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TESI DI DOTTORATO DI RICERCA

ANALYSIS AND CHARACTERIZATION OF MICROPLASTICS THROUGH VIBRATIONAL SPECTROSCOPIC TECHNIQUES FOR ENVIRONMENTAL MONITORING

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To my dear family…
It's our PhD…

Acknowledgments

The most heartfelt part of my PhD....

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This PhD may appear to have been completed in three years, but as I write this line, I am reminded of my parents' sacrifices and hard work of twenty-eight years.

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-Monika

SUMMARY

The pinnacle of technological advancements, especially plastic, has become one of the greatest environmental challenges that the earth has ever dealt with. In the face of ground-breaking versatility, plastic litter has marked its presence from the highest peaks to the deepest points in the oceans. Microplastics (MPs) are plastic particles with a size of less than 1 mm along their longest dimension, originating from a wide array of sources. The current public awareness of MP pollution is based on a huge amount of scientific research completed and published over the last fifteen years, which has just recently been highlighted by the media. It's been a protracted process that began with isolated examinations carried out by researchers who were ordinarily working in various fields of study but recognised the threat's potential. MPs are not traditional chemical contaminants, but rather a complex array of manmade detritus made up of various sizes, polymers, chemical additives, and sorbed pollution. The MP study is still in its infancy stage since it continues to be hampered by a lack of defined protocols and methodologies for investigating MPs in the laboratory. The use of MPs in laboratory research necessitates precise particle characterization to link the impacts of microplastics to their characteristics. To understand microplastic transit, deposition, and toxic effects, it is vital to distinguish between MP particles and those that are not. This thesis has primarily focused on the application of a new technology for analyzing MPs, based on Near-Infrared Spectroscopy (NIRs). As revealed by the bibliometric analysis of characterizing MPs by Fourier-Transformation Infrared Spectroscopy (FTIR) and Near-Infrared Spectroscopy, NIRs have only lately been applied, notably in the form of the miniaturized spectrometer (NIRs). Although NIR spectroscopy has been used as a standard tool for online quality assurance in food manufacturing and pharmaceuticals for decades, its ability to analyse microplastics in various environmental matrices has only recently been recognized. The workflow of this thesis begins with the use of handheld MicroNIR to analyse urban plastic garbage and construct an in-house NIR spectrum library, showcasing the usage of portable technology in the recycling sector. Because most libraries are constructed with virgin polymers, spectral shifts caused by MPs degradation are frequently missed. As a result, a new, more durable library searching algorithm capable of dealing with the difficulty of comparing degraded MPs to pristine polymer references was necessary. The second section discussed the significance of using reference materials in MP research and compares three techniques for producing MPs for research laboratories. This work will make it easier to comprehend the morphologies of MPs produced from the same parent particle using diverse techniques in a short period, allowing MP research to accelerate. The third section is based on the proof-of-concept study to analyse mixtures of microplastics through a handheld Near-Infrared Spectrometer. Given that, this study has proven the possibility of a portable tiny near-infrared spectrometer (MicroNIR) paired with chemometric methodologies for the measurement of secondary MPs mixes created at a laboratory scale for the first time. Extraction and purification are followed by identification and quantification in the MP analysis. The extraction of MPs from any environmental matrix is the most important phase since it is controlled by the matrix type and microplastic's size, shape, and density. As a result, the extraction technique should be tailored to the type of matrix under consideration. Following this idea, a comprehensive description of microplastic extraction processes distinguished by environmental matrix is offered at the end of this thesis in the form of a review. With concluding remarks, the final chapter gives a glimpse into the study's future prospects.

DECLARATION OF SCIENTIFIC CONTRIBUTIONS

The present thesis includes articles that have been published in cooperation with co-authors, with my own contributions declared as follows:

CHAPTER 1: THE PILOT

1.3 The Global Vision: Results of VAMAS Survey on the issues of Microplastics
The concept was designed by Prof. Laura E. Depero. The questionnaire was designed by Monika Rani under the guidance of Dr. Stefania Federici and Prof. Laura E. Depero.

Federici, Stefania, Monika Rani, and Laura Eleonora Depero. "Results of VAMAS Survey Regarding Microplastic Issues." (2021).

CHAPTER 3: THEORITICAL SETUPS

3.3 Bibliometric Analysis on Influx of NIR Technology in Microplastic Analysis
The concept was designed by Monika Rani. The literature search, review and data analysis were done by Monika Rani. The work was supervised by Dr. Stefania Federici and Prof. Laura E. Depero

CHAPTER 4: THE FOUNDATION

ON BUILDING OF IN-HOUSE NIR REFERENCE SPECTRA LIBRARY WITH POST-CONSUMER PLASTIC WASTE USING HANDHELD MICRO-NIR
The formal investigation and Near Infrared Measurements were done by Monika Rani and Claudio Marchesi. The NIR data analysis was done by Monika Rani and Chemometric analysis was done by Claudio Marchesi. The manuscript was written by Monika Rani and Claudio Marchesi while Dr. Stefania Federici and Prof. Laura E. Depero supervised the work.

Rani, Monika, et al. "Miniaturized near-infrared (MicroNIR) spectrometer in plastic waste sorting." Materials 12.17 (2019): 2740.

CHAPTER 5: THE GROUNDWORK

ON PREPARATION OF TRUE-TO-LIFE MICROPLASTICS IN LABORATORY
The concept and elaboration of the work was done by Monika Rani, supervised by Dr. Stefania Federici. The literature search, Fourier Transform Infrared Spectroscopy Measurements and Optical Image acquisition and analysis were done by Monika Rani. The X-Ray Diffraction measurements and analysis were done by Dr. Annalisa Zacco.

In submission.

CHAPTER 6 : THE TURN-ARY PLOT

ON THE USE OF HANDHELD MICRONIR FOR MICROPLASTICS ANALYSIS IN TERNARY MIXTURE:
The formal investigation and Near Infrared Measurements were done by Monika Rani and Claudio Marchesi. The NIR data analysis was done by Monika Rani and Chemometric analysis was done by Claudio Marchesi and Monika Rani. Raman measurements were made by Irene Vassillini. The manuscript was written by Monika Rani and Claudio Marchesi while Dr. Stefania Federici and Prof. Laura E. Depero supervised the work.

Submitted

CHAPTER 7: THE REVIEW
ON THE EXTRACTION OF MICROPLASTICS FROM COMPLEX ENVIRONMENTAL MATRICES.
The literature search, manuscript preparation was done by Monika Rani, while Dr. Stefania Federici supervised the study.

In submission.

APPENDIX E: **Smart Storage of Samples**

The samples from chapter 4 were stored in a Smart Storage book by Monika Rani for future references.

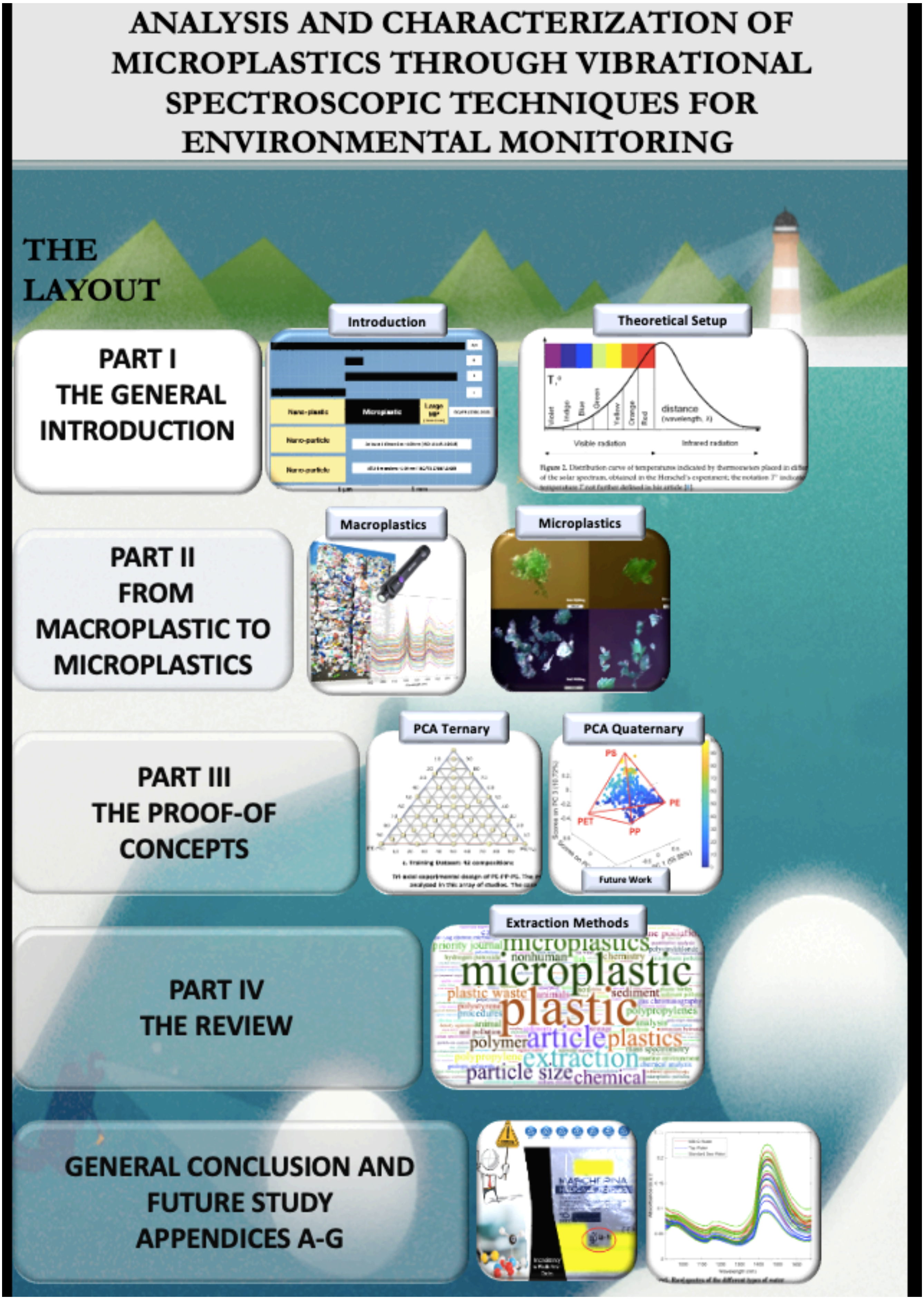
APPENDIX F: **Vibrational Spectra of Environmental Samples from Naples**

The sampling was done by SIRIMAP Project Partners of University of Brescia and Vibrational Spectral Acquisition (Micro Raman and Micro NIR) was done by Monika Rani.

APPENDIX G: FTIR and Optical Analysis of Microplastic Samples from Sardinia Island, Italy

The sampling was done by SIRIMAP Project Partners of University of Brescia and FTIR spectra acquisition and Optical Images were acquired by Monika Rani and Claudio Marchesi

Graphical Abstract



ANALYSIS AND CHARACTERIZATION OF MICROPLASTICS THROUGH VIBRATIONAL SPECTROSCOPIC TECHNIQUES FOR ENVIRONMENTAL MONITORING

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PART 1: GENERAL INTRODUCTION

CHAPTER 1:THE PILOT
INTRODUCTION

“While some might regard the global rise of plastic as a fantastic economic success story, others see an environmental tragedy- Roland Geyer, Earth and Plastic[1].”

1.1 Environmental Research on plastics: Status Quo

On 22 April, 1970, first Earth Day was observed. The seed idea behind celebrating the Earth Day was to provide voice to the burgeoning environmental concern, putting environmental issues, front and centre. We have, without a doubt, made significant progress in tackling a variety of serious environmental challenges such as acid rain and ozone hole depletion since the first Earth Day. However, other more insidious concerns have surfaced, ranging from climate change to global biodiversity loss; the alarms are becoming louder, and the brink is becoming closer, as mounting data shows planetary-scale human perturbations of the Earth System. One such major issue that has kept scientists on the notch is plastic pollution and the forces that are driving it appear to be intensifying.

The advent of synthetic and durable materials known as plastic, along with an increasing human population, has resulted in a fast increase in man-made debris levels in rivers, along beaches, and in the wider marine ecosystem over the last sixty years. Plastic litter pollution of the aquatic environment is a sad sign of the Earth's development into the Anthropocene epoch[2]. The first modern plastic, 'Bakelite,' synthesized in 1907[3] was result of serendipity and humans have now generated more than 8.3 billion tons of plastic, according to a 2015 research[4]. It has also been documented[5] that the amount of plastic created between 2002 and 2015 was exactly the same as the amount produced between 1950 and 2002. According to the etymology, the term plastic is derived from the Greek word plastikos, which means "capable of being moulded." Plastic, according to the International Union of Pure and Applied Chemistry (IUPAC)[6], is "a general word used in the case of polymeric material that may incorporate additional components to improve performance and/or lower costs." Despite their low cost, each of these polymers is a carefully designed material with specific physical attributes. Plastic items have displaced and replaced many materials such as glass, wood, paper, stone, and many others due to various factors such as light weightiness, high strength to weight ratio, bio inertness, low cost, and durability, and this is why the plastic industry has grown from producing 1.5 million tons in 1950 to 367 million metric tons in 2020, a tenfold increase in fifty years, with Asia accounting for lion’s share (51%) (China-32%, Japan-3%), followed by NAFTA (19%, North American Free Trade Agreement), Europe (15%), the Middle East and Africa (4%), Latin America (4%), and the Commonwealth of Independent States (3%)[7]. Most petroleum-based polymers are expected to be very resistant to microbial breakdown. As a result, the bulk of plastics created today are expected to endure for a very long period in the environment. Furthermore, the negligent disposal of plastic garbage, particularly in developing world, exacerbates the accompanying environmental difficulties. In numbers, only 18% of plastic garbage is recycled globally, while the remaining 24% is burnt. The remaining 58% are either landfilled resulting in soil infertility, or enter the natural environment, where plastics build and linger for lengthy periods of time[8].

Polymeric materials disintegrate into smaller particles (mesoplastics, microplastics, nanoplastics) after they have served their purpose, regardless of how they were obtained and/or made, and to the average consumer, their preparation is redundant; only the usage matters. Generally speaking, the synthetic plastic polymers (Figure 1) are classified into two types: Carbon-Carbon (C-C) backbone polymers and heteroatomic Carbon-Oxygen backbone(C-O) polymers. The first class includes Polyethylene (PE), Polyvinyl Chloride (PVC), Polystyrene

(PS) and Polypropylene(PP) accounting for 77% of the total market share while whereas heteroatomic polymers, such as Polyethylene Terephthalate (PET) and Polyurethane (PU), account for 18% of the total share. The irony is that the backbone of most plastics, as mentioned earlier, is organic hydrocarbon chain, which comprises of many repeating units formed from monomers. However, when extensively used plastics are discharged into the environment, they seldom decompose spontaneously. Obviously, every organic polymer may be quickly charred; but, in the absence of thermal degradation, the physical and microbiological surroundings attack the polymer chain and commence a process to split it into smaller pieces irrespective of its structure. The only difference is the amount of time this operation will take in the specific setting[9]. The duration is dictated by the structure of the polymer. It might take a few days to months to a few years. The plastics' resiliency and impermeability make them suitable for applications such as food packing, sterile medical purposes, and construction, among others, but they also make them exceptionally long-lasting when discarded. This is rather predictable, given that one of the key reasons for many polymers' popularity and extensive use is their outstanding stability and endurance[9]. The discarded plastic is either disposed of by burning, which emits hazardous gases and persistent organic pollutants (POPs) such as dioxins into the atmosphere, or by dumping it in landfills or water columns. Even if plastic does ultimately biodegrade, it will first break down into tiny particles, resulting in 'microplastics.' These have a distinct and major set of consequences explained in further sections.

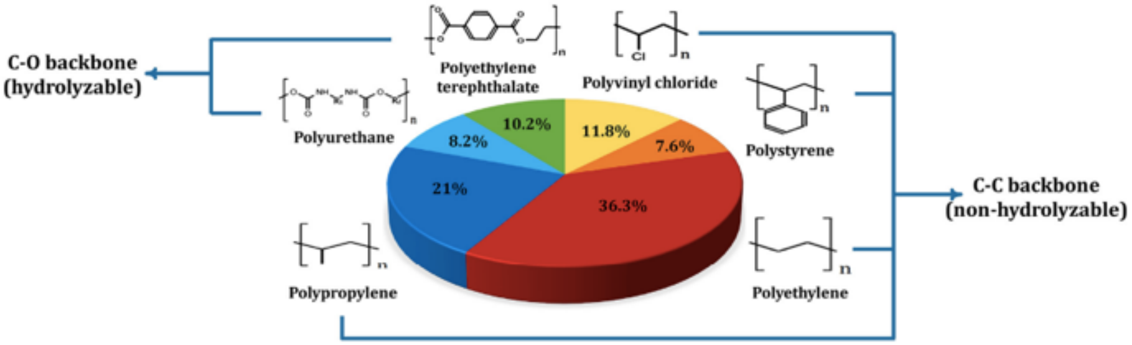

Figure 1: The synthetic plastic types and their market share percentages (Image from: https://www.sciencedirect.com/science/article/pii/S0048969721016582?casa_token=9sR52gfHegAAAAAA:tE5MvxguS1rrOHrx1SUPwY0kXIsfXvGfUpPLoz4dXgCqMWwSRzyBlEyui4d4ufWWEevk7HWqAUc)

The adaptability of plastic, as well as demand from a rising 'throw-away' society, have driven this remarkable surge in plastic production resulting in plastic being declared as a pollutant internationally, even after making our lives easier with an array of benefits. While accurate figures are difficult to come by due to a variety of factors such as the constant inflow of pieces, the ongoing breakup of larger pieces into smaller ones, and variable levels of buoyancy for different types of plastics and plastic products, scientists' estimates are alarming. Various studies[4,10] have attempted to quantify the quantity of plastic waste that enters our seas each year. According to the UNEP[11] (United Nations Environment Programme), the figure is over 8 million tonnes, or around 3% of total yearly plastic trash. Other research[12] have proposed higher figures or a range of figures of up to 11.8 million tonnes of plastic, or the equivalent of a full garbage truck per minute, enter our seas each year. Another analysis put the figure anywhere between 5 and 13 million tonnes[13].

Out of the plastic litter entering the oceans, the plastic material with dimension less than 5 mm, vaguely called microplastic is of utmost importance to study for two reasons: the first that these tiny particles are bioavailable to aquatic animals throughout the food web and the other one being their large surface area which makes the waterborne pollutants to be adhered to them. The International Organization for Standardization (ISO)[14] defines Microplastic as “any water-insoluble plastic particle with its longest dimension between 1 um and 1 mm". In addition, based on dimension, ISO classifies large microplastics as any water-insoluble solid particle with dimension between 1 mm and 5 mm. Entering of these toxic particles in the food chain is a potential source of bioaccumulation[15]. These are created through the weathering of bigger plastics, or by being applied directly as pre-consumer plastic, or by being employed as abrasives like those found in some cosmetics. Microplastics are very difficult to track, and thus they have the potential to have a greater influence than bigger plastics. With their high surface area-to-volume ratio, they may be able to make toxins more accessible to wildlife and the environment than bigger plastics.

Researchers from all around the world are reporting[16] on the impact of plastic trash on both the terrestrial and marine environments. However, the predominance of MPs in the ocean has resulted in numerous threats to aquatic animals' habitats and lives, making them the most vulnerable because they ingest these useless particles (MPs) directly from the water, passing them higher up the food chain through bioaccumulation and biomagnification. Blue mussels obtained in the Mediterranean Sea contained 0.2-0.5 microplastics/g by weight, according to De Witte et al[17]., Van Cauwenberghe[18]. Microplastics have a deleterious impact on both physical and chemical aspects due to their capacity to absorb and collect a small number of contaminants, such as persistent organic pollutants (POPs), through a process known as partitioning[19]. The size, density, and color of these double-edged swords all influence the absorption of MPs in oceans. These plastic bits, for example, have colors that attract species that consume them because they can't tell the difference between manufactured particles and their prey. Plankton, which is food for many aquatic species, combines with these microscopic particles, making distinction more difficult and ingestion probable, according to Moore[20] and Ivar et al[21]. According to a research conducted on birds in China, 28 pieces of microplastic ranging in size from 0.5 to 8.5 mm were detected in terrestrial birds[22].

Reports suggest that the use and disposal of one-time use plastic materials such as in packaging, microbeads in cosmetic products, contribute majorly to accumulation of litter[23] in the oceans and this business is growing by 3% every year. Marine and coastal environments provide a variety of valuable services and benefits to humans as well as other vertebrate and invertebrate animals. While there is just one global ocean, the massive body of water that spans 71% of the Earth is separated into five distinct zones based on geographic, historic and scientific reasons. These are namely: The Atlantic, Pacific, Indian, Arctic, and Antarctic ocean. Interestingly, these basins have become the offshore plastic accumulation zones, Pacific ocean now being identified as The Great Pacific Garbage Patch (GPGP)[24], anticipated to cover 1.6 million square kilometres, twice the size of Texas and three times the size of France, estimated to contain 1.8 trillion pieces of plastic. To put this staggering figure into perspective, it equates to 231 pieces of plastic for every person on the earth. It's largely made up of plastic litter, and it includes anything from giant abandoned fishing nets to plastic bottles to small plastic particles (or 'microplastics'). 'Plastic soup' is the term for this sort of material in the waters.

The words microplastics and plastic trash in general are frequently confused in the public eye, as well as in research, which is particularly perplexing when addressing the effect, risk assessment, and analytical aspects of microplastics. To that purpose, the next section (1.2) provide a full definition and description of analytically significant characteristics with the results of survey conducted during this work (section 1.4).

1.2 Microplastics

1.2.1 Definition: An evolutionary Process

The word 'microplastic' was originally used in a report released by the US Air Force Materials Laboratory in 1968 to characterize the deformation of plastic material, measured in microinches per inch, when an external stress is applied to it[25]. Since then, scientists all around the globe have used this phrase to refer to the dimensions of the plastic fragment. The finding of microscopic fragments of plastic in the marine environment in 1972, when vast amounts of little plastic chunks (0.2 - 0.5 cm) were discovered floating on the surface of the Sargasso Sea[26], was the spark for this transformation. Following that, oceanographers began to gather evidence of plastic contamination in aquatic environments. During the early stages of discovery, scientists saw microplastics as buoyant plastic pellets, with little regard for particle size or content. While both big and tiny plastic particles have been researched from their inception, scientists immediately focused on the large plastic particles. Later on, the term “plastic particles” was coined to describe these micro-sized plastic fragments.

Microplastics research in aquatic environments took another 3 decades to get off the ground. For the first time in 2004, the term "microplastics" was used by Thompson et al.[27], with no formal definition, to describe granular and fibrous plastic pieces with a diameter of roughly 20 um found in UK beach and sea collecting programs. In the years that followed, many scholars came up with their own definitions of microplastics. Arthur et al.[28] proposed a size restriction to the first phrase in 2009, referring to microplastics as "plastic particles less than 5 mm." This term was revised in 2011, when Cole et al[29]. separated primary (made to be of microscopic dimensions) and secondary (created to be of larger dimensions) microplastics based on their origin (resulting from degradation and fragmentation processes in the environment). Microplastics were defined by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP)[30] as "plastic particles with a diameter of less than 5 mm, including particles in the nano-size range (1 nm).", and it aided in the global diffusion of the term. Andrady[38] suggested adding the term "mesoplastic" to the scientific nomenclature, in order to distinguish between small plastics visible to the human eye and those visible only with the use of microscopy. Furthermore, the same author, from the dimensional point of view, has inserted other 3 terms: mesoplastic (500-5 μm), microplastic (50-500 μm) and nanoplastic (<50 μm), each with its own set of physical characteristics and biological impacts. The ISO technical report[14] published in February 2020 (ISO/TR 21960:2020 Plastics — Environmental aspects — State of knowledge and methodologies) has, finally, given the definition for MP, large MP and nanoplastics.

It defines microplastics as any solid plastic particle insoluble in water with any dimension between 1 μm to 1000 μm (=1 mm). Large MP instead are defined as any solid plastic particles insoluble in water with any dimension between 1 mm and 5 mm. Nanoplastics are all those plastic particles under 1 μm.

But there are some researchers that still identify with other definition of microplastics[31]. (Section 1.4)

1.2.2 Origin and Classification

Microplastics (MPs) are evident as minute colorful plastic fragments in the sand on beaches. Before delving into microplastics analysis, it's vital to know where these colorful bits and pieces typically originate. Microplastics in the aquatic environment are regarded to be a heterogeneous collection of particles with varying shapes, colors, sizes, and chemical composition. By bulk (kg/km^2), macro-sized litter (> 5mm) accounts for the majority of plastic in the marine environment, but micro-sized trash accounts for a higher proportion by quantity (items/km^2). During the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris in 2009, the scientific community agreed to differentiate between two main types of sources of microplastics[28]. Borrowing terminology from atmospheric sciences, it was chosen to classify microplastics as "primary" if they are intentionally produced for direct use or as precursors to other products such as in cosmetics or air blasting technology, and "secondary" if they are formed in the environment from the breakdown of bigger plastic components or according to IUCN are primarily the result of improper waste management during the disposal of plastic-containing products. Microplastics are thus classed as primary or secondary microplastics based on their origin. However, some environmentalists[32] define these classes differently: primary microplastic particles are defined as particles created through technical processes and entered the respective ecosystem in that form, whereas secondary microplastic particles are generated from larger particles within the respective system. Thus, tyre wear and abraded fibers from garments are classed as primary microplastic particles since they entered the environment—particularly aquatic systems—with this form already. This difference based on origin is useful since it identifies moderation techniques to reduce their environmental impact. The pace of formation of secondary microplastics is most likely determined by the plastic's properties, the level of weathering, and the energetics of the local environment.

Primary Microplastics

According to International Union for Conservation of Nature[33], the annual global release of primary microplastics into the ocean is estimated to be 1.5 million tons. Land-based activities are responsible for the vast majority (98%) of primary microplastics losses[33]. Only 2% of share is generated by maritime activities. The significant proportion of these particles are caused by the laundry of synthetic textiles and the abrasion of tyres when driving. Most product emissions to the oceans (49%) are caused by product use or maintenance (28%). Road runoff (66%), wastewater treatment systems (25 %), and wind transfer are the primary sources of these plastics entering the ocean (7%)[33].

These plastics are commonly used in facial cleansers and cosmetics, as plastic powders in molding, or as a compressed air cleaning medium. Scrubbers, used in exfoliating cleansers and face scrubs, have mostly supplanted the natural components that were formerly employed. Since its patent in cosmetics in 1980[34], the use of cleansers containing exfoliating plastic material has expanded considerably. These plastics, which are commonly sold as "microspheres" or "micro-exfoliants," can vary in shape, size, and composition depending on the product. Gregory et al.[35], for example, reported the inclusion of polyethylene and polypropylene granules (5 mm) as well as polystyrene spheres (2 mm) in a cosmetic product. Fendall and Sewell[36] detected a wide range of irregularly shaped microplastics in another cosmetic product, typically 0.5 mm in diameter with a size of 0.1 mm. Spherical or cylindrical virgin resin pellets (usually 2-5 mm in diameter) are commonly utilized as "raw material" for the production of many types of plastic products during the production and transport of plastic material of the basic resin[37]. Although their inclusion in this group has been criticized[38], these can also be termed main microplastics. Microbeads are part of the larger issue of microplastics, and it is estimated that cosmetic product sources account for 0.1 to 4.1% of marine microplastic pollution in Europe, equating to 2,461 to 8,627 tonnes of plastic discharged into the marine environment every year[39]. Air-blasting technique also benefits from the use of microplastic particles (0.25–1.7 mm) composed of acrylic, melamine, or polyester in the "media blasting" process to remove rust and paint off machinery, motors, and boat hulls[29].

Secondary Microplastics

When plastic waste breaks down into smaller pieces as a result of a comprehensive consequence of biological, mechanical, and chemical processes such as wind, exposure to UV radiation of light, water current, and tidal waves, the structural integrity of the particles decreases, and bond cleavage and breakage occurs, resulting in the formation of secondary microplastics. The plastic debris on beaches has a high availability of oxygen and direct exposure to sunlight causes it to degrade rapidly, turning it brittle over time, and causing[20,38] cracks and "yellowing". With loss of structural integrity, these plastics are increasingly sensitive to fragmentation resulting from abrasion, wave action and turbulence. This process is still ongoing with fragments getting smaller and smaller over time until they become microplastic in size[36,40]. In the low energy marine environment of the benthic zone, the rate of biodegradation of plastics will be substantially slowed by a dramatic drop in temperature as one descends in depth but new processes emerge in the water, namely: (1) biodeterioration, the alteration of the polymer's mechanical, chemical, and physical characteristics caused by the development of microbes on or inside the polymer's surface; (2) bio-fragmentation, the action of microbes on polymers to convert them into oligomers and monomers; (3) assimilation, where microorganisms obtain the required carbon, energy, and food supplies from polymer fragmentation and convert plastic carbon to CO_2, water, and biomass. And the most efficient of which are mechanical abrasion by sediments and fragmentation in the sea swash and wave breaking zone, particularly during stormy occurrences. It is noted that microplastics may further disintegrate into nano-scale plastic particles with lower bound size, since the least size currently identified in the marine environment is 1.6 um[41].

1.2.3 Physical Properties

Microplastics are dynamic in nature, and their behaviour varies based on physical characteristics such as particle density, shape, and size. Their density, polymeric chemical composition, and morphologies determine whether they may float, suspend, or sink in riverine habitats. These particles have been shown to separate in the water column based on their density. MPs with densities less than that of river water often float or remain suspended, but greater density microplastics are non-floating particles that tend to settle on the river bed. Even the most widely used polymers, such as polyethylene (PE), polypropylene (PP), and some kinds of polystyrene (PS), which are less dense than saltwater, can sink , due to aggregation, biofilm growth, degradation, and flocculation processes, plastic particle density fluctuates with residence time in the riverine environment. This is hardly unsurprising given that these materials accounted for 74% of global plastic production in 2015 and are often employed in goods with a short life cycle[8]. The specific density of plastic particles varies greatly depending on the polymer and manufacturing technique. The following types of plastics dominate the market: *1*-PETE (Polyethylene terephthalate), *2*-HDPE (High Density Polyethylene), *3*-PVC (Polyvinyl Chloride), *4*-LDPE (Low Density Polyethylene), *5*-PP (Polypropylene), *6*-PS (Polystyrene) , *7*-OTHER. PE, PP, PS are the most common polymer types found in aquatic settings (Table 1).

Table 1: Most common plastic types found in marine environment

IURC Code	Polymer Type	Density (g/cm^3)
1	PET	1.37–1.45
2	HDPE	0.94-0.97
3	PVC	1.16–1.58
4	LDPE	0.91-0.93
5	PP	0.83-0.85
6	PS	1.03-1.06
7	OTHERS (PU)	1.2, 1.17-1.28

Particle form and surface area are also important factors in the movement of microplastics, since the high surface area to volume ratio of fibers, films, and foams indicates aggregation and sink behavior as compared to larger plastic particles. Hoellein et al.[42] found that uniformly formed plastic particles, such as polypropylene pellets with the lowest surface area to volume ratio, can travel long distances, but randomly shaped plastic fragments had the shortest transit length in streams. It is suggested that the shape[43] of plastic fragments depends on the fragmentation process as well as residence time in the environment: sharp edges might indicate either recent introduction into the sea or the recent break-up of larger pieces, whilst smooth edges are often associated with older fragments that have been continuously polished by other particles or sediment. These characteristics are significant on the one hand for a broad knowledge of microplastic behaviour and destiny in the marine environment, and on the other hand, they may give a variety of parameterizations that are necessary for future calculations.

1.2.4 End of life = Start of Impact

The deepest parts of the ocean are equivalent to the world's tallest peaks. The Mariana Trench is so deep that Mount Everest would fit inside it with a mile to spare, according to popular marine science study[44]. And plastics now persists in these highest peaks to the deepest points in explored, majority being reported along the coastlines. After it has served its purpose for a common man, the life of the plastic material used comes to an end but it has become evident that there is no such thing as "end of life" for plastics since they persist and contaminate long after their intended usage. Large amounts of plastic garbage are easily evident. Studies have revealed significant social, economic, and environmental consequences. These vary from wildlife ingestion, damage, entanglement, or asphyxia to economic consequences for tourist destinations and maritime sectors. On 10 January 1992, a cargo ship carrying 28000 bathtub ducks fell off in the Pacific ocean, these ducks were located in 2003 and 2007 on the shores of Australia, UK and South Africa[45]. This perfectly illustrates how marine pollution at one point can affect the other one across the globe

Microplastics are not easily visible to the human eye but their impact on the flora and fauna are now evident. They act as vectors for chemicals in marine organisms, including plankton species[38]. MPs transport pollutants over large ocean areas[46] and contaminate marine biota when ingested[15]. By eating contaminated MPs, organisms are sensitive to physical damage and doses of pollutants that were not previously accessible from other matrices, such as seawater and sediments.

The ingestion of MPs has been demonstrated in various marine organisms with different feeding strategies. This phenomenon can negatively affect both the feeding activity and the nutritional value of a plankton-based diet, particularly in those species that cannot discriminate the food source. The plankton species, in fact, form the foundation of every food net, and the transfer of contaminants results everywhere within it. This can affect even those species that are not marine like humans.

Primary producers are well known to absorb MPs and related organic pollutants[47,48] therefore, bioaccumulation can occur in large predators, including larger species such as Mysticeti[49], or between primary and secondary consumer. Potentially, low-density, and high-density MPs are ingested when present in the marine environment and are available to a wide range of organisms that can passively or actively ingest MPs. Those species that live in industrialized areas are more exposed to high concentrations and may be more contaminated. However, the hypothesized amount of contaminants varies between fragments within the same zone, consequently, the toxicity of pollutants and incorporation into body tissues varies for each biological species[21].

The numerical increase of MPs can intensify the possibility of mixing with surface food sources and can affect the ability of fishes that feed on plankton to distinguish between plastic and their natural food[50].

The high ratio of MPs to plankton potentially influences the marine biota, so that the neustonic zooplankton itself or their predators can encounter MP fragments in terms of risk[29,51]. Boerger et al.[50] suggested that the high ratio of neustonic plastics to zooplankton, which involves relatively large numbers of MPs, may cause confusion with natural fish food in the Central North Pacific Gyre. This ratio indicates the level of interaction between neustonic zooplankton and MPs and the level of potential contamination for the consumer[52]. Consequently, a strong negative impact of MPs for consumers at the higher levels of the marine food chain would be expected[51]. Plastics are involved in 92% of all contacts between marine biota and garbage[53] . Plastic pollution affects at least 233–267[54] marine macro fauna species, such as turtles and sea birds, according to estimates and extensive data shows that detrimental consequences at the sub- and organismal level can be deadly[54].

Recent studies suggest the potential role of MPs as carriers of chemical pollutants, either used as additives during the synthesis of polymers, or directly absorbed by seawater[55,56,40]. Absorption reduces the transport and diffusion of contaminants, emphasizing particular affinities between contaminants and polymers. Teuten et al.[15] ,in fact, underlines how hydrophobic organic contaminants have a greater affinity for plastic materials such as polyethylene, polypropylene, PVC. Polychlorinated biphenyls (PCBs) are a group of organic compounds that were once used as insulating and coolant fluids, as plasticizers and stabilizing additives in PVC, flame retardant compounds (prior to the introduction of BFR as a flame retardant) and electronic components[57]. Mato et al[58]. identified PCBs, nonylphenol and DDE on polypropylene resin pellets collected in Japanese waters at concentrations similar to or higher than those found in sediments. In a further experiment, virgin resin pellets were shown to absorb contaminants from seawater over a 6-day exposure period. The results of an experiment conducted by Teuten et al.[15] showed that PCBs were transferred to the shearwater chicks from contaminated plastics by ingesting fish. The PCB concentrations present were three times higher than in the control group. These results confirm that Persistent Organic Pollutants (POPs; including PCBs) are transferred to organisms through plastics. Many POPs are considered toxic, inducing endocrine alterations, mutagenesis and / or carcinogenesis, and can lead to bio-amplification in higher trophic organisms[27].

Phthalates, in particular, are a class of chemicals commonly used to soften hard plastics. Di-2-ethylhexyl phthalate (DEHP), is the most abundant in the environment, and is rapidly metabolized in organisms as the primary metabolite MEHP[59] (mono-2-ethylhexyl phthalate). Most of the chemicals that are absorbed by PBT or phthalates can adversely affect marine organisms through the alteration of the endocrine system and the subsequent vitality of the population.

Bioplastics research has grown tremendously in response to the plastic challenge. Biodegradable polymers, such as PLA, PBS , are frequently viewed as a feasible alternative to regular plastics. They can, however, be a source of microplastics[27]. Biodegradable plastic materials are often made of synthetic polymers mixed with biodegradable botanical polymeric materials such as starch or vegetable oils, or with specific compounds meant to improve decomposition kinetics. These systems are predicted to degrade in industrial composting plants under adequate heat, humidity, and ventilation conditions if properly disposed off. However, there is just a partial degradation; although components such as starch disintegrate, the synthetic polymers remain undisturbed. Furthermore, in the relatively cold coastal habitats and in the utter lack of terrestrial organisms, the decomposition rates of even degradable bioplastic components will be significantly prolonged, increasing the likelihood of plastic fouling and, as a result, reducing UV permeation, which is required for the degradation process. When decomposition happens, microplastics are discharged into the marine environment, adding to seawater contamination. Indeed, bioplastics and biodegradable systems are interesting, but they are still a long way from fixing the problem.

1.3 The Global Vision: Results of VAMAS Survey on the issues of Microplastics

As stated, an explosion of research has been devoted in the last years to detect, identify and quantify the microplastics, hidden not only in the oceans but also in the world’s rivers, lakes, air, soil as well as food and organisms. There are currently no standardised and unambiguous methods for sampling MPs in the various environmental matrices, even if a lot of work has been done[60,61]. During the last 2 years (namely, 2019 and 2020)[31], more than 230 reviews have been published about different topics related to MPs and NPs. The scientific community is rarely involved in the standardisation process since usually this is not considered part of the research. However, scientists should participate in this process when the assessment of experimental procedures is required. Indeed, a technical standard must correspond to the best practice assessed by experts, who must compare the results through inter-laboratory tests and understand the differences among results obtained by different techniques, different instruments, different laboratories. Standards can be used for proficiency tests to guarantee the reliably of the data, a mandatory requirement to any kind of analysis, and to help the operator to improve the laboratory performances. Moreover, the standards are relevant for the decision-makers, and scientists have the responsibility to give a contribution to their validation and assessment. In such a complex scenario, it is necessary to know the matrix (Chapter -extraction for details) to be monitored and to carry out the proper sampling. Knowledge of the sources, levels, environmental fate of MPs and the models are based on sampling and analysis, and reliable data are mandatory for the assessment of the management options

The standardisation process for the definition of material properties or new techniques is always based on research results (Figure 2). A new method then must be assessed first by intra- and then interlaboratory test to define the best procedure. After that, the standardisation process inside can begin. The standardisation is a mandatory step in the case of issues related to society, in particular in the case of health and environmental normative.

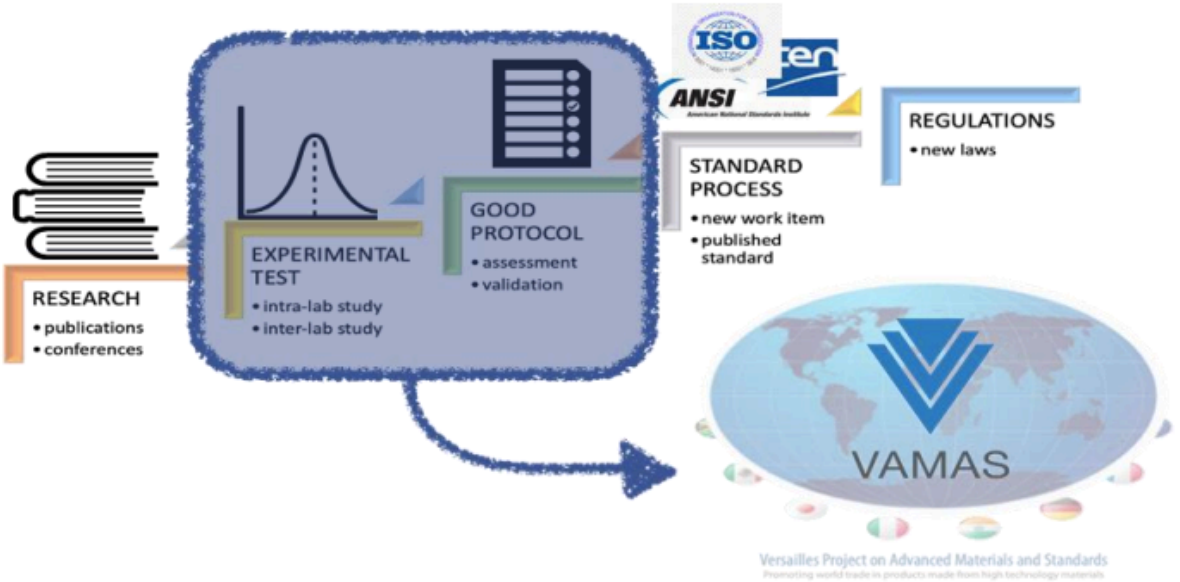

Figure 2: The Standardisation Process.

In the year 2020, a survey was proposed to the scientific community in collaboration with VAMAS (Versailles Project on Advanced Materials and Standards) to collect information about experts who work and plan to work on MP issues. 390 experts from 46 countries answered the survey.

Some of the questions with their results are discussed below.

1. What is the correct definition of microplastics?

When standardisation procedures have to be assessed, definitions have to be clear and clarified as definition is part of the process of identifying the materials of interest. Regarding microplastics, there are different definitions on the web (also described in the section 1.2) and in the scientific publications. Thus, the first question of the survey regards the definition of MPs. Six definitions were proposed to choose from as shown in Figure 3.

As it can be concluded from Figure 3, the majority of the expert chose option E as the best definition for MPs. Interestingly, the percentage of experts choosing different definition is independent by the country.

The ISO technical report[14] published in February 2020 has given the definition for MP, large MP and nanoplastics . In Figure 4, the comparison of the survey results and the ISO definition is shown. The term "large microplastic" is introduced for a particle with size between 1 to 5 mm. Remarkably, following the ISO definition, 200 nm particles is defined as nanoplastic, but not as nanoparticle.

It is thus evident that despite all of this attention and research, there is still no clear agreement on a definition that is broad enough to include all of the necessary elements for describing "microplastics." This technicality creates various methodological issues, and it is common to hear authors express their fear of comparing research while reading review papers on microplastics. Although such discussions may appear to be purely semantic in origin, they have far-reaching implications in research as well as the establishment and enforcement of laws, mandates, and standards.

2. What are the key criteria for defining microplastic?

Experts were given choices between size, shape, origin or other factors to choose from. As expected, the majority chose as key criteria the size. In particular, 255 experts choose only the size. However, 27 experts choose the origin as the only key criterion Figure 3. Some of them suggested other criteria's that should be considered: colour, texture, biodegradability, composition. Colour however is not considered important in characterizing microplastics because colour differentiation is arbitrary and cannot help with visual identification of microplastics on their own[62].

When taking legal or voluntary steps to limit microplastic emissions, a definition is required, thus it is important to figure out the key criteria's to be included in a definition as it provides statutory stability and allows for effective evaluation of microplastic contamination trends as well as comprehensive assessment of the consequences of policy initiatives.

3. What properties of microplastics are more/less critical for the environment?

The choices given were: chemical, physical, biological, mechanical, biodegradable or thermal property. The properties were rated from most relevant(6) to least relevant (0). Most of the experts chose biodegradability as the most critical properties to be considered, followed by chemical and biological features. Mechanical and thermal properties are those considered less critical from most of the experts(Figure 5).

Some experts, answering the question gave interesting suggestions regarding other critical properties that have to be considered namely:

- Capacity to adsorb persistent organic pollutants (POPs);
- Transportation mechanisms;
- Biofilm formation around micro-particles;
- Degradation path;
- Absorption by living beings, ecotoxicity;
- Chemical additives in plastic production.

4. What are the characterisation techniques used by the experts for analysing microplastics?

As expected by the bibliometric analysis, the most used technique to characterise MPs is the infrared spectroscopy, with 111 experts declared to use it already. However, it has been recently outlined that most studies cannot be replicated due to missing experimental details. Other techniques used by many experts are Raman spectroscopy and electron microscopy, followed by DSC,TGA, ICP and Chromatography (Figure 6).

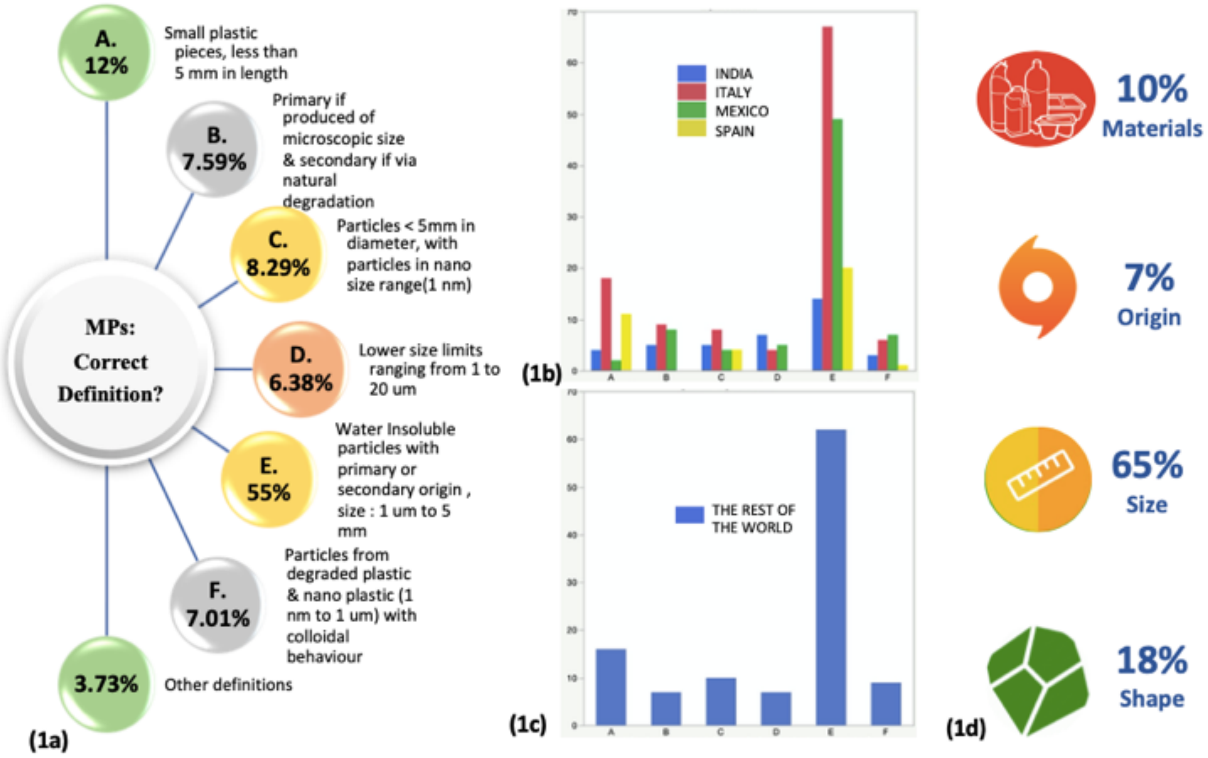

Figure 3: (a) The correct definition of MPs as chosen by experts from proposed definitions.
(b, c) The percentage of experts choosing different definition is independent by the countries
(d) The key criteria according to the experts to define MPs.

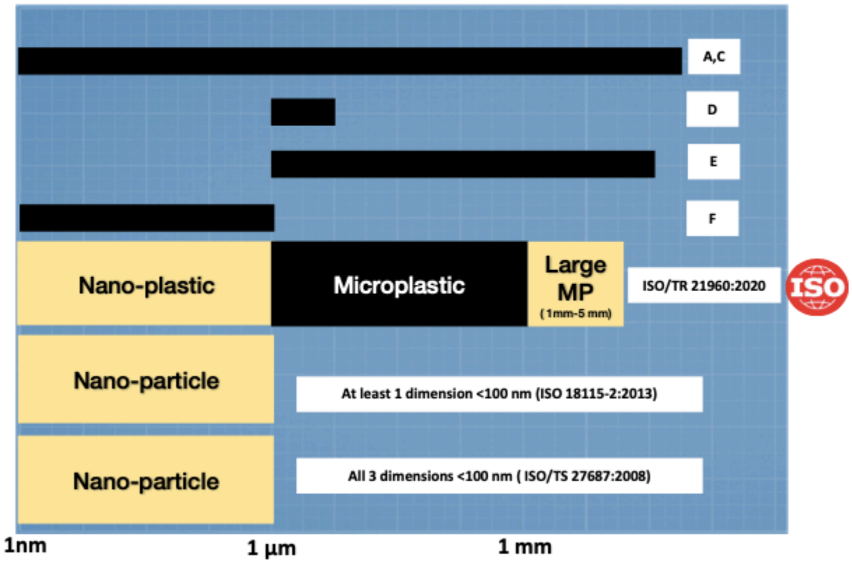

Figure 4: ISO defines MPs as water insoluble plastic particles & size between 1um-1mm.
The above graph shows the comparison of the ISO defined MPs & definition according to the experts who answered.

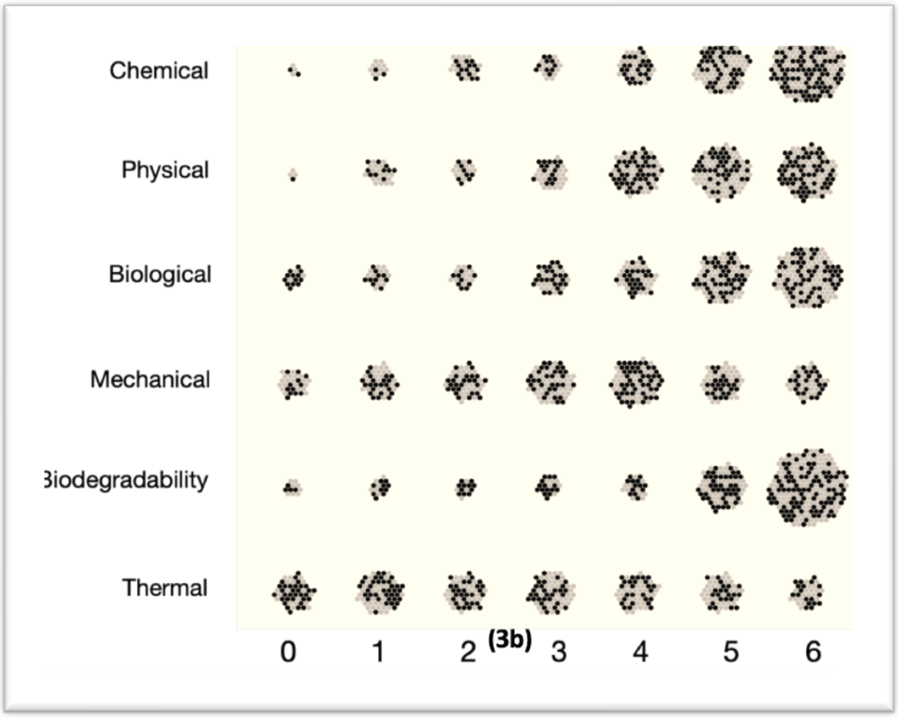

Figure 5: Properties of MPs more/less critical for the environment. (The black dots represents the experts with experience in the given field).

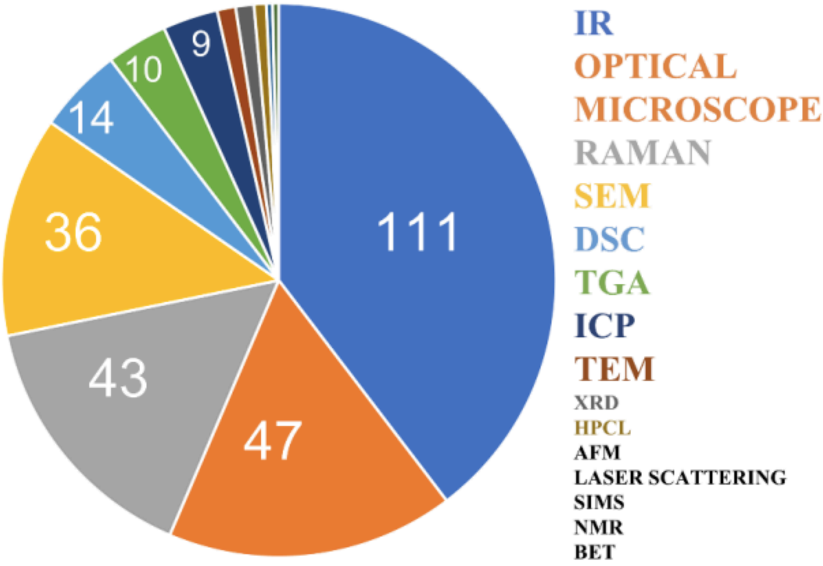

Figure 6: Spectroscopy Techniques used by experts who answered to characterize MPs**.**

The scientific community involved in MP and NP issues is growing fast since potential drawbacks for the environment and possible for human health are relevant. To have reliable data are mandatory for the society and, in particular, for the decision-makers. Inter-laboratory study to assess the protocols for sampling, detection and modelling the phenomena should be a priority to the standardisation. The evaluation of the real dangers should be assessed and declined in legislation acts that should be shared by all the countries to be effective.

