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IMPROVING AND EVALUATING METHODS OF THE ANALYSIS OF MICROPLASTICS FROM ENVIRONMENTAL SAMPLES

MASTER'S THESIS

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Abstract

The objective of this thesis was to improve the methodology in the studies of the microplastics (MPs) in the laboratory and from environmental samples. The main application for this purpose was by the use of a quality assurance and quality control (QA/QC) protocol and by the development of the standard reference materials (SRMs) for a range of MPs (beads, fibers, car tires) as a method validation which is the lack in many MPs studies.

Experiments were created aimed at quantifying laboratory-based contamination sources and from environmental resources as water. The results in the studies of the lab-based contamination sources for MPs in the analytical laboratory, the analysis for the SRM and the application of QA/QC and validation of methods for MP in tap water revealed different outcomes. The results were also considered related to the own characteristics of the sample. However, in all studies was detected the presence of MPs, suggesting contamination in the samples.

Despite the challenge of the study, due to the easy contamination, for example, from dust deposition in the laboratory atmosphere, the objectives of this work were achieved. This study demonstrated that the use of QA/QC protocol and the application of SRM should be included for a better method performance of MPs samples in order to provide valuable information to assess and to validate the measurement of MPs.

Keywords: Microplastics, Standard Reference Material, Method Validation, Quality Control, Quality Assurance, Laboratory-based contamination.

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Oslo, June 2019

Natália de Paiva Lôpo Ferreira

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Abbreviations

DDTs - Dichloro-diphenyl-trichloroethane

FT-IR – Fourier-transform Infrared Spectroscopy

GC – Gas Chromatography

mFT-IR – Micro Fourier-transform Infrared Spectroscopy

MPs - Microplastics

MS – Mass Spectrometry

NIVA – Norwegian Institute for Water Research

NOAA – National Oceanic and Atmospheric Administration

OM – Organic Matter

PAHs - Polycyclic Aromatic Hydrocarbons

PBDEs - Polybrominated Diphenyl Ethers

PCBs - Polychlorinated Biphenyls

PE - Polyethylene

PET - Polyethylene Terephthalate

PP - Polypropylene

PS - Polystyrene

PUR - Polyurethane

PVC - Polyvinyl chloride

RO – Reverse Osmosis

SRM - Standard Reference Materials

QA – Quality Assurance

QC – Quality Control

UV – Ultraviolet

WWT – Wastewater Treatment

1. Introduction

The broad occurrence of microplastics (MPs) in the environment has recently become an issue of major societal and scientific concern (Thompson et al., 2009). MPs enter the environment through multiple sources and processes, most of which are still uncharted and poorly understood. The definition of MPs is in itself the subject of an ongoing debate. Attention on this type of pollution arose from early observations of the ubiquitous occurrence of small debris from plastic litter in the ocean. In 2003, microliter with sizes of 63-500 μm was used to describe the marine plastic fine fraction, for the first time (Gregory et al., 2003). In 2004, the term "microplastics" became popular and was mentioned for the first time to describe particles smaller than 20 μm in their major dimension. In 2008, in a meeting organized by the NOAA – National Oceanic and Atmospheric Administration of the United States, decides that MPs should have a dimension of <5 mm. Until now, this is the most broadly used definition (Hartmann et al., 2019). Such a definition is, however, very comprehensive and captures an incredibly diverse group of materials with the only common characteristic of being made of anthropogenic polymeric materials. This lack of classification and nomenclature contributed to a certain extent to the disharmonized development of the research field, especially, concerning the focus of monitoring activities and the targets for chemical analyses and detection. Most of the existing datasets from different research groups are of difficult comparison because of poor classification and also the use of not yet validated analytical methods. These problems represent also hindrances for the development of environmental protection regulation.

The definition of MPs solely based on the dimensional boundary is of little usefulness. Shape, composition, and diversity of MPs ageing status represents key factors to consider when classifying them, setting the target for analytical measurements, presenting results and defining environmental quality standards (Rocha-Santos et al., 2015). MPs are also classified according to their origin, as primary and secondary (Cole et al., 2011). Primary MPs include intentionally produced materials with dimensions, commonly, smaller than 5 mm (e.g. plastic pellets, microfibers, beads, some toys). Secondary MPs are created after the degradation of larger plastic items in the environment or during use as a consequence of the natural or induced fragmentation or degradation processes (Barnes et al., 2009). Some of the most common polymer environmental MPs are made of polypropylene,

polyethylene, polystyrene, polyethylene terephthalate, and polyvinyl chloride (Andrady, 2011). In addition, the chemical additive can be present that can alter the properties of MPs. Their morphological and physical characteristics are obviously very variable too, with shapes including fibers, beads, fragments, films, etc and densities varying essentially from 0.8 or less kg L^{-1} to 2.8 kg L^{-1} (Eriksen et al., 2013). This complexity of chemical composition, morphology and physical properties have implications for the particles' environmental fate, behavior and biological activities. Degradation and environmental transport are strongly influenced by the morphological and physical properties, and degradation is also influenced by procedures used to analyze MPs in environmental samples.

The most common techniques for the analysis of MPs are visual microscopy and the identification of the chemical composition of MPs by infrared (IR) spectroscopy through Fourier-transform infrared (FT-IR) or by the RAMAN spectroscopy (Song et al., 2015). These technologies do not evaluate the mass composition of the MPs, but they determine the chemical composition and the amount in a given sample. Other technologies have been used to target the mass of MPs in environmental samples. For example, pyrolysis-gas chromatography (GC) or mass spectrometry (MS)-based methods (Frias et al., 2010; Nuelle et al., 2014) have been applied and are currently under development in many laboratories in Europe and beyond. The level of comparability across different methods has never been really assessed. Similarly, the performance of different analytical methods has never been adequately addressed. This largely derives from the lack of standard reference materials (SRM) for MPs that can be used to cross-validate and compare different methods.

Through available techniques, many studies have diversely highlighted the presence of MPs in different environmental compartments including marine, river/lake and drinking waters (Klein et al., 2015; Driedger et al., 2015; Free et al., 2014; Schymanski et al., 2018). Also, in air, sediments, and soil (Dris et al., 2016; Claessens et al., 2011; Rillig et al., 2017). Despite the increasing number of reports on MP occurrence in environmental samples, very little information is provided on the reliability of the measurements as the assurance and control measure to ensure the good quality measurement is not yet consolidated or is even overlooked by many authors.

Developing validated methods for the measurement of MPs in environmental and biological samples is essential to build a better understanding of their sources, fate, and

impacts (Sundt et al., 2014). Methodologies both for sampling and analyses lack the necessary level of standardization. Establishing proper SRM for method validation is crucial (Qiu et al., 2016). As MPs found in the environment are very heterogeneous in nature and composition, this makes data of most available reports difficult to compare. With the aim to contribute to improve this situation, this thesis work is done to evaluate and to improve some of the MP analytical methods based on the quality assessment by laboratory blanks and certified reference materials for different MP materials (beads, fiber, car tires).

2. Theoretical background

2.1 A short history of plastic and its use

The need for developing new materials to reduce dependence on natural raw materials led to the synthesis of the first synthetic plastic (Bakelite) in 1907 by Leo Baekeland (Reboul, 1998). This thermoplastic was created with the purpose of replacing the natural plastic insulator, shellac, because of the high demand from energy sources in that period (Science History, 2016). The development of the material had enormous consequences on the history of human society and technology. The use of plastic in the transport or electronics revolutionized the way people live (Rosato, 2011, p. 3). Plastics are currently found in everyday life of technology and industrial production: transport, packaging (including food packaging and preservation), medicine, clothing, cabling, insulation, toys, etc (Hamaide et al., 2014, p. 24).

Plastics are versatile, light, motile, water-resistant, resistant to weathering, mouldable and even printable. Because of these characteristics materials can be deployed in a multitude of different applications (Mills et al., 2005). Such a great success brought the global production estimated to 1.5 million tons in 1950 to currently 300 million tonnes per year (Hamaide et al., 2014, p.18). This is bringing upon a new form of environmental pollution. Because of its resistance to the bio, chemical and photodegradation plastic persist over a very long time and accumulates in the environment. The inappropriate management of plastic items during consumer and post-consumer phase produce pollution harmful to biota and humans. Inadequate form of management, such as poorly controlled combustions can generate hazardous substances too and can pollute the air, water, soil, and vegetation. These contaminants include dioxins and furans from the combustion of plastic or the leaching of many different chemical additives (such as plasticizers and flame retardants, many of which were recognized as endocrine disruptors) intentionally used in the formulation of plastic items (Barnes et al., 2009). Despite humanity is increasingly aware of the scale of this environmental problem, both global production and plastic waste mismanagement are in a global exponential increase.

Plastic is even divided into two major categories: thermoplastics and thermosets (Busse et al., 2013, p. 181). Because of their physical-chemical properties (thermoplastics

being more easily recyclable then thermosets) they generally undergo different end of life processes (Subramanian et al., 2017, p. 9). Polymers with the largest production volumes are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PUR) and polyethylene terephthalate (PET). Hidalgo-Ruz (2012) also described that the plastic most commonly found in the environment are in fact polyethylene, polypropylene, and polystyrene.

Plastic waste mismanagement represents a major source of plastic litter and MPs to the environment. A study evaluated that countries with a high rate of waste mismanagement (mostly located in Asia continent) (Jambeck et al., 2015) represent a major global contributor of oceanic plastic pollution. From these sources, marine plastic pollution can be transported globally and impact remote pristine environments. A dramatic example is the Henderson Island in the remote South Pacific, that because of its location and a combination of the influence of marine currents and winds is the target of a large quantity of remotely transported plastic litter impacting its coastline and its biota. Henderson Island is considered to have the highest accumulated and the highest density of plastic litter and MPs in the world with debris up to 671.6 items/m^2 (Lavers et al., 2017).

While a large part of plastic pollution reaches the ocean, major sources are on land, especially concentrated in highly populated areas. Freshwater, soils, and sediments represent major storage compartment that can both accumulate and release plastic pollution. It is estimated that from these terrestrial sources/repositories between 4.8 to 12.7 million metric tons enter the ocean every year (Jambeck et al., 2015). MPs found in the oceans can originate from the fragmentation of mismanaged plastic litter, but there are important direct primary and secondary MPs sources also. Households and industrial laundry, car tire consumption during use, fragmentation of painting and industrial blasting activities represent important examples (Boucher et al., 2017).

In the following sections of the background, more detailed information on these sources of MPs will be provided and the challenges inherent to the determination of MPs in environmental samples described in detail. Considering such complexity of sources, and typology of plastic pollution, planning concrete actions for sound global management appears to be a challenging task. Building a solid frame for the quantitative observation of

plastic environmental pollution is a key step. This thesis work wants to contribute to this specific development.

2.2 Definition of MPs

MPs are generally defined as plastic particles smaller than 5 mm, incorporating both intentionally produced items (pellets or beads) and fragment/debris from the decomposition/fragmentation of larger plastic items. As anticipated earlier the debate about MP definition is still open. Some authors have provided different working definitions. For example (Browne et al., 2007) described MPs as plastic particles ranging 1 – 1000 μm, (Ryan et al., 2009) defined MPs to be less than 2000 μm and (Costa et al., 2010) less than 1000 μm. Moore (2008) also use the definition of MP being less than 5000 μm. Many other authors (Desforges et al., 2014), reported MPs as a size between 1 – 5000 μm and this definition is currently the most used. Also, as anticipated earlier, a unique classification frame is missing to cluster environmental MPs based on their size, color, shape (fragments, fibers, pellets, beads, and foams) composition and origin (Bråte et al., 2018).

Such a lack of standardization resulted in a lack of SRM and uniformity in analytical methods. For example, the selection of filtration meshes, or sieve meshes for the separation of MPs from water or sediments is strongly influenced by the definitions. Also, the presentation of results based on the total number of particles is of little utility when their characteristics, composition, and shape are not described uniquely. Sometimes even the definition of what actually plastic is a matter of debate. For example, rubber debris was not considered “plastics” by some authors (Hartmann et al., 2019). There is, therefore, a clear need to establish a frame and SRM to endorse the development of standardization among research laboratories engaged with the measure of MPs in the environment. Such a step will be crucial for the improvement of data quality, the formulation of environmental quality standards, and ultimately the protection of the environment.

2.3 Major sources of MPs

In this section, different sources of MPs to the environment will be inventoried and discussed, based on information extracted from recent literature. Table 1 presents a summary of estimated emissions of MPs from major sources drawn from the situation in the Nordic Countries.

Table 1. Estimated emission of MPs from different major sources based on assessments from Lassen et al., 2015; Magnusson et al., 2016; Sundt et al., 2014.

	Emission of MPs per million inhabitants tones	
	Minimum	Maximum
Road wear and abrasion of car tires	1000	1500
Wear and tear of materials used in fishery and aquaculture	25	100
Artificial turf	230	400
Laundry dust	20	200
Erosion of building coatings	10	25
Personal care products	6	6
Household dust	0,02	2

2.3.1 Personal care consumer products and other sources of primary MPs

Many primary MPs are produced for the formulation of different personal care products and cosmetics. Examples are toothpaste and scrubs/cleaner products. The relevance of these products as sources of environmental MPs is already documented in many articles. For example, a recent study (Cheung et al., 2017) estimated that the total releases of primary MPs from personal care products from China average 209.7 trillion microbeads or (306.9 tonnes) per year (with 80 percent from the wastewater treatment (WWT) and 20 percent from direct sources). These estimates are often obtained by crosslinking sales volumes and population distribution data.

Another study looked at the release of MPs from consumer products to wastewater in the Netherlands. In this case, the total release of primary MPs from consumer products resulted in a concentration of 0.2 μg L^{-1} to 66 μg L^{-1} in a sewage treatment effluent (Wezel et al., 2016).

Beyond those from personal care products, other environmental sources of primary MPs include several industrial processes where beads used in industrial blasting processes,

plastic pellets, or fragments originating from manufacturing processes can be released to the soil or water environments.

2.3.2 Secondary MPs from ageing of plastic items

Deterioration and ageing of plastic macroscopic items is an important secondary source of MPs (Li et al., 2016). A number of different processes contribute to such a source. Accurate estimates of MP generation rates from each of these processes are far from being settled. MP generation depends on the type of source material, its use, the ageing process under different conditions, and the type and amounts of chemical additives present in the plastics (Law et al., 2014).

Major sources of secondary MPs from land-based activities relate to industrial, commercial, and domestic processes. Residential, urban and industrial areas are hot spots of these sources (Barnes et al., 2009). Some studies show that the degradation of plastics on land can take decades or centuries. Where no mechanic abrasion drives the process, photodegradation represents a fundamental trigger of plastic ageing. When high radiation exists together with oxygen, the process is named thermo-oxidation. Other drivers of ageing are the leaching of additive chemicals that protect the polymer from UV radiation or confer them mechanic resilience. All together these processes trigger the inception of fragmentation of large plastic items to MPs. Degradation can also be induced by microorganisms (Andrady et al., 2015, p. 147).

Formation of secondary MPs is however enormously accelerated when plastic materials are directly subjected to mechanical abrasion. This can occur during the use of plastic items or can naturally happen in the environment. For example, the wash-out of plastic litter on beaches and their interaction with the sandy sediment represents an effective process producing secondary MPs from the larger plastic litter present in the ocean (Zarfl et al., 2011). In the following section, major processes underpinning the formation of secondary MPs are described.

2.3.3 Tire of vehicles in a traffic road

Another important source of MPs to the environment is the release of rubber debris from the consumption of vehicles' car tires. Tires are made of a complex mixture of chemicals and rubber of both natural and synthetic origin. Some studies assessed the emission/discharge and fate of tire particles occurring in the aquatic environment. Tire wear is expectedly the major source of MPs in many developed countries. It is estimated that in average a single car tire emits 0.81 kg of debris in the small microscale per year. 5-10 percent of these particles are estimated to reach the ocean. There is a lack of knowledge on the behaviour and transport pathways in the environment, as well as on the characteristics and size of tire debris released to the environment, as well on the impact, they can cause to biota (Kole et al., 2017). There is currently insufficient empirical evidence experimentally confirming this assessment as no successful method to detect car tire debris in environmental samples exists (Wagner et al., 2018).

Drawing from tire life cycle assessments for Norway, Sweden and Denmark based on considerations on tire usage by different vehicle categories, numbers of vehicles, and the average loss of weight of the tire during their lifespan it is estimated that between 1000 and 1500 tonnes of car tire debris per million inhabitants are released every year (Table 1; Lassen et al., 2015; Magnusson et al., 2016; Sundt et al., 2014).

2.3.4 Laundry (Households) - cleaning of synthetic fibers: textiles

Many studies documented the release of synthetic fibers from laundry (Hernandez et al., 2017; De Falco et al., 2018; Åström et al., 2018). These studies are based on the detection of microfibers in the washing machine wastewater after washing commercially available textiles both during household washing or industrial washing. The temperature, the time and the type of wash can have a great impact on the quantity of the release of the fibers from the machine. MPs released in this way encompass mostly synthetic fibers of polyester, polyamide, viscose, nylon or acrylic.

During WWT, most of these fibers are removed from effluent and are retained in the sewage sludge. In many countries' sewage sludge is used as fertilizer over agricultural soils. These fibers are therefore emitted to a large proportion to terrestrial environments (Nizzetto

et al., 2016). Direct emissions to water occur also in the case of discharges of untreated wastewater, or as a consequence of inefficient WWT (Henry et al., 2019). Releases of microfibers from the laundry are influenced by the condition of the washing (temperature, time, use of detergents, etc.). It has been suggested that by changing washing methods, a reduction of microfiber releases can be achieved (Salvador et al., 2017).

2.3.5 Wear and tear products in aquaculture/mariculture, agriculture and fishery

Aquaculture and fishery activities include many uses of plastic such as fishing nets, buoyant material or net cages. Those are also an important source of plastics and MPs to the ocean. In coastal China, for example, this source is estimated to account for up to 5-12 particles/m^3 in seawater and 1000-3000 particles/kg in marine sediments (Chen et al., 2018).

Plastics/MPs usage in agriculture is documented from the practice of plastic mulching. These films are often made of polyethylene (PE) with the addition of UV protective filters, other polymers, and additives. The degradation of this material is slow, however, fragmentation of mulching films has been observed and believed to represent a major direct source of MPs into the soil and consequently river and the ocean (Steinmetz et al., 2016a). The alternative is the use of the biodegradable mulching in agriculture. More studies are still needed to better characterize this potentially very important source of MPs (Steinmetz et al., 2016b).

2.3.6 Wastewater treatment

Wastewater treatment plants (WWTPs) convey large amounts of primary and secondary MPs. Sewers convey MPs and other microparticles sources in conurbations including industrial districts, domestic wastewater and surface runoff (Mrowiec, 2018).

Recent studies assessed that wastewater effluents can release up to 4 million MPs per day, mostly fibers and fragments (Mason et al., 2016; Mahon et al., 2017). Effective WWT results in sequestering MPs to the sewage sludge. This is often used as a fertilizer in agriculture, bringing a new source of this pollution directly to the soil (Nizzetto et al., 2016).

2.4 MPs occurrence, transport and behavior in the environment

Ongoing developments in the techniques for the detection and quantification of MPs in environmental samples have resulted in an increasing number of reports on MP contamination in different environmental compartments and regions. Most frequently analyzed matrixes include marine water, sediment, soil, and biota. MPs occurrence in environmental samples is usually reported in terms of the number of particles per unit of surface area (e.g. in case of soils or sediments) or volume and mass. Monitoring of MP concentration in the environment is the key to assess pressure on and exposure of biota (Wardrop et al., 2016), investigate processes controlling MP fate, transport and behaviour in the environment (Paula et al., 2018) and ultimately inform risk assessment and eventually, environmental protection.

The marine environment is the ultimate sink for plastic pollution including litter and MPs (Yu et al., 2018). It is believed that, as the rate of plastic pollution release to the environment is exponentially growing, the burden of plastic in the environment will continue to increase. As most of the plastic pollution releases occur on land, freshwaters rivers act as important transport medium of MPs to recipient marine waters. Floods can have a great influence on the movement of the MPs from soil to rivers and further transport via rivers (Horton et al., 2017). Intense flooding has been shown to remobilize a large amount of plastic temporarily accumulated in river sediments (Hurley et al., 2018). Model exercise has also shown that hydrologically driven soil erosion controls the transfer of particles from soil to streams (Nizzetto et al., 2016).

In the sea, plastic litter and MPs with a density similar or lower than that of water may undergo long-range marine transport with sea currents and winds, while heavier particles my sink and be incorporated within sediments. During this transport litter and particles can change their properties (i.e. shape and density) as they may undergo fragmentation and degradation, operated by the environment or may serve as a substrate for the growth of organisms that can ultimately change their environmental behaviour. Marine deep water sediments are regarded as the ultimate sink for this pollution (Siegfried et al., 2017).

