LABPLAS

Horizon 2020
European Union Funding
for Research & Innovation

# Land-Based Solutions for Plastics in the Sea

*This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003954*

## D3.2 Guideline methods for MP determination in atmospheric samples

**Due date of deliverable: 30/11/2022**

**Actual submission date: 28/11/2022**

Horizon 2020
European Union Funding
for Research & Innovation

LABPLAS

Horizon 2020
European Union Funding
for Research & Innovation

## PROJECT INFORMATION

**Project number**: 101003954

**Project acronym**: LABPLAS

**Project full title**: Land-Based Solutions for Plastics in the Sea

**Call**: H2020-SC5-2018-2019-2020 submitted for H2020-SC5-2020-2 / 03 Sep 2020

**Topic**: CE-SC5-30-2020 – Plastics in the environment: understanding the sources, transport, distribution and impacts of plastics pollution

**Type of action**: RIA – Research and Innovation Action

**Starting date**: June 1st, 2021

**Duration**: 48 months

**List of participants**:

| Nº | Participant name | Acronym | Country | Type |
|---|---|---|---|---|
| 1 | UNIVERSIDADE DE VIGO | UVI | SPAIN | HES |
| 2 | UNIVERSIDADE DA CORUÑA | UDC | SPAIN | HES |
| 3 | BUNDESANSTALT FUER GEWAESSERKUNDE | BfG | GERMANY | RTO |
| 4 | LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA | INL | PORTUGAL | RTO |
| 5 | KATHOLIEKE UNIVERSITEIT LEUVEN | KUL | BELGIUM | HES |
| 6 | HELMHOLTZ ZENTRUM FUR OZEANFORSCHUNG KIEL | GEOMAR | GERMANY | RTO |
| 7 | NATIONAL OCEANOGRAPHY CENTRE | NOC | UNITED KINGDOM | RTO |
| 8 | SORBONNE UNIVERSITE | SU | FRANCE | HES |
| 9 | OPEN UNIVERSITEIT NEDERLAND | OUNL | NETHERLANDS | HES |
| 10 | LEIBNIZ INSTITUTE FOR BALTIC SEA RESEARCH | IOW | GERMANY | RTO |
| 11 | ASSOCIACAO PARA O DESENVOLVIMENTO DO ATLANTIC INTERNATIONAL RESEARCH CENTRE | AC | PORTUGAL | RTO |
| 13 | BASF SE | BASF | GERMANY | LE |
| 14 | TG ENVIRONMENTAL RESEARCH | ER | UNITED KINGDOM | SME |
| 15 | CONTACTICA S.L. | CTA | SPAIN | SME |
| 16 | STICHTING EGI | EGI | NETHERLANDS | Non-P |
| 17 | RADBOUD UNIVERSITEIT | RU | NETHERLANDS | HES |
| 18 | UNIVERSIDADE FEDERAL DO PARÁ | UFPA | BRAZIL | HES |

## DELIVERABLE DETAILS

| | |
|---|---|
| **Document Number:** | D3.2 |
| **Document Title:** | Guideline methods for MP determination in atmospheric samples |
| **Dissemination level** | PU - Public |
| **Period:** | PR1 |
| **WP:** | WP3 |
| **Task:** | Task 3.2 |
| **Status:** | Final |
| **Author:** | UNIVERSIDADE DA CORUÑA |
| **Reviewers:** | bfg German Federal Institute of Hydrology |
| **Recommended citation format** | 2022. *Guideline methods for MP determination in atmospheric samples*. Deliverable 3.2, LABPLAS Grant Agreement No. 101003954 H2020-SC5-2020-2 |
| **Abstract:** | This report corresponds to Deliverable 3.2, *Guideline methods for MP determination in atmospheric samples*, resulting from Task 3.2 of the LABPLAS project.<br>It describes the analytical procedures for field sampling, sample preparation, identification and quantification of microplastics (MPs) and small microplastics (SMP ≤ 100 µm) in atmospheric samples. Per LABPLAS' main goals, harmonized methods to determine MPs in atmospheric samples are proposed. They are intended as a comprehensive approach to allow reliable comparisons between results, and they consider field sampling, sample treatment and final measurement. |

| Version | Date | Comments |
|---|---|---|
| 1.0 | 28/11/2022 | Initial version - Guideline methods for MP determination in atmospheric samples |

**Disclaimer**

The views and opinions expressed in this document reflect only the authors' views, and not necessarily those of the European Commission.

