Review

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Abstract: Micro-nano-plastics (MNPs) are an important constituent of atmospheric aerosol. However, there is still no standard procedure for their sampling and size fractionation, which is an obstacle to the aggregation and critical analysis of results obtained by different research groups. This review focuses on the sampling and fractionation methodologies used for MNPs. Moreover, a straightforward optimized methodology for the sampling and fractionation is proposed.

Keywords: microplastics; nanoplastics; atmosphere; aerosols; sampling procedure; size fractionation procedure

1. Introduction

Microscopic particles in the atmosphere, contrary to the large ones visible to the human eye, negatively impact human health by imperceptibly and constantly exposition due to inhalation [1]. In the case of synthetic plastic materials with sizes smaller than 5 mm, which include a large subclass of textile fibers, it has become one of the currently major environmental challenging problems due to their widespread occurrence, which are known as microplastics (MP) [2]. MP with a size smaller than 1 micrometer constitutes a sub-group known as nanoplastics (NP) [3]. Because in this review, we are considering both MP and its lower-size sub-group NP, the abbreviation for micro-nano-plastics, MNP, will be used.

In the middle of the twentieth century, the plastics industry expanded, and since then, fifteen new classes of polymers have been discovered and synthesized in large quantities [4]. Plastic production surpassed most of the other man-made materials and, currently, plastic materials are ubiquitous in the world [5]. The major application of plastics is packaging, which results in an enormous increase of plastic waste to be processed when efficient solid waste management exists, or end up in randomly scattered environmental contamination when no environmental regulation exists.

Although the types of polymers that constitute MNPs can vary with the environmental compartment and the collection region, the most common are: polyethylene (PE), polypropylene (PP), polymethyl methacrylate (PMMA), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polystyrene (PS) [6].

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Besides the chemical polymer that names the plastic material, constituted by a repeating monomer unit, "plastics" include additives such as antioxidants, plasticizers, flame retardants and surfactants, and many other trace substances related to their manufacturing (catalyst, solvents and lubricants) and by-products, breakdown products and contaminants [7–9]. These substances will readily leach from the plastic material, and some have been shown to be toxic *in vitro* [8]. When MNPs are in the atmosphere, they can adsorb toxic aerosols, and behave similarly to particulate matter (PM), which constitutes well-known human health risk factors, due to their pollutants content (for example, highly toxic heavy metals and polycyclic aromatic hydrocarbons, PAH) [1].

The persistence of MNPs in the air, coupled with long-distance transport, resulted in their accumulation in the food chain, and now they are already found inside human bodies [10,11]. Taking into consideration their trace chemical content and adsorption capabilities of environmental pollutants, including some persistent organic products (POP) that are regulated by the Stockholm Convention, it is becoming urgent the establishment of regulatory issues by governments and environmental agencies [12]. Indeed, until now, only California (USA) regulates the presence of MPs in ecosystems and drinking water [12]. However, there are still no standards for the collection and analysis of MNPs. In the case of atmospheric MNPs, the discussion about sampling is still very fuzzy.

In a recent review about the classification of MPs by infrared spectroscopy [13], several critical questions were raised about the results obtained. Indeed, the direct comparison of the results described in each paper can be compromised due to a lack of a standard protocol for sampling, fractionation and analysis. Also, the use of the global statistical analysis of the different datasets is challenging.

The entire MNPs analysis chain can be described as a general three-step process (Figure 1), where standardized protocols should be implemented to promote regulatory worldwide monitoring and comparison.