1.4 Importance of Reference Materials

New data on the destiny of microplastic waste emerges from both field and laboratory research on a regular basis. Researchers have until now mostly concentrated on ambient plastic particles; however, research using specified microplastic particles as the sample input are few. Furthermore, a comparison of those studies reveals a disparity between the particles discovered (e.g., in the environment) and those employed for future research (e.g., exposure studies). Consequently, a growing number of academics are using MPs as input samples in various investigations, rather than only finding and characterizing microplastic particles prevalent in the environment. Generally speaking, this is not an easy task to perform, characterising MPs come with multidimensional challenges such as evaluating particle shape, size, recovery rates, water susceptibility, aging-modified particle surfaces (additional OH, C=O, and COOH), optimizing extraction techniques, and subjecting animals to microplastic particles[63]. Environmental MP-like reference particles, on the other hand, are scarce. This has been also reported in a recent study done by Rujiter et al.[64] where they reviewed 105 MP studies to assess their effect on marine organisms and developed 20 quality criteria's divided among four main categories (particle characterization, experimental design, applicability in risk assessment, and ecological relevance), and reported the maximum score to be 44.6% on average.

Reference materials are essential for confirming the consistency and accuracy of analytical results across multiple sites and over time. Regardless of the application sector, using reference materials to ensure the accuracy and comparability of the results is a constant thread. In the present day research of microplastics, NIR technology combined with chemometrics is an uprising tools. New models are being built and tested and the question of using more and more test materials becomes relevant as they are used for method validation, calibration, estimate of measurement uncertainty, and are a significant instrument in realizing a number of elements of measurement quality. MPs found in the environment vary vividly from the post-consumer plastic waste in terms of size, shape, degradation level. Generally they have to be extracted using various methods based on density separation mostly. The results of MP analysis are highly dependent on sampling location and time. Global comparability and traceability of MP studies is still missing by large. The results that come from the analysis of MPs are not only confined to one sector of identifying that from what type of polymer has been identified, rather this being a chemically, physically and biologically interrelated area of study, the measurements performed must yield reproducible results. Thus, the use of relevant test materials becomes incredibly important. They ensure the quality of the measurement results.

Another challenge in creating MP reference materials is the large number of morphologies that must be included. This is especially problematic if the particles are being utilized to build image-based approaches. Particles for various shapes (fragments, spheres, films, fibers) must be supplied, ideally in the same sample, in order to create acceptable morphological characterisation methods. Each approach has its own set of advantages and disadvantages, and ongoing attempts are being made to enhance existing methods and develop new ones with the goal of increasing throughput, detection limit, and repeatability. Grinding is often used to generate the currently available MP standard particles (1 m1 mm). Special cryomills are state-of-the art. But this equipment is not available in every laboratory, it disconnects makers from users, making rapid technique development difficult. Some easier methods based on ultrasonication in alkaline conditions (KOH) have also been tested but KOH has been marked to cause irritation and is corrosive in nature. Another fact that comes into play is the reproducibility that allows responsible decision making and opens the path to expansion of protocols. As a result, there are several unanswered questions in this field that must be studied and debated and the aim of this work is to close the gap by stepping further.

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CHAPTER 2
THE DIRECTION

" *To achieve great things, two things are needed: a plan and not quite enough time- Leonard Bernstein*".

2.1 Aim of the work

Developments of analytical techniques in the field of microplastics environmental pollution are well reported in literature. Nevertheless, the use of miniaturized and portable instruments has gained popularity only in the last years and there is an urgent need of harmonized and shared protocols to guarantee the capabilities of these ultra-compact instruments to routinely analyse microplastics. This thesis is primarily concerned with development of new analytical approaches with chemometric tools (from Chem4Tech Laboratory, University of Brescia) in environmental samples using miniaturized technology and establishing the efficacy of portable instrument in microplastic community for characterisation of microplastics. The other aspect deals with the identification of main challenges and barriers that hinder the characterisation of plastic in mixed waste and microplastic in oceans.This study can disclose a new accessible way for the identification and quantification of environmental MPs. It also intends to shed the light on importance of reference materials for building protocols for characterizing MPs. To attempt this endeavour, the first step lead to the development of in-house library of NIR spectra of polymers, collected from a recycling plant in Bergamo, Italy (Chapter 4), followed by the fragmentation of plastic items collected in laboratory to synthesise true-to-life microplastics with different techniques (Chapter 5). In the next step, ternary (Chapter 7) mixtures of microplastics were made to test the limits of the handheld instrument used in this work. Towards the end, the author reviewed the extraction methods of microplastics from complex environmental matrices (Chapter 7)

The results here are of immediate importance for data standardization to build reliable databases and to perform quantitative analysis but are indeed poised to impact also for a fast, reliable, and in-situ environmental microplastics identification.

2.2 Outline of the thesis

This PhD thesis is divided into three parts, with 08 Chapters in total.

Chapter 4,5,7- constitute complete scientific works that can be read independently. As a result, readers who read this thesis cover to cover may detect certain repetitions in the chapters.

Chapter 1 offers a general introduction to the thesis. It presents status of the microplastics in general, potential sources and classification, environmental concerns and risks. It also presents the results of VAMAS survey regarding Microplastic Issues.

Chapter 2 outlines the objective and structure of the thesis.

Chapter 3 introduces the need for characterising the microplastics and defines the importance of compact technology and reference materials in the field of MPs. It also provides theoretical fundamentals of infrared spectroscopy with special focus on Near-Infrared Region and microplastics. Some basic principles of chemometrics are also discussed. This section also serves as introductory practice-oriented literature, containing guidelines for the analysis of microplastics using infrared spectroscopy. Towards the end, a bibliometric analysis of the influx of Near-Infrared Technology has been provided.

Chapter 4 leads to making of in-house library of polymers using miniaturized handheld MicroNIR and tests the efficacy of this instrument on direct use of post-consumer plastic waste.

Chapter 5 deals with the different types of fragmentation procedures for making true-to-life microplastics using three different techniques.

Chapter 6 demonstrates, for the first time, the feasibility of a handheld miniaturized near-infrared spectrometer (MicroNIR) coupled with chemometric methods for the quantification of secondary MPs mixtures produced at a laboratory scale.
Chapter 7 presents a complete guide for the extraction procedures of microplastics from complex environmental matrices.
Chapter 8 discussed the general conclusion and future prospective of this thesis.
Appendices A-H lists the publications, conferences, awards and extra research work related to microplastic carried out in these 3 years.

CHAPTER 3
THEORITICAL SETUPS

3.1 On the challenges of characterizing MPs

"…characterization of polymers is inherently more difficult than that of other materials. Polymers are roughly equivalent in complexity to, if not more complex than, other materials, at every physical level of organization from microscopic to macroscopic…- F. W. Billmeyer Jr. (1976): J. Polym. Sci.: Symp. (1976) 55: 1-10[1]."

The recognition and demonstration of the large molecular weight and long-chain nature of polymers can be regarded to have started the characterisation of these substances. But why is it critical to examine the chemical makeup of microplastics in environmental samples? The reasons are manifold:

(1) First, it is critical to ensure proper nomenclature, which is a scientific premise that extends beyond microplastics pollution study[2].

(2) Second, the diversity and complexity of plastic sources, usage patterns, emission paths, and material qualities are mirrored in the diversity of MP particles, which display a wide range of physical, chemical, and biological properties (e.g., size, shape, density, polymer type, surface properties, etc.).

(3) Third, plastics research is critical, owing to the inherent properties of plastic component polymers, such as permanence in the environment, which does not always apply to other anthropogenic particles.

(4) Fourth, current scientific debate on microplastic contamination issues is centred on their relatively long persistence in the environment, and only if reliable methods for identifying plastic particles are used will the microplastic research field improve its credibility, withstand the test of time, and become a permanent research field.

(5) Fifth, The critical understanding of the polymer class is a pre-requisite for recovering, recycling and monitoring purposes.

The goal of the characterization is to obtain information not only on the type of material, but also on particle sizes and MP abundances in environmental samples. Despite its similarities, each polymer has distinct physical qualities in terms of flexibility and density thus monitoring of MPs in diverse biotic and abiotic environmental matrices is required to determine the level of contamination, flux, and potential of organism exposure. The introduction of synthetics and the emergence of petrochemicals as reliable and cost-effective feedstock for polymers set the stage for the phenomenal growth and development of polymeric materials throughout the previous century whereas semi-synthetic polymers derived from nature also contain a part of synthetic polymers. Apart from the fact that they are either entirely or partially synthetic, they are resistant to most forms of breakdown in the environment, making microplastics research attractive. There are a plethora of anthropogenic particles that fit within the microplastic size classification and can be classified as plastic particles < 1 mm in diameter. They may also qualify for the definition of microplastics because they exhibit other characteristics that allow them to be successfully sampled using the sophisticated methods used to collect microplastics in the environment. Cotton and wool fibres, paint chips, and spheroidal carbonaceous particles (SCPs; a separate kind of black carbon) are examples of these particles, all of which constitute environmental contaminants to some extent. To avoid such misidentifications, classification of the polymer type has become a non-negotiable requirement for microplastics research and the microplastic scientific community to attain a degree of investigative maturity[2].

Unfortunately, many of the approaches that are accessible are not suitable to all polymer systems[3]. The practical issue, however, is not so much the availability of characterization tools as it is their application to the circumstance at hand in an economically feasible and scientifically valid manner.

3.2 Current Technologies for Characterizing Microplastics

The polymer characterization technique categories are: chemical, electrical, mechanical, molecular, physical, rheological, spectroscopic, thermal property, thermal transition and viscoelasticity. In case of MPs, the characterisation is divided into two steps: physical description (size, shape, colour) by visual methods and chemical segment to confirm the nature of the particle under observation. Visual observation with optical microscope is one of the basic ways of analysis and could only be useful for some large colourful MPs and pre-production resin pellets with sizes ranging from 2–5 mm hat can be identified with the naked eye but this method cannot be seldom relied on as there are positives for the introduction of sampling error, bias or omission of particles, resulting in many qualitative rather than quantitative. The variations in the results can be attributable to the operators' varying degrees of expertise and weariness. Although most particles in the size range of <_1mm may be identified by microscopy, particles in the sub-hundred micron size range (<_100 um) with no color or conventional form are difficult to characterize as plastics with certainty[4]. Previous research[5,6] found that incorrect identification of plastic-like particles by microscopy was frequently greater than 20_%, and greater than 70_% for transparent particles; these findings were validated by later spectroscopic analysis. However, this method acts a supporting tool to narrow down the choice of samples being analysed.

Scanning electron microscopy (SEM) may provide pictures of plastic-like particles that are exceedingly clear and high in magnification and the elemental composition of the same item can be determined using energy-dispersive X-ray spectroscopy (EDS)[7]. The elemental composition with high resolution image can be useful for separating carbon- dominant plastic from inorganic material. But SEM-EDS is costly and takes a significant amount of time and effort to prepare and examine samples, limiting the number of samples that can be handled. The approach is suggested for observing the morphological diversity and elemental analysis of particular plastic particles. These strategies, however, have disadvantages such as slow speed and low reliability due to errors induced by analyst proficiency. The use of staining dyes is a low-cost approach of facilitating visual identification as in Fluorescence Microscopy. Dyes like Nile Red and Rose Bengal have been tested so far. Nile red has been designated as the most promising staining protocol, thanks to its high recovery rates of MPs[8,9] but it still has significant limitations, such as co-staining of leftover natural lipids or organic components with the MPs, making it unable to discriminate MPs from other materials and changes in the hydrophobicity of plastics caused by surface contaminants might modify the color of Nile Red released[10]. Unsatisfactory findings[11,9] have also been observed in relation to Rose Bengal, and a complete digestion stage is required to avoid MPs staining owing to biofilm remnants.

While there is no one-size-fits-all solution, many strategies may be employed to put together the entire picture, each with its own set of limitations and benefits. Methods such as X-Ray detection, Raman micro spectroscopy, Gas Thermogravimetric analysis coupled to solid-phase extraction (TGA-SPE) followed high-temperature gel permeation chromatography (HT-GPC) for quantification, hyper-spectral imaging methods, vibrational spectroscopic methods are reported in the literature[12,13,14]. But there occur several drawbacks while using few of them; TGA-SPE, and GC/MS are destructive, HT-GPC requires sample pre-treatment, X-Ray detectors only can detect PVC among the mixed waste of polymers and the purity and speed of sorting is unsatisfactory due to mechanical singularity inadequacy in the conveyor system at high speed. Gas chromatography with mass spectrometry (GC/MS) and its variants, such as pyrolysis (py-GC/MS) or thermal desorption (TDS-GC/MS), are among the prominent mentions for analyzing MPs that are too small particularly when they exist in complex environmental samples where a thorough cleaning process is done to eliminate organic debris and concentrate plastic material and these methods are able to identify the additives in the polymers as a result of the decomposition product. But as mentioned, every method has their set of pros and cons, this approach has certain drawbacks too, such as the restricted sample mass that must be employed, the difficulty in physically moving the hand-picked particle into a pyrolysis cup, the lengthy analysis time, the destructive character of the procedure, the similar pyrolysis product of polymers[15]. Previously, thermal examinations for bulk samples could not offer information on the amount, size, and shape of MPs. With advancement in research, there are few studies that have presented the use of a thermal degradation methodology for characterizing and quantifying microplastics have largely focusing on method development rather than use for environmental monitoring, but this area is still in its infancy.

Another thermoanalytical technique, DSC can be useful for detecting certain main microplastics for which reference materials are available, such as polyethylene microbeads. Although DSC analysis is easy and quick, it has limits when it comes to distinguishing microplastics from diverse polymer compounds in environmental samples. Polyethylene and polypropylene have been identified by DSC, however due to overlapping phase transition signals, polyvinylchloride (PVC), polyamide (PA), polyester (PES), polyethylene terephthalate (PET), and polyurethane (PU) limits the technique[16]. It appears that these approaches are too new to be used for regular analysis of microplastics in environmental samples, but they might be useful for screening bulk samples or further investigations of microplastics that have not yet been thoroughly characterized by spectroscopy. Table 1 gives an overview of the characterization techniques in MP analysis.

Table 1: Characterization technique of MP analysis.

Technique	Definition	Property Investigated
Microscopy	***Analyses substances using a microscope.***	***Shape, Size and Number of Particles***
Stereo Optical	• Reflected Light as illumination. • 3-d Colored Images. • Low magnification as compared to SEM.	Particles from 5mm to 20 um
Fluorescence	• Uses Visible light for illumination • Use of Dyes (fluorescent material) • 3-D Images	A dye colors transparent fragments.
Electron (SEM)	• Electron Illumination. • 3-D Black & White Image. • High Magnification & Resolution • Sample Coated in Gold for electrons to bounce off and give viewers the image of external surface of sample.	Particles from 20 um to 100 nm
Vibrational Spectroscopy	***Analyses the surface of a material using optical technology***	***Chemical Composition of polymer***
FTIR	• Infrared Radiation as illumination. • Spectra is produced based on interaction of IR light with vibrations of molecule, instead of images.	Identification of polymer type and its composition
Raman	• Monochromatic laser as illumination source.	Identification of polymer type and its composition

	• Interaction of light with molecule to induce a deformation of its electron cloud and obtain a spectra.	
Light Scattering	***Analyses power spectral density of the surface morphology during growth or sputter-etching.***	***Size and number of particles.***
DLS	• Dynamic Light Scattering • Measures the particles suspended within a liquid. • DLS measures Brownian motion and relates this to the size of the particles in submicron range • Utilises a technique where the intensity fluctuations in the scattered light are analysed and related to the diffusion of the scattering object.	Particles from 0.1 nm to 10 um
NTA	• Nanoparticle Tracking Analysis • Utilizes the properties of both light scattering and Brownian motion in order to obtain the nanoparticle size distribution of samples in liquid suspension. • Utilises the trajectories of individual scattering objects observed under a microscope and their displacement related to each object's size.	Particles from 30 nm to 2000 nm.
Thermoanalytical	***Analysis using heat based on the unique physical and chemical properties of each substance***	Chemical composition of polymer
Thermogravimetric Analysis	• Measures weight changes in a material as a function of temperature (or time) under a controlled atmosphere.	Identification of polymer type and its composition
Pyro-GC/MS	• method of chemical analysis in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry.	Identification of polymer type and its composition

The most common sorting techniques are based on vibrational techniques - Infrared and Raman spectroscopy. Raman spectra has abundance of sharp and well-defined bands but a highly optimized and sensitive instrument is needed to detect the Raman information[17]. Also while studying the chemically identical samples that only differ in particle size distribution, Raman spectra is largely unaffected[17]. Thus, during this work the sensitivity of Raman spectroscopy will be enhanced through multiple scattering phenomenon and optical resonances with the use of nanostructures. Spectroscopic techniques based on infrared region have also been used to study the plastic. However, there is still no universally accepted method to effectively characterize, determine the particle size, shape, number, and morphology of the MPs particles.

The majority of microplastic identification is done using spectroscopic approaches such as infrared or Raman spectroscopy. Spectroscopy uses light to analyze materials by describing the energy transfer between light and matter based on the scattering of light. Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions[18] whereby a molecule scatters incident light from a high intensity laser light source. Most of the scattered light is at the same wavelength as the laser source and does not provide useful information – this is called Rayleigh Scatter. A Raman spectrum features several peaks, showing the intensity on y-axis and the Raman shift on x-axis. Each peak corresponds to a specific molecular bond vibration, including individual bonds such as C-C, C=C, N-O, C-H etc., and groups of bonds such as benzene ring breathing mode, polymer chain vibrations, lattice modes, etc. The Raman shift, measured in cm^{-1}, is the energy difference between the incident (laser) light and the scattered (detected) light. This difference is then only connected to the energetic properties of the molecular vibrations studied and hence independent of the laser wavelength. The vibrations of certain distinct subunits of a molecule, called its functional groups, will appear in a Raman spectrum at characteristic Raman shifts. Such a shift is similar for all molecules containing the same functional group. Using these characteristic shifts makes it possible to relate the spectrum of an unknown compound to a class of substances[19] The benefit of Raman spectroscopy is that it can detect particles as tiny as 20 micron[20] due to the small diameter of the laser beam in Raman spectroscopy. Nonetheless, additives, oils, or organic material adhering to the sample might produce interference and significantly alter the polymer's range[11,21,22]. Another benefit of Raman spectroscopy is its larger spectrum range, which may approach 50 cm-1, allowing inorganic substances to be identified. Because Raman is not affected by water also, it is a viable alternative for in-situ measurements in wet conditions. Furthermore, Raman microscopes lack rapid detectors such FPA. Thus, Raman mapping is done by measuring regions point by point, being called a time consuming method. As mentioned earlier, this technique is based on scattering of light, the scattering of photons is quite weak- one in a million photon is scattered to obtain Raman spectra. As a result, for an optimal signal, a very high laser intensity is necessary, which, on the other hand, might damage sensitive samples such as very minute MP particles. Moreover, fluorescence can be induced by the laser light source, resulting in a strong background in the spectrum that overlaps with the Raman peaks but this can be handled with some other techniques such as by subtracting the fluorescence background after simultaneous excitation with two laser light sources with slightly different wavelength[23].

Based on metal nanostructured metal substrate, Surface-enhanced Raman scattering (SERS), an extension of Raman method, is a well-established measuring technique for detecting micrometre-sized materials. Due to electromagnetic field enhancement effects and plasmonic charge transfer behaviour of metallic nanostructures, the SERS signal is enhanced by several orders of magnitude compared with the conventional Raman signals. Xu et al. successfully employed this technique to detect MPs <1 um in the environment but sample preparation is required here because MPs must be in the proximity of the metal structure. There[24] are other variant so Raman technique such as CARS (coherent anti-Stokes Raman scattering microscopy) but as these approaches need expensive equipment and knowledge, they have not acquired as much traction in MP research as classic vibrational spectroscopies.

Infrared spectroscopy, the most adopted method is discussed in detail in the next section.

3.3 Infrared Spectroscopy of Microplastics: Brief Overview

Molecules can absorb electromagnetic radiations of many wavelengths, but here the discussion is restricted within the range of 2 and 50 um., range of infrared radiations.

3.3.1 Beginning Beyond Red: Infrared

The term “infrared” means “beyond red” and its significance dates to 1800 when Sir John Frederik William Herschel’s was conducting experiments to modernise the construction of his telescope. He was seeking for a filtering substance that would allow him to lessen the brightness of the Sun's picture in his telescope during solar studies. He was fascinated by the fact that some of the samples communicated little solar heat, others transmitted a lot, while evaluating a variety of samples of coloured glass that showed a comparable drop in brightness. He noticed the temperature effects of specific sun spectrum regions of light. Herschel discovered that thermometers put outside the visible spectrum's red boundary showed a larger increase in temperature than thermometers placed inside specific visible areas of the spectrum. He called this newly found radiation "invisible rays of the Sun," "dark heat," or "invisible thermometrical spectrum”. It was eventually dubbed infrared radiation because its spectrum extended beyond the visible spectrum's red limit[25]. Herschel's experiment yielded a distribution curve of temperatures indicated by thermometers positioned in different colors of the solar spectrum (Figure 1).

Infrared (IR) spectroscopy is well suited for qualitative examination of polymer starting materials and final products, as well as quantification of components in polymer mixes and in-process sample analysis. Passing infrared light through a sample and calculating what proportion of the incident radiation is absorbed at each energy level is a typical way to acquire an infrared spectrum. Put simply, it is the measurement of absorption of various IR frequencies by a sample placed in the path of an IR beam. The study and interpretation of the IR spectra of polymers can be done in two ways: empirical and conceptual. The empirical interpretation is based on the idea of macromolecule atomic groupings vibrating virtually independently. It primarily gathers data on the polymer's chemical composition. The conceptual treatment focuses on a comprehensive examination of the IR spectrum in terms of polymeric system vibrational behavior and intermolecular forces. In both the cases, vibrational behavior is of utmost importance.

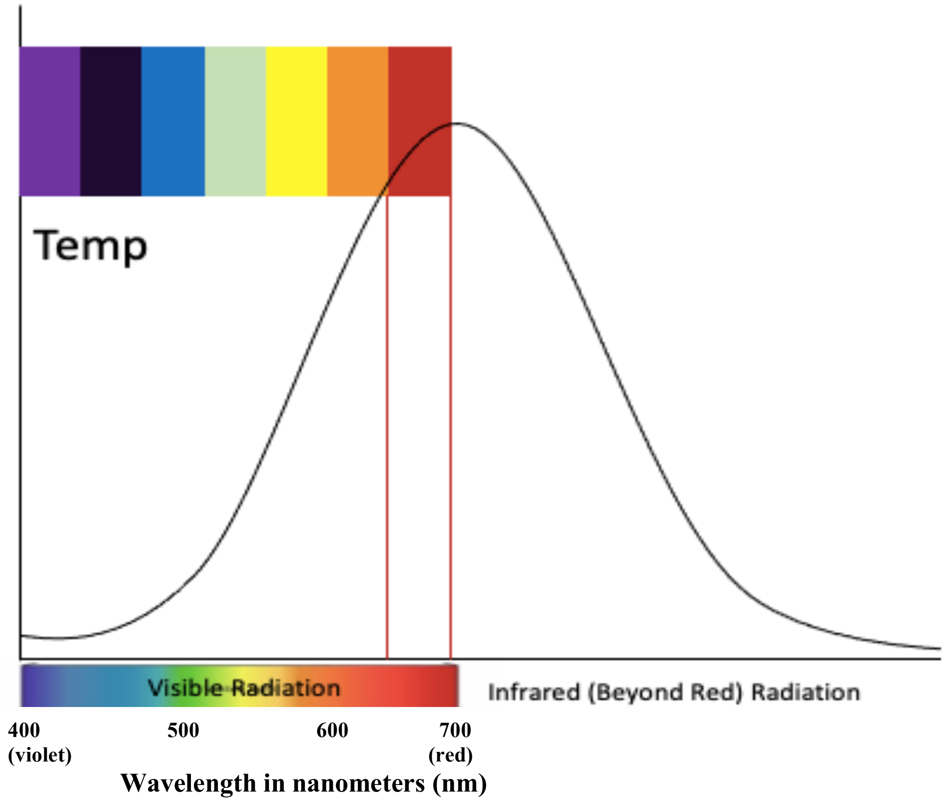


Figure 1: Distribution curve of temperatures indicated by thermometers positioned in different colors of the solar spectrum; the notation degrees of temperature[26] was not further described in his essay.

3.3.2 Principles of Infrared Spectroscopy

Chemical bonds are not stiff and inflexible components but are capable of a multitude of vibrations. In this perspective, molecules appear to act like balls connected by springs, with the balls representing atoms and the springs indicating chemical bonds. Most of the infrared radiation is thermal energy which induces strong molecular vibrations For the absorption of the light photon, the frequency of the photon must be equal to the energy difference between two vibrational states of the given bond and thus specific bonds absorb specific frequencies. Furthermore, a method for successfully transferring energy to the molecule must be identified (e.g.: by exerting physical contact or using a magnet for magnetic field). This transfer happens for molecular vibrations via molecular polarity, which is when IR radiation interacts with a vibrating molecule only when the vibration is accompanied by a change in the dipole moment (u).

$$\frac{\partial \mu}{\partial x} \neq 0$$

And this forms the basis of IR spectroscopy, i.e., for a molecule or functional group to absorb IR radiation, it must have a net change in dipole moment during vibration. The strength of light absorption is largely determined by the degree of this dipole moment change inside the vibrational transition. Only heteronuclear, diatomic molecules display (vibrational-spectral) transitions between photons of light and molecule vibrations, according to the above mentioned equation. The lightest atom is the hydrogen atom. Thus, hydrogen bonds (C–H, N–H, O–H, and S–H) have the highest vibrations and consequently, polymers basic carbon-hydrogen backbone are mostly characterised using infrared spectroscopy.

As the simplest vibrating system (Figure 2) , these molecular vibrations may be represented using the classical physics paradigm of the harmonic diatomic oscillator. Two vibrating masses coupled by a spring with a given force constant, k , changes the internuclear distance. Hooke's Law is used to calculate the frequency *v* of this motion using a specific force constant *k*, which characterizes the bond strength, and the corresponding masses *m* or reduced mass, respectively.

Hooke's Law:

$$v = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}} \qquad (1)$$

where v = fundamental vibration frequency,
k = force constant, and
μ = reduced mass.

The reduced mass, $\mu = \frac{m1 * m2}{m1 + m2}$, where m1 and m2 are the component masses for the chemical bond under consideration

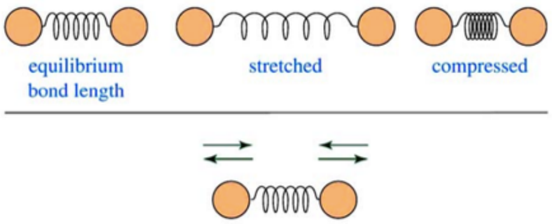


Figure 2: A simple vibrating system

This simple equation connects the strength (or springiness) of a covalent bond between two atoms (or molecular fragments), the mass of the interacting atoms (molecular fragments), and the frequency of vibration. Because frequency depends on the chemical characteristics of the vibrational group, this basic theory incorporates the main premise of infrared spectroscopy. These molecular vibrations produce spectral fingerprints and thus are useful in distinguish the compounds in the entire supply chain that are added to polymers while adding value to the final product in form of additives, fillers etc. In layman's terms, this equation explains partly that why different functional group absorb different frequencies and we are able to distinguish between different types of structures. The remains are explained by the anharmonicity explained in further sections.

The second prerequisite for infrared absorbance is that the energy of the light impinging on a molecule must equal a vibrational energy level difference within the molecule.

$$\Delta E_{vib} = hcW$$

ΔE_{vib} = vibrational energy level difference in a molecule.
h = Planck's constant (Joule-sec)
c= speed of light (cm/sec)
W= wavenumber in cm^{-1}

If a photon's energy does not match the condition in this equation, it will be transmitted by the sample; however, if the photon's energy does fulfil the criterion in this equation, it will be absorbed by the molecule.

Modes of vibration

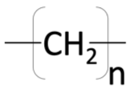
After defining the grounds for the simple vibration of an atomic bond, we must consider the molecule as a whole. In a vibrational spectrum, each absorption corresponds to a normal mode which depends on the internal degree of freedom, deduced from individual point group of the molecule under investigation.

The frequencies of the overall vibrations are particularly sensitive to the length or overall shape of a large structure and provide useful information on polymer chain conformations. It is quite trivial to conceive an unlimited number of vibrations, which would result in a completely chaotic model for interpretation. Instead, the model is characterized in terms of a minimal set of basic vibrations based on a three-dimensional set of coordinate axes known as the normal modes of vibration.

Each atom has 3 degrees of freedom, which correspond to the 3 Cartesian coordinates needed to define its location in the molecule. As a result, the molecule possesses 3n degrees of freedom. Among the 3n, 3 are used to describe translation modes and three are used to describe rotation modes (2 if the molecule is linear). Each molecule thus has 3n-6 (for non-linear) or 3n-5 (for linear molecule) modes of vibration. Stated earlier, atoms in molecules shift relative to one another, causing bond lengths to change or one atom to move out of its current plane. This is a description of stretching and bending motions, which are referred to collectively as vibrations and are the most common types of vibrational modes.

Stretching is the vibration of the bond along the plane, causing the interatomic distance to vary periodically -symmetrically (in-phase) or asymmetrically (out-of-phase). Bending vibrations are distinguished by changes in the angle between two atoms in the plane (scissoring and rocking) and out of the plane (wagging and twisting).

The interesting fact about polymers is that they are made up of series of identical chemical structures that form a chain. One of the most common MPs found in the environment, polyethylene (PE) has the repeat unit of methylene ($-CH_2-$) group. The chemical structure of PE is:



$$\left(-CH_2- \right)_n$$

Chemical Structure of Polyethylene

n means there are n number n of repeat units in a row comprising the polymer chain.

When we look at the vibration modes of $-CH_2-$ inside a carbon chain of polyethylene, we identify six modes (2 stretching and 4 bending modes), shown in Figure 3 below. Within a polymer, there are typical carbon backbone vibrational groups such as CH, CH_2, CH_3, CO, CN, and so on, and each of them performs typical vibrational modes with certain frequencies listed further.

3800-2700cm^{-1}: C-H, O-H, N-H
2300-2000cm^{-1}: C≡C, C≡N
1900-1500cm^{-1}: C=C, C=O, C=N, N=O
1300-800cm^{-1}: C-C, C-O, C-N

The observed stretching modes have frequencies that are somewhat lower than those obtained by Hooke's law (2926 and 2853cm-1 versus 3040cm-1) due to the environment of the C-H liaison, which Hooke did not account for. Also, the values of the reduced mass of the groups -OH, -CH and -NH are rather similar, the spectral information is determined mainly by the value of k, which depends not only on the length and strength of the bond, but also from the surrounding environment, thus creating differences in energy absorption for each link and are used in the interpretation of a spectrum

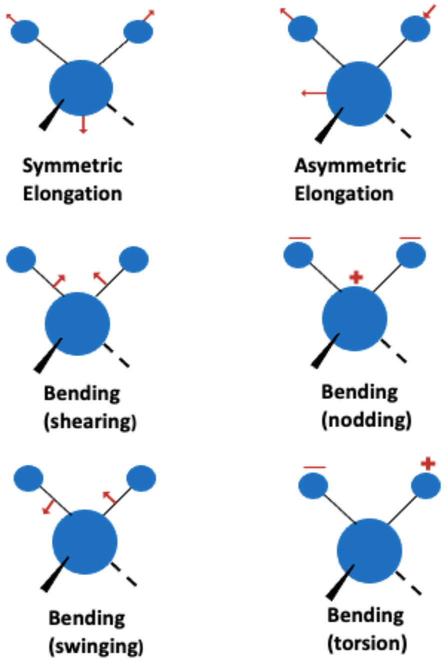


Figure 3: The six vibrating modes of CH_2

From harmonious to inharmonious nature of the molecules.

Until now, we have been considering the vibrations in a harmonic fashion which means when a chemical bond stretches, the two atoms attached to it move in phase with each other, with same amplitude and pass through their equilibrium position at the same time. t. Mechanical anharmonicity, i.e., the loss of equidistance between the distinct energy levels, and electrical anharmonicity, i.e., the change of the equation of moment dipolar electric, both move the system away from ideal conditions.

In case of harmonic potentials, the vibrational states are equidistant and have energy levels E given by:

$$E = \left(v_i + \frac{1}{2}\right) hv \text{ , } v_i = 0,1, 2, 3\ldots \text{ (only integer values)}$$

v = classical vibrational frequency of the oscillator.
h = Planck's constant

For anharmonic oscillator, corrected anharmonicity term is:

$$E_{\mathrm{v}} = hv_e\left(v + \frac{1}{2}\right) - hx_e v_e \left(v + \frac{1}{2}\right)^2 \quad (2)$$

Where $x_e v_e$ defines the magnitude of anharmonicity .
The anharmonic effects are amplified in a polyatomic molecule, where the reciprocal impacts between atoms increase exponentially as the vibrations are no longer independent of each other and can interact among themselves.
For a simple harmonic oscillator, the rule that defines the transition of the photon from a lower to higher vibrational energy state is :
Δv = ±1, i.e. the vibrational quantum number changes by unity.
Due to the introduction of anharmonicity this rule changes to Δv = ±2, ±3…
The absence of harmonicity is particularly noticeable in bonds involving hydrogen molecules, which have a very little mass. As a result, vibrations with larger amplitude and more intense absorption bands are produced. These phenomena have the greatest impact on the near-infrared spectrum as the changes in the selection rules from Δv = ±1 (fundamental vibration) to ±2, ±3 leads to overtones and combination bands which forms the basis of Near- Infrared Spectroscopy.

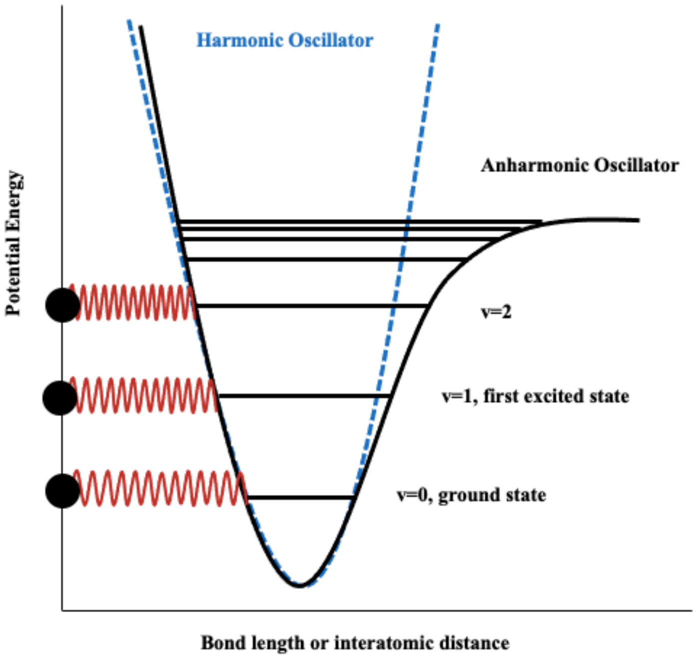


Figure 4: Potential Energy Diagram Comparison of the Anharmonic and the Harmonic Oscillator.

The Infrared Spectrum

A spectrum is a representation of what part of electromagnetic radiation is absorbed or emitted by sample under investigation. A graph, a diagram on a computer screen, or even a list of wavelengths and intensities could be used to illustrate the data. An infrared spectrum is a plot of measured infrared radiation intensity versus wavenumber. The three important aspects of a Infrared Spectrum are: peak position, peak height and peak width (Figure 5). All these three together are a major source of distinguishing various functional groups from each other.

Peak Position

The peak position is based on the variables involved in equation 1:

$$v \propto \frac{1}{\sqrt{u}} \text{ and } v \propto \sqrt{k}$$

force constant (k) and reduced mass (u). The above relations suggests that higher the force constant, higher will be the peak position. And force constant is the property of the strength of the chemical bond, so stronger bonds will have higher peak positions than weaker ones. And the molecules with heavier atoms will have peaks at lower position as the relation is inverse between reduced mass and vibrational frequency. And because no two chemical compounds in the universe have the same force constants and atomic masses, each chemical substance's infrared spectrum is unique.

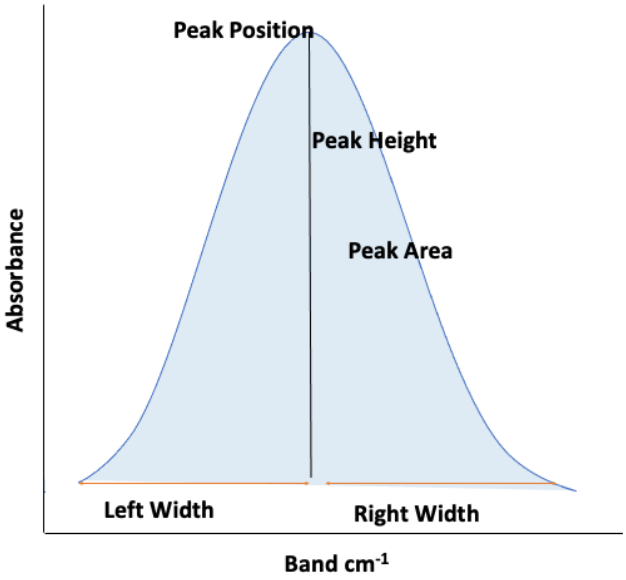


Figure 5: Parts of a spectrum**.**

Peak Height and Intensity

One of spectroscopy's capabilities is the potential to estimate the amount of absorbing material in a sample. The concentration of a molecule present in the sample leads to variation in peak height of the spectra. This leads to Lambert Beer's Law, which relates a signal's absorbance to the concentration of absorbing species, implying that we're interested in the line's (peaks') height. The version of Beer's Law considered here is:

$$A = £lc$$

where, A = absorbance

l = path length

£ = molar absorptivity of the sample

c = concentration of the absorbing species.

Absorbance is measured as peak height, peak height ratio, area under peak and peak area ratio in the IR spectra.

The molar absorptivity, £, is the intrinsic property of every chemical entity that describes how strongly the species absorbs light at different wavelengths. At some wavelength, £ is large and light is absorbed while at some wavelength £ is small and very little or no light is absorbed. Thus £ is a function of wavelength. Since absorptivity is indeed a physical constant for a given pure molecule at a given frequency, it must be connected to the chemical structure of the molecule. The other two parameters, path length and concentration are determined experimentally. The pathlength is just the sample thickness as travelled by the infrared beam. From quantum mechanics, the intensity of IR bands is proportional to the square of the change of the molecule's dipole moment caused by vibrations.

$$\varepsilon = \left(\frac{\partial \mu}{\partial x}\right)^2$$

For example, in the IR spectra of polystyrene below (Figure 7), different vibrations of the different functional groups give rise to different band intensities. The most intense band is at 698 cm^{-1} due to bending of benzene ring. At 1600 cm^{-1}, there is a weak band due to stretching of C-C bonds of benzene ring.

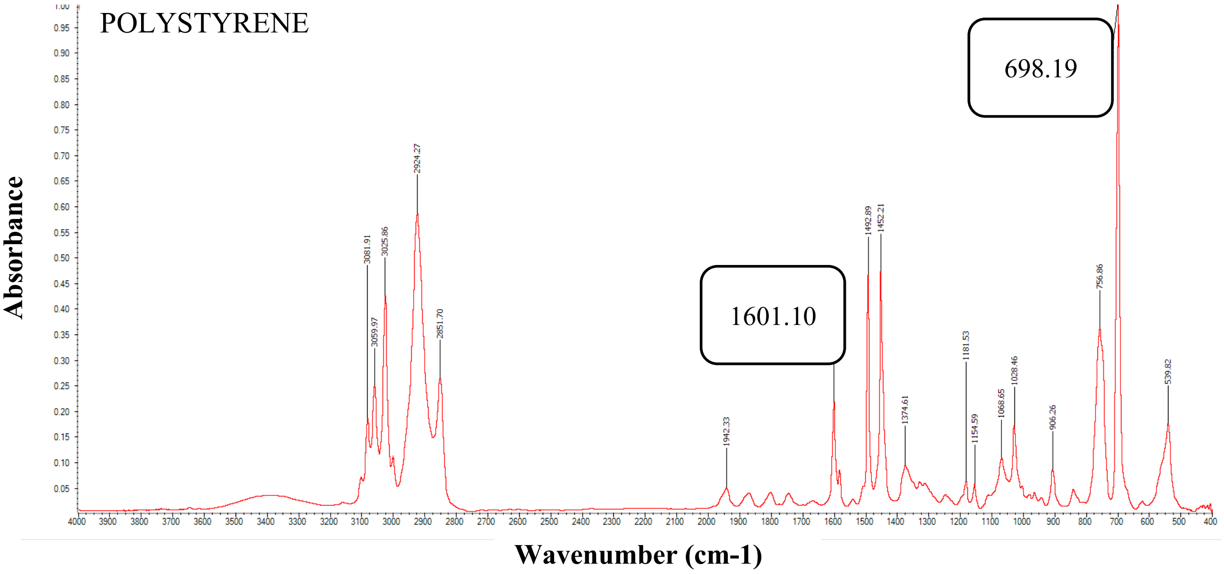


Figure 6: IR Spectral of Polystyrene taken in Chem4Tech Lab

Peak Width

The widths of infrared peaks vary widely across the spectrum of known compounds. Some functional groups produce IR bands that are so wide that the width of the bands alone indicates the presence of that functional group in the sample. The answer is found in the strength of the intermolecular interactions of the two entities. When intermolecular interactions are strong, the strength of the intermolecular bonds changes throughout a wide range, resulting in a large number of energy states. Each of these states absorbs infrared light at slightly different wavenumbers, resulting in broad peaks.

Regions of Infrared Spectrum

The infrared part of the electromagnetic spectrum is typically separated into three distinct regions(Figure 7) based on the frequency of the infrared (IR) radiation relative to the visible spectrum (400-800 nm).

- **Far-IR region[1]** from 400-10 cm^{-1}(~25-1000 μm wavelength) – farthest from the frequency of visible light;
- **Mid-IR region [2]** from 4000-400 cm^{-1} (~2.5-25 μm wavelength);
- **Near-IR region [3]**: 12500-4000 cm^{-1} (~780-2500 nm wavelength) – closest to the frequency of visible light;

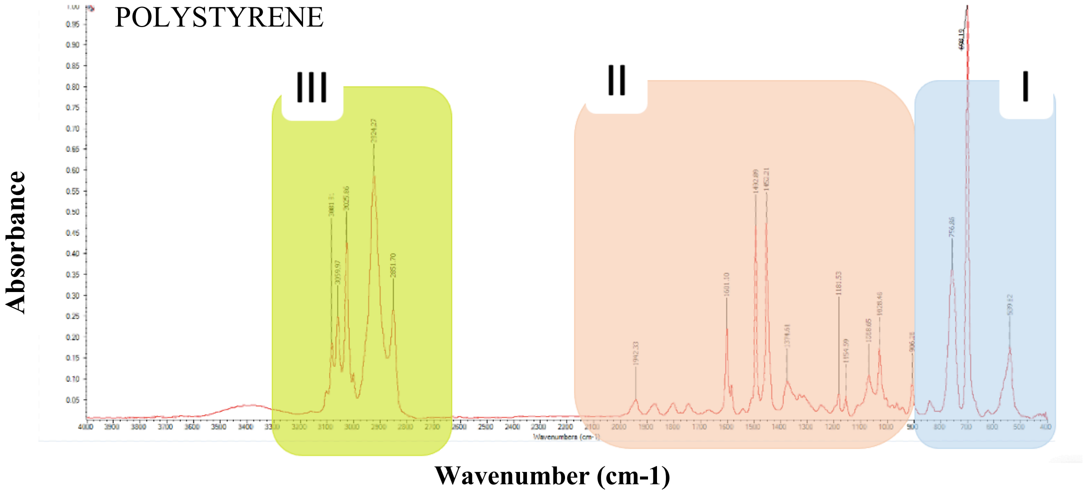
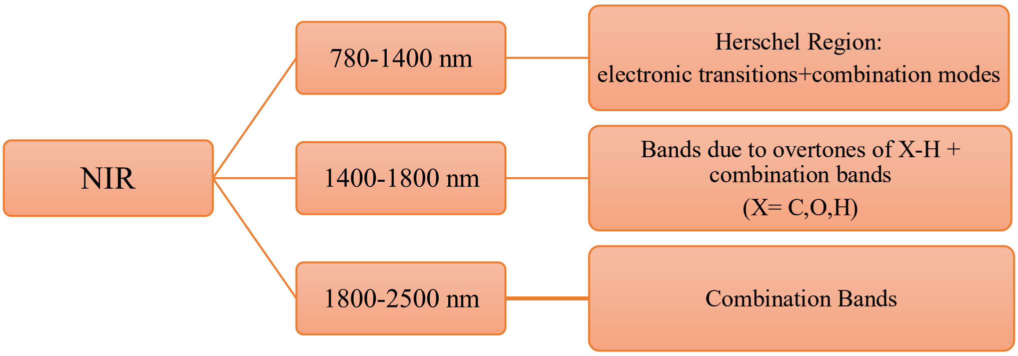


Figure 7: The distinct regions of IR spectrum.

3.3.2.1 Near-Infrared Spectroscopy: The Study of Overtones and Combination Bands

Anharmonic effects govern the major characteristics of the resultant bands (overtones and combination band), wavenumbers, and intensities, with inter-mode anharmonicity having the most significant influence.

From figure 4, when a molecule undergoes a vibrational spectroscopic transition between the v = 0 and v = 1 levels, the transition is referred to as a fundamental transition, and the accompanying IR peak is referred to as a fundamental peak. Fundamental bands are naturally intense, account for the majority of the peaks visible in mid-IR spectra. Any spectroscopic transition from v = 0 to v = 2,3,4... or higher results in an overtone band. The "first overtone" is a v = 0 to v = 2 transition that occurs at approximately twice the wavenumber of the fundamental and the area approximately often 10–100 times less powerful than fundamental bands. When a molecule absorbs IR light, more than one vibration can be stimulated at the same time, in addition to overtones. Because they require the activation of a mixture of vibrations, these IR characteristics are known as combination bands. A combination absorption only requires one of its vibrations to be infrared active, thus NIR spectroscopy can make measurements of absorptions, which would normally be thought of as being only Raman active[27]. This is apparent from some of the applications in the polymer industry and was demonstrated by the work of Barton et al.[28] in two-dimensional spectroscopy, which linked spectra from Raman and NIR measurements of the same samples. These signals have relatively low intensities in traditional molecular vibrational spectroscopy, but they are very important in microplastics investigation. This is due to the huge number of identical vibrational groups inside a polymer, which increases the likelihood of these transitions occurring. In this regard, for polymer identification NIRS distinguishes itself from other types of vibrational spectroscopy (mid-infrared, MIR; far-infrared, FIR) in which the primary chemical information is derived from fundamental vibrational transitions.



NIR ACTIVE GROUPS

All of the NIR bands involve CH stretching mode at 3312.36 nm. This result indicates that only the molecular vibrations with high frequency fundamental bands, such as CH, NH and OH and C=O stretching bonds, contribute to prominent overtone and combination bands in the NIR spectrum. The predominant near-infrared spectral features include:

a. Major vibrations[29]
    i. Methyl C-H stretching vibrations
    ii. Methylene C-H stretching vibrations
    iii. Aromatic C-H stretching vibrations
    iv. O-H stretching vibrations
b. Minor vibrations
    i. Methoxy C-H stretching vibrations
    ii. Carbonyl associated C-H stretching
    iii. N-H from primary and secondary amides (Both alkyl, and aryl groups)
    iv. N-H from primary, secondary and tertiary amines
    v. N-H from amine salts.

Identification of plastics is mainly based on the stretching vibration modes of the CH, CH$_2$ and CH$_3$ groups between roughly 1.1 and 1.25 m that correspond to the second overtone, the first overtone range between 1.65 and 1.7 um, and the combination bands, respectively[30]. Thus, it can be marked that in the NIR region, we predominantly see the result of vibrations of light atoms that have strong molecular bonds. If the chemical bond is weak, or the atoms are heavy, the vibrational frequency is low and its overtone will not be detectable in the NIR. Thus, we primarily see chemical bonds containing hydrogen attached to atoms such as N, O, C, thereby limiting the chemical structures that are simple ones that are common in many organic compounds. To sum up, if a sample contains molecular bonds such as C-H, N-H, O-H or S-H and if the concentration of the analyte exceeds about 0.1% of the total composition, then it is very likely that the analysis with NIRS would be feasible

Comparing the diversity of NIR and MIR region

Measuring MPs has been carried out by number of researchers, mainly in laboratory conditions. Thus, analyzing such research is usually difficult since they differ greatly in terms of the technology utilized (MIR/NIR) and how it is performed, i.e., in the laboratory or in the field. Both Near-Infrared (NIR) and Mid-Infrared (MIR) spectroscopy are extremely appealing for plastic identification since they are low-cost and simple to utilize. During early stage of microplastic identification methods, the mid-infrared range (FTIR) was most commonly used part because practically every substance may provide a mid-infrared spectrum and analysis using these systems is particularly frequent in the field of microplastic characterization. NIR spectral characteristics have traditionally been employed for waste plastic identification since early 90's but for MP identification, this technique is still in its infancy, with first publication reported in 2016 as per SCOPUS database.

The key distinction between the two spectral ranges is that absorption in the mid-infrared corresponds to fundamental bands of molecular vibrations, whereas absorption in the near-infrared corresponds to overtones and combinations of these fundamental band. As a result, absorption coefficients (also known as extinction coefficients, specifies how far light of a specific wavelength may reach into a substance before being absorbed.) in the NIR region are substantially less, allowing light to permeate materials more easily, while on the other hand, because of the number of combination and overtone bands, the NIR spectrum is substantially more cluttered. The interesting part ass will be detailed further below, is that the majority of the uniqueness of NIRS stems from the exclusivity of the corresponding spectral band positioned between visible and infrared (near-infrared or NIR; 800–2,500 nm; 12,500–4,000 cm1).If sample contains molecular bonds such as C-H, N-H, O-H or S-H and if the concentration of the analyte exceeds about 0.1 % of the total composition, then it is very likely that the analysis with NIRS would be feasible. Another aspect to consider is that light dispersion is substantially stronger in the NIR region than in the MIR range. As a result, elements that affect light diffusion, such as physical structure (aggregate size, porosity), as well as the presence of water, which modifies the refractive index and hence light diffusion, would have a considerably greater impact on NIR spectra[31]. The practical consideration is that NIR requires less sample preparation than MIR and is better suited for in-field analysis, with lower specificity requirements, whereas MIR usually has higher specificity and repeatability but requires more sample preparation. Even if polymers are coated in thick biofilms or other pollutants, they may be examined. As a result, NIR approaches are often more robust than MIR methods[32].

The advantages[29] touted for NIR measurements over other vibration techniques have proven themselves true throughout the 1980s up until today, they include: (1) C-H associated vibrational information is repeated 8 times from 690 nm to 3000 nm; (2) Simple harmonics may be selected or more information rich combination regions; (3) Low cost instruments with high signal-to-noise (SNR) are simple to make and typically exhibit signal-to-noise ratios (SNR) of 25000-100000:1; (4) High NIR throughput is possible, even when employing low cost fiber optics; (5) Variable pathlengths for industrial use are possible, typically 1 mm to 10 cm or more using different NIR spectral regions; and (6) NIR Light penetrates plant and animal tissue easily for biomedical applications (when using 900 nm and longer). Table 1 shows the relative intensities of C-H stretch bands for infrared and the various NIR overtone regions (first through fourth overtone). This repetitive information gives a great deal of flexibility for pathlength selection and information content

According to the current level of science and technology, the limitation of use of NIR spectroscopy in MP characterization lies in its limited sensitivity due to highly overlapped overtones and combination transitions which explains why NIR spectroscopy is rarely used in this study field. If these challenges are solved, NIR spectroscopy has the potential to be a powerful technique for analyzing microplastics. One important thing to note is that The NIR is generally plotted with wavelength because the areas with larger absorbance bands are more spread out.

This PhD work has aimed at reducing this very gap in the following steps:

1) Building an in- house library of NIR spectra of post-consumer plastic waste.

2) Using the above said library to study microplastic pieces fragmented in laboratory, also aiming at developing the importance of reference material in microplastic studies.

3.3.3.2 Bibliometric Analysis: Influx of NIR Technology in Microplastic Analysis

Since the publication of the first paper[34] motivating research on Microplastics in 2004 by Prof. Richard C. Thompson, research in this field has grown exponentially in the last few years. Analysis of microplastics has become increasingly common, with vibrational spectroscopic methods being preferred the most. While with time, new technologies come up compensating the drawbacks of the pre-existing methods. According to recent literature[32], handheld NIR spectrometers are being used for the analysis of diverse polymer types and this technique has made its way in analysing microplastics quite recently. Near-infrared (NIR) spectroscopy has grown in popularity as a non-destructive analytical technique, and it has become the tool of choice in a variety of sectors[35]. A noteworthy adoption of this technology stems from its good synergy with the autonomous, portable spectrometers that are capable of on-site analysis used in monitoring environmental pollutant, especially microplastics. Although multiple review articles have been published regarding microplastic research[36], only a few[37,38] have performed bibliometric analysis. Emerging fields are frequently monitored through bibliometric analysis, a statistical method for finding and assessing publication trends applied to a collection of related literature. Bibliometric analysis has already been used to spot trends in emerging disciplines like nanoparticle and nanomaterial toxicity.