## TABLE OF CONTENTS

## LIST OF FIGURES

## ABBREVIATIONS AND ACRONYMS

| Abbreviation / Acronym | Description |
|---|---|
| ATR-FTIR | Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy |
| FPA | Focal Plane Array Detector |
| FTIR | Fourier-Transform Infrared Spectroscopy |
| IR | Infra Red |
| LDIR | Laser Direct Infrared chemical imaging |
| MPs | Microplastics |
| AMPs | Atmospheric microplastics |
| MPF | Microplastics free |
| NIR-HIS | Near Infrared-Hyperspectral Imaging |
| PA | Polyamide |
| PE | Polyethylene |
| PET | Polyethylene terephthalate |
| PS | Polystyrene |
| PVC | Polyvinyl chloride |
| Py-GC-MS | Pyrolysis Gas Chromatography coupled to Mass Spectrometry |
| QA/QC | Quality Assurance/ Quality Control |
| QCL | Quantum Cascade Laser |
| SDS | Sodium Dodecyl Sulfate |
| SEM | Scanning electron microscopy |
| SEM-EDS | Dispersive Energy X-ray Spectroscopy |
| SMP | Small Microplastics ( ≤ 100 µm) |
| SOPs | Standard Operating Procedures |

# 1 INTRODUCTION

Environmental information about airborne MPs is very limited and few studies have been published until today. Therefore, there is still a lack of standardized sampling and identification methods. In this task, several approaches for air sampling will be evaluated to assess their utility for MP determination.

Dris et al. (2015) reported the first work on atmospheric (or airborne) MPs, termed AMPs. Since then, more atmospheric studies were published (Munyaneza et al., 2022), both in urban and suburban areas (Can-Güven, 2021) and natural areas (Beaurepaire, 2021). There, it was found that the main sources of MPs seem to be roads and traffic, plastics from oceanic emissions, wear and tear from agricultural plastics and dust generated in urban environments.

The sampling systems used to monitor AMPs can be passive (gravity-based) and active (pumping-based). The first was used in most publications because they are much simpler and cost-effective. Therefore, we expect that passive deposition sampling methods will be validated and implemented in LABPLAS due to their suitability, simplicity of use for field sampling and lower cost.

Digestion protocols for atmospheric samples need to be standardised, taking into account the cost, time, and preservation of MPs. Acid digestion is not recommended because they destroy many types of MPs (Enders et al., 2017; Pfeiffer and Fischer, 2020). Researchers recommend three options: alkaline digestion (due to its low-cost and moderate risk of MPs degradation (Prata et al., 2019)); oxidative digestion (employing $H_2O_2$ (Treilles et al., 2020)) and enzymes (so far, the safest approach to preserve polymers, but of high cost and very time-consuming (Löder et al., 2017)).

The most commonly recommended analytical techniques to characterize AMPs are infrared (IR) and Raman spectroscopy, although they are time-consuming for monitoring studies. That problem is addressed partially by the use of tunable quantum cascade lasers (QCL) operating in the medium IR region. These systems can scan particles very fast (ca. 4 s/particle), saving much time (Hildebrandt et al., 2020). However, due to their novelty, setting parameters and transference protocols of the suspicious particles to the reflective slides need some study and optimization.

This deliverable summarises the overall analytical approach resulting from Task 3.2 of the LABPLAS project, including atmospheric deposition sampling -using passive samplers' devices-, a sample treatment process and a measurement approach to determine MPs in atmospheric samples. The proposed guideline methods will be validated and updated after their implementation during LABPLAS field sampling campaigns. These results have been presented in the Micro 2022 Meeting (López-Rosales et. al, 2022b) and a paper is in preparation, which is planned to be published in a peer-reviewed scientific journal in 2023.

## 2 SAMPLING

Most passive samplers are based on a funnel-bottle bulk collector. This was first used by Dris et al. (2016; 2015) for AMPs monitoring in Paris and other authors developed similar systems in other parts of the world (Dos Santos Galvão, 2021). These systems allow for easy comparisons amongst each other (Can-Güven, 2021), but sampling time and the surface of sampling are very variable across studies.

Two passive samplers based on atmospheric bulk deposition (dry + wet) were tested (Figure 1):

- Depobulk® sampler, a standardised device with a 22 cm-diameter glass funnel and a 10 L glass collection container.
- Custom-built sampler, Spanish EnviroPlanet Network, with an 11 cm-diameter metallic funnel and a 2 L opaque glass collection bottle.

