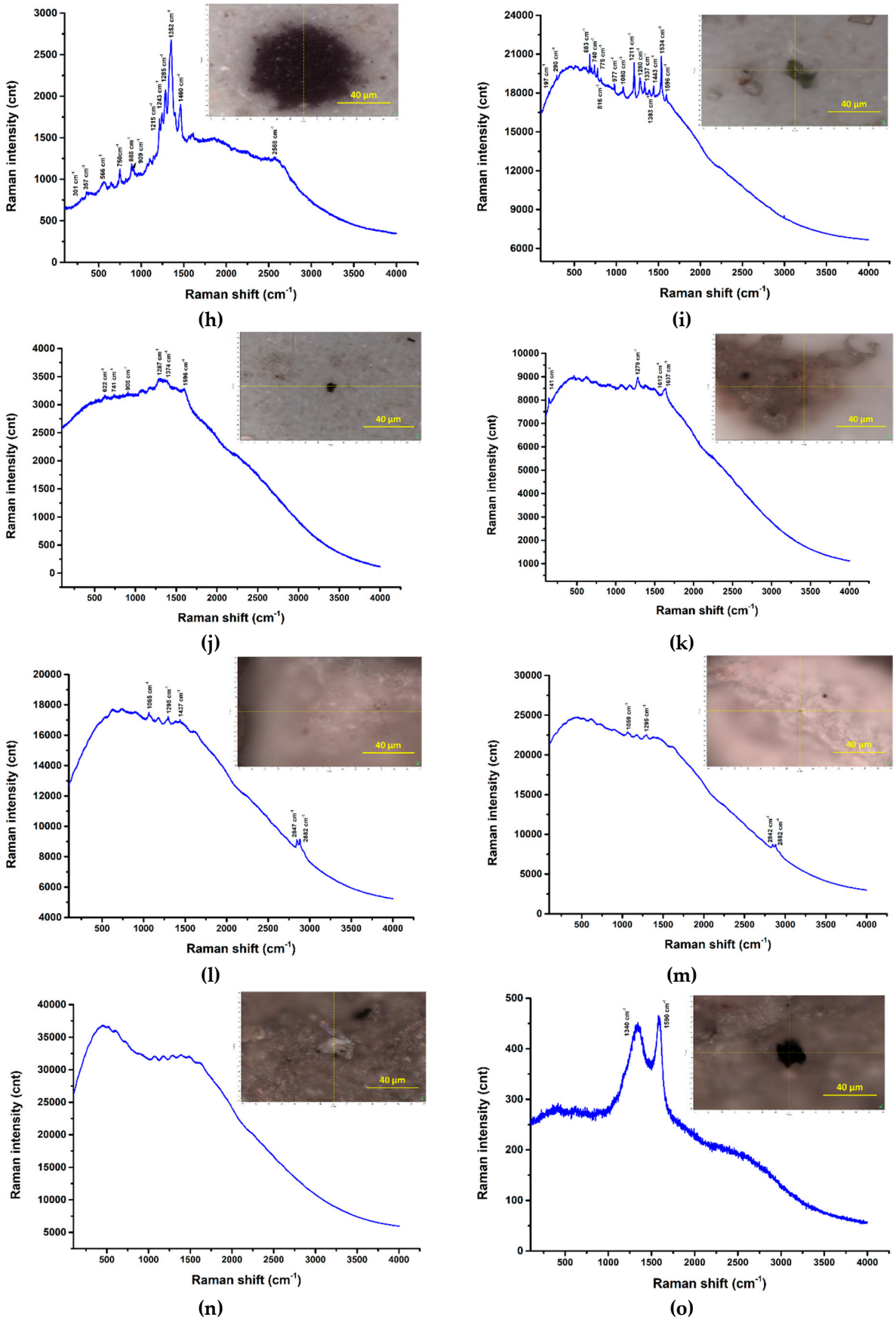

Figure 24. µRaman spectra recorded at the highlighted locations – surface water samples: **(a)** filter membrane spectra; **(b)** UP Cluj-Napoca WWTP 20µm –potentially PP among others [54]; **(c)** UP Cluj-Napoca WWTP 20µm – a C compound among others [55]; **(d)** UP Cluj-Napoca WWTP 20µm –

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potentially TiO_2 (anatase phase), white pigment [54]; **(e)** UP Cluj-Napoca WWTP 20µm- potentially α-Fe_2O_3 or $FeO(OH)_nH_2O$, red pigment; **(f-g)** UP Cluj-Napoca WWTP 200µm – no clear Raman bands, only fluorescence; **(h)** DW Cluj-Napoca WWTP 20µm - potentially PP among others [54]; **(i)** DW Cluj-Napoca WWTP 20µm - potentially PP among others [54]; **(j-k)** DW Cluj-Napoca WWTP 20µm - a C compound among others [55]; **(l-m**) DW Cluj-Napoca WWTP 200µm – clear evidence of PE [54] **; (n)** UP Floresti 200µm – no clear Raman bands; **(o)** UP Floresti 200µm - a C compound [55].