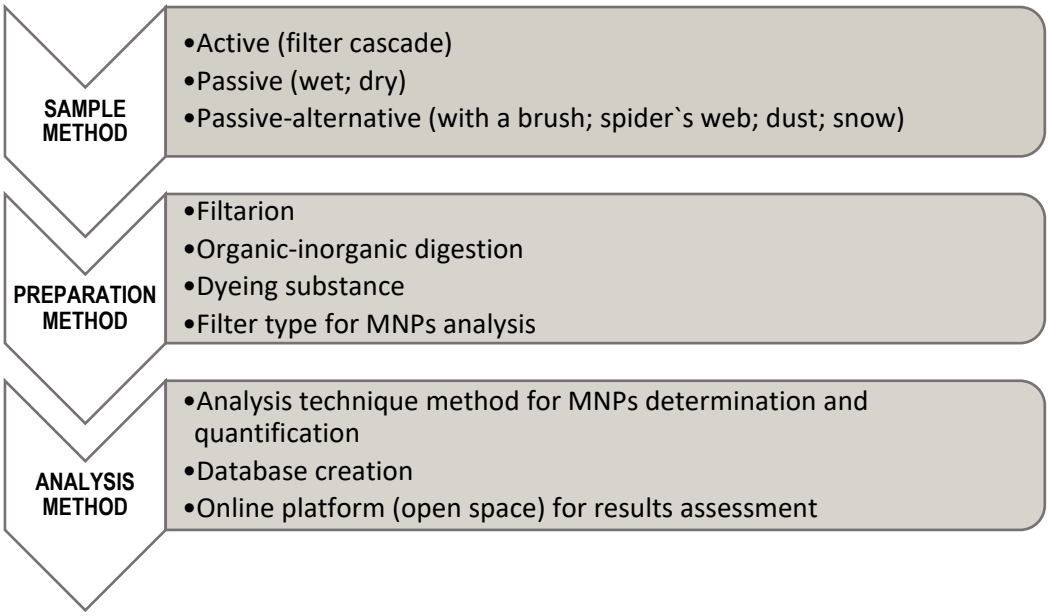

Figure 1. The three steps in the MNPs analysis chain.

This review will focus on the sampling procedures of MNPs present in the atmosphere and cover papers published between the years 2020 and 2022. Considering the information found in the literature, a sampling/fractionation protocol is proposed that has been developed by us for the sampling of MNPs in the air of Porto (Portugal) since April 2022.

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2. Sources and fate of atmospheric MNPs

2.1. Sources

MNPs sources are classified as primary or secondary [14,15]. Primary sources correspond to the manufacturing and manufactured products containing plastic or made of plastic, such as packing, home appliances, toys, synthetic fabrics, abrasives, paints, and cars. Secondary sources originate from plastic breakdown by human activity, resulting in micro and nano secondary plastics, and from degradation under natural environment factors (weathering), such as temperature or UV-radiation, where microscopic plastics are resized into nanometric fragments. Figure 2 represents the environmental fate of MNPs and associated contamination sources, from plastic production to micro and nano fragments.

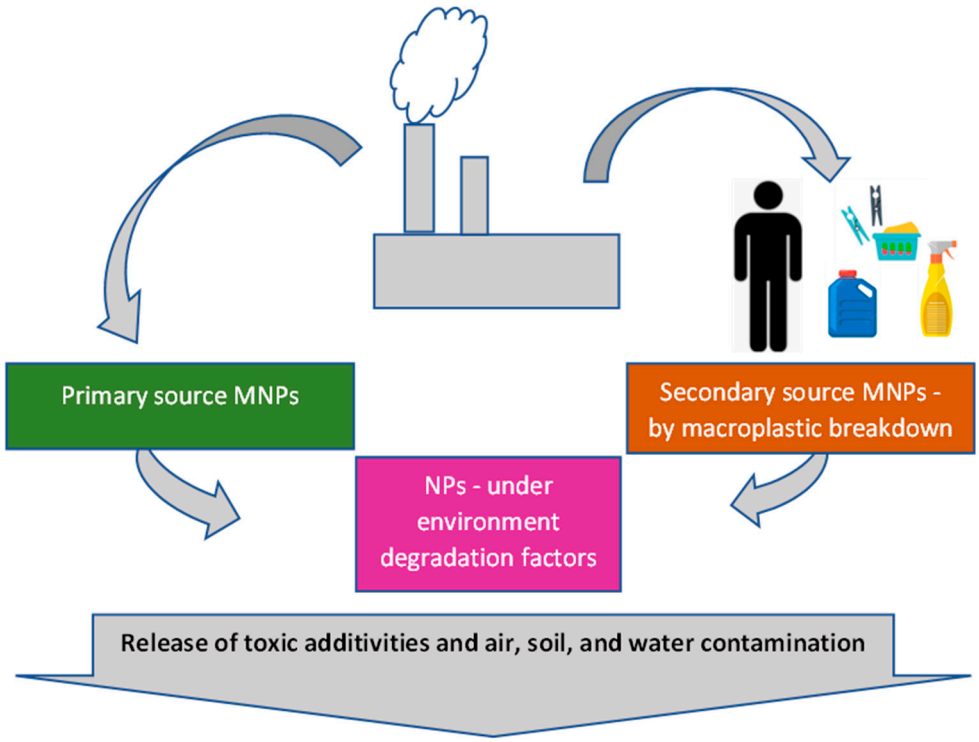

Figure 2. Plastic sources and the fate of MNPs in the environment. MNPs are generated from primary (industries) and secondary sources (breakdown of plastic materials used by consumers), with subsequent degradation into MNPs under weathering processes. MNPs can occur in all environments and bioaccumulate in the food-chain, with the release of the toxic substances that are adsorbed to them.

2.2. Plastic pollution

Since the studies of references [16,17], reporting the presence of MPs in global environments and their causing problem, several papers followed describing MNPs in freshwater, marine, and terrestrial aquatic environments [17–19], in flora and fauna [20], their atmosphere and cryosphere transporting to and within the Arctic [21], and negative impacts on ecosystems and climate change [22]. More recently, greater concerns have been raised towards human health since there was described the occurrence of MNPs in blood [23], lung tissue [24], breast milk [25,26], placentas, meconium, and infant feces [26].