The height of the opening area of the collector shall be at least 1.5 m above ground, to avoid sample contamination due to ground during heavy rains. The sampling time was set at 1 month to total deposition (dry and wet).

UDC was in charge of the comparative studies, where several devices of each type were employed, the variability in the final results within each type of sampler is quite high, so, no significative statistical differences (95% confidence level) between the two types of samples were encountered. The study was repeated in 3 different months. Therefore, it seems that –with the available comparative data- both systems can be proposed to perform monitoring studies.

Nonetheless, it is simpler to work with EnviroPlanet samplers as they only need 3 L of Milli-Q water to be washed and to get good MPs recoveries. They also contain less organic material and, so, simplify the subsequent digestion process.

## 3 SAMPLE PREPARATION

Three protocols are suggested here, each with good recoveries for the most common polymers (PS, PP, PVC, PE, PET, PA particles and PET fibres) (Figure 2). Out of them, **protocol 2** appears as the more suited one for most airborne samples due to its simplicity. But it is worth noting that when very high contents of organic matter are present (e.g. the presence of bird excrement in the collection funnel) **protocol 3** may be required.

**Protocol 1: Samples with low to moderate organic matter content.**

| Steps | Specifications |
|---|---|
| Step 0:<br>Wash all funnel contents into the collection bottle<br>Step 1:<br>Sample filtration | Water samples are vacuum-filtered through stainless steel filters (47 mm diameter and 20 µm pore size). Rinse the funnel and bottle with 0.1 % Triton X100 or Tween 80 solution for better MP recovery.<br>(Several filters could be required depending on the sample). |
| Step 2:<br>Sodium dodecyl sulfate (SDS) treatment | Add 100 mL 2 % (w/v) SDS solution to the filters and incubate for 24 h at ≤ 40 ºC and agitation 130 U/min. |
| Step 3:<br>Oxidative treatment with $H_2O_2$ | Add 100 mL 30 % of $H_2O_2$ (v/v) (final concentration of 15 % $H_2O_2$ should not be exceeded). Add gradually (5 mL-10 mL and wait for foam diminution). Incubate for 24 h at ≤ 40 ºC and 130 U/min. |
| Step 4: Filtration | The digested solution is vacuum-filtered through stainless steel filters (47 mm diameter and 20 µm pore size). |
| Step 5: Identification / Quantification | Proceed according to section 4. |

**Protocol 2: Samples with high contents of organic matter.**

| | Specifications |
|---|---|
| Step 0:<br>Wash all funnel contents into the collection bottle<br>Step 1:<br>Sample filtration | Water samples are vacuum-filtered through stainless steel filters (47 mm diameter and 20 µm pore size). Rinse the funnel and bottle with 0.1 % Triton X100 or Tween 80 solution for better MP recovery.<br>(Several filters could be required depending on the sample). |
| Step 2:<br>Alkaline-oxidative treatment (KOH and NaClO) | Add 150 mL of a mixed solution of 10 % (w/v) KOH and 15 % NaClO.<br>Incubate at ≤ 40°C at 24 h and agitate at 130 U/min |
| Step 3: Filtration | The digested solution is vacuum-filtered through stainless steel filters (47 mm diameter and 20 µm pore size). |
| Step 4: Identification / Quantification | Proceed according to section 4. |

**Protocol 3: Samples with very high organic matter content.**

| | **Specifications** |
|---|---|
| Step 0:<br>Wash all funnel contents into the collection bottle<br><br>Step 1:<br>Sample filtration | Water samples are vacuum-filtered through stainless steel filters (47 mm diameter and 20 µm pore size). Rinse the flask with 0.1 % Triton X100 or Tween 80 solution for better MP recovery.<br>(Several filters could be required depending on the sample). |
| Step 2:<br>Alkaline treatment with KOH treatment | Add 100 mL of 10 % (w/v) KOH and 0.1 % (w/v) surfactant (Triton X100 or Tween80).<br>Incubate at ≤ 40°C and agitate at 130 U/min |
| Step 3:<br>Oxidative treatment with $H_2O_2$ | Add 100 mL of 30 % $H_2O_2$ (v/v) (the final concentration of 15 % $H_2O_2$ should not be exceeded). Add gradually (5 mL-10 mL and wait for foam diminution). Incubate for 24 h at ≤ 40 ºC and 130 U/min. If necessary, repeat the $H_2O_2$ addition/replacement every 24-48 h until the digestion is finished. |
| Step 4: Filtration | The digested solution is vacuum-filtered through stainless steel filters (47 mm diameter and 20 µm pore size). |
| Step 5: Identification / Quantification | Proceed according to section 4. |