Physical/chemical and biological degradations of plastics/MPs are obviously also important sink processes. Their rates and mechanisms are, however, not clear and whether they can result in a next generation of larger number of smaller particles as a result of the fragmentation of larger items is still under debate. As already anticipated, UV radiation can play a crucial role in the prime breaking down of polymers, especially of those that have lost their protective chemical additives. Thermo-oxidative degradation (oxidation in the air), hydrolytic degradation (reaction with water) and biodegradation by microorganisms (Nithin et al., 2017, p. 240) can also be accounted among the mechanisms controlling the environmental breakdown of MPs. These degradation processes are often studied in the laboratory by measuring molecular weight changes of polymers and molecular mass distribution, as well as morphological changes (Tosin et al., 2012).

It is believed that degradation and generally sink processes are "slow process" if compared to the high rate of release of plastics to the global environment. Currently, there is little consensus on the total burden of MPs already present in the environment. Some considerations or estimations were done, suggesting figures in the order of a few million tonnes per year been released globally. Only 2% of this is originated from direct releases at sea (Boucher et al., 2017). The flows of total emissions/discharges are expectedly in an exponential growth.

2.4.1 Contamination in marine environments

The total load of plastic to the ocean is in an increasing phase (Gesamp, 2015). Several monitoring studies have been conducted on the occurrence of MPs in marine environments from different locations. Some of these studies in marginal seas of highly anthropic regions have even shown an extremely high level of contamination. For example, in the Adriatic Sea, surface water was assessed to host an average of 315,009 MPs or litter items per km^{-2} with most of them being PE. In the Northeast Atlantic Ocean (Lusher et al., 2014) an average concentration of 2.46 particles per m^3 was observed. Most updated global scale estimations suggest that there are between 93,000 to 236,000 tons of floating plastic in the global ocean (Van et al., 2015).

Several studies analysed plastic fragments from the open ocean and from remote and urban beaches. Some of them have also focused on chemical contaminants associated with

these plastics showing that they can convey several polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dichloro-diphenyl-trichloroethane and its metabolites (DDTs), polybrominated diphenyl ethers (PBDEs), alkylphenols and bisphenol (Hirai et al., 2011).

2.4.2 Freshwater ecosystems

Freshwater system recipients of MPs releases tend to have a high concentration of MPs. An early study in Lake Hovsgol/Mongolia (a mountain lake with a small surface area and long hydraulic residence time) found an average MP density of 20,264 particles per km^2 in the lake. Fragments of plastic and films were the most abundant contaminants. Such pollution was linked to inefficient WWT in the area (Free et al., 2014). MPs were also analyzed in the Laurentian Great Lakes of the United States. Between 0 and 450,000 plastic per km^2 of lake surface were counted (Driedger et al., 2015). Such a large variability in determined concentrations was related to the distribution of anthropic areas along the coasts of the lake.

In South Korea, the Nakdong River was monitored for MPs and PES fibers, PE fragments, paint particles (alkyd), and styrofoam (expanded PES) were detected with the highest frequency. Concentrations in the range of 0.6–860 particles/m^3 were measured (Lee et al., 2013).

Estuaries are also particularly interesting locations for MP accumulation. For example, in the Tamar Estuary - Southwest England, the first European assessment of MP releases from riverine transport to the ocean was conducted. Surface water contamination was accessed and it was found that over 82% of the samples contained MPs (Thompson, 2014).

2.4.3 Soil

An increasing number of reports highlight the soil contamination by MPs. Road and urban run-off, the application of contaminated sludge to agricultural soils, irrigation with poorly treated or untreated wastewater or contaminated river water, the mismanagement of mulching films in agriculture and potentially another type of depositions (including

atmospheric depositions) serve as main mechanisms for soil contamination 15/06/2019 10:41:00. Most of the plastic retained in soil can persists for decades or longer.

2.4.4 Sediments

Many reports have described MP contamination in marine sediments. For example, along the Belgian coast, up to 390 particles, kg^{-1} dry sediment were detected with occurrence verified in the large majority of samples (Claessens et al., 2011). Another study, in South Africa, counted an average of 700 and 3308 ± 1449 particles m^{-2} of sandy sediment (Nel et al., 2015).

The analysis of sediment concentrations in areas directly impacted by industrial activities shows a clear link between anthropogenic drivers and MP contamination. A study conducted in Alang-Sosiya, India in proximity of a ship breaking facility, highlighted extremely high level of sediment contamination averaging 81 mg of small plastics fragments per kg of sediment with PU, nylon, PS, and PES being the most abundant polymers detected (Reddy et al., 2006). Not only in higher activities concentration, but far in the deep-sea sediments in remote open sea locations of the Atlantic Ocean and the Mediterranean were also detected MPs (Van et al., 2013).

2.4.5 Atmosphere

The occurrence of airborne MPs has been confirmed by monitoring studies both indoors and outdoors (Dris et al., 2016). MPs from textiles in indoor and outdoor atmospheric environments were detected. Indoor concentrations ranged between 1.0 and 60.0 fibers/m^3. Outdoor concentrations were found to be significantly lower (0.3 and 1.5 fibers/m^3). 33% of the detected fibers were polymeric (Dris et al., 2017).

The atmosphere can serve as a transport compartment for MPs. To date report on MP in the air are still scarce, however, as indoor environments are reportedly particularly exposed, airborne MPs can represent an important/predominant exposure patterns for humans.

2.5 Challenges for the analysis of MPs

Despite the growing abundance of reports on environmental contamination by different MPs, existing dataset is biased by a general poor description of QA/QC measures, method validation, assessment of laboratory blanks and assessment of analytical recoveries. This makes the comparison of results from different studies a difficult task.

Factors affecting measurement quality include contamination of samples during analysis, unavailability of matrixes for field and laboratory blanks, lack of certified reference materials and reference samples for testing method performance. Analytical methods differ among environmental matrixes and even different protocols have been deployed for analyses of similar matrixes. Such a lack of standardization is one of the important issues hindering the development of the research field. Considering that there is a very large social and political interest surrounding the MP issue and that large amounts of financial resources are already placed for MP monitoring to develop standardization measures is one of the most urgent steps to undertake.

The following section will present some of the commonly adopted methods for the sampling and analyses of MPs in different environmental matrixes and highlights major uncertainties and lack of adequate QA/QC measures. A review of the analysis used for detection and quantification of MPs is available from (Hidalgo-Ruz et al., 2012). They listed and reviewed the three fundamental steps involved with these analyses, including:

1) Sampling approaches are selective sampling (which is done directly from the field), volume-reduced sampling (preserving the sample of interest and volume reducing) and bulk sampling (no reduction of sample volume);

2) Four sample preparation steps (density separation, filtration sieving, and visual sorting);

3) Chemical composition determination.

2.5.1 Sampling and pre-processing of solid samples (sediments/soils)

Methods for the sampling of sediments for MPs can involve different approaches depending on the location or the depth. Bottom sediments could be collected by a box corer or samples of the surface could be scooped out using iron spoons or non-plastic material. Then the sediments are prepared, the samples are dried and quantified (Qiu et al., 2016). As sediments are a non-homogeneous matrix and MPs may not follow a homogeneous distribution within the sampled volume, homogenisation of the samples is a particularly crucial step that can significantly affect analyses results. To this regard, the use of all-metal sample splitter (Figure 1) is recommended with repeated processing of dry sediments to ensure complete homogenization.

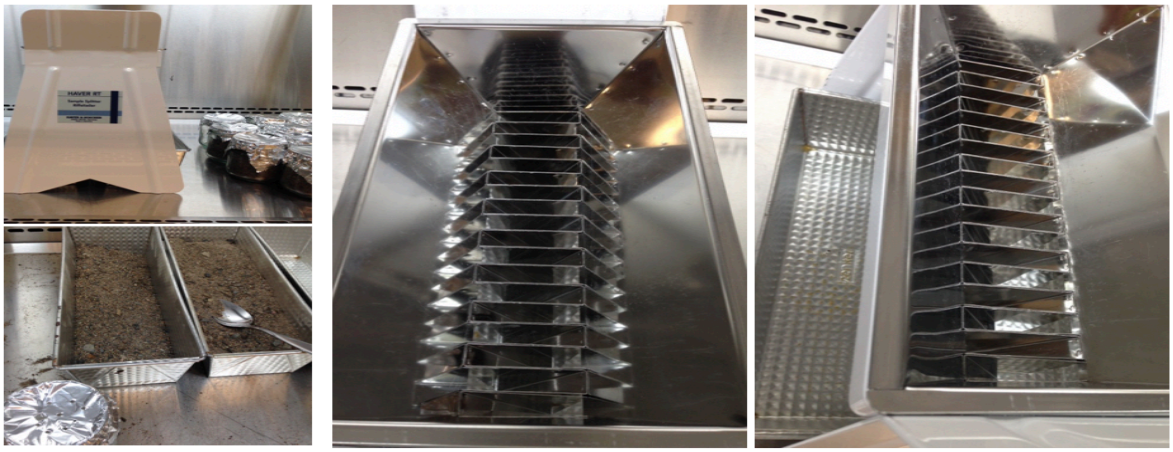

Figure 1. Sample Splitter equipment showing how the sediments were homogenized with the help of two containers.

Analyses of solid complex samples typically include the following steps (Figure 2)

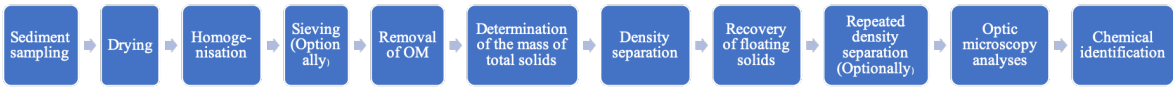

Figure 2. The sequence of a method to study sediments samples for MP extraction.

These steps can be carried out in many different ways, and there is generally significant disagreement between procedures adopted by different research groups. For example, different temperatures have been used in order to dry the samples. In some case temperatures up to 70-100 °C (Dubaish et al., 2013; Ivar do Sul et al., 2009) were used. Other authors operated at a temperature of 60 °C or lower (Nuelle et al., 2014). Very high

temperature can affect the characteristics of MPs in the samples. Therefore, more recent studies have recommended using temperatures not exceeding 40 °C. Some works do not even mention drying temperatures (Claessens et al., 2011; Nel et al., 2015; Reddy et al., 2006).

The sample homogenization step is often missing in the description of analytical methods. However, as both in soils and sediments the distribution of MPs can vary both longitudinally and vertically, special care in homogenising the samples are recommended.

Concerning the step of OM removal, different reagents and conditions have been used. A recent study systematically compared the efficacy and possible artifacts associated with the use of different protocols for OM removal from complex samples (Hurley et al., 2018). Four main protocols were assessed: oxidation using H_2O_2, Fenton's reagent, and alkaline digestion with NaOH and KOH. Eight common polymer types were used to assess the influence of reagent exposure on particle integrity. Organic matter removal efficiencies were also assessed per each protocol. Fenton's reagent under a controlled reaction temperature was identified as the optimum protocol. All other methods showed signs of particle degradation or resulted in an insufficient reduction in organic matter content. Many previous reports have adopted OM removal protocols that were indeed incompatible with MP integrity. Once isolated, the particles can be identified and quantified by spectroscopy (FT-IR or Raman Spectroscopy).

Whilst MPs particles in liquid samples can be extracted through filtration, the solid components of soils and sludges require additional processing steps before MPs can be efficiently extracted. Particles can be extracted from sediments using a density separation procedure; however, the OM found in soils or sludge often has a density similar to that of the target microplastic particles, e.g. soil organic matter (SOM). Hence, this step will not effectively extract MPs in this case. OM removal is required prior to density separations. For the density separation, four chemicals are usually used: H_20_2 (hydrogen peroxide), $FeSO_4.7H_20$ (ferrous sulphate heptahydrate), H_2SO_4 (sulfuric acid) and NaI (sodium iodide) (Kedzierski et al., 2017; Mausra et al., 2015).

Each density extract will be a liquid sample containing microplastic particles. To isolate the particles for analysis, the samples need to be filtered. The usual is by vacuum

filter (Büchner or Nalgene) and the samples are pass through 47 mm Whatmann glass fiber filters (Hidalgo-Ruz et al., 2012).

2.5.2 Collection and analyses of water samples

A method for the analysis of MPs in water samples is described by the Marine Debris Program from the United States which involves the study of plastic debris as suspended solids (Mausra et al., 2015). The methods will consist of filtration of the solids through filters or membrane with a specific size cut-off. When large volumes are required, such as during the sampling of marine waters, manta net or phytoplankton nets have been used. For smaller volumes or water samples with low particulate content, vacuum or pressure filtration through the circular membrane can be used. Typically used membranes include nylon with size cut-off typically ranging 50-350µm (for particle-rich water samples) or glass fiber filters with size cut-off down to 0.5 µm for cleaner samples and smaller volumes. It is often recommended to avoid, as much as possible plastic components in the filtration system. After the solids have been transferred to the membranes the sample is dried and the sample is analysed.

2.5.3 Determination of the composition of MPs by FT-IR

Many authors opted for visual characterization of MP through optic microscopy, before addressing the samples to chemical analysis through, for example, IR or Raman Spectroscopy. The visual screening is highly subjective steps that require qualified and experienced personnel. IR or Raman spectrometry is applied for the qualitative and quantitative determination of MPs. Spectroscopy is a term that describes the area of science that studies the interaction of different radiations (Skoog et al., 2007, p. 132). In recent year Fourier Transform IR spectroscopy have become a preferred approach for MP determination.

Atoms and molecules have specific states of peripheral electrons. The vibrational state of molecules relate to their internal energy is correlated with interatomic vibrations (Skoog et al., 2007, p. 147). The ground state is considered to be the lowest energy state and the higher energy is considered as an excited state. For this reason, infrared spectroscopy utilizes the interaction of infrared light with the vibrating of the molecules.

Fourier Transform Infrared Spectroscopy (FT-IR) work through the infrared radiation that is absorbed by individual target particles, however, some of the incident radiation is transmitted through the particle. The result of this interaction is the spectrum (radiation X frequency) of the molecule, measured by the detector. The interferometer is responsible to accelerate the process of scanning in the FT-IR spectrometry by measuring the infrared frequencies at once. The interferogram signal is created and contains the information of the infrared frequency coming from the source. The identification of the composition is made by the frequency spectrum. Before the spectrum result, a calculation is performed by the Fourier transformation via computer (Thermo Scientific, 2013) (Figure 3).

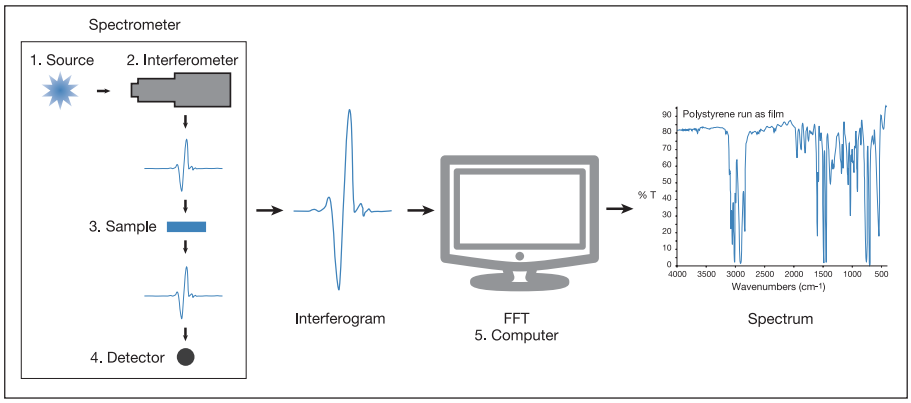

Figure 3. Full components of the sample analysis using FT-IR. From: Thermo Scientific index, 2013.

As MPs are made of polymeric molecules with repeated functional groups linked with a carbon backbone it is possible to do the identification by this method. The general idea of the use of infrared spectroscopy is that the frequency is linked to the chemical properties of the vibrational groups. Fourier transformation facilitates the identification of such periodicities and represent an ideal approach to the identification of polymeric structures through their unique vibrational characteristics defined by specific frequencies (Renner et al., 2017, p.2).

The characterization and quantification of MPs by the FT-IR (FT-MIR) requires first the analysis of the data based on the quality and to check the result. The next step is the evaluation of the FT-MIR spectra in order to analyze the results of MPs (polymer

differences) to obtain information about the MPs and environmental sources. Figure 4, shows a common reference for different types of polymers (Renner et al., 2017, p.48).

There are several advantages of using FT-IR to analyse MPs, such as the high speed of the measurements, all the frequencies analysed at the same time, highly sensitive equipment to detect MP, mechanical simplicity, self-calibration, and a non-destructive technique. So, the FT-IR is suitable for recognizing the unknown substance and to quantify the components in a sample. For these reasons it was used to identify MPs in this thesis's work. (Thermo Scientific, 2013).

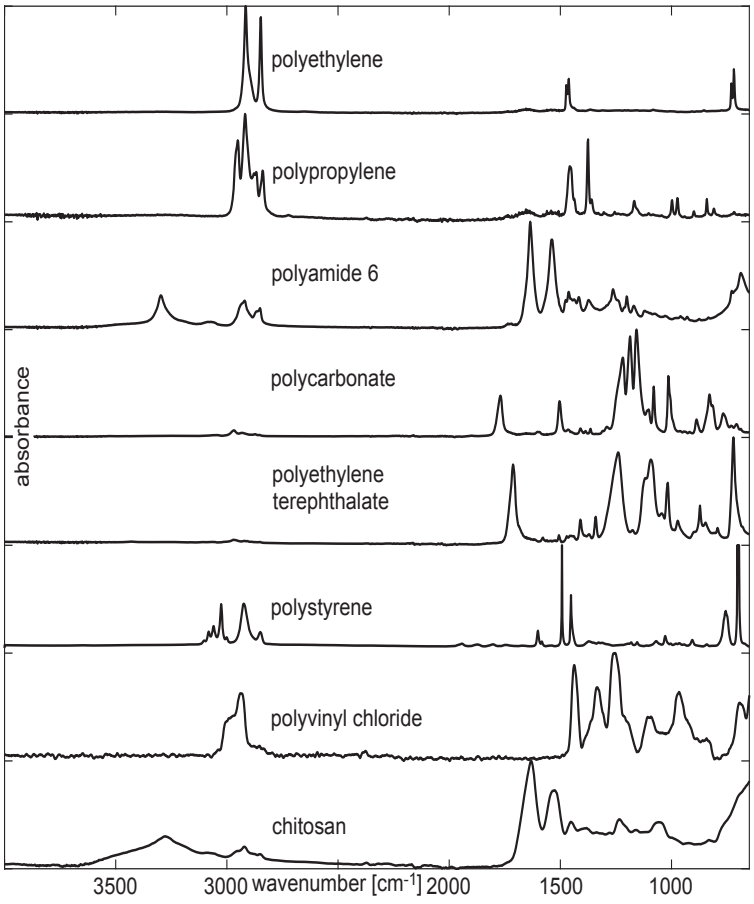

Figure 4. FT-MIR spectra reference for different types of polymer for analysis of MPs. (Chitosan as a natural biopolymer's representative). From: Thermo Scientific index, 2013.

2.6 Criticalities in the analyses of MPs in environmental samples

As anticipated earlier, current approaches to the determination and quantification of MPs in environmental samples lack a sufficient focus on QA/QC measures. The following criticalities are identified:

1) Lack of analytical SRM. As a first instance, laboratories engaged with these analyses are prevented from fully validating analytical methods because of the lack of SRM. SRM for selected major groups of MPs are necessary for determining analytical recovery performances (e.g. through the inclusion of positive control during the analysis of different batches of samples), and also to cross-validate measurements among different laboratories. The lack of SRM for MPs originates essentially from the still insufficient level of standardization and classification of MPs. MPs present in the environment are highly heterogeneous in terms of size, shape, color, chemical composition and physical properties (e.g. density). In order to validate a generic analytical method, a broad range of SRM for MPs is necessary, albeit, not available so far.

2) Lack of adequate assessment of laboratory-based contamination sources during sampling processing and analyses. As seminar studies show, the indoor atmosphere can be a major source of MPs such as fibers or small fragments. The use of a clean room for the analyses of MP is not yet consolidated. Therefore, many research groups operate in relatively uncontrolled laboratory atmosphere. To our knowledge, no study has so far included adequate laboratory blanks during the analyses of MPs in environmental samples, in order to rule out possible laboratory-based contamination. This step appears to be particularly crucial for the analyses of samples were low contamination profile is expected, such as, for example, samples of outdoor air, or drinking water samples.