Raman spectroscopy was commonly used for the MPs detection in water, sediment, and biota systems, as evidenced in the literature [6,50]. However, its utility is still in its early stages of development concerning the identification of small MPs particles, particularly those less than 500µm [1,51]. The configuration of equipment has a considerable impact on the analysis outcomes, respectively the laser excitation wavelength employed. The most frequently utilized laser wavelengths were 532 nm, 632nm and 785 nm, the latter ones being used also in our study. The main problem with 532 nm and 632nm laser sources is that there are some analysts or impurities with fluorescence emission that interfere with the detection of MPs particles. The presence of noise background and fluorescence is characteristic of the existence of organic and inorganic materials [53]. As can be seen, all the Raman spectra shown in Figure 24 shows significant fluorescence signal, which may have covered the signal of possible micro-plastic materials present in the analyzed regions. Also, the choice of acquisition times and laser power could increase the signal intensity and can reduce the noise ratio.

The results of spectroscopic analysis can also be significantly influenced by the preparation of sample before analysis. The recorded Raman signals, potentially originating from the plastics present in the sample are not easily identifiable based on existing literature data; some suggestions were offered, but the peaks obtained do not perfectly fit the different plastic materials common in such environments [1]; in any case, it must be considered that this behavior can be justified by the probable presence of different pigments or contaminants. According to existing literature, the primary limitations of Raman spectroscopy are the fluorescent compounds (such as pigments, degradation compounds, organic residues) as well as the size of MPs [56]. Other sources of interference are linked to the reagents used in MPs separation, such as H_2O_2, $FeSO_4{\cdot}7H_2O$ and NaCl [33] or even the specific type of filter membrane. Filter membranes based on cellulose esters appear to introduce interference in the Raman detection of plastic polymers. Additionally, factors like substrate thickness and the distribution of the particles on the filter can compromise the accuracy of Raman detection [57].

According to the literature, the utilization of 30%H_2O_2 in the digestion process can lead to reductions in Raman peaks of certain compounds such as polyamide or color changes [58].

3.4. Fourier transform infrared spectroscopic analysis (FT-IR)

Fourier Transform Infrared (FT-IR) spectroscopy employs infrared radiation to irradiate polymer particles and subsequently analyses the wavelengths reflected from these particles to determine their composition. Using the *Cary 630 FT-IR Spectrophotometer* the presence of PS in foam spheres sized 3-5 mm (Figure 25) and PE in fragments smaller than 5mm (Figure 26) was confirmed, which aligns with findings similar to [51].

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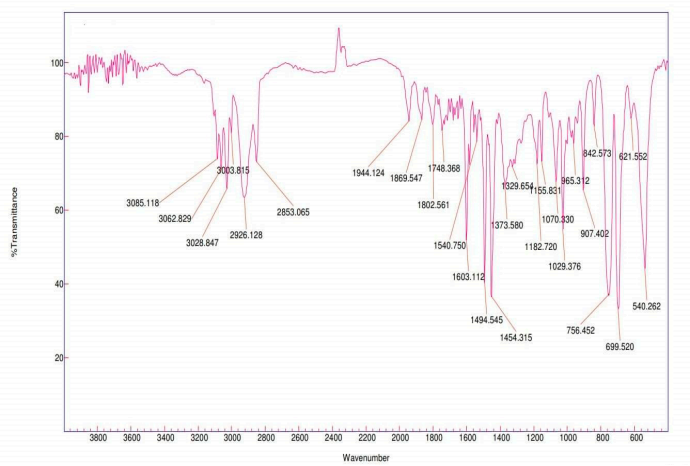

Figure 25. The infrared spectra of PS foam 3-5 mm (floating MPs).