MNPs accumulation in the human body has different pathways: inhalation, water ingestion, and food ingestion where MNPs bioaccumulates [27]. MNPs pollution can also contribute to climate change, with concerns regarding MNP pollution can also contribute to climate change, with concerns regarding reproducing/existing conditions of flora and fauna living forms related to increase of

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temperature and change in the precipitation regime due to radioactive forcing, but socio-economic factors for humans also [28,29]. Based on the articles analyzed in this section, we consider the more complex concern the relation between MNPs environmental pollution and the potential effects on flora and fauna as well on different aspects for humanity, from health to socio-economic factors. Due to a lack of comprehensive literature, the impact of MNPs on climate change should be addressed with precaution regarding existing/reproducing conditions of living forms (Figure 3).

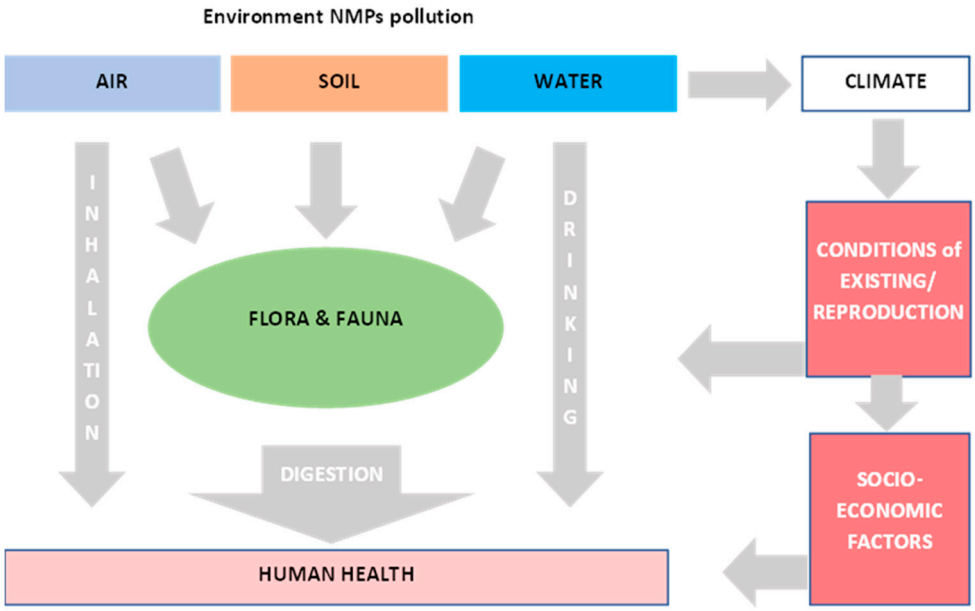

Figure 3. MNPs different routes for human-health exposure by inhalation, water ingestion, and daily uptake of contaminated food. Climate changes due to environmental pollution by MNP show-up questions related to nature conditions for reproduction or the existence of living beings and socio-economic factors for humans.

2.3. Atmospheric microplastic: first studies

The attention of scientists to atmospheric Airborne Microplastic was given in a first study from 2015 [30], where it was described the presence of MPs in the atmosphere of Paris (France) with a total fallout in the 100–5000 µm range. In 2017, concentrations of non-fibrous microplastics and fibres were reported in the atmospheric fallout in Dongguan city (China) from 175 to 313 particles.m^{-2}.day^{-1}, being identified three different polymers, i.e., PE, PP and PS [31]. After these first studies, the number of papers about atmospheric MPs and articles reporting distinct analysis techniques and sampler collector types increased.

The effort to analyze atmospheric MNPs, due to its size and air dilution factor, turns out to be challenging since analysis techniques and sample preparation methods employed in soil or water environmental contexts may not be directly transposed. Concerns regarding the detection limits of the sampling equipment, sample representativity, probable loss of some microplastic parts or fractions, or sample contamination by the lab air during the analytical procedure can hamper reliable results.

Moreover, sample treatment preparation could be associated with MNPs degradation by chemical and biological parts.

3. Sampling of atmospheric MPs

3.1. Sampling methods

Sampling strategies in atmospheric MPs studies are based on collecting suspended aerosols and deposited particles, usually performed by active samplers and passive collectors.

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Aerosol samples are generally collected with a pump-powered total suspended particulate (TSP) air sampling system with a known flow rate for a determined amount of time, allowing for proper calculation of airborne particulate concentrations per unit volume [72,95]. Suspended particles are captured through a filter membrane. Membrane filters used are mostly made up of glass-fiber (44%), PTFE (20%) or aluminium (8%), with a pore size between 0.22 and 5 µm (Table 1, Figure 4). Some studies use active sampling through a cascade impactor for collecting size-fractionated aerosol samples in different filters [47]. This device allows the determination of the MPs concentration in each size fraction, avoiding overlapping.