3) Lack of control over the reproducibility of measurements. Very rarely routine monitoring studies routinely adopt a protocol that allows for replicate analyses of a single samples. As a result, very little is known about the variability of results

associated with the laboratory practice. Also, as the distribution of MPs in environmental samples (both solid or liquid) is not even homogeneous, one can expect a "sampling effect" on the results, whereby subsampling from a large volume of sample (e.g. a small aliquot of a large sediment sample) may result in considerably underestimating or completely missing the MPs that are present in the sample in smaller numbers, while overestimating the most frequent ones. To our knowledge, no study has addressed this effect.

4) Lack of a consolidated approach to the determination of method detection limits. Most reports on MPs in environmental samples have presented results without specifying the detection or quantification limits. These limits are typically obtained by analyzing laboratory or field blanks (or negative controls). The method detection limit can be defined as the smallest detectable number of a MP of a given shape and composition that is significantly higher than its number in the negative control.

2.7 Objective of the research

Given these premises, the scope of this thesis is to quantitatively assess the challenges concerned with the accurate analysis of MPs in different environmental samples. The study aims to closely support the development of SRM for a variety of different MP types and to support development/employment of state of the art assurance and control (QA/QC) measures to ensure measurement quality. The specific objectives are:

- To assist the analysis of SRM used in a future interlaboratory cross-validation exercise by evaluation of the quality and purity of these SRMs pills;
- To evaluate the possible influence of laboratory-based contamination on measurements results;
- To contribute with useful data and suggest good practices for development of strict QA/QC;

- To validate procedures for MP measurement in environmental samples (using tap drinking water as case).

3.Method

3.1 Outlines of method section and experimental approaches

The first part of the method section presents the approach adopted to address several endpoints of QA/QC assessment in MP analyses. These include:

i) Assessment of quality during the production of certified standard reference materials (SRM) necessary for validating analytical protocols and results;
ii) Assessment of the quality of SRM for different types of MPs;
iii) A first full validation of MP measurement in tap water samples.

The last part of the method section presents in detail the general protocols for the detection and characterization of MPs and basic quality assurance and control measures adopted.

3.2 Approach to the assessment of quality during the production of SRM

The scope of this part of the study is to support the development of the first line of certified SRM currently ongoing at NIVA. The SRM assessed in this study are in the process of being used for an international laboratory intercomparison/intercalibration exercise. The initiative is organized by NIVA, Vrije University Amsterdam and the spinoff company QUASIMEME, and involve about 50 laboratories around the world committed to the analyses of MPs. To my knowledge, no similar exercise has previously been conducted. Assuring an adequate level of quality for the SRM used in this exercise is, therefore, instrumental for the development of the field and for the quality of future data from many laboratories and monitoring studies worldwide.

The approach to the study of SRM quality includes 3 phases:

i) Assessment of potential sources of contamination in the laboratory environments where SRM are produced (preparation laboratory) and validated (analytical MP laboratory);

ii) Assessment of potential sources of contamination from the ingredient used for the formulation of SRM;

iii) Assessment of reproducibility and contamination background in SRM that will be used in the international laboratory intercomparison study.

3.2.1 Description of MP SRM

Since SRM is neither currently available to support the development of analytical methods for MP detection in environmental samples nor to validate protocols and individual measurements, NIVA is developing an original line of different types of SRM in order to fill this gap. This includes fragments of a range of polymer types, fibers and car tires debris. These materials are produced starting from raw materials and inducing accelerated fragmentation through controlled mechanical stress. The methods used to generate the particles are covered by industrial confidentiality and it is not relevant for the present study to describe. Here it is focused on assessing the quality of SRM during their incorporation into a special carrier that allows delivery of a controlled and certified number of particles to a given sample. NIVA has developed a carrier in the shape of an effervescent pill that can encapsulate a defined number of MP (Figure 5).

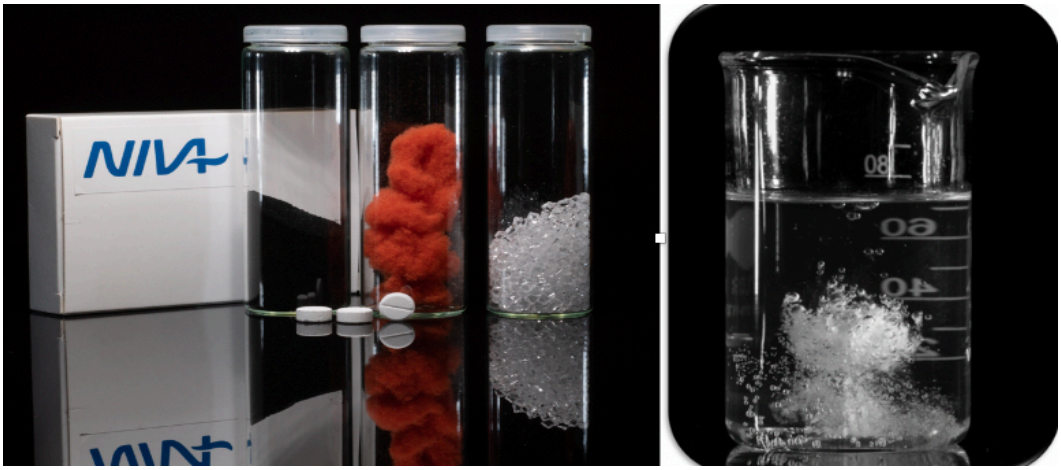

Figure 5. Illustrative material of the new NIVA MP SRM and the concept of the effervescent pill as a carrier of certified numbers of particles.

Since MP in the scale of 0.020-5 mm is present in liquid samples as suspension, dosing and delivering certified numbers of these particles to a sample is a difficult task. Particles are not homogeneously distributed in the liquid and they often interact electrostatically with vessel sidewalls. For these reasons the concept of the effervescent pill

carrier was invented, where a certified number of particles are mixed with pill ingredients in powder form and delivered in "solid state" to the users. Once added to any sample containing even a relatively small amount of water, the pill rapidly dissolves quantitatively delivering the SRM to the sample.

The specific scope of this part of the study was to assess the potential sources of contamination during the preparation of these SRMs, both originating from the laboratory environment or present at sources in the different ingredient used in the formulation of the carrier pill. The formulation of the pill is covered by industrial confidentiality. It contains four ingredients at specific proportions. For the aim of this study, the ingredients were simply indicated as illustrated in table 2.

Table 2. Characteristics of the Ingredients used in SRM.

Ingredient encrypted identifier	Status	Amount in a single pill (g)
Ingredient 1	Solid (powder)	2
Ingredient 2	Solid (powder)	1,2
Ingredient 3	Liquid	0,5
Ingredient 4	Liquid	0,2

Encased materials include PET fibers, PP, PE and PET fragments, car tire debris, all in the range of 50 to 500 μm. During the development of the pills, two batches of ingredients from different suppliers were used. A goal of this study was to analyse whether these batches were carrier of MP contamination that will interfere with the SRM, reducing their quality, or hindering the possibilities of using these SRMs for method validation and QA/QC.

Also, the laboratory environment where this SRM is produced can represent a potential source of contamination from atmospheric deposition during the different steps to produce the pills. It was one scope of this study to detect the presence of such contamination.

3.3 Assessment of potential lab-based contamination sources

The preparation of the carrier pills and the certification of SRM they contain include several steps conducted in different locations in both a preparation laboratory and a MP analytical laboratory. This study was conceived to keep track of the potential influence of laboratory atmosphere deposition during the preparation and certification.

i) Preparation steps

The preparation of the SRM includes the following steps and locations:

- Grinding of powder ingredients (preparation laboratory bench)
- Weighing of the chemicals in the weighing room
- Mixing of ingredients (preparation laboratory bench)
- Desiccation (dessicator)
- Pressing of the pills (preparation laboratory bench)

ii) Validation steps

- Microscopy bench (MP analytical laboratory)
- FT-IR bench (MP analytical laboratory)
- Lab shelf (MP analytical laboratory)
- Laboratory entrance door (Left side) (MP analytical laboratory)
- Laboratory entrance door (Right side) (MP analytical laboratory)
- Chemical storage shelf (MP analytical laboratory)
- Laboratory glassware shelf (MP analytical laboratory)

The influence of laboratory atmospheric deposition of MP as a possible source of contamination of the SRM was assessed using deposition analysis in the locations listed below (Figure 6).

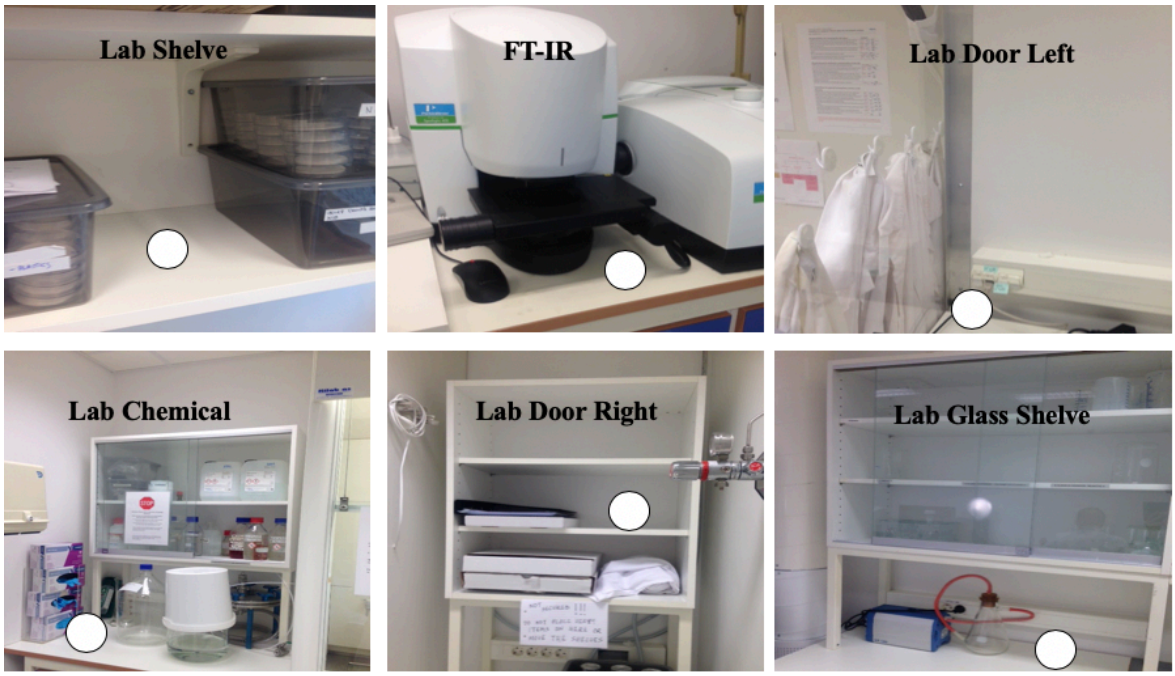

Figure 6. MP's analytical laboratory and the area planned for the studies with the Petri dishes.

Petri dishes (47 mm) containing a Whatman glass fiber filter papers (47 mm) were used as deposition collector. They were inspected at optical microscopy prior to deployment to exclude contamination. Glass fiber filters were also individually carefully inspected prior to deployment. They were humified with filtered reverse osmosis (RO) water to increase the trapping efficiency of deposited particles. This step is relevant because the particles contained in the atmosphere can adhere to the filter more easily when this is moist.

A total of six Petri dishes were used in the MP analytical laboratory and three in the preparation laboratory. Depositors consistently lasted twenty-four hours. This step was repeated four times in order to get information on temporal variability in atmospheric MP levels. After, the Petri dishes were immediately capped and stored (sealed) in the analytical laboratory until the microscopy and FT IR analysis was carried out.

3.4 Contaminations in SRM formulation ingredients

MP contamination potentially occurring in SRM could originate from debris, fragments or fibers present in origin in the ingredients used for making the effervescent pills.

The aim of this study was to check the occurrence of such contamination and inform SRM developers on the selection of adequate reagents (Figure 7).

All materials entering in physical contact with the samples (Petri dishes and glass fiber filters) were inspected at the optical microscope to exclude prior contamination. Then, the filters were weighted for different ingredients. For instance, Ingredient 1 was weighted 20g, for the Ingredient 2 (10g), and for the Ingredient 3 (15g) and Ingredient 4 was 0.5g in the weighing room. Individual ingredients were then completely dissolved in 500 mL of filtered RO water in a Duran glass bottle (500ml). The solution was then filtered through the 47 mm glass fiber filters. Filters were then transferred to inside a desiccator where they were dried at 40 degree Celsius for a period of twenty-four hours. Dry filters were then transferred into the Petri dishes and stored sealed until the microscope analysis.

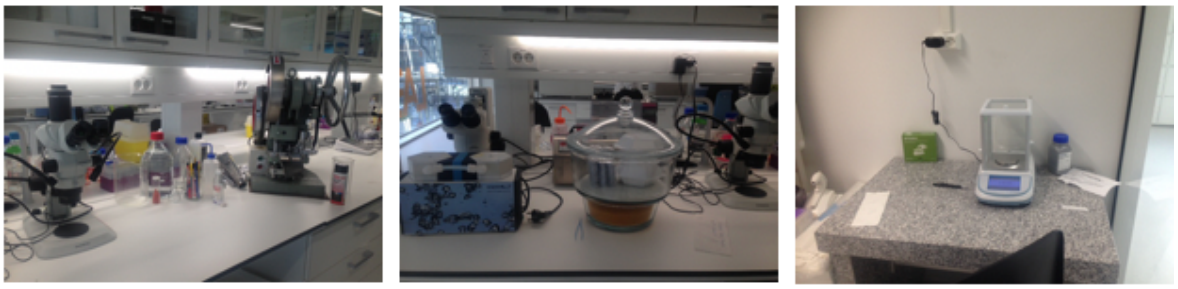

Figure 7. Benches laboratory preparation for the SRM's pills and local inspection.

3.4.1 Quality analysis during SRM development and production

During the thesis work, the development of SRM for MPs was carried out and materials were used in the study to validate methods through the determination of analytical recoveries (see below). As SRM were not available at the inception of this work, it was a goal of this thesis to contribute to the characterization and validation of SRM for different MPs. The SRMs carried in effervescent pills (see above) allows easy and quantitative addition to the samples and the SRM pills were therefore analyzed qualitatively and quantitatively for the consistency in the number of SRM MPs and for the presence of impurities.

Pills are prepared by adding MPs SRM to one of the ingredient and mix the composit with the other ingredient before pressing the pills inside a mode. During the development phase the focus was to obtain pills containing a consistent number of MP SRMs. In order to

do so an heuristic approach (trial and fail) was carried out, whereby different batches were sequencially prepared by changing the total amount of MP SRM added to the pill ingredient mix, or changing the homogenization method. After each batch of about 50 SRM pills was prepared, 20 % of it (i.e. 10 pills) were dissolved in 50 mL of filtered RO water. This suspension was filtered through a 47 mm glass fiber filter. The filter was transferred into a Petri dish and dried into a drying cabinet before analyses. Analyses were carried out in optic microscopy where the counts of MP of different polymers and possible impurities were determined. The end points of the analyses was to determine mean and variability of the number of different MP SRM added to the pill. NIVA set a quality standard goal of at least 10 particles per pill and a relative standard deviation smaller than 15%. The pills contained MP SRM for the following MPs typologies: car tire debris with dimensions between 180 – 425 µm, fragments of PVC with dimensions 250 – 300 µm, beads of polyethylene (PE) with 425 – 500 µm and PES fibres with dimensions between 100 – 1000 µm.

3.5 Drinking Water samples

A number of reports have provided data on MP contamination in drinking water (Pivokonsky et al., 2018; Schymanski et al., 2018; Mintenig et al., 2019). None of these reports have employed a comprehensive evaluation of the measurement quality. Information of the following aspects are often missing method validation with the positive control (e.g. analysis or SRM), assessment of recovery, sound assessment of laboratory and field blanks, data on measurement reproducibility or definition of method detection limits.

The method described here for the analysis of drinking water samples aims at covering this gap and provide a full assessment of measurement quality. The choice of analysing drinking water as an experimental matrix for the scope of this study derives from the need to be able to handle highly challenging samples from the analytical point of view, which drinking water represents. We in fact expect to find little or none MP contamination in treated drinking water. Such a condition brings the need for the highest standard of quality assurance, especially regarding the use of blanks.

In this experiment, a filtration system was constructed to hold a nylon membrane with a mesh size of 10 μm mesh and directly connected to a tap. The endpoints of the experiment are:

i) To study the variation and the reproducibility of the MPs measurement in drinking water samples;

ii) To study the influence on the filtered volume on the result of the measurement. It is expected that in samples with expected very low level of contamination the selection of sampled volume is a crucial factor for the determination of MPs;

iii) To study recovery using SRM addition as a positive control;

iv) To study blank contamination.

3.5.1 Experimental design

Measurements of particles in drinking water were repeated for three types of samples characterized by the different filtered volumes: 10 L, 100 L, and 1000 L. Per each volume measurements were repeated in triplicates to assess reproducibility. Three analysis of positive control was included by spiking the membranes with the SRM described above. Three negative controls (blanks) were included to characterize the background contamination and determine method detection limits. The negative control choice in the figure below was random. A total of 15 samples (including positive and negative controls) were processed (Figure 8).

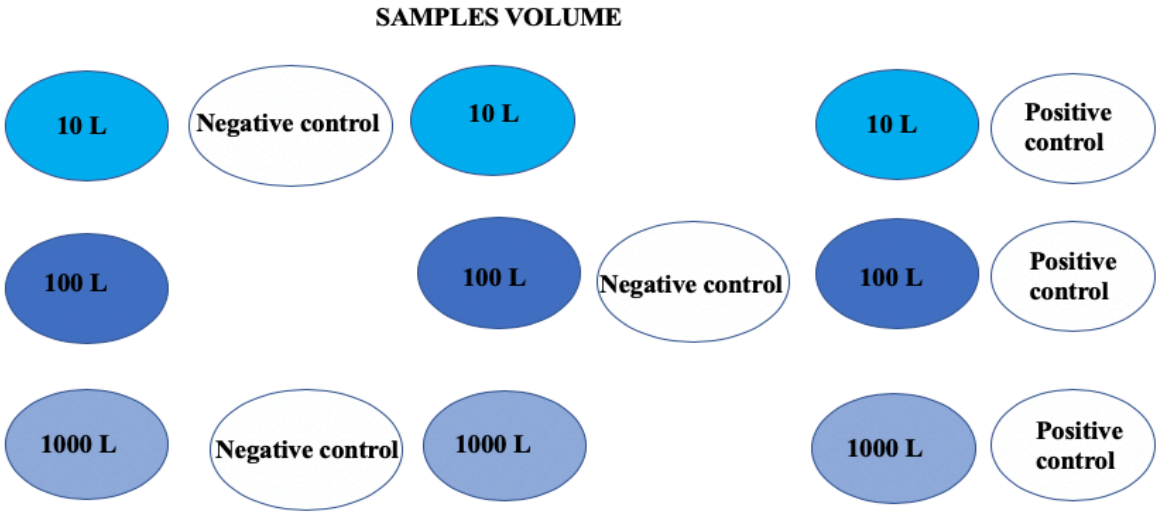

Figure 8. Negative control and positive control for each of the sample volumes.

3.5.2 Sampling and sample handling

Water flow was checked before and during the sampling using a chronometer and a 1 L graduated cylinder. A flow rate of 1L per 15 second (0.066 L/s) was selected for all sample volumes. Based on this setting the total filtration time for the 10 L, 100 L, and 1000 L was determined. The flow was checked regularly during the sampling to ensure accuracy on the sampled volume. Based on this setting the following parameters were set for the sampling (Table 3).

Table 3. Water flow parameters for collecting the membrane samples.

Sampled volume	Sampling time	Flow period (s/L)	Flow rate (L/s)
10 L	145 s (2min 25s)	14,53	0,0688
100 L	1470 s (24min 30s)	14,69	0,068
1000 L	14619 s (4h 3m 39s)	14,61	0,0684

Filtration was processed using a stainless-steel filter holder (Figure 9) with a nylon membrane (mesh 10 μm, diameter 320 mm, Sterlitech Corporation, Kent, WA, USA). This was directly connected to the tap water supply using gas-tight coupling.

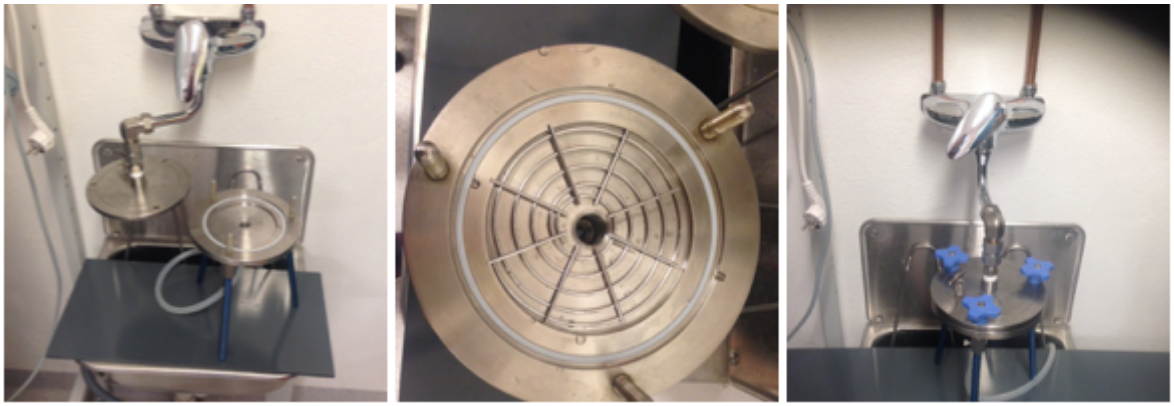

Figure 9. Filtration system for drinking water analysis samples.