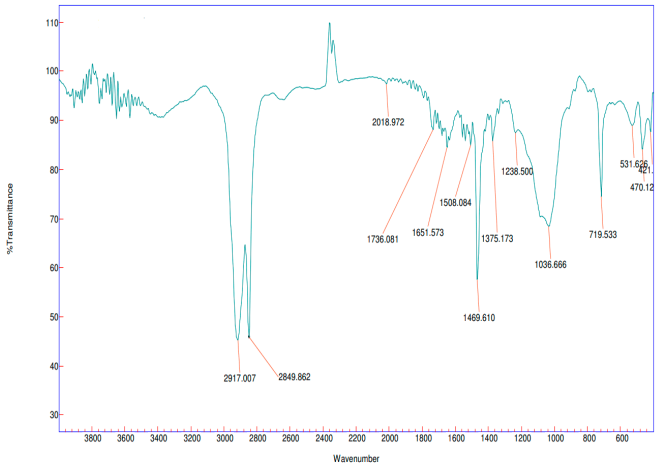

Figure 26. The infrared spectra of PE fragment <5 mm (floating MPs).

However, this technique also has limitations: i) it can identify quantitatively larger samples ≥2 mg; ii) it is possible to identify the samples that can be mortared for pilling, it could not be applied to foil-type microplastics.

The molecular structure of PS contains CH_2 groups and a benzene ring. The saturated C-H bonds show peaks below 3000 cm^{-1}, while the unsaturated C-H bonds originating from the benzene ring result in peaks above 3000 cm-1. According to [59], the peaks around ~3081, ~3059, ~3025 cm^{-1} are specific for aromatic C-H stretches, while peaks at 2923 cm^{-1} and 2850 cm^{-1} correspond to asymmetric and symmetric CH_2 stretches. Peaks at 1600 and 1492 cm^{-1} are associated with the aromatic ring, 756 cm^{-1} relates to the out-of-plane C-H bending in the aromatic ring and 698 cm^{-1} indicates the bending of the aromatic ring. These same peaks were observed in our study in the case of PS foam collected from the surface of water (Figure 25). Peaks at 3060 and 3026 cm^{-1} along with other peaks at 1600, 1492 and 1452 cm^{-1} that confirm the presence of benzene rings in the molecule, were also identified in the study of [60] for PS.

In case of PE fragment, peaks at 2917, 2849, 1469, 1375 and 719 cm^{-1} were observed (Figure 26), specific for CH_2 asymmetric C-H stretch, CH_2 symmetric C-H stretch, CH_3 umbrella mode and CH_2 rock may be similar with the results of [61].

The ***ATR-FTIR method*** using the ***Perkin – Elmer Spectrum Two IR spectrometer*** was able to detect small particles (<500 μm) of MPs from the filter surface**.** Two types of MPs, PP and PE were detected. The measurements in filtered surface water samples showed: UP Floresti – 200 μm – PP; DW Cluj Napoca WWTP – 200 μm – PE; UP Cluj Napoca WWTP - 200 μm and 20 μm – PE; DW Cluj Napoca WWTP - 20 μm- PE (Figures 27–30, table S2-5).

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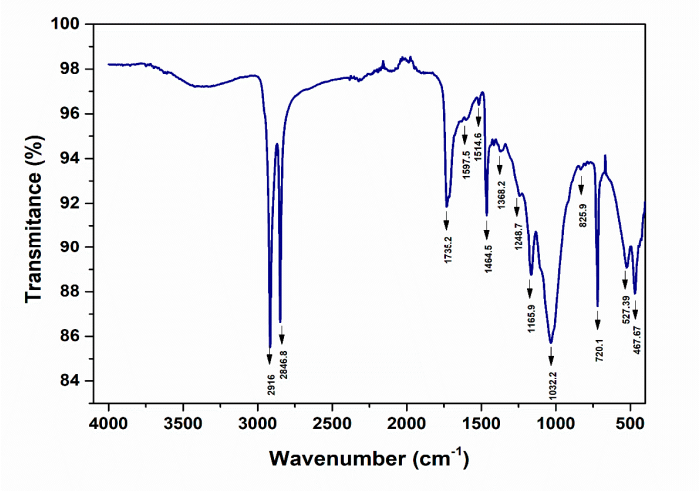

Figure 27. ATR-FTIR spectra – PE- UP Cluj Napoca WWTP 200 µm (water sample).

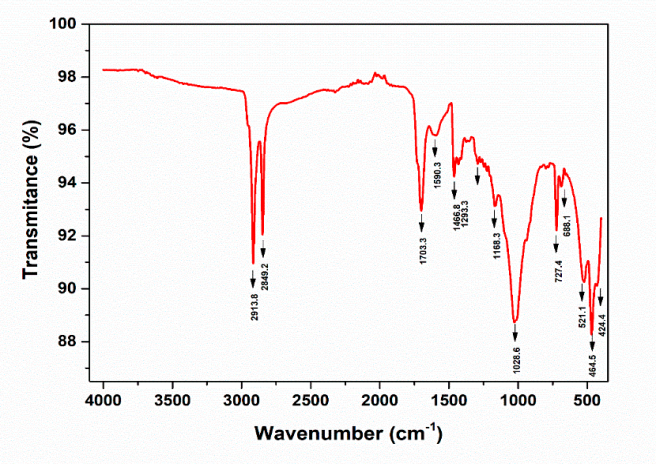

Figure 28. ATR-FTIR spectra - PE-DW Cluj Napoca WWTP 200 µm (water sample).