# 4 IDENTIFICATION AND QUANTIFICATION OF MICROPLASTICS

In the following, some broadly used and accepted characterization methodologies are depicted. These are not the only possible options, but the ones to be used in LABPLAS:

## 4.1. FTIR:

A first, preliminary step is recommended before proceeding with the true characterization of the particles by FTIR. It consists of the visual inspection of the filters for the presence of MPs using a stereomicroscope:

- Particles >500 µm: Manually pick up them using micro tweezers and place them in glass vials or a Petri dish. Then, each of them can be characterized straightforwardly by macro-ATR-FTIR. Identification of the corresponding spectrum must be done by comparing it with spectral libraries.
- Particles <500 µm: micro spectrometry FTIR (µ-FTIR) is required here. Any of the commercially available instruments can be used (single-point detector, a linear array of detectors or focal plane array (FPA) detectors). If possible, measure all particles directly on the filter (although particles up to 80 µm can be picked up manually with micro tweezers quite easily and located in holders to measure them). If the filter contains too many particles, a filter sub-sampling strategy should be applied. In general, between 33% and 50% of the filter has to be measured using different patterns, as presented elsewhere (Brandt et al, 2021; López-Rosales et al. 2022c). Identification of the corresponding spectrum must be done by comparing it with spectral libraries.

## 4.2. QCL-based IR:

The particles present in the filter have to be transferred to the surface of the reflective slides (usually, Kevley slides). This can be done in different ways but try to avoid the use of aliquots as they lead to highly variable results. An aliquots-free procedure was presented elsewhere with good results (López-Rosales et al., 2021). In brief, remove the particles from the filter using 50 mL of 96 % ethanol into a glass tube with a bottom collection space and sonicate it for 15 min, ≤ 40 °C. Then, wash the filter with another 10 mL of ethanol (5 mL/side) and remove it. Evaporate the solvent gently to ca. 1.0 mL in an automatic evaporation system (40 ºC, 180 rpm) employing a pressure gradient. Sonicate the small remnant volume for 10 s and collect it with a micropipette, pour carefully the suspension into the reflective slide and dry gently. Wash the glass tube with 20 mL of ethanol and repeat the evaporation and transfer processes (two times) to ensure particles are not lost (Figure 3).

Insert the reflective slide in the QCL-based system for IR chemical analysis. If possible, measure all particles on the slide. Identification of the corresponding spectra must be done by comparing them with spectral libraries. We suggest accepting positive matches only when the match index (or a similar denomination) is, at least, 0.9 (or 90 %).

### 4.3. NEAR-INFRARED HYPERSPECTRAL ANALYSIS (NIR-HSI)

If required, particles with sizes between 100 µm to 300 µm can be visualized easily under a magnification glass or stereomicroscope and can be picked up manually to a glass mounting slide for subsequent NIR imaging. In general, it would be recommended to skip this stage to avoid errors and submit all the filters to the imaging process. Then, raw data are processed using currently a custom-built particle-search program followed by a trained machine-learning algorithm for polymer identification.

### 4.4. Thermoanalytical methods: PYROLYSIS-GC-MS (PY-GC-MS)

Qualitative identification and mass quantitation of extracted particles can be done using Py-GCMS measurements. For this, a multi-shot pyrolyzer (EGA/Py-3030D) working at 600 ºC coupled to a GC/MS-MS system is recommended. The extracted particles retained on the filter are weighed into a pyrolysis cup. The identification of the single polymers present in the environmental samples will be done with polymer-specific marker compounds and indicator ions. To get mass quantification, individual calibration curves have to be generated on a per–polymer basis.

## 5 SAMPLE PRESERVATION AND HANDLING

Samples must be collected and preserved so that degradation and contamination are minimized. During storage, samples must be kept at low temperatures (i.e., 4 °C to -20 °C). Samples must also be protected from direct sunlight and/or strongly bright light.

## 6 CROSS-CONTAMINATION CONTROLS AND QA/QC

Most recommendations given below were extracted from the literature where more details can be found. We present here a summary:

- Procedural blanks should be done to ensure they are free of microplastics in the size range of interest. Their final subtraction is recommended to get the final results. The overall procedural blanks should also control contamination of the laboratory air and field sampling and transport.
- Ultrapure water and microplastics-free water (MPF-water) should be used to prepare all solutions and washing. Water filtration before use is recommended.
- All glassware material should be washed with a 15 % HCl solution, for 24 h, and rinsed with a 1:1 mixture of ethanol (96%) and water before using it. Calcinate metallic filters ≥ 450 °C for at least 3 h. Avoid using plastic items whenever possible.
- Cover all glassware and working items while not in use with clean glass or clean aluminium foil, and store them upside down.
- Use a laminar flow cabinet/dedicated workspace for processing samples in the laboratory.