In more specific terms, it is a quantitative analytical method that uses mathematics and statistics to identify journals, authors, institutions, universities, and countries' scientific activity. It can assist researchers assist in determining national and international contributions to the development, hot topics discussed by researchers, and research gaps in the field. The findings could be useful in future study and decision-making.

NIR technology has been used in plastic sorting since 1990s[39] but it has been recently[40] utilised in microplastic research, especially owing to the development of handheld devices.

This section explores the influx of Near Infrared Technology in Microplastic analysis through bibliometric analysis.

Examining the scientific literature on this current subject reveals a remarkable increase in the number of publications focusing on microplastic debris and their characterisation methods based on infrared spectroscopy over the last several years. The year-wise distribution of publication output revealed the progress of MPs research over time. After the term microplastic was introduced in 2004, it took almost a decade for microplastic problem to gain the attention of the world when plastic was declared as pollutant globally in 2011 by UNEP[41] and the severity of its impact came to be reserached on the marine habitat that publications rose exponentially in the last decade. Methodologies have also evolved significantly, and the original technique provided in Thompson's study has been significantly improved afterwards. Similarly, for a long time, visual observation was the most generally used way of identifying plastic particles, although chemical characterisation using spectroscopic approaches is now often utilized. But even today the most used technique for characterisation is infrared spectroscopy[42].

According to analysis from the bibliometrix R package[43], FTIR use in microplastic research has grown at a 90.19 % annually in terms of scientific production from 2016 to 2021, and in the first six years (2010-2015), the annual growth rate was 24.57 %, while NIR research in the MP field has grown at the rate of 83.84 % annually from 2016 to 2021, indicating the start of an impressive growth in publications in this field (Figures 8-9).

In the year 2021, 18 articles claiming the use of NIR technology in MP analyses were published after the first publication in 2016, whereas only 6 articles stating the use of FTIR technology in MP analyses were published in 2015, after it was first used in 2010 to analyse MPs. Since the field of NIR in MP analysis is still in its early stages, the scientific contribution is predicted to rise year after year, as evidenced by the results of the study.

It is also clear that the number of publications (Table 2) in both domains has expanded significantly in recent years, demonstrating the progression and development taking place in this field of study.

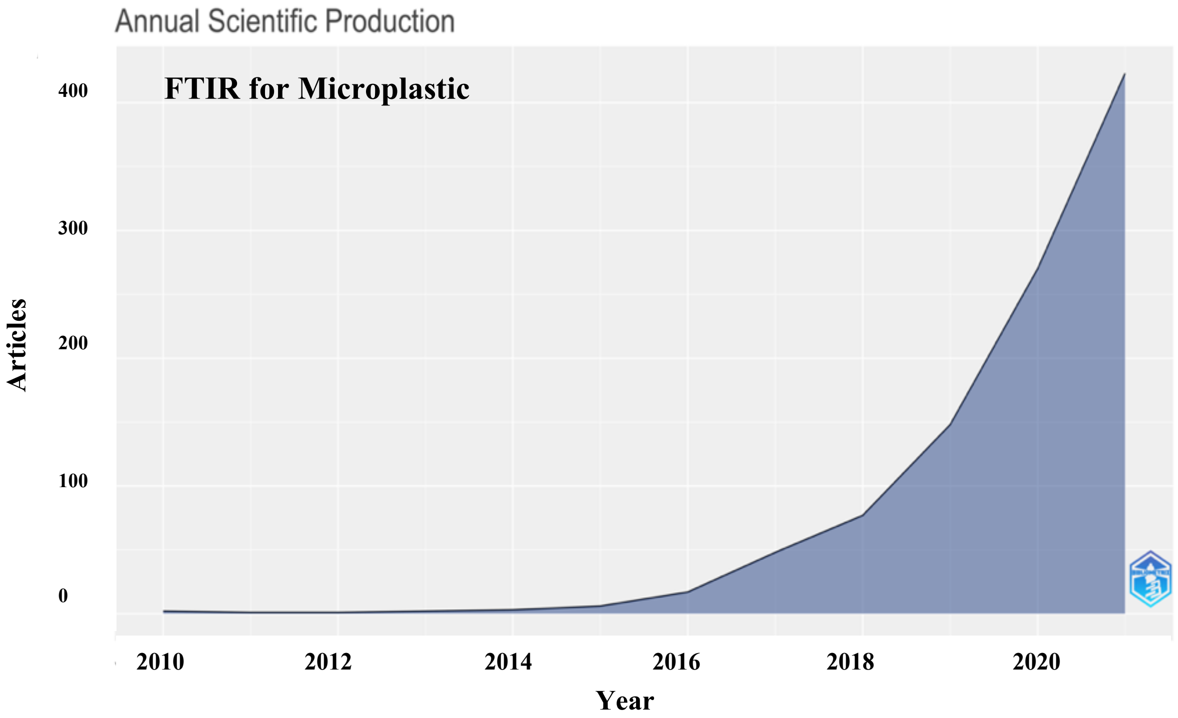


Figure 8: Annual Scientific Production of the publications mentioning use of FTIR technology to analyse Microplastics.

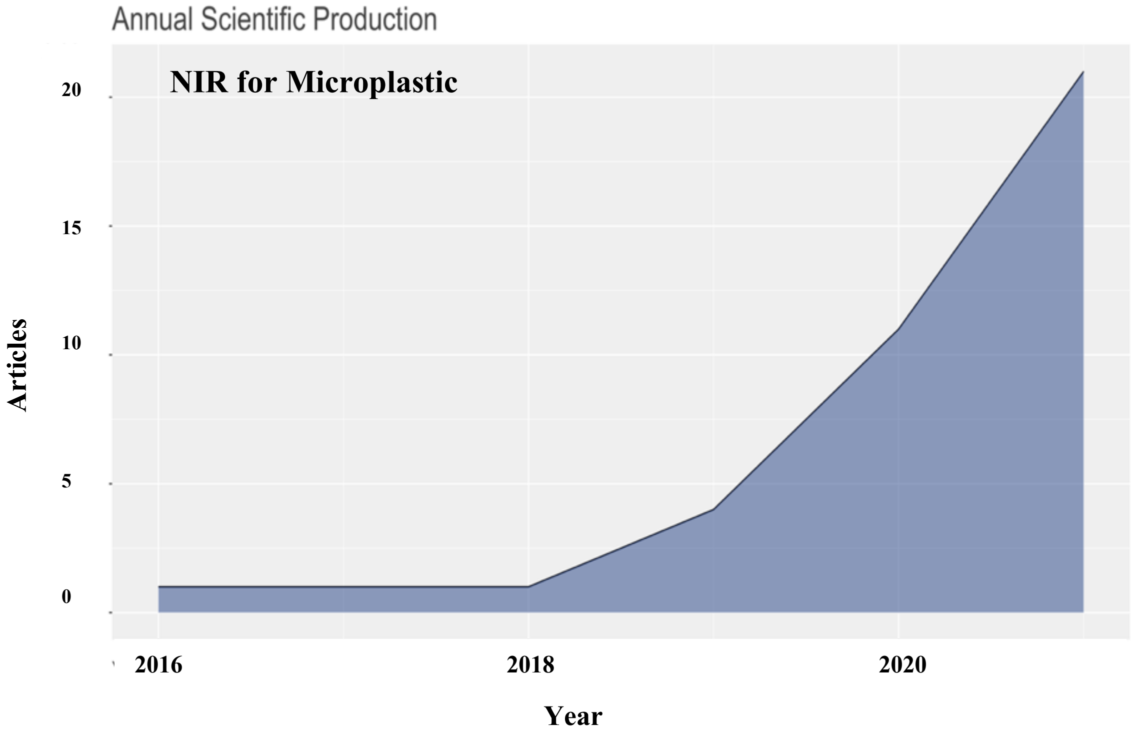


Figure 9: Annual Scientific Production of the publications mentioning use of NIR technology to analyse Microplastics.

Table 2 shows the average number of citations received by MPs papers using FTIR and NIR technique per year. This figure publication's annual influence on the field. The results shows that the only single publication in 2016, which appears to be the beginning of the field and the, received an average number of 7.83 citations and the second publication in 2018 has 20 citations. This implies that the work done by the authors of these publications had a good impact in the use of NIR technology for MP analysis. Inspite of COVID-19 pandemic, the average citation per year for MP papers with use of NIR increased from 4.25 to 13.41, while with the popularity of FTIR technqiue for MP analysis, the average ciation decraesed from 31.85 in 2018 to 21.22 in 2019. This indicates the growing use and popularity of NIR technqiue among researchers in microplastic community. And the decrease in average citations and publications in both domains since 2020 is attributed to the COVID -19 pandemic.

Table 2: The average number of citations received by analysis of MP with FTIR and NIR papers per year. This metric measures the impact of a publication on the field each year.

S.No.	**Year**	**Number of publications**	**FTIR+ MP Average citation per year**	**Number of publications**	**NIR+ MP Average citation per year**
1	2010	2	40.7	-	-
2	2011	1	69.45	-	-
3	2012	1	19	-	-
4	2013	2	85.94	-	-
5	2014	3	40.95	-	-
6	2015	6	45.90	-	-
7	2016	17	24.75	1	7.83
8	2017	48	30.42	1	20
9	2018	77	31.85	1	4.25
10	2019	148	21.22	4	13.41
11	2020	270	14.24	11	6.36
12	2021	423	7.75	21	4.19

3.3 Active Journals

Since 2010, studies on MP analysis with FTIR have been published in 196 journals, while studies on MP analysis with NIR have been published in 20 journals. Table shows the results of the top ten most relevant sources focused on producing publications on the use of FTIR and NIR to analyse MPs.

This result is based on Scopus data retrieved in January 2022 (Table 3). Out of the top 10 journals, 6 journals publish the articles from both domains. Among them, the journal "Marine Pollution Bulletin" resulted the first-ranked with 231 articles published on the topic of FTIR use, accounting for 19.06 % of the total number with 11149 citations, while for NIR use, the number was only 5 until December 2021 accounting for 12.8 % of the total publications with 135 citations of the journal. This low number of articles on NIR use in MP research is comprehensible given that only 39 articles have been published since the first article on MP analysis utilizing the NIR wavelength range was published in 2016. The usage of NIR technology in MP research analysis is indicated by the top six journals being common in both application areas. This suggests that the NIR technology is being given relevant importance in microplastic research since the very beginning.

Table 3: List of Top 10 Journals Publishing FTIR and NIR-Related literature

S.No.	FTIR+ MP	JOURNAL IF	NIR+ MP	JOURNAL IF
1	Marine Pollution Bulletin	5.54	Marine Pollution Bulletin	5.54
2	Science Of The Total Environment	7.963	Analytical Methods	2.896
3	Environmental Pollution	8.071	Chemosphere	7.086
4	Chemosphere	7.086	Environmental Pollution	8.071
5	Journal Of Hazardous Materials	10.39	Science Of The Total Environment	7.963
6	Water Research	11.24	Journal Of Hazardous Materials	10.39
7	Environment Science and Technology	9.028	Water Research	11.24
8	Environmental Science and Pollution Research	4.223	ACS Applied Materials and Interfaces	9.229
9	MethodsX	3.641	Applied Sciences (Switzerland)	2.736
10	Environmental Toxicology and Chemistry	3.742	Chemosensors	3.293

Thematic Map

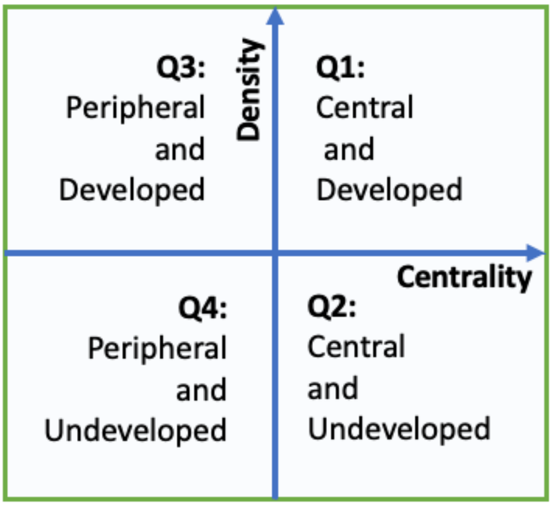
Thematic maps are simple to use plots that allow us to analyze themes based on the quadrant in which they are placed according to their centrality and density rank values along two axes. A basic thematic map looks like in the figure shown below.

(Q-1) Upper-right quadrant: motor themes – These are well-developed themes that are essential to the research field's structure and are characterized by high centrality and density.

(Q-2) Lower-right quadrant: basic themes- Clusters are linked by numerous keywords but are very different from each other. Here promising or past themes that are part of the discipline

(Q-3) Upper-left quadrant: extremely specialized/niche themes- These are well developed and very specialised themes, but marginal in the overall field.

(Q-4) Lower-left quadrant: emerging or vanishing themes- These themes may evolve to the right, gaining centrality and evolve upwards, gaining density. They might be at the origin of new trends oe development within this field.



Fgure 10: Basic themes of a thematic map.

The *centrality* of a network is a measure of its interaction with other networks and is regarded "as a measure of the relevance of an issue in the evolution of the entire study field under consideration"[44]. The *density* identifies the degree of development of a theme and assesses the internal strength of the network. The number of occurrences of the keywords in the cluster, and thus the number of related publications, determine its size. The software-generated label corresponds to the dominant keyword. For this analysis, the two types of literature downloaded (FTIR-related literature, NIR-related literature) were combined.

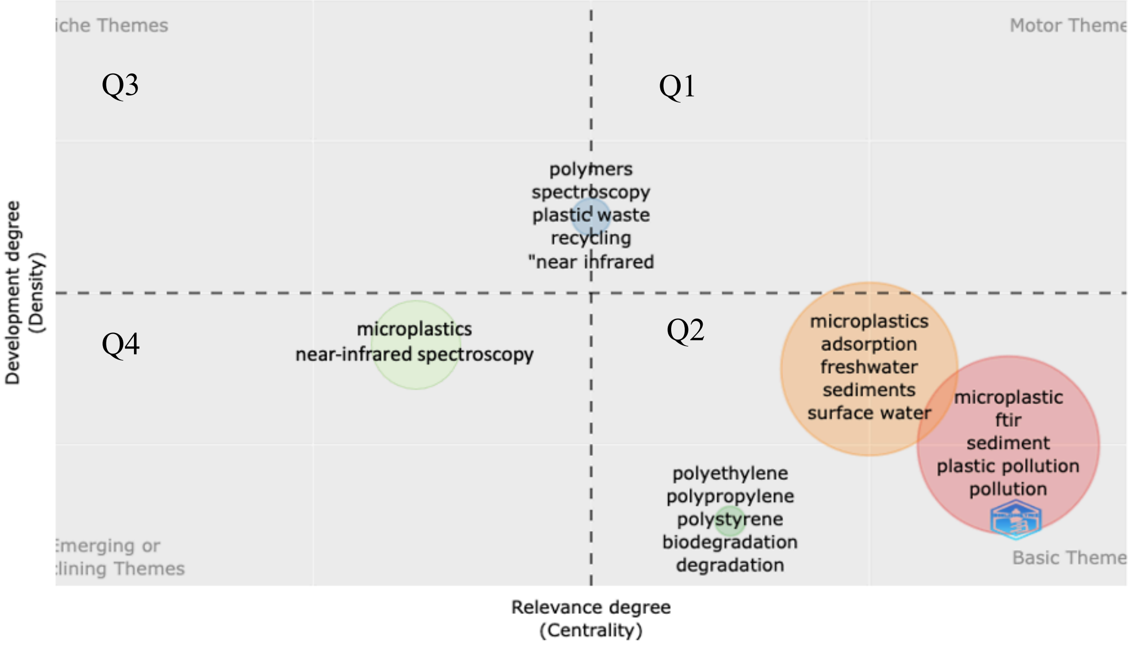


Figure 11 : Thematic Map: FTIR and NIR related literature combined.The main theme is in Q1, followed by highly developed and specialized themes that connect to the main theme in Q2, fading or emerging themes in Q3, and foundational and transversal themes in Q4.

The following observations are made from this thematic map (Figure 11):

1. Polyethylene, Polypropylene and Polystyrene (Q2, basic theme) are the most studied microplastics and this result is consistent with the fact that these polymers form the major part of the plastic waste.
2. Notably from the figure, a theme such as microplastics in freshwater and sediments on the edge of Q1 (motor theme) and in Q2 (basic theme), is capable of structuring the research field. In other words, microplastics in marine environment is the leading theme withing the field, probably because MP research has been focused in marine environment since the very beginning. In the Q2 region, FTIR with microplastic and plastic pollution is seen, and it is a given that in the second quadrant promising and past themes that are part of the research field are found and FTIR has been used since the very beginning of the microplastic research.
3. In between Q1 (motor theme) and Q3 (niche theme), there is a cluster of near-infrared, plastic waste, recycling, polymers and spectroscopy, predicted to move in Q1 in upcoming years, given the increasing popularity of NIR technology in plastic characterisation.
4. According to this analysis, we identified microplastics and Near Infrared Spectroscopy as emerging topics (Q4), the underlying theme of this paper.
5. It is evident from Q3 that there are no fully developed themes in the field of microplastic analysis, either through FTIR or NIR method, and it is very well known that there are no

standard methods to analyse MPS, however vibrational spectroscopy-based methods are highly preferred.

For a better understanding of the use of NIR in MP analysis, the thematic map was only confined to NIR-related literature (Figure 12). The result is shown below. As seen in the keyword analysis of NIR-literature, near-infrared hyperspectral imaging is an emerging topic in this field.

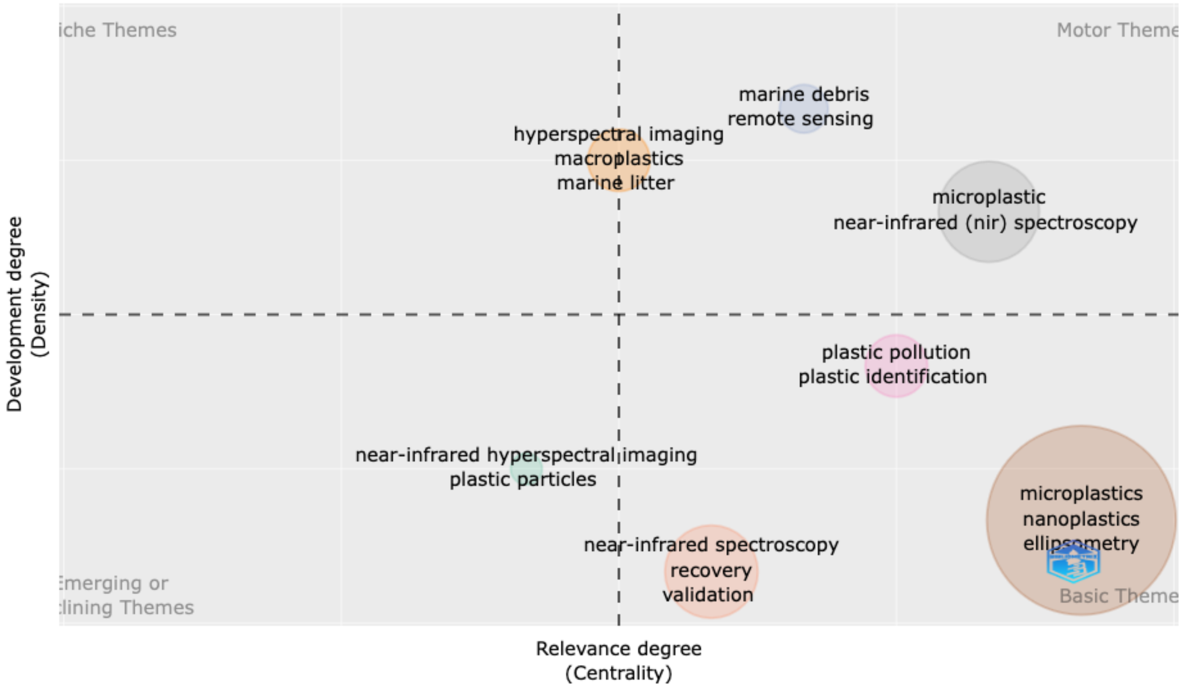


Figure 12 : Thematic Map: NIR related literature combined.The main theme is in Q1, followed by highly developed and specialized themes that connect to the main theme in Q2, fading or emerging themes in Q3, and foundational and transversal themes in Q4.

The research on microplastics is currently particularly contentious due to the wide range of research methods used. The current study was carried out to give a snapshot of the use of Near-Infrared Technology to characterize microplastics compared to the use of Fourier-Transform Infrared Spectroscopy. Identifying the most influential papers, authors, and countries in microplastic research can help with regulatory decisions, research technique, and definitions. FTIR has been linked with the MP analysis since the very beginning, while NIR has been recently introduced (from 2017) in this field. This theme is still in its infancy stage but is gaining popularity not only because of its benefits, such as portability and comprehensive measurements, but also because of the updated technology. Since the inception of NIR technology in MP research, it has grown at a breakneck pace. Many industrial businesses are now participating in the development of instruments based on NIR technology, ranging from on-line analysis in factories to sensors in handheld devices, which is noteworthy. It is also worth mentioning that according to the citation analysis of journals, "Marine Pollution Bulletin" came in first among scientific publications that published works on this topic, as it accepts FTIR for MP analysis and the scientific production is geographically polarised within European Union (EU).

3.2.3 Instrumental Aspects: Importance of Compact Technology

It has now been very well established that the polymers can be identified with both Mid-Infrared and Near-Infrared Spectroscopy. But it is always not so simple to measure microplastics as they come in different shapes, size, color, especially covered with organic matter when environmental samples are measured. When compared to the spectrum library, FTIR spectroscopy, based on MIR is a particularly strong approach for characterizing known and undiscovered organic molecules. Because of its lengthy history of polymer identification, FTIR has gained favor as an effective tool for studying MP. For various applications, multiple variants of FTIR devices, notably transmission, reflection (specular and diffuse), and attenuated total reflection (ATR)—with their related limits, are available. Aside from its capabilities, the choice of the FTIR instrument is heavily influenced by the size of the MPs.

ATR with a tabletop FTIR spectrometer is the most cost-effective and user-friendly apparatus. It has been used to characterize MPs, particularly when analyzing bigger particle sizes (≥1 mm) or confirming the correctness of other analytical procedures. ATR measurements, on the other hand, are time-consuming and need a bigger particle size for ease of handling and sample preparation. Furthermore, surface alteration of samples might affect the quality of the acquired signal, perhaps limiting the percentage confidence in identifying environmental MPs from spectra libraries. Another limitation is that even FTIR is a nondestructive technique, environmental samples being brittle are sometimes broken by the ATR tip.

Based on the facts discussed above, it appears that current identification approaches are still restricted to the laboratory, where sample type and size are known in advance and measurement circumstances are reasonably steady but thanks to the various types of portable and handheld commercial spectrometers are available as a result of the development of tiny size light sources, detectors, and optical components, as well as the requirement for field measurements. Over the past decade, the miniaturized vibrational spectrometers have gained popularity as they allow real-time assessment with no or minimal sample pre-treatment, but the commercial availability has only begun in the last few years, creating a new population of users taking measurements in the field by non-technical personnel that were previously conducted in the laboratory by professionals. The use of these portable spectrometers with computational tools has shown a great potential in the analysis and classification of the most common polymer[32]. Although there has been some research carried out on the use of micro spectrometers for the study of polymers, controlled studies are still required. It has now become critical to expand the capabilities of these miniaturized instruments to routinely analyze the composition of MPs due to the recent explosion in this field. These procedures can assist with building up where material may have come from and could distinguish added substances that themselves could present negative natural impacts.

NIRS, unlike MIR is classified as an indirect or secondary approach. Their spectra must be compared to primary or reference techniques in order to acquire information. As a result, mathematical methods are used to the spectra in order to create an NIR "predictive model" that can predict the composition of the studied material. This is addressed in the next section.

3.2.4 The Basics of Chemometrics

Qualitative and quantitative near infrared (NIR) spectroscopy techniques frequently require the use of multivariate calibration algorithms and statistical methodologies to describe the NIR spectral response to chemical or physical features of the materials used for calibration, as this method generates massive volumes of data that is difficult to analyze manually by professionals. In contrast, spectra can be manually evaluated based on the researcher's expertise by studying the typical form of the complete spectrum and identifying specific vibrational bands. This approach, however, requires a high level of specialist knowledge and, due to its time requirements, it is not appropriate for large sample throughput analysis and to take advantage of NIR spectroscopy, the analyst must also overcome sensitivity and selectivity constraints caused by the NIR region's relatively weak and largely overlapping spectral bands. As a result, it is even more critical to invest time in designing appropriate and realistic data treatment procedures, as well as documenting them in the same depth as other experimental phases. Chemometrics aid in completing such a difficult process by absorbing data and revealing important information.

Traditional statistical methods only consider a single variable, such as the intensity value at only a single wavenumber. NIR spectra, on the other hand, convey a fingerprint-like signature of substances, resulting in incredibly complicated spectra. To process, classify, or quantify NIR spectroscopical data, multivariate statistical analysis, also known as chemometric approaches, are thus necessary. Unlike traditional statistical methods, which are often insufficient for accurate and detailed interpretation of results, chemometrics allows valuable information to be extracted from a wide range of complex data sets and makes hidden relationships between variables easier to detect.

In the analysis of NIR data through chemometrics, the first general step includes mathematical pretreatment that enhances and organizes the information related to the chemical variation.

This structuring or pre-treatment serves a number of purposes:(1) enhancement of a weak absorption band that is convolved with nearby spectral data, either intrinsically or due to instrument restrictions. (2) elimination of random noise and (3) decreasing the light scatter effects, caused when the size of the particle in the sample matches the magnitude of the spectroscopic wavelength. These undesirable fluctuations frequently account for a large portion of the total variation in the sample set and can be noticed as changes in baseline (multiplicative effects) and other non-linear phenomena. Thus, the use of pre-processing techniques is critical for preparing data for further analysis (exploratory, regression, or classification analysis). In general, the goal is to change the data in a way that better adheres to Beer's law.

Typically, the optimal pre-treatment is unknown in advance leaving the analyst with the responsibility of manually searching for the pre-treatment that yields the lowest residual (NIR minus reference) results. The two most common pre-processing approaches in NIR spectroscopy (in both reflectance and transmittance mode) are scatter-correction algorithms and spectral derivatives. The goal of scatter correction methods is to minimize the impact of scatter between samples. Derivatives, on the other hand, have the capacity to remove baseline effects as well as any offset discrepancies between the data. Scatter pre-processing techniques includes Standard Normal Variate (SNV), Normalization, Multiplicative Scatter Correction (MSC), Extended MSC (EMSC), Inverse MSC, Extended Inverse MSC, and de-trending[33]. The spectral derivative correction uses first, second, Norris-Williams and Savitzky-Golay derivatives. In this thesis, SNV and second order derivatives have been used (chapter 4, and 6 for further details). The explanation of the above mentioned treatments is beyond the scope of this thesis. If required, to obtain a deeper understanding of chemometrics, the readers are encouraged to look in the given references

After the pre-processing is done, there are two types of chemometric methods used: unsupervised and supervised procedures. Unsupervised techniques look at a dataset's structure based on its measurements. It identifies clusters without making any assumptions about the number or type of classes. Unsupervised approaches are beneficial for exploring the data structure, identifying likely outliers, and condensing big complex data sets into a series of smaller, more manageable chunks. Principal component analysis is one of the most widely used unsupervised techniques (PCA) and cluster analysis (CA). These unstructured techniques are frequently used to reduce dimensionality of the original data, locate internal trends in the dataset, or identify the major factors.

The chemometric techniques that have been used in this work are described in the subsequent chapters at the point-of-use.

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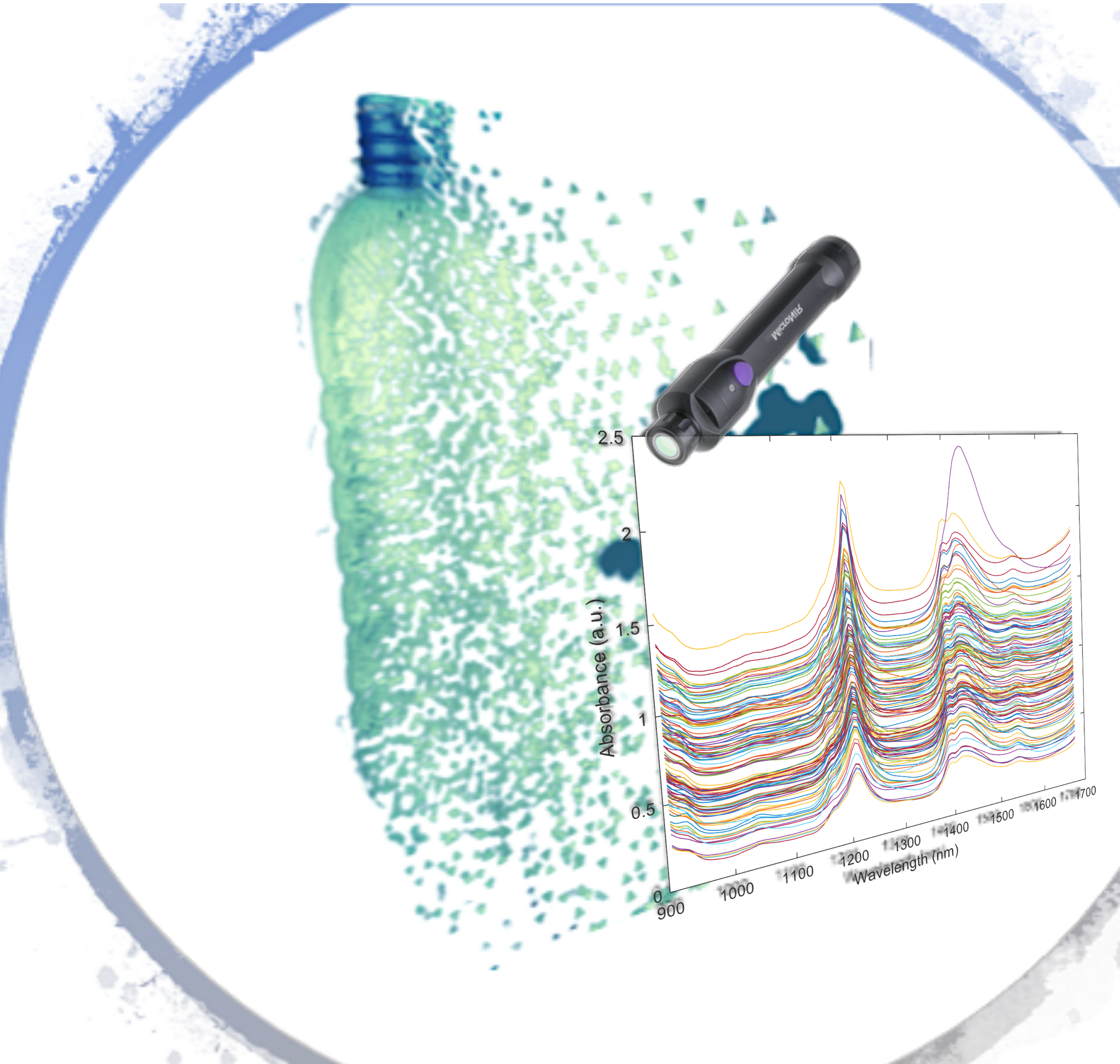
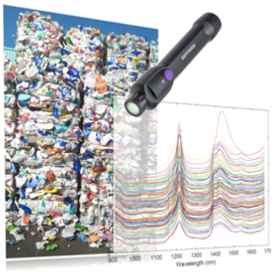
PART 2:
FROM MACROPLASTICS TO MICROPLASTICS



CHAPTER 4

THE FOUNDATION

ON CHALLENGING A MINIATURIZED HANDHELD MICRONIR FOR POST-CONSUMER PLASTIC WASTE SORTING



4.1 Abstract

Valorisation of the urban plastic waste in high-quality recyclates is an imperative challenge in the new paradigm of the circular economy. In this scenario, a key role in the improvement of the recycling process is exerted by the optimization of waste sorting. In spite of the enormous developments achieved in the field of automated sorting systems, the quest for the reduction of cross-contamination of incompatible polymers as well as a rapid and punctual sorting of the unmatched polymers has not been sufficiently developed.

In this work, a miniaturized handheld near-infrared (NIR) spectrometer was used to successfully fingerprint and classify different plastic polymers. The investigated urban plastic waste comprised polyethylene (PE), polypropylene (PP), poly(vinyl chloride) (PVC), poly(ethylene terephthalate) (PET), and poly(styrene) (PS), collected directly in a recycling plastic waste plant, without any kind of sample washing or treatment.
The application of unsupervised and supervised chemometric tools such as principal component analysis (PCA) on the NIR dataset resulted in a complete classification of the polymer classes. In addition, several kinds of PET (clear, blue, coloured, opaque, and boxes) were correctly classified as PET class, and PE samples with different branching degrees were properly separated.

4.2. Introduction

The huge amount of urban plastic waste and the continuous growth in human plastic consumption require a high valorisation of the collected waste in the direction of a whole-system economic sustainability. By the numbers, European plastic production reached almost 60 million tonnes in 2016, and 335 million tonnes globally (Source: PlasticsEurope Market Research Group (PEMRG) /Conversio Market & Strategy GmbH). In this framework, over 8.4 million tonnes of plastic waste were collected in order to be recycled inside and outside the EU[1]. The recycling of polymer waste has valuable environmental benefits due to the substitution of primary production, and a key role in the improvement of the recycling process is exerted by the optimization of waste sorting[2,3].

Generally, automated sorting processes are widespread in plastic recycling facilities[4]. These systems are usually based on vibrational spectroscopy techniques for polymer identification[5,6,7,8,9] and camera systems for optical recognition of clear and coloured products[10,11]. Other sorting technologies include UV–Vis spectroscopy[12] or mass spectroscopy (Py-GC/MS) for identification[13], hyper-spectral imaging methods[14], X-ray detection for the separation of specific containers[15], fluorescence spectroscopy for identifying halogens and heavy metals[16] , and laser-induced plasma spectroscopy to detect additives[17]. This approach has increased the purity of the output plastic over the years, reaching a high percentage of recyclates in the production of secondary materials. Nevertheless, there are severe limitations of these techniques in handling mixed plastic, which requires additional sorting elsewhere and can affect the quality of the recyclate, if not properly assigned. The positive cost/benefit balance can take place only if the separated fractions of polymers match a high purity grade, fulfilling the market requirement of high recyclate quality. Therefore, a key step in post-consumer recycling is reducing the cross-contamination of incompatible polymers[10].

Besides the automated sorting of the plastic stream, manual sorting is still necessary for the full separation of the collected waste. However, manual sorting based only on plastic code recognition is inefficient and time consuming. In some cases, plastic goods can be badly crushed or have missing or unreadable codes. Manufacturers can also use different polymers for the same product, depending on marketing choices. In addition, the high turnover rate can be affected by an improperly trained or unskilled workforce.

In this scenario, the possibility of exploiting a well-established technique for polymer identification combined with a miniaturized, portable, low-cost, and real-time spectrometer for local and punctual semi-automated sorting is highly desirable. Urban plastic waste normally contains plastics such as polyethylene (PE), polypropylene (PP), poly(vinyl chloride) (PVC), poly(ethylene terephthalate) (PET), and poly(styrene) (PS). These polymers have characteristic near-infrared (NIR) spectral fingerprints that can be used for accurate sorting and separation[5].

The first near infra-red (NIR) sorting systems were used to process recyclable materials in the early 1990s. Now, NIR technology has found its way into almost all areas of sorting recyclable materials. The progress in miniaturization technologies has generated specific instrumental features that have been reported in polymer identification applications, but results have been reported only for a selection of polymers from commercial libraries, and are not fully representative of real plastic waste[18].

In the present work, we challenged a compacted and miniaturized NIR spectrometer for a rapid identification of urban plastic waste collected directly from a selection and recycling plant, without any sample treatment and to build an in-house NIR spectral database since the majority of commercially available spectra libraries are based on pure, clean, and non-degraded references, identification of some environmental samples may be hampered.

We collected a large amount of plastic samples directly from the plant and we created a robust database of NIR spectra. Different chemometric tools were applied for data evaluation of all the recorded samples. Principal component analysis (PCA) for preliminary data exploration and partial least squares-discriminant analysis (PLS-DA) as supervised pattern recognition were applied to fingerprint and classify the different plastic polymers.

4.3. Materials and Methods

4.3.1. Polymer Samples Collection

Plastic samples were collected at the selection division of the recovery and recycling plant Montello SpA (Bergamo, Italy), which receives post-consumer plastic in the form of urban waste for recycling. In total, 250 samples belonging to various classes of polymer were used The polymers considered were: several kinds of PET (clear, blue, coloured, opaque, and boxes), PE with different branching degrees (low-density polyethylene, LDPE, and high-density polyethylene, HDPE), PVC, PP, and PS (Figure 1). The collection included bottles, containers, and packets of different shapes, sizes, and colours. The samples were flattened or partially flattened at the sampling site, and were not washed prior to testing. Black samples were not considered, due to the very low reflectance in the NIR spectral region and the low signal-to-noise ratio of NIR sensors, which hamper successful black polymer spectral acquisition[19].

Figure 1: Systematic organization of the collected urban plastic waste for laboratory measurements

4.3.2. NIR Measurements

Spectra acquisition was realized using MicroNIR On-site (Viavi Solutions Inc., CA, United States in reflectance mode without any sample preparation. The instrument is a miniaturized palm-sized portable spectrometer of about 250 g in weight, less than 200 mm in length, and 50 mm in diameter. This handheld spectrometer employs a linear variable filter (LVF) as dispersing element, in contrast to traditional diffraction-based spectrometers (see Figure 2 for an instrument operating scheme). The controlling parameters for spectral data collection were set at 10 ms integration time and 50 scans, resulting in a short measurement time of 0.25 s. Not less than five replicates in different positions were acquired by a point-and-shoot technique, in order to minimize effects driven by the non-uniformity of samples. A total of 1303 NIR spectra were collected. A dark and a reference scan were carried out approximately every 10 min. Data acquisition was realized through MicroNIR™ Pro v3.0 software (Viavi Solutions Inc., CA, United States).

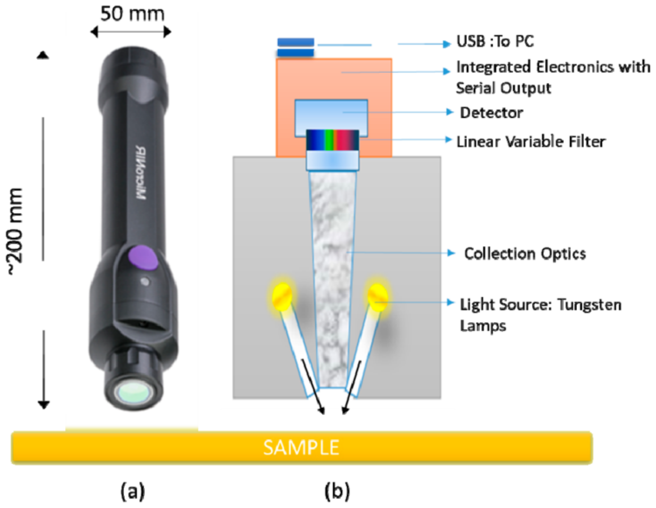


Figure 2: (**a**) Miniaturized near-infrared (microNIR) device and (**b**) instrument operating scheme.

4.3.3. Signal Pretreatment

Signal correction methods are mathematical tools employed to remove additive and/or multiplicative effects on spectroscopic data, which often affect the performance of chemometric analysis[20]. These phenomena are typical of light scattering effects, which induce a photon loss (addictive effect) and an increased path length (multiplicative effect), among others. These methods are "row-wise", and therefore the correction is made sample-by-sample. This is in contrast to mean centring and autoscaling, which are "column-wise" treatments. In most cases, the combination of the two types of method is required in order to make a satisfactory data analysis[21]. In this study, spectra were collected into a single 1307 × 125 matrix (samples × scans), and several pre-processing tools were applied to the raw data. The best result was obtained by applying Savitzky-Golay second-derivative procedure of seven data points and a second-order polynomial followed by standard normal variate (SNV). Moreover, after the preprocessing, spectra were normalized by mean centring.

4.3.4. Data Analysis

Different chemometric tools were applied in order to have a correct data evaluation of all the analysed samples. The first step consisted of an exploratory analysis by PCA aiming to explore the data structure. PCA was carried out with a total of 1303 NIR spectra for all five polymer classes. Subsequently, PLS-DA was used as supervised pattern recognition with the aim of separating the different commodities, and it was performed on a total of 1287 NIR spectra (16 spectra were removed as outliers). All calculations were performed using MATLAB (R2018a) with chemometric toolboxes[22,23].

4.4 Results and Discussions

For building our in-house library of NIR spectra, plastic samples were collected from a recycling plant in the form of post-consumer plastic waste since majority of commercially available spectra libraries are based on pure, clean, and non-degraded references, identification of some environmental samples may be hampered.

4.4.1 Spectral Analysis

NIR spectroscopy has the key advantage of being a rapid-response analytical tool, recording spectra with no prior manipulation and predicting physical and chemical parameters from a single spectrum[24]. In the NIR region, the absorption bands occur due to overtones or combination bands of mainly carbon–hydrogen vibrations and oxygen-hydrogen vibrations. A correct band assignment is challenging because it may be due to different combinations of fundamental vibrations. Moreover, overtone vibrations are highly overlapped[25].

The NIR reflectance spectra of the five polymers (i.e., PET, PP, PVC, PE, and PS) in the range 900–1700 nm, obtained by the MicroNIR On-site, are shown in Figure 3.

In the absorbance spectra (Figure 3a) the principal absorbance band for PET was found at 1660 nm due to the first overtone of C–H stretching[26] , with two other small peaks around 1130 nm and 1415 nm. In the case of PVC, this main peak was shifted and was not recorded in the instrumental operating window, and only the two small peaks around 1190 nm and 1420 nm were seen[26] . The same behaviour appeared for PE and PP, assigning two bands around 1210 nm and 1430 nm for PE and around 1190 nm and 1400 nm for PP. PS exhibited a dominant band around 1675 nm and two small peaks around 1140 nm and 1205 nm[5] . Figure 3b refers to the corresponding spectra shown in Figure 3a after pre-treatment by second derivative and SNV.

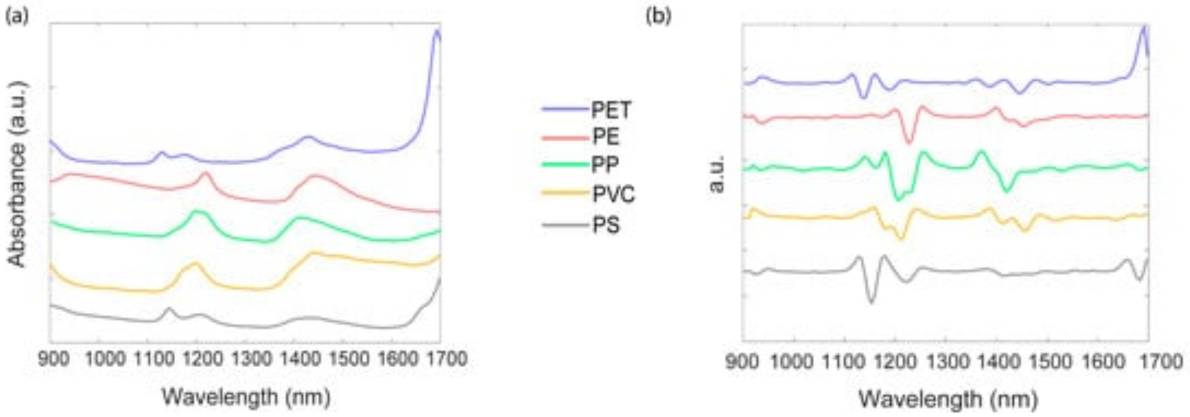


Figure 3: Near-infrared (NIR) spectra of five classes of plastics: (a) representative raw spectra of the five classes; (b) corresponding spectra after pre-treatment by second derivative and standard normal variate (SNV). PE: polyethylene; PET: poly(ethylene terephthalate); PP: polypropylene; PS: poly(styrene); PVC: poly(vinyl chloride).

4.4.2 Effect of Colour and Contaminants on NIR Spectra

As the samples were not washed prior to acquiring the NIR spectra, it was evitable to understand the effect of contaminants and colour on the same. In general, by comparing the spectra of samples made of one type of polymer, a variation in the absorbance values was noted and this was ascribed to variations in sample thickness[5] . Liquid residuals, pieces of labels, and environmental dust were largely present on the surface and inside the samples. Nevertheless, NIR spectra showed only a variation in the total absorbance values, attributable to variations in sample thickness, according to Lambert–Beer law[27] .The overall form of the spectra was preserved, concluding that dust or liquid contaminations randomly characterizing each sample did not affect the quality of NIR spectra[28].
(Figure 4).

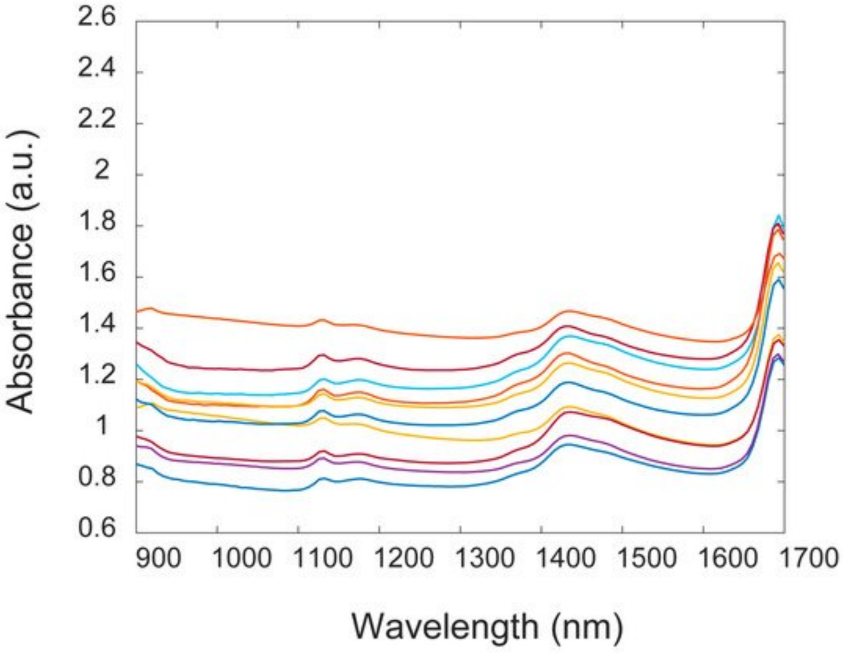


Figure 4: Representative NIR spectra of coloured PET samples.

In addition, NIR spectra of samples of different colours did not display considerable differences (Figure 5), meaning that the colorants present in the plastic resin matrix did not significantly influence the NIR spectra. Moreover, no differences in band positions were found when studying the spectra of several kinds of PET (clear, coloured, opaque, box, and blue) implying that the spectra can be successfully exploited to discriminate PET samples, despite their differences. For this reason, hereinafter, only one PET class is considered.

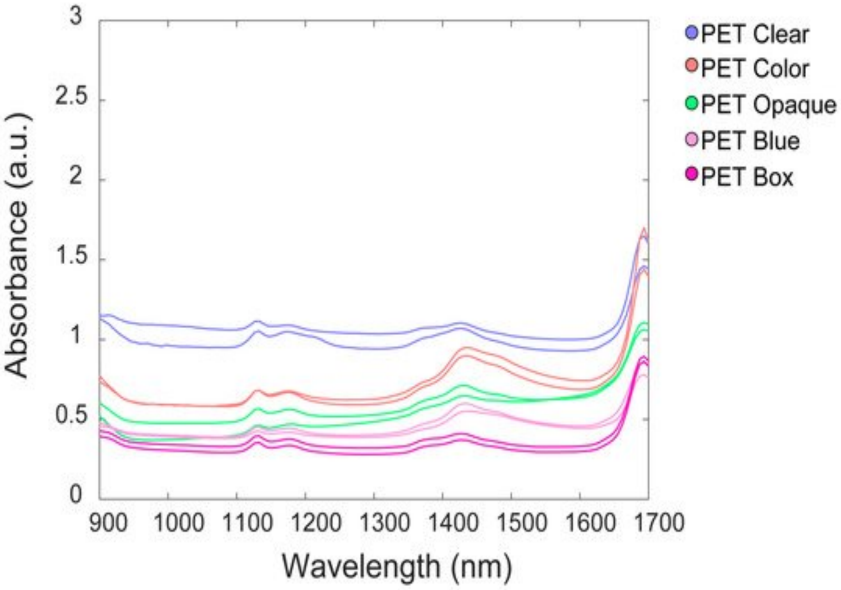


Figure 5: Representative NIR spectra of two random samples for each kind of PET commodity.

4.4.3. Principal Component Analysis

Principal component analysis is a very powerful chemometric tool for analysing data structure[29]. The aim of PCA is to extract the information encoded in a certain number of variables into a smaller set of new orthogonal variables called principal components. PCA calculation was performed over the entire spectral region and using all collected spectra. The first three components accounted for 87.11% of the total variability (PC1 56.75%, PC2 15.63%, and PC3 14.73%). Figure 6 displays the score plots of PC1 vs. PC2 (Figure 6a) and PC1 vs. PC3 (Figure 6b). In the score plot displayed in Figure 6a clear separation between the five commodities can be seen. Indeed, in the new space determined by the first two components, polymers formed very tight and homogeneous clusters. Along the first component, a clear differentiation between PET, PVC, PP, and PE was highlighted, while the second component allowed the differentiation of PS from the other polymers, and PE from PP. On the other hand, Figure 6b shows the score plot of PC1 vs. PC3. In this case PET was differentiated from the other polymers along PC1, while PC3 distinguished between the remaining sets of polymers.

Furthermore, the results of PCA applied to the (Figure 7) confirming the interpretation of the band assignment of NIR spectra

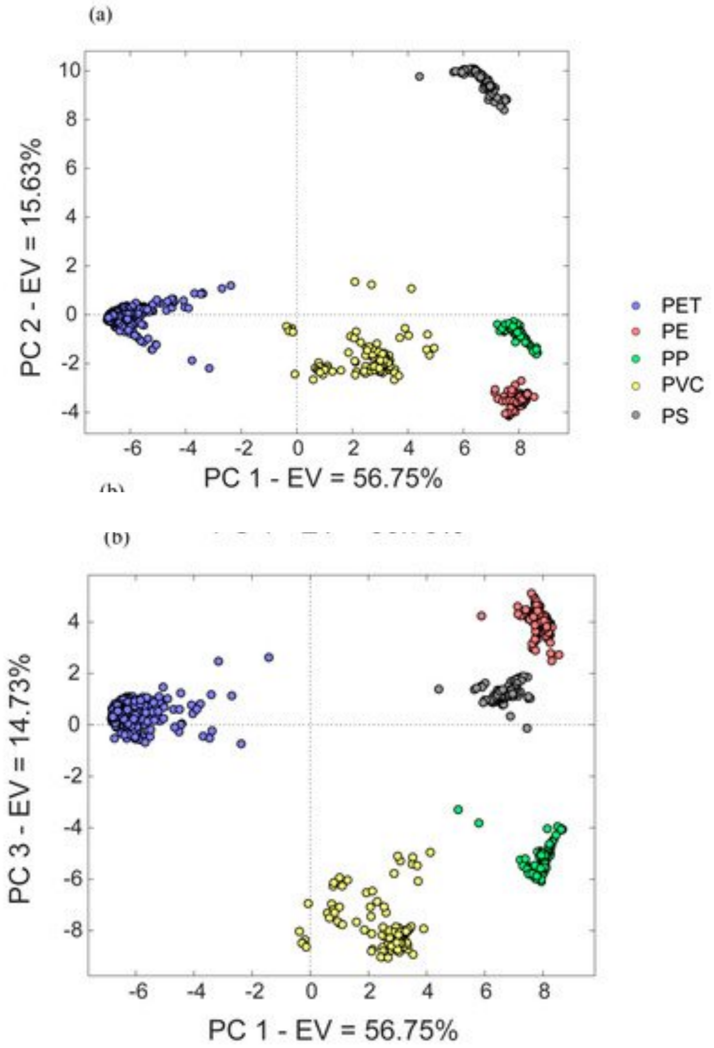


Figure 6: Results of principal components analysis carried out with spectral data of the different commodities. (a) The score plot of the first two components is shown, as well as (b) the score plot of PC1 vs. PC3 EV: explained variance.

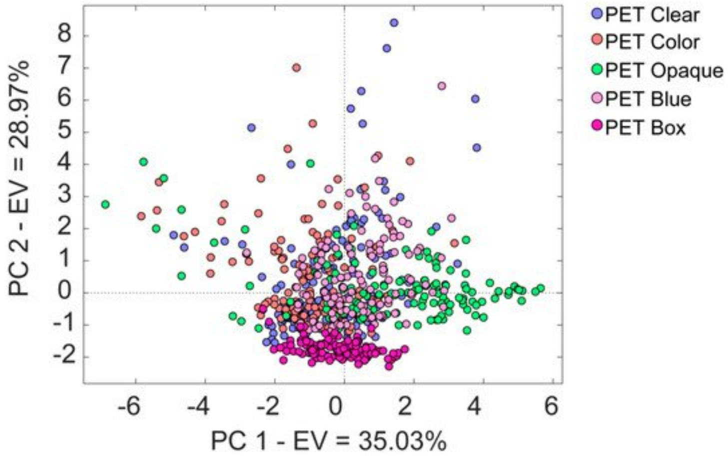


Figure 7: Principal component analysis (PCA) analysis of several kinds of poly(ethylene terephthalate) (PET) samples for a total of 659 spectra considered. EV: explained variance.