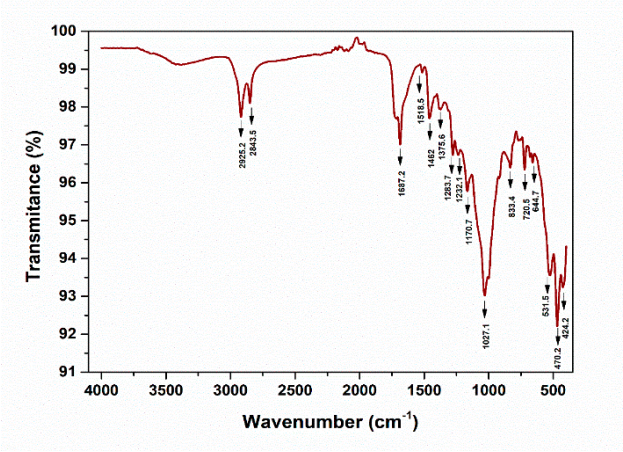

Figure 29. ATR-FTIR spectra – PE -DW Cluj Napoca WWTP 20 µm **(**water sample**).**

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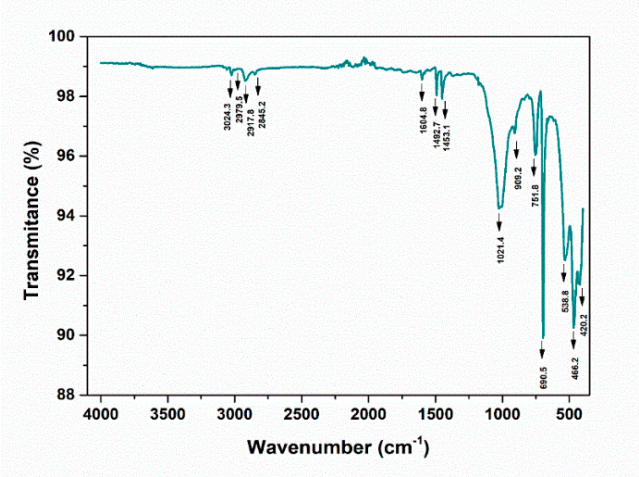

Figure 30. ATR-FTIR spectra-PP-UP Floresti 200 µm (water sample).

The peaks around ~2955, ~2848, ~1467 and ~720 cm^{-1} correspond to PE. Meanwhile, PP can be identified by its strong absorption bands at 2845, 2917 and 2979 cm^{-1}.

The infrared spectra of the filter paper are presented in supplementary material (Figure S4 and table S1). Literature reported that cellulose ester filters reveled peaks at 900, 1300, 1400 cm^{-1}. This aspect made it difficult for the MPs identification.

The employed spectral techniques Raman and FT-IR enabled the estimation of qualitative identification of MPs smaller than 500µm. According to the literature, these methods are complementary [53]. The results showed that both methods confirm the identification of the same type of polymers. The presence of MPs (such as PE, PS, PP and PC) in various forms and sizes is linked to their use in food packaging, bottles, toys, textiles and building materials.

4. Conclusions

The issue of MPs contamination is gradually being recognized in order to highlight its effects on the environment and human health. Scientists are engaged in debates regarding new analytical control and monitoring methods. The objective of the paper was to explore existing methods for the identification and characterization of MPs. Both field and laboratory activities were conducted to emphasize the problem of rivers contamination with MPs. The main challenge was to determine the presence of MPs using the available characterization and identification methods and research infrastructure. The approach included: i) organizing an environmental sampling campaign in the North-West area of Romania, specifically in the Somesul Mic River section from Floresti to Cluj-Napoca. This campaign aimed to reveal the occurrence MPs contamination in the surface water, sediment, and wastewater. (ii) Carrying out laboratory experiments to isolate and characterize MPs from the collected samples; iii) Exploring the possibilities of identifying MPs through the use of spectroscopic techniques, namely Raman and FT-IR. By combining these approaches, the research aimed to shed light on the extent of MPs contamination and contribute to the development of effective identification and monitoring methods.