- Use 100 % cotton lab coats whenever possible, avoiding synthetic fibre clothes underneath to minimise airborne particle contamination. If a synthetic garment is worn, register its colour and date, to trace potential operator contamination.
- Appropriate QA/QC for analytical methods should include replicates, blank controls, calculation of recovery rates using either 'clean' solutions or spiked samples, and consideration of uncertainties/confidence levels. Spectral identifications should be done against reference libraries including virgin and environmentally aged materials. Try to avoid low matches (even when they are in the 70 – 80 % match, they can be defective) and inspect visually and critically.

## REFERENCES

Beaurepaire M, Dris R, Gasperi J, Tassin B. Microplastics in the atmospheric compartment: a comprehensive review on methods, results on their occurrence and determining factors. Current Opinion in Food Science 2021;41:159–68. https://doi.org/10.1016/j.cofs.2021.04.010.

Brandt J, Fischer F, Kanaki E, Enders K, Labrenz M, Fischer D. Assessment of subsampling strategies in microspectroscopy of environmental microplastic samples. Front. Environ. Sci., 8 (2021), https://doi.org/10.3389/fenvs.2020.579676

Can-Güven E. Microplastics as emerging atmospheric pollutants: a review and bibliometric analysis. Air Qual Atmos Health 2021;14:203–15. https://doi.org/10.1007/s11869-020-00926-3.

dos Santos Galvão, L., Fernandes, E.M.S., Ferreira, R.R., dos Santos Rosa, D., Wiebeck, H., 2022. Critical steps for microplastics characterization from the atmosphere. Journal of Hazardous Materials 424, 127668. https://doi.org/10.1016/j.jhazmat.2021.127668

Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B. Microplastic contamination in an urban area: a case study in Greater Paris. Environ Chem 2015;12:592–9. https://doi.org/10.1071/EN14167.

Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Marine Pollution Bulletin 2016;104:290–3. https://doi.org/10.1016/j.marpolbul.2016.01.006.

Enders K, Lenz R, Beer S, Stedmon CA. Extraction of microplastic from biota: recommended acidic digestion destroys common plastic polymers. ICES J Mar Sci 2017;74:326–31. https://doi.org/10.1093/icesjms/fsw173.

Hildebrandt L, Gareb F, Zimmermann T, Klein O., Emeis KC, Proefrock D and Kerstan A. Fast, Automated Microplastics Analysis Using Laser Direct Chemical Imaging.(2020). Agilent Application Note. https://www.agilent.com/cs/library/applications/application-marine-microplastics-8700-ldir-5994-2421en-agilent.pdf

Löder MGJ, Imhof HK, Ladehoff M, Löschel LA, Lorenz C, Mintenig S, et al. Enzymatic Purification of Microplastics in Environmental Samples. Environ Sci Technol 2017;51:14283–92. https://doi.org/10.1021/acs.est.7b03055.

Lopez-Rosales A., Andrade J.M., Grueiro-Noche G., Fernandez-Gonzalez V., Lopez-Mahía P., Muniategui-Lorenzo S. Development of a fast and efficient method to analyze microplastics in planktonic samples. Marine Pollution Bulletin, 168:112379. 2021. https://doi.org/10.1016/j.marpolbul.2021.112379

López-Rosales A, Andrade J, Fernández-González V, López-Mahía P, Muniategui-Lorenzo S. A reliable method for the isolation and characterization of microplastics in fish gastrointestinal tracts using an infrared tunable quantum cascade laser system. Marine Pollution Bulletin 2022;178:113591. https://doi.org/10.1016/j.marpolbul.2022.113591.

López-Rosales A, Pazos-Espiñeira C, Pena-Pais Y, Fernández-Amado M, Andrade JM, López-Mahía P, Muniategui S. 2022b. Characterization of Atmospheric Microplastics in a Sub-urban area. Poster presentation at MICRO 2022 (Plastic pollution from MACRO to Nano), 14-18 November 2022 - Online. https://www.micro.infini.fr/IMG/pdf/426898_adrian_lopez-rosales.pdf

López-Rosales A, Andrade JM, López-Mahía P, Muniategui-Lorenzo S. 2022c. Development of an analytical procedure to analyze microplastics in edible macroalgae using an enzymatic-oxidative digestion. Marine Pollution Bulletin, 183,114061, 2022. https://doi.org/10.1016/j.marpolbul.2022.114061.