4.4.3.1 HDPE versus LDPE

The discrimination between HDPE and LDPE is an important issue in terms of plastic recycling[3]. PE is largely used in the food packaging sector, thanks to its remarkable properties in terms of mechanical and optical performances, water-vapor-resistance, and heat-sealing attitude. The amount of side branches that are attached to the main PE polymer chain determines the morphological properties, which in turn affects optical, physical, and thermal properties[30,31]. NIR spectroscopy successfully discriminates between HDPE and LDPE[32,33] even if severe limitations can occur depending on the characteristics of the measuring instruments[28]. In the sampling campaign, we collected 25 samples of HDPE and 25 samples of LDPE. Raw NIR spectra did not show any significant feature to discriminate the two plastics, nor did a clear separation in the score plots resulting from the previous PCA analysis (Figure 8). However, by limiting the PCA analysis to the PE samples, a separation between HDPE and LDPE was obtained; the first two components explicated 61.22% of the total variability (PC1 43.22%, PC2 18.00%), confirming the successful application of NIR spectroscopy in discriminating these two polymers. Figure 8 displays the score plots of PC1 vs. PC2.

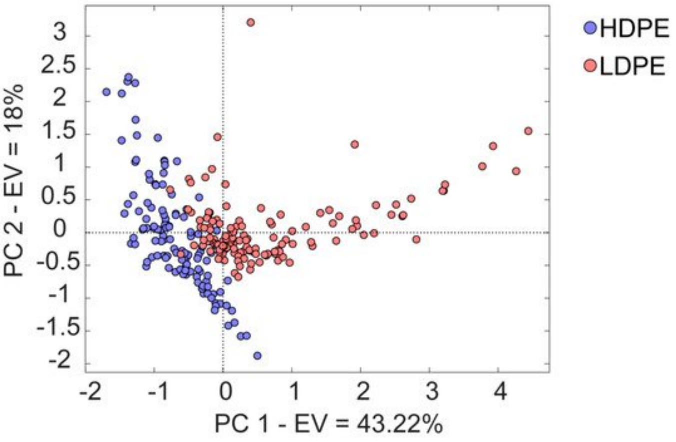


Figure 8: Results of principal components analysis carried out with spectral data of polyethylene (PE) class polymers. Score plot of the first two components is shown.

4.4.4 Partial Least Squares-Discriminant Analysis

After the exploratory PCA analysis, a supervised classification tool was applied in order to distinguish the different plastic classes. In PLS-DA, the well-known PLS regression algorithm is modified with a classification goal. The response variable is categorical, reflecting the belonging class of the statistical units. PLS-DA returns the prediction in a vector of size equal to the number of classes in the predictor variables, with values ranging from 0 to 1[34].

Validation tools are very useful methods in chemometrics, and they are used to verify the capability of the model prediction. Before the model calibration, data were split into training and test sets containing 901 and 386 samples, respectively. The training set was used to calibrate the model both in fitting and in cross-validation, while the test set was employed only at the end of the procedure in order to evaluate the true predictive capability of the calibrated model[22].

During the calibration, the selection of the optimal number of latent variables (LVs) is a crucial point, which is performed based on the cross-validation procedure[22]. This should allow the optimization of the complexity of the multivariate model according to the predictive capacity of the model itself. Cross-validation is generally carried out by dividing the training set into different cross-validation groups, and during each round one group is removed from the training set. Over the rounds, the model is calibrated on the remaining training samples and then used to predict samples of the cross-validation group. The cross-validation procedure was based on a Venetian blinds approach with 10 groups. In the Venetian blinds procedure, the calibration group and the cross-validation group are selected by choosing every n-th sample from the dataset starting from the first one[22]. The selection of the number of cross-validation groups was set to 10, as a reasonable number in order to avoid the so-called “overfitting” (i.e., the overestimation of the predictive capability of the model). Figure 9 shows the error rate (Figure 9a) and the degree of not assigned samples as a function of the number of latent variables (Figure 9b). The optimal number of LVs was set to 6 (explaining 94% of the total variability and 0.0099% of not assigned samples), which was associated with the minimum error rate and, simultaneously, with the minimum percentage of not assigned samples. Four LVs reached the same error rate, but 0.019% of not assigned samples.

After the determination of the optimal number of LVs, the calculation of the PLS-DA model was performed selecting six latent variables and 10 cross-validation groups for internal validation. In Table 1, the two confusion matrices achieved in fitting and in cross-validation were presented. Both in fitting and in cross-validation, the performance of the model was very high, proved by the correlation between observed and predicted classes. All the investigated samples were correctly designated, and only four samples in fitting and five in cross-validation were not assigned.

Table 1. Confusion matrices obtained from the PLS-DA model, both in fitting and in cross-validation (based on Venetian blinds with 10 groups). The “Not Assigned” column contains samples which were not assigned to any of the considered classes.

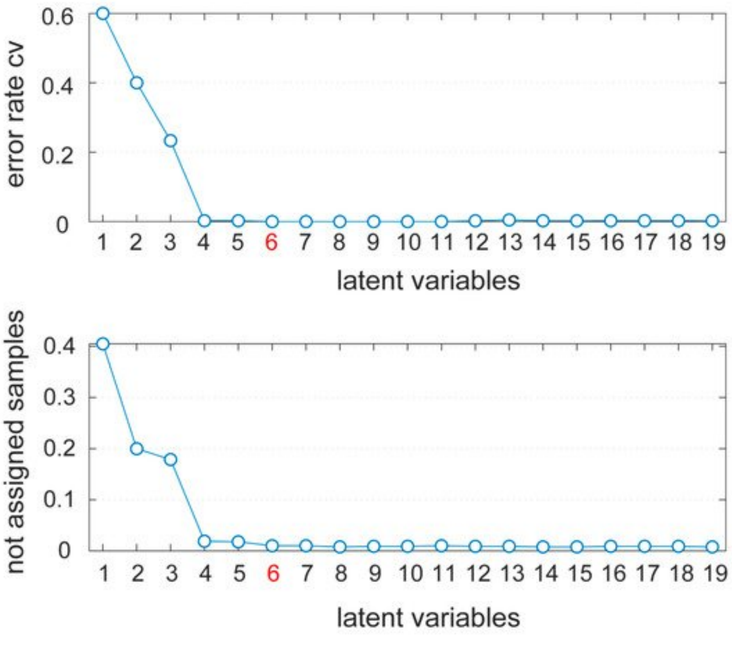


Figure 9: Error rate (a) and not assigned samples (b) as a function of latent variables calculated in the partial least squares-discriminant analysis (PLS-DA) model. Six latent variables (LVs) was the optimal number, marked in red, with 0.0099% of not assigned samples.

Table 1. Confusion matrices obtained from PLS-DA model, both in fitting and in cross-validation (based on venetian blinds with 10 groups).

Experimental Class	Calculated Class PET	PE	PP	PVC	PS	Not assigned
Fitting						
PET	459	0	0	0	0	2
PE	0	181	0	0	0	1
PP	0	0	82	0	0	0
PVC	0	0	0	84	0	1
PS	0	0	0	0	91	0
Cross-Validation						
PET	460	0	0	0	0	1
PE	0	181	0	0	0	1
PP	0	0	82	0	0	0
PVC	0	0	0	82	0	3
PS	0	0	0	0	91	0

The performance of the classification model could be also evaluated by considering the classification parameters derived from the confusion matrix: sensitivity (Sn), specificity (Sp), and non-error rate (NER). These parameters represent the ability of the model to correctly identify the samples of the given class, the ability of a classifier to reject the samples of other classes, and the average of the class sensitivities, respectively[35]. Table 2 collects the parameters of the PLS-DA model. In both fitting and cross-validation, NER, Sn, and Sp were equal to 1, meaning that 100% of the polymers were correctly classified.

Table 2. Classification parameters (non-error rate (NER), class sensitivity (Sn), and specificity (Sp)) calculated in fitting and in cross-validation.

Table 2. Classification parameters (Non-error rate, class sensitivity and specificity) calculated in fitting and in cross-validation.

		PET		PE		PP		PVC		PS	
	NER	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp
Fitting	1	1	1	1	1	1	1	1	1	1	1
Cross-validation	1	1	1	1	1	1	1	1	1	1	1

Furthermore, we analysed the confusion matrix and the classification parameters for the test set, used as an additional trial to validate the model. Table 3 shows the confusion matrix derived from the test set; again, 100% of the samples were classified, and only four samples were not assigned. Table 4 summarizes the classification parameters obtained from the confusion matrix, with the NER, Sn, and Sp of each class equal to 1.

Table 3. Confusion matrix obtained from the PLS-DA model fitted on the validation set. The "Not Assigned" column contains samples which were not assigned to any of the considered classes.

Table 3. Confusion matrix obtained from PLS-DA model fitted on the validation set

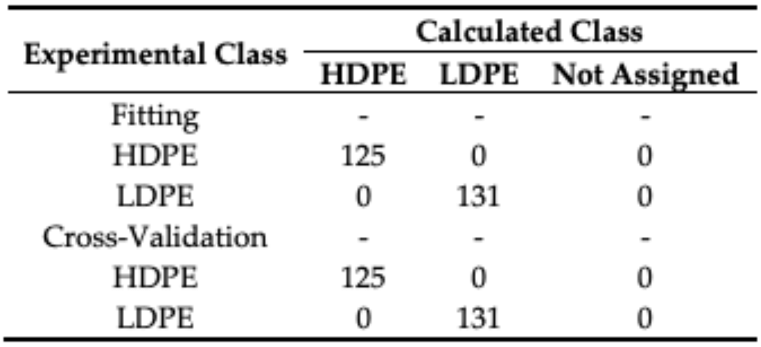
Experimental Class	Calculated Class					
	PET	PE	PP	PVC	PS	Not assigned
PET	197	0	0	0	0	1
PE	0	78	0	0	0	1
PP	0	0	33	0	0	1
PVC	0	0	0	35	0	1
PS	0	0	0	0	39	0

Table 4. Classification parameters (non-error rate (NER), class sensitivity (Sn), and specificity (Sp)) calculated on the validation set.

		PET		PE		PP		PVC		PS	
	NER	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp
Test	1	1	1	1	1	1	1	1	1	1	1

In the case of the restricted data set composed of HDPE and LDPE spectra, the optimal number of LVs was set to six, assigning 90% of total variability (see Figure 10). With respect to the analysis of the polymer classes, the degree of not assigned samples as a function of the number of latent variables was equal to zero, meaning that all the samples were assigned to a class. After the determination of the optimal number of LVs, the calculation of the PLS-DA model was performed, selecting six latent variables and 10 cross-validation groups for internal validation. Both in fitting and in cross-validation, all the investigated samples were correctly designated (see Table 5 for the two confusion matrices achieved in fitting and in cross-validation)..

Table 5. Confusion matrices obtained from PLS-DA model, both in fitting and in cross-validation (based on venetian blinds with 10 groups) restricted to PE class



Experimental Class	Calculated Class		
	HDPE	LDPE	Not Assigned
Fitting	-	-	-
HDPE	125	0	0
LDPE	0	131	0
Cross-Validation	-	-	-
HDPE	125	0	0
LDPE	0	131	0

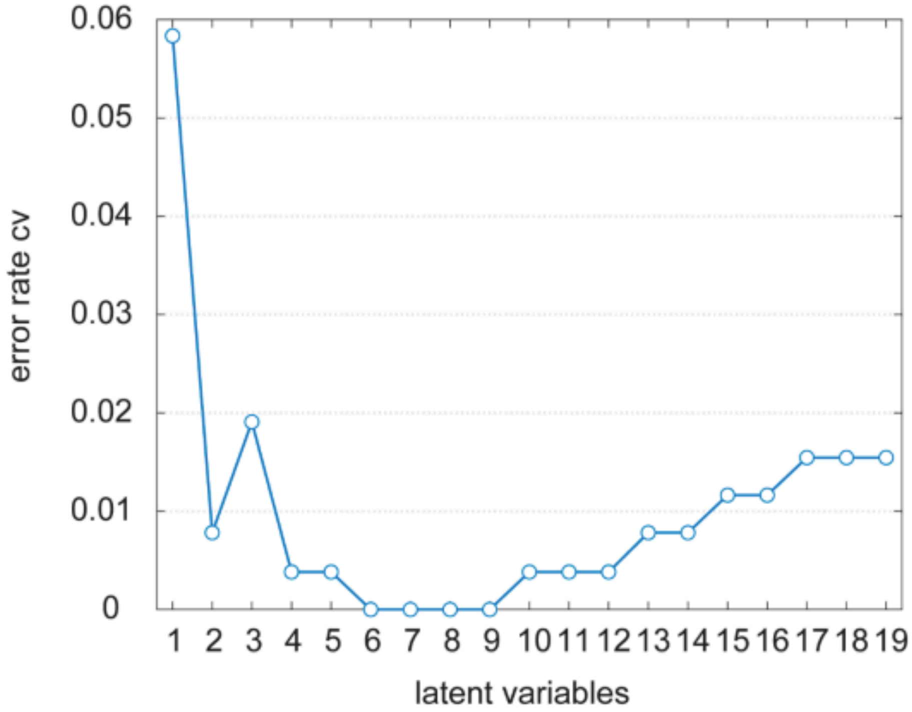


Figure 10: Error rate as a function of latent variables

4.5 Conclusion

In the present study, the use of a handheld NIR spectrometer combined with robust chemometrics tools to fingerprint different urban plastic waste collected directly from a recycling plant was presented. Plastic samples included clear, blue, coloured, opaque, and boxes of PET, PE with different branching degrees (HDPE and LDPE), PP, PVC, and PS. In a first step, PCA was applied to NIR spectra of all polymer classes, and polymers were correctly separated in clusters. Moreover, by limiting the analysis on PE samples, HDPE and LDPE were correctly discriminated.

In addition, by comparing samples of different colours, no differences in band positions were found, suggesting that colorants present in the plastic resin matrix do not significantly influence NIR spectra. Moreover, even if samples were not washed or treated prior to the analysis, NIR spectra were not affected by dust contamination or liquid residuals, meaning a successful application directly to the urban plastic waste.

After the exploratory PCA analysis, PLS-DA was applied. Before the model calibration, data were split into a training and test set, using the training set to calibrate the model. In both fitting and cross-validation, the performance of the model was very high, proved by the correlation between observed and predicted classes. All the analysed samples were also correctly designated in the case of the restricted data set composed of HDPE and LDPE spectra.

The results show promising outcomes in order to improve the reliability and efficiency of the manual sorting of plastic waste, increasing the volume of recycled plastic and the management of multi-component polymer types.

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CHAPTER 5: THE GROUNDWORK
ON PREPARATION OF TRUE-TO-LIFE MICROPLASTICS IN LABORATORY

5.1 Abstract

Microplastics are a pervasive and diverse contaminant with the potential to influence ecosystem characteristics and processes. MP research is critical for determining their potential effects on the environment and human health. The laboratory and field research on MPs necessitate the use of the smallest particles, which are frequently difficult to create and thus the advancement in this sector is dependent on the availability of engineered microplastics similar to those present in the marine environment. Plastic fragmentation is particularly tough because most plastic brands are strong and resistant to mechanical force. We investigated the three most common cryogenic grinding techniques, as well as the resulting products and the technology's advantages and limitations. Furthermore, the linkage between the different techniques employed and shape of microplastics obtained in this work is explored.

5.2 Introduction

Microplastic particles (MPs) are a significant hazard to the environment. Despite being spotted in 1970s, their study is still in the infant stage, be it the sampling, extraction or identification methods. The validation of analytical techniques for measuring microplastics (MP) is complicated by a widespread paucity of reference materials. To perform proper study, it is obvious that particles that mimic those present in the environment must be used. The validation of analytical methods for measuring microplastics (MP) is hampered by a general lack of reference materials; as a result, while the presence of MPs in marine environments has been well documented[1], a legitimate and authentic analytical technique to sample, analyse, and quantify MPs is long awaited. Plastic deteriorates when it ages in the environment, resulting in secondary MP fragmentation. Methods for detecting secondary MP must be established using relevant reference materials[2,3]. The vibrational spectra of the particles may not match standard databases if Raman or infrared (IR) spectroscopy is employed for examination, as they may include hydroxy, carbonyl, and carboxy groups in addition to the pure polymer spectrum, as a result of environmental ageing. Nevertheless, reported investigations on the effects of plastic litter on marine biota have nearly all used pristine[4] plastic materials that are uniform in polymer type, size, shape, and chemical makeup. Thus, the test materials should be such that they have the same features as environmental MP, such as a wide size range and a variety of forms (fragments, spheres, films, fibres) from a wide range of consumer products. However, former studies have only concentrated on producing MPs from only a specific method[5,6] and these research used distinct experimental setups, the results cannot be compared, and so no conclusions can be formed about how effects may differ. In the study here presented study, we have compared the effect of three fragmentations techniques, namely through, an Immersion Blender, an Ultracentrifugation Mill and a Ball Mill producing secondary microplastics as a step towards preparing more realistic reference materials, named here "true-to-life" materials and at the same time the samples can be used for future experimental work. The technique of grinding and milling plastics is considerably older, dating back to at least the 1970s. The true-to-life microplastics were imaged using optical microscope for their shape and surface morphology and XRD measurements were performed for analysing the effect of three methods on change of crystallinity. FTIR measurements were performed to study the change in the chemical structure, if any. According to optical microscopy study, fragments are the most common form for all polymers, however fibres and spheroids were also present.

As a result, we concluded that microplastics have different shapes depending on the type and the fragmentation method. Furthermore, existing literature[7,8] has shown that particles of various sizes and shapes accumulate at different levels in organisms, so even particles with the same composition and concentration can have varying toxicities. With this work, it will be easier to comprehend the morphologies of MPs produced from the same parent particle using various techniques in a short period of time, allowing MP research to be accelerated. Our study has focused on the varying shaped achieved with the three grinding techniques.

5.3 Materials and Methods

5.3.1 Base Material

The work has been focused on the production of MPs starting from daily used disposable plastic items representative of the polymers find in environment: a blue polystyrene plate (PS, specific gravity 1.04-1.1 g/cm^3), a green polyethylene shampoo bottle (HD-PE; specific gravity 0.94-0.98 g/cm^3) and a transparent polypropylene cup (PP; specific gravity 0.83-0.92 g/cm^3) and a milky translucent sheet (LDPE). The samples were chosen that could be fragmented mechanically in variety of ways (flexible LDPE film, Solid HDPE Springing PP, Britttle PS). They have a short lifetime and a high contribution to the content of the global plastic waste (56%). Recent meta-analysis also identified PE, PP and PS as the most abundant polymers in the marine environment, although their distribution in the open ocean and especially its interior was poorly constrained. Since the density, surface chemistry, degree of crystallinity, and presence of chemical additives and plasticizers all impact the fate of MP in both the test system and the environment, it's critical to give as much information as possible about the polymer composition. Taking into account this aspect, both high density and low density polyethylene's were fragmented.

5.3.2 Fragmentation techniques

With the goal of producing secondary true-to-life MPs, we picked mechanical fragmentation as one of the ways available to imitate the mechanical action of waves or collisions with other objects that can occur in seas. By this process it is possible to produce materials with different dimensions and shapes, reflecting the heterogeneity shown by the environmental particles. The fragmentation was performed using three different techniques: a food processor (immersion blender), ultracentrifuge milling and ball milling.

Figure 1 shows the schematic representation of the three techniques.

Step 1

The first step was same for all the three techniques: removing the labels (Figure 1, Step 1), washing the samples with Milli-Q water (Millipore Direct-Q™ 5) (Figure 1, step 1), drying and cutting them into small pieces using a pair of steel scissors (Figure 1, step 2). And freeze drying the samples for 30 minutes in Liquid Nitrogen (LN_2). In general, embrittlement with LN_2 improves the grinding process by counteracting the frictional heat effects.

The subsequent steps are described for each technique as follows.

Step 2

Manual Fragmentation

After the samples were kept in LN_2 for half hour, size reduction was achieved by pulsing the Immersion Blender for 5 minutes with intervals of 30 seconds to avoid thermally altering the plastics at maximum speed. The fragmented plastic was sieved through 500-µm mesh sieve (Sieve from Fisher Scientific, UK) to get the desired MPs fraction.

Ultracentrifugation Milling (UC Mill)

The cut up samples were embrittled in liquid nitrogen for 30 minutes, then transferred into an ultracentrifugal mill (Retsch ZM 200 with a combined cyclone) previously cooled with liquid nitrogen and blended under a continuous flow of liquid nitrogen (LN_2) until completely fragmented. The uninterrupted cooling was necessary to avoid overheating the rotor and consequently damage the polymeric particles. The mill was equipped with a 500 um filter, so the sample underwent a fractionation step. The grinding was done at 18000 rpm The plastic powder produced was collected and stored glass bottles. The pre-cooled plastic samples were spoon-filled into the grinding chamber with liquid nitrogen.

Ball Milling

The samples were kept in LN_2 for 30 minutes and dried before transferring into stainless-steel grinding jar with 50 ml volume and 4 stainless-steel balls (25 mm diameter) at different frequencies for different samples. The steel balls were also immersed in liquid nitrogen for about 10 min. After filling the sample vial with the cut up plastic, the sample jars were kept in LN_2 for 15 minutes as it is worth noting that the temperature of the vial increases during the milling process. The milled plastics were then sieved through a 500 um sieve and stored in a falcon tube. All milling runs in the ball mill were performed with 2 g plastic samples.

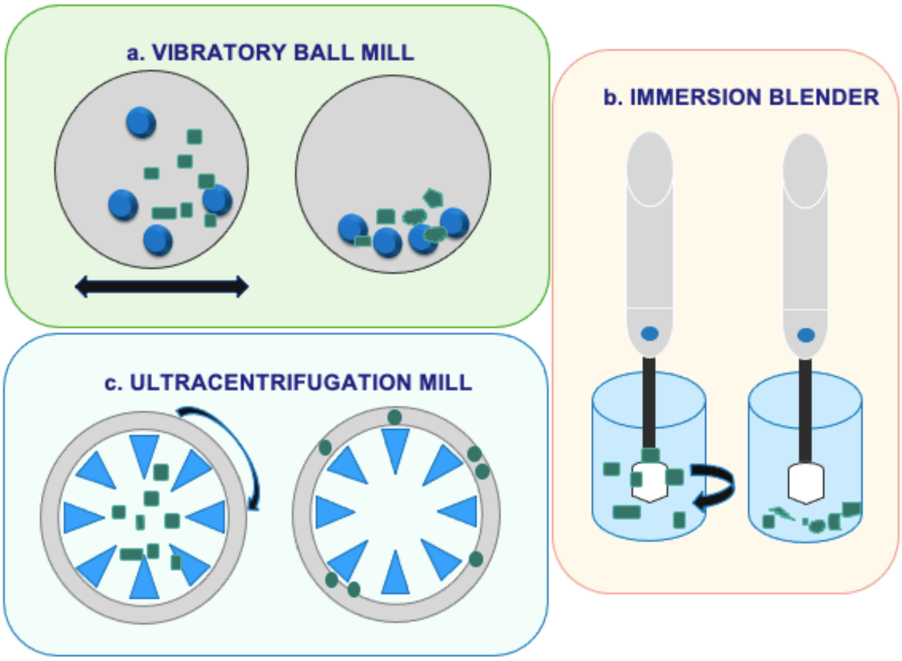


Figure 1: The schematic representation of types of milling techniques

5.3.3 Characterisation Methods

Optical Analysis

A stereo microscope equipped with a HD Digital Camera (MZ16-A, Leica Microsystems) was used to record optical images of the MPs fragmented with all three techniques.

XRD Analysis

XRD analysis were performed by PANalytical X’Pert Pro diffractometer (PANalytical, Almelo, The Netherlands) equipped with an X’Celerator detector and Cu anode (CuKα = 0.15406 nm), operating at 40 kV and 40 mA.

FTIR Analysis

Infrared spectra were recorded for all samples in ATR mode using a Nicolet iN10 MX FT-IR spectrometer (Thermo Scientific, USA). Spectra were recorded in the range 650–4000 cm^{-1} with a resolution of 4 cm^{-1}. Background spectra were collected before every sample, and all spectra were corrected by subtraction of atmospheric H_2O and CO_2 signals. Acquisitions were performed with the OPUS software (Version 6.0, Bruker Optics, Germany).

5.4 Results and Discussions

The basis of empirical scientific knowledge is controlled variation as it enables for precise control of decision-making conditions and in case of polymers, laboratory experiments are a prominent approach for expanding knowledge on their fragmentation methods. This is because the fragmentation process and its consequences are impacted by a variety of components and external environmental influences, whereas laboratory circumstances allow for more focused research. It is impossible to assess the correctness of the procedures used in the absence of certificate reference materials. The major purpose of this study is to study the effects of different macroplastic fragmentation processes, which are most routinely employed in laboratories to create microplastics for relevant test materials. Microplastics are normally produced when some plastics are exposed to the environment and degraded. We used a three different milling methods to prepare microplastics in a short time to mimic the physical degradation of the environment without the use of any toxic substances.

This study is one of its kind to compare the three mechanical fragmentation methods. Only new polymers were used in the studies to ensure that the results were comparable. Influence of plastic weathering, degree of crystallinity and structural changes were studied. We believe that reporting the qualitative aspects of the behaviour of the plastics/products chosen for this inquiry is crucial. This may facilitate the application of the obtained results to other polymers/objects with comparable (e.g., brittle, plastic, elastic) qualities, as well as demonstrate how (and how rapidly) the features of distinct polymers change with fragmentation method. All the three procedures acted differently on different kinds plastics.

5.4.1 Preparation of Microplastics

Cryogenic grinding is a technique for comminuting brittle materials that takes advantage of their brittleness at low temperatures. Cooling the material below the glass state temperature is simple because liquid nitrogen has a boiling point of 77K. Furthermore, because liquid nitrogen is an inert gas, it can prevent materials from oxidizing during high-speed comminution. The mechanical reaction of polymers becomes increasingly rigid, stiff, and brittle when they are cooled from ambient temperature to cryogenic temperatures. In all the three fragmentation methods liquid nitrogen was used.

There are two types of cryogenic grinding processes for polymeric materials using liquid nitrogen: one way is to instantly freeze the plastic sample to the glass state temperature with liquid nitrogen. Another technique is to crush the polymer to a specified size at room temperature and then freeze it. When comparing the two approaches, the second is more cost-effective and energy-efficient.

Different plastic items (flexible LDPE film, Solid HDPE, Springing PP, Brittle PS) commonly used on daily basis were used as starting material for preparing the microplastics. The time required for each sample to fragment through each procedure is described in the table below:

Table 1:
Process variable of the different fragmentation methods used.

S.No.	Sample	HAND BLENDER		UC MILL		BALL MILL		
		Rep*	Time	Rep*	Time	Rep*	Time	Frequency
1	LDPE	5	5 min	1	3 min	6	5 min	30 Hz
2	HDPE	2	5 min	1	3 min	5	5 min	30 Hz
3	PP	2	5 min	1	3 min	2	5 min	30 Hz
4	PS	2	5 min	1	3 min	2	5 min	30 Hz

***Repetitions required to complete the fragmentation procedure with use of LN_2 & just enough sample for characterisation purposes.**

While all plastics fragmented with different time requirements, the breakdown of plastic depended on the type of plastic. Out of all the plastics fragmented, Polystyrene was the easiest sample to fragment with all the three methods and required the least amount of mechanical stress to shed it into microplastic pieces. This observation is also consistent with the findings reported[9] in the literature. The easy fragmentation of PS can also be explained on the basis of the elongation percentage (Table 2). If a polymer's elongation decreases, the rate of rupture and fragmentation increases under external stress. PS is a hard plastic with lowest elongation % among the polymers used here. PP, LDPE and HDPE have high elongation % and low Young's modulus due their structure and branching and thus were comparatively difficult to fragment. The most difficult polymer to work with each kind of method were the LDPE samples, being flexible and elastic. It was expected as the crystallinity level in LDPE is poor because chain branching (both long and short) disrupts polymer chain crystallization and thus poor embrittlement in at low temperature. Thus the PE class needed to be milled more than the other plastics used in this work. The LDPE-material of the samples became exceedingly soft, virtually inelastic ,wrinkled, and fatigued toward the end of grindings

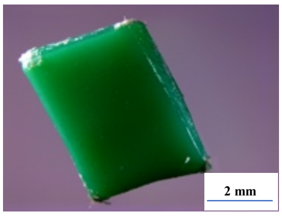
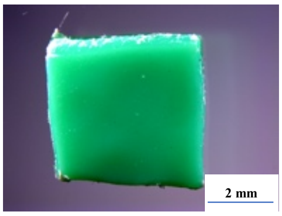
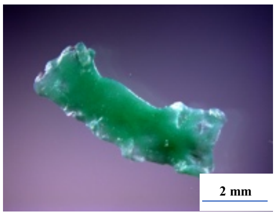
Table 2: Elongation %age and Young's Modulus of PS, HDPE, LDPE and PP

S.No.	Sample	Youngs Modulus	Elongation	Conclusion
1	Solid PS	3250 MPa	1-4%	PS with lowest elongation has highest fragmentation rate.
2	Flexible LDPE	300 MPa	200-600%	Due to the lower crystallinity, the Young's Modulus is also lower and thus high elongation. This makes LDPE very ductile, even at low temperatures.
3	Hard HDPE	1000 MPa	500-700%	Long chain or short chain branching decreases the crystallinity and leads to a lower Young's Modulus , higher ductility, and lower density. HDPE has a low degree of branching and a high crystallinity, leading to the highest density among the PE types and a relatively high Young's Modulus.
4	Springing PP	1325 MPa	200-500%	PP has higher Young's Modulus and intermediate Elongation %, It was easier to fragment than PE class but difficult than PS sample.

The % elongation is a property of the material, which provides a value of its ductility. Lower the elongation %, easier is to fragment the plastic. Young's modulus is a measure of the stiffness of an elastic material. Higher the Young's modulus, higher is the brittleness of a polymer, thus easier to fragment.

The other aspect of this study was to assess the difference in the fragmentation methods in terms of time requirement for each method. It was indeed easier to fragment the plastics using UC mill followed by Immersion Blender and Ball Mill, respectively. For each type of sample, ball milling required more than two repetitions (Table 1), and there is a clear change in morphology with longer milling times when ball milling the material. As an example, the five stages of HDPE milled through Ball mill are shown in Figure 2. Except for LDPE, 4 balls were utilized to grind the samples in ball milling, but 6 balls were required for LDPE crushing. Increasing the number of balls also increased the number of collision events and just enough sample was obtained to suffice for the characterisation purposes

It's worth noting that in the case of the Immersion Blender and Ball Mill, a considerable portion of partially comminuted pellets remained at the end of the experiment and had to be sieved out. These data show that particles fractionate irregularly due to the crude processing approach, resulting in the creation of particles of myriad of shape and morphology described in the next section.

Stages of Ball Milling : HDPE			
Cycle No.	1	2	3
Time lapsed	5 min	10 min	15 min
Frequency	30 Hz	30 Hz	30 Hz

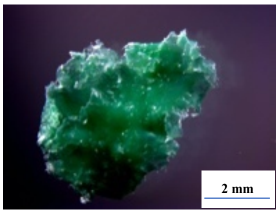
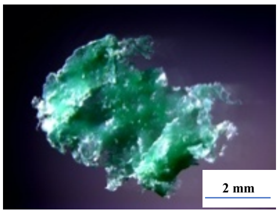
Stages of Ball Milling : HDPE		
Cycle No.	4	5
Time lapsed	20 min	25 min
Frequency	30 Hz	30 Hz

Figure 2: The process of fragmentation of HDPE through ball milling, mimicking[10] the physical degradation of polymers in the environment.

5.4.2 Optical Analysis

The most visible distinction between primary and secondary MPs is their shape. They are generally categorized as fragments, fibres, films, foam, and beads. These various forms are relevant to material science and can be utilized to investigate interactions with other components, particle mobilities, and degradation processes and to build adequate morphological characterisation methods, and particles of various forms (fragments, spheres, films, fibres) must be provided, ideally in the same sample, as a true sample would present all morphologies simultaneously.

Examining small particles under a microscope is the first step towards characterizing them. When compared to particle size, the impact of particle shape has received far less attention[11] . The shape factors are closely related to their impact on the environmental matrix and organisms feeding on them[12,13,14]. The form of plastic pieces is influenced by the fragmentation process as well as the length of time they spend in the environment:

Apart from size, milling also alters the surface roughness and shape of particles. Surface roughness also influences deposit development; flat surfaces do not allow growth to adhere as easily as textured surfaces[15]. There are numerous factors that influence biofouling, but one of the most important is the material's surface characteristics.

5.4.2.1 Shape of Microplastics

All the microplastics tried to remain close to the shape of their parent material but a noticeable sphericity in UC milled particles is seen. A microbead like structure of HDPE and PS are noticeable whereas a fibrous structure of UC milled PP particles is evident (Figure 4c-7c). UC milled LDPE remained in the shape of its parent particle. The UC mill reduces the residence time of the material in the milling unit, and therefore reduces the chances of degradation. The material is fractionated by striking on the rotor blades, and the particles are crushed further between the rotor and the sieve in the milling unit. This extra shearing effect is thought[16] to be the cause of the particles rounding off in the UC mill. As a result, few particles have a fairly spherical morphology. Because PS is brittle, not all particles are formed in this manner; some tend to fall apart, resulting in the inconsistencies seen in Figures 4. The rationale for the fibrous shape of PP is not known yet, which is a matter of further investigation.

5.4.2.2 Effect of Residence Time: Surface Morphology

There is a significant difference in the surface morphology of the samples fragmented through all the three techniques as evident from the microscopic images of mechanically milled particles from the same parent particle. Each sample type's representative images are shown below. Investigating the effect of UC milling on all the samples milled, all the four samples presented a comparatively smoother surface, followed by immersion blender fragmentation and ball milling resulted in pockmarked surface in all the four samples as seen in Figure 3-6. This can be explained on the basis of residence time of the particles in each setup. Higher is the residence time of sample in the mill, higher is the degradation caused. The samples spent the maximum time inside ball mill, resulting in maximum disintegration of the samples while UC mill required minimum time, accounting for the smooth surface of the MPs (Figure), while hand blender method produced MPs with surface texture with surface texture that fell somewhere between UC and Rotor Mill. Smooth edges are typically associated with older pieces that have been continuously polished by other particles or sediment, whereas sharp edges are frequently associated with newer fragments that have been continuously polished by other particles or silt. Thus, to prepare MPs as close as to the environmental MPs in a short time, these techniques are useful.

The efficiency of ball milling with hard little spheres can be compared to what can happen on seashores, where hard sand grains impact meso- and microplastics at low speeds but for incomparably longer periods of time[17]. In the ball milling process, the breakage occurs when the elastic stress of the particle reaches a critical level and further the fractionation is mediated by the production of incipient cracks as a result of high local stress. Cracks induced by ball milling process could be the reason for increased porosity in the sample[18]. These pronounced cavities explains the affinity of marine plastics for different microbes and their tendency for biofouling[19]. From a scientific standpoint, it could also be interesting to create samples with regulated porosity and this could be potentially used to study the adsorption of pollutants on microplastic particles.

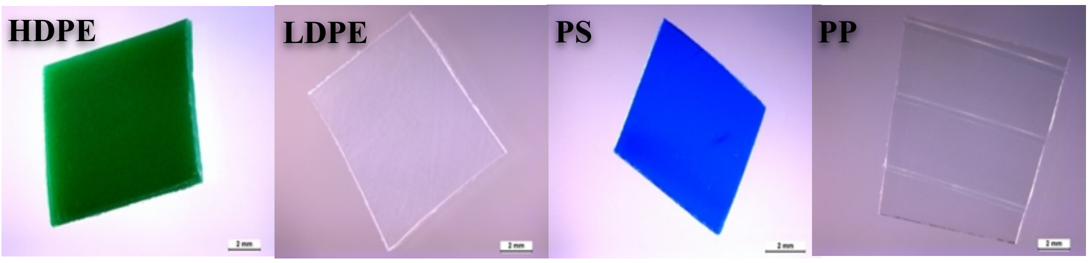


Figure 3: Raw Plastic samples

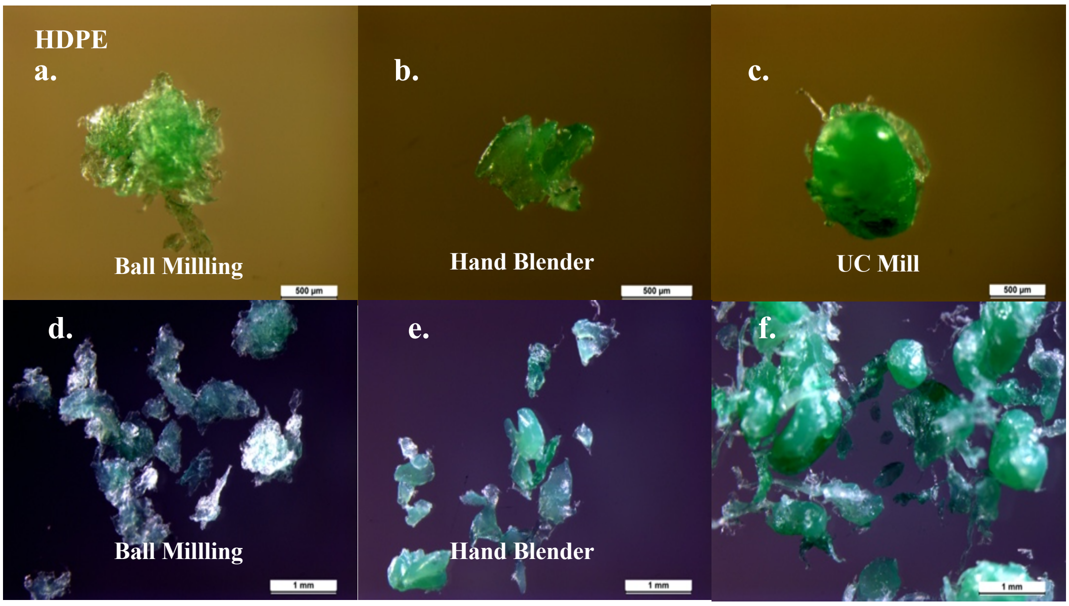


Figure 4: HDPE plastic samples after a) Ball Milling b)Immersion Blender c)UC milling; a-c: 500 um ; d-f: 1 mm.

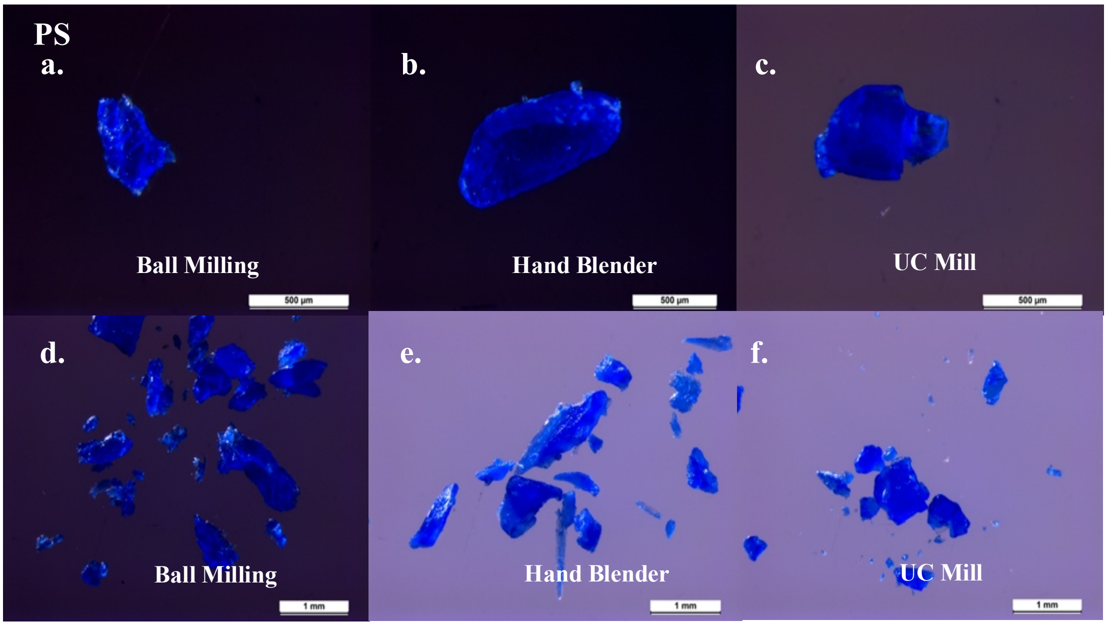


Figure 5: PS plastic samples after a) Ball Milling b)Immersion Blender c)UC milling a-c: 500 um ; d-f: 1 mm.

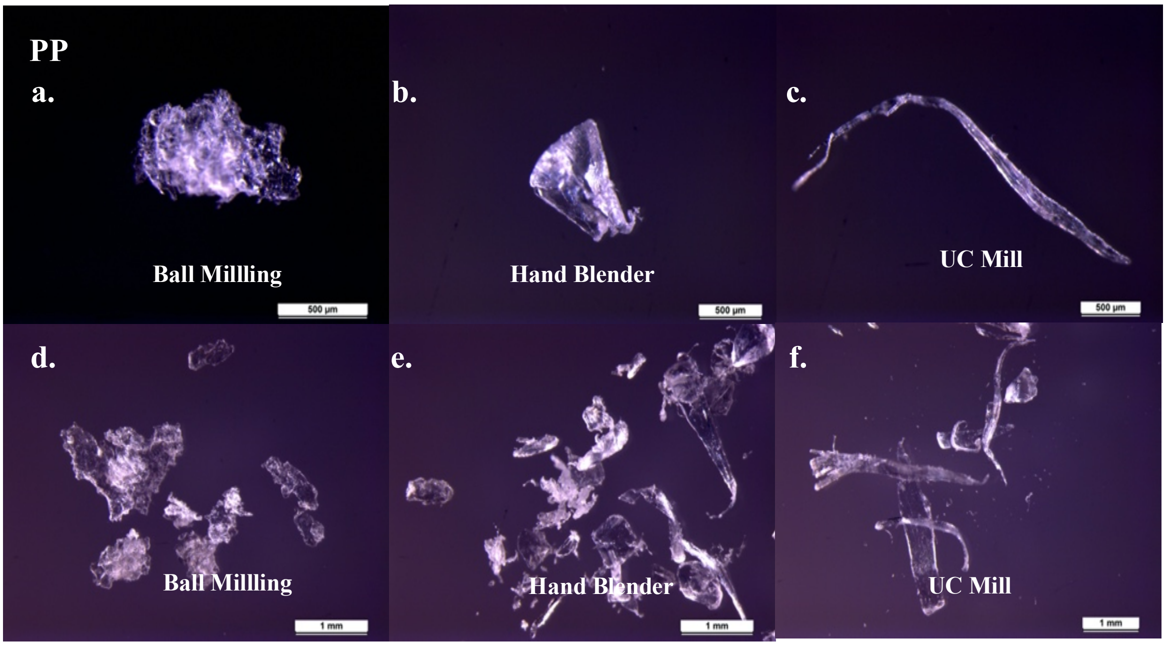


Figure 6: PP plastic samples after a) Ball Milling b)Immersion Blender c)UC milling a-c: 500 um ; d-f: 1 mm.

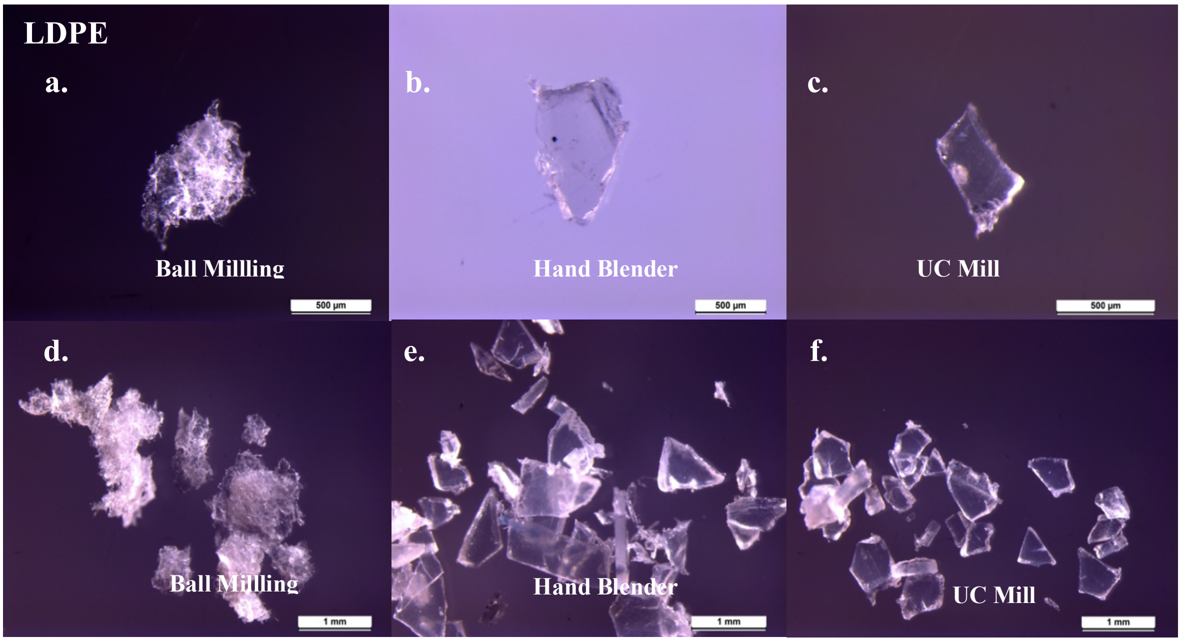


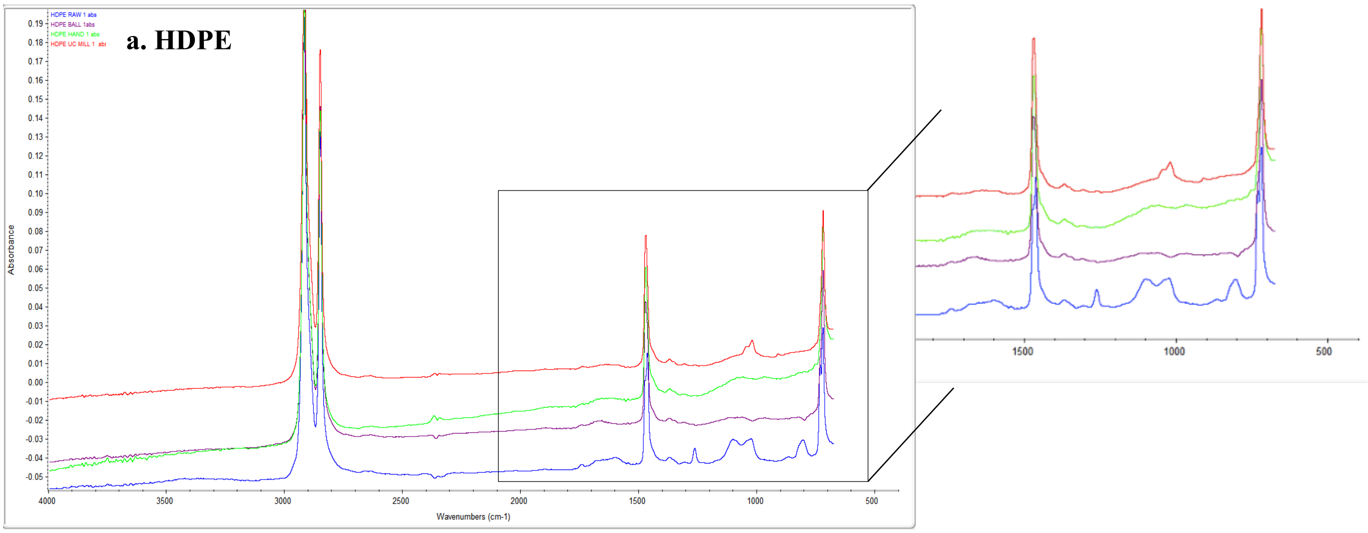
Figure 7: LDPE plastic samples after a) Ball Milling b)Immersion Blender c)UC milling a-c: 500 um ; d-f: 1 mm.

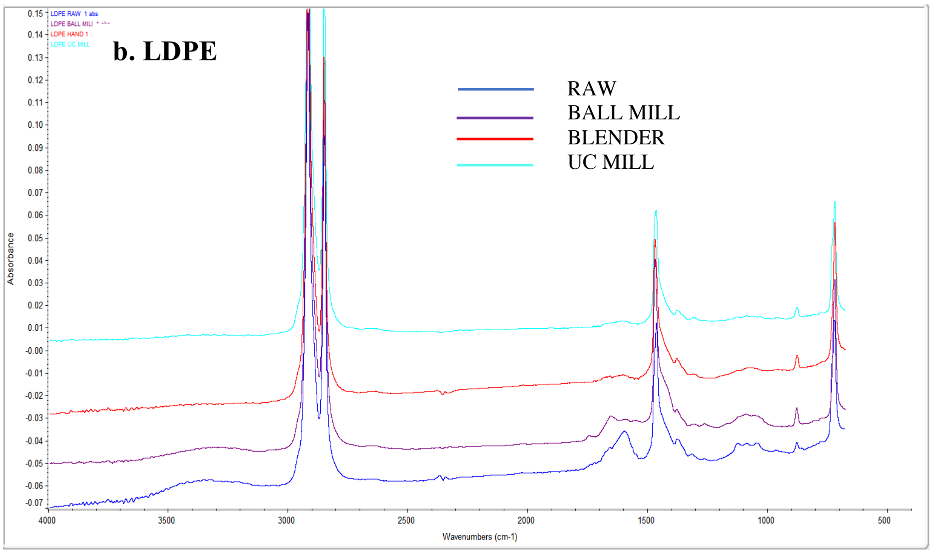
5.4.3 FTIR Analysis

ATR-FTIR was used to determine whether the MPs produced from a parent plastic showed any chemical changes. ATR-FTIR spectra obtained for all three milling methods for each sample are shown below (Figure 8).

All material types, excluding HDPE, had consistent key ATR-FTIR peak locations from the original polymer pellets to the final milled plastic particle using all three processes, suggesting that milling procedure here had no effect on the composition of the MPs obtained. However increased residence time (24hrs) or milling at room temperature may lead to depolymerisation of some polymer chains or decrease in the crystallinity[20].

In the case of HDPE, disappearance of peaks at 1098 cm^{-1} and 1023cm^{-1} in the milled samples is seen (Figure 8a). These peaks are due to C-O stretch and in the molecular structure of HDPE the repeating unit -(CH2-CH2)n- , has no C-O group, thus these peaks are due to the additives present in the parent HDPE sample as virgin HDPE pellets were not used. During all the grinding processes, the additive must have been eliminated, accounting for the disappearance of the peaks at 1098 cm^{-1} and 1023cm^{-1}. This effect was more pronounced in the ball milling and hand blender spectra of raw HDPE due to their increased residence time inside the milling jar, unlike in the UC mill.





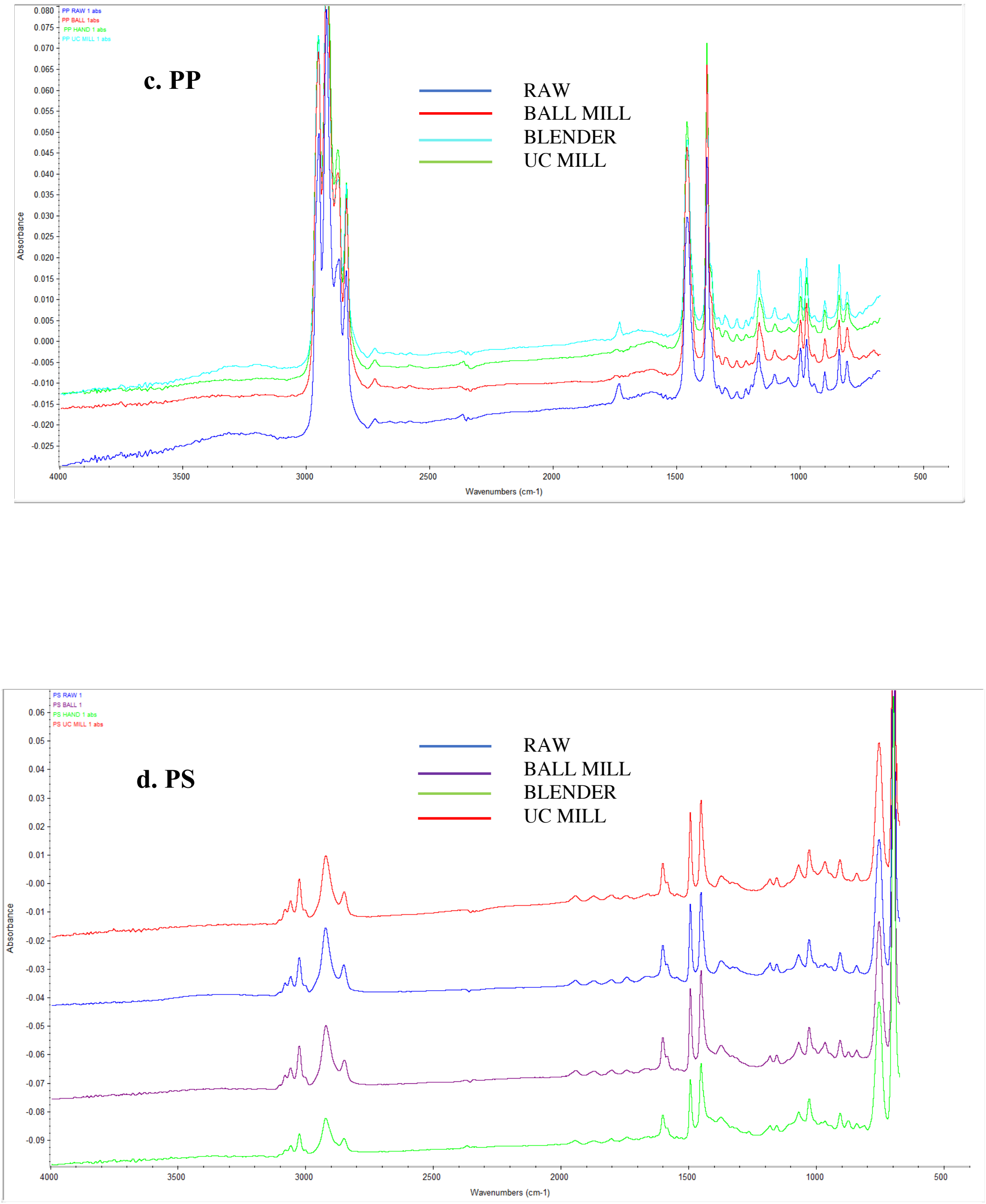


Figure 8: FTIR spectra of a) HDPE; b) LDPE; c) PP; d) PS.