The filter holder was carefully rinsed with filtered RO water and flushed with tap water for at least 5 minutes before each sampling section using a spare membrane before each sampling section. The membrane used for the sample filtration was rinsed thoroughly with filtered RO water by pouring water on both sides. Once washed, the mesh was stored in an aluminium foil. Before sampling the mesh was inspected under the microscope to ensure no visible particles were present. As under the microscope only a small area of the

filter could be inspected at any time, the entire surface was scanned while covering the remaining with aluminum foil to avoid deposition of particles in the laboratory atmosphere to contaminate the mesh. Any detected particles at this stage were removed with tweezers.

Before the mesh was added to the filter holder, the inner surfaces of the filter holder were rinsed with filtered RO water. Care was taken to minimize the time the mesh was directly exposed to the laboratory atmosphere. After the sampling was completed, the water source was turned-off, the filter holder was opened, the mesh removed and folded as quickly as possible in the aluminium foil. This was labelled and transferred in a drying cabinet.

The particles on the mesh were transferred to the glass fiber filter by holding them vertically and raised with filtered RO water in a filtration process. Each side of the mesh was washed. Then these glasses fiber filters were stored in a Petri dishes to further analysis. This process was done in the Sterile Cabinet, to avoid any contamination to interfere with the results.

3.5.3 Procedure for processing the negative controls (blanks)

For the processing of negative controls, the same procedure as described for the sample was adopted, with the only difference that no water was passed through the mesh.

3.5.4 Procedure for processing the positive control (recovery test)

The positive control was spiked with NIVA SRM. Pills were hand made specifically for this study. Used SRM materials were: car tires debris with dimensions between 180 – 425 µm, fragments of PVC with dimensions 250 – 300 µm, beads of polyethylene (PE) with dimensions 425 – 500 µm and PES fibres with dimensions between 100 – 1000 µm (Figure 10). A total of 40 of each particle were manually transferred to the pill ingredients (10 of each polymer type). During the processing of the positive control, the SRM pill was added on the top of the filtering membrane. The system was flushed with the same tap water used for as samples in order to make the pill reacting and releasing the SRM to the mesh. (See section 3.9 for further details on recovery assessment). Simulated sampling process with 10L, 100L and 1000L tap water was carried out to obtain the positive controls. These control samples were analyzed as done for real tap water samples.

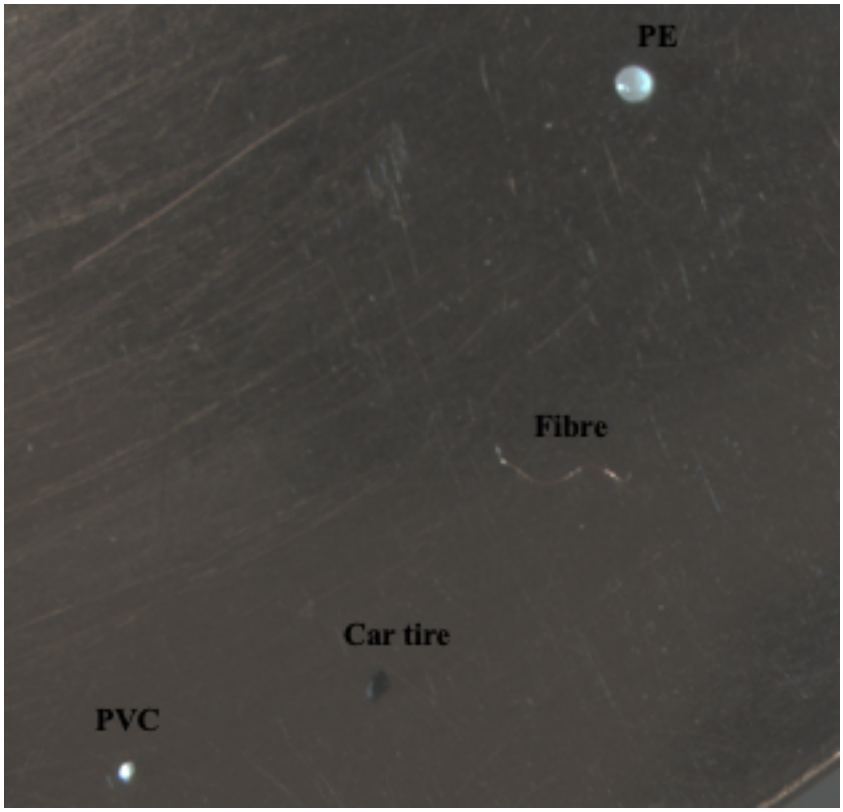

Figure 10. The reference material used for recovery assessments (positive control).

3.6 Visual microscopy analysis

All the preparation steps described so far delivered samples of isolated MPs deposited over a 45 mm glass fiber filter. These filters were then analysed in optic microscopy (Model: Nikon SMZ745T Coupled with the camera: Infinity 1-Lumenera; Eyepiece zoom: C-W10xB / 22, amplification of 1 during analysis). The image processing software was Infinity Analyze. All the filter surface was inspected for the presence of any solid. At each visual detection, a unique identifier code was attributed to individual particles and their position in the filter marked. The dimensions of the particles were measured using the image processing software. In particular, the longer and shorter dimension of the particle were measured. Other notes, including information on morphological characterization and the color, were recorded for each individual particle. The morphological labelling considered the characteristics shown in Table 4.

Table 4. Morphological classification and description of the particles studied.

Morphological classification	Description
Fiber	Extremely elongated shapes with the longer dimension exceeding by several folds the shorter ones, whereby the shorter dimension does not exceed 10μm.
Fragment	Irregularly shaped particles. Often with evidences of fractures or erosions.
Bead	Spherical microparticles.
Film	Fragment with a flat appearance.

3.7 Identification of particle chemical composition (FT-IR)

Each individual particle detected during the optic microscopy analysis was analysed by FT-IR (Model: PerkinElmer, FT-IR Microscope, Spotlight 400) (Figure 11). The identification of the chemical composition of the particle occurs when the spectra of the unknown chemical are compatible with the Spectra of the chemical particle in the FT-IR library. The source of this library comes from three different bases. First from the commercial library named Perkin Elmer: Polymer Starter Pack. Second, a commercial Polymer library called BASEMAN library. The last library identified as IN-HOUSE has two different sources that have the origin from different laboratory contamination sources that contain in the laboratory made with polymers, for example, adhesives, glues, rubber or any utensils that contain polymers in its composition. The other origin is related to the reference polymers as textiles from different clothes as viscose, polyester or epoxide. These libraries together in the FT-IR helps to amplify the level of the identification in the samples.

Figure 11. Microscope accoupled with the camera and the FT-IR.

3.8 Adopted quality assurance and quality control (QA/QC) measures

This section describes QA/QC measures and precautions common to the analyses of air, water, and sediments. The possible occurrence of contamination from laboratory-based sources was a constant concern. At any analytical step (e.g. during the preparation of the samples and the analysis) verifications were undertaken to ensure the laboratory materials (such as glass fiber filter, Petri dishes, jars, funnels, and tubes) were particle free. These controls were undertaken by carefully inspecting these materials using the microscope. Detected interfering particles were either removed mechanically or rinsed away through filtered RO water. Also, all the utensils or tools were washed with filtered RO water.

Special precaution was taken by wearing clean laboratory clothes and lab-coats at any access to the laboratories. For this, a lint roller was often used. Whenever possible, filtration and density separation steps were conducted in a laminar flow sterile cabinet to minimize contamination from the laboratory. Finally, access to MP laboratory was restricted only to trained personnel.

3.9 Definition of analytical recoveries and method detection limits

Special samples, named here as “positive” and “negative” controls were used in the study to determine recovery efficiency and method detection limits, respectively. It is

important to recall that these measures are still commonly not encompassed in most published studies on MP assessment in environmental samples. It is, in fact, the central focus of this thesis to investigate the quality of measurements through definition of such a QA/QC metrics.

Recovery of the positive controls based on the addition of a known number of SRM particles to the sample matrix were after the analytical determination of the sample calculated as:

Equation 1. Recovery equation

$$R = \frac{MP_{exp}}{MP_{SRM}} \cdot 100$$

where MP_{exp} is the number of the SRM MP obtained from the measurement of the positive control, and MP_{SRM} is the number of MP present in the SRM pill.

Results from blank samples (negative controls) were used to determine the method detection limits. Method detection limits were derived per each category of MP resulting from the combination of the morphological labelling obtained from the optic microscopy analysis, and the result of FT-IR. Based on this approach a value of detection limit for fibers, fragments, beads and film of any type of polymer identified by the FT-IR analysis was derived. Method detection limit was calculated per each individual category of MPs as the mean of the results in the negative controls plus their standard deviation. If no detection was achieved in the negative control samples, then the method detection limit for a given MP category was set equal to zero.

4. Results

This section will present the results in the following order:

- The study of the lab-based contamination sources for MP in the analytical laboratory;
- The result of lab-based contamination in MP standard reference materials (SRM) (atmospheric deposition in the SRM preparation laboratory and analysis of MP in an ingredient used in the formulation of SRM pills);
- Application of QA/QC and validation of a method for MP in tap water through membrane filtration;

4.1 Lab-based contamination sources for MP in the analytical laboratory

4.1.1 Results from the microscopy analysis

The Petri dishes collected from all the areas of the study in the laboratory were first analysed by microscopy for the determination of the presence of particles and the initial qualitative screening. In the total 76 particles were observed in the filters. Table 5 summarize these results by the monitored area. The highest concentration was discovered close to the FT-IR. The lowest was found in the area where chemicals are stored (Lab Chemical).

Table 5. Summary of the results for laboratory atmospheric deposition by laboratory area.

Location	Quantity
Lab Door Left	8
Lab Door Right	16
Lab Chemical	2
FT-IR	33
Lab Glass Shelve	11
Lab Shelve	6

Figure 12 shows the details and the average of the particles by monitored area and the standard deviation of repeated observations (N=4). More precisely, as, the observations in each monitored area were not conducted, simultaneously, but in four different periods, the error bars depicted in the figure represent the variation of atmospheric particle deposition in the laboratory over time. Overall, a consistent number of particles were observed in subsequent observations. The variance was larger for the FT-IR area.

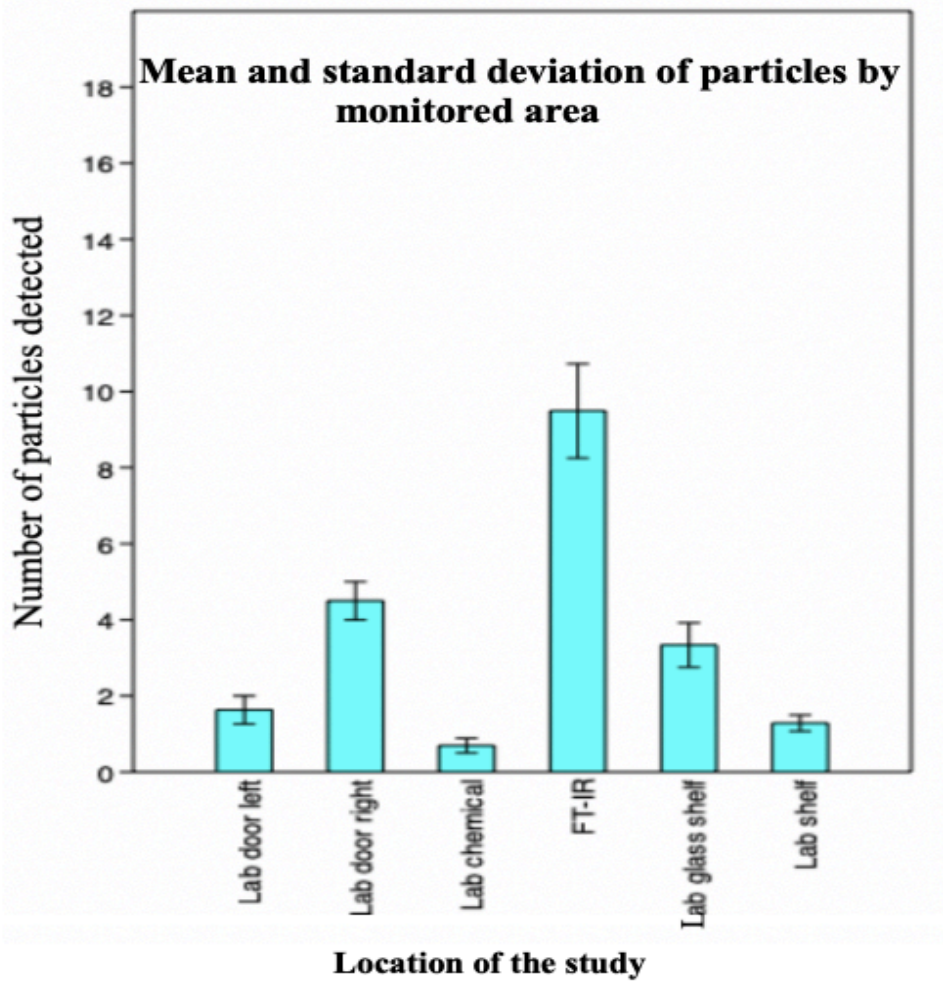

Figure 12. Average and standard deviation by each location related to the particles detected.

The microscopy analysis of Petri dishes revealed the presence in the laboratory atmosphere of a variety of particles with different characteristics and colors. Table 6 shown an average of single particles dimensions. Figure 13 depicts examples of the most common types observed. These particles in μm dimension were then selected to be analyzed in the FT-IR for chemical identification.

Table 6. Summary table of physical attributes of MPs observed in laboratory atmospheric deposition.

Type	Major dimension (µm)	Estimated minor dimension (µm)	Number of particles observed
Fibers	346,78	32,89	69
Fragments	284,97	27,81	7

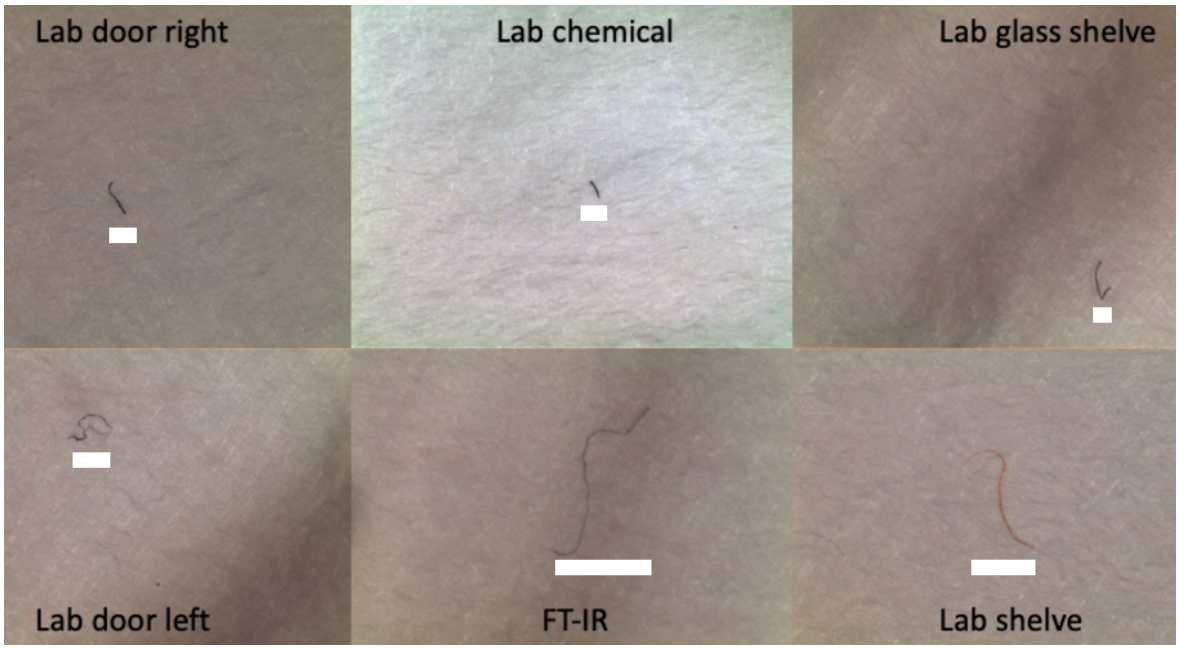

Figure 13. Examples of MPs observed in laboratory atmospheric depositions. Only particles identified as polymeric following FT-IR analyses are shown. Scale bar represents 1 mm.

4.1.2 Laboratory atmospheric deposition results from the FT-IR

Table 7 summarizes the FT-IR results on deposition measurements, reporting the chemical identity of the prevalent particle types observed in different locations in the laboratory. Most detected particles were cellulose. They were therefore not classified as synthetic polymers. However, occurrence of polymeric particles was verified in several cases. Prevalent detected polymers were polyester, polyacetal, viscose, acrylic fabric, polyisoprene chlorinated and epoxide (Table 7).

Table 7. The results for polymers in each location.

Location	Non-polymers detected	Polymers detected
Lab Door Left	cellulose	polyester
Lab Door Right	cellulose	polyacetal, viscose, acrylic fabric
Lab Chemical		viscose
FT-IR	cellulose	epoxide, polyisoprene chlorinated, viscose
Lab Glass Shelve	cellulose	epoxide, polyisoprene chlorinated, viscose
Lab Shelve	cellulose	

Figure 14 represents the relative proportion of particles detected in laboratory atmospheric depositions. Most of the observed particles were cellulose, followed by viscose. Traces of polyacetal, acrylic fabric and polyisoprene chlorinated were observed with a frequency of about 1%.

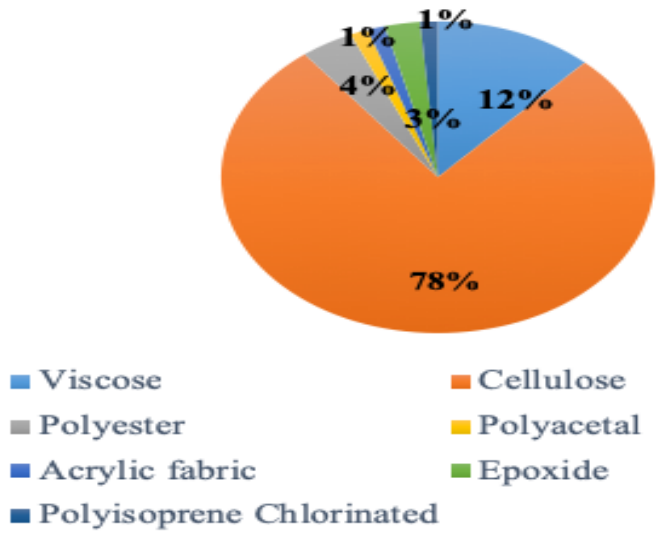

Figure 14. Percentage of the particles detected on the FT-IR.

4.2 Lab-based contamination of MPs in standard reference materials (SRMs)

4.2.1 Deposition of airborne MP in the SRM preparation laboratory

Six Petri dishes were deployed in two different benches (as described in the methodology) and in the mass balance room of the laboratory for the preparation of MP SRM. From the analysis of optic microscopy, no particles as cellulose or polymers were

observed in any of the samples, suggesting ideal conditions in the laboratory for the preparation of SRM.

4.2.2 MP contamination in SRM ingredients

Individual chemical ingredients for the formulation of MP SRM were analyzed both by optic microscopy and FT-IR. In summary, significant contamination was found in the chemical ingredients used in the formulation of SRM for MPs. As shown in Figure 15, a total of 53 particles were counted in the microscope in samples of the 4 ingredients, and particles were found in all 4 ingredients.