In the case of passive sampling methods, atmospheric particulate matter fallout is collected in glass or metal containers, which consist of a funnel on a bottle for wet deposition and a beaker or barrel for dry deposition. Deposited particles are collected by rinsing the device with ultrapure water and filtering. Some studies collect the deposited dust over a clean glass petri dish [42,44,58,69] or in specific areas using bristle brushes and metal dustpan [49,50,66] or a vacuum cleaner [89,90]. In addition, plant leaves [81], and spider nets [45] are also used as samplers for atmospheric MNPs deposition studies. Only recently, the Norwegian Institute of Air Research (NILU) designed a stainless-steel collector considered by ISO as an international reference collector for atmospheric MNPs fallout. The duration of passive sampling can range from days to months (Table 1). The deposition area is an important factor in calculating the number of MPs atmospheric deposition per unit area [30,32]. For deposited dust, the MPs abundance is measured in units per gram of dust-fall [49,50,66,72].

Atmospheric fallout samples are highly influenced by local weather phenomena, elevation, human activities, and population, so comparing the data reported in different areas is difficult. Moreover, the different sampling methods, with diverse data units, make it complex to evaluate and compare the global atmospheric MPs pollution.

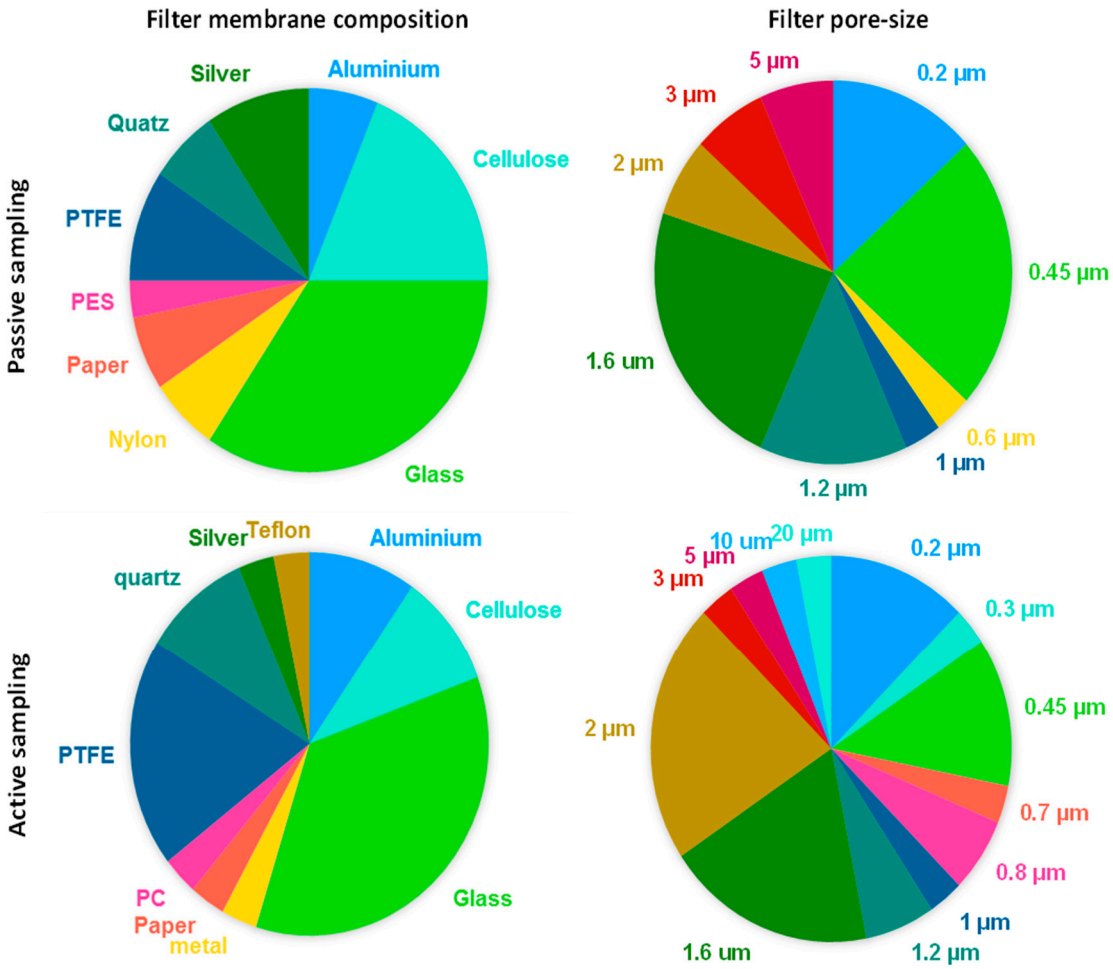

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Figure 4. Different filter membranes composition and pore size used in atmospheric microplastic sampling reported in studies from 2020 to 2022.