5.4.4 XRD Analysis

The results of the XRD analysis are discussed below:

Material	**Sample**	**Treatment**
Polypropylene	PP-Macro	-
	PP-I	Grinding with immersion blender
	PP-M	Cryo-grinding with ultracentrifugal mill

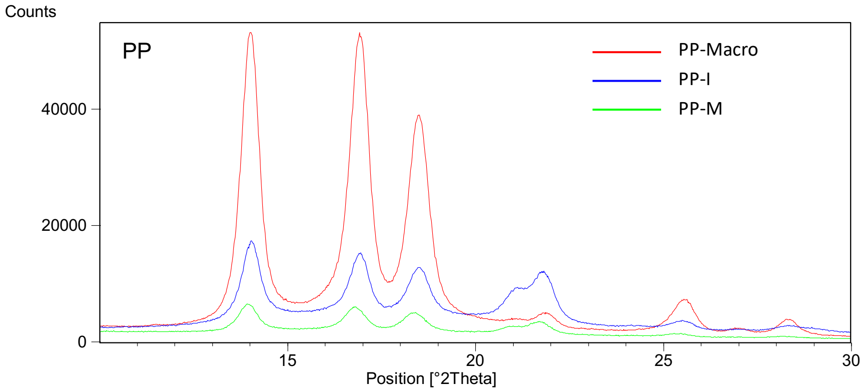


Figure 9 : XRD spectrum of the PS-Macro sample and of the PP-I and PP-M ground samples

The spectra show a difference in the relative intensities of the peaks in the three samples.
In the PP-Macro sample the first three peaks have a much greater intensity than the others due to a preferential orientation confirmed by the 2D image of figure 9, where it is noted that the Debye rings show non-uniform and marked intensities only along some directions .
With the grinding, the fragments of the material arranged randomly in the sample holder made it possible to obtain a 2D spectrum (figure 10) with more homogeneous intensities, which are reflected in the spectrum of figure 1 with a variation of the relative intensities with respect to the PP-Macro sample.
The width (identified by the FWHM value) and the position of the peaks remain almost unchanged, indicating that there is no evident structural modification of the material.
Despite the presence of high intensity peaks, the presence of an amorphous component is visible.

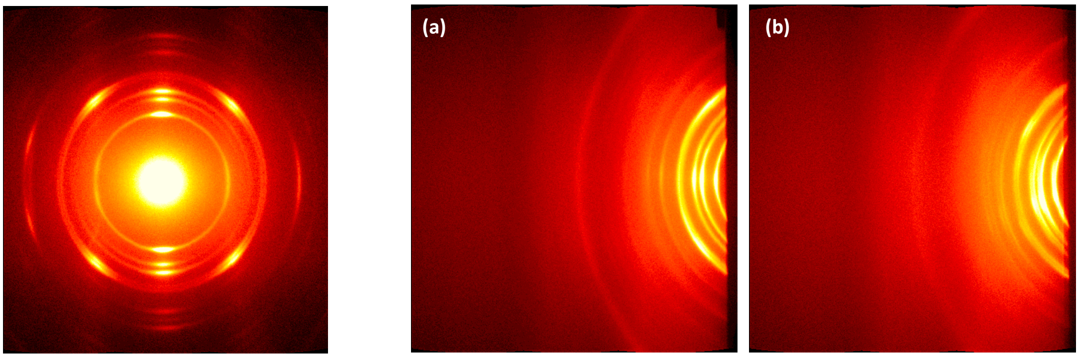


Figure 10 : Two-dimensional spectrum obtained with μXRD analysis in transmission of the PP-Macro sample with (a) PP-I and (b) PP-M.

Material	Sample	Treatment
Polystyrene	PS-Macro	-
	PS-I	Grinding with immersion blender
	PS-M	Cryo-grinding with ultracentrifugal mill

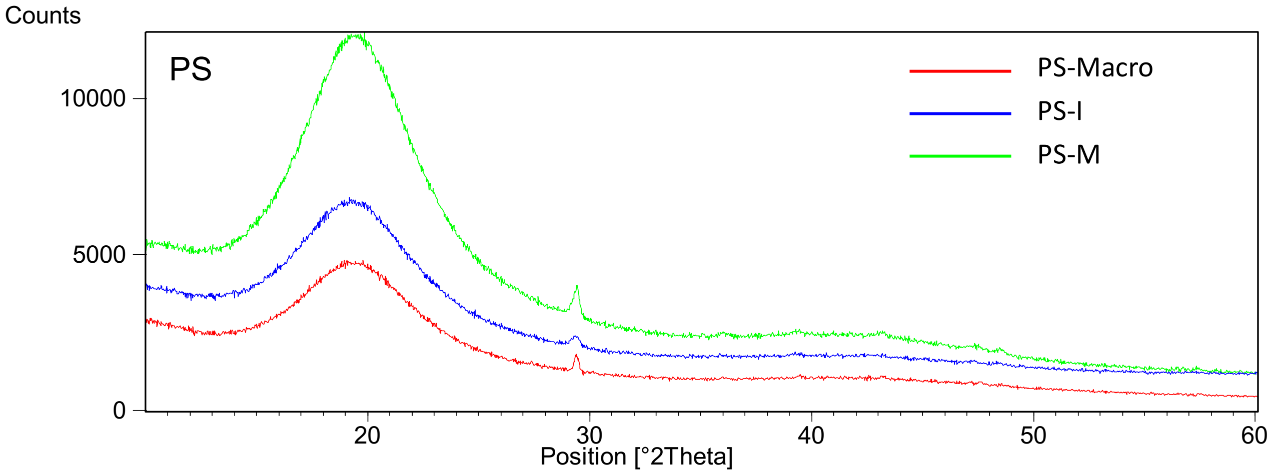


Figure11 : XRD spectrum of the PS-Macro sample and of the PS-I and PS-M ground samples

The structure of the material is completely amorphous in all three samples. We distinguish the presence of a single peak at 29.5 ° 2θ. From the images of the two-dimensional spectra it can be seen that the ring associated with the peak is not continuous but formed by spots uniformly distributed along the ring itself. The peak could be associated with the calcite used in the material as an additive in small percentages, such as not to allow the formation of a continuous ring.

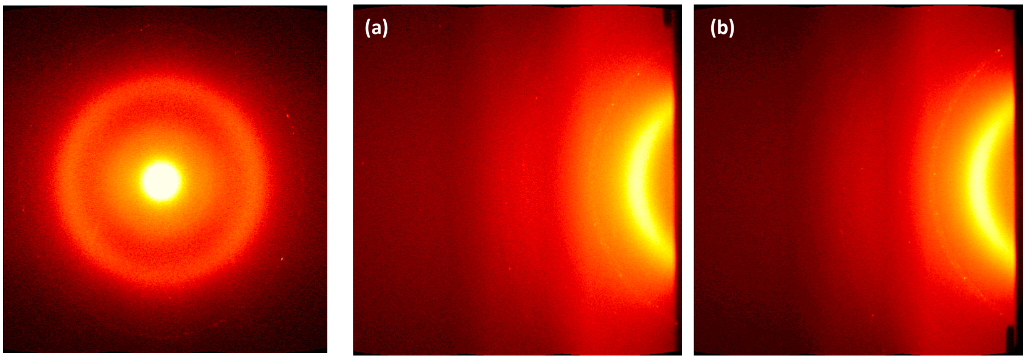


Figure12 : Two-dimensional spectrum obtained with μXRD analysis in transmission of the PS-Macro sample with (a) PS-I and (b) PS-M.

Material	Sample	Treatment
Low density polyethylene	LDPE-Macro	-
	LDPE-I	Grinding with immersion blender
	LDPE-M	Cryo-grinding with ultracentrifugal mill

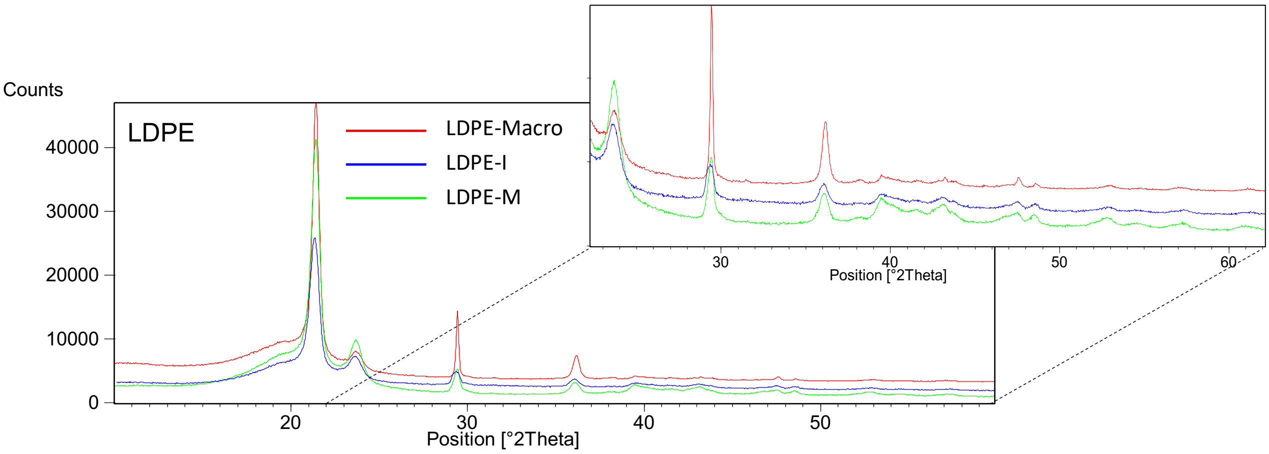


Figure13: XRD spectrum of LDPE-Macro sample and LDPE-I and LDPE-M ground samples

Observing Figure 13, the same peaks are identified in the three different samples, which also approximately maintain the relative intensities. The LDPE-M sample shows an increase in intensity of the peak at 21.4 ° 2θ, very pronounced also in image (b) of figure 14, plausible with a possible non-uniformity of crushing in the different crystallographic directions. As in the PS material, also in this material we note the presence of calcium carbonate, characterized by a main peak of very high intensity at about 29.5 ° 2θ and by a Debye ring formed by uniformly distributed spots. Probably in this case the percentage of the additive is higher.
From the evaluation of the amplitude of the two main peaks at 21.4 and 23.7 ° 2θ there is a slight increase in the ground samples, probably due to small distortions of the lattice during the crushing process. There is also an amorphous phase in the material.

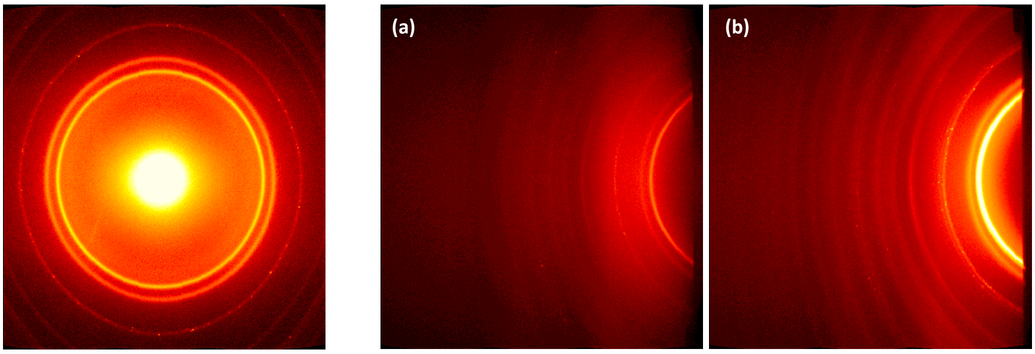


Figure14 : Two-dimensional spectrum obtained with μXRD analysis in transmission of the LDPE-Macro sample with (a) LDPE-I and (b) LDPE-M.

Material	Sample	Treatment
High density polyethylene	HDPE-Macro	-
	HDPE-I	Grinding with immersion blender
	HDPE-M	Cryo-grinding with ultracentrifugal mill

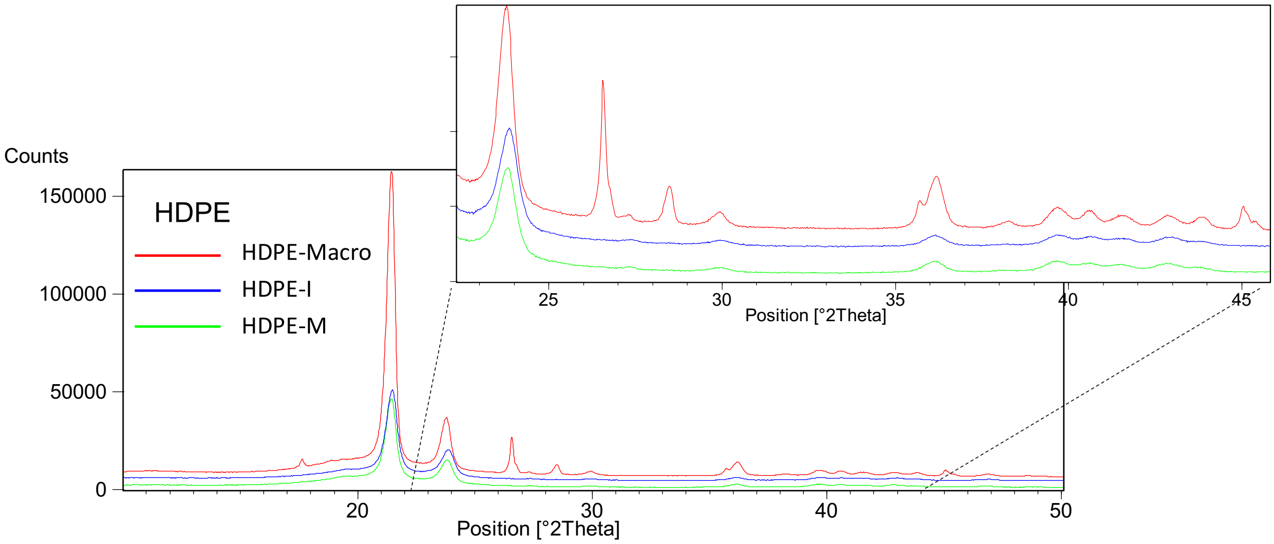


Figure 15: XRD spectrum of HDPE-Macro sample and HDPE-I and HDPE-M ground samples

In this case, unlike other materials, the disappearance of some peaks in the ground samples is evident. The peaks not present in the ground samples could be associated with a crystalline phase of an additive which in the crushing phase came out of the matrix and then eliminated by sieving.
Also visible in the spectra is the presence of an amorphous phase.

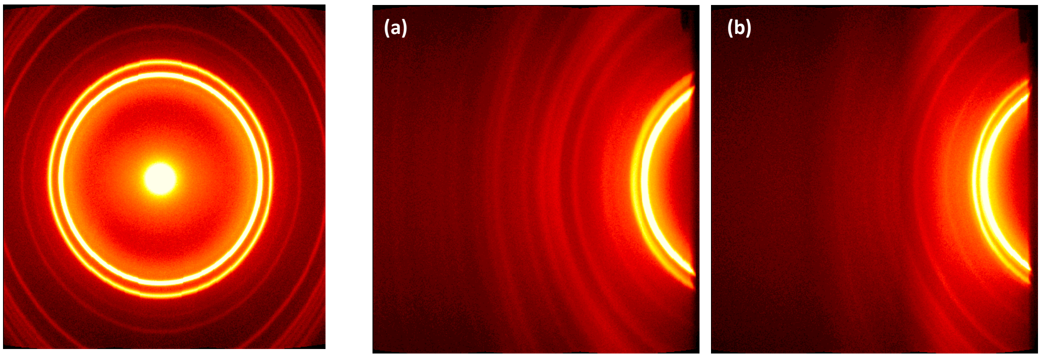
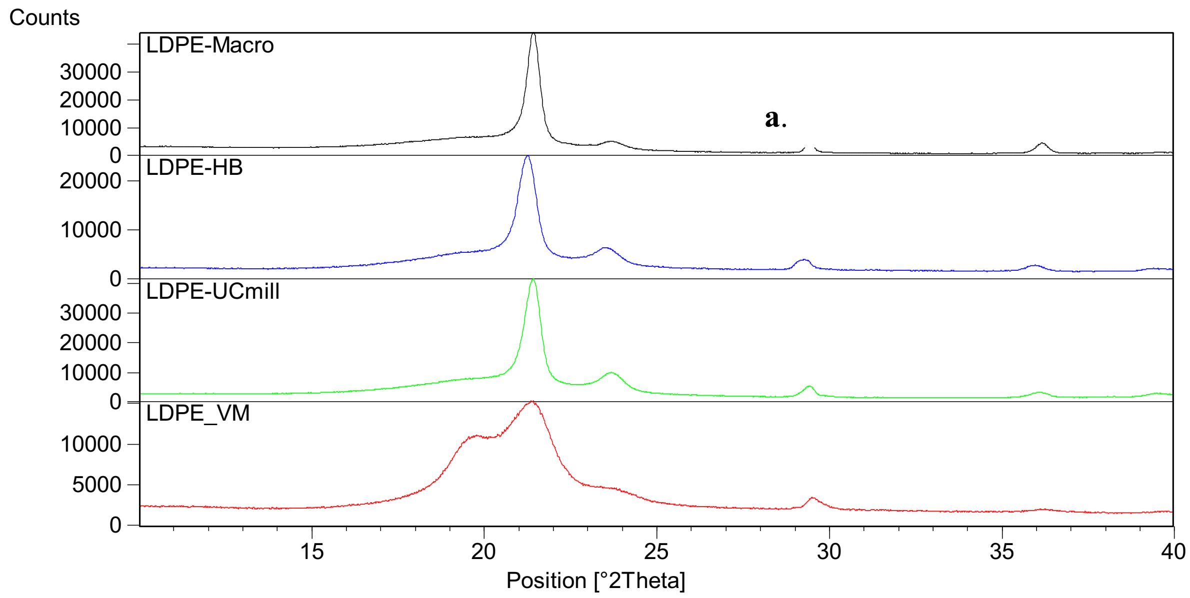
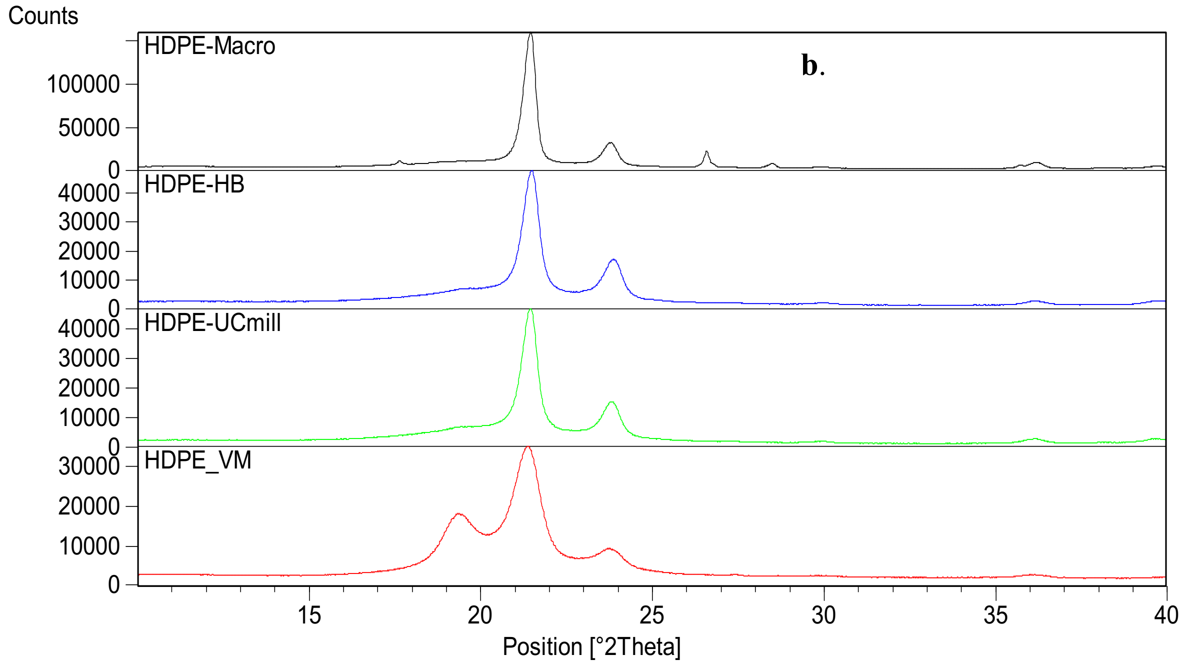


Figure 16: Two-dimensional spectrum obtained with μXRD analysis in transmission of the HDPE-Macro sample with (a) HDPE-I and (b) HDPE-M.

To conclude the characterization of the ground samples with the different techniques, XRD measurements were carried out on the plastic samples treated with the Restch MM400 vibro-mill.

The following figures 17(a-d) show the spectra (-VM) of the new measurements compared with the spectra of the original samples (Macro) and treated with other grinding techniques (HB - Hand Blended, UCmill - UltraCentrifuge mill).





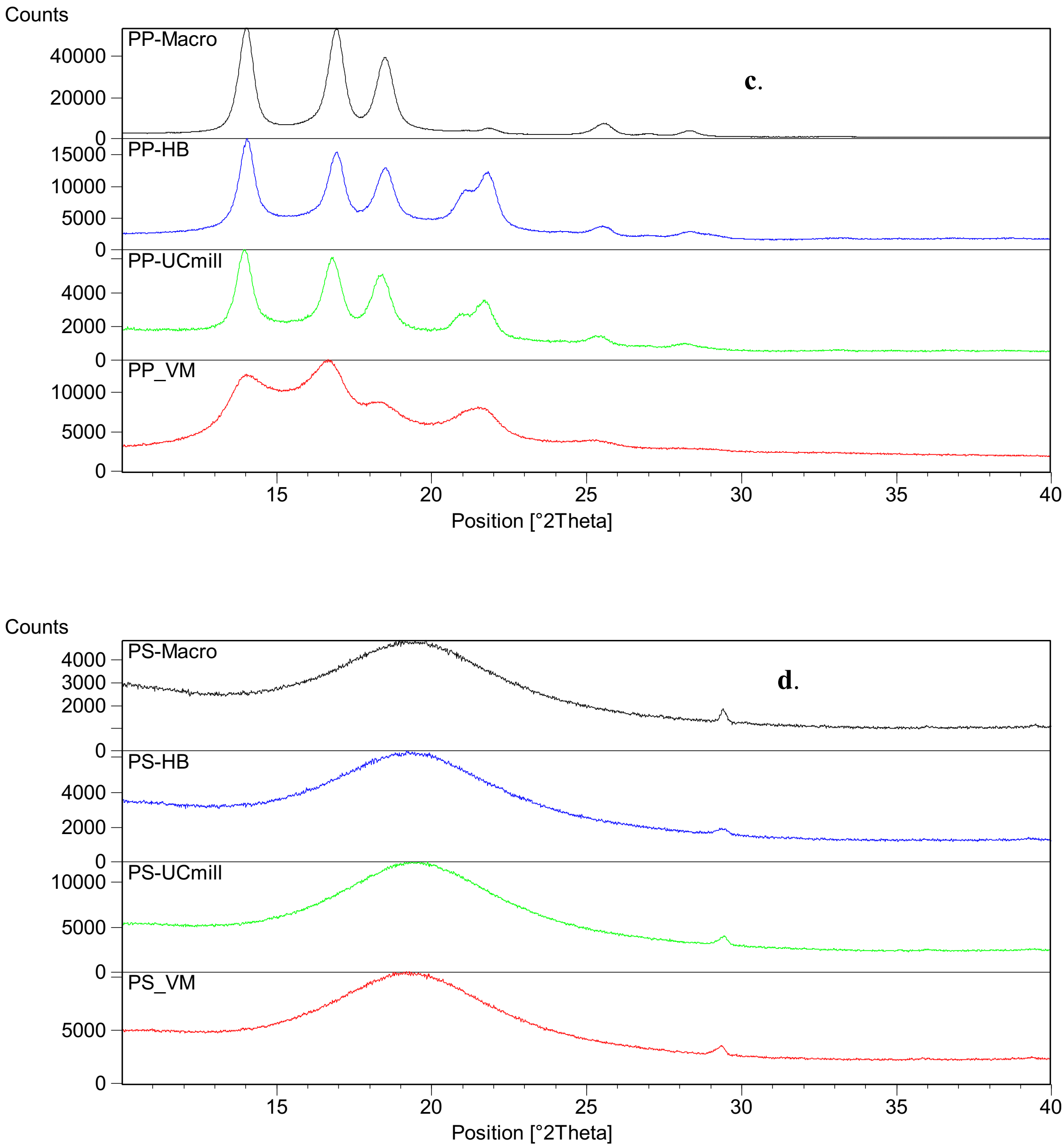


Figure 17 (a-d): Comparison of XRD Spectra of Parent sample and MP Samples fragmented through UC Milling, Vibrational Milling(VM) and Hand Blender Method.

5.5 Conclusion

The use of macroplastic litter to produce more environmentally relevant microplastic reference materials appears to be a vital step toward reducing the current gap between experimental lab investigations and observations made in the natural environment[21]. We studied how plastics can change the shape when they are milled through different methods, namely using an Immersion Blender, an Ultracentrifugation Mill and a Ball Mill under cryogenic conditions. We analysed the shape of the plastic through optical imaging and changes in structure and crystallinity through FTIR and XRD measurements, respectively. However the authors have not studied the influence of working parameters on the size evolution. Following observations were made:

1. The efficiency of a process is significantly influenced by the type of plastic used and the grinding/cooling intervals. For optimal performance, each plastic requires its own customized procedures and operating conditions.
2. No change in the composition of the milled MPs was seen, except in the case of HDPE, where FTIR and XRD peaks present in parent sample were eliminated in the milled sample, attributed to the loss of additive present in HDPE and plastics that have been exposed to the environment have been shown to have changed crystallinity as a result of polymer breakdown[22,23]. Thus, MPs produced through these grinding methods are equivalent to the environmentally weathered plastics.
3. The research requirements define the use of the method for producing MPs. For producing MPs with smooth texture, UC mill is the most efficient process to employ, followed by the use of Immersion Blender. Ball milling, on the other hand, can be useful for studying the degradation process by changing the oscillation frequency and time, as proven by the case of HDPE fragmentation.
4. Furthermore, we infer that the shape of milled plastic varies depending on the type of plastic and the technique used. UC mill is capable of producing fibrous and microbeads like shape. Ball milling displayed an exceptional reduction in crystallinity, suggesting severe degradation
5. The production of a homogenous microplastic mixture is conceivable through the three techniques, according to the comparison of different methodologies used, which is a first step toward reducing the gap between laboratory investigations with pristine materials and realistic scenarios with worn microplastic.

It should be noted that the only one sample per class was studied. It is out of scope of this work to study multiple samples per class for more which is a topic of further research. The microplastics employed in our study were not directly exposed to the environment, thus microorganisms, contaminants, and so on were not present but it should be noted that microplastic collection from the marine environment in substantial numbers for use in laboratory investigations is not possible, as it would require extraction and cleaning techniques that could alter physicochemical qualities and is time consuming. These findings however can be helpful in studying the impact of the varying shapes and texture of the MPs on the environment in a timely manner. Furthermore, this paper may show that the shape of the degraded debris differs depending on the type of plastic and can be an indicative of future research into shape analysis of microplastics exposed to the environment and for developing and validation of standardized test procedures for evaluating the biological impacts of plastic particles and chemicals leached from weathering plastic in model organisms[21].

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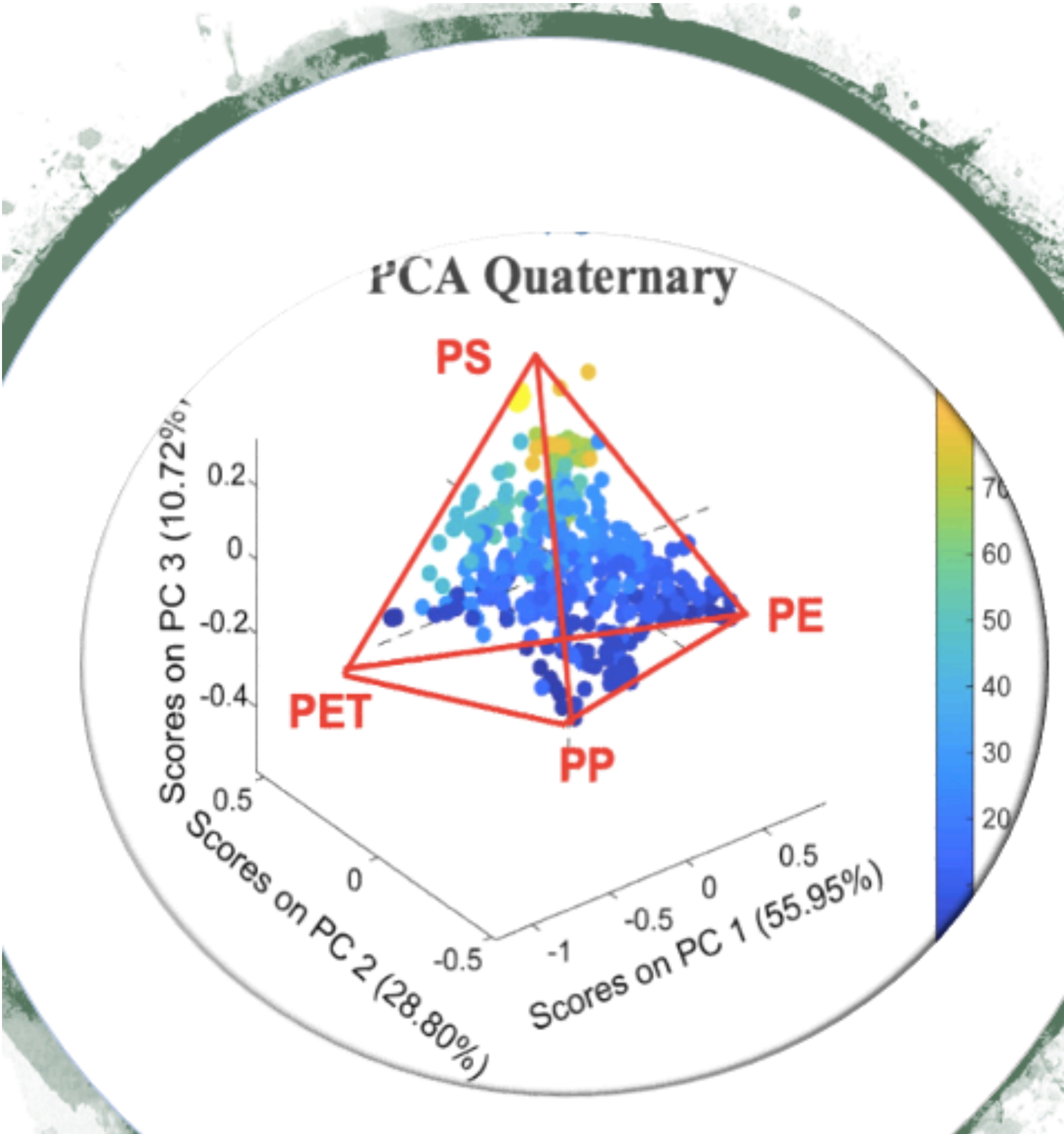
PART 3:
THE PROOF-OF CONCEPTS



CHAPTER 6 : THE TURN-ARY PLOT
ON THE USE OF HANDHELD MICRONIR FOR QUANTIFICATION OF TERNARY MICROPLASTICS MIXTURES

6.1 Abstract

In the last years, portable spectrometers have gained popularity thanks to the excellent capability for fast and on-site measurements. Ultra-compact spectrometers coupled with chemometric tools have shown great potential in the polymer analysis, showing promising applications in the environmental field. Nevertheless, systematic studies are still required, for the identification and quantification of fragments at the microscale. This study demonstrates the feasibility of a Miniaturized Near-Infrared (MicroNIR) spectrometer coupled with chemometrics for the quantitative analysis of microplastics. Samples were mixtures of the three most common polymers of daily used plastic items (polypropylene, polyethene, and polystyrene) mechanically fragmented. The chemical nature of samples before and after fragmentation was checked by Raman spectroscopy. 63 different mixtures were prepared: 42 for the training set and 21 for the test set. Blends were investigated by the MicroNIR spectrometer, and the dataset analysed using Principal Component Analysis (PCA) and Partial Least Square (PLS) Regression. PCA score plot showed a samples distribution consistent with their composition. Quantitative analysis by PLS showed the great capability prediction of the polymer's percentage in the mixtures, with R^2 greater than 0.9 for the three analytes and a low and comparable Root-Mean Square Error. The findings show that this study is a proof-of-concept for employing a portable tool in conjunction with chemometrics to quantify the most abundant forms of microplastics found in the environment.

6.2 Introduction

Degradation of environmental compartments by plastic litter, especially microplastics (MPs), i.e., any water-insoluble solid plastic particle with the longest dimension in the range of 1 μm through 1000 μm[1] is an unfortunate indication of the advancement of Anthropocene age on the Earth[2]. MPs consist of heterogeneous collection of particles differing in size, shape, morphology, composition, and other characteristics. Owing to factors such as light weightiness, high strength to weight ratio, bio inertness, low cost and durability, plastic has displaced and replaced many traditional materials like glass, wood, paper, stone, leading to rise in its production from 1.7 million tons in 1950 to 368 million metric tons in 2020, Europe accounting for 16% of the total production[3]. Only a small percentage (~9%) of plastics is recycled and the rest ends up as trash[4]. Due to the lack of appropriate and sufficient waste management strategies, plastic waste is discarded either in water bodies, natural environment, open landfills, or dumps. It is subjected to degradation due to UV radiations, wind, physical abrasion by water, leading to its fragmentation in tiny particles, so-called secondary MPs, to distinguish from microbeads, micro flakes, or nurdles MPs present in various products such as cosmetics or paintings, so-called primary MPs. The ubiquitous distribution of plastics and MPs and their resistance to biological and chemical decay has adversely affected the environment, especially aquatic life. According to the International Union for Conservation of Nature (IUCN 2018)[5], at least 14 million tons of plastic makes its way into the oceans every year constituting 80% of total marine garbage, and marking their presence from limnic waters to deep sea-sediments. A study by Lebreton et al.[6] indicates that, even if all plastic production is stopped globally with immediate effects, the level of microplastics in the ocean could still double by 2050. Out of the plastic litter entering the oceans, the MPs are even more dangerous due to their bioavailability to aquatic biomass and their large surface area that facilitates the adhesion of waterborne pollutants[7]. Water-insoluble plastic items with dimension > 5 mm account for major portion of plastic in the marine environment by mass (kg/km^2), but micro-sized debris is responsible for largest proportion by number i.e. items/km^2 (GESAMP)[8]. Studies done by De Witte et al.[9] and Van Cauwenberghe[10] demonstrated that blue mussels collected in Mediterranean Sea contained 0.2-0.5 microplastics/g by weight. The negative effect of MPs is not only limited to physical parameters but extends to chemical factors due to their ability to adsorb and accumulate contaminants such as Persistent Organic Pollutants (POPs)[11]. Moore[12] and Ivar et al.[13] reported that plankton, i.e., food for many aquatic animals, mixes with these small particles, thus making differentiation more complex and ingestion possible.

According to ASTM D7611 / D7611M – 20[14], plastics are marked with resin identification codes (RIC) inscribed in a triangle signifying the chemical composition of the principal polymer. These codes categorize thermoplastic resins and are used for identification of recyclable harmonious materials. They are: #1-PETE (Polyethylene terephthalate), #2-HDPE (High Density Polyethylene), #3-PVC (Polyvinyl Chloride), #4 -LDPE (Low Density Polyethylene), #5-PP (Polypropylene), #6-PS (Polystyrene), #7-OTHER. However, the codes are often missing from the recovered plastic and it is impossible to identify MPs as the standard size of the code is ≥ 12mm [14], and the polymer must be identified by chemical testing. When the polymer is known, it is possible to trace the fate of MPs in the environment, leading to more effective waste management and recycling strategies.

Even though the presence of MPs in marine environments[15,16], surface waters[17] and sediments[18] is well reported, a legitimate and authentic analytical technique to sample, analyse, and quantify the MPs is still in progress. Among the characterization techniques largely adopted in the field of polymers, vibrational spectroscopic techniques are the most commonly used for the identification and characterization in both near and middle infrared ranges, together with Raman spectroscopy[19]. The latter gives information about molecular backbone structure, crystal lattice, and symmetrical non-polar groups, whereas Infrared Spectroscopy provides information about hydrogen bonds and asymmetric polar groups[20]. Thus, the information provided by these two techniques is complimentary up to some extent[20]. Over the past decade, the miniaturized spectrometers have gained popularity as they allow real-time assessment with no or minimal sample pre-treatment. Nevertheless, the commercial availability, ensuring low cost instruments with respect to the benchtop equivalents[21], has only begun in the last few years, creating new less skilled users able to perform in situ measurements that were previously conducted only at laboratory conditions . Several types of portable and handheld devices for MPs analysis in complex environments were recently reviewed[22] and mostly based on FTIR spectroscopy, Raman spectroscopy[23–26] and their combinations, hyperspectral imaging and other novel optical methods[22]. Within these devices, the use of portable equipment in the near-infrared region, is prevalently assessed in pharmaceutical[27] quality control[28,29] and for polymer type identification of macroplastics[30,31]. Although NIR spectrometers have proven to be an effective tool for MPs analysis, with advantages in terms of high-throughput results and easy handling[32,33], the ability of the portable instruments in this area is still in its infancy.

There is an urgent need of harmonized and shared protocols to guarantee the capabilities of these miniaturized instruments to routinely analyse MPs. Standardized data will allow to build reliable databases and to perform quantitative analysis. However, most of the libraries are built with virgin polymers and spectral changes induced by MPs deterioration are sometimes overlooked. As a result, it is critical to develop new library searching algorithms that are more robust and capable of handling the challenge of comparing degraded MPs to virgin polymer references[21]. In this study, for the first time we demonstrated the feasibility of a handheld miniaturized near-infrared spectrometer (MicroNIR) coupled with chemometric methods for the quantification of secondary MPs mixtures produced at a laboratory scale. We chose a ternary model with three polymers (PE, PP and PS) that are the most abundant part of plastic waste and considered the most representative environmental plastic litter[34,35].

Daily life plastic products commonly found in urban waste plastic were mechanically fragmented in a controlled way to obtain true-to-life microparticles of PE, PP, and PS, mimicking the environmental weathered particles. The "true-to-life" approach allows to build a trustworthy database that fulfils the requirement of using controlled and reproducible samples (mandatory need for the realization of models and databases), but also closer to the environmental counterpart.

True-to-life MPs are not uniform in terms of size and shape, and additives present in the starting macro pieces can give a different and peculiar chemical identity to the fragmented MPs, reflecting the broad heterogeneity of environmental MPs[36]. The library of polymers developed in our previous work about the characterization of urban plastic waste via MicroNIR[30] was employed here to validate the MicroNIR spectra of the micro-sized samples. The information from the raw data was extracted through chemometrics by using Principal Component Analysis (PCA). Finally, Partial Least Square Regression (PLS-R) method was used to predict the percentage of each component in the mixtures, providing qualitative and quantitative predictions of unknown mixtures of MPs.

6.3. Materials and Methods

6.3.1. Microplastics preparation

MPs were produced at the laboratory scale by mechanical fragmentation of commercial day-to-day items using an immersion blender (Braun MQ3038 700-watt hand blender) with wet grinding technique. The procedure is sketched in Figure 1. MP samples originate from the following products: chewing gum bottles (HD-PE; specific gravity 0.94-0.98 g/cm^3), single-use beverage glasses (PP; specific gravity 0.83-0.92 g/cm^3) cutlery items (PS; specific gravity 1.04-1.1 g/cm^3)[37]. After removing the labels, the samples were washed with Milli-Q water (Millipore Direct-Q™ 5) (Figure 1, step 1) and cut into small pieces using a pair of steel scissors (Figure 1, step 2). Each component was shredded separately with Milli-Q water as a cooling and lubricating agent. The blender was turned off after every 10 minutes to abstain from overheating, for a total operating time of two hours per sample. The resulting grinded plastic was kept for drying at room temperature for 24 hours covered with a finely perforated sealing film to avoid entry of any contaminant particles. In the final step, the dried fragmented plastic was sieved through 500-µm mesh sieve to get the desired MPs fraction.

Figure 1. Scheme of microplastics preparation.

6.3.1.1 Environmental Sample Collection Campaign

To compare the MPs fragmneted from laboratory samples to the field-collected samples, a sampling campaign was done in the University of Brescia and the Valley of Mompiano (45°34′04″N 10°14′10″E) on 29 November 2021. NIR spectra of samples as such was collected and after cleaning the samples, they were framented through UC-mill procedure described in Chapter 5 to prepare microplastics.

6.3.2. Dataset construction

The distinctive arrangement of the MP mixtures was prepared according to the ternary composition plot displayed in Figure 2a. The triangular diagram was developed with a conventional spreadsheet (Microsoft Corporation, Microsoft Excel)[38]. Each polymer is displayed in the mass percentage plot with a 10-by-10 increment from 0% to 100% (Figure 2). The corners of the triangle represent 100% single polymer, i.e., single composition MPs; binary mixtures are along the borders, and the ternary MP compositions are inside the triaxial plot. We tested 63 mixtures (Figure 2a); 42 data points were selected for the training set (Figure 2b) and the remaining 21 compositions were used for testing the regression model (Figure 2c).

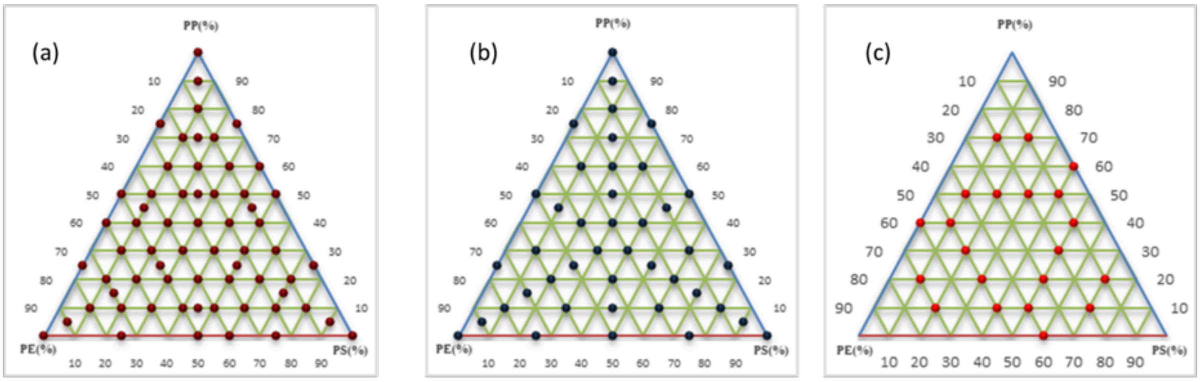


Figure 2. Triaxial experimental design of PE-PP-PS mixtures. The points indicate the samples composition. Each polymer component has a 10-by-10 increment from 0% to 100%. (a) Complete data set of 63 compositions; (b) Training set; (c) Test set.

6.3.3 Otpical Microscopy

A stereo microscope equipped with a HD Digital Camera (MZ16-A and MC190, Leica Microsystems) was used to record optical images of the secondary MPs in the different mixtures.

6.3.4 Raman measurements

Single polymer MP preparations were checked for composition by means of vibrational spectroscopic methods (NIR and Raman) and compared with the parental macro pieces to exclude chemical modifications induced by the mechanical fragmentation.

Raman spectra were acquired with a LabramHR-800 micro-Raman (Horiba, Jobyn-Yvon), equipped with a He–Ne laser source (632.8 nm), a 50x microscope objective (BX41; Olympus Optical Co. Ltd.) and a CCD detector (Wright Instruments Ltd.). All spectra were acquired under the same conditions, with acquisition time of 60s.

6.3.5 Near-Infrared Spectra Measurements

Miniaturized MicroNIR OnSite-W spectrometer (Viavi Solutions) along with a customized sample holder designed ad hoc (Figure 3) were used for spectra acquisition of mixed MP samples. MicroNIR is a compact device weighing about 250g and it non-destructively evaluates samples with no or minimal sample pre-treatment. It is equipped with Linear Variable Filter (LVF) as the dispersive element, coupled directly to a linear detector array (128-pixel InGaAs photodiode array), which works in the wavelength range 950-1650 nm. Two tungsten light bulbs are integrated as light sources. Based on the thickness of the LVF, light with explicit wavelength can only pass through, making each pixel of the detector respond to a particular wavelength. The spectra were collected at a nominal spectral resolution of 6.2 nm in diffuse reflectance mode. For each measurement, 70 mg of the plastic mixture was used, with an estimated error over replicates < 1%.

63 mixtures were obtained with the composition shown in Figure 2, 42 were used for training the model and 21 for testing it. To broaden the spectral variability and enhance representativeness for the regression model, for each sample five replicates for the training set and three replicates for test set were recorded, for a total of 210 spectra for the training set and 63 spectra for the test set. A reference and a dark measurement were recorded approximately every 10 minutes from a white reference provided along with the spectrometer. Each scan had an integration time of 12.6 ms with spectrum averaged over 100 scans.

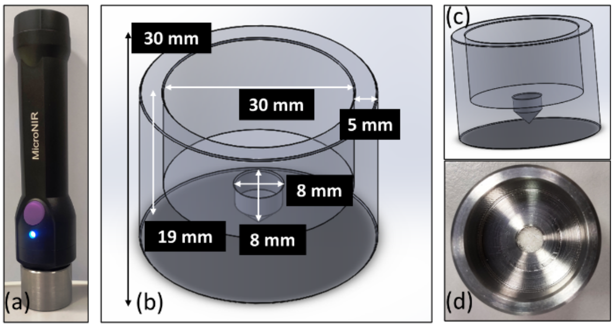


Figure 3. (a) Miniaturized MicroNIR spectrometer coupled with the customized sample holder. (b) Sample holder: (c) lateral and (d) top view.

6.3.6 NIR spectra pre-treatment

The NIR spectra of training set were retrieved in ASCII file, collected in a single matrix 210 x 125 (Samples x Wavenumbers), and then imported in MATLAB environment for the analysis. Before applying statistical tools, spectra were treated by Savitzky-Golay second-derivative technique of seven data points with second order polynomial and Standard Normal Variate (SNV). These are typical pre-processing tools for infrared spectra adopted to erase the multiplicative and additive scattering effects, which often alter the performance of statistical analysis[39]. Finally, the spectra were normalized by column mean-centering. The same pre-processing was also applied to the 63 spectra of test set.

NIR spectra acquired on single MP compositions were validated through the library of polymers developed in a previous work[30].

6.3.7 Multivariate Data Analysis`

PCA was carried out as multivariate technique to identify the structure of the training data. After PCA, PLS-R was performed to relate the amount of the three different polymers in the PE-PP-PS mixtures with NIR spectra. All calculations were executed in MATLAB 2019a (The MathWorks, Inc, Natick, MA USA.) by means of PLS-Toolbox (Eigenvector Research, Inc. Manson, Washington).

6.4 Results and Discussions

6.4.1 Mechanical Fragmentation

Physical degradation, together with photo-chemical degradation, and biodegradation by organisms are the most important environmental degradation pathways for larger plastic items towards smaller particles. Mechanical degradation is a critical abiotic process in marine environment, mainly due to wave and currents action and to abrasion by sand and other materials. When creating an analytical technique, several features should not be overlooked and should be deemed "verified" before being used on real samples[40]. With the precise aim to obtain true-to-life reference materials to apply chemometrics for a quantitative analysis of ternary mixtures of MPs we decided to mimic the breakdown activity by means of mechanical fragmentation, obtaining size-reduced fragments of parental plastic items.

After the fragmentation procedure described in the experimental section, the optical microscope images showed plastic fragments with at least one dimension less than 500μm (Figure 4), ensuring that MPs were obtained. The images also show the presence of very different shapes, from spheroidal-like structures to fibers, mirroring the broad features found in the environment [41,42].

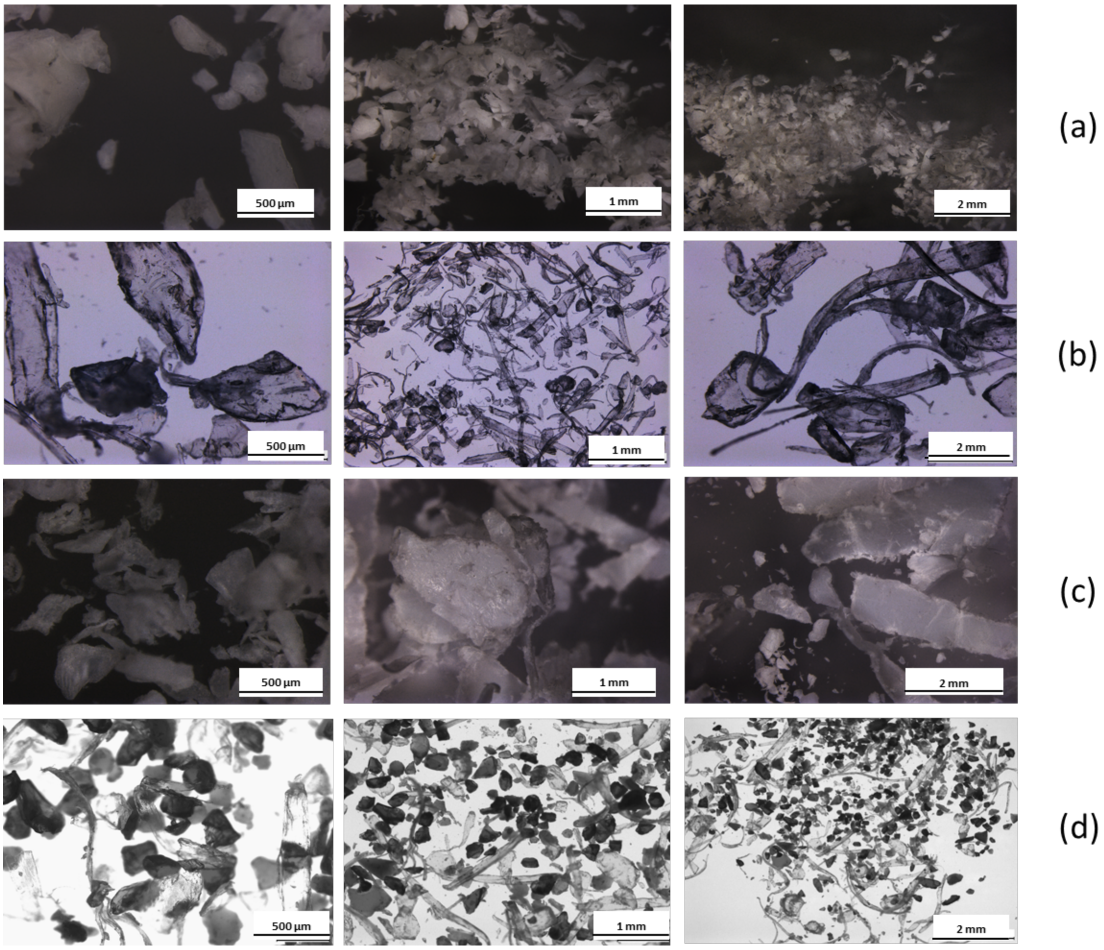


Figure 4. Optical microscope images of fragmented MPs: (a) PE; (b) PP; (c) PS; (d) PE-PP-PS mixture

6.4.2 Comparison of laboratory fragmented microplastics and environmental samples (macro and microplastics)

To further validate/confirm the nature of the microplastics fragmented in laboratory, called true-to-life MPs, we have compared them with the real environmental samples. A total of 21 samples coming from a collecting campaign were analysed through MicroNIR and classified using the PLS-DA previously calibrated. From these only those samples that were predicted as PP, PS and PE were selected and fragmented further. NIR spectra of these samples were compared with the pure compound of MPs mixtures using PCA with the pre-treatment described in section 2.6. The results are shown in Figure 5 in which is represented the score plot of the first two principal components. The "true-to-life" microplastics and those fragmented from the real environmental samples are closely related as the different types form tight homogeneous clusters. The variability factor is due to the type of polymer and not by the type of plastic used for fragmentation.