The investigations revealed the widespread presence of MPs of various forms, types, and sizes along the course of the Somesul Mic River in the Floresti-Cluj-Napoca section. Both large (1-5 mm) and small (<1 mm) MPs were observed, taking on shapes such as fibers, fragments, foam, foils, and spheres, and exhibiting a range of colors including red, green, blue, purple, pink, white, black, transparent, and opaque. Based on their spectra and literature data, the identification of PE, PP, and PS was confirmed in all samples. Due to the lack of quantitative determinations, the differences between the sampling points was not possible. Based on the stereomicroscopic investigations could be highlighted a greater abundance of MPs in sediments compared to the water. The inadequate waste management measures and lack of public awareness contribute to the persistence and amplification of MPs contamination in river areas. This has been highlighted through the field images of the banks of Somesul Mic River.

Raman techniques using the NRS-7200 Raman Spectrometer and/or LabRam HR800 system and ATR FT-IR method using Perkin – Elmer Spectrum Two IR spectrometer were useful for application in MPs identification from environmental samples. The identification of MPs particles smaller than 500 μm to 20 μm could be able using μRaman or ATR FT-IR method but with some limitations concerning the sample preparation, interferences reduction, availability of polymer spectra database and human training. Also the microscopic investigation showed the abundance and diversification of MPs, being a method that complete the research of MPs contamination.

Currently, the monitoring MPs in environmental samples is a time-consuming and costly process without standard protocols that required experienced researchers and new field and laboratory equipment's.

It is becoming increasingly apparent that MPs have become a part of our daily lives, and the effects of their presence are starting to manifest. The slogan "*our waste on our plate*" is becoming truer as the impact of MPs on the environment and even our own consumption becomes more evident.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1. Photos taken during the sampling campaign on Somesul Mic River; Figure S2 Images of the river banks taken at the sampling points along the Somesul Mic River; Figure S3 Visualization of plastic particles - large, micro and macro floating MPs separated from Somesul Mic River (surface water and sediment); Figure S4 Visualization of plastic particles in the influent of Cluj-Napoca WWTP; Figure S5 SEM images of suspected MPs - decomposing floating MPs; Figure S6 SEM images of suspected MPs without exposure to digestion treatment, scale bar = 500 μm, 50 μm, 20 μm; Figure S7 ATR-FTIR spectra of the ester cellulose filter membrane; Table S1. Assignment of the ATR-FTIR band exhibited by the filter membrane; Table S2. Assignment of the ATR-FTIR band exhibited by the UP Cluj-Napoca WWTP 200 μm (PE) - Figure 27; Table S3. Assignment of the ATR-FTIR band exhibited by the DW Cluj-Napoca WWTP 200 μm (PE) - Figure 28; Table S4. Assignment of the ATR-FTIR band exhibited by the DW Cluj-Napoca WWTP – 20 μm (PE) - Figure 29; Table S5. Assignment of the ATR-FTIR band exhibited by the UP Floresti 200 μm (PP) - Figure 30 [59,62–69].

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References

1. Nava V, Frezzotti ML, Leoni B. Raman Spectroscopy for the Analysis of Microplastics in Aquatic Systems. *Appl. Spectrosc.* **2021**, *75(11)*, 1341-1357.
2. Rodriguez, Ferdinand. "plastic". *Encyclopedia Britannica*, 22 Jun. 2023, https://www.britannica.com/science/plastic. Accessed 9 October 2023.

