OCCURRENCE AND TYPE OF MICROPLASTICS PRESENT IN ISHMI RIVER WATER AND SEDIMENTS: AN OVERVIEW OF METHODOLOGY FOR SAMPLING AND ANALYSIS

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ABSTRACT

In the past decade, microplastic waste in marine and freshwater ecosystems has received increasing attention. The presence of floating plastic debris, including microplastics (MPs), has been documented since the early 1970s, not only in the North Atlantic, North Pacific, and South Pacific Oceans but also in the Mediterranean Sea. In response to this, the United Nations Environment Program has identified plastic pollution as a critical environmental issue. Alongside climate change, microplastic pollution is emerging as a serious challenge with the potential to significantly affect biodiversity and human health. In Albania, an estimated 198 tons of low-density and 111 tons of high-density plastic waste are produced daily, comprising about 14% of the country's total waste. Due to its durability, plastic can persist in lakes and seas for hundreds of years, eventually breaking down into micro- and nanoplastics, which can infiltrate living organisms and enter the food chain. While most microplastic studies have focused on marine environments, research on riverine systems remains limited. This study addresses this gap by investigating the presence of microplastics in the water and sediments of the Ishmi River, which is considered Albania's most polluted basin by the National Environmental Agency. The Ishmi River, 74 km in length, flows through

Rinas and Fushë Kruja before reaching the Adriatic Sea at Cape Rodoni. The primary sources of pollution in the river are plastic and microplastic waste, stemming from household products, local activities along the river basin, and natural processes. This study presents an analytical methodology for sampling and analyzing microplastic pollution, including the steps of collection, chemical treatment, density separation, and sample filtration. The objective was to adopt protocols that isolate microplastics from large amounts of organic matter in the riverine environment, while preserving their structure. The identification of microplastics was performed using optical microscopy, FTIR, and Raman Spectroscopy.

***Keywords*:** Microplastics, sampling, spectroscopy, freshwater ecosystem

1. INTRODUCTION

A substantial volume of plastic waste has infiltrated aquatic ecosystems due to extensive production and inadequate waste management practices (Filgueiras et al., 2019). Within these environments, plastic waste undergoes fragmentation, producing micro-sized particles such as fragments, fibers, spheroids, granules, pellets, and flakes, ranging in size from 0.1 to 5000 μm, collectively known as microplastics (MPs) (Adomat *et al*., 2021a). Plastics are extensively used in various industries, with packaging accounting for the largest share at 40%. Unfortunately, around 70% of all produced plastic ends up as waste, with only 9% being recycled (Maddela *et al*., 2023). The ecological ramifications of microplastics are profound, affecting degradation processes, environmental interactions, and their potential impact on the food chain and human health (Vethaaka *et al*., 2021; Maddela *et al*., 2023).

Plastic pollution is a significant issue in Albania. According to a recent World Health Organization (WHO) report, Albania is among the most problematic countries, with 73% of its plastic waste left untreated (WWF, 2019). This places Albania among the top four Mediterranean countries with the highest levels of untreated plastic waste, alongside Montenegro, Egypt, and Libya. Moreover, the average amount of total marine litter in Albania's coastal area is estimated to be 0.7 kg at a depth of 241–244 meters, with 0.2 kg of that being plastic (Gjyli *et al*., 2020; Kolitari *et al*., 2020). To date, there are only 2-3 studies that provide data on plastic pollution in the Albanian marine ecosystem, with a similarly limited number of studies on microplastic pollution (Aliko *et al*., 2022).

This study aims to introduce a sample preparation technique designed specifically for the analysis of microplastics in river waters. The correct

application of this technique was crucial to ensuring accurate and reliable data, involving the effective selection and isolation of microplastics while minimizing contamination (Löder *et al*., 2015). The study critically assessed the methodologies used to gauge microplastic pollution in the Ishmi River environment (Marine Debris Program, 2015; Faruk Çullu *et al*., 2021). The primary focus of this paper is on presenting analytical methods, including a review of non-selective sampling approaches, laboratory processing, and the techniques applied for microplastic analysis.