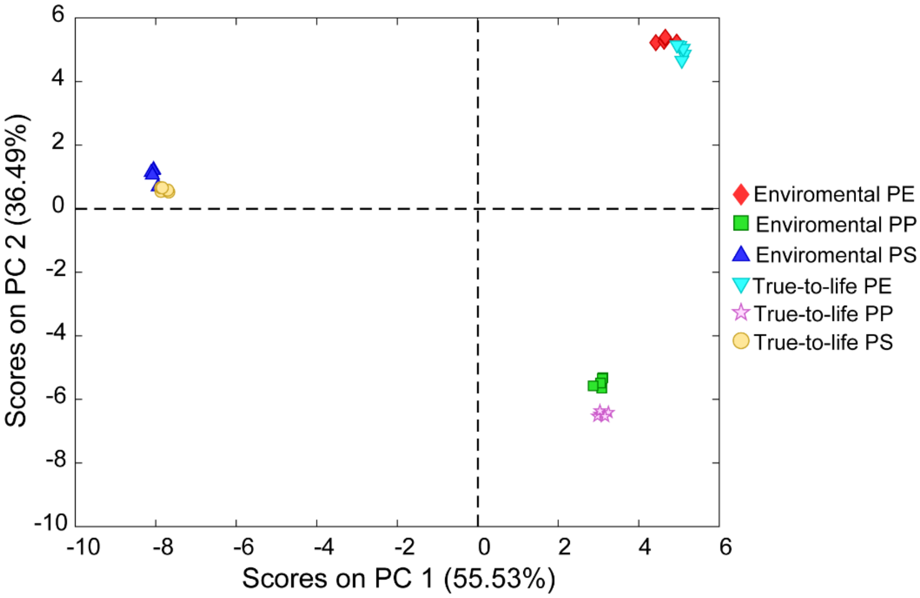


Figure 5. Score Plot of PCA of environmental Microplastics and laboratory fragmented microplastics

6.4.3 Raman measurements of single composition of MPs

Raman spectroscopy is a powerful and recognized tool for the identification of polymers[19]. The chemical nature of samples was checked by acquiring Raman spectra on macro samples, and after the fragmentation procedure for PE, PP, and PS single composition samples. The three polymers most found in the environment were chosen. The polyolefins PE and PP are referred in literature as the most abundant polymers in beach litter[43] and, generally, in global marine debris[44], possibly reflecting the distribution of plastics demand[45]. They are described as dominant polymers also in North Atlantic sediments[46] and ingested plastics in different species in North Sea[47]. PE and PP, together with PS are also reported to be the most abundant microplastics in the Mediterranean coastal waters[48] and, globally, in freshwater and estuaries[49].

Figure 6 shows the characteristic spectra of the polymers and the comparison with the measurements performed on MPs. Spectral differences among the three polymers are evident and they are not affected by the fragmentation procedure. PE is characterized by Raman bands at 1461 and 1439 cm^{-1} for CH_2 scissoring, 1416 cm^{-1} for CH_2 wagging, 1298 cm^{-1} for CH_2 twisting, 1131 and 1063 cm^{-1} for C- C stretching modes[50]. The main features of PP are concentrated in the region between 600 and 1600 cm^{-1}. A very strong band at 809 cm^{-1} is attributed to CH_2 rocking and C-CH_3 stretching[51]. The other characteristic bands are at 973 cm^{-1} due to C-C stretching and CH_3 rocking, 1330 cm^{-1} for CH bending and CH_2 twisting, 1167 cm^{-1} for C-C backbone stretching[51,52]. The Raman breathing mode of PS aromatic carbon ring appears at 1000 cm^{-1}. This band is not affected by polymerization, and it is used as the PS reference peak. The band at 621 cm^{-1} is due to ring deformation mode. The band at 1031 cm^{-1} is ascribed to C-H in-plane deformation, 1157 cm^{-1} to C-Ph asymmetric stretching, and 1604 cm^{-1} to ring skeletal stretch[53]. The C-H vibrations of PS appears in two bands. The first band around 2900 cm^{-1} is due to aliphatic carbon chain and the second band at 3050 cm^{-1} corresponds to carbon atoms in aromatic ring [54].

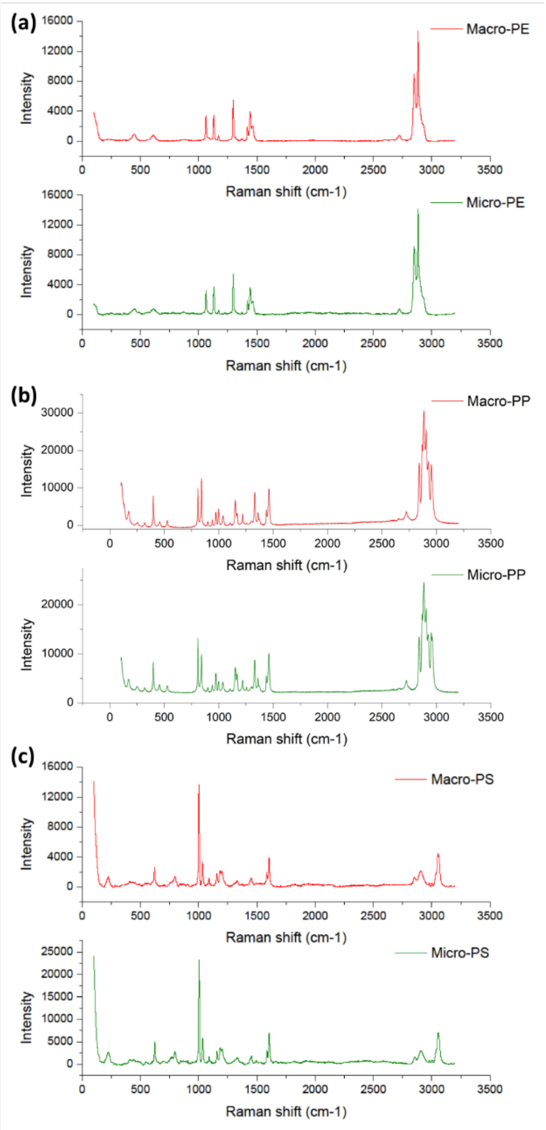


Figure 6. Micro-Raman measurements on single polymer composition before and after fragmentation for (a) PE, (b) PP, and (c) PS.

6.4.4. NIR Analysis of single MP compositions and MP mixtures

NIR region serves as a highly transmitting window for the region of forbidden transitions in the neighbouring Mid Infrared, Far Infrared, and UV-Visible regions. The predominant NIR active groups are Oxygen-Hydrogen (O-H), Carbon-Hydrogen(C-H), Nitrogen-Hydrogen(N-H). One of the reasons for ascription to functional groups with hydrogen atom is that the anharmonic constant of X-H bond (X=O, C, H) is large and X-H stretching vibrations have higher frequency[55]. NIR region mainly consists of overtones and combination bands arising from stretching and bending combinations in the same group[56].

The NIR reflectance spectra of each polymer are shown in Figure 7a-c and the main band assignments are listed in Table 1.

Table 1. NIR Band Assignments of PE, PP, and PS.

Polymer	IR Band (nm)	Band Assignment
PE	211 217 391/1416 168	Methylene C-H;2nd overtone C-H stretch[57] C-H combination band CH_2 symmetric stretch, 2nd
PP	193 211 391/1397	Asymmetric methyl C-H stretch:2nd overtone Asymmetric methylene C-H stretch:2nd overtone Methyl and Methylene (C-H) combination
PS	205 639 391	Aromatic C-H Stretch; 2nd overtone Stretching Vibrational mode of C-H Aromatic C-H Stretch; 1st overtone C-H combination;2nd combination region

NIR spectra acquired on each polymer were tested through the application of the Partial Least Squares – Discriminant Analysis (PLS-DA) model previously developed based on a robust library of NIR data collected on urban plastic waste[30] and correctly classified (see Supplementary Materials Figure S1), confirming the polymers identification and providing a further external validation in the MPs region of the model. Ternary MP mixtures were created according to the triangular diagram described in the experimental section. NIR spectra were recorded coupling the use of the custom-made sample holder that allows the loading of a small number of MPs, facilitating the mixing operation inter-replicates, guaranteeing the homogeneity and representativeness of the mixtures. A typical spectrum of a MPs mixture is reported in Figure 6d to single polymer MPs, showing the resulting combination of the single component bands, and giving a distinct spectroscopic fingerprint. However, the simultaneous spectroscopic determination of the subtle variations arising from different concentrations is challenging for a direct spectra analysis. To overcome these limitations, multivariate methods were used, extracting qualitative and quantitative information from the spectra.

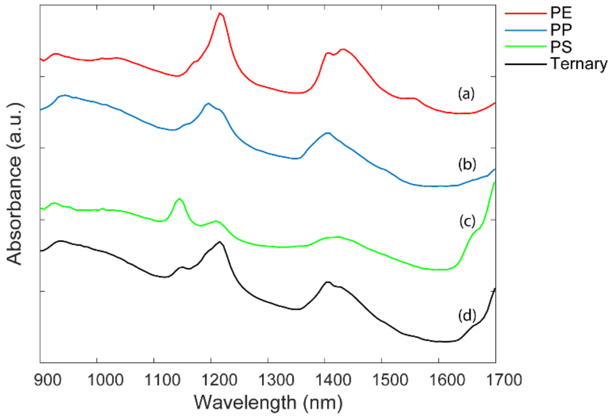


Figure 7. NIR spectra of (a) PE, (b) PP, (c) PS, and (d) PE-PP-PS mixtures, 40%, 30% and 40%, respectively.

6.4.5. Principal Component Analysis of MPs Mixture

PCA is an unsupervised pattern recognition technique characterized by the projection of the data in a new subspace defined by the linear combination of the original variables [58]. These are called Principal Components (PCs) and they are constructed according to the explained variance; the first one explains the largest variance, the second-one the second-largest variance and so on. PCA is often applied to spectroscopic data, and recently it has shown a great capability for exploratory analysis of MicroNIR spectra of waste plastics[30]. Performing PCA, the treated 210 spectra were analysed to give a description of the main variation within them. The calculation was carried out over all spectral regions. The results agree with the nominal composition of the mixtures, as shown by the score plot of the first two components in Figure 8, where samples are highlighted in a coloured scale, from blue (0%) to yellow (100%), based on the percentage content of PE. PC1 and PC2 explain together 89.55% of the total variability (PC1 52.43% and PC2 37.12%). The remaining variance to be explained is related to a non-systematic variation, i.e., spectral noise. This is confirmed by the scree plot and cumulative-variance plot[59], clearly illustrating that two PCs are adequate for the computation (**.** The main significant finding is the matching of the triangular plan and the score plot; samples are distributed inside a triangle whose borders represent the binary mixtures and the inner part the ternary ones. Furthermore, the three vertexes represent the single components. PCA shows a great capability in retrieving the original mixture composition, reflecting scores linearly related to the percentage concentration of microplastics.

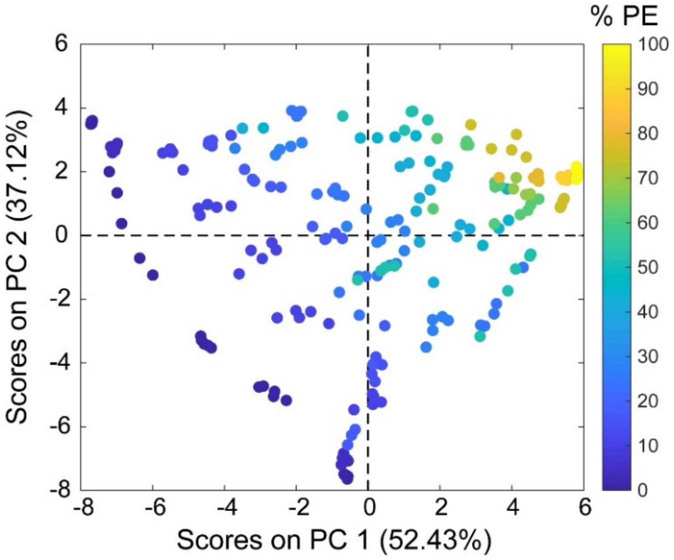


Figure 8. Score plot (PC1 vs PC2) of PCA performed on 210 spectra of the training set. Samples are colored according to the percentage content of PE.

6.4.6. Quantitative Analysis

After PCA, PLS regression was applied to quantify the percentage content of the three polymers in the MPs. PLS is a well-known regression technique for multivariate data. It allows to model the relationships between a matrix of predictors X and a vector response Y by modelling their structure. PLS is based on Latent Variables (LVs), which are linear combination of the original variables, whose direction is the maximum correlation with considered response Y[60]. In details, PLS-1 algorithm was carried out, meaning that the concentrations of PE, PP and PS have been predicted one by one. Before applying PLS, the average of the five replicates was calculated and the model was performed over all spectral region with same pre-processing used for PCA, i.e., 2nd derivative and SNV followed by mean-centering. The most crucial step in PLS is to find the number of optimal Latent Variables (LVs). This was carried out by the cross-validation procedure; the number of LVs was chosen according to the minimum Root Mean Squared Error in Cross Validation[60] (RMSECV) (see Figure 4S of Supplementary Materials for the plots of RMSECV as a function of latent variables for the three analytes). In particular, the cross-validation procedure was based on a Venetian blinds approach with 5 groups. Finally, PLS-R models was tested to quantify the percentage of PE, PP, and PS of the 63 spectra of the test set. Also in this case, the average of the three replicates was calculated. In Figure 9 the comparison of predicted and experimental values for the test set distributed in the triangular diagram is displayed, indicating a satisfactory degree of matching of the experimental points with the predictions.

The calibration results are summarized in Table 2, in which are displayed the number of LVs, regression coefficient (R^2), and RMSE, evaluated both in calibration, cross validation, and on the test set. With respect to calibration and cross-validation, PLS showed a high performance. The results between and among the models were balanced. For the three analytes R^2 exceed 0.9 and RMSE were low and comparable to each other. Moreover, with respect to the singular models, the regression parameters calculated in calibration and in validation were comparable to each other, meaning that no overfitting was expressed, with models reliable and stable. These results confirmed a high predicting level for the three analytes in the unknown mixtures. Again, R^2 are higher than 0.9 with low RMSE; so, all the three polymers have been quantified in an equal and precise way. Figures 10a-f shows predicted and the measured values for three analytes, both for training and test sets. The plots show the homogeneity in the choice of the test set, and the satisfactory level of prediction of the unknown concentrations

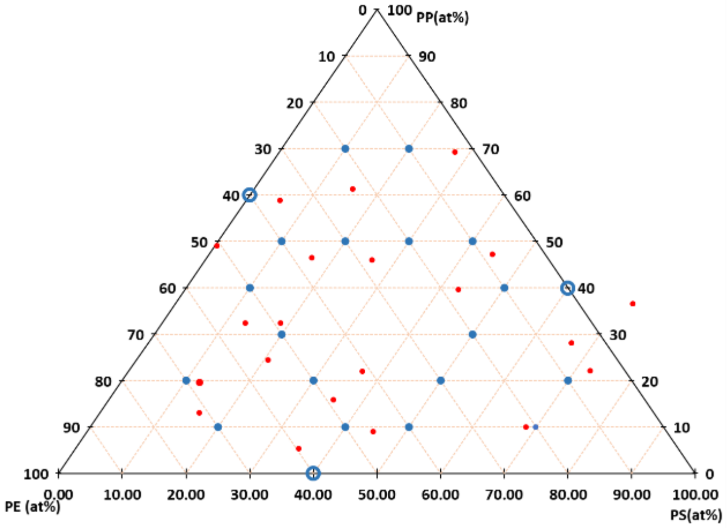


Figure 9. Triangular diagram displaying experimental points (blue) versus predictions of the test set (red).

Table 2. Results of PLS-R calibration models.

				R^2			RMSE
Analyte	Vs	al	V	est	al	V	est
E		.96	.94	.96	.25	.49	.73
P		.96	.94	.94	.02	.54	.65
S		.98	.97	.94	.96	.32	.65

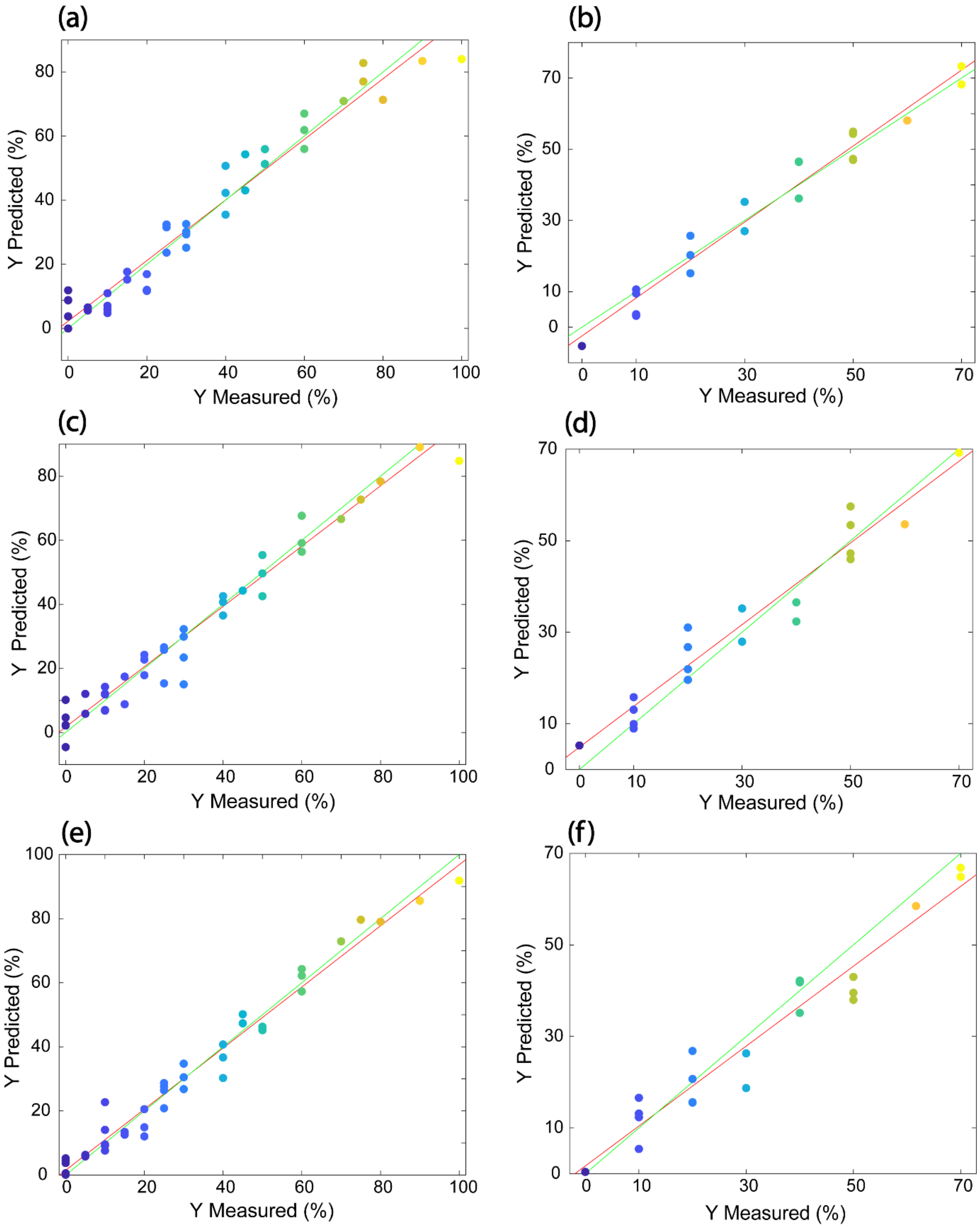


Figure 10. PLS output for PE. (a-b), PP (c-d), PS (e-f) for training (a-c-e) and test set (b-d-f).

6.5. Conclusions

In the present study, the integration of a portable near-infrared spectrometer with a robust chemometric approach to quantify true-to-life MP mixtures was presented. PCA was applied with a very good efficiency to NIR spectra of PE, PP, and PS mixtures, locating all the samples inside a triangle, reflecting the triangular diagram of the initial compositions. Then, PLS regression was applied to quantify the content of the three different MPs, providing predicted values of concentrations for PE, PP, and PS characterized by R^2 are higher than 0.9 with low RMSE, evaluated both in calibration, cross validation, and on the test set. The model developed on true-to-life MPs opens the possibility to apply such algorithms to environmental samples and has the merit to fill in the gap between lab-scale and the environment. From an environmental point of view, the evaluation of the MPs as can be possibly found in the environment, for example, diluted in water or dispersed in soil, could be of utmost importance. In addition, the investigation of the minimum concentration detectable, the interference of water in the spectral information, and the possibility to set up drying protocols to be able to apply the model on samples from the environment will set the future development of these predictive models. Though in theory, PLS operates well if the matrix of external samples contains the same components as the calibration matrix. The results here indicate that this work is a proof-of concept of using a handheld instrument coupled with chemometrics to quantify the most abundant types of microplastics found in the environment. The dataset with more polymer types must be studied which is beyond the scope of this work. These results will serve as the basis of the building blocks of this new method.

In conclusion, in this paper we validated the use of portable spectroscopy coupled to chemometrics for MPs quantification in a model system that mimics the most common types of polymers that can be found in environmental microplastics. In addition, morphological characterization of the realized true-to-life MPs showed fragments faithfully representing the broad features of the naturally occurring MPs. These conditions, together with the choice of the most abundant polymers found in the environment, highlighted the crucial role that this approach can play in future developments of fast, reliable, and in-situ analyses for the quantification of environmental microplastics.

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PART IV: THE REVIEW

CHAPTER 7: THE REVIEW

A COMPLETE GUIDE TO MICROPLASTICS EXTRACTION METHODS FROM COMPLEX ENVIRONMENTAL MATRICES

7.1 Abstract

Sustainable development is a big challenge that our world is facing. In recent years, the presence of a class of contaminants known as microplastics (MPs) has been identified as a significant pollutant with the potential to harm human health and animals. These small plastic particles have been found in every matrix on the planet, with aquatic habitats serving as the ultimate sink for scattered MPs. The challenge to extract microplastics from environmental matrices is an actual and imperative issue. One of the primary lines of research in environmental chemistry is the development of simple, rapid, low-cost, sensitive, and selective analytical methods for the extraction and identification of microplastics in the environment. The present review aims to describe the developments in microplastic extraction methods from complex environmental matrices, with special attention given to each method. All methodologies (new, old, proof-of-concept) are discussed and evaluated for their potential usefulness to extract MPs from various biological compartments for the sake of progress and innovation. This study concludes with the existing issues and future research goals that will guide us down the path of combating microplastics pollution.

7.2. Introduction

With science and innovation present in the DNA of plastic, the plastic industry has become crucial for any country's economy. With its unparallel features such as low-cost production and availability, durability, high strength to weight ratio, plastic has become indispensable product in our day-to-day life, allowing us to meet myriad of our aesthetic and functional demands. According to a report published in Plastics Product Global Manufacturing Global Market 2017[1], the plastic business is growing by 3 % every year. But the disposal of the post-consumer plastic waste has been a global concern from the beginning of the plastic era. The mismanaged plastic waste is discarded off in the water bodies and landfills, where it is subjected to either physical, chemical or biological degradation processes, which transforms it into smaller fragments called microplastics (MPs).

The International Organization for Standardization (ISO)[2] defines Microplastic as "any water-insoluble plastic particle with its longest dimension between 1 um and 1 mm". They are further classified as primary MPs when produced intentionally in form of pellets, granules, fibers or powder in the industries for use in personal care products and are called secondary MPs when fragmented due to external forces (UV degradation, wind, water current, washing of clothes). In addition, based on dimension, ISO classifies large microplastics as any water-insoluble solid particle with dimension between 1 mm and 5 mm. After Prof. Richard Thompson published the first report on microplastics in sediments in 2004[3], for many years, the study of microplastic was confined to the marine environment, focusing on their occurrence in the oceans and along the beaches with very few studies reporting the interaction with aquatic animals. But later the researchers began to examine the other environmental compartments and found that these tiny pieces are present far and wide, ranging from Arctic Sea ice to hadal sediments of Mariana Trench[4], from drinking water to waste-water and from soil to sewage sludge.

As plastic do not behave the same way in the different matrices, it is fundamental to trace their origin, path, and fate for the understanding of the recovery strategies, their effect on flora and fauna. Interaction of plastic debris with the animals is sabotaging them in two ways: a) physically via ingestion or entanglement and b) chemically due to the presence of additives, adsorbed Plastic organic Pollutants (POPs). The adverse effects of MPs can be based on various factors such as chemical composition, dosage, size, color etc. Though the harmful effects of macroplastics have been widely reported by the scientific community, more information is still needed in microplastic toxicity[5]. And this can be attributed to the lack of standardization methods while working with Microplastics.

From local to global level, researchers have been widely reporting about the presence, sampling[6], extraction methods, characterization techniques of MPs, especially in the aquatic environment but the bottom line remains intact: no harmony in the protocols to either sample, extract or characterize the microplastic in any given environmental matrix. The following constraints come into play while working with microplastics:

1. The varying size range that has been used by different researchers: The ambiguity in the size range used by the researchers has led to the use of different methods to study the MPs.
2. The other roadblock is the unavailability of every technique in all the laboratories analyzing MPs. Since the scientific horizon is widening at a great pace, new techniques come in the market which makes the standardization almost an impossible task. Furthermore, for certain establishments, the large monetary and personal investment required for the efficient and delicate separation approach is prohibitively expensive, leading to the use of simpler and less precise procedures instead.
3. The third hurdle is the complex environmental matrix. For example, working with sewage sludge or wastewater requires many complex steps just to extract the MPs which in turn require more chemicals, increasing the cost of the overall procedure. Not every laboratory can afford to work with the expensive methods.

Thus, owing to the above-mentioned factors and the conditions favorable for a given researcher or group, an array of methods is present and an absolute method for the complete analysis of microplastics is yet to be developed.

The basic approach towards any MP study is sampling, extraction, characterization, and quantification. The amount of information that has been accumulated since United Nations Environment Programme (UNEP) declared MPs as worldwide pollutants[7] in 2011 is dramatically lower than the number of articles floating, owing to unhomogenized protocols. With ISO defining MPs and NPs after 16 years of wait when the problem of such small-scale plastics was identified in 2004, a new direction has been given to the research community. As a result, the science of MPs is once again in its infancy, now with one defined variable, i.e., the size. Being said that we as a group (actively working with the MPs)[8,9] believe that the main challenge comes with the extraction of the microplastic, as the plastic behavior changes with the changing environmental profile and each matrix must have its own set of protocols for more effective and prudent analysis. Thus, this paper aims to bring the various extraction methods that are frequently used in the scientific community and the modifications done in them for all the possible matrices: Water (fresh water, drinking water, and wastewater), Soil, Sediment, Sludge, Biological Tissue, and Air.

7.3. Methods and Approach

The aim of this work is to review the recent advancements in the extraction methods of microplastic from various environmental matrices. The microplastic analysis is based on extraction and purification followed by identification and quantification. Extraction of MPs from any environmental matrix is the most crucial step as it influenced by the size, shape, and density of both the matrix type and microplastic. It thus follows that the extraction process should be differentiated based on the type of matrix being analyzed. The MPs must be isolated from the environmental matrix to simplify the further processes. There are many articles present highlighting the sampling, extraction process for either of the complex matrix. Also, owing to the various factors: funding, demographics, laboratory resources, interest of the matrix, new protocols go on developing with time. We aim to bring together the state-of-art for all the matrices in a compact and concise manner. The review is divided into two sections: First section traces the origin of the original method that has been modified over the course of years and the second section explains in detail the different extraction methods currently in use divided by the different environmental compartment.

7.3.1 Literature Search

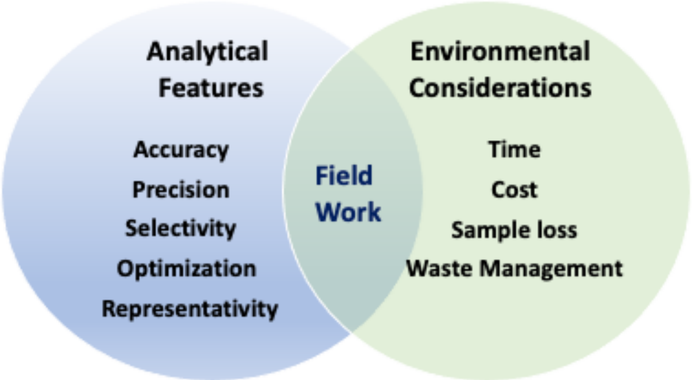
A wide search of peer reviewed articles was performed using two online publication databases: SCOPUS and Web of Science. The search was restricted to articles in the English language .For selection of articles for this systematic review For a more systematic review, a bibliometric analysis was performed in this work using Biblioshiny platform. Here more than 80 papers are reviewed published between 2004 to 2021, with special attention to recent publications. The following keywords, in combination with *extraction* and *microplastic* were used during the database search: "sediment*", "soil*", "water*", "sand*", "cosmetic", "biota", "air".

For the first screening, Bradford's law was used as a criterion to refine the dataset using R project and Biblioshiny[10]. Thereupon, the full texts of the articles, selected based on the title and abstract, were studied to shortlist the ones which met the selection criteria and could be included in the review. The reference indices of peer-reviewed publications were likewise investigated, and potentially relevant studies not found in online databases were added manually.

The critical analytical method is commonly used to discover the most significant authors, their partnerships, keywords, or nations that have worked on related research topics. A few publications have been published in recent years on the presence, content, distribution, and contamination of MPs in various environmental matrices but in a scattered manner. To our knowledge, there are no evaluations that comprehensively illustrate the technology for detecting and extracting MPs giving equal importance to each environmental matrix. This article fills up the gaps by discussing technology for detecting and extracting MPs in the different matrices collectively.

7.3.2. Environmental-Analytical Holistic Perspective

The understanding of the fate and effects of microplastic can be made through two paths: first through Environmental Chemistry approach, in which the effect of various matrices of nature (air, water, soil, biota) is studied and the second is via Analytical Chemistry, where analytical methods are developed for solving the problems related with microplastics.



The development of new sampling, extraction, and user information exploitation strategies through field sampling research, as well as the revolution in analytical methodology provided by the development of flow analysis concepts and process analysis strategies, have provided a link between modern instrumentation and social or technological environmental issues in recent years. Regardless of the appropriate analytical approach for MP detection, a demanding element that has been extensively explored is the extraction and isolation of plastic particles originating from varied and complicated environmental matrices. It is an essential level of environmental protection that has typically been associated with compliance and/or exposure monitoring operations for environmental pollutants. Physical methods of analysis have advanced to the point that they now offer an astounding variety of tools for determining a wide number of elements and compounds at very low concentration levels.

From analytical point of view, the defining attributes of a technique that are considered when the analyst is actively looking for the most effective method in order to obtain depth information from a system are accuracy, diversity, precision, specificity, and sensitivity. In this context, quantitative properties should be permanently considered as the guide for sample assessment and comparison between analytical approaches and practices. That being said, in real time, there are certain practical elements to consider, such as the time necessary to do sample analysis, the expenditure, user friendliness, including side-effects, which is an essential environmental consideration. The Environmental perspective in Analytical Chemistry is a modern chemistry compromise, but it should be carefully studied to ensure the long-term evolution of our field.

In this review, new methods, specifications for extraction of MPs from various matrices are presented in an integrated environmental approach as a problem-solving strategy centred on the development of efficacy of the various techniques but also speed and cost. Technological and industrial concerns, as well as environmental, health, and social issues, have been identified as challenges for which the chemist must pick the most effective instrument and devise an acceptable approach.

7.3.3 Importance of Matrix Selection

Microplastics have migrated over the world and may now be found in almost every environmental compartment. The matrix has a considerable influence on the test method choice, therefore for the same analyte in sample matrix, the technique and its operative stages could be completely different. The purpose of matrix understanding is to guide the selection of monitoring activities based on data objectives and available resources in order to ensure the harmonisation and consistency in the protocols being built for the standardisation purposes. The sample matrices should be chosen depending on the goals of the monitoring exercise, with certain matrices being more important than others.

It is undeniably difficult to design a novel method for determining which polymers, often degraded, are present, their concentration, size, shape, and color in environmental matrices that differ in origin and physical properties. In terms of DoE (design of experiment), in order to forecast the outcome of the experiment, it is required to identify the variables influencing the resultant data, which requires a thorough understanding of the habitat, feeding niche, and so on, or the "environment," of the sample site. The broad category of environmental compartments include- Water, Air, Soil/Sediment, Biota. The main reason for designing distinct matrices is that the chemical nature of these matrices is heterogeneous, resulting in a wide range of characteristics, functions, and interactions with contaminants. For example beaches, coastlines, benthic marine sediments, rivers, and lakes are all examples of sediments that can be distinguished by their environment[11]. Microplastics have been majorly studied in the aquatic environment, especially surface waters but the reality is that the variation in the hydrodynamic profiles of water bodies contributes to difference in the properties of microplastics. For instance, rivers have unidirectional current with a relatively high average flow velocity ranging from 0.1 to 1 m/s. Lakes have a modest average current velocity of 0.001 to 0.01 m/s. Groundwaters have a fairly consistent flow pattern in terms of direction and velocity(10^{-10} to 10^{-3} m/s.). Biota can be classified according to their taxonomic group or their ecology. The development of flora and fauna, particle movement in any given region is influenced by these varied profiles of various matrices which affects the sampling and extraction methods.

Lessons learnt from assessing microplastics in water or biota samples apply only to a limited extent to soils. Soils are made up of unconsolidated organic and inorganic matter material that forms as a result of the interaction of water, air, and organisms on the earth's surface. It serves as a barrier between distinct environmental compartments in terrestrial ecosystems (the lithosphere, hydrosphere, biosphere and atmosphere). The methods used to extract MPs from soil and sediment may differ dramatically. Organic matter removal is not often performed when studying mineral-dominated sediments such as sandy beach deposits, but it is usually a required processing step for low-energy estuary, lacustrine, or pelagic sediments, and it is required when evaluating soil. Because of the variability of soils and the features of MPs, separating MPs from a soil system is more difficult than doing so in a water environment.

The requirements for specific data can be made obvious if the nature of the study is clear, resulting in less uncertainty or omission in the sampling technique used. The extraction of microplastics from environmental matrices is a field with enormous promise for future method development.

7.3.4. The Original Methods

Visual sorting, sieving, or filtration are regularly used methods for separating bigger microplastics from fine sediments (mud or silt). Small microplastics, on the other hand, can be more difficult to separate, especially from finer sediments, with the structure of the microplastic determining separation ability. We hereby review the methods for separation of

1.Density Separation: Developed by Prof. Richard C. Thompson and team in 2004[3] in which he isolated MPs from sediment using a concentrated saline solution. This method is only suitable for polymers with density lower than the hypersaline brine.

Many methods and devices namely, Munich Plastic Sediment Separator (MPSS), Elutriation technique, Froth-Flotation Method use the principle of density separation to work. These will be discussed in detail in the later sections.

2. Oxidative Digestion[12]: Liebezeit, G. Dubaish, F. 2012. Microplastics in beaches of the East Frisian islands Spiekeroog and Kachelotplate. Bull. Environ. Contam. Toxicol. 89, 213-217

3. Alkaline Digestion[13]: Enders, K., Lenz, R., Beer, S., Stedmon, C.A., 2017. Extraction of microplastic from biota: Recommended acidic digestion destroys common plastic polymers. ICES Journal of Marine Science 74, 326–331

4. Enzymatic Digestion[14]: Cole, Matthew, et al. "Isolation of microplastics in biota-rich seawater samples and marine organisms." Scientific reports 4 (2014): 4528.

5. Oil Extraction Protocol[15]: Based on the oleophilic properties of polymer, this method was developed by Crichton in 2017.

6. Pressurized Fluid[16]: Developed by Fuller and Gautam in 2016. PFE is based on the use of solvents at subcritical temperature and pressure conditions, principally for the recovery of semi volatile organics from solid materials.

7. Electrostatic Separation[17]: Initially tried by Richard C. Thompson in 2011, it only worked for extracting spiked samples in sediments, it failed for other matrices but in 2018, Felsing et. al developed a Korona-Walzen-Scheider (KWS) electrostatic separator to extract MPs from quartz and beach sand.
8. Magnetic Separation[18]: Introduced by Grbic et al. in 2019, this method is based on exploiting the hydrophobic surface of plastics to magnetize them to isolate MPs from soil.
These methods are the basis of all the new protocols that have been developed so far. As a matter of fact, not all methods can be applied to every section of the ecosystem. Thus, we have categorized the extraction processes based on the type of matrix we deal with.

7.4. Extraction Methods

Environmental supervision is essential to guarantee an ecosystem's well-being and protection. Ecosystems are dynamic, including a variety of processes, inputs, pollutants, and living organisms. Nature is made up of various sectors, each of which has its own impact and thus must be measured in a unique manner. Extraction is a method for segregating plastic from other contaminants or getting plastic from a polluted medium. Separation, on the other hand, is the process of sorting or dividing plastic into various types. As a result, keeping track of an environmental system is no easy feat. Water, sediment/soil, air, and biota are the four major environmental compartments within which specific matrices can be targeted for monitoring. The goal of monitoring a certain matrix should be properly recognized.

7.4.1 Extraction from the 3 ‘S’s: Sediment, Sand, Soil.

Due to the vivid, vast accumulations of plastic in the oceans and the effects on marine creatures, the most attention and research on plastic trash has been focused on marine settings. Whereas plastic trash collects and spreads in water according to currents and weather patterns, little is known about the role of soils and sediment in the movement, processing, and disposal of plastic garbage. Terrestrial ecosystems (sediments, soil, compost, sand), which are especially prone to plastic contamination, are relatively unexplored and are likely key drivers in the production and spread of microplastics. The extent to which plastic pollution have settled and accumulated in these matrices is critical for understanding their ecological impacts, because the presence of microplastics can be detrimental to organisms. Researchers all throughout the world have used a wide range of sample processing and analysis procedures, which makes comparisons and magnitude estimates difficult, also posing a substantial difficulty in developing a thorough perspective of microplastics in soils and sediments.
Sediments are considered to be the long-term sinks of the MPs[19]. Sediments are deposited in a variety of sites by wind and runoff water. Because lake sediments and deep-ocean sediments are the least disturbed, climatologists rely on them the most to study climate change. They have records for 99 % of geological history[20]. Willis et al.[19] have reported microplastics to be present in sediments from pre-plastic age. The best strategy to extract microplastics from a vast number of sediments is one of the primary concerns in microplastic investigations of sediment samples. The development of a reliable method for identifying microplastics in sediments is crucial for better understanding the distribution, mass, and ecological effects of microplastics in both marine and freshwater ecosystems.
A recent study found to contain 165 MP particles per kg of sediment[21]. The first ever mention of occurrence of microplastics in beach sediments date backs to 1977 when a survey conducted by Murray[22] on 300 beaches of New Zealand recorded the presence of virgin polyolefins in form of virgin plastic pellets; however, it was only after two decades, that microplastics were extracted and quantified for the first time in 2004[3] from beach sediments To date various methods, ranging from one step extraction to complex methods based on the oleophilic, electrophilic or magnetic properties have been developed. The extraction of microplastics from

soil is more complex than that from sediment and sand, owing to heterogeneity of the soil, thus organic matter digestion methods are more prevalent after the first step of extraction is done through the procedures explained below. Soil organic matter is a heterogeneous mix of chemical components. Although the precise chemical compositions have remained a source of contention, it is widely assumed that humic compounds are composed of polyphenols, peptides, lipids, and polysaccharides. We will hereby review the various methods used and developed by independent researchers and some authoritative bodies.

7.4.1.1 Pre- Extraction: Sieving & Filtration

Sieving

MPs can be separated from the soil, sand or sediments into granulometric fractions by sieving[23]. It is a pre-treatment procedure that is typically used to eliminate particles larger than 5 mm. In several research, MPs of a certain size were recovered by sifting in the field[24]. When plastic particles are visible to the human eye and are larger than 5 mm, this technique is recommended (mesoplastics). Because soil is likely to be moist due to a variety of circumstances, samples should be dried prior to analysis which may improve microplastic recovery in soils where they are bound by organic matter.

Filtration

When the goal mesh size is crucial, filtration is a technique of separating a solid phase from a liquid phase using a filter baffle. This method could be employed at many stages of sample processing, such as after density separation or chemical digestion, as well as at the start of the sampling procedure. Traditional filtration procedures, such as filtration under vacuum or membrane filtration, are utilized in the laboratory for analysis[25]. Alumina, ceramics, and polycarbonate are some of the materials used in membrane filters. Currently, glass fiber membranes, cellulose acetate membranes, cellulose nitrate membranes, polycarbonate membranes, nylon membranes, and alumina membranes are the most common filter membranes used in MP analysis[26]. All of these filters have benefits and drawbacks, but there has yet to be a clear process for using a homogenous filter material. Similarly, the filter mesh size ranges from hundreds of micrometers to tenths of micrometers.

7.4.1.1 Density Separation Using Hypersaline Solutions

Density separation is particularly effective for samples with substantial volumes of sand or sediment[27]. The removal of the sample matrix, whose preparation process is significantly dependent on the sample type and available resources, is the first necessary step for the determination of microplastics in marine samples. The two primary phases that are shared by all extraction techniques are generally the ones that require adaption to the examined matrix. The first stage is density separation, in which MPs are isolated from their matrix by utilizing density differences between an extraction solution and MP polymers. The second stage involves oxidation of organic debris, which floats with separated MPs, making MP extraction and detection difficult. This method of extracting MPs based on the differences in density of the polymer and the flotation solution can be regarded as the ground method for most of the research that has been going on for the last two decades. It involves placing materials of varying densities in a liquid of intermediate density, where the less dense material floats and separates from the denser sinking material. Changing the density of the liquid, usually a saline solution allows particles of different densities to float relative to the density of the solution. The average density of sediments and soil varies[28] from 1.70 g/cm^3 – 2.65 g/cm^3, the density difference between the lighter microplastics and the heavier sand or sediments can be utilized to separate them. NaCl, being cost-effective and environmentally beneficial solution, has been the most widely used brine solution. The MSFD Technical Subgroup on Marine Litter[29] has also suggested this method for extracting microplastics from intertidal deposits. NaCl, on the other hand, produces a solution with a density of only 1.2 g/cm^3, preventing the separation of denser polymers. However, because soil particles may greatly adsorb or embed MPs, such a system may require precise tweaking. For robust isolation of different forms of MPs from soils, two processes are vital. The extraction of all MPs from mineral particles by density separation is the first step, whereas the digestion of organic matter is the second step. When the density of MPs is uncertain, higher-density salt solutions are required for MP extraction and flotation. Thus, researchers are experimenting with different salts listed in Table 1 to separate microplastics.

Table 1 Microplastic extraction abilities of different hypersaline solution

Salt	**Density g/cm3**	**PET**	**HD PE**	**PVC**	**LD PE**	**PP**	**PS**	**PA**	**Remarks**	**Price €/kg**	**Ref**
		1.32-1.41	0.94-0.96	1.14–1.46	0.91-0.92	0.85-0.92	1.04-1.08	1.12-1.15			
Milli-Q water	1	-	-	-	+	+	-	-	1. Easy to use 2. Low recovery rate.	-	30
NaCl	1.2	-	+	±	+	+	+	+	1. Easy to use, Non-Toxic 2. Low Recovery Rate, Requires multiple washings.	3.00	3
$ZnCl_2$	1.5–1.8	+	+	+	+	+	+	+	1. Reusable 2. Corrosive, strong foaming with organic samples 3. Toxic to aquatic life	137.00	31
NaI	1.55–1.8	+	+	±	+	+	+	+	1. Reusable 2. Reacts with cellulose fibers, Hygroscopic, Multi-Step Method 3. Eye irritant	70	32
Sodium Polytungstae	1.4–1.65	+	+	+	+	+	+	-	1. Eye irritant 2. Toxic to aquatic life	671	33
Sodium Dihydrogen Phosphate	1.4-1.45	+	+	+	+	+	+	-	1. Hazard free 2. Heating is required to achieve desired density	54	34
$CaCl_2$	1.3–1.35	+	+	+	+	+	+	-	1.Organic matter settles slowly due to high viscosity 2. Ca^{2}+ caused flocculation of organic substances through ion bridging, thus not suggested for organic rich samples 3. Eye irritant	81.20	35

$ZnBr_2$	1.7	+	+	+	+	+	+	+	1. Toxic to aquatic life, Eye irritant	205	36
NaBr	1.37	+	+	+	+	+	+	+	1. Eye irritant	138.00	37
Li_2WO_4	1.62	+	+	+	+	+	+	+	1. Recommended by NOAA	195.00	38
KI	1.7	+	-	+	-	+	-	+	1. Eye irritant 2. Toxic to aquatic life	306.00	39
HCOOK	1.5	+	+	+	+	+	+	+	1. Reusable	83	40
NaCl/NaI	1.2/1.8	+	+	+	+	+	+	+	1. High recovery rate. 2. Pretreatment required	73	41

Density separation method may look like an easy approach but, the extraction is influenced by number of factors listed below:

(1) Organic matter: More complex matrices such as soil, sludge, are heterogenous solid combinations of minerals with varied particle sizes and organic matter in various states of degradation. Given the small size and wide variety in shape of the particles, accurate and absolute extraction of microplastics from soil matrixes has proven difficult. Density separation is a typical method for extracting microplastics from soil but pre-treatment in form of organic matter removal through digestion methods is required. (Described in later sections).

(2) Wetting agents: Regardless of having the same resin identification number (RIC), studies have shown how the same polymer can include different additives or wetting agents, which affects its density and subsequent separation by considerably reducing the floatability. Wang et al.[42] reported that there is significant difference in the floatability of virgin polymer resins and post-consumer plastic waste. The floatability of the plastics decreases with the increasing concentration of the wetting agent.

(3) Hazardous/Toxic Salts: Certain salts have concerns due to the related costs and hazards, even though they allow for the separation of denser polymers (Table 1). Some regulatory bodies such as Globally Harmonized System of Classification and Labelling of Chemicals (GHS)[43] by United Nations discerns between two distinct signal phrases that pertain to two levels of hazard severity: Signal word "danger" is often reserved for the more serious hazard categories, while "warning" is reserved for the less serious. The signal words "danger" are appended to $ZnBr_2$, $ZnCl_2$, and NaI, chemicals that cause skin, ocular, and respiratory irritation. Use of NaI has been suggested in various studies because of its_reusability, high density, possibly in combination with separation columns. Based on particle number, NaI solution provides good recoveries, but this is highly dependent on the type of plastic. Several chemical combinations for density extraction procedures have caused difficulties in deciding on the best approach. The GHS system identifies $CaCl_2$ and $Na_2WO_42H_2O$ as "warning" substances.

(4) 3R's: Repeatability, Reproducibility and Representativeness:Numerous authors have attempted to establish their own ways but have failed to implement those that have previously been devised. The scientific endeavor to develop analytical methodologies for MPs extraction has increased in recent years. For instance, in the work of Daz-Jaramillo et al.[44], when a known amount of MPs is spiked in a natural clean sand, extraction efficiency is studied. This study contains laboratory blanks, replicates, and the detection of false positives by fulfilling selectivity, matrix effect assessment, repeatability, and offering an error on the method requirements. According to Ogonowski et al.[45] 2021, studies with anthropogenic and natural microplastics are not representative, and the results frequently contradict those of other researchers.

(5) Dimension: Veronica Nava and Barbara Leoni[46] have recently reported and tested three protocols for the extraction of MPs from sediments. The duo tested two protocols based on use of density separation (NaCl extraction followed by use of NaI in first method and NaI extraction followed by centrifugation) on three size classes (3.08 ± 0.31 mm, 1.41 ± 0.23 mm, 0.42 ± 0.08 mm) and reported that the % recovery differed depending on the particle's dimensional class. Indeed, regardless of the methodology or polymer used, microplastic retrieval was inversely linked to dimensional class, with the number of particles recovered from the sediment matrix being less for the smallest dimensional classes. In contrast, 100% recovery was achievable for the largest particles, i.e., those with the greatest dimension in the range of 2–5 mm. As a result, the size of the microplastics might have a big impact on healing.
When providing statistics on microplastics, all these above factors should be taken into consideration. Indeed, monitoring in organic-rich particle matrices like soil is still in its early stages, and methods for microplastic separation are still being debated. As a result, future studies should include analytical control when separating and quantifying microplastics from sediments and soils in order to provide uncertainty measurements about the data reported
(6) Overlapping densities: Following density separation using salts with higher densities, some fractions of thermoset plastics types, such as PET/PVC, LDPE/PP and HDPE/PP, must still be separated. The difficulty is that PVC and PET cannot be separated in this manner because their density ranges overlap[47]. The densities of the polymers are likewise quite near in the case of PE/PP, making it impossible to differentiate them based on density.
(7) Equipment building: Liu et al.[37] have created a system particularly intended for the extraction of microplastics from soil samples, consisting of an acrylic glass cylinder with an aeration disc at the bottom and two rows of 5 mm holes at the top which works on density separation, vacuum filtration and a solution recovery step. For ten different kinds of microplastics(PA, PC, PP, ABS, PE, PS, PMMA, POM, PET, and PVC), recovery rates of more than 90% have been observed. However, the separating cylinder is constructed of Plexiglas(PMMA), which is a significant disadvantage of the stated technique. Abrasion generated by stirring coarse soils may result in an overestimation of PMMA contamination in the samples; hence, a nonplastic substance should be employed, or PMMA should be omitted from the analysis.

All of the density separation methods now in use have drawbacks. In order to select the most appropriate chemical, the performance of the techniques, the environmental effects, and the cost effectiveness must all be addressed. Even though high-density salt solutions can be more successful in separating small microplastic fibers with high density, but they have little effect on bulk or fragment microplastics but major effect on the environment if used in bulk. Same observation has been made by Li at al.[48] and he explains that this is most likely because bulk and fragment MPs have a bigger surface area and can float up even in low-density salt solutions, whereas fibers with high densities require a high-density flotation solution to separate. The major share of the plastic waste comes from PP, PS and PE classes. According to the 2020 Report by Plastics Europe-Facts[49], these three classes account for more than 50% of the plastic demand. After evaluating the separation efficiency of several common solutions, Scheurer and Bigalke still recommend sodium chloride as the density solution because the authors believe that classes with high density (PVC and PET) play a minor role in the large microplastic fraction of their samples and will not cause too much deviation in the results in Europe. As a result, the selection of separation solutions must be done in compliance with local plastic demand circumstances. When the overall image of microplastic dispersion is still hazy, a denser separation solution is preferable. Use of NaI has been suggested in various studies[32,41] because of its_reusability, high density, possibly in combination with separation columns. Based on particle number, NaI solution provides good recoveries, but this is highly dependent on the type of plastic. Several chemical combinations for density extraction procedures have caused difficulties in deciding on the best approach. Recent studies[15,35] have proposed the use of calcium chloride ($CaCl_2$; 1.30–1.35 g ml^{-1}) as a safer, more cost-effective alternative. However, the large amounts of organic matter that may co-occur with microplastics in some environmental samples create an additional impediment to clean-up and enumeration using density-based techniques. But $CaCl_2$ is not suited for the separation of organic-rich materials, according to Scheurer and Bigalke's[50] research. Organic matter flocculates because Ca^{2+} may bridge the negative charge of organic molecules. As a result, the filter may be covered in a thick brownish substance that obstructs the measurement.Low-density polymers such as PE, PP, and PS can be separated from soil mineral matrix using deionized water and saturated NaCl solution, which are inexpensive, readily available, and environmentally friendly.

Recycling of salt solutions

Recycling, reuse, and reclamation of the hazardous waste is required for both expensive and environmentally toxic density separation solutions. Some methods of recycling of salts for density separation have already been documented. The NaI solution's reuse process[32] has been extensively researched. It can be recycled up to ten times through rinsing and evaporation stages without any chemical contamination and significant loss in a cost-effective manner (3.7 €/kg). As a result, the use of NaI is recommended because it is environmentally friendly and can be recycled multiple times as long as it is not used with a cellulose filter. The recycling process described in this study allows for the recovery of 95 % of the NaI salt after each usage. According to the findings of Rodrigues[51], $ZnCl_2$ solution may be reused at least five times while maintaining an efficiency of more than 95%. He reported that this salt solution is the most cost-effective approach for isolating MPs from aquatic samples. A saturated potassium formate (K(HCOO)) solution[52] may be filtered after use for density separation in aquatic environment, making it conveniently reusable. In Air-Induced Flow technique by Nuelle et al.[53], which uses NaCl for pre-extraction to reduce the initial sediment sample bulk and NaI for subsequent flotation of microplastics, was shown to be efficient for extracting common polymer types from marine sediments, including higher density polymers. NaCl was used for six repetitions in the pre-extraction step and NaI was reused for upto five times. Whilst most studies observed cost and material savings, it is vital to note that recycling and storage involve additional labor, materials, space, and energy.