3.2. Sample preparation

Standardized methods for MNPs sample preparation have not been established, so the herein-described treatment procedures were chosen depending on the degree of contamination of the sample with plant debris, tissues, pollen, algae, insects, and inorganic material, which had to be removed before analyses. On the whole, collected samples must go through several purification processes for MNPs concentration: sieving, filtration, digestion and density separation.

In active sampling, suspended particles were collected directly through filtration within the sampler device, and the MNPs were identified without a purification process in 74% of the reviewed papers. Only works with sampling times longer than 24h (7 out of the 26 studies; 26%; Table 1) were treated with H_2O_2 to remove the interference of organic impurities, three of which were subjected to density separation (Table 1).

In the passive sampling methodology, the collection time is longer than the active sampling, so organic matter accumulation is higher, and MNPs purification treatments are more frequent (44% of reviewed studies; Table 1). First, the samples may be sieved using deionized water to remove large impurities using stainless steel mesh with 1 or 5 mm pore size (17.6% of reviewed studies). Then, the sample is filtered to concentrate it in a membrane filter. Different filter membrane compositions with diverse pore sizes were used (Table 1 and Figure 4). Glass-microfiber, cellulose, PTFE and silver were the most used filter composition, and the most selected filter pore sizes were 0.45 and 1.6 μm (Table 1 and Figure 4).

From the atmospheric MPs studies reviewed (2020-2022), only 21 performed treatments for organic matter elimination. Usually, the use of oxidants (H_2O_2), acids (HNO_3, HCl), alkalis (KOH, NaOH) and enzymes have been pointed out in the scientific literature to remove organic matter from the atmospheric particle samples [40]. However, in the 21 studies, H_2O_2 was the most chosen as a digestion treatment, with 80% of the studies using it at 30%. The digestion time and temperature were different (ranging from 1 h to 8 d at room temperature to 70 °C; Table 1), which may be related to the organic matter content in the sample itself. Compared with H_2O_2, the Fenton reagent (H_2O_2 30% with $FeSO_4$) might be more efficient at digesting organic matter [92,93] and was used in three out of the reviewed studies [45,47,83]. Some studies suggest that using H_2O_2 at 30% can affect the MPs by decolorization, making further detection of MNPs difficult [40,94], and recommend reducing from 30 to 15% the concentration of H_2O_2 used in the digestion protocol [92]. Only one work followed this recommendation and employed 15% H_2O_2 [73].

The last step in MNPs purification is separating them from high-density impurities such as mineral matter by density separation. Different solutions with diverse densities have been used, such as sodium chloride (NaCl), sodium iodide (NaI), and zinc chloride ($ZnCl_2$). The different densities of the separation solutions (NaCl, 1.2 g cm^{-3}; NaI, 1.6 g cm^{-3}; or, $ZnCl_2$, 3 g cm^{-3}) have a direct effect on the flotation of different MNPs due to the densities of the plastics (MNP density is between 0.8 - 2.4 g cm^{-3}) [56,94,95]. The higher density MNPs [polyester, 1.77 g cm^{-3}; polyvinyl alcohol (PVA), 1.61 g cm^{-3}; or polytetrafluoroethylene (PTFE), 2.2 g cm^{-3}] can be underestimated in NaCl density separation process. These fractions may remain non-buoyant in NaCl solution. Although $ZnCl_2$ solution is considered the most effective method for separating multiple microplastic particles [56], it is the less commonly used due to its environmental toxicity [96]. Based on the reviewed literature, NaI is more environmentally friendly and highly efficient for collecting denser polymers [92,97]. Only 22% of the reviewed studies purify the MNPs by density separation, using NaCl, NaI, and $ZnCl_2$ as separation solutions (Table 1).

Notably, most of the reviewed studies carried out no particle size separation before the MNPs detection and identification. After sample preparation, different sizes of particulate matter (between 1 to 5000 μm) were accumulated in the same filter. Consequently, small particles can be overlapped with larger ones, underestimating the number of MNPs in the samples.

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Table 1. Articles about microplastic sampling (2020-2022).