Using Image-J software, microplastics were classified by size and type. The study also evaluated the presence of various microplastic polymers through Fourier Transform Infrared (FTIR) and Raman spectroscopies, complemented by microscopic examinations

2. MATERIALS AND METHODS

2.1 Sample collection in river water and sediments

Collecting river water samples for microplastic pollution analysis requires meticulous planning to ensure accurate and representative results (AMP *et al*., 2020). Before selecting the sampling stations, it was essential to gather information on the Ishmi River, including its characteristics and potential sources of microplastics in the area. Three stations were selected along the river based on their potential for microplastic contamination (Diku *et al*., 2020), representing urban, industrial, and rural zones.

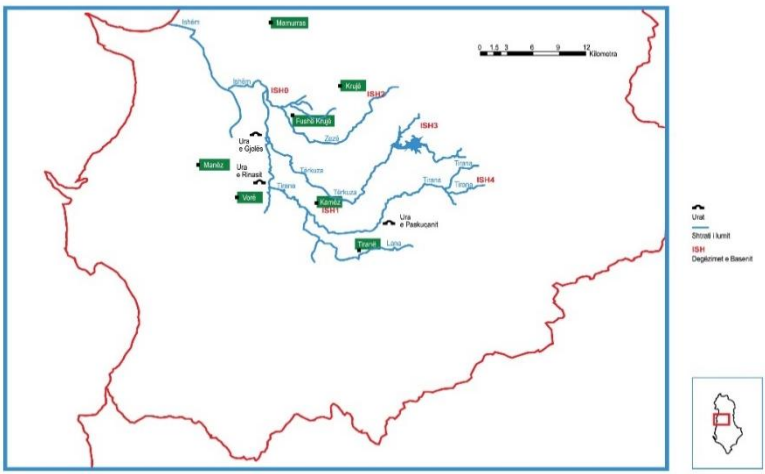


Fig.1. Map of bridges where the samples were taken.

Before sampling, all equipment was sterilized, and cotton clothing and laboratory gloves were worn to prevent contamination. At each station, 2-3 liters of water samples were collected from the coastal areas, with the third station located along the riverbanks and at the river's center. Sterilized glass bottles were used to collect surface water at approximately 10 cm depth (Zheng *et al*., 2021). Sampling took place in June 2023.

For accurate estimation of microplastic concentration in sediment samples, 2-3 kg of sediment was collected from a depth of 1 to 5 cm (Prata *et al*., 2019). All samples were transported to the laboratory and stored at 4°C until further processing.

2.2 Microplastic Separation from samples

The process of microplastic separation is a pivotal stage, crucial for ensuring the precision and reliability of the analysis, thereby aiding in a deeper understanding of microplastic pollution in aquatic environments. Separation of samples (Prata *et al*., 2019) is performed for three main purposes: i) to concentrate microplastics from a larger water volume, facilitating more accurate detection and analysis. ii) to eliminate other organic and inorganic materials present in the sample, and iii) to enhance the concentration of microplastics, allowing for a clearer visual examination under a microscope, aiding in their accurate identification and classification.

The separation of microplastic particles from denser matrices is achieved through flotation, using saturated salt solutions with high density.

2.3 Water samples

To minimize the influence of organic matter in the water sample, 30% H_2O_2 was added (Li *et al*., 2021) to the glass bottle containing the water sample. The bottle was then placed in a constant temperature oscillation box and vibrated at 100 r/min at 70°C for 24 hours. After this treatment, the supernatant was extracted and mixed with NaCl in a ratio of 1 liter to 300 grams and placed on a constant temperature magnetic plate for an additional 24 hours.

To reduce sample volume and improve efficiency, the entire sample was transferred to a separatory funnel and allowed to sit for 24 hours. After decanting the larger particles and separating densities, only the top 1/5 of the water column (surface part) was filtered using a vacuum pump with 2.2 μm filter paper. Prior to analysis, the filter papers were placed in an oven and dried at 30°C for 48 hours.