Advancements in Density Separations: New Methods and Customised Sediment Separators

The innovation of density separation instruments is also improving the capabilities of researchers to separate low-density microplastics and microplastics < 500 µm. To overcome the limits of the separation solution and improve separation efficiency, multi-stage flotation-based separation techniques have been adopted which is based on the relative low density of plastic[53]. An effective approach for removing microplastics from soils using the flotation principle necessitates the consideration and balancing of environmental impact and operational expenses, both of which are dependent on the stability of the flotation solution.

Imhof et al.[31] developed the Munich Plastic Sediment Separator (MPSS). He employed pine oil in conjunction with a froth conditioner to increase wetting, lower surface tension, and facilitate the detachment of plastics from sediment in deionized water, resulting in low recovery rates (55.0 %), particularly for high density plastics. The gadget can separate large-volume samples and can analyze up to 6 kg of samples in one run. Microplastics have been successfully isolated from estuary silts and clay, as well as coarse beach sand, using this procedure . Coppock et al.[54] devised a Sediment-Microplastic Isolation (SMI) unit with a 95.8% extraction effectiveness for varied density microplastics. Using a plexiglass cylinder with a jagged edge overflow structure as the air flotation unit, Han et al.[41] constructed a device for recovering microplastics from soil and sediment samples, with typical microplastic recovery rates of 90% or greater. The JAMSTEC microplastic sediment separator (JAMSS) unit by Nakajima[55] (2019) and his group is a compact portable glass separator which uses NaI as the floatation media. This separator is simple to clean and reuse, enabling for the quick separation of microplastics from sediments, with microplastic recovery rates ranging from 94 to 98%. The standard decanting method, for example, the use of a beaker, is basic in design but microplastic adherence to the interior of the container is an issue when the medium is moved, resulting in a relatively poor recovery rate. However, the majority of the devices indicated above are complicated and require particular customisation, resulting in significant processing costs. Furthermore, for total separation, certain devices have many operating phases, which are labor-intensive and time-consuming as only little amount of sample can be treated at one time. Furthermore, using huge amounts of flotation liquid comes at a considerable expense and poses an environmental risk.

7.4.1.2 Elutriation

Elutriation is a method that employs a stream of gas or liquid to separate particles depending on their size, shape, and density in a direction that is usually opposed to the direction of sedimentation. The basic concept came from the realm of biology for extracting meiofauna from sediments. However, the extraction of microplastics is more challenging than that of live creatures, requiring the calculation of fluid velocity as a function of particle and fluid characteristics. Sample preparation, on the other hand, may be time consuming and requires pre-separation into the necessary size range. Classens et al.[56] modified it to extract microplastics as a first step from beach sand before density separation using NaI solution. A continuous flow of filtered water through a 15 cm column, containing pre-washed sediment sample (500 mL), from below agitates the sediment sample, dislodging the lighter particles trapped between sediment grains. The MPs are caught in the overflow by a very tiny filter at the top. Even though NaI is an expensive salt, by incorporating this elutriation phase, the amount of NaI required is lowered by at least 97 %.

In another similar study, Zhou et al.[57] designed an elutriation system on the lines of Classen's' device to maximize microplastic recovery efficiency by varying water flow and column diameter. He reduced the column height to 50 cm from 147 cm in Classesn's device and width between 5.06 -10.16 cm. To test the device's performance, the scientists used a control sample of 500 mL of sand and 50X5 mm plastic fragments. Under the restrictions of his model, the highest anticipated recovery of microplastics was 50 %. However, the system was simple to use and could be put together using common household items, which is an advantage over other filtering techniques for removing plastic pollution, such as triboelectrostatic separation, which does not operates in humid environments like those found near beaches.

Though the authors optimized the flow rate of water to ensure maximum extraction effectiveness, the sand recovery yield was still high. To overcome this short-coming, a pre-size fractionation (63 um-2 mm) of the sample could increase the method's adaptability. Based on this key note, Kedzierski[58] used granulometric(particle size sub division) approach to optimize the elutriation process with advantages including (1) the capacity to handle a large quantity of sample in a single run, (2) excellent recovery and viability, (3) speed, and (4) repeatability. He later with his team also developed a numerical model to determine the elutriation velocity of the fluid and reported that to limit the sand suspension during elutriation; particle velocity (based on duration of elutriation and height of column) must be tailored according to particle size. This approach, however, was invented and refined solely for sand sediments. Its efficiency is anticipated to be poorer in fine and/or organic matter-rich sediments that can agglomerate and/or react with plastics[59].

Table 2 : Extraction of microplastics based on elutriation technique

Author	Height	Width	Sieve size	Optimal Conditions	Sample amount	Saline Solution	Removal Efficiency	Ref
Classens	147 cm	15 cm	Top: 1mm Bottom: the 35 μm mesh has the function of a sample holder, supported on a 1 mm mesh.	Flux of 300 L/h of water for 15 min.	500 mL	NaI	93-98%	[56]
Zhou	50 cm	5.06-10.16 cm	Top: 3mm	385 L/h and 5.06 cm in column width for 10 min		-	50%	[57]
Kedzierski	186 cm	106 mm	Top: 63 and 32 μm					[58]

7.4.1.3 Pressurized Fluid Extraction (PFE)

PFE has been presented as a viable solution in the quest for alternatives to extract MPs in high purity from oils, sediments, and wastes. The particle size of the microplastics has little bearing on this separation approach, and even submicron particles can theoretically be studied[16] . A solvent extraction approach has been recently reported as a new methodology for detecting microplastics. This technique was introduced in 1995 by Dionex Corporation at the Pittcon Conference. The first honor[60] was its recognition as an official US Environmental Protection Agency (USEPA) method for detecting persistent organic contaminants in environmental solid samples. Since then, it has been employed in many routines for the determination of organic contaminants in complex matrices. This technique uses organic solvents to extract solid or semi-solid materials. High pressures (3.5-20 MPa) and elevated temperatures (313-473 K) are employed to keep the organic solvents in a liquid condition above its boiling point while increasing the kinetics of the extraction process once the temperature is high enough. The solvent extraction process enables matrix removal and MP enrichment to be completed in a single fully automated stage.

In 2016, two researchers from Australia, Fuller and Gautam[16], used PFE in combination with gravimetric quantification to measure microplastics from industrial soil and municipal waste material. This procedure involves two steps: static extraction followed by dynamic extraction. To eliminate all semi volatile organic substances (fats, oils), methanol was used at 100 °C during the first extraction phase. The microplastic fraction was recovered using dichloromethane (DCM) at 180 °C in the second extraction from the residual matrix. DCM extracts were collected, evaporated to dryness, and gravimetrically quantified. This approach performed well for several plastic kinds, including PE, PVC, PP, with average recoveries ranging from 84_% to 94_% despite the presence of wide varieties of physicochemical pollutants. Though they retrieved, plastic particles as fine as 30 um successfully, this approach has following drawbacks: (1) it is limited in its ability to offer size information, (2) the method is destructive which leads to morphological changes in the MPs, thus hampering the studies to aid in source contribution , (3) this does not provide information with plastic size > 30um, and (4) residues from materials containing various types of microplastics will have complicated FTIR spectra, which may necessitate spectral deconvolution skills or sophisticated methods to identify constituent polymers. The simplicity, affordability, speed, and uniformity in reporting concentration findings, as well as the possibility of automating the extraction component, are all advantages of this approach and is thus one of the most promising ways for plastic separation from soil samples.

In the second study of this kind, Dierkes et al.[61] combined Presuurized Liquid Extraction (PLE) and GC-MS pyrolysis technique for the quantification of MPS by using a less toxic and less volatile Tetrahydrofuran (THF) for the dissolution of the most abundant MPs (PE, PP, PS) and reached efficiency of 80 % in soil and sediment matrices., Pyr-GC-MS is destructive technique based on the polymer pyrolysis, which results in chemical bond breaking and formation of low molecular weight moieties from the non-volatile polymer. Thermal degradation products can be cryo-trapped, sorted, and identified based on their mass spectra. Identification is accomplished by comparing the retention time and mass spectrum to polymer standards or by using spectral libraries. Even though this technique has the benefit of high selectivity specificity, and provides a simplified overview of polymer types represented by a common chemical backbone exhibited by basic polymer clusters when a detection threshold is exceeded, it requires high level of equipment maintenance because the relatively heavy moieties resulting from polymer breakdown might condense in the capillary between the pyrolysis chamber and the GC, causing clogs and cross contamination.

In a more recent study, Okoffo et al.[62] further optimized this technique to analyze MPs in Australian biosolids using double shot Py-GC–MS. His method requires no pre-extraction clean-up phase or sample pre-treatment. PE, PVC, PP, PS, and PMMA mass concentrations ranged from 0.1 to 4.1 mg/g dry weight (dw) across all samples, with a total plastic concentration of 2.8 to 6.6 mg/g (dw).

7.4.1.4 Magnetic separation

The magnetic field, which is a controlled force source, has received a lot of interest in separation research. When looking for suitable removal methods, one of the most easily scaled ways is the use of magnetic adsorbents. It employs magnetic seeds and acid in conjunction with an external magnetic field to increase separation speed. Polymers are hydrophobic, thus hydrophobic magnetic materials can be utilized to adsorb them and subsequently collect them for removal.

Grbic et al.[18] synthesised hydrophobic iron nanoparticles (Fe-NPs) that bond to plastic through silanization and facilitate magnetic recovery, as part of a new approach for magnetic extraction of MPs from sediment and water samples. The technology is based on the usage of tailored iron nanoparticles that bind to polymers and allow magnet recovery. These customized NPs with a high surface area to volume ratio are treated with a silane with extended hydrocarbon tails to improve hydrophobicity and sorption to the MPs. Magnetic extraction was shown to be more effective in removing small microplastics. The recovery rate for sediments as reported by Grbic et al.[18] was poor because soil particles prevent Fe nanoparticles from colliding with microplastics due to relatively lower surface area to volume ratio of the MPs. Furthermore, if lipophilic chemicals or biota are present in soil samples, nonspecific binding of nanoparticles will greatly limit the impact. As a result, he claimed that this technology may be more suited post density-separation or digestion and for drinking water treatment. In this regard, relatively high values were obtained (93% for water samples, 78% for sediment samples), and the technology might be applied to future MP research.

In yet another recent study, Rhein et al.[63] affirmed that tiny MP particles may be recovered successfully from dilute solutions using magnetic filtering. This has been discussed in the next section of this article. Even though magnetic separation procedures do not modify the sample's structural integrity, many gaps are seen during separation. As a result, most studies have emphasized the use of extraction as a pre-treatment step rather than as a solitary separation approach.

7.4.1.5 Electrostatic Separation

Electrostatic separation is a potential approach that has been used in the processing of plastic trash. A critical aspect in attempts to simplify MP separation is that the sample bulk be lowered and biological components eliminated without affecting the particle characteristics. A reduction in sample mass would result in a large reduction in the amount of chemicals required for subsequent processing. Based on this theory, Felsing et. al.[17] proposed Korona-Walzen-Scheider, a device used in the recycling business that takes advantage of the electrostatic characteristics of plastic. The idea behind this method is based on the varied electrical conductivity of the sample particles, with mineral particulates in soil and sand being more conductive than polymers in general. A high-voltage electrical field (max. 35 kV, DC) between the grounded drum and an above-mounted rake-shaped electrode charges the particles. Mineral particles that are more conductive discharge faster and leap off the drum, with the separating flap directing them into the "sediment container." Less conductive polymers discharge more slowly and remain stuck to the revolving drum, only to detach later and fall into a separate collection container. Here the use of well dried and unconsolidated samples is a must; otherwise, the separator will not be able to separate the components because the presence of water alters the electrostatic behavior of particles which is also a time-consuming step lasting up to few days.

Electrostatic separation developed as an environmentally benign method among all the separation procedures since it permits the generation of fractions enriched in the elements of interest from biomass particles ranging in size from 10 to 500 m but in a recent validation study by Enders, some of the drawbacks of KWS are highlighted. The sample drying process is tedious, and parameters such as humidity prior to freeze drying is difficult to manage. Another limitation is in the soil observation data indicates that the presence of tiny particles and agglomerates (as is characteristic for soils) in samples restricts the use of KWS. Electrostatic separation of microplastics samples need further treatment processes such as density separation and digestion[64]. However, one crucial element to consider when comparing electrostatic separation experiments is that the mineral composition has a significant influence on the recovery rates. Electrostatic separation does not require any chemical treatment, although it is best suited for big samples, but sediment loss is a drawback.

In a study concerning removal of polystyrene nanoplastics (PNPs) from aqueous solutions, fly ash modified with Fe ions has been tested and the adsorption-desorption setup by Zhao et al.[65] showed that there are strong interactions between the PNPs and the new magnetic material synthesized with reusability up to 4 times. Another point of stress is that because cohesive soil samples invariably form agglomerates and microplastics in the soil are not easily liberated, the applicability of this approach for soil has to be determined.

7.4.1.6. Oil Extraction Protocol

The oil separation method is based on the oleophilic properties of plastic polymers. Oils have hydrophobic characteristics that can assist remove plastics from environmental samples and increase recovery rates. MPs can be retrieved through the water-soil interface since the bulk of soil or sediment particles are hydrophilic. Although recovery rates have varied between investigations, they have been shown to decrease surface tension and aid in the removal of plastics from sediment samples. Even with high-density polymers, the oil–polymer clusters have a lower total density than water. The non-polar lipophilic component of these long-chain aliphatic hydrocarbon-dominated fatty acids can attach non-polar lipophilic carbohydrate surfaces of synthetic polymer fragments in a quasi-micellar way due to their large molecular weight. In the density separation, NaCl being environmental friendly is not suitable for all polymer types, Zinc chloride ,one of the promising candidates for extraction of MPs is toxic to aquatic environment.

However, combining the non-toxic nature of oil and the fact that it does not depend on specific density as most density based separation method works, in 2017 Crichton proposed a new method for separation of MPs from soil using the oleophilic properties of polymers. The outline of this method consists of shaking the dry sediment with water, followed by addition of few millimetres of oil, placing the mix in a shaker for about 30 seconds to allow the sediment and plastic to stick with oil. Water is decanted followed by a vacuum filtration step. The oil layer is next filtered, and the filters are treated with reagent to eliminate any oil residues that may interfere with future examination. For the samples rich in biomass, it has been suggested by the author to perform digestion before oil extraction protocol. According to Crichton et al.[15] , recovery rates range from 90–100% for all seven investigated virgin polymer types, outperforming two density separation techniques with NaI and $CaCl_2$. The technique is straightforward, safe, inexpensive, and time-efficient, This method has been recently validated by Crew et Aa.[66] for studying the variation in the distribution, abundance and diversity of microplastics across St. Lawrence river. However, this procedure may limit the recovery rates due to one of the following reasons: 1) All the MPs are not transferred to the oil phase and may remain in the sediment. The flotation of MPs from the environmental matrix to the oil–water interface is the focus of oil–based extraction. Some oil-extracted MPs may be contaminated by unwanted particles or lost at the oil–water interface due to the difficulty of adequately isolating oil–water boundaries.. 2) The MPs remain intact on filters, glass surface while transferring leading to underestimation. 3) Recovery rates decrease while working with real environmental samples. 4) Due to reagent alcohol, MPs (particularly PE and PS) have high static electricity during examinations 5) After the rinsing step with alcohol, some of the MPs remain in the oil, adding another point to underestimation. Further research attempted to increase the overall performance of this system by adjusting many phases.

To minimize the shortcomings of the first proposed method, different groups have tested different oils listed in Table 2. Microplastics have also been extracted from soil and compost samples using olive oil. Without the need for oxidation, the oil separation method can be utilized to extract a variety of soil types. The recovery rate from low-density polymers to high-density polymers is likewise significant, with an average of over 90%. In yet another experiment[67] , the recovery rate was boosted by adding a drop of olive oil to the sodium chloride solution, increasing the rates from 64% to 80%. When compared to other salt solutions, oil extraction is both cost-effective and environmentally benign. It should be emphasized that oil extraction necessitates a detergent cleaning phase.

For a wide spectrum of polymers, the optimum oil should have a strong affinity between oil and each micro-polymer. The viscosity, density, and surface tension of the oil determine the oil's attraction for MPs. Higher the viscosity,better is the interaction between the oil and the micro-polymer. Among the oils listed in Table 2, Castor oil[68] has the highest viscosity of 580 cP in comparison with the natural plants oils listed. The effectiveness of utilizing oil to extract MPs from a solid matrix is heavily reliant on the use of an appropriate agitation rate. A very recent study[69] has created a highly selective microplastic separation technique that consists of two phases, both of which include hydrophobic interactions, namely interactions between MPs and oil in the first step and interactions between oil and a hydrophobic/magnetic a polydimethylsiloxane (PDMS)-coated nickel (Ni) foam adsorbent in the second step. This[69] work tested two vegetable oils (sunflower & canola oil) and mineral oil, highlighting the superiority of mineral oil in extracting MPs from soil.

Mel Constant[59] , by comparison of the different extraction methods that combines OEP and density separation, has shown that there is no robust protocol for extracting all types and forms of microplastics from fine sediments, and that research efforts to develop a reliable method must take into account the interaction of MPs with other particles as well as the electrostatic properties of MP. The relatively small sample size of sediment that may be analyzed per study, which hampers representativeness, is a limiting issue in the impending separation of sediment samples with this technology. Extrapolation of determined MP concentrations to a larger volume increases the uncertainty. Interestingly, oil separation is a time-saving and environmentally beneficial process that deserves to be developed further as an alternate separation method.

Table 3: Summary of oils used for extracting microplastics

Oil Type	Viscosity	Matrix	Separator	Amount of Oil	Sample Mass	Extraction time	Polymer Type	Mean Recovery Rate	Ref
Canola Oil	86 cP	Aquatic Sediment	Separatory Funnel	5 mL	50 g	90 to 168 minutes per sample	EPS, PVC, ABS, PA, PES	96.1% ± 7.4	15
Canola Oil	86 cP	Fluvial/artificial sediments (enivironmental samples)	Sediment Microplastic Isolator	10 mL	10 g	15 minutes for water 45 minutes for sediment	EPS, CoPA, PA6, PE, PP, PS, PVC, PVDC, , PET, synthetic rubber	85.8%	70
Castor Oil	580 cP	Marine beach sediments Agricultural soil Marine suspended surface solids Fluvial suspended surface solids	Separatory Funnel	10 mL	1-10 g	-	PP, PS, PMMA and PET-G) spiked samples	99% ± 4 95% ± 4%	68
Olive Oil	84 cP	Soil Compost	Cylinder	3 mL	25 g 10 g	-	PE, PS,PVC,PC,PET PU	90% ±2% to 97% ± 5%	71
NaCl + Olive Oil	-	Sediment Water	Custom Glassware with Peristaltic pump	1 drop oil with 20 g w.w. NaCl	20 g	-	PP, PE, PA, EPS, PET	82%	72
Mineral Oil	95-100 cp	Sea sand Agricultural Soil Sea Sediment	-	0.25 g	2g	10 seconds	PP, PS, LDPE, HPDE,PET, PVC, PTFE	99%	69

7.4.2 Water Samples

The early evaluations of plastic in the coastal and marine environment were based on things floating on the surface or immediately under the ocean's surface. Most investigations on plastic contamination have been undertaken on the ocean surface to date. This is likely because nearly half of all plastic manufactured has a lower density than saltwater and is projected to float at sea, as well as the fact that water is one of the easiest and cheapest subjects to research. The other reason could be an attempt made by National Oceanic and Atmospheric Administration (NOAA)[38] at standardization of microplastics sampling methodologies, which focuses on water and sediment matrices. However, this recommended methodology can only be used to determine several plastics with sizes ranging from 0.3 to 5 mm, such as PE (0.91–0.97 g m/L), polypropylene (PP) (0.94 g m/L), polyvinyl chloride (PVC) (1.4 g m/L), and polystyrene (PS) (1.05 g m/L), which limits the range of microplastics detected in water samples. This technical memorandum[38], on the other hand, may be viewed as a first step toward the much-desired standardization of sampling and sample processing procedures for microplastics in water and sediments.

When we discuss water as an environmental matrix, we must use the plural since we are dealing with two separate forms, surface water and groundwater. Surface water may be found in rivers, lakes, seas, and oceans, whilst groundwater can be found in aquifers. Drinking water and wastewater are surface and groundwater categories based on their usage. In terms of matrix and organic composition, surface area, MPs sources, and sizes, urban waters differ from marine waters. The distribution of microplastics in the water column is driven by their physical characteristics (density, shape, size), environmental factors (biofouling, water current, waves, hydrodynamic profiles, and density). Freshwater and saltwater have different densities of 1.00 g/cm^3 and 1.03 g/cm^3, respectively, which could contribute to different distributions of microplastics in the water column in each system. Wastewater is yet another type of water that has been studied for presence of MPs. Freshwater samples are frequently richer in natural particles than surface saltwater, confounding sample analysis even further. Large amounts of microplastics are detected daily in wastewater treatment plants (WWTPs)[73] because the plant only impedes microplastics and tiny plastic particles in the aeration ponds, but a significant amount of microplastics escape through the filter and be released into the freshwater environment. To identify microplastics in these complex (i.e., with numerous types of matrices) and organic-rich environmental matrices, appropriate analytical techniques are required. In many instances, a combination of density and digestion methods (explained later) is used to eliminate both mineral and biogenic organic substances from the sample. The major source of drinking water for humans is freshwater (surface and groundwater). Despite the fact that the sampling processes in freshwater and marine settings are comparable, the quantities of organic and inorganic compounds in the samples differ significantly. Extraction of microplastics from water sources should be done according to the nature of the water, such as, for drinking water, bottled water, simple filtration or sieving followed by staining are enough while for samples with remains of organic matter, digestion method based on the content of organic matter, which is related to the source of water.

Water Volume Reduction Method

The volume of water samples collected fluctuates between studies; only a part of the samples is of interest and must be saved for subsequent processing. Large amounts of water are often filtered through nets to get these water samples. Thus, it should be the first step towards extracting microplastics from water samples with large volumes of water. Size-based separation procedures, such as sieving and filtering, are widely employed to remove microplastics from environmental samples or to minimize sample volume. Although the implications of sieving on polymers are still rarely discussed, sample transference across sieves may lead to microplastic loss, fragmentation, or cross-sample contamination. Water samples can be sieved first using 500 mesh sieves. For complicated and organic-rich water samples, centrifugation might be a useful alternative to sifting as a water volume reduction technique but again, processing samples takes a long time, making the approach inappropriate for marine and estuary solutions that need high sample quantities. For laboratory analysis, centrifugation has been already applied for waste-water bio solids, snow, vegetal rich samples, and biota studies[74]. After volume reduction, the treatment must be determined by the source of water.

7.4.2.1 Clean water samples: From drinking water to seawater

Intense research is being performed in the area of MPs separation from water to develop standard separation methods with the highest yield as first MPs were isolated from the marine waters and water is vital for life on earth, be it aquatic organisms, land animals, birds, or humans. The majority of drinking water—whether from a tap, a well, or a bottle—comes from surface or groundwater. Almost all drinking-water supplies obtained from surface water are filtered, and some are further treated. Samples coming from drinking water, bottled water,

sometimes water samples from places with few biological particles, tertiary treated wastewater or recycled water, freshwater are thus counted as relatively low organic matter samples for the purpose of this review and sometimes seawater samples too, as the sampling here is dependent on the location, weather, water-column depth. Adsorption, coagulation, membrane filtration, oxidation, and microbial degradation are the most prevalent ways for removing MPs from these types of samples and require minimal or no pre-treatment (in case of bottled water) and extraction here can be done with careful sieving or vacuum filtration. Samples that have little amount of organic matter present in them, can be isolated with density separation described in previous section and if required followed by digestion methods explained in next section.
In recent years, there has been a surge of interest in the study of micro/nanomachines. New technologies based on the removal of oil spill in water environment are also being applied to capture microplastics in the same league[75]. One such technology uses hollow micro submarines, based on sunflower pollen grains, carbon nanotubes. These cooperative micro swimmers may successfully catch, move, and release microplastics by combining the rolling and wobbling modes, resulting in microplastic elimination. Given the importance of modern technologies in addressing present concerns, UK government has funded[76] a concept based on hybrid green hydrogen and battery power to collect microplastics as the cargo submarine cruises between Scotland and Northern Ireland.

A fresh concept[77] for identifying the polymer kinds of microplastics in the field utilizing density separation with non-hazardous reagents has been demonstrated recently. The study mainly addresses the issue faced by scientists working in remote places in the field who lack access to analytical equipment (e.g., spectroscopy) to determine the kind of polymer in a microplastic sample. The discovered method employs ethanol, distilled water, and sugar combinations to produce a variety of solutions with varying specific densities. Polymer samples are then categorised based on their ability to float or sink in specific solutions. The approach proved successful in distinguishing seven common polymers taken from the water and shoreline of Samos, a Greek island in the Aegean Sea. For example, in a 7:11 ratio of EtOH/H2O can distinguish between LDPE and HDPE based on their floatability in the given solution.
Though, future studies need to be carried out in more realistic settings that mimic the plastics prevalent in marine habitats and seawater samples. Furthermore, additional study is required to expand this process to include alteration of the same polymers and new polymer kinds. Nonetheless, this is a fantastic beginning point for additional discussion and research into more ecologically friendly and cost-effective methods of identifying microplastics. In another new type of study, a proof-of-concept of using superconducting magnets for collecting MPs from sweater has also been proposed.

7.4.2.2 Wastewater

Wastewater and sludge fall under this category. They are more difficult subjects to analyze than other samples because of dense matrix of organic components, bacteria, and inorganic particles bonded together by biopolymers and has a high affinity for most polymer surfaces. Many methods for analyzing MP concentrations in water and sludge have been developed, but the knowledge is still in developing phase. Microplastics frequently circumvent treatment units, infiltrating and accumulating in the aquatic environment, whereas big plastic particles are efficiently removed during wastewater treatment. A considerable number of water treatment plants are located near the ocean and saltwater, resulting in a substantial source of microplastic discharge.

6.2.2.1 Conventional Wastewater Treatment Systems.

To remove particles, organic materials, and, in some cases, nutrients from wastewater, traditional wastewater treatment uses a combination of physical, chemical, and biological processes and activities. In order of increasing level of processing, general terminology used to describe different degrees of treatment include preparatory, primary, secondary, tertiary, and/or advanced wastewater treatment. Despite the fact that facilities prominent in WWTPS, such as ultrafiltration, membrane filtration are not especially intended to extract MPs from wastewater, excellent removal efficiency are attained. According to the stage-wise removal of microplastics in wastewater treatment plants, usually 35–59% of microplastics are removed during preliminary treatment and 50–98% are eliminated during primary treatment[78]. At this step, the principal method for removing microplastics is the skimming and settling of entrapped microplastics during gravity separation. Despite excellent overall removal percentages, the large volumes of water processed result in estimates of plastic particles entering the environment every day with treated effluent from a single facility. In these treatments, the type of the MPs has also been hypothesized to have a role in removal efficiency, with primary MPs being more successfully removed than secondary MPs since the former is often formed as microbeads, whilst the latter is more likely to be textile fibers or other debris.

New Techniques For Removal Of Microplastics

Ferrofluid based Separation

Grbic et al[18]. published a research on the removal of microplastics from water using a magnetic extraction approach (explained above). The novelty of this approach was that while dealing with small-sized microplastics, the extraction efficiency was higher because more Fe nanoparticles could be bound per unit mass of plastic. The advancement of this technology is nano-ferrofluids which are colloidal compositions comprising of single-domain magnetic nanoparticles in a liquid carrier and this this approach has piqued the interest of the industrial and academic scientific communities during the last several decades because of their large surface areas that can efficiently bind contaminants, especially in removing oil spills from water and now tested for removal of nano and microplastics in WW. The formulation of ferrofluid involved has been tested[65] by mixing a lubricating oil and fly ash (FA) to modify the iron nanoparticles, by two independent authors. Zhao et. al[65] modified FA with Fe nanoparticles by co-precipitation method for removal of Polystyrene nanoplastics from sewage as a new eco-friendly adsorbent. The author reported a strong interaction between Fe modified FA and PS NPs, highlighting the reusability of this new magnetic materials up to 4 times. However, this method was only tested for PS NP and tests for MPs still need to done. In a similar kind of study by Hamzah et. al[79], tested four types of oil (cooking oil, used cooking oil, lubricating oil and used lubricating oil) for making ferrofluid to isolate MPs from laundry wastewater. His testes were based on the hypothesis that if oil can trap all microplastics, magnetite can remove a significant amount of microplastics suspended in water. According to the results, the production of ferrofluid using lubricating oil resulted in the maximum microplastic extraction efficiency (99% PET removed from synthetic WW & 64% PET removed from laundry WW) related to the stable features of a comprehensive mixture of hydrocarbons. This method needs more validity as the size of the PET taken was 2mm, and thus should be further tested with actual MPs smaller in size.

Photocatalytic micromotors

Micromotors are miniature self-propelled machines that have caught the interest of academics all over the world due to its applicability in oil removal, metal/metalloids removal and organic matter degradation. Wang et al.[80] demonstrated the robust photocatalytic removal of polystyrene microplastics from wastewater using an Au-decorated TiO2-based micromotor under UV light. Because they are photocatalytic, these micromotors can move in water due to photocatalytic reactions on the particles, thus requiring no fuel. The shortcoming of this method however is the lack of selectivity of micromotors for varied spectrum of microplastics.

There are some other methods that have been tried such as use of bioinspired moleculs for MP removal, made up of combination of organic and inorganic molecular framework, with an inclusion unit (IU) , an alkoxysilyl functionalized bioinspired component and a capture unit (CU) to catch MPs and NPs in IU based on based on hydrophobic and Van Der Waals interactions, and remove trapped NPs/MPs by a sand filtration device[81]. As no experimental work has been done on this technology, this method lacks practicality, in future exploiting this adaptable host-guest system to protect filtration membranes against NPs/MPs can be however considered.

7.4.3 Biota

The bioavailability of microplastics to marine species is one of the most significant environmental dangers linked with them. The extent to which biota consumes microplastics is critical for determining and monitoring 'excellent environmental status' for plastic pollution. A number of studies have indicated that microplastics are consumed by marine biota. As a result, researchers must be able to reliably isolate, identify, and count microplastic waste eaten by or entangled with biota. MPs. In recent years, a growing variety of procedures, including as dissection, depuration (in particular cases)[82], homogenization, and tissue digestion, have been proposed and modified, with the choice of one or more approaches being primarily driven by the research subject being investigated.

Digestion has become the most commonly used method in recent years for microplastic isolation from biota tissue. Many digesting methods have been devised, including bases like NaOH or KOH, acids[83] like HNO_3, HNO_3, HCl, $HClO_4$; enzymes[84] and oxidants[85] such peracids, and sulfuric acid (Table 4).

Several methods for dissolving organic material with acids have been introduced into microplastic research. Strong oxidizing agents have usually been used in digestion; however, synthetic polymers can be destroyed or harmed by these chemical treatments, especially at higher temperatures. Acids can destroy biogenic substances with a high level of efficiency (94–98%), but they can also dissolve polymers. Although acidic digestion affects numerous polymers, it should be avoided and utilized with extreme caution when other approaches fail.

KOH is effective at breaking down fish tissue but saponification frequently complicates traditional potassium hydroxide (KOH) digestion methods in lipid-rich (fat) tissues[86]. This reaction results in a glycerol and fatty acid (soap) solution, which can encase microplastics, preventing their recovery, and clogging filter resulting in lower filtration efficiency, or completely stopping it. To lessen the impact of lipids on microplastic extraction, additional method stages that do not interfere with microplastics (e.g. ethanol) are necessary. This approach has been widely used for water and sediment samples.

While enzymatic digestion is a fresh separation approach in MP research, chemical treatments are the most commonly used method because to their lower cost and relatively good recovery rates (explained in detail in next section).

Another popular method for confirming MP ingestion in marine biota (larger field collected organisms)[89] is to dissect the gastrointestinal tract (GIT) and chemically degrade the tissue, leaving only indigestible remnants, including putative microplasics. The digestive system can be isolated and kept for big species, whereas tiny organisms can be studied fully, depending on their size.

Table 4: Digestion methods for extracting MPs from marine biota

S.No.	Reagent	Organism	Exposure time	Temp	Ref.
1	Pancreatic Enzyme & tris	Bivalve Tissue	12 hrs	37.5 ℃	[84]
2	HNO_3:H_2O_2 65% :30% 4:1 by volume	Mussel	30 min	50 ℃	[83]
3	10 M NaOH 5:1 (w/v)	Mullet	48 hrs	60 ℃	[87]
4	HNO3: NaClO 1:10 by volume	Herring	5 min	Room temp	[88]
5	H2O2 (30%)	Bivalves	65 ℃	24 h-48 h	[85]

7.4.4 Organic Matter Removal Methods

One of the most difficult aspects of microplastic detection is distinguishing between plastic and naturally occurring bits. Removal of organic matter from the complex matrices is indispensable as without this step it would not be possible to isolate the desired end product because even after routinely used isolation procedures, similar seeming remnants of shells, plankton, vegetation, or deposits are still present in the sample due to the hydrophobic nature of plastics, increasing the effort for microplastic particle recognition by optical or spectral analysis methods. Biogenic and non-biogenic components of the sample must be eliminated without degrading microplastics to reduce labor and error in microplastic analysis. As the size range of MPs vary from anywhere between 1 mm and 1 um, the smaller sized particles are more pronounced and have higher impacts as stated earlier, and because they have greater surface area, MPs with small diameter are more sensitive to digestion methods than the larger ones. When studying mineral-dominated sediments like sandy beach deposits, organic matter removal is not often done, but it is usually a required processing step for low-energy estuary, lacustrine, or pelagic sediments, and it is required when examining soils, sludge or compost. It's usually done after density separation, when the sample has been reduced to only microplastics and lower density organic materials however some studies have done it first[90]. Though the order in which these steps are completed had no effect on recovery efficiency in spiked samples, it may result in greater microplastic recovery in soils where they are trapped within organically bound soil aggregates. Thus, the nature of the sample, the analyte, the availability of reagents, and the equipment typically play a critical role in the digesting technique selection since it helps to pick the optimal conditions ideal for yielding the largest yield of extractable metals. The following factors must be optimized: 1. Digestion reagents (acid, base, oxidants, enzyme); 2. Temperature of digestion (room temp, high temp.); 3. Digestion Period (hours, days); 4. Digestion steps (1,2 or more); 5. Concentration of reagents. All methodologies to which plastic samples are submitted must not change their properties: weight, quantity, or shape.

7.4.4.1 Acid Digestion Methods

To decompose organic materials in various samples, acids such HNO3, HCl have been used in open or closed systems, in combination with high pressure and temperature, to oxidize substances resulting in molecular cleavage. The matrix influences the efficiency of biogenic organic matter decomposition. Strong hot nitric acid solution has been shown to effectively remove organic matter from biota tissues[91], mainly made up of carbohydrates, proteins, and fats, also liberating trace elements from many materials in form of highly soluble nitrate salts. But the use of nitric acid was found to be unsuitable when MP extraction was done in mangrove vegetal litter[92] as it has a degrading effect on polyamide, polystyrene, nylon fibers and its tendency to change the color of some plastics such as PE, PP, PVC. When combined with other acids, oxidizing power of HNO3 can be boosted. For example, although hydrochloric acid is a non-oxidizing acid when used independently, it becomes a potent oxidizing agent when combined with nitric acid (aqua regia) in a certain proportion (HCl: HNO3, 1:3). This is due to the fact that the products of their reaction, nitrosyl chloride and chlorine, are powerful oxidizing agents. It has been reported to be less aggressive than nitric acid at almost same concentration Acid treatment has the advantage of destroying some inorganic particles such as carbonate (particularly in sediments)[91]. As a result, acid digestion is not suggested for biogenic organic material in sediments and water samples, unless the sample matrix has a high concentration of calcareous compounds.

Sulphuric acid[93] has also been used for carrying out acidic digestion, also to avoid precipitation of calcium carbonate in the beach sediment samples in combination with hydrogen peroxide to form peroxymonosulfuric acid, which oxidized organic residues. But the sample has to be stirred for one week.

In spite of the above-described shortcomings of acid digestion methods, the International Council for the Exploration of the Sea (ICES)[94] considers acid destruction method to be the most suitable for digestion of tissues as it not only digests the tisues, but also removes all other organic material, leaving only silica (e.g. sand particles) and plastic particles. The approach suggests using a 4:1 combination of HNO3 and HClO4 as digesting agents and has marked the importance of further improvements as nylon fibers are degraded through this method.

To improve the purification of microplastic derived carbon, a new thermos analytical approach has been employed to remove organic matter, followed by an acidification procedure that removed inorganic carbon (e.g., carbonate minerals and shells) using >85% phosphoric acid[95].

7.4.4.1 Alkaline Digestion

Alkaline digestion is another type of digestion experimented for removal of organic matter. However, depending on the reagent and incubation temperature, results may vary. For example, Hurley et al.[96] reported substantial degeneration in polyethylene terephthalate (PET) and polycarbonate (PC) MPs after digestion with NaOH (10 M) at 70 °C. This impact was less noticeable in when 1 M NaOH was used, with evidence of 'peeling' (PET) and the development of a matte texture in PC.

Alkaline hydrolysis is good at degrading proteins, it is widely used to remove microplastics from biota. The optimal approach for extracting microplastics from biota has been identified as 10% KOH at 60°C[96]. Cellulosic and chitinous material, on the other hand, are resistant to KOH and NaOH treatment and may be present in both sludge and soil. Furthermore, alkali-insoluble humins are frequently the most common organic portion in soils. This explains why NaOH and KOH have poorer removal efficiency Another innovative strategy is to eliminate cellulosic-rich sample components using a urea solution with thiourea and NaOH; however, this method requires the use of the microplastic degrading NaOH. Also this method requires another oxidative digestion step. And this has been reported[97] recently that a mix of urea:thiourea: NaOH can dissolve cellulosic materials, but with its own sets of pros and cons.

According to the findings of Pfeiffer and Fischer[91], digestion processes that use alkaline protocols are also less suited for use on sediment and water samples. These samples are distinguished by larger quantities of cellulose, hemicellulose, and lignin from plant remains that are only slightly modified by alkaline digestion. Furthermore, NaOH and, to a lesser extent, KOH have an effect on PET. PET makes up the majority of fibers in environmental samples and, as such, should not be omitted when adopting treatments that cause significant degradation.

A mix of 30% KOH and NaClO in ratio of 1:1 has been reported[13] to be the most appropriate due to its widespread availability and excellent digestive capabilities, and advocated as a rapid, economical, and effective digestion approach for sediment samples.

7.4.4.3 Oxidative Digestion

Oxidizing agents are attractive possibilities for the most efficient digestion of a variety of sample matrices as it can digest grease, cellulose, fat, and chitin shells. Oxidation using hydrogen is a typical method for eliminating organic molecules from environmental matrices, especially from soil samples. The commonly used oxidizing reagents is Hydrogen Peroxide. H_2O_2 may also aid in the filtering and identification of MPs. Despite this, the effectiveness of H_2O_2 has been questioned[96]. Many polymers, although not all, are resistant to hydrogen peroxide treatment. For example, polyethylene (PE) and polypropylene (PP) particles 1 mm in size, a size loss of 6.2 % was reported for extraction in marine sediments. Cole et al.[14] discovered that only 25% of biogenic material was eliminated after 7 days of treatment with 35% H2O2 at room temperature. Some experiments have used higher temperatures during peroxide oxidation to shorten reaction time. Sujathan et al.[98], for example, utilized 30% H_2O_2 at 70 °C to reduce the reaction time to around 12 h. While 70 °C is lower than the continuous operation temperatures (COTs) for the majority of popular polymer types, the authors cautioned that PMMA particles may be damaged. A modified strategy employing lower temperatures may be able to address this problem, however the impact on reaction time must be evaluated. Temperature regulation plays a significant role in digesting the unwanted matter. For example, at room temperature, the use of oxidants such as H_2O_2 has only a modest influence on synthetic polymer weight and size but can cause discolouration. An increase in temperature to 70–100 °C, on the other hand, is believed to induce a considerable reduction in weight and size of PA[96].

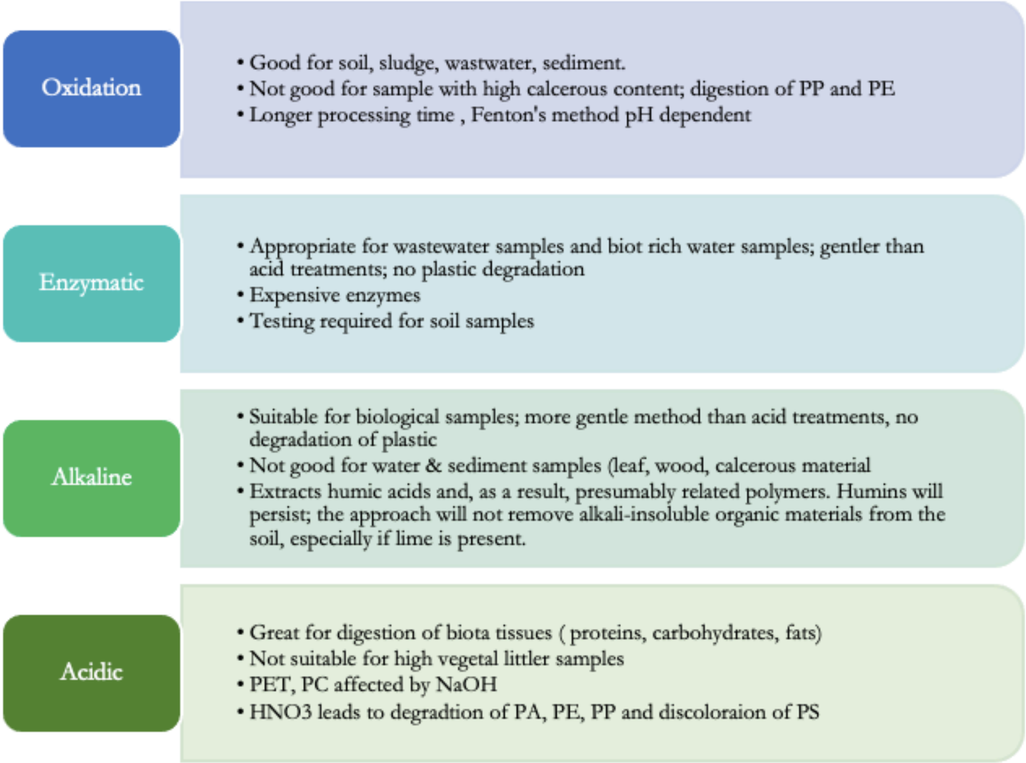
The wet peroxide oxidation (WPO) method is among the most successful ones for digesting organic matter in soil, sludge, wastewater and sediments. Furthermore, the reaction is faster than standard H2O2 oxidation, often requiring less than 1 hour to handle wastewater samples. It entails the use of a combination of H_2O_2 and ferrous ion, Fe^{2+}, known as the Fenton's reagent. This method has been recommended by NOAA that employs 30% H2O2 and heats the sample solution to 75 °C. The ferrous ions catalyze the breakdown of H2O2 and the formation of radicals, which function as powerful oxidants. Plastics can tolerate peroxide oxidation, whereas organic matter degrades at 75 °C [38]. The protocol's viability is mostly determined by the management of the pH (optimal at 3.0) and the reaction temperature (40 °C). When the pH range surpasses 5–6, this approach may be deleterious in carbonate soils owing to the development of Fe(OH)3 precipitates. However, when using H2O2 or Fenton's reagent for the digestion of organic-rich materials, significant temperatures may arise in the absence of external supply due to exothermic oxidative processes. Temperatures above 40°C or 50°C are not suggested based on the results by Pfeiffer and Fischer[91]. This can be accomplished by lowering the concentrations and/or immersing the sample beakers in a cooling water bath. Application in various dosages may also be tried for improved performance. In another study[74], Fenton's reagent with NaCl has been reportd to reduce organic matter in estuarine samples by 98% with almost no effect on polymers.

Digestion time is in oxidizing methods is crucial, as it governs how long the matrix is exposed to the oxidizing acid. Exothermic reactions can be accelerated by increasing the period of exposure, which increases the extent of solubilization of the polymer of interest from the biological matrix. The normal time to complete a wet digestion via open vessel digestion is 1 to 2 hours, however this might vary depending on the circumstances. As a result, it is critical to pick the most effective digesting temperature and duration to get the best element recovery. Given the importance of time in decision-making and protocol selection, as well as the demand for procedures that do not underestimate the number of MPs, microwave-assisted wet digestion (MAWD) has been suggested as a viable strategy for the breakdown of biological tissue, with relatively quick and high efficiency[99].

7.4.4.4 Enzymatic

Enzymatic degradation is another promising method for removing organic waste, which employs physiologically specialized techniques of hydrolyzing proteins and breaking down tissues. Enzymes, as opposed to chemical digesting procedures, are less dangerous to human health but assure no microplastic loss, degradation, or surface change. They are mostly employed alone, but sometimes in combination with other digestion methods.Proteins, lipids, and carbohydrates are examples of organic materials that can be precisely eliminated. Cole et al.[14] proposed the use of a single enzyme (proteinase-K) to separate MPs from saltwater samples containing a high concentration of planktonic species. Although a high level of separation was achieved (97%), a notable disadvantage of the method is the high cost of the enzyme that is often utilized in molecular biology procedures. Meanwhile, low-cost enzymes have been effectively utilized to remove plastic particles from mussel tissue samples such as lipase, amylase, chitinase, and cellulose throughout the breakdown process.

Loder et al.[100] appear to have created the most thorough enzyme-based technique. The Basic Enzyme Purification Protocol (BEPP) consists of a series of purification stages that include sodium dodecyl sulfate (SDS) treatment, cellualse enzymatic digestion, and peroxide oxidation. Because it includes protease and lipase, the enlarged version (Universal Enzyme Purification Protocol (UEPP)), looks to be more appropriate. The lipase treatment aims to break down lipids in samples that often contain a lot of them, such as tissues from various biota.



Oxidation
- Good for soil, sludge, wastwater, sediment.
- Not good for sample with high calcerous content; digestion of PP and PE
- Longer processing time , Fenton's method pH dependent

Enzymatic
- Appropriate for wastewater samples and biot rich water samples; gentler than acid treatments; no plastic degradation
- Expensive enzymes
- Testing required for soil samples

Alkaline
- Suitable for biological samples; more gentle method than acid treatments, no degradation of plastic
- Not good for water & sediment samples (leaf, wood, calcerous material
- Extracts humic acids and, as a result, presumably related polymers. Humins will persist; the approach will not remove alkali-insoluble organic materials from the soil, especially if lime is present.

Acidic
- Great for digestion of biota tissues (proteins, carbohydrates, fats)
- Not suitable for high vegetal littler samples
- PET, PC affected by NaOH
- HNO3 leads to degradtion of PA, PE, PP and discoloraion of PS

Figure 2: Summary of digestion methods used for removal of organic matter.

Table 4: Organic removal methods used in various studies

S.No.	Matrix	Method	Main composition	Temp	Digestion Time	Remarks	Recovery	Ref.
1.	Organic rich freshwater	Density Separation with ZnCl2 + centrifugation	7% NaClO	50 ℃	6h – 12 h	Nylon : digestion LDPE: centrifugation	94%	74
2.	Mussels	Oil Extraction with NaCl	30% H2O2	60 ℃	40 h	Study needed for small microplastics Only done for PP, PVC, PET	95%	101
3.	Vegetal rich clayey sediment	Density separation with ZnCl2	30% H2O2	70 ° C 100 ℃ 100 C°	1 h 3 h 7 h	Muti digestion steps High temprture can degrade MPs	-	92
4.	Algae, Driftwood, Feathers		H2O2+Fe	50 ℃	1 h	Good for plant removal organic matter Cellulose acetate is degraded	65.9%	102
5.	Fish, Muscle		KOH	50 ℃	1 h	Good for animal tissues Cellulose acetate is degarded LDPE mass loss	58.3%	102
6.	Edible salt	Density separationwith NaI + centrifugation	H2O2	65 ℃ 30 ℃	24 h 48 h	MPs lost during centrifugation	95%	103

Now that all the digestive methods have been taken into account, it is vital to mark that the composition of the matrix must be given preference, as different reagents behave differently with the constituents of the given matrix because, for example, organic matter to be removed is connected to marine creatures or biogenic tissues, the methods used may not be efficient in circumstances where vegetal matter predominates such as in soil or sediment as they consists of humic substances which are complex polymers created from the breakdown products of plant and animal leftovers' chemical and biological degradation. They're dark-colored, acidic, and mostly aromatic compounds with molecular weights ranging from a few hundred to tens of thousands. They may be divided into three major groups:

1) Humic acid - soluble in dilute alkaline solutions, although acidification of the alkaline extract can precipitate it.
2) Fulvic acid - soluble in alkaline solutions, but acidification makes it soluble.
3) Humin cannot be recovered from soil or sediment using dilute acid or alkaline solutions.

The sediment matrix has more or less the same composition a, except it has higher calcareous concentration, often found in shallow waters near land but even among sediments from various sources or water samples from various marine or freshwater settings, each sample is unique. Calcareous compounds show very little reactivity towards oxidizing agents and bases, but are easily dissolved by both HNO3 and HCl tested at all concentrations and temperatures as tested by Pfeiffer and Fischer[91] . In order to get the optimum digestion outcomes while also considering the best possible preservation of the targeted plastic particles, all methods are recommended to be tested for extraction efficiencies in laboratories before and during use, as efficiencies can vary between personnel and the method used. This frequently necessitates a multi-stage approach involving the successive application of various digestive solutions. In most cases, interim washing is required between the individual doses, which increases the danger of contamination.

According to the literature, any method that uses aggressive reagents at high temperatures, and prolonged digestions will harm MPs.

The reviewed studies shows that the studies employing may be accurate in terms of polymer identification, but yield may be skewed in terms of size estimates. Thus, different chemical treatments can degrade or injure synthetic polymers in different ways, with enzymatic treatment causing less damage but not being widely employed due to the expensive cost of enzymes. The chemical resistance data can be referred before selecting any chemical. Here a part of the chemical resistance is shown[104]. For other reagents, the complete chart can be found elsewhere[104].

Table 5: Chemical Resistance Data of Common Plastics

S.No.	Reagent	%	PA	PC	PE	PET	PP	PS	PVC
1	HNO3	20	-	R	R	R	PR	R	R
2	HNO3 @ 50°C	70	NR	NR	NR	NR	NR	NR	NR
3	HCl	35-36	NR	NR	R	NR	R	PR	R
4	HCl @ 50°C	35-36	NR	NR	R	NR	R	PR	PR
5	H2SO4	30	-	R	R	R	R	R	R
6	H2SO4	95-98	NR	NR	PR	NR	PR	NR	PR
7	H2SO4 @ 50 °C	95-98	NR	NR	NR	NR	NR	NR	NR
8	KOH	30	R	NR	R	NR	R	R	R
9	KOH	50	R	NR	R	NR	R	R	R
10	KOH @ 50 °C	50	PR	NR	R	NR	R	R	PR
11	NaClO	30	NR	R	R	R	R	R	R
12	NaOH	30	R	NR	R	NR	R	R	R
13	NaOH	50	R	NR	R	NR	R	R	R
14	NaOH @50 °C	50	-	NR	R	NR	R	R	R
15	H2O2	30	NR	R	R	NR	R	R	R
16	H2O2	90	NR	R	RR	R	R	R	NR
17	H2O2 @ 50 °C	90	NR	R	R	-	PR	R	NR

PR: partially resistant
NR: not resisistant

Table 6: Cost of reagents used in organic matter removal.

S.No.	Reagent	Cost
1	HNO3	$38.00/ l
2	HCl	$36.00/l
3	H2SO4	$25.56/l
4	KOH	$85.72/ kg
5	NaClO	$24.00/ l
6	NaOH	$62.63/ kg
7	H_2O_2	$27.67/ l
8	Protease A,	$48.34/kg
	Lipase FE-01	$48.34/kg
	Amylase TXL	$36.50/ kg
	Cellulase TXL	$43.95

R: resistant to degradation from chemical

7.5 Recommendations

The chemical composition of air, soil, sediment and water is heterogeneous, resulting in a wide range of characteristics, functioning, and interactions with contaminants.

With all the methods described in this review, a comprehensive recovery of all the spiking microplastics across all size classes was not attainable by one single method and it is now evident that the type of matrix should be prioritized in deciding the extraction procedure as each environmental compartment has its own complexity.

Each isolation technique has its own advantages and disadvantages, and ongoing attempts are being made to enhance existing methods and develop new ones that may increase throughput, detection limit, and repeatability. When selecting an analytical technique, there are four primary criteria to consider: i) examine the whole technique; ii) evaluate the major figures of merit of the various accessible methods; iii) adapt methodology to channels and instrumentals; and iv) construct integrated strategies and while providing statistics on microplastics, these should be taken into consideration. The ideal option is to test, and employee and intelligent mix of data acquired from various approaches.

Choosing effective techniques for microplastic separation must consider both the complexity of the sample and the complexity of the needed processes. As a result, before processing a matrix, researchers must evaluate its performance.

It is suggested to take replicates of water, sediment, soil and biota from different geographical landscapes and environment to increase the comparability and repeatability even among same kind of matrix. Digestion modules, which frequently require case-by-case customization, are an excellent illustration of methodological heterogeneity dependent on sample type. A combination of different protocols should be tried and tested. Figure 3 presents a summary of extraction methods by sample type.

Also, a technical data sheet can be a powerful tool that the author recommends, as also recently suggested by a team of 23 researchers[105] to be to build a database of all the research that is going on in microplastic community. And in this technology driven times, an application based on this sheet is could be a game changer.