Ref.	Sampling method	Filter type	Filter pore size µm	Sampling collect time	Digestion	Temperature/time	Sieving
[34]	Passive	PTFE	0.45	2018; 1 month	H_2O_2 ; 30%	RT / 7 days	---
[35]	Active/Passive	---	---	2019; ---	---	---	---
[36]	Passive	Glass-fiber	1.6	2019 - 2020; 3 - 48 days	---	---	---
[37]	Passive/Snow	CN; Glass-fiber	0.45; 1.2	2019; 1 time	Fenton's reagent	45ºC / 2 - 3 hours	---
[38]	Passive	---	---	2019 - 2020; 1 month	HF	---	---
[39]	Active	Glass-fiber	1.60	2017; 24 hours	---	---	---
[40]	Passive	CN; Glass-fiber	12; 1.6	2018 - 2019; 24 hours	---	---	30
[41]	Passive	Quartz-fiber	1.6	2019 – 2020; ---	---	---	---
[42]	Passive	CN	3	---	TWEEN	20 (0.1%)	---
[43]	Active	CN	5	2020; 48 hours	H_2O_2 ; 30%	40ºC / 2 hours	20 µm
[44]	Passive	Silver-fiber	0.45	2021; 24 hours	Washing with Ethanol		---
[45]	Passive	PTFE	0.45	2017 - 2019; 1week-1month	---		---
[46]	Passive	PTFE	0.45	2019; 30 min	H_2O_2 ; 30%	55ºC / 24 hours	---
[47]	Passive	Glass-fiber	1	2020, ---	Fenton's reagent ($FeSO4 + H_2O_2$)	---	---
[48]	Passive	Nylon-fiber	0.22	2021; 24 h	---	---	---
[49]	Active/Passive	Aluminum Oxide	0.2	2018; 3 hours; 1 month	Fenton's reagent ($FeSO_4 + H_2O_2$); +Enzymatic digestion	40ºC / 2 hours	500 µm
[50]	Active/Passive	Glass-fiber	1.6	2020; 12 hours	---	---	---
[51]	Passive/Dust	Silver-fiber	0.45	---	H_2O_2 ; 30%	24 hours	---
[52]	Passive/Dust	Paper	2	2019; ---	H_2O_2 ; 30%	RT /10 days	5 mm
[53]	Passive	---	---	2020; 1 week	---	---	---
[54]	Active/Dust	Paper	---	2019; each 7 days	H_2O_2 ; 30%	RT / 8 days	5 mm
[55]	Passive	CN	0.45	2018 - 2019; 96 hours	H_2O_2 ; 30%	60ºC / 48 hours	0.2-5 mm
[56]	Active	PTFE	2	2020; 24 hours	---	---	---
[57]	Active	Glass-fiber	0.3	2019; 24 hours	---	---	---
[58]	Active	---	---	2021; 6 hours	---	---	---
[59]	Active	Aluminum Oxide	0.22	2020 - 2021; 4 hours	HCl; pH3	24 hours	---
[60]	Active/Passive	Quartz-fiber	2.2	-	H_2O_2 ; 30%	RT / 24 hours	---
[61]	Active	Glass-fiber	1.6	2019 - 2020; 24 hours	---	---	---
[62]	Active/Passive	Glass-fiber	3	2019; 12 - 24 hours	---	---	---
[63]	Passive	CN	0.45	---; 22 - 40 days	H_2O_2 ; 30%	RT / 24 hours	---
[64]	Passive	MCE	5	2019; 7 days	H_2O_2 ; 30%	55ºC / 3 days	---
[65]	Passive	Glass-fiber	1.2	2020; 6 days	---	---	---
[66]	Active	Glass-fiber; PTFE	0.7; 0.45	2019; 2 - 3 days	H_2O_2 ; 30%	70ºC / 1 hour	---
[67]	Active	PTFE	2	---; 24 hours	H_2O_2 ; 30%	RT / 1 day	---
[68]	Passive/Dust	---	---	2020	---	---	5-1mm
[69]	Active	Glass-fiber	1.6	2017; 24 hours	---	---	---
[70]	Active	Teflon; Silver-fiber	0.2; 1.2	24 hours	---	---	---
[71]	Passive/Dust	Glass-fiber	0.6	30 days	---	---	n/a
[72]	Passive	Glass-fiber	1.6	2017 - 2018; 1 - 8 days	---	---	---
[73]	Passive	Glass-fiber	1.6	2018 - 2019; 1 year; 3 - 4 days	Bioenzym SE/F + H_2O_2	40ºC / 48 hours	1mm
[74]	Passive/Dust	CN	1.2	1 day	H_2O_2 ; 30%	---	---
[75]	Active	Quartz-fiber; Glass-fiber	2.2; 1.2	2020; 24 hours	H_2O_2 ; 15%	RT / 8 days	---
[76]	Active	PTFE	---	2019; ---	H_2O_2 ; 30%	---	---
[77]	Active	Quartz-fiber; PTFE;	10; 0.45; 0.2	2018; 8 days	H_2O_2 ; 30%	55ºC / 7 days	---