Munyaneza J, Jia Q, Qaraah FA, Hossain MF, Wu C, Zhen H, et al. A review of atmospheric microplastics pollution: In-depth sighting of sources, analytical methods, physiognomies, transport and risks. Science of The Total Environment 2022;822:153339. https://doi.org/10.1016/j.scitotenv.2022.153339.

Pfeiffer F, Fischer EK. Various Digestion Protocols Within Microplastic Sample Processing—Evaluating the Resistance of Different Synthetic Polymers and the Efficiency of Biogenic Organic Matter Destruction. Front Environ Sci 2020;8. https://doi.org/10.3389/fenvs.2020.572424.

Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Science of The Total Environment 686, 131–139. https://doi.org/10.1016/j.scitotenv.2019.05.456.

Treilles, R., Cayla, A., Gaspéri, J., Strich, B., Ausset, P., Tassin, B., 2020. Impacts of organic matter digestion protocols on synthetic, artificial and natural raw fibers. Science of The Total Environment 748, 141230. https://doi.org/10.1016/j.scitotenv.2020.141230.

## ANNEX FIGURES


Figure 1.- Proposed atmospheric bulk deposition samplers (wet and dry).

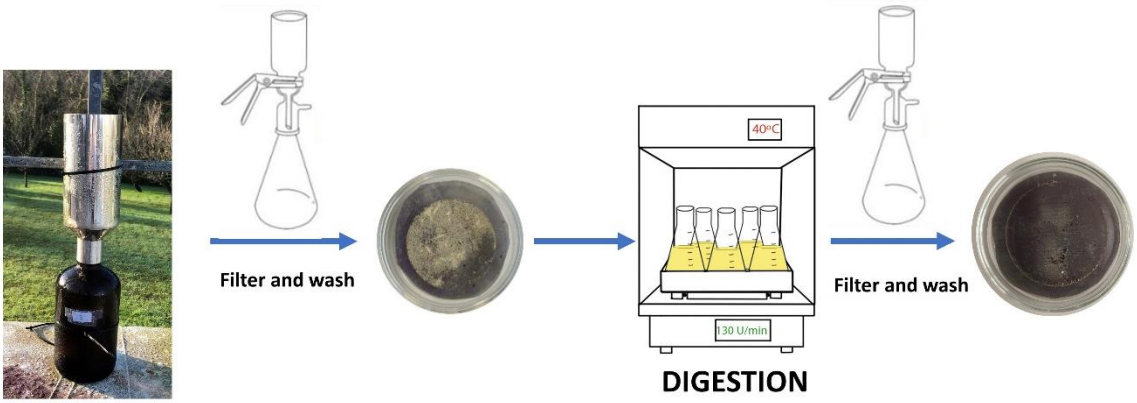


Figure 2.- Protocol applied to identify suspected microplastics. Obtaining a clean filter.

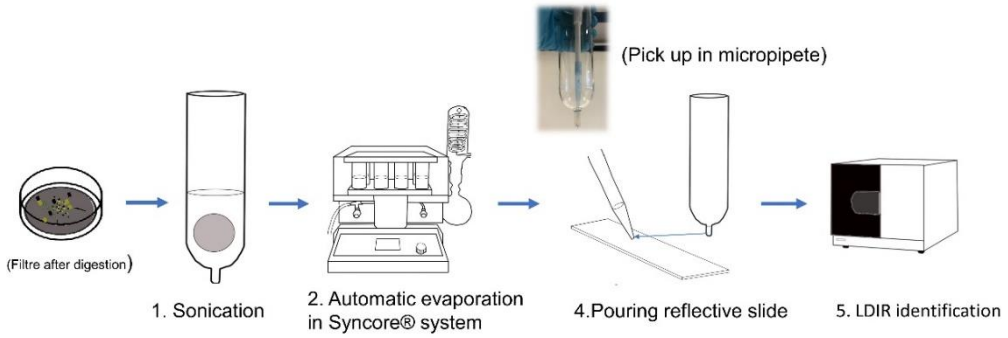


Figure 3.- Protocol applied to identify suspected microplastics. Particle transference protocol to reflectance platforms employed in quantum cascade laser-based systems.