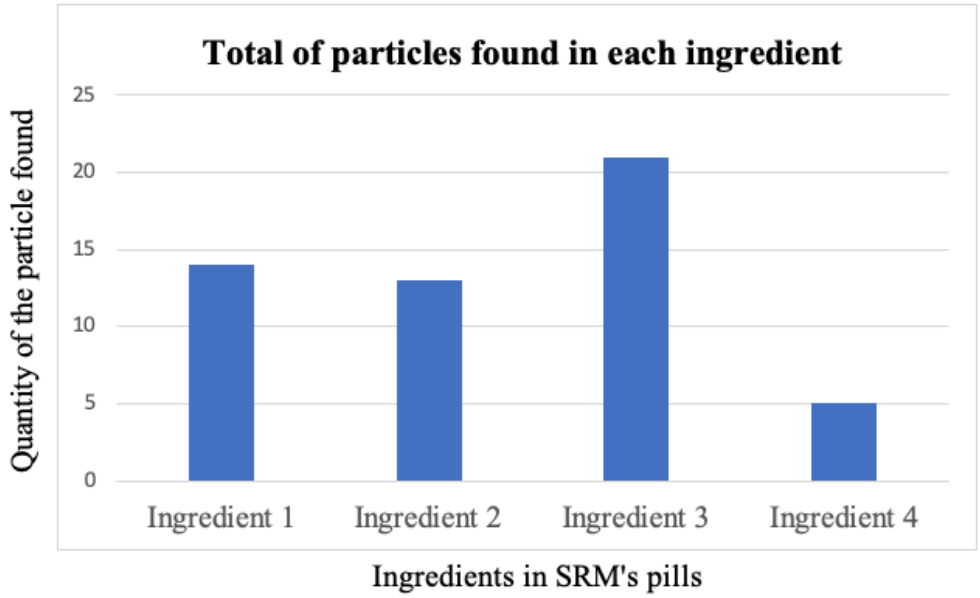

Figure 15. Quantity of particle detected in each ingredient by microscopy analysis.

Ingredient 1 and 2 are the most abundant in the formulation of the effervescent pills used to carry the certified MP SRM. In these ingredients was measured a similar number of potential contaminations. In the Ingredient 3 was found the highest concentration and less in the Ingredient 4. Figure 16 displays an example of particles typology most frequently observed in the Ingredient for the formulation of SRM. Particles in the form of fibers were most frequently observed, with different lengths (ranging 1 – 4 μm) and different colors.

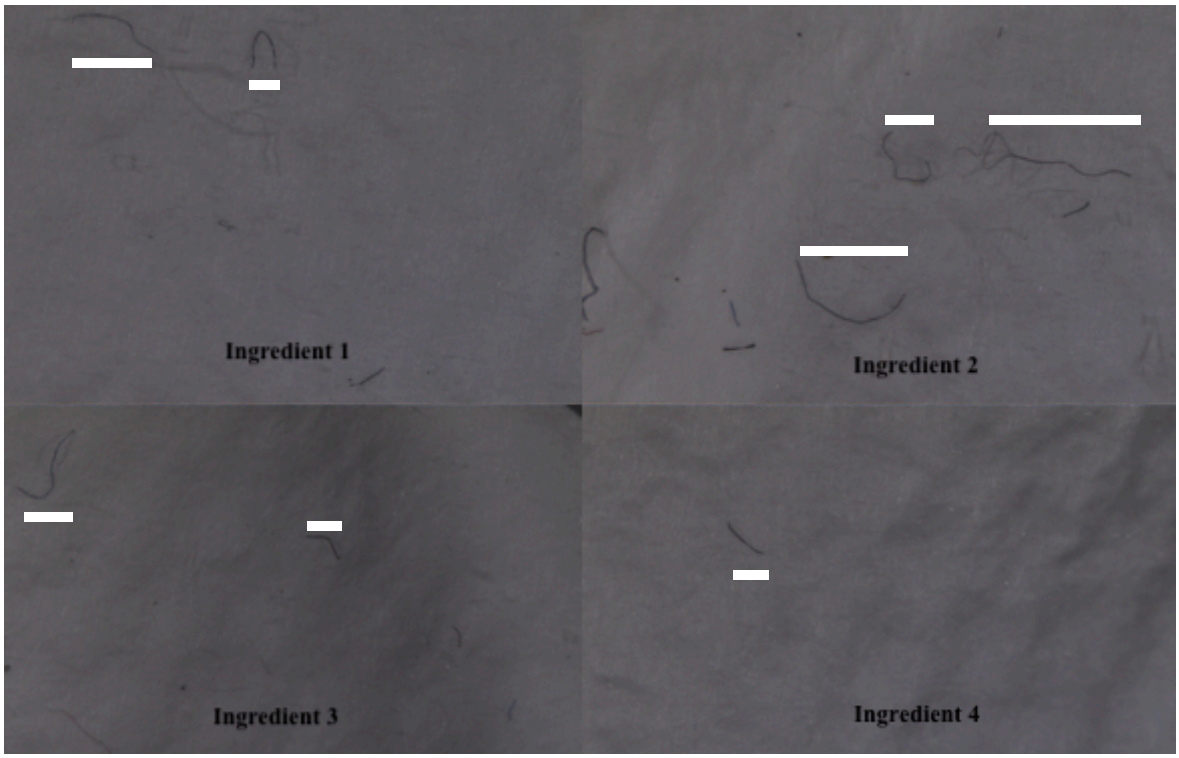

Figure 16. dentification of particles in the glass microfiber filter. Scale bar represents 1mm.

4.2.3 Results from the FT-IR analysis

Identification of individual particle chemical composition was carried out by FT-IR showed that prevalent contaminants were cellulose fibers or fragments in all ingredients (Figure 17) Ingredients 2 and 4 had the largest variability in terms of particle composition. Polymeric particles were observed in most ingredients, particularly, in Ingredient 4 in which epoxide, viscose, and acrylic fabric fibers and fragments were detected. Ingredient 1 and 3 had a lower variability in the composition of contaminants, again with cellulose fibers being the most frequently detected particles.

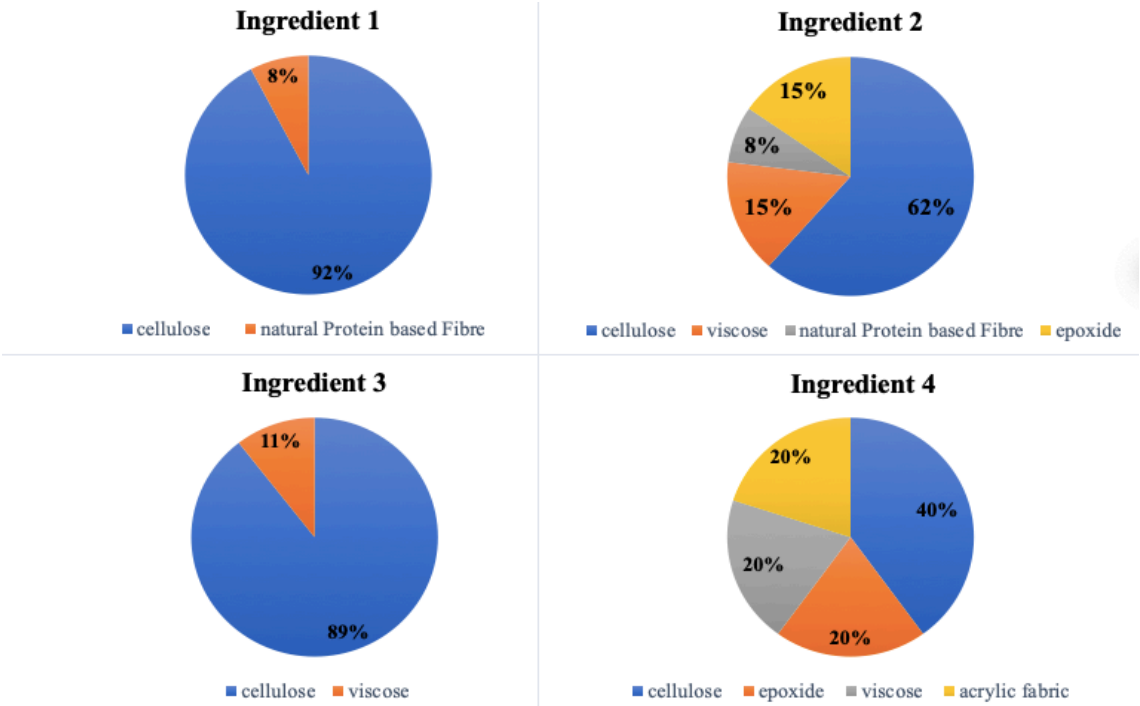

Figure 17. Detection of different particles and polymers in the first analysis of the ingredients.

4.2.4 Frequency estimation of the presence of contaminants in a single SRM pill

Based on the results on contamination in the ingredients used in the formulation of SRM pills the frequency of finding a particle inside a single individual pill was estimated as follows:

Equation 2. Estimation of contamination in a single SRM pill

$$F = \sum_{i} \frac{m_i}{M_i} \cdot n_{p,i}$$

Where m_i (g) is the mass of a given ingredient i used in the formulation of an individual SRM pill, M_i (g) is the mass of the ingredient i analyzed and $n_{p,i}$ is the total number of particles detected in the ingredient i sample. The sum aggregates F from the different ingredients. Table 8 report the results of this elaboration.

Table 8. Estimation of the frequencies of finding contamination in SRM pills: 1st stock of ingredients.

	Mass of ingred. in a SRM pill (g)	Total Particles	Cellulose	Viscose	Epoxide	Acrylic	Natural proteic fiber	Un-identified
Number of detected particles								
Ingredient 1	2	14	12				1	1
Ingredient 2	1.2	13	8	2	2		1	
Ingredient 3	0.5	21	17	2				2
Ingredient 4	0.2	5	2	1	1	1		
Calculated frequencies								
Ingredient 1		0.56	0.48				0.04	0.04
Ingredient 2		0.31	0.19	0.05	0.05		0.02	
Ingredient 3		0.21	0.17	0.02				0.02
Ingredient 4		0.02	0.008	0.004	0.004	0.004		

The results from the analysis of the first batch of ingredient provided evidences of unsatisfactory level of purity in the SRM. It was opted therefore to use a new batch of the individual ingredients with a higher level of nominal purity. This was analyzed in a similar way as done for batch 1 and results show a considerably lower amount of contaminant. A total of 7 particles were counted in the microscope in samples of the 4 ingredients, as shown in Figure 18.

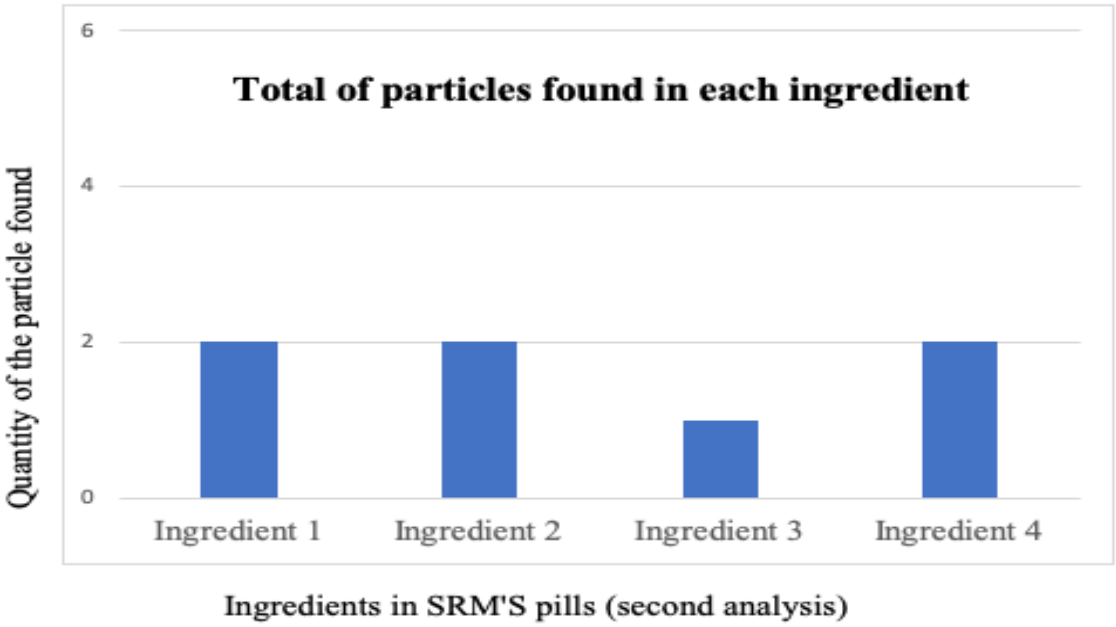

Figure 18. Identification of particles by microscope in the second analysis of the ingredients for a SRM production.

In this case, Ingredient 1, Ingredient 2 and Ingredient 4 had the same amount of impurities. In Ingredient 3 only one particle was found. Also, in this case contamination by fibers with lengths from 1 – 4 μm and different colors was prevalent (Figure 19).

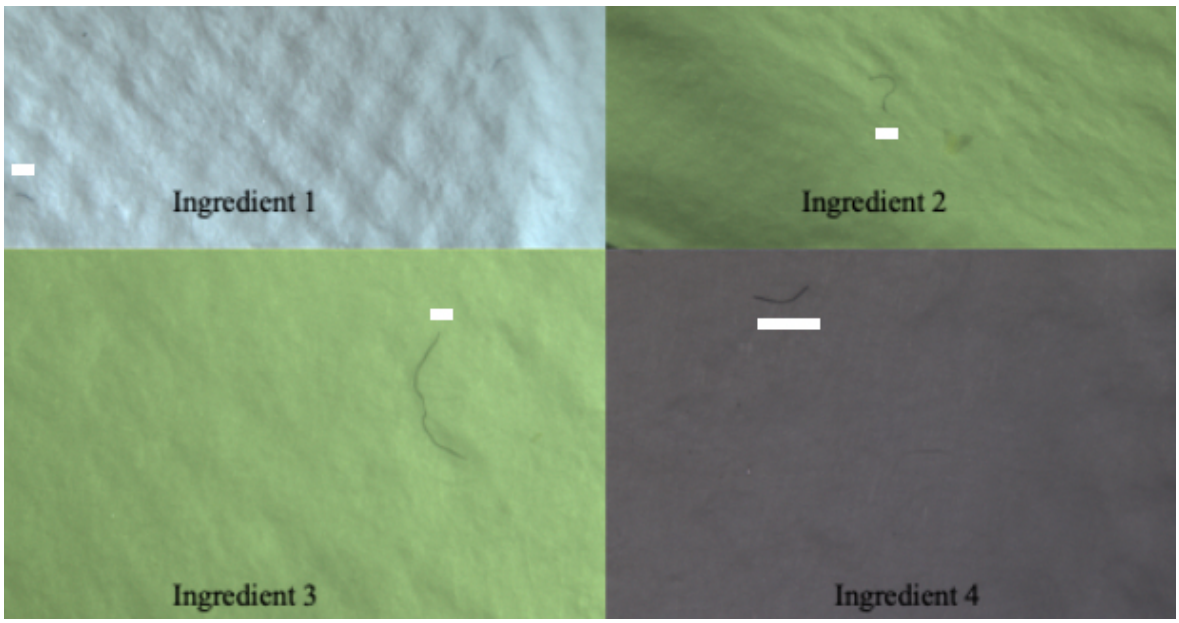

Figure 19. Identification of particles in the glass microfiber filter. Scale bar represents 1mm. Second analysis for SRM pills productions.

4.2.5 Results from the FT-IR

Identification of these particles was performed by FT-IR. In this case, the prevalent contaminants were cellulose. No polymers were detected (Figure 20).

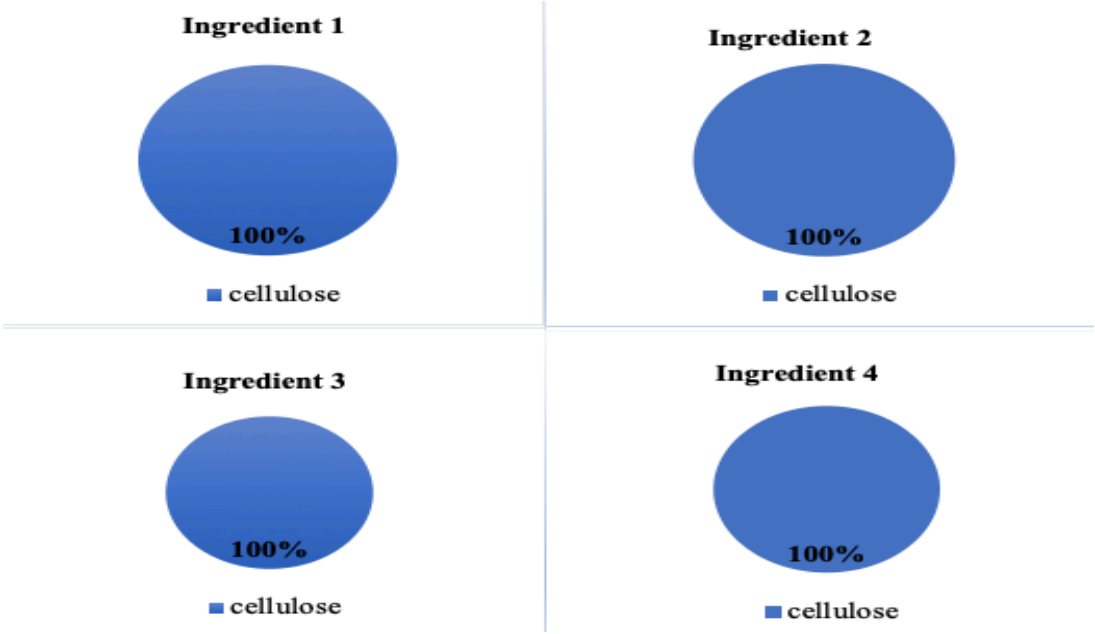

Figure 20. Percentage of particles found in the second analysis on FT-IR for SRM's pills production.

4.2.6 Estimation of the new frequency of contamination in one SRM pill

Based on the same calculation presented in the section 4.2.4, a new frequency was calculated to find interferent particles in the SRM was estimated, showing a considerably lower chance of meeting a potentially interferent particle in all ingredients in the SRMs (Table 9).

Table 9. Estimation of the frequency of finding contamination in SRM pills: 2nd batch of ingredients.

	Mass of ingred. in a SRM pill (g)	Total particles	Cellulose
Number of detected particles			
Ingredient 1	2	2	2
Ingredient 2	1.2	2	2
Ingredient 3	0.5	1	1
Ingredient 4	0.2	2	2
Calculated frequencies			
Ingredient 1		0.08	0.08
Ingredient 2		0.048	0.048
Ingredient 3		0.01	0.01
Ingredient 4		0.008	0.008

4.3 Validating the analysis of MP in tap water by membrane filtration

4.3.1 Optical microscopy results for tap water samples and negative controls

This section includes results from optical microscopy for triplicate analyses of tap water samples at different volumes (10L, 100L, and 1000L), as well as the results for negative control (NC) samples (i.e. laboratory blanks) and the positive control (PC) (i.e. recovery assessment). Table 10 summarizes all the particles counted in each batch of samples. Throughout this study, 38 particles were found in real tap water samples. The number of detected particles did not co-vary with the analysed volume of the sample. The variance among replicates was relatively small as shown in Table 10.

Table 10. Samples of water with volume in liters and number of particles.

	10 L	100 L	1000 L
W1	4	3	4
W2	7	3	5
W3	6	4	2
NC	9	4	4

Figure 21 shows the average number of particles in the three tap water volume groups and the degree of variation between them expressed by standard deviation. Comparison with table 10 shows that all the three mean particle values are slightly lower than the NC values. The measure was made with the volumes to study the influence or not of the volume with the number of particles obtained in the positive control. As mentioned before, the 10L was found with more results.

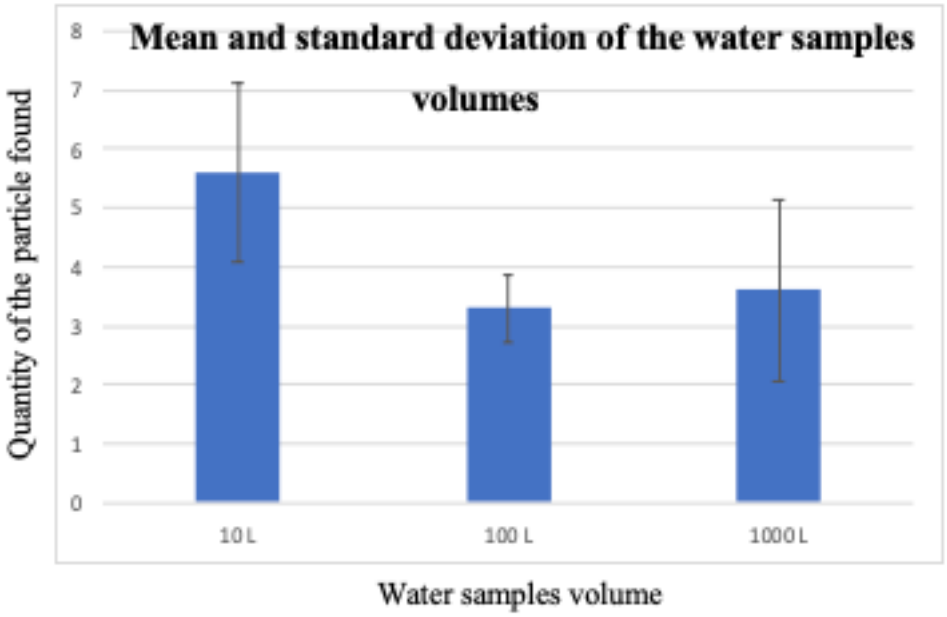

Figure 21. Results of median and standard deviation of particles in different water samples volume.