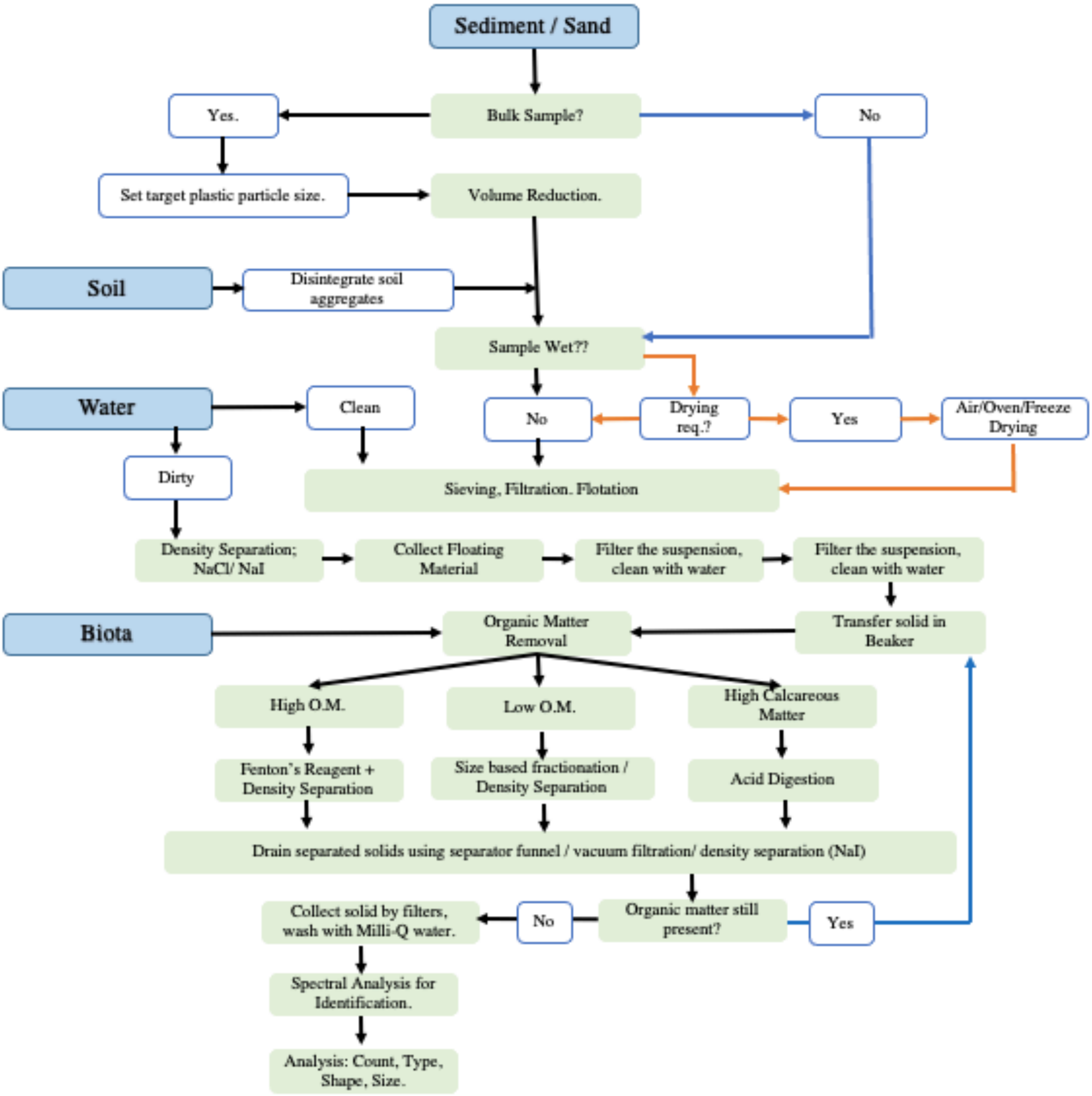


Figure 3: A possible routemap for extraction of microplastics with reference to different environmental compartments.

7.6 Future Research

The search of the analytical literature here reveals that there are numerous proposed methods for carrying out a given experiment, and this proliferation of methods, which in theory is a sign of our discipline's health, can have disastrous consequences for those who try to apply a method to solve a specific problem without taking the time to weigh all of the benefits and drawbacks of the various methodologies. It has to be highlighted that substantial progress has been achieved in MPs research in terms of their analysis, interactions with other pollutants, toxicological effects, and removal by various treatment methods, there are still numerous gaps. The focus on microplastics as a novel contaminant has resulted in a considerable rise in worldwide funding in this emerging study sector during the last decade as can be estimated from the number of publications in this sector are rapidly increasing, but knowledge is not keeping pace, there is repeatability without reproducibility. This is logical because microplastics have no defined characteristics and are present almost everywhere, from sea to snow, from food to feces with no definite shape, size, or color.

The optimal standardization procedure is determined by the data set as well as the norms of the specific field of study. In the case of microplastics, there is huge variation across space and time. It should also be acknowledged that MP research has come a long way in a short span of time, with no standardization in sampling, extraction, and identification methods. But the same has produced a massive number of results that seems to be incomparable. This review highlights the diversity in the extraction methods of microplastics from all environmental compartments. This clearly resulted in a non-gold standard approach that can be followed to reach the desired outcome. A set of guidelines should be laid in terms of matrix and location combined, that should include time, size, quantity, and other factors expressed here. Before designing the method, following key points should be given utmost importance

- Goal of study
- Scientific hypothesis
- Experimental factors: dependent and independent variables, repeatability, reproducibility
- Time efficiency
- Cost of the procedure, reagents.
- Environmental health: toxicity of reagents
- Sample size
- Large scale applicability
- Long-term goal
- Qualitative and quantitative aspects

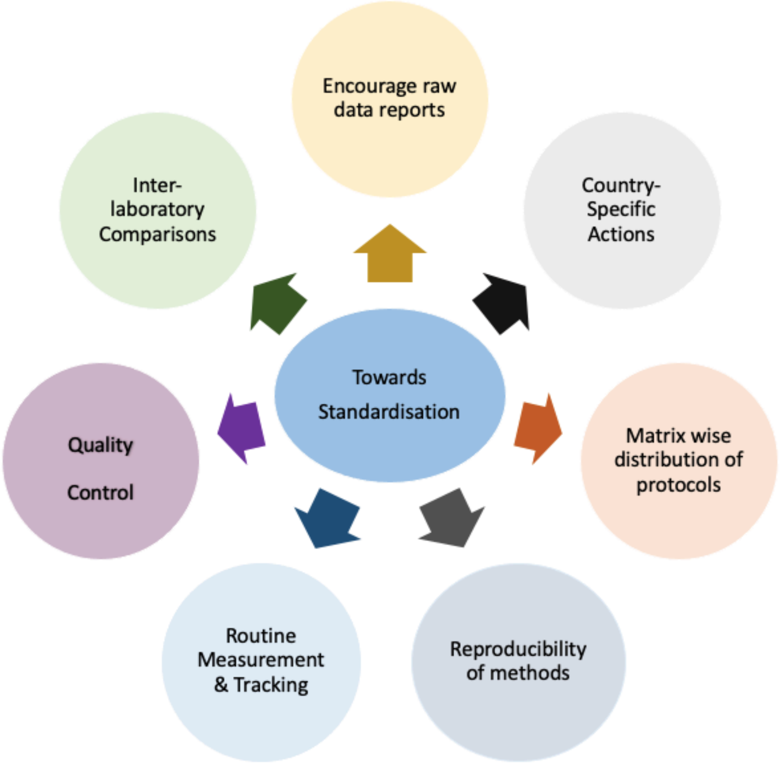


Figure 4: Recommendations for future research

It is recommended that raw data should be shared among researchers on a common platform, ticking all the boxes laid by the Cowger et al.[105] as it assures that the findings can be replicated as microplastic research is highly variable across space and time. It could possibly lead us one step closer to finding methods that are scalable, time and cost-efficient. Having said that, it is also advised that proof-of-concept studies should be encouraged as strategic framework to help environmental and analytical researchers in making decisions about future research and monitoring in order to develops better models to address the implications of microplastic research.

According to researchers, by 2050, a century after commercial manufacturing began, the total amount of plastics produced will have topped 25 billion tonnes. Plastics demand in low- and lower-middle-income countries is one-fifth that of their wealthier counterparts. In other words, established economies create more rubbish than emerging economies, closing the gap between countries' net contributions to the problem of plastic pollution[106]. To put it another way, in the next 35 years, we will make and sell twice as much plastic as we did in the previous 65. Considering these parameters and the difficulties related with microplastic study and a realization that plastic buildup in the ecosystem will eventually have an influence on our environment, it is necessary to build a sustainable relationship the use of plastic with global international collaboration.

7.7 Conclusion

In this review, we have provided a comprehensive description of the methods used for extracting microplastics based on types of environmental matrices. Although the bulk of the techniques are utilized in comparable ways in many almost all types of matrices, it is crucial to note that even little changes in the supplied approach can have a significant impact on the outcomes. The general need for improved environmental analytical methods has resulted in the launch of a new generation of extremely sensitive analytical equipment as well as the development of novel MP extraction procedures.

Given the complexities of soil, sediment, and wastewater matrices, all the steps that lead to MP analysis are quite arduous. Many alternative methodologies have been developed to answer various concerns concerning microplastic contamination, including origins, transportation, and environmental destiny, as well as human and wildlife consequences. Despite the fact that certain technologies are still in their early stages, we can observe that automation is continually expanding the breadth of such devices' uses in environmental study in academic and commercial contexts. Although sample(matrix) processing is essential in practically all analytical methods, it is often overlooked as a significant phase in analytical chemistry, with major attention focused on the determination step. This idea of priorities is all too visible in the equipment and investment plans of many analytical laboratories. However, a positive trend in recent years indicates a greater understanding of the fundamental relevance of matrix processing in the pursuit of high-quality analytical results and valid conclusions.

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CHAPTER 8 : GENERAL CONCLUSION AND FUTURE OUTLOOK

8.1 CONCLUSION

This thesis covered three different aspects of polymer study: Macroplastics, Preparation of Microplastics and Analysis of Mixture of Microplastics.

It is well known that[1], of the two vibrational spectroscopy approaches (Raman and Infrared Spectroscopy (IR)), IR is the most commonly used in the study of MPs. The application of Near Infrared technology in microplastic analysis was evaluated in the first phase (Chapter 3), and it was determined that NIR technology is an emerging subject in the study of Microplastics (MPs).

The primary distinction between NIR spectroscopy and other vibrational spectroscopic methods is that only overtones and combination transitions are seen in the NIR spectral range (800-2500 nm). These are "forbidden" transitions, and the frequency of such transitions occurring is substantially lower than that of fundamental transitions (i.e., the most relevant in IR and Raman spectroscopy), which is immediately measured as a much lower absorption index of a material in the NIR range[2] (800-2500 nm). This results in a deeper penetration of NIR radiation under the sample surface (from a few millimetres to a few centimetres), allowing NIR spectroscopy to investigate a bigger sample volume.

The use of portable equipment in the NIR region, is prevalently assessed in pharmaceutical[3] quality control[4,5] and for polymer type identification of macroplastics[6,7]. Although NIR spectrometers have proven to be an effective tool for MPs analysis, with advantages in terms of high-throughput results and easy handling[8,9], the ability of the portable instruments in this area is still in its infancy. Miniaturized molecular spectroscopy techniques combined with chemometric analysis have positioned themselves as viable 'green' alternatives for field applications allowing quality assurance, authentication, and detection of MPs by producing a distinctive chemical 'fingerprint' with unique signature profiles.

There is an urgent need for standardized and shared protocols to ensure that these small equipment can routinely analyse MPs. Standardized data will enable the creation of dependable databases as well as quantitative analysis. However, because most libraries are constructed using virgin polymers, spectral shifts caused by MPs degradation are sometimes ignored. As a result, developing new library searching algorithms that are more resilient and capable of dealing with the difficulty of comparing degraded MPs to virgin polymer references is crucial.

In this scenario, NIR Technology was used as the primary characterisation method to study commingles plastic waste (Chapter 3) and micro-plastics (Chapter 4, 9), in combination with a miniaturized, portable, low-cost, and real-time spectrometer. In Chapter 3, the development of NIR spectral library to fingerprint post-consumer plastic waste has provided unique detection capabilities in order to improve the reliability and efficiency of the manual sorting of plastic waste, increasing the volume of recycled plastic and the management of multi-component polymer types.

Many studies observed an overall lack of uniformity throughout the literature search, and the validation of analytical procedures for quantifying microplastics (MP) is hindered by a widespread scarcity of reference materials. To conduct a proper research, particles that mimic those found in the environment must be employed. Almost all of the documented studies on the effects of plastic litter on marine biota used pristine[10] plastic objects that were consistent in polymer type, size, shape, and chemical composition. The test materials should have the same characteristics as ambient MP, such as a wide size range and a variety of morphologies (fragments, spheres, films, and fibres) derived from a variety of consumer products.

As a result, in the second phase, we compared the effects of three fragmentation techniques, namely, an Immersion Blender, an Ultracentrifugation Mill, and a Ball Mill producing secondary microplastics, as a step toward preparing more realistic reference materials, dubbed "true-to-life" materials. Furthermore, previous research[11,12] has revealed that particles of various sizes and shapes accumulate at different levels in organisms, implying that even particles with the same composition and concentration can have varied toxicities. We came to the conclusion that microplastics come in a variety of shapes depending on the type and manner of fragmentation. It will be easier to understand the morphologies of MPs created from the same parent particle using multiple procedures in a short amount of time as a result of this work, allowing MP research to accelerate.

To routinely analyse MPs, in the third phase (Chapter 7), a ternary mixture of the most common MPs found in the environment (polyethylene, polypropylene, and polystyrene) was qualitatively and quantitively studied through chemometric tools applied on NIR spectroscopic data of these mixtures. The model established on real-world MPs paves the way for such algorithms to be used to environmental samples. The examination of MPs that may be present in the environment, for example, diluted in water or scattered in soil, could be critical. Furthermore, the examination of the least detectable concentration, the incorporation of new polymer types, the interference of water in the spectral information, and the feasibility of setting up drying protocols should be taken into account. This research can be used as a foundation for the future development of these predictive models. These results are of immediate importance for data standardization to build reliable databases and to perform quantitative analysis, but are indeed poised to impact also for a fast, reliable, and in-situ environmental MPs quantification.

In the last experimental section of this thesis, a protocol was provided to fasten up the NIR spectral acquisition of the MPs found in marine environment.

In this thesis, we developed a proof-of-concept model for studying MP mix through a handheld MicroNIR instrument. This proof-of-concept modeling approach is critical for linking laboratory investigations with environmental samples. This method aids in illustrating the local effects of a global problem, and the modeling exercise is also beneficial in identifying data and research gaps that may be filled to better describe the situation.

This framework can be used to assist managers in making decisions about future research and monitoring in order to develop the model and better address the possibilities of NIR technology in MP research.

8.2 MOVING FORWARD IN MP RESEARCH : EXTENDED VISION

ON EXTENSION OF THE TERNARY MIXTURE TO QUARTENARY MIXTURE AND ANALYSIS THROUGH MICRONIR

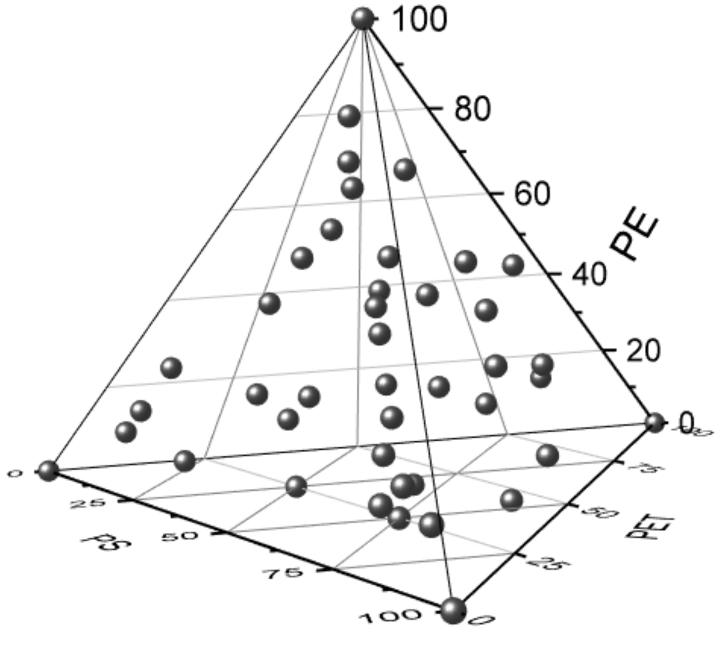
The number of papers on compact NIR spectrometers in the literature is quickly expanding presently (Chapter 3, Section: Influx of NIR technology in MP Analysis), demonstrating the sharp edge in application of this technology to conventional spectroscopy, which is limited to laboratory use. However, the significant step toward shrinking necessitates the implementation of new technological solutions, which clearly impacts the performance of portable NIR spectrometers.

Once a reliable calibration model is established that links the measured NIR spectra to a given sample property (e.g., concentration of a selected compound or a group of compounds available from reference analysis), rapid and efficient spectral measurements can replace the less efficient analytical method in subsequent routines. As a result, the greatest benefit from using NIR spectroscopy is in analyses that involve a large number of samples with reasonably consistent attributes.

For a more realistic approach, the long-term goal of this thesis is to build a model that incorporates all polymer kinds according to IURC code. To accomplish this, the ternary plot was expanded to a quaternary plot, followed by the addition of real environmental samples, as MPs are covered with organic materials in the environment, as well as non-plastic samples.

Construction of Quaternary Composition Plot

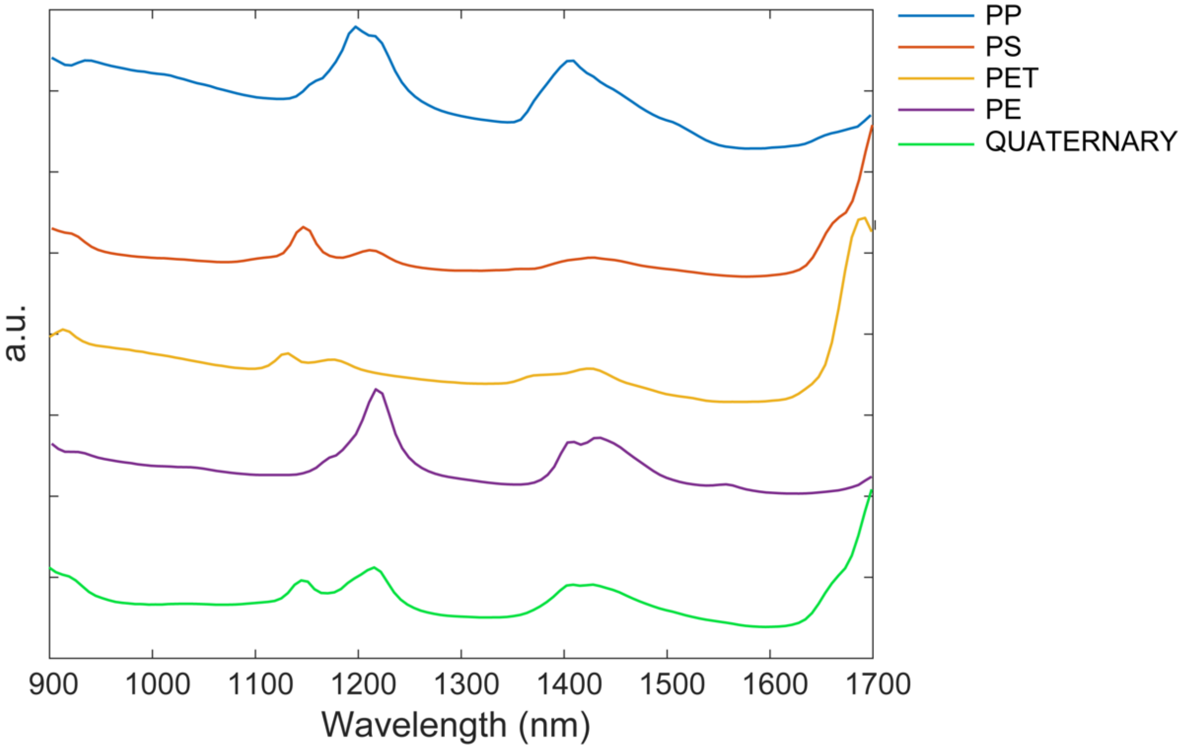
Polyethylene (PE), Polystyrene (PS), Polypropylene (PP), and Polyethylene Terephthalate were all present in the quaternary plot (PET). The unique layout of the MP mixtures was created using the quaternary composition plot shown in Figure. OriginLab was used to create the tetrahedron. In total, 60 compositions have been made, out of which 40 data points were selected for the training set and the remaining 20 compositions were used for testing the regression model.



The Near-Infrared Spectral Measurements, NIR Spectral Pretreatment and Multivariate Data Analysis were carried out according to the procedures described in Chapter 6, Section .

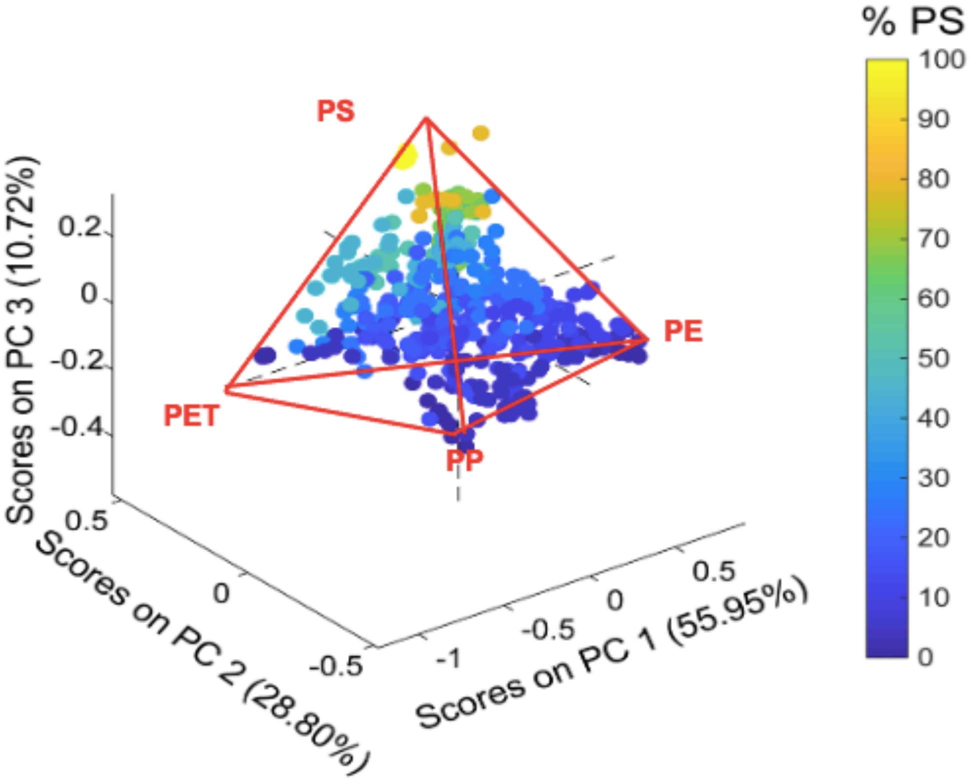
The Preliminary Results of the quaternary plot experiment are shown below:

a) NIR Spectra



b) Principal Component Analysis:

The main significant result is the reflection of the ternary plan in the score plot.



c) PLS-R Results

Table 1: Results of PLS-R

	R^2	
Analyte	**Test**	**CV**
PP	**0.94**	**0.93**
PS	**0.91**	**0.94**
PE	**0.94**	**0.94**
PET	**0.97**	**0.94**

The initial results pertaining to the investigation of mix of four types of MPS: PE, PP, PET and PS are quite satisfactory. This has encouraged us to apply this model to real environmental samples for a more realistic approach, which covers a part of the long term objective of this thesis.

8.3 References

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Appendix A :

Aim:

1) To evaluate the NIR spectral differences among Standard Sea Water, Milli-Q Water and Tap water.

2) To evaluate the drying time of polymers (PE, PP, PS, PET) and differences in the NIR spectra of Wet and Dried polymers

Introduction

Water is a strong absorber in the infrared (IR), including the NIR area spectrum, and the signature from water dominates the near infrared spectra of materials with high water contents (> 80 percent). Water absorption characteristics can be detected at 970, 1200, 1450, and 1950 nm as a result of absorption by O-H bonds in water. The characteristics at 1450 and 1950 nm are the most noticeable.

Microplastics are most prevalent in marine environment and during extraction process from other environmental matrices (soil, sediments, sludge), water is used to clean the samples in the final stage in almost all the processes to clean them before analysing.

In this study, three types of water have been checked for their NIR spectra, namely: Standard Sea Water (SSW), Tap water collected from Chem4Tech Laboratory and Milli-Q Water as water from different sources have different properties. Salt water is heavier than tap water, if measured in "per unit volume" of water. Because salt water has a higher density than tap water, a volume of salt water is heavier than an identical volume of tap water. Tap water is relatively pure, containing only trace levels of mineral salts and even trace amounts of biological debris. Water solutions that are extremely rich in dissolved salts have substantially higher densities than pure or tap water. Milli- Q water on the other hand is the water that has been purified to the highest levels of purity for all contaminant types, including organic and inorganic compounds, dissolved and particulate matter, volatile and non-volatile, reactive and inert, hydrophilic and hydrophobic, and dissolved gases.

The time taken to dry by Polystyrene (PS), Polyethylene Terephthalate (PET), Polyethylene (PE) and Polypropylene (PP) was also analysed.

Materials and Methods.

Water Types

To evaluate any differences, at a spectral level, between three different types of water were tested: Standard Sea Water (prepared according to ASTM D-1141 – 98 (Reapproved 2003), Tap Water (obtained from Chem4Tech Laboratory, University of Brescia) and Milli-Q Water (obtained from).

Microplastic Preparation

In this work, four types of plastics were used: Polypropylene (PP), Polystyrene(PS), Polyethylene Terephthalate (PET), and High-Density Polyethylene (HDPE)). The MPs used in this study were prepared according to the fragmentation methods described in chapter 6.

Near-Infrared Spectra Measurements

Miniaturized MicroNIR OnSite-W spectrometer (Viavi Solutions) was used for spectra acquisition of mixed MP samples. MicroNIR is a compact device weighing about 250g and it non-destructively evaluates samples with no or minimal sample pre-treatment. It is equipped with Linear Variable Filter (LVF) as the dispersive element, coupled directly to a linear detector array (128-pixel InGaAs photodiode array), which works in the wavelength range 950-1650 nm. Two tungsten light bulbs are integrated as light sources. Based on the thickness of the LVF, light with explicit wavelength can only pass through, making each pixel of the detector respond to a particular wavelength. For

This study was performed by acquiring 10 replicates for each water type and 10 instrumental repeats for each replicate, for a total of 300 spectra collected. Spectra were collected via the portable MicroNIR (Viavi Solutions, USA.)

Data Analysis

The NIR spectra of all the samples were retrieved in ASCII file, and then imported in MATLAB environment for the analysis. The information was extracted from the raw spectra by use of Principal Component Analysis (PCA). The PCA was performed after having appropriately pre-treated the data. Three types of pre-treatment were evaluated: Standard Normal Variate (SNV), SNV + First Derivative and SNV + Second Derivative. These are typical pre-processing tools for infrared spectra adopted to erase the multiplicative and additive scattering effects, which often alter the performance of statistical analysis.

Results and Discussions

a. Comparison of different water types.

SSW (standard sea water) contains a variety of salts in varying concentrations. For example, there is Magnesium Chloride, Calcium chloride and other salts present. Milli-Q water is mineral-free, whereas tap water contains calcium, magnesium, and copper minerals. To see if there are any spectral differences in the NIR spectra of the three types of water, Figure 1 shows the NIR spectra of the three types of water. The NIR spectra revealed no differences in band position, only in band intensity.

Figures 2, 3, and 4 depict the PCA results. In all three examples, there is no difference between the two types of water; the variability shown in this research is most likely attributable to drop size.

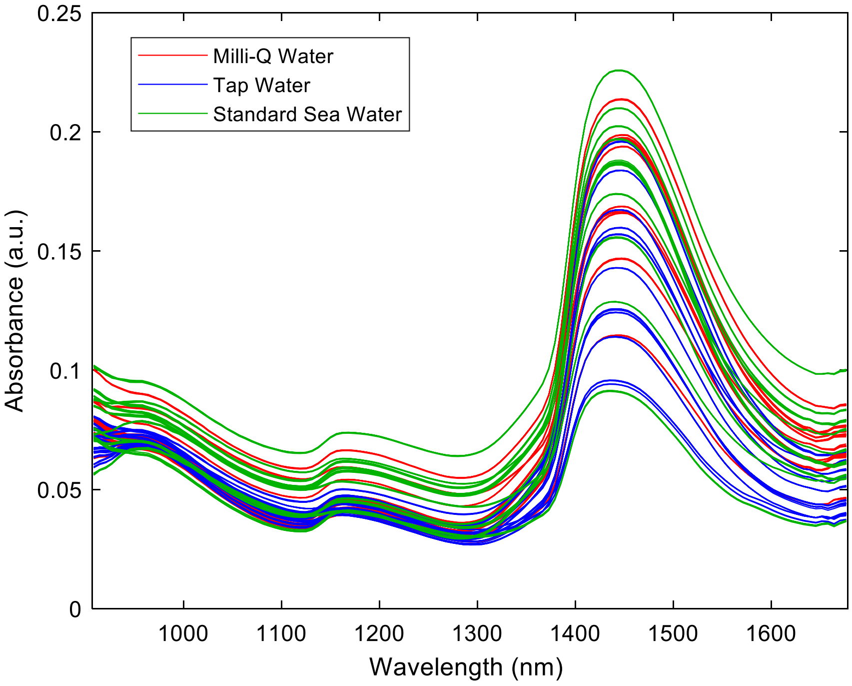


Figure1- Raw spectra of the different types of water

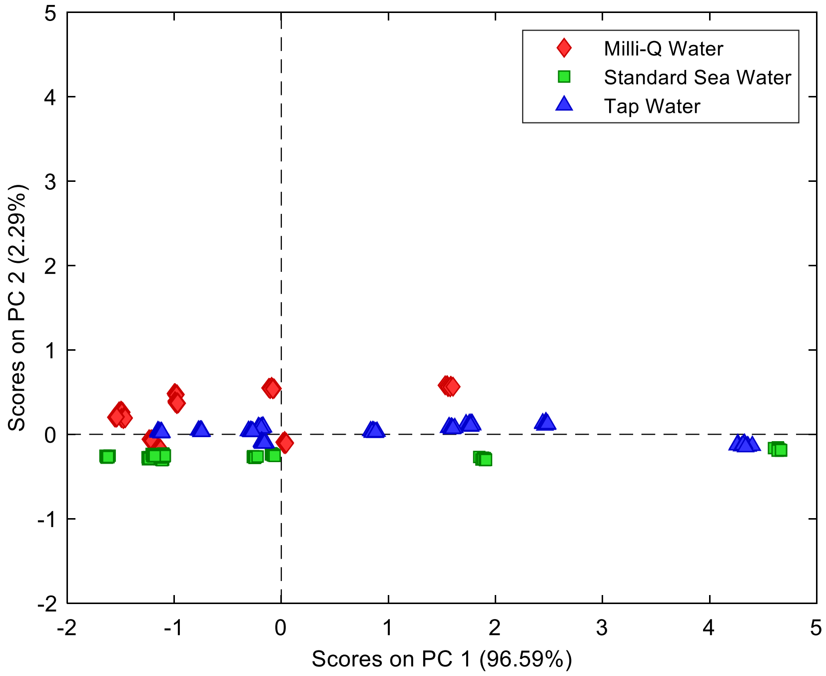


Figure2- PCA carried out on the spectra of different types of water. Pretreatment: SNV

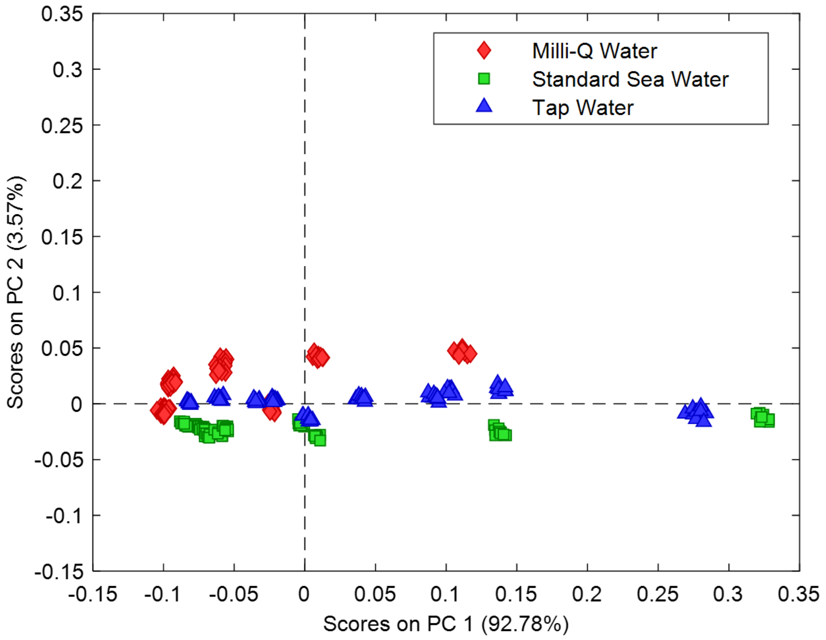


Figure3- PCA carried out on the spectra of the different types of water. Pretreatment: SNV + first derivative

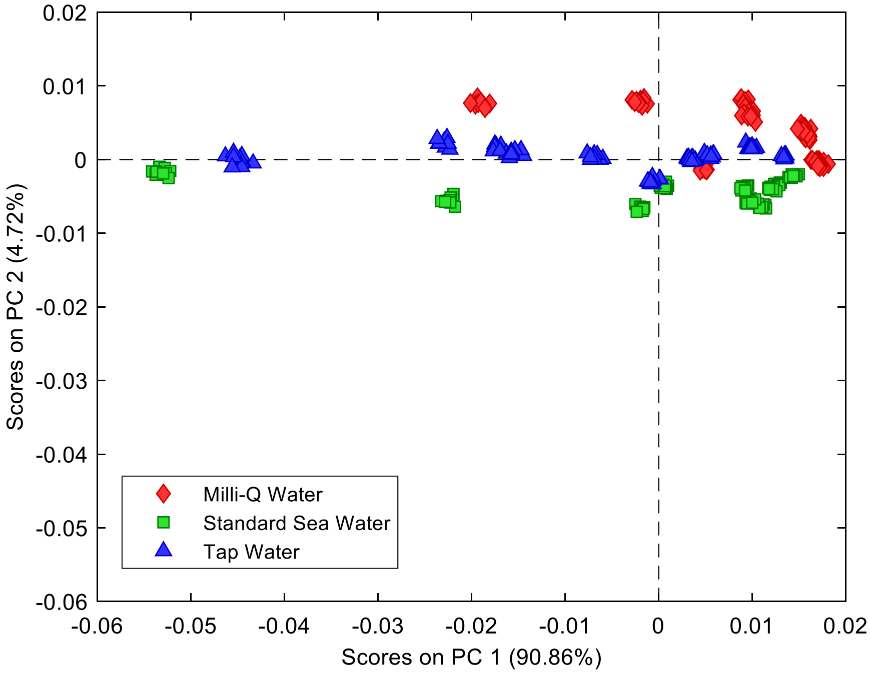


Figure4- PCA carried out on the spectra of the different types of water. SNV pretreatment + second derivative

Based on the results obtained in the previous work, we wanted to continue using the same approach with some changes, taking into account the following parameters:
Degree of water complexity:

- Standard Sea Water

Drying time:

- T0 Minutes
- T5 Minutes
- T10 Minutes
- T15 Minutes
- T20 Minutes

As for the temperature, it was decided to use that of the environment (25 ° C). As will be seen later, it was not essential to wait 20 minutes for complete drying of some polymers; instead, 10 - 15 minutes sufficed. Furthermore, the drying time is highly dependent on the weather.

Standard sea- water

Following the fragmentation protocol described in Chapter 6, microplastics of PP, PS, PE and PET were generated, and wetted with Milli-Q water following the following protocol:

- ✓ NIR spectra acquisition of completely dry microplastic samples
- ✓ 50 mg of polymer wetted with 50 ml of water in a beaker
- ✓ Leave the sample in water for five minutes and mix, if necessary
- ✓ Filtration with the use of a vacuum pump, after weighing the filter
- ✓ Wet filter weighing with microplastics on top
- ✓ Spectrum acquisition on microplastic samples directly on filter (We consider this as T0).
- ✓ Leave the filter in the sun (or in any case away from sources of humidity), and acquire spectra of the sample every 5 minutes until you notice the disappearance of the water-related peak (very intense band1400-1600 nm).

NIR Spectra of PP, PS, HDPE and PET wetted with SSW

MP samples were wetted with 15 ml of water, and the spectrum was obtained after 15 minutes, from the samples transferred to a metal sheet, instead of directly taking the spectrum from the filter. This method was chosen because there was no sun during the day and the humidity was 50%, and the spectra taken directly on the filter after 15 minutes still contained the water peak. Furthermore, the spectra acquisition time was limited to 15 minutes.

Figure 7 shows the NIR spectra of PS, PP and PE acquired following this protocol. The difference between the spectrum acquired after 15 minutes directly on the filter and that acquired on the metal sheet was noted, and water peaks from all the three polymer disappeared after 15 minutes of drying on a humid day.

As for PET, on the other hand, as shown in figure 8, the drying time was extended to 20 minutes, since even if the sample was transferred to the foil, the water peak was still present. The 1400 nm region contains the 1st overtone of the O-H stretch in water and a strong peak around 1450 cm^{-1} indicates the presence of methylene groups (CH_2), as in PET. Thus it took more time NIR to capture the spectra of PET wetted

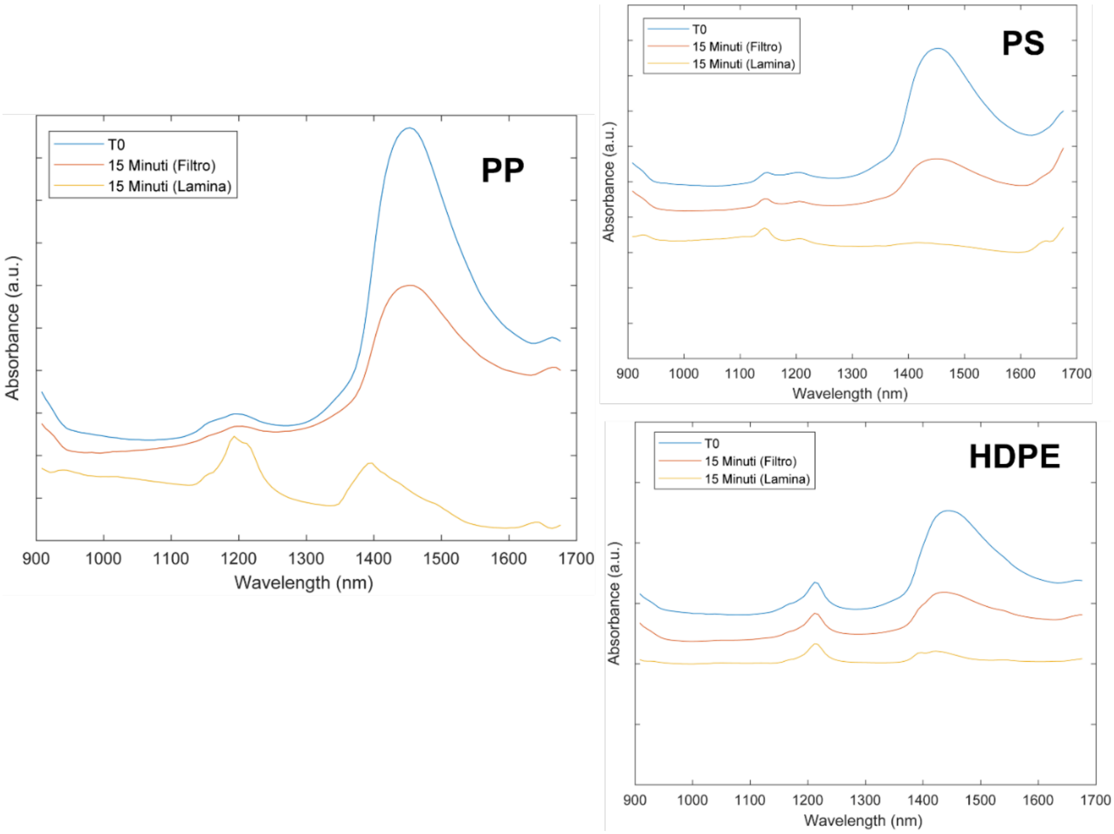


Figure 5- NIR spectra of laboratory produced microplastics of PP, PS and HDPE wetted with standard seawater. Spectra were acquired immediately after wetting, after 15 minutes on filter, and after 15 minutes on metal sheet

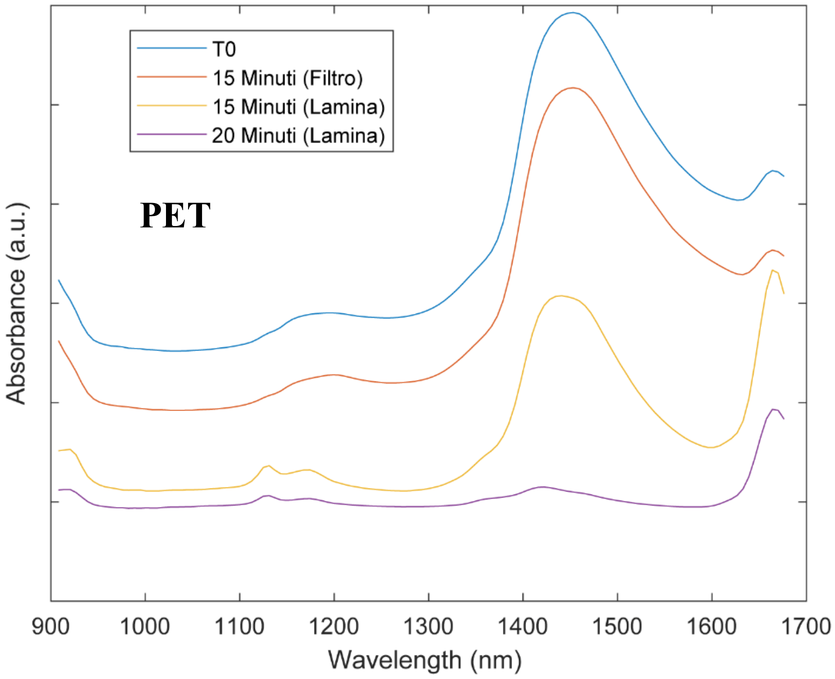


Figure6- NIR spectra of laboratory-produced PET microplastics wetted with standard seawater. Spectra were acquired immediately after wetting, after 15 minutes on filter, on metal sheet and after 20 minutes on metal sheet.

Taking all these considerations into account, it was decided to use 20 minutes as the ideal drying time, for future tests when we will be dealing with mixtures of microplastics instead of pure components.

Conclusion

Near-infrared measurements of water are perhaps the most prevalent (NIR). Water is simple to evaluate from an analytical standpoint due to its comparatively high signal compared to the hydrocarbon background. The purpose of this work was to detect the minimum time required for capturing a NIR spectra of wetted microplastic. The analysis of plastics, after extracting from water can be done after a minumum time of 20 minutes, even on a humid day.

APPENDIX B

Contributions at International Conferences : Posters and Oral Presentations

S.No.	Conference	Place	Contributi
1	VISPEC Summer School on Vibrational Spectroscopy	University of Brescia	Poster
2	International Conference On Microplastic Pollution in the Mediterranean Sea	Hotel la Residenza, Capri	Poster
3	Milan Polymer Days-2020	Online (Milan)	Poster
4	Global Research & Innovation in Plastics Sustainability (GRIPS 2021	Online	Poster
5	Virtual Conference Microplastic 2020	Online (India)	Oral
6	PolyChar 2020- International Polymer Characterization Forum	Online (Venice)	Oral
7	ICMOPP- International Conference on Microplastics and Ocean Plastic Pollution	Online (London)	Oral

MINIATURIZED HANDHELD MICRONIR - A TOOL FOR MICROPLASTIC ANALYSIS

Characterization of Macro and Microplastics using Near Infrared Technology and Chemometrics

APPENDIX C
LIST OF PUBLICATIONS

S.No.	Title	Status
1	Miniaturized Near-Infrared (MicroNIR) Spectrometer in Plastic Waste Sorting Monika Rani, Claudio Marchesi, Stefania Federici et al.	Published
2	Results of VAMAS Survey Regarding Microplastic Issues Stefania Federici, Monika Rani, Laura E. Depero.	Published
3	Compact holographic microscope for imaging flowing microplastics Teresa Cacace, Simona Itri, Monika Rani et al.	Published
4	Quantification of ternary microplastic mixtures through an ultra-compact near-infrared spectrometer coupled with chemometric tools Claudio Marchesi, Monika Rani, Stefania Federici et al.	Submitted
5	A Complete Guide On the Extraction Methods of Microplastics in Complex Environmental Matrices : A Review Monika Rani, Claudio Marchesi, Stefania Federici et al.	In Submission
6	Laboratory Insights and Comparison of Fragmentation Methods to Produce Microplastics Monika Rani, Annalisa Zacco, Stefania Federici et al.	In Submission
7	A Bibliometric Analysis on the Influx of Near Infrared Technology in Microplastic Research Monika Rani, Stefania Federici, Laura E. Depero.	In Submission

APPENDIX D

Inconsistency in Plastic Resin Code Label

During a sampling campaign in Chem4Tech Laboratory for producing microplastics from macroplastics, a sample labelled Polypropylene was found to be Low Density Polyethylene.

This inconsistent labelling of plastic objects can ruin a whole batch of recycling plastic products as the monomer unit of PP and LDPE differ from each other.

It is always thus important to confirm the type of sample being used for the experiment. This discrepancy in the label was found with the library of MicroNIR spectra built in Chapter 4 and further confirmation by Fourier-Transformation Infrared Spectroscopy.

APPENDIX E
Smart Storage of Samples

Plastics are here to stay for centuries. To serve as a reference library for future research work, a library of the samples collected from the recycling plant of Montello (Chapter 4) were cut into small pieces and stored in the Smart Storage Book.
Total samples catalogued : 250

SMART
STORAGE OF
SAMPLES

APPENDIX F

Raman and NIR Identification of Samples from environmental sampling campaign

32 environmental samples collected from the beach of Naples by the partners of SIRIMAP project were analysed through micro-Raman by Ostec and micro-NIR by Viavi Solutions.
The library developed in Chapter 4 was also tested for its performance and the samples were identified as follows through all the three methods:

HDPE	**5 samples**	**LDPE**	**20 samples**
PVC	**2 samples**	**PS**	**1 sample**
PP	**1 samples**	**Metal**	**1 sample**

[illegible]

ENVIRONMENTAL
SAMPLES-NAPLES

APPENDIX G

FTIR Analysis of Samples form Sardinia

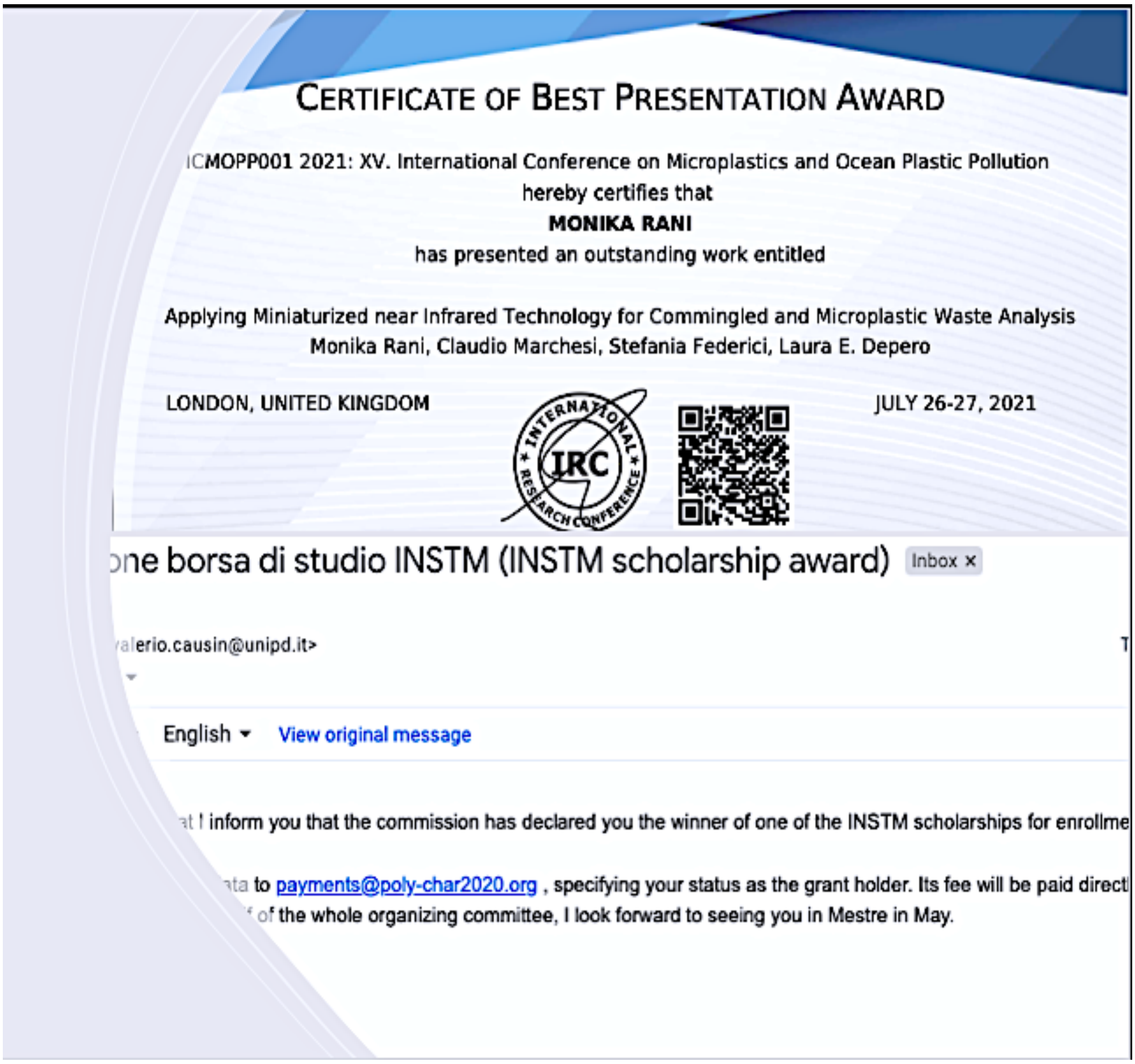
447 microplastics samples by our SIRIMAP partners from different marine environment sites (Sardinia) were extracted through vacuum filtration and analysed through FTIR spectroscopy.

The following table shows the location of the environmental sample collection and subsequent samples analysed. LDPE was the major form of microplastic found in these samples.

S.NO.	Bottle Name	Total Samples	MPs (PE, PP, PS, PVC,PET)	Others	Unrecog-nized
1	**Sinis C-1**	18	14	4	0
2	**Sinis C-2**	29	25	3	1
3	**6 Tavolara C**	11	4	6	1
4	**9 Orosf C**	35	27	7	1
5	**12 Villasimius C**	26	2	20	4
6	**15 Cagliari C**	46	36	4	6
7	**18 C. Tevlada C**	24	19	4	1
8	**24 C. Caccia C**	191	159	19	13
9	**27 Castelsardo C-1**	33	20	11	2
10	**27 Castelsardo C-2**	09	03	5	1
11	**27 Castelsardo C-3**	25	14	4	7
	TOTAL	**447**	**323**	**87**	**37**

APPENDIX H
Awards And Recognition

S. No	Type	Working Title	Conference
1.	Scholarship for POLYCHAR2020	Analysis of Micro and Macroplastics using Miniaturized Handheld MicroNIR	Poly-Char 2020 [Venice] – International Polymer Characterization Forum
2	Best Presentation Award	Applying Miniaturized Near Infrared Technology For Commingles and Microplastic Waste Analysis”	International Conference on Microplastics and Ocean Plastic Pollution to be held in London, United Kingdom - 2021



CERTIFICATE OF BEST PRESENTATION AWARD

ICMOPP001 2021: XV. International Conference on Microplastics and Ocean Plastic Pollution
hereby certifies that
MONIKA RANI
has presented an outstanding work entitled

Applying Miniaturized near Infrared Technology for Commingled and Microplastic Waste Analysis
Monika Rani, Claudio Marchesi, Stefania Federici, Laura E. Depero

LONDON, UNITED KINGDOM

JULY 26-27, 2021

ne borsa di studio INSTM (INSTM scholarship award)

alerio.causin@unipd.it>

at I inform you that the commission has declared you the winner of one of the INSTM scholarships for enrollme

ata to payments@poly-char2020.org , specifying your status as the grant holder. Its fee will be paid direct
of the whole organizing committee, I look forward to seeing you in Mestre in May.