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		Aluminum Oxide					
[78]	Active	Glass-fiber	1	2020; 24 hours	---	---	---
[79]	Passive	PES	0.45	2017 - 2019; 1 - 2 month	---	---	---
[80]	Active	Glass-fiber	1.6	2019; 8 hours	---	---	---
[81]	Active	---	---	---	---	---	---
[82]	Active	PTFE	2.0	2017; 24 hours	---	---	---
[83]	Active/Dust	MCE	0.8	2018; 6 - 8 hours	---	---	---
[84]	Passive	Glass-fiber	---	2018; ---	---	---	---
[85]	Active/Passive	Glass-fiber	1.6	2018 – 2019; ---	H_2O_2 ; 30% +$FeSO_4$(0.05 M)	---	---
[86]	Passive/Snow	PTFE	0.2	2017, ---	---	---	---
[87]	Passive	Glass-fiber	1.6	2017 - 2018; 1 month	---	---	---
[88]	Active	PC	0.8	2016; 12 - 24 hours	---	---	---
[89]	Active	Glass-fiber	1.6	20219; 10 - 48 hours	---	---	---
[90]	Passive	Aluminum Oxide; Silver-fiber	0.2; 1.2	2018; 3 - 4 days	---	---	---
[91]	Passive	Nylon-fiber	100	2017; 1 minutes	H_2O_2 ; 30%	RT / 1 week	75 µm
[92]	Passive	---	---	2010 -2014	---	---	150 µm
[93]	Passive	Cellulose	5	2019; 24 hours	---	---	---
[94]	Passive	Glass-fiber	1.2	2017 - 2018	---	---	2 mm
[95]	Active	Glass-fiber	1.6	2018; 1 hour	---	---	---
[96]	Active	Glass-fiber	1.6	2019; 1 hour	---	---	---
[97]	Active	Glass-fiber	1.6	2018 - 2019; 4 - 24 hours	---	---	---
[98]	Active	Glass-fiber	1.2	2019; 48 hours	H_2O_2 ; 15%	RT / 8 days	---

* CN – cellulose nitrate; MCE – mixed cellulose ester; PC – Polycarbonate; PES – Polyethersulfone; PTFE – polytetrafluoroethylene polymer.

4. Sampling of atmospheric MNPs

In order to quantify and characterize MNPs in the atmosphere, the first step is to perform a sampling campaign. Depending on the sampling equipment used, the sampling time reported in the literature varied from 30 minutes to one year (Table 1). The longer the sampling, the higher the probability of clogging issues. Because the size of this type of aerosol varies from the nanometer scale up to the millimeter scale, their simultaneous analysis is impossible. Consequently, after bulk sampling, it is necessary to perform a size fractionation into several homogeneous sub-fractions. Moreover, there are other types of aerosols in the atmosphere, and the MNPs analysis can only be made if the other aerosols are separated from MNPs or destroyed. The conservation of the MNPs under different sub-fractions may prevent interaction between MNPs and/or the potential pollutants associated with matrix compounds connected with the larger particles allowing further reliable analyses of these pollutants.

Here we propose a size fractionation procedure based on a sequence of sieving and filtration unitary operations. A sample collected with no-plastic passive samplers (wet or dry deposition by force of gravity) is washed and sequentially passed through a series of sieves and membrane filters (Figure 5).

(i) The dry or wet deposit in a collector (Norwegian Institute of Air Research - NILU) is washed with pure water and transferred to a dark glass vial.

(ii) A cascade of metallic sieves (125, 63, and 25 µm) is used to remove large organic matter. The two bigger mesh sieves (125 and 63 µm) are used to minimize clogging. The mesh size sieve of 25 µm is used to retain pollen.

(iii) After sieving, cellulose acetate (CA) or cellulose nitrate (CN) membrane filters with a pore size (12, 1.2 µm) and 0.2 µm aluminium oxide membrane filters are used to separate different size fractions of MNPs. The membrane filters with a pore size of 12 and 1.2 micrometres were chosen to evaluate the size of microplastics that are considered respirable PM_{10} and $PM_{2.5}$ fractions. Membrane filters with pore size 0.2 micro are used to retain nano-size fractions by more accurate and sensitive techniques.

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(iv) The sieves and their content are placed in beakers with 200 mL of H_2O_2 (15%) for a period of 12 hours (overnight) at 50 C°. After organic matter digestion and, eventually dispersing of agglomerated particles, the sample is dispersed by ultrasounds and will go again through the cascade of sieves and filters.

It is important to mention that, due to the complex matrix and existence of many non-soluble and non-miscible compounds with water in the sample removed from the collector/sampler, washing the equipment in each step with plenty of water is crucial to obtain reliable values.