4.3.2 Optical microscopy results for positive controls

The results of the positive control analyses are depicted in table 11. While a total of 10 MP of each individual SRM type were added to each sample, the recoveries showed some variability essentially depending on the MP type.

Table 11. Reference materials detected in different volumes.

Positive Controls			
	Polymers detected		
	10 L	100 L	1000 L
Car tires	3	10	30
PVC	4	8	6
Beads PE	3	7	7
Orange fibers	8	10	10

Figure 22 shows the calculated average recovery efficiency for different MP types based on optical microscopy. Largest variability was observed for the car tire debris with recovery, in the 1000 L case exceeding 100%. The source of additional particles is here unknown – it may in principle have come from the tap water, the SRM, atmospheric contamination in the laboratory, or it can have been a misjudgement of the type of material in the optical microscopy analysis (see below).

Relatively low average recovery (50%) was observed for PE beads while it was 60% for PVC fragments. Higher recoveries (e.g. over 80%) was observed for the fibers.

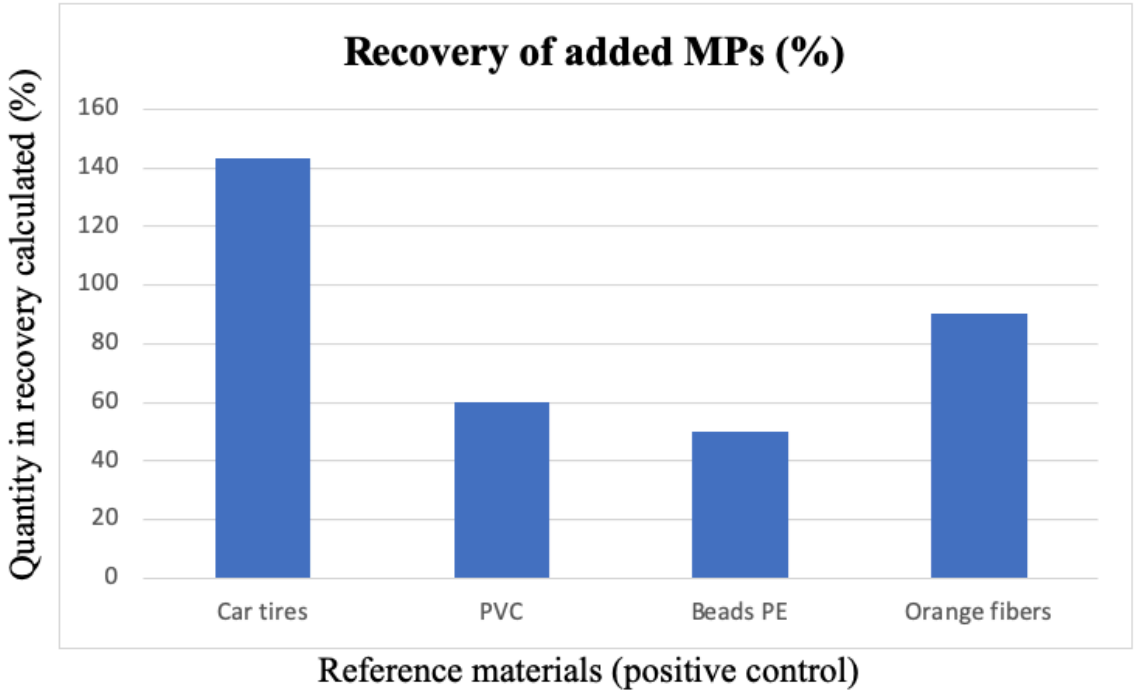

Figure 22. Recovery of the reference materials used as a positive control.

Figure 23 shows results from the microscopy analysis. Particles of different materials in many cases have very similar appearance in microscopy. FT-IR analysis is, therefore, important to correctly classify the detected particles. In this Figure 23, it is possible to see the higher abundance of tire particles observed in the 1000 L positive controls.

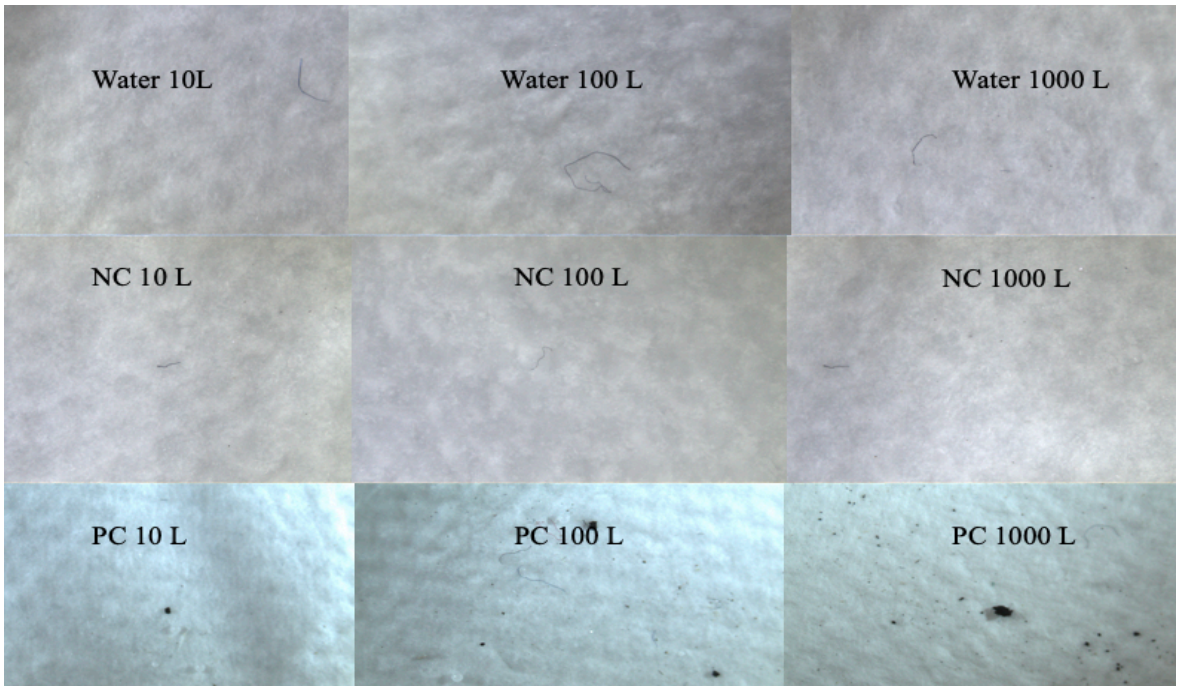

Figure 23. Microscope identification of the samples and the positive control quantities.

FT-IR results on the positive controls are displayed in table 12. Notably, impurities were found in both the negative control and the positive controls, mainly classified as cellulose fibers, epoxide and viscose were the only two anthropogenic MP detected as impurities in the negative control. Unfortunately for the 1000L positive control particles were lost during the transfer to the FT-IR detector. The lack of analysis in FT-IR could have resulted in misjudging impurities as car tire debris, determining therefore the very high recovery value.

Table 12. Results of the analysis on FT-IR in different water volumes and NC.

Identified particles (in parentheses are indicated the particles recognized as SRM)			
Sample	**10 L**	**100 L**	**1000 L**
W1	(styrene isoprene), cellulose	cellulose	cellulose
W2	cellulose	cellulose	cellulose, (fibre-viscose)
W3	cellulose, (viscose)	cellulose, (viscose)	unknown
NC	cellulose, (epoxide, viscose)	cellulose	cellulose

By figure 24 it can be compared all the volumes and the different samples concerning the percentage of each polymer found for the water membrane analysis and not for the negative control, since only one source of the polymer was detected in the NC (10L) – epoxide and viscose. Cellulose was the most predominant in all cases. Styrene isoprene and viscose were detected in the analysis of 10L. Viscose in the analysis of the 100L. Also, fibre-viscoce in the analysis of 1000L. These particles related to the SRMs shown other source of contamination, since the SRMs that were used are not correlated. For the positive control, only the analysis in the microscope was evaluated, because it was already known the polymers used in this study, so the identification on FT-IR was not necessary.

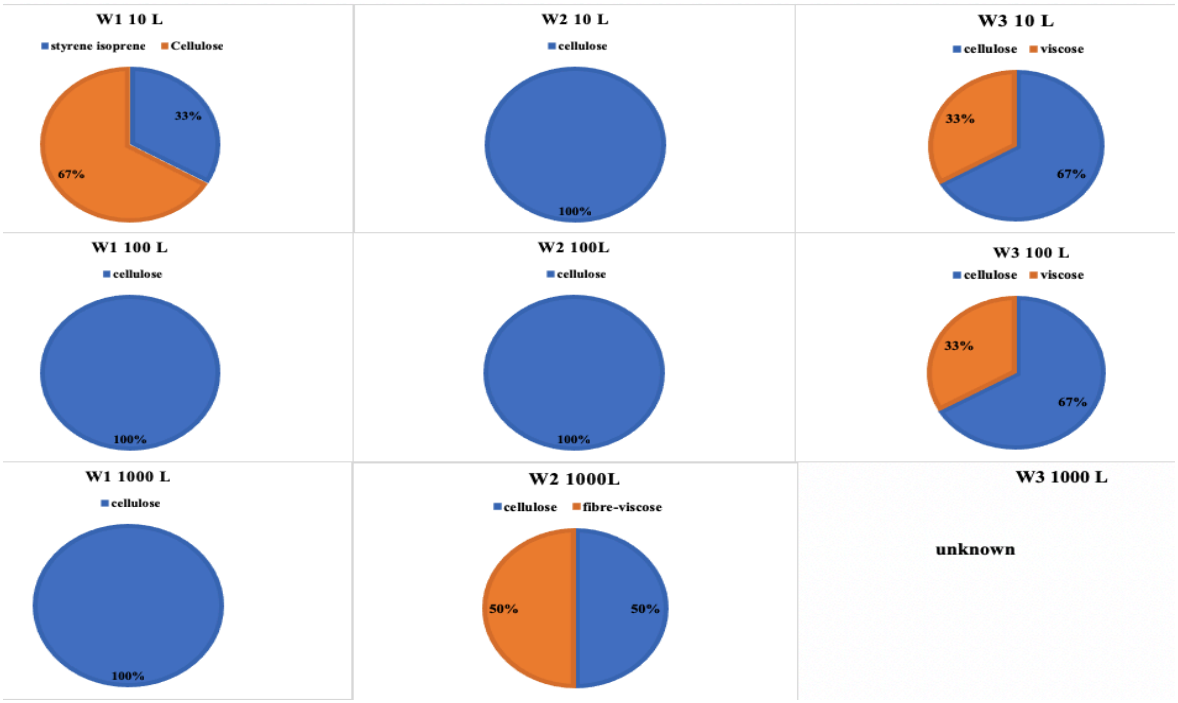

Figure 24. Results for FT-IR results on the positive and negative controls.

5. Discussion

5.1 Laboratory-based contamination sources

The assessment of laboratory-based sources of MP contamination is, to my knowledge, the first of its kind. If conducted it has at least not been included in any of the papers reviewed for this thesis (see references in introductory chapters). This result confirmed the occurrence of MP, especially microfibers, in atmospheric deposition in the laboratory where MP is analysed. The studied laboratory is equipped with controlled ventilation of filtered air and a marginal positive pressure. This standard set-up is conceived to protect the laboratory environment from poor air quality conditions from outdoor or adjacent indoor spaces. This measure, however, does not protect this working environment from the influence of endogenous sources of MP contamination. These can be, for example, appliances of the laboratory that can release MP to the air, or particles conveyed to the laboratory by the laboratory personnel. In the studied laboratory basic precaution is regularly taken to avoid such contamination including:

- maintaining, as much as possible a confined environment in relation to other areas of the laboratory;

- the use of the lint roller before accessing the laboratory by users;

- restricting access to a limited number of users.

The results presented here suggest these simple precautions are insufficient to guarantee a MP-free atmosphere, and therefore, to fully protect samples' integrity during the analysis. The scale of contamination measured here is fortunately unlikely to produce significant changes in MP measurements in highly concentrated samples, as, based on the present result, only a limited number of airborne particles are expected to deposit on the samples while they are analysed, for example, under the microscopy or during FT-IR analysis. However, when very "clean" samples are under the analyses (such as drinking water or pristine marine waters), the observed lab-based contamination can be of particular concern. In this case, the use of laboratory blanks is strongly recommended. The deposition of even a small number of airborne MP could significantly alter measurement results. Petri

dishes similar to those used in this study can be conveniently deployed side-by-side the real samples during the time they are directly exposed to the laboratory atmosphere, as a quality control measure for validation of the measures and the quality of the data.

The results of laboratory-based contamination highlighted the occurrence of temporal changes and a variable of contamination across different locations in the laboratory. This variability can help to identify the drivers and origins of the laboratory contamination. To interpret it can be considered such factors as the number of people using the laboratory at a given time, the location in relation to the aeration system or the laboratory entrance door can be considered. NIVA MP laboratory is a relatively small space where 4 among researchers, students, and technical staff can work simultaneously with a total number of different laboratory users with 6 professionals. This laboratory can, therefore, be defined as an intensively used space.

The influence of human presence in the laboratory emerges from the difference in results of the deposition in location “Lab door right” and “Lab door left” (Figure 6). The “Lab door right” is a transit zone that anyone using the laboratory will frequent often, while the location named “Lab door left” is adjacent, but it is screened from the transit area by a plexiglass sheet, in order to limit potential contamination from the transit zone to the microscope location. “Lab door left” has a relatively lower contamination than “Lab door right”. This result support the expectation that simple measure to prevent MPs from laboratory base sources (such as plexiglass screens), can effectively be set in place to limit (albeit not eliminating) the chance of sample contamination.

Another interesting result is the one of the areas labelled “Lab chemicals”, the storage location for reagents used in the laboratory. In this case, the usage of the laboratory and in particular activities requiring frequent use of the reagents (e.g. during the analyses of sediments or soils) may induce a resuspension of dust and MPs deposited in the cabinet. During the period of the laboratory deposition monitoring, no intense use of these chemicals was carried out. This can explain the low amount of MP found in the filter (Figure 12).

The sampling location named “Lab shelf” is the area where samples are stored in a safe box and it is generally a relatively “inactive” area of the laboratory, which is also

reflected by a low number of particles (Figure 12). Deposition measurements in the above shielded or inactive areas showed the lowest contamination levels.

The highest lab-based contamination was recorded in the proximity of the FT-IR instrument where an average of 10 particles was observed in the different sampling periods. This is according with the FT-IR station being one of the more crowded and active locations in the laboratory. Unfortunately, the long exposure time of samples to the atmosphere during FT-IR analyses may thus result in a risk of contamination from lab-based sources. This would be particularly critical for samples with a low level of contamination such as drinking water or marine waters, where the deposition of few particles from the laboratory atmosphere could significantly alter the measurement result.

Future work concerned with the identification of laboratory-based sources of MP could be conceived, for example, at the scope evaluating the laboratory contamination profile after structural changes or following upgrades in the infrastructure. A more thorough characterization of laboratory contamination can lead to identifying sources and eliminate them. It is recommended that investing adequate time to characterize the laboratory contamination profile when setting up a new laboratory for MP analyses is a useful and important exercise. Our simple study gives an impression that MP contamination is variable but rather omnipresent, including the air and solid surfaces of indoor laboratory environments.

Many analytical procedures in MP laboratories are conducted in laminar flow cabinets, however, not all crucial steps, including microscopy analysis and FT-IR analyses can be conducted in such a cabinet. A costly alternative to work in good laboratory conditions is to conduct the MP analyses inside clean or ultra clean rooms. This will assure a higher level of quality control and lower detection limits. However, it will also considerably increase the costs for setting up and running the MP laboratory. Such a solution may not be viable for many of the recently established MP laboratory in Europe. Moreover, improvement of the ventilation system of the laboratory can be useful, provided an adequate check on the effectiveness of air filtering and substitution of filters is performed regularly. Plexiglass screens can be used to protect specific areas, despite they will obviously not completely eliminate contamination.

Based on the observation regarding influence of human activity in the lab it will also be important with precautions lab practices. Regular cleaning performed by specially trained staff can be of great help. Trained staff is important because inappropriate cleaning could actually increase MP contamination (e.g. from the use of poor-quality cleaning wipes). It is also a good practice to cover, whenever possible, the samples with aluminium foil (a measure regularly employed at NIVA laboratory) and to use as much as possible the laminar flow cabinet.

In contrast to the results obtained for the intensively used MP analytical laboratory, indoor atmospheric deposition in the SRM preparation lab provided evidence of a much cleaner environment. This is a newer laboratory much less frequented. These results confirm that the number of users and the frequency of their access in the laboratory can influence the potential for sample contamination.

5.2 Contamination of MPs in SRM formulation ingredients

Two batches of ingredients used for the formulation of SRM were analysed. The first batch showed that different ingredients contributed differently to the background impurities of SRM. The highest particle contamination was observed in Ingredient 3, followed by the Ingredient 1, then Ingredient 2 and finally Ingredient 4 (Figure 15). Cellulose fibers were the most abundant of the observed particle contaminants, in other words non-plastic particles, but anthropogenic polymeric particles were also detected with certain frequencies. Cellulose fiber are nature-derived materials. Although they would not interfere with the counting of MP following characterization in FT-IR or spectroscopic technique, they could be misjudged as MP by an inexperienced operator in optic microscopy analysis.

In addition, independently from the nature of the contamination, the SRM background impurity must also be minimized. It was revealed by the FT-IR analysis that improvements were achieved in the purity of these pills in the second batch, and it can indicate that similar measures as made in the production of the second batch is generally necessary in order to produce these pills as reference material for MPs with less impurities. In ingredient 1, cellulose and natural fibre were detected, which beside being an SEM impurity also can be because of the contamination from the use of a laboratory coat or another natural component. However, in Ingredient 2, Ingredient 3 and Ingredient 4 it was

detected polymers (viscose, epoxide or acrylic fabric). Based on these analyses result the frequency and thereby the probability to find impurities in MP SRM was high, meaning that it is likely that at least a single contaminant particle will be present in any SRM pill. In our lab we judge this to be unacceptable, which lead us to substitute the batch of ingredient with a new one with a higher quality level.

The new upgraded batch of ingredients was anayzed with very positive results. Only rare cellulose particles were detected in the samples. No anthropogenic polymer was found. The frequencies in our measurements, and thus the probability of finding any particulate impurity dropped for all four ingredients and almost 10 folds for the average of them in the new batch. We believe this made an important contribution to improve the quality of SRM that is being used in the current international inter-laboratory calibration exercise.

5.3 Validation and quality assurance during the analysis of drinking water

The analyses of the different volume of tap water (10L, 100L, 1000L did not provide significantly different results). All together the measured MP levels were not significantly different from the level measured in the negative controls (or field blanks; Table 10.) The measurement showed generally good reproducibility, judged by the low standard deviations (Figure 21). These results clearly indicate that there were no detectable MPs of any type in up to 1000 L of the measured water and since the detected particles were similar for all three tap water volumes we tend to believe that the detected particles were the result of background contamination associated with the analytical method. Hence, the water itself was not significantly contaminated and the detected particles were likely originated before. Detected particles were just originated from the background contamination from the filtration unit and that accumulated during the analysis steps.

Expectedly, with the choice of drinking water as a method validation matrix, the need for a high level of quality control was clearly emphasized. Because of the omnipresence of MP particles, to measure false positives can be an obvious pitfall. Here, the correct application of negative controls prevented this to occur. In relation to analytical quality the use of adequate negative control can thus contribute to properly formulate method detection limits and avoid the occurrence of false positive signals. Based on the lack of difference

from the negative controls, it can in this study be concluded independently from the analyzed volumes that there was non-significant MP contamination measured in tap water. However, in order to contribute on this basis, it must also be shown that the measurement system is working adequately. This is done by the positive control which is discussed in the following.

The inclusion of the positive control can further confirm the quality of the measurements, fully validating the analysis quality. Positive controls are used to calculate analytical recoveries of MP added as SRM. A result of recovery equals to 1 (=100%), means a perfect recovery. In this study, different recoveries were found for different types of MPs. In the figure 22, the results for recovery calculation is depicted. While there obviously was a difference in the recovery results of different type of SRM it is possible to calculate and aggregated recovery number, taking into account the total number of particles added as in the positive control compared to those experimentally detected. For the 10 L positive control a total recovery of 45% was obtained. While for the 100 L positive control 87% and for 1000 L positive control 132,5% recovery efficiencies were calculated. The impossibility of comparing these results with any of the previous studies makes it difficult to evaluate how good or satisfactory this recovery is. It shows us that the measurement system is working, and it gives us a certain idea about variation, which is quite high, but it will require more studies of similar kind to conclude and utilize this result further.