3. Geerdes Z., Hermann M., Ogonowski M. et al. A novel method for assessing microplastic effect in suspension through mixing test and reference materials. *Sci Rep*. **2009**, *9*, 10695.
4. Hurley R., Woodward J. and Rothwell J. J. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat. Geosci*. **2018**, *11*, 251–257.
5. Cózar A., Echevarría F., González-Gordillo J.I, Irigoien X., Úbeda B., Hernández-León S., Palma A.T, Navarro S., García-de-Lomas J., Ruiz A., Fernández-de-Puelles M.L and Duarte C.M. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci*. **2014**, *111*, 10239–10244.
6. Park H., Park B. Review of Microplastic Distribution, Toxicity, Analysis Methods, and Removal Technologies. *Water*, **2021**, *13*, 2736.
7. ISO/DIS 24187:2022, Principles for the analysis of microplastics present in the environment;
8. Jadhav E.B., Sankhla M.S., Bhat R.A., Bhagat D.S. Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. *Environ. Nanotechnol. Monit. Manag*. **2021,** *16*, 100608.
9. Boucher J. and Friot D. Primary Microplastics in the Oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN. **2017**, 43.
10. Available online: https://www.who.int/news/item/03-03-2020-shortage-of-personal-protective-equipment-endangering-health-workers-worldwide (Accessed in 10.08.2023).
11. Martinho S.D., Fernandes V.C., Figueiredo S.A., Delerue-Matos C. Microplastic Pollution Focused on Sources, Distribution, Contaminant Interactions, Analytical Methods, and Wastewater Removal Strategies: A Review. *Int. J. Environ. Res. Public Health.* **2022**, *19*, 5610.
12. Available online: https://waterquality.danube-region.eu/wp-content/uploads/sites/13/sites/13/2019/11/EUSDR_20191.pdf (Accessed on 11.08.2023).
13. Schrank I., Löder M.G.J., Imhof H.K., Moses S.R., Heß M., Schwaiger J. and Laforsch C. Riverine microplastic contamination in southwest Germany: A large-scale survey. *Front. Earth Sci.* **2022**, *10*, 794250.
14. Liu R.-P., Li Z.-Z., Liu F., Dong Y., Jiao J.G., Sun P.-P., El-Wardany R.M. Microplastic pollution in Yellow River, China: Current status and research progress of biotoxicological effects. *China Geol.* **2021,** *4 (4)*, 585-592.
15. Scherer C., Weber A., Stock F., Vurusic S., Egerci H., Kochleus C., Arendt N., Foeldi C., Dierkes G., Wagner M., Brennholt N., Reifferscheid G. Comparative assessment of microplastics in water and sediment of a large European river. *Sci. Total Environ*. **2020**, *738*, 139866.
16. Gerolin C.R., Nascimento Pupim F., Oliveira Sawakuchi A., Grohmann C.H., Labuto G., Semensatto D. Microplastics in sediments from Amazon rivers, Brazil. *Sci. Total Environ*. **2020**, *749*, 141604.
17. Kiessling T., Knickmeier K., Kruse K., Brennecke D., Nauendorf A., Thiel M. Plastic Pirates sample litter at rivers in Germany – Riverside litter and litter sources estimated by schoolchildren. *Environ. Pollut.* **2019**, *245*, 545-557.
18. Huang S., Peng C., Wang Z., Xiong X., Bi Y., Liu Y., Li D. Spatiotemporal distribution of microplastics in surface water, biofilms, and sediments in the world's largest drinking water diversion project. *Sci. Total Environ*. **2021**, *789*, 148001.
19. Semmouri I., Vercauteren M., Van Acker E. et al. Presence of microplastics in drinking water from different freshwater sources in Flanders (Belgium), an urbanized region in Europe. *FoodContamination*. **2022**, *9*, 6.
20. Ziani K., Ioniță-Mîndrican C.-B., Mititelu M.; Neacșu S.M., Negrei C., Moroșan E., Drăgănescu D., Preda O.-T. Microplastics: A Real Global Threat for Environment and Food Safety: A State of the Art Review. *Nutrients.* **2023**, *15*, 617.
21. Available online: https://cdn.who.int/media/docs/default-source/wash-documents/microplastics-in-dw-information-sheet190822.pdf; (Accessed on 13 August 2023).
22. Mason S.A., Welch V.G., Neratko J. Synthetic Polymer Contamination in Bottled Water. *Front Chem*. **2018** *6:407*.
23. Chaudhari S., Samnani P. Determination of microplastics in pond water. *Mater. Today: Proc.* **2023**, *77*, 1, 91-98.
24. Pojar I., Stănică A., Stock F., Kochleus C., Schultz M., Bradley C. Sedimentary microplastic concentrations from the Romanian Danube River to the Black Sea. *Sci Rep*. **2021**, *11(1):2000*.
25. Pojar I., Kochleus C., Dierke G., Ehlers M.S., Reifferscheid G., Stock F. Quantitative and qualitative evaluation of plastic particles in surface waters of the Western Black Sea, *Environmental Pollution*, 021, 268, Part A, 2, 115724.
26. Ding L., Mao R. f., Guo X., Yang X., Zhan, Q., et al. Microplastics in surface waters and sediments of the Wei River, in the northwest of China. *Sci. Total Environ*. **2019**, *667*, 427–434.
27. Global Plastics Outlook: Policy Scenarios to 2060, OECD Publishing, **2022,** Paris.
28. Available online: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/ (Accessed in 10.08.2023).