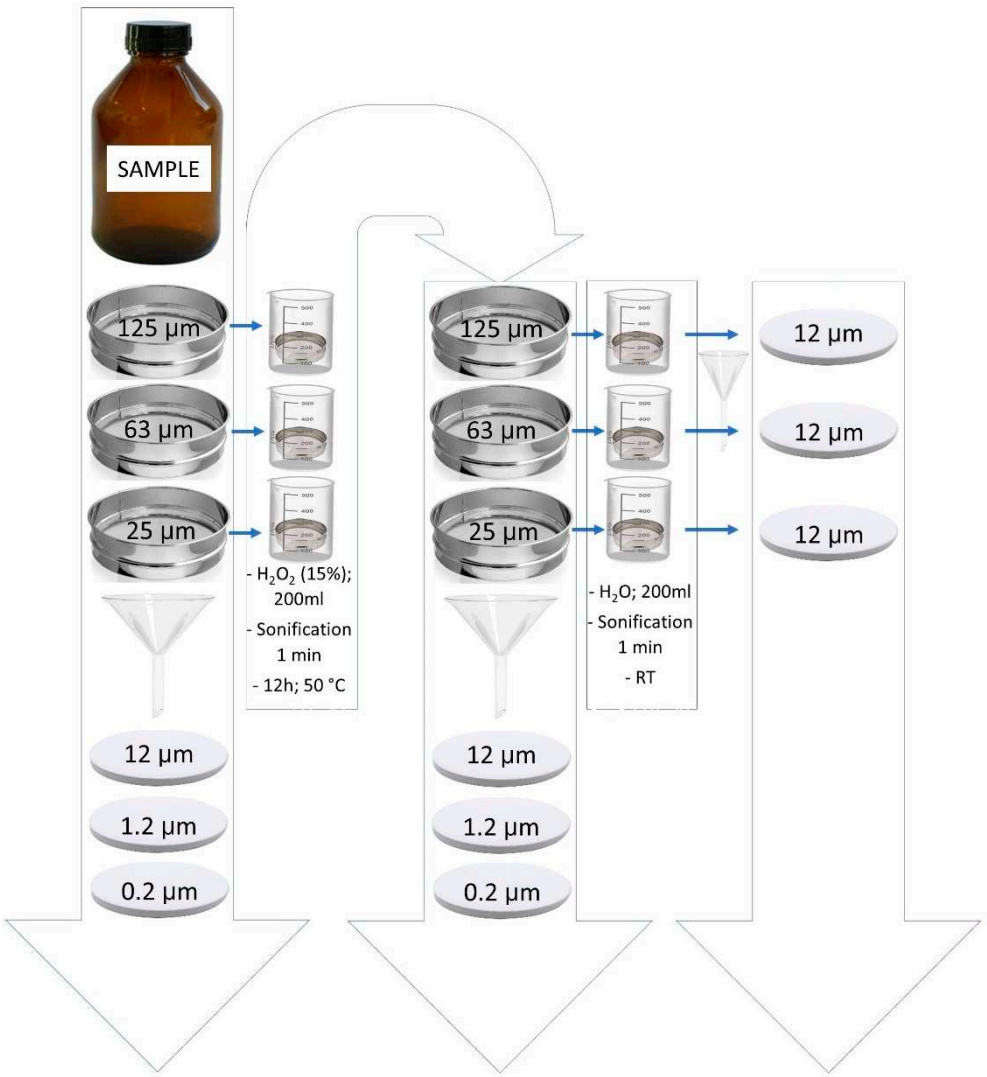

Figure 5. Scheme of different filter membranes and sieves used in atmospheric microplastic sampling preparation by filtration from passive collector.

5. Perspectives

The lack of standardization in sampling and analysis protocols is a significant issue that has been raised in several studies about MNP. In the future, it would be essential to establish a standardized protocol to allow for the comparison of results obtained by different researchers. This could be achieved through the development of an international standard protocol that outlines the sampling and analysis procedures for MNP in the atmosphere. Also, available open-access databases for MNP identification would be helpful.

The current methods for the detection of MNP in the atmosphere have limitations. They are not specific to MP, and they cannot detect small particles. In the future, new and more sensitive methods should be developed that can detect MNP in lower concentrations and smaller particle sizes.

To reduce the amount of atmospheric MNP, it is essential to understand the sources and transport of these type of aerosols. The sources of atmospheric MNP include vehicle emissions, industrial emissions, and waste disposal sites. Once the sources of MP are identified, it will be possible to develop strategies to reduce the amount of MP emitted into the atmosphere. Additionally, understanding the transport of MP in the atmosphere will help to determine where the highest concentrations of MP occur, allowing for targeted strategies to be developed to reduce MNP pollution.

There is growing concern about the impact of atmospheric MP on human health. It is essential to investigate the impact of atmospheric MP on human health to determine the extent of the problem and develop strategies to reduce the impact of atmospheric MP on human health. This could involve epidemiological studies to determine the association between atmospheric MP exposure and adverse health outcomes. Also, samplers simulating human inhalation can help study possible impacts of MNP on human health.

Overall, the future directions for research on atmospheric MP are diverse and challenging. However, it is essential to continue to investigate this problem and develop strategies to reduce the amount of MP in the atmosphere. By working together, researchers can develop new and innovative solutions to this problem and create a cleaner and healthier environment for all.

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