2.4 Sediment samples

The sediment samples were dried in an oven at 70°C and then sieved through a 500–75 μm mesh for 30 minutes (Claessens *et al*., 2011; Adomat *et al*., 2021b). The sieved samples were diluted in a saturated NaCl solution and left on a magnetic plate for 72 hours to ensure thorough homogenization. To minimize the sample volume, the mixture was transferred to a separatory funnel and allowed to settle for 24 hours. After decanting, only the top 1/3 of the water column (surface portion) was treated with 30% H_2O_2 at 60°C on a magnetic plate (Klein *et al*., 2015). After 48 hours, the solution was transferred to the separatory funnel again and left to settle for another 72 hours. Following this second decantation, the remaining water column was filtered using a vacuum pump through 2.2 μm filter paper. The filtered sample was then placed in a covered Petri dish and dried in an oven at 30°C for 72 hours.

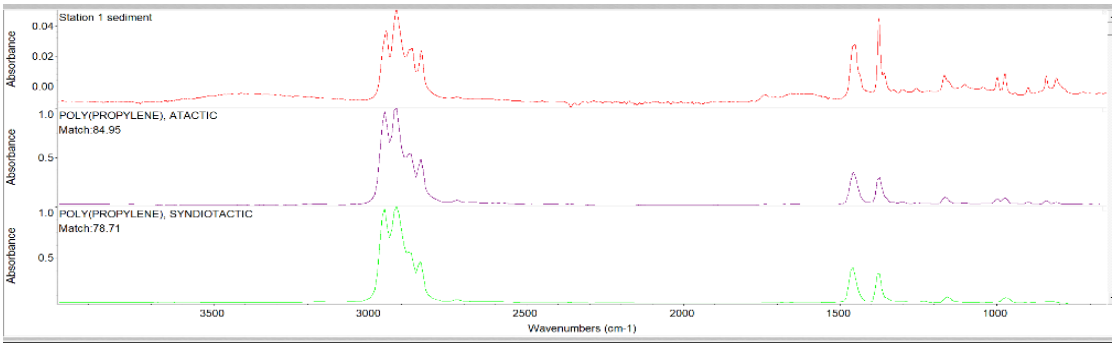
3. RESULTS

3.1 Visual and chemical identification of Microplastics

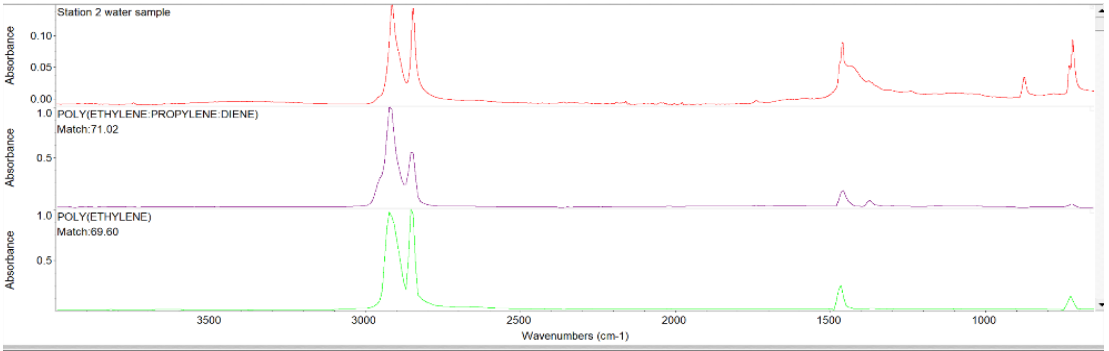
Over the past decade, FTIR imaging has emerged as one of the most effective techniques for analyzing microplastics. This method allows for the rapid recognition, counting, identification, and classification of particles and fibers based on their spectral information (Janice Brahney, 2021).

Initially, the filter was examined using an optical microscope (Kozo XJPG304, Sony TCC-8.1, version 7.3.1.7, 40x100 zoom) (Fig. 2). Detected microplastic waste was pinpointed with coordinates and subsequently analyzed using a FTIR Spectrometer Nicolet 6700 (Fig. 3). The resulting images provided clear information on the presence of microplastic polymers such as polystyrene and polyethylene.