29. Uddin S., Fowler S.W., Saeed T., Naji A., Al-Jandal N., Standardized protocols for microplastics determinations in environmental samples from the Gulf and marginal seas. *Mar. Pollut. Bull.* **2020**, *158*, 111374.
30. Wagner M, Scherer C, Alvarez-Munoz D, Brennholt N, Bourrain X, Buchinger S, et al. Microplastics in freshwater ecosystems: what we know and what we need to know. *Environ Sci Eur*. **2014**, *26(1)*, 12.
31. Asifa A., Afroza A.L., Md Nazrul I., Md Morsaline B., Shaikh T.A., Md Moshiur R., Sheikh M.R., Microplastics Pollution: A Brief Review of Its Source and Abundance in Different Aquatic Ecosystems, *Journal of Hazardous Materials Advances*, 2023, 9, 100215, https://doi.org/10.1016/j.hazadv.2022.100215.
32. Ashkan J., Microplastics in the urban atmosphere: Sources, occurrences, distribution, and potential health implications, *Journal of Hazardous Materials Advances*, 2023, 12, 100346, https://doi.org/10.1016/j.hazadv.2023.100346.
33. Enders K., Lenz R., Stedmon C.A., Nielsen T.G. Abundance, size and polymer composition of marine microplastics >= 10um in the atlantic ocean and their modelled vertical distribution. *Mar Pollut Bull*, 2015, 100, 70- 81.
34. Eriksson C, Burton H. 2003. Origins and biological accumulation of small plastic particles in fur seals from macquarie island. Ambio 32:380-384.
35. Cox K.G., Covernton G.A., Davies H.J, Dower J.F., Juanes F. and Dudas S.E. *Environ. Sci. Technol.* **2019,** *53* (12), 7068-7074.
36. Meyers N., Catarino A.I., Declercq A.M., Brenan A., Devriese L., Vandegehuchte M., De Witte B., Janssen C., Everaert G., Microplastic detection and identification by Nile red staining: Towards a semi-automated, cost- and time-effective technique. *Sci. Total Environ.* **2022,** *823*, 153441.
37. Cowger W., Gray A., Christiansen S.H., DeFrond H., Deshpande A.D., Hemabessiere L., Lee E., Mill L., Munno K., Ossmann B.E., Pittroff M., Rochman C., Sarau G., Tarby S., Primpke S. Critical review of processing and classification techniques for images and spectra in microplastic research. *Appl. Spectrosc.* **2020**, *74*, 989-1010.
38. Bianco V., Memmolo P., Carcagnì P., Merola F., Paturzo M., Distante C., Ferraro P. Microplastic identification via holographic imaging and machine learning. *Adv. Intell. Syst*. **2020,** *2*, 1900153
39. Available online: https://www.epa.gov/system/files/documents/2021-09/microplastic-beach-protocol_sept-2021.pdf (Accessed on 13 August 2023)
40. Guidelines for sampling microplastics on sandy beaches. Rocha International Sampling Guide, **2018**, A Rocha International, London.
41. Filipa Bessa et al. Technical Report. Harmonized protocol for monitoring microplastics in biota. JPI-Oceans BASEMAN project. **2019.**
42. ISO/TR 21960:2020, Plastics — Environmental aspects — State of knowledge and methodologies.
43. Masura, J., et al. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48. **2015**
44. Schrank I., Möller J.N., Imhof H.K., Hauenstein O., Zielke F., Agarwal S., Löder M.G.J., Greiner A., Laforsch C. Microplastic sample purification methods - Assessing detrimental effects of purification procedures on specific plastic types. *Sci. Total Environ*. **2022**, *833*, 154824.
45. Al-Azzawi M.S.M., Kefer S., Weißer J., Reichel J., Schwaller C., Glas K., Knoop O., Drewes J.E. Validation of Sample Preparation Methods for Microplastic Analysis in Wastewater Matrices—Reproducibility and Standardization. *Water*. **2020**, *12*, 2445.
46. Kärrman A., Schönlau C., Engwall M., Exposure and Effects of Microplastics on Wildlife - A review of existing data, 2016-03-01, Funded by Swedish Environmental Protection Agency, MTM Research Centre School of Science and Technology Örebro University, Sweden.
47. Wu X., Zhao X., Chen R., Liu P., Liang W., Wang J., Teng M., Wang X., Gao S. Wastewater treatment plants act as essential sources of microplastic formation in aquatic environments: A critical review. *Water Research.* **2022**, *221*, 118825.
48. Gerdts, G. (ed.). Defining the BASElines and standards for Microplastics ANalyses in European waters. Project BASEMAN Final report. JPI-Oceans BASEMAN Project. **2019**, 25.
49. Blair R.M., Waldron S., Phoenix V.R. et al. Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. *Environ Sci Pollut Res.* **2019**, *26*, 12491–12504.
50. Joshy A., Krupesha Sharma S.R, Mini K.G. Microplastic contamination in commercially important bivalves from the southwest coast of India. *Environ. Pollut.* **2022**, *305*, 119250.
51. Mariano S, Tacconi S, Fidaleo M, Rossi M and Dini L. Micro and Nanoplastics Identification: Classic Methods and Innovative Detection Techniques. *Front. Toxicol.* **2021**, *3:636640*.
52. Schwarzer M., Brehm J., Vollmer M., Jasinski J., Xu C., Zainuddin S., Fröhlich T., Schott M., Greiner A., Scheibel T., Laforsch C. Shape, size, and polymer dependent effects of microplastics on Daphnia magna. *J. Hazard. Mater*. **2022**, *426*, 128136.