It must be emphasized that large part of the variability observed among the different positive controls was influenced by the results of car tires reference materials. These are notoriously challenging particles to detect as they have irregular shape, black color and are not identified by any spectroscopic technics as they tend to be coated with black carbon. Very importantly the results of recovery for the fibers was considered good. All together, these results confirm that MP can be quantitatively analyzed in environmental samples when the application of strict QA/QC measures is implemented in analytical protocols. More data of similar kind will hopefully contribute to a future higher precision regarding determination of concentration values and detection/quantification limits, and to expectations regarding positive control recovery.

5.4 Discussion on general FT-IR results

The identification of the particles detected in any of the different experiments conducted during this thesis is discussed here. Detected materials included cellulose, polyester, polyacetal, viscose, acrylic fabric, epoxide and polyisoprene chlorinated. These essentially represented the background contamination profile building up during the analysis in NIVA laboratory.

Cellulose $(C_6H_{10}O_5)_n$ is a natural organic compound. It originates mostly from wood pulp and cotton. It has different uses as in paper industry, clothes, and plastics. Related to clothes, cotton, linen, or jute cellulose is an important source, constituting almost of 90% (Brigham, 2018; O'Connor et al., 2014). Considering, that the laboratory coat is made with cotton, the highest concentration found in all the samples can be expected from the use of the laboratory coat in the laboratory. It is not surprising that such a natural fiber was frequently detected in the blanks and samples. This highlight on the one side, how the operator itself can cause contamination of the samples under analysis, and on the other side the sensitivity of the method in picking up even small level of background contamination. This is not trivial if one considers that detection is essentially carried out by searching for microscale particles manually under a microscope.

Polyester was frequently detected as laboratory-based contamination. This is a synthetic polymer. It is frequently used as a fiber in clothes or other textiles (Sillanpää et al., 2017). Being an important source of MPs, clothing based on synthetic fibers should be avoided in MP analytical laboratories.

Viscose is a semi-synthetic polymer largely used in the clothing industry. It is used as an alternative to cotton as it is derived from cellulose fibers (Salvador et al., 2017). It usually presents in textile in mixture with other synthetic fibers. This compound was detected in the sample from many laboratory locations particularly in those from "Lab Door Right", the "FT-IR area" and the "Lab Glass Shelve". Viscose particles were found in the SRM formulation ingredients: Ingredient 2, Ingredient 3 and Ingredient 4. In the analysis of MP in tap water, most in the volume of 10 L (2 times), 100L (1 time) and in the volume of 1000L (1 time) viscose was the most common MP detected across all the samples.

Acrylic is a synthetic polymer also used in fabrics and common in clothing especially blended with natural wool or cotton (Napper et al., 2016) Acrylic-based MPs were detected in two locations of the analytical laboratory and in some of the Batch 1 ingredients used for the formulation of MPs SRM.

Polyacetal (Polyoxymethylene) is also an oil-derived polymer mainly used in engineers' components such as eyeglass frames or lock systems, as well as in mechanical gears, sliding and guiding elements or in insulators or connectors (Lüftl et al., 2014, p.2). This source was found only at the MP's laboratory at the "Lab Door Right" adding to the suggestion of this place as one of the "hotspots" in the lab. Its occurrence could be linked to the mechanical erosion of larger items during the use of laboratory, appliances or other items. Most of the laboratory bench surface, walls, containers, etc. are made of plastics, and this type of contamination appear difficult to avoid. Only working in a highly controlled ultra-clean laboratory seem to result in minimizing this source of contamination.

Epoxide based particles were detected at MP's laboratory on the "FT-IR" and in the "Lab Glass Shelve". Since epoxide is a chemical group used as a reagent for the synthesis of a range of different products (Nakano et al., 2007) it is difficult to trace the presence of these contaminant particles to their potential source. In the production of the chemical ingredients, this element was also detected, in Ingredient 2 and Ingredient 4. The reasons for this are not known.

Polyisoprene chlorinated is a polymer derived from isoprene. The use includes adhesives, sealant or rubber bands (Ciullo et al., 1999). These polymers were discovered at the MP's laboratory on "FT-IR" and in the "Lab Glass shelve" location only. It can be hypothesized that this contamination derived from laboratory appliances, but it is not possible to conclude based on the present data.

Styrene Isoprene is a copolymer. They are frequently used for injection-molded parts, adhesives, sealants, gasket materials, rubber bands (Polymer Data Base, 2019). This copolymer was only detected in the sample of 10L (one time). If this rather low frequency of occurrence is representative in MP labs or in tap water can only be concluded after more data becomes available.

6. Conclusion

The main conclusion from this thesis is that MPs are ubiquitous in laboratory environments, and strict quality assurance and control measure (QA/QC) are necessary to ensure good analytical results and avoid false positives. In this study, interfering airborne MPs from lab-based sources were detected in indoor atmospheric depositions in all locations of a MP analytical laboratory. Laboratory blanks used for support analyses of real samples are also conveying significant quantities of interfering particles. In order to keep these potential sources of contamination under control, a further improvement in the methodology and the development of adequate SRM was necessary. The thesis contributed to fill this gap, with some fundamental seminar work. The overall scope of the thesis was to support the development of stricter criteria for MP measurement quality and the development of standard reference materials (SRM), along with approaches to validate measurement results.

The thesis presents, to my knowledge, the first assessment of laboratory-based contamination sources looking at the deposition of airborne particles from the laboratory atmosphere. Despite the controlled ventilation system of the laboratory, MP contamination was observed in all monitored locations. It was identified a variability across the locations that reflected the use of the laboratory and the frequency at which a given instrument, tool, or reagent is used. Fibers emerged as the main contaminant in the laboratory atmosphere, suggesting that clothing of laboratory users can serve as a source. The results suggested that some basic precaution (such as the use of Plexiglas screens or limiting access to the laboratory) can result in better conditions for MP analysis. Other useful measures to reduce laboratory contamination include frequent cleaning with adequate materials and the use of non-plastic tools when dealing with the samples. Although the observed contamination is unlikely to affect measurements of samples carrying a high number of particles, they can interfere with the quantitative analysis of "cleaner" samples such as drinking water, marine water or air. As a conclusion, the study demonstrates the need for using laboratory blanks when analysing "low-contamination" samples.

A very important section of this work was the support to the development of SRM for MP. To this regard this work contributed to the development of the SRM that is currently used in the first international interlaboratory calibration exercise. As such, this result will influence the quality and development of good laboratory practice in many international MP

laboratories. During this part of the study, the occurrence of interferent particulate impurities in the formulation ingredient for the SRM was addressed and characterized. The work contributed to the choice of higher-grade ingredients for the SRM formulation that considerably reduced the background contamination. The four ingredients analysed in each batch were mostly contaminated by cellulose fibers, although polymeric particles were also detected.

This thesis has closely supported the development of a new concept where MP SRM are carried in effervescent pills. Beyond the analysis of impurities and contamination in the SRM formulation, the work carried out within this thesis focused also on the reproducibility of the number of MP SRM included in each pill. After a trial and fail reiterative process, SRM with a relative standard deviation lower than 15% was achieved per any of the MP SRM types. To this regard, improved homogenisation of the ingredient and the reference MP was instrumental, as well, as the increase of the total number of MP SRM over 20 units per pill.

Concerning the validation and quality assurance during the analysis of drinking water, it was found that no detection of MP in up to 1000 L in drinking water was achieved. However, MP and other types of particles were present in the samples. This is a situation that can easily produce false positives and induce a misled interpretation of the results. In this study, however, the thorough deployment of QA/QC measures allowed to prevent such a risk. Adequate laboratory blanks showed that the observed contamination was originated from the laboratory and the materials used for sampling, and not from the sample. The analysis was also fully validated through the use of positive controls where MP SRM were intentionally added to the second set of samples. Despite the large variabilities in the recovery efficiency of some MP type (i.e. car tire debris). For polymeric fragments and synthetic textile microfibers recovery efficiencies ranging 50-85% were obtained.

This study demonstrates the need for solid quality assurance and control measure to prevent external contamination sources interfering with the analyses results. Previous studies have conducted an assessment of drinking water starting from 1 L samples. Analyses were conducted without these precautions and generate questionable results that had high resonance in the scientific for and the media.

FT-IR analysis is an essential step to identify MP as plastics. Most frequently observed particle contamination in laboratory blanks are cellulose fibers. This is not plastic. However, many anthropogenic polymers and materials were identified too, such as polyester, polyacetal, viscose, acrylic fabric, epoxide and polyisoprene chlorinated. For correct identification of these particles optic microscopy analysis is insufficient. FT-IR instead provide the necessary complementary information about the characteristics and a potential source of the contamination.

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Appendixes

Appendix A Physical measurements of the ingredients, as longitude and latitude

Appendix B Spectra of the polymers detected on the FT-IR

Appendix A includes the physical measurements of the ingredients and features such as particles relate to the location, round/time of the study, number of the particles counted, the polymer type and maximum/minimum short axis of the particles. Lost particles mean that the particles identified in the microscope were not identified in the FT-IR due to the loss of the particle in the sample when using tweezers.

In the study of the contamination in the MP's analytical laboratory, it is also including data of the average, the sum, the standard deviation, the maximum, the minimum and the coefficient of variation in percentage of the particles studied.

Location	Round	Particle	Polymer type	Max. long axis	Min. short axis
Lab Door Left	1	1	cellulose	203.378 µm	27.186 µm
		2	cellulose	111.348 µm	23.763 µm
	2	1	cellulose	163.063 µm	29.279 µm
		2	polyester	246.346 µm	23.693 µm
		3	lost	266.491 µm	28.357 µm
	3	1	cellulose	241.597 µm	25.936 µm
		2	lost	73.357 µm	25.775 µm
		3	cellulose	440.904 µm	25.936 µm
	4				

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
2	8	1.26	3	2	63%

Location	Round	Particle	Polymer type	Max. long axis	Min. short axis
Lab Door Right	1	1	lost	132.493 µm	20.976 µm
		2	cellulose	97.341 µm	24.635 µm
		3	polyacetal	88.181 µm	35.806 µm
		4	lost	202.938 µm	22.713 µm
		5	cellulose	238.737 µm	39.436 µm
		6	cellulose	475.531 µm	27.543 µm
	2	1	fibre	82.675 µm	16.526 µm
		2	cellulose	316.957 µm	33.508 µm
		3	fiber	298.434 µm	34.511 µm
		4	viscose	937.905 µm	36.593 µm
		5	lost	219.456 µm	25.393 µm
		6	cellulose	139.157 µm	33.166 µm
		7	cellulose	598.768 µm	44.837 µm
		8	viscose	2367.946 µm	29.279 µm
	3	1	cellulose	1336.949 µm	36.451 µm
		2	acrylic fabric	1642.883 µm	24.622 µm
	4				

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
4	16	5	8	2	125%

Location	Round	Particle	Polymer type	Max. long axis	Min. short axis
Lab Chemical	1	1	lost	69.171 µm	11.017 µm
		2	viscose	121.104 µm	19.862 µm
	2				
	3				
	4				

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
0.5	2	0	2	2	178%

Location	Round	Particle	Polymer type	Max. long axis	Min. short axis
FT-IR	1	1	cellulose	551.001 µm	22.206 µm
		2	epoxide	1051.674 µm	22.713 µm
		3	lost	119.212 µm	23.533 µm
		4	cellulose	935.080 µm	29.665 µm
		5	cellulose	207.253 µm	17.636 µm
		6	fibre	108.537 µm	16.526 µm
		7	lost	210.664 µm	22.206 µm
		8	cellulose	966.725 µm	30.422 µm
	2	1	cellulose	896.264 µm	21.512 µm
		2	cellulose	148.068 µm	47.066 µm
		3	cellulose	179.178 µm	44.497 µm
		4	cellulose	542.744 µm	27.680 µm
		5	cellulose	795.938 µm	43.024 µm
		6	cellulose	220.482 µm	22.034 µm
		7	cellulose	1076 µm	56.782 µm
		8	cellulose	464.633 µm	23.533 µm
		9	cellulose	1088.818 µm	22.713 µm
		10	cellulose	375.948 µm	29.279 µm
		11	cellulose	243.519 µm	33.166 µm
		12	cellulose	561.298 µm	29.792 µm
	3	1	cellulose	87.976 µm	31.037 µm
		2	cellulose	865.899 µm	34.701 µm
		3	polyisoprene chlorinated	863.060 µm	47.962 µm
		4	lost	45.564 µm	45.014 µm
		5	viscose	254.757 µm	29.669 µm
		6	cellulose	506.691 µm	27.339 µm
		7	fibre	659.941 µm	23.233 µm
		8	cellulose	104.421 µm	32.219 µm
		9	lost	132.121 µm	34.581 µm
		10	cellulose	192.301 µm	56.764 µm
		11	epoxide	117.734 µm	41.26 µm
	4	1	fibre	2788.261 µm	72.274 µm
		2	lost	177.012 µm	29.669 µm

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
8.25	33	10.73	12	2	130%

Location	Round	Particle	Polymer type	Max. long axis	Min. short axis
Lab Glass Shelve	1	1	cellulose	24.790 µm	359.380 µm
		2	cellulose	82.184 µm	37.463 µm
		3	viscose	145.624 µm	49.746 µm
		4	polyester	79.234 µm	29.669 µm
		5	natural fiber	50.327 µm	32.219 µm
		6	polyester	645.161 µm	14.409 µm
		7	cellulose	475.779 µm	35.645 µm
	2	1	cellulose	768.214 µm	17.420 µm
		2	cellulose	127.481 µm	13.772 µm
	3				
	4	1	cellulose	1152.165 µm	23.233 µm
		2	cellulose	498.673 µm	18.452 µm

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
2.75	11	3.92	7	2	142%

Location	Round	Particle	Polymer type	Max. long axis	Min. short axis
Lab Shelve	1	1	cellulose	1792.453 µm	43.112 µm
		2	cellulose	1356.786 µm	72.716 µm
		3	cellulose	616.929 µm	49.271 µm
	2	1	cellulose	1233.921 µm	25.984 µm
	3				
	4	1	fibre flax	296.502 µm	20.377 µm
		2	cellulose	704.021 µm	37.463 µm

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
1.5	6	1.07	3	1	71.3%

The result of lab-based contamination in MP standard reference materials (SRM) included Part 1 of the first experiment with the chemical related to physical characteristics (maximum long axis and minimum short axis). Furthermore, Part 2 with the same analysis but with the new chemicals.

PART 1

Ingredient 1

Particle	Polymer type	Max. long axis	Min. short axis
1	lost	164.32 µm	14.67 µm
2	cellulose	257.71 µm	19.89 µm
3	cellulose	346.89 µm	18.55 µm
4	cellulose	5.51 µm	8.32 µm
5	cellulose	607.79 µm	10.15 µm
6	cellulose	72.11 µm	10.5 µm
7	cellulose	82.63 µm	20.8 µm
8	cellulose	75.59 µm	27.18 µm
9	cellulose	89.86 µm	24.19 µm
10	cellulose	299.29 µm	8.16 µm
11	cellulose	110.78 µm	16.08 µm
12	cellulose	377.53 µm	34.29 µm
13	cellulose	150.45 µm	19.95 µm
14	natural protein based fibre	102.29 µm	18.09 µm

Ingredient 2

Particle	Polymer type	Max. long axis	Min. short axis
1	viscose	37.91 µm	2.89 µm
2	viscose	157.67 µm	5.15 µm
3	zein purified	329.47 µm	8.32 µm
4	cellulose	47.15 µm	4.14 µm
5	cellulose	72.72 µm	4.11 µm
6	cellulose	310.36 µm	2.75 µm
7	epoxide	177.18 µm	6.73 µm
8	cellulose	63.88 µm	5.4 µm
9	epoxide	72.98 µm	5.28 µm
10	cellulose	34.42 µm	4.46 µm
11	cellulose	44.53 µm	4.00 µm
12	cellulose	46.07 µm	5.99 µm
13	cellulose	55.33 µm	4.32 µm

Ingredient 3

Particle	Polymer type	Max. long axis	Min. short axis
1	cellulose	19.06 µm	8.25 µm
2	cellulose	19.97 µm	4.06 µm
3	cellulose	98.98 µm	4.89 µm
4	cellulose	184.86 µm	5.42 µm
5	cellulose	226.63 µm	4 µm
6	lost	63.86 µm	5.55 µm
7	cellulose	31 µm	5.24 µm
8	cellulose	158.88 µm	5.09 µm
9	viscose	105.26 µm	6.78 µm
10	cellulose	107.91 µm	5.08 µm
11	lost	88.13 µm	3.64 µm
12	cellulose	71.5 µm	5.9 µm
13	cellulose	251.94 µm	5.54 µm
14	cellulose	96.18 µm	3.75 µm
15	cellulose	160.17 µm	9.69 um
16	cellulose	98.23 µm	5.49 µm
17	cellulose	22.98 µm	8.18 µm
18	cellulose	337.8 µm	7.13 µm
19	cellulose	192.51 µm	3.05 µm
20	viscose	83.07 µm	7.13 µm
21	cellulose	661.14 µm	5.16 µm

Ingredient 4

Particle	Polymer type	Max. long axis	Min. short axis
1	cellulose	143.04 µm	3.86 µm
2	cellulose	81.58 µm	3.42 µm
3	epoxide	94.96 µm	3.86 µm
4	viscose	73.34 µm	4.45 µm
5	acrylic fabric	148.98 µm	4.85 µm

PART 2

Ingredient 1

Particle	Polymer type	Max. long axis	Min. short axis
1	cellulose	243.81 µm	3.34 µm
2	cellulose	108.73 µm	4.01 µm

Ingredient 2

Particle	Polymer type	Max. long axis	Min. short axis
1	cellulose	20.21 µm	4.24 µm
2	cellulose	65.21 µm	6.44 µm

Ingredient 3

Particle	Polymer type	Max. long axis	Min. short axis
1	cellulose	75.04 µm	4.05 µm

Ingredient 4

Particle	Polymer type	Max. long axis	Min. short axis
1	cellulose	34.95 µm	3.47 µm
2	cellulose	164.77 µm	3.44 µm

In the study of the application of QA/QC and validation of a method for MP in tap through membrane filtration, the data of the average, the sum, the standard deviation, the maximum, the minimum and the coefficient of variation (in percentage) of the particles were also included. The features of each particle related to the maximum long axis and the minimum short axis, as well.