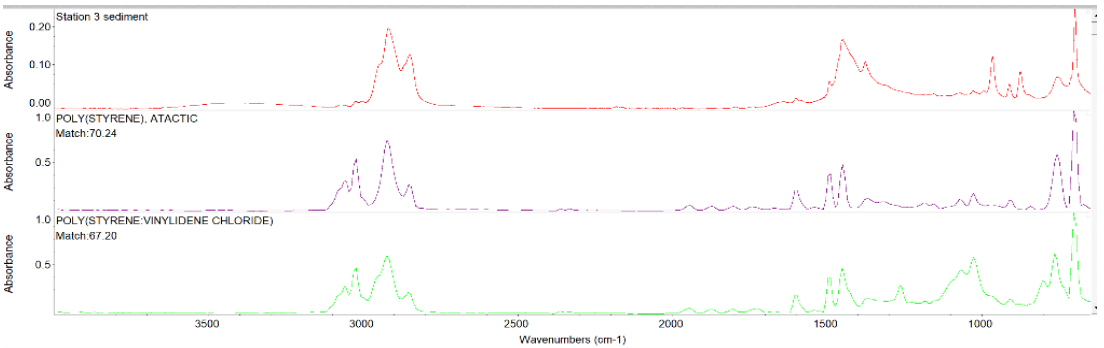
Fig.2. Images of MP waste in samples



	Index	Match	Compound Name	Library Name
1	41	84.95	POLY(PROPYLENE), ATACTIC	Hummel Polymer Sample Library
2	38	78.71	POLY(PROPYLENE), SYNDIOTACTIC	Hummel Polymer Sample Library
3	39	62.35	POLY(ETHYLENE:PROPYLENE)	Hummel Polymer Sample Library

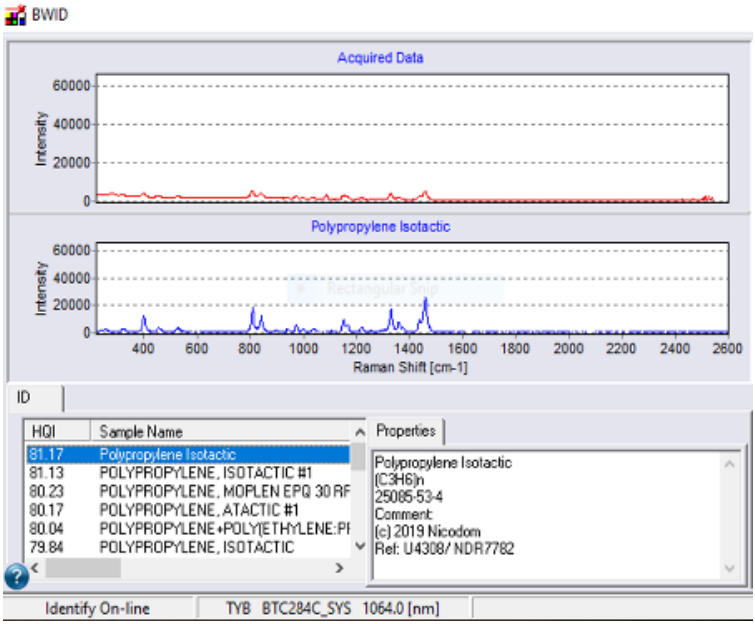


	Index	Match	Compound Name	Library Name
1	32	71.02	POLY(ETHYLENE:PROPYLENE:DIENE)	Hummel Polymer Sample Library
2	7	69.60	POLY(ETHYLENE)	Hummel Polymer Sample Library
3	39	56.84	POLY(ETHYLENE:PROPYLENE)	Hummel Polymer Sample Library



	Index	Match	Compound Name	Library Name
1	46	70.24	POLY(STYRENE), ATACTIC	Hummel Polymer Sample Library
2	47	67.20	POLY(STYRENE:VINYLIDENE CHLORIDE)	Hummel Polymer Sample Library
3	25	64.00	POLY(BUTADIENE) + NAPHTHENIC OIL	Hummel Polymer Sample Library

Fig 3: Examples of the FTIR spectra of the three microplastic particles found in the water and sediment samples, which were measured by micro-FTIR spectroscopy: upper panel polypropylene, second panel polyethylene, third panel polystyrene.