53. Rytelewska S., Dąbrowska A. The Raman Spectroscopy Approach to Different Freshwater Microplastics and Quantitative Characterization of Polyethylene Aged in the Environment. *Microplastics.* **2022**, *1*, 263-281.
54. Sharma S., Chio C., Muenow D. Raman Spectroscopic Investigation of Ferrous Sulfate Hydrates**, 2006**,*37*.
55. Scardaci V.; Compagnini G. Raman Spectroscopy Investigation of Graphene Oxide Reduction by Laser Scribing. *C* **2021**, *7*, 48.
56. Primpke S., Christiansen S.H., Cowger W., de Frond H. et al. Critical Assessment of Analytical Methods for the Harmonized and Cost Efficient Analysis of Microplastics. *Appl. Spectrosc*. **2020**, *74(9)*, 1012–1047.
57. Leung M. M.-L., Ho Y.-W., Lee C.-H., Wang Y., Hu M., Kwok K. W. H., Chua S.-L., Fang, J. K.-H., Improved Raman spectroscopy-based approach to assess microplastics in seafood, *Environ. Pollut*. **2021,** *289*, 117648.
58. Karami A., Golieskardi A., Choo C.K., Romano N., Ho Y.B., Salamatinia B. A high-performance protocol for the extraction of microplastics in fish. *Sci. Total Environ*. **2017**, *578*, 485-494.
59. Smith B.C. The Infrared Spectra of Polymers II: Polyethylene. Spectroscopy. **2021**, *36 (9)*, b, 24–29.
60. Herman V., Takacs H., Duclairoir F., Renault O., Tortai J.H., Viala B. Core double-shell cobalt/graphene/polystyrene magnetic nanocomposites synthesized by in situ sonochemical polymerization. *RCS Advenced*. **2015**, *5(63)*, 51371–51381.
61. Smith B.C. The Infrared Spectra of Polymers III: Hydrocarbon Polymers. *Spectroscopy.* **2021**, *36 (11)*, a, 22–25.
62. Käppler A. et al. Analysis of environmental microplastics by vibrational microspectroscopy: FT-IR, Raman or both? *Anal. Bioanal. Chem*. **2016**, *408(29)*, 8377–8391.
63. Sharma N., Sharma V., Jain Y., Kumari M., Gupta R., Sharma S. K. and Sachdev K. Synthesis and Characterization of Graphene Oxide (GO) and Reduced Graphene Oxide (rGO) for Gas Sensing Application. *Macromol. Symp.* **2017**, *376(1)*, 1700006.
64. Nallasamy P., Anbarasan P.M., Mohan S. Vibrational Spectra and Assignments of cis- and Trans-1,4-Polybutadiene. *Turk J Chem*. **2002**, *26*, 105 – 111;
65. Gong Y., Li D., Fu Q., and Pan C. Influence of graphene microstructures on electrochemical performance for supercapacitors. *Prog. Nat. Sci.: Mater. Int.* **2015**, *25(5)*, 379–385.
66. Syakti A.D. et al. Simultaneous grading of microplastic size sampling in the small islands of Bintan Water, Indonesia. *Mar. Pollut. Bull.* **2018,** 137, 593–600.
67. Chirea M. et al. Gold nanowire networks: Synthesis, characterization, and catalytic activity. *Langmuir.* **2011,** 27(7), 3906–3913.
68. Krylova V. and Dukštienė N. Synthesis and characterization of AG2S layers formed on polypropylene'. *J. Chem*. **2013**, 1–11.
69. Bhattacharya S.S. and Chaudhari S.B. Study on Structural, Mechanical and Functional Properties of Polyester Silica Nanocomposite Fabric. *Int. J. Pure Appl. Sci. Technol.*, **2014**, *21(1)*, 43-52.