Sample	Round	Particle	Polymer type	Max. long axis	Min. short axis
10L	1	1	styrene isoprene	28.31 µm	4.44 µm
		2	cellulose	94 µm	3.56 µm
		3	cellulose	28.48 µm	6.45 µm
		4	lost		
	2	1	cellulose	142 µm	3.9 µm
		2	cellulose	345 µm	4.78 µm
		3	cellulose	28.73 µm	3.24 µm
		4	cellulose	405 µm	3.43 µm
		5	cellulose	19 µm	3.45 µm

		6	cellulose	322.84 µm	4.35 µm
		7	lost		
	3	1	cellulose	55.31 µm	5.67 µm
		2	cellulose	71.81 µm	4.43 µm
		3	viscose	71.02 µm	4.53 µm
		4	lost		
		5	lost		
		6	lost		

Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
5.6	17	8.16	7	4	145%

Sample	Round	Particle	Polymer type	Max. long axis	Min. short axis
	1	1	cellulose	216.95 µm	4.35 µm
		2	cellulose	86.07 µm	5.46 µm
		3	cellulose	23.01 µm	3.46 µm
	2	1	cellulose	265.87 µm	3.23 µm
		2	cellulose	152.39 µm	4.35 µm
100L		3	lost		
	3	1	cellulose	78.23 µm	2.34 µm
		2	viscose	100.36 µm	5.64 µm
		3	cellulose	262.34 µm	6.1 µm
		4	lost		

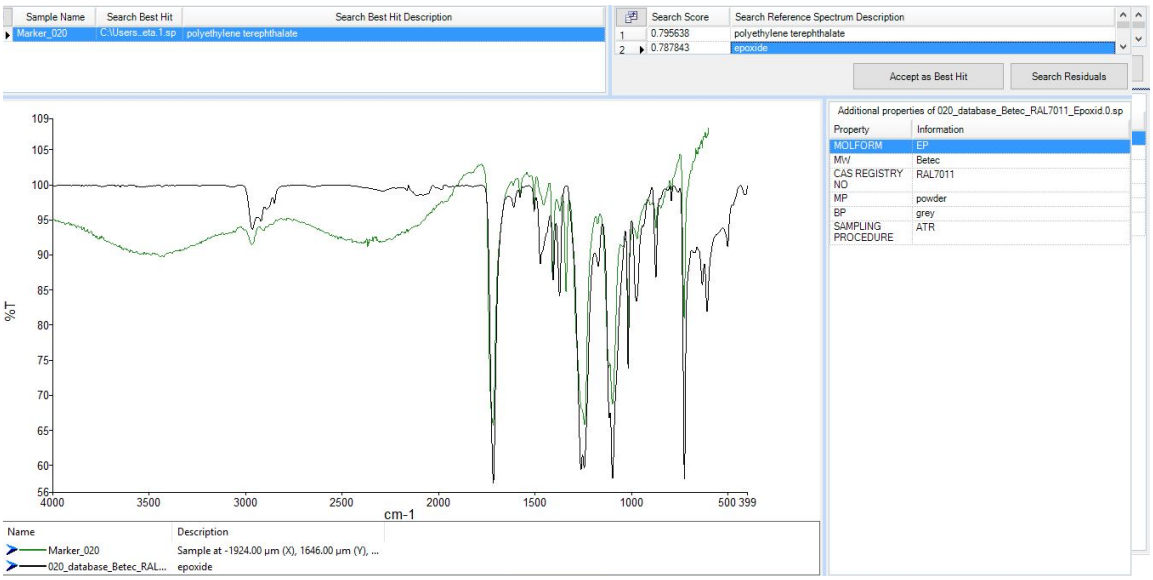
Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
3.3	10	3.29	4	3	99.6%

Sample	Round	Particle	Polymer type	Max. long axis	Min. short axis
	1	1	cellulose	127 µm	5.43 µm
		2	cellulose	109.04 µm	3.56 µm
		3	cellulose	82.48 µm	4.06 µm
		4	cellulose	44.58 µm	3.46 µm
	2	1	cellulose	13.54 µm	5.4 µm
1000L		2	fibre-viscose	55.06 µm	4.5 µm
		3	lost		
		4	lost		
		5	lost		
	3	1	lost		
		2	lost		

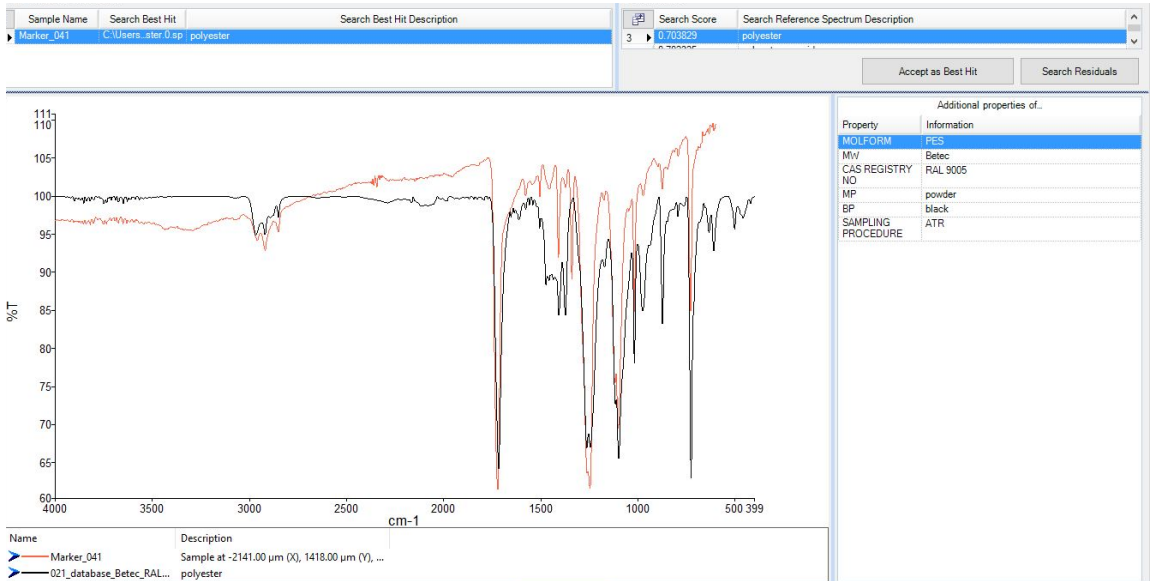
Average	Sum	STDEV	Maximum	Minimum	Coefficient of variation (CV) %
3.6	11	3.93	5	2	109%

Application of QA/QC and validation of MPs in a real sediment sample.

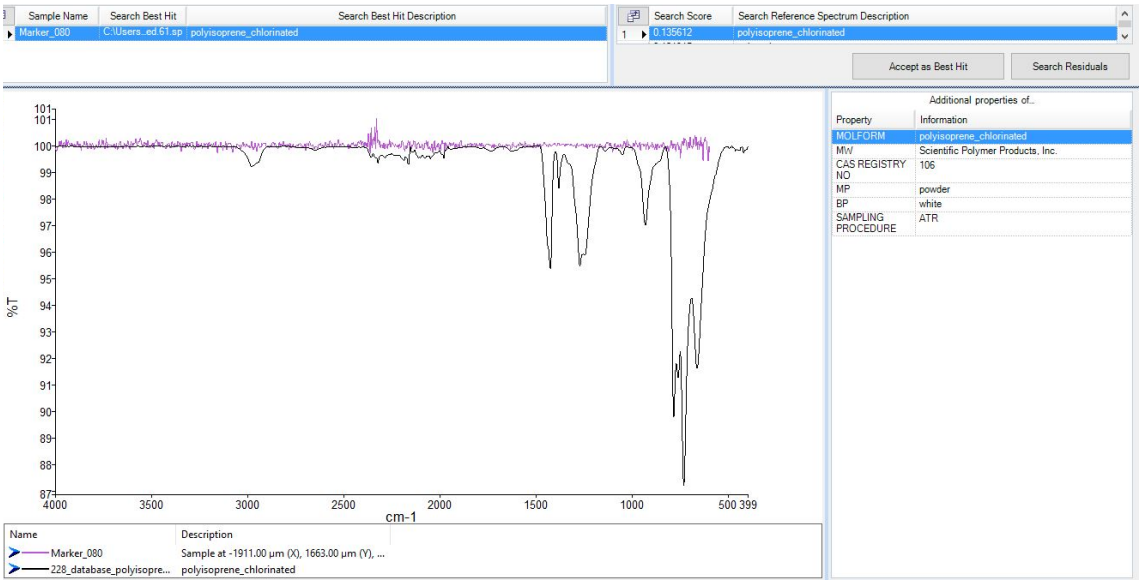
Appendix B shows the spectra of the polymers detected on the FT-IR. A total of eight polymers were detected: cellulose, acrylic fabric, styrene, epoxide, polyacetal, polyester, polyisoprene, and viscose. As illustrated in the pictures the identification of the chemical happens when the Spectra of the unknown spectra has the same level as the peak of the Spectra that is added in the library. Being almost identical or very similar, the particle is finally, identified. This identification, usually, is very fast in the system of the FT-IR. However, some Spectra was not well compatible, for instance, in the Spectra of the polymer of the styrene, polyacetal, and polyisoprene.



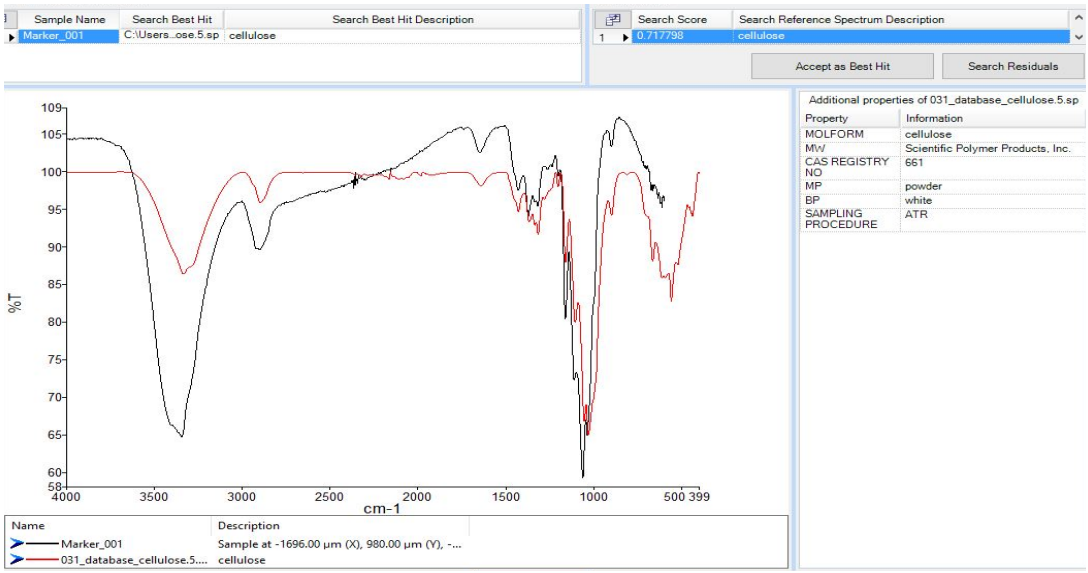
Epoxide



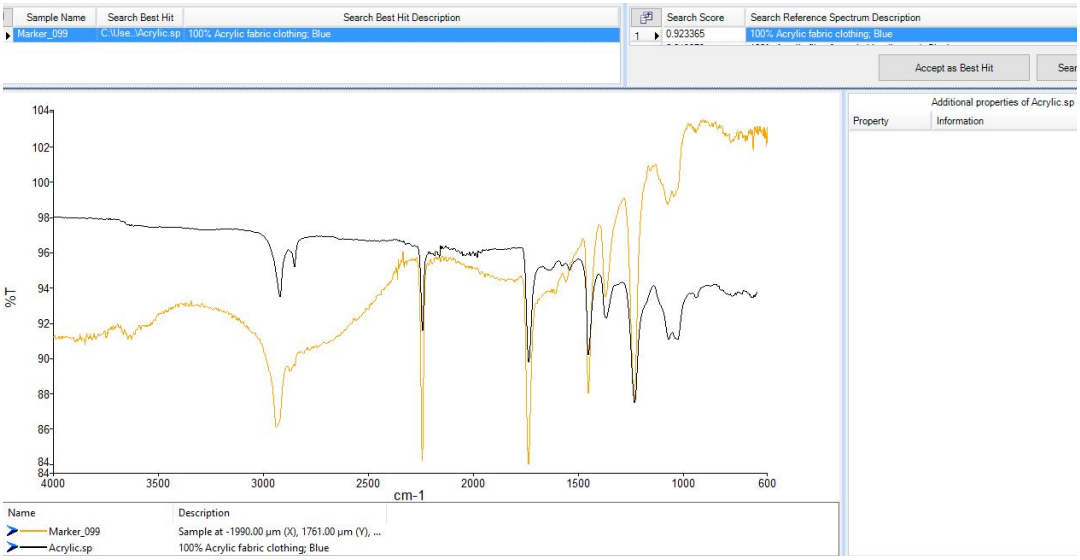
Polyester



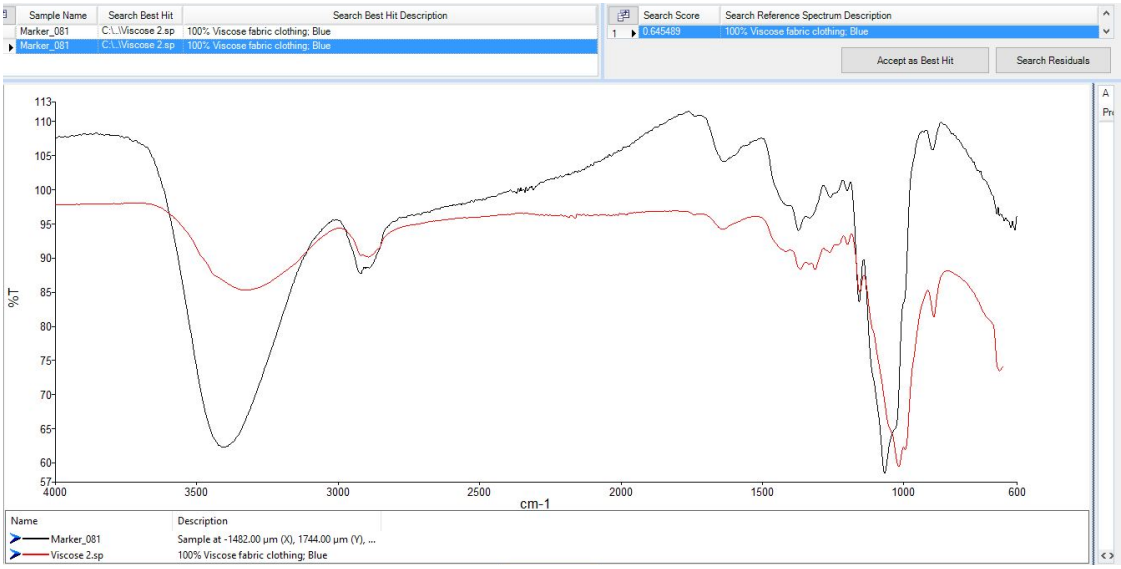
Polyisoprene Chlorinated



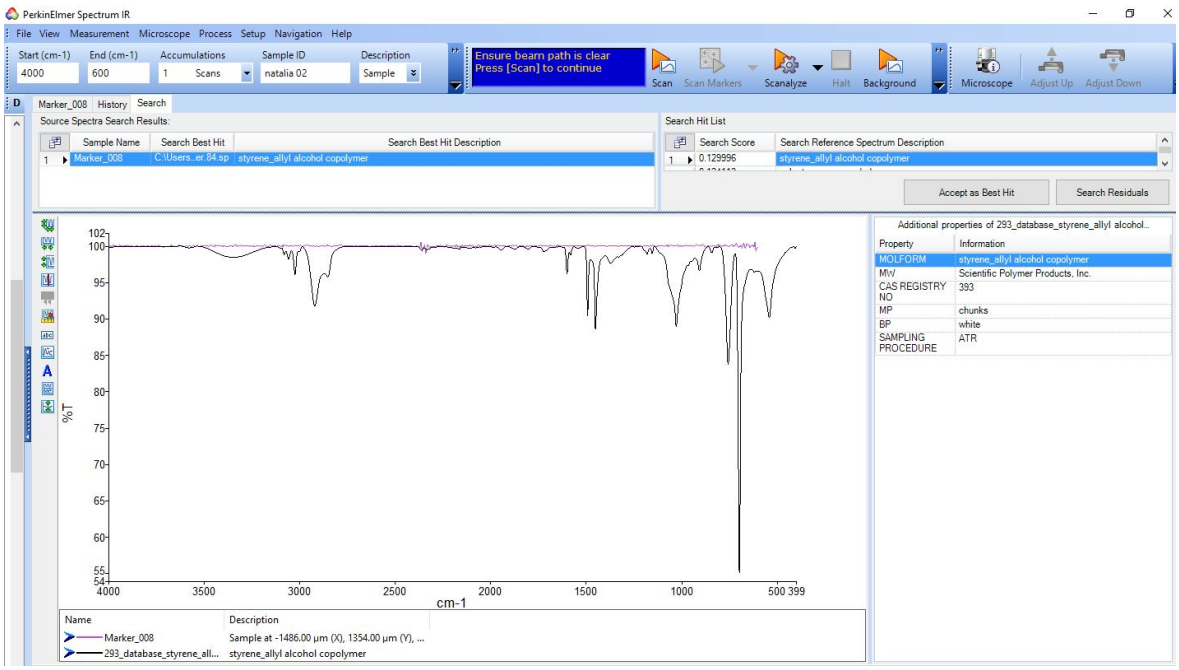
Cellulose



Acrylic Fabric



Viscose



Styrene

The next tables are related to further investigation of the pills (SRM) and background calculation, including mean and the standard deviation. As shown below for the PET was necessary an increase in the particle to get a lower percentage of the standard deviation. The other tables refer to the PVC, PS and the blanks (pill weight, (mg) number of the particles detected and the background is also included). Last a full analysis of the mixture in the pills (SRM) is presented with a final standard deviation of 10,99%, considered a good result for this work.

	Pill weight (mg)	Particles	Background
1	0,498	7	4
2	0,499	3	4
3	0,498	11	3
4	0,492	3	5
5	0,493	6	2
6	0,493	9	3
7	0,489	6	5
8	0,499	8	4
9	0,5	4	2
10	0,499	9	4
Mean	0,496	6,6	3,6
SD	0,003858612	2,716207	1,0749677
RSD	0,78%	41,15%	29,86%

Batch 1 - standard method

	Pill weight (mg)	Particles	3ackground
1	0,501	21	6
2	0,5	9	4
3	0,5	23	5
4	0,495	14	7
5	0,5	11	4
6	0,501	14	2
7	0,503	19	6
8	0,496	16	3
9	0,5	22	1
10	0,501	11	7
Mean	0,4997	16	4,5
SD	0,002406011	5,011099	2,068279
RSD	0,48%	31,32%	45,96%

Improved mixing

	Pill weight (mg)	Particles	3ackground
1	0,501	16	3
2	0,499	25	6
3	0,499	21	5
4	0,501	18	5
5	0,501	29	5
6	0,498	16	3
7	0,501	30	3
8	0,503	24	5
9	0,503	22	4
10	0,5	19	4
Mean	0,5006	22	4,3
SD	0,001646545	4,988877	1,05935
RSD	0,33%	22,68%	24,64%

Mixed for longer, and small increase in particles

	Pill weight (mg)	Particles	Background
1	0,492	58	2
2	0,493	41	4
3	0,498	44	4
4	0,498	48	6
5	0,497	49	4
6	0,496	55	2
7	0,501	54	2
8	0,502	42	3
9	0,497	43	3
10	0,501	64	3
Mean	0,4975	49,8	3,3
SD	0,003308239	7,714344503	1,251665557
RSD	0,66%	15,49%	37,93%

Improved mixing and significant increase in particles

PET

	Pill weight (mg)	Particles	Background
1	0,49	27	5
2	0,489	26	0
3	0,484	25	1
4	0,497	41	2
5	0,487	25	3
6	0,489	24	2
7	0,494	24	3
8	0,492	23	2
9	0,486	26	1
10	0,484	30	4
11	0,493	25	2
12	0,487	28	1
13	0,488	28	3
14	0,491	27	3
15	0,492	25	2
16	0,489	30	1
17	0,485	30	3
18	0,486	27	2
19	0,485	29	2
20	0,476	29	3
Mean	0,4882	27,45	2,25
SD	0,004549147	3,845366	1,164157703
RSD	0,93%	14,01%	51,74%

PVC

	Pill weight (mg)	Particles	Background
1	0,5	21	3
2	0,496	26	3
3	0,497	26	1
4	0,497	21	2
5	0,496	25	3
6	0,498	21	7
7	0,496	26	3
8	0,501	24	3
9	0,496	26	5
10	0,5	20	4
Mean	0,4977	23,6	3,4
SD	0,001946507	2,547329757	1,646545205
RSD	0,39%	10,79%	48,43%

PS

	Pill weight (mg)	Particles
1	0,497	1
2	0,496	3
3	0,499	2
4	0,5	2
5	0,5	3
6	0,495	5
7	0,5	4
8	0,494	3
9	0,494	0
10	0,495	3
11	0,492	1
12	0,469	5
13	0,497	2
14	0,496	3
15	0,5	1
16	0,496	5
17	0,496	2
18	0,497	2
19	0,495	4
20	0,494	3
Mean	0,4951	2,7
SD	0,006568666	1,41793
RSD	1,33%	52,52%

Blanks

	Pill weight (mg)	Particles	(PS)	(PVC)	(PET)	Background
1	0,497	20	8	9	3	3
2	0,499	25	7	11	7	4
3	0,495	25	6	11	8	1
4	0,498	19	6	11	2	3
5	0,5	32	13	12	17	5
6	0,493	20	10	6	4	4
7	0,489	17	5	10	2	3
8	0,492	16	4	9	3	4
9	0,497	23	7	14	2	5
10	0,493	29	13	8	8	2
Mean	0,4953	22,6	7,9	10,1	5,6	3,4
SD	0,003497618	5,189733	3,142893	2,233582	4,695151	1,26491106
RSD	0,71%	22,96%	39,78%	22,11%	83,84%	37,20%

	Pill weight (mg)	Particles	(PS)	(PVC)	(PET)	Background
1	0,561	70	25	31	14	8
2	0,5	52	22	20	10	2
3	0,501	51	26	19	6	5
4	0,498	66	19	41	6	3
5	0,497	64	28	32	4	6
6	0,494	61	24	26	11	6
7	0,496	62	29	27	6	7
8	0,499	60	25	29	6	5
9	0,5	51	18	24	9	4
10	0,498	58	30	20	8	4
Mean	0,5044	59,5	24,6	26,9	8	5
SD	0,019995555	6,536224	4,005552	6,773314	3,018461713	1,825741858
RSD	3,96%	10,99%	16,28%	25,18%	37,73%	36,51%

Increased particle numbers

Mixture