BWID
Acquired Data
Intensity
60000
40000
20000
0
Polypropylene Isotactic
Intensity
60000
40000
20000
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400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600
Raman Shift [cm-1]
ID
HQI Sample Name
81.17 Polypropylene Isotactic
81.13 POLYPROPYLENE, ISOTACTIC #1
80.23 POLYPROPYLENE, MOPLEN EPQ 30 RF
80.17 POLYPROPYLENE, ATACTIC #1
80.04 POLYPROPYLENE+POLY(ETHYLENE:PI
79.84 POLYPROPYLENE, ISOTACTIC
Properties
Polypropylene Isotactic
(C3H6)n
25085-53-4
Comment
(c) 2019 Nicodom
Ref: U4308/ NDR7782
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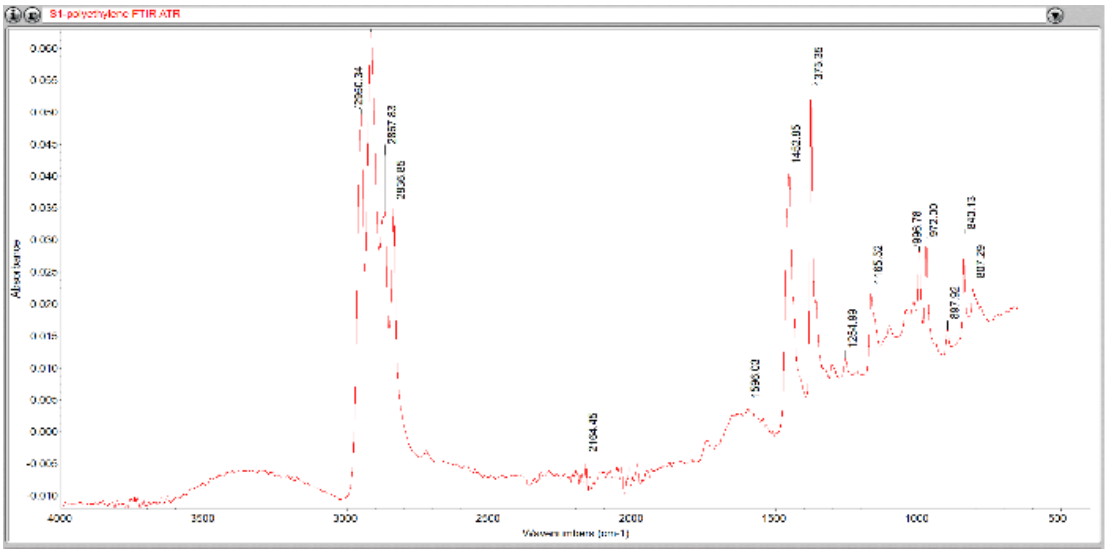
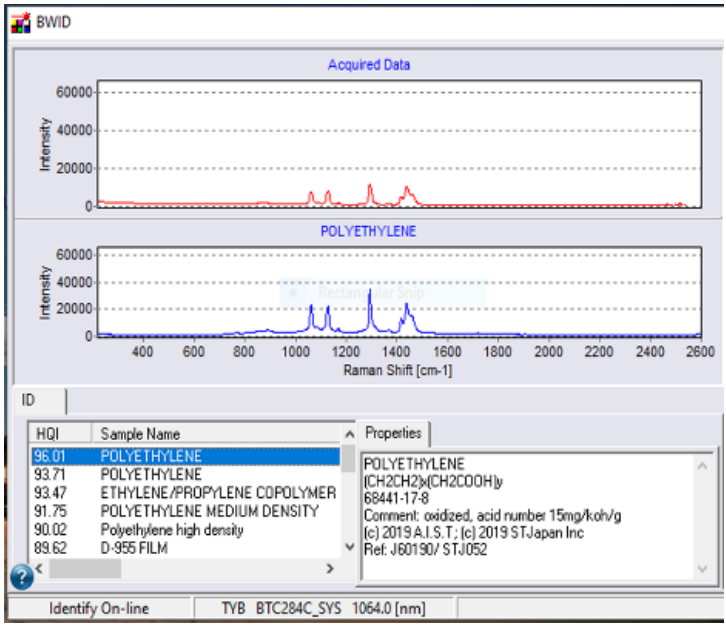
TYB BTC284C_SYS 1064.0 [nm]


S1 polypropylene FTIR ATR
Absorbance
4000 3500 3000 2500 2000 1500 1000 500
Valores enteros [cm-1]



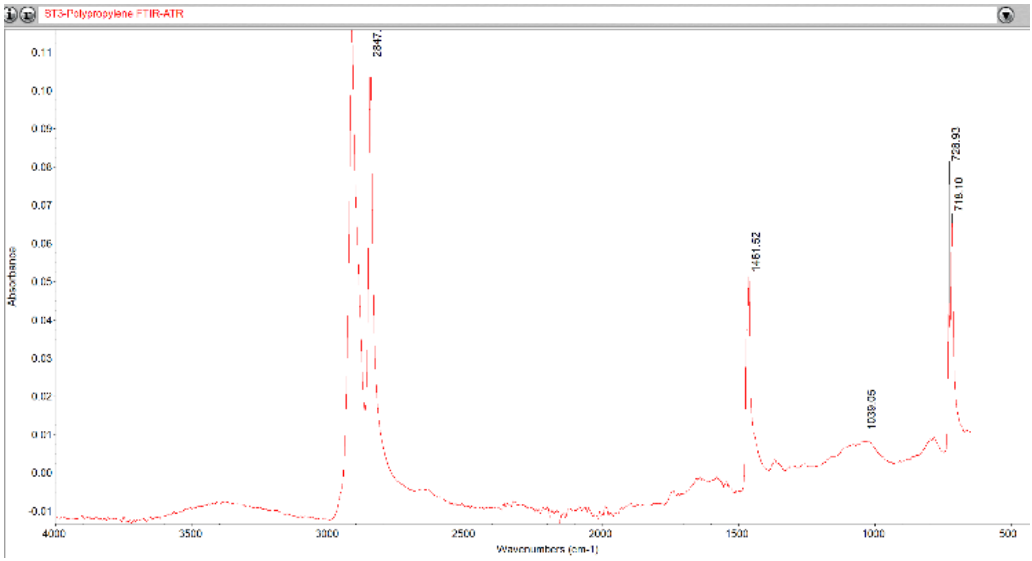


Fig.4. Raman Spectra (left), FTIR Spectra(right) of polypropylene and polyethylene of the same samples.

3.2 FTIR and Raman analysis

To assess the quality of the obtained results, the samples were also analyzed using Raman spectroscopy. The results from both spectroscopic techniques—FTIR and Raman—were consistent, confirming the presence of the same polymers in the samples (Fig. 4). These techniques, being complementary, provided a robust validation of the microplastic polymer identification.

4. Discussion

Due to the techniques used for extracting and purifying the samples, it was expected that they would primarily contain tiny fragments, fibers, particles, and other low-density organic materials. However, plastic fragments were notably scarce, comprising only 20.8% of the particles analyzed. Plastic fibers made up 23%, while particles constituted 22.9%. Our manual screening of bright-field microscopic images for areas with heterogeneous particle appearance revealed a moderate overall abundance of microplastics in the samples.

First, it is noteworthy that a significant portion of the particles were quartz, which was unexpected given that these were supposed to be excluded during density separation. The presence of substantial quantities of sand grains raises questions and suggests a need for further investigation. To address this, it is advisable to use a higher-density salt, such as NaI instead of NaCl, to improve the qualitative differentiation between microplastic waste and the remaining solution.

Second, literature on water sample collection indicates various methodologies, emphasizing the importance of sampling time and river water velocity. Using a Manta net could enhance the capture of a representative sample of microplastic pollution by ensuring a more comprehensive collection of waste abundance (Pasquier *et al*., 2022).

5. CONCLUSION

This case study highlights the essential role of spectroscopic techniques, such as FTIR and Raman spectroscopy, in microplastics analysis. FTIR and Raman spectroscopy have proven to be highly effective for confirming the polymer composition of microplastic particles. The microscopic method used to evaluate the residues was successful, as microplastic particles were clearly visible and identifiable. The analysis

revealed that the most common microplastics were fibers ranging from 100 to 200 µm in size, while fragments were predominantly smaller than 100 µm². The polymers identified in the samples included polypropylene, polyethylene, polystyrene, polyvinyl chloride, and polybutadiene. FTIR spectroscopy offers a broad range of wavenumbers, including fingerprint regions and single bonds, providing comprehensive polymer characterization. Conversely, Raman spectroscopy excels in providing detailed information in the fingerprint region, complementing the data obtained from FTIR.

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