A METHODOLOGY FOR SAMPLING, ANALYSIS AND IDENTIFICATION OF

MICROPLASTICS IN RIVERS

LIBERA
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HUNT

A METHODOLOGY FOR SAMPLING, ANALYSIS AND IDENTIFICATION OF MICROPLASTICS IN RIVERS

DRAFTING AND COLLABORATING TEAM

David León Muez. *Asociación Hombre y Territorio, HyT.*

Patricio Peñalver Duque. *Asociación Hombre y Territorio, HyT.*

Eduardo Franco Fuentes. *Asociación Hombre y Territorio, HyT.*

Eduardo Benfatti. *Asociación Hombre y Territorio, HyT.*

Laura Comes Aguilar. *Asociación Hombre y Territorio, HyT.*

Laura Mazuecos Heredia. *Asociación Hombre y Territorio, HyT.*

Carlos Ciudad Trilla. *SEO/BirdLife.*

Miguel Muñoz. *SEO/BirdLife.*

Sara Güemes. *Ecoembes.*

Aída Fernando de Fuentes. *Ecoembes.*

Laura Serrano Martín. *Departamento de Biología Vegetal y Ecología. Universidad de Sevilla.*

Rubén Parrilla Giraldez. *Servicio de microanálisis, Centro de Investigación Tecnológica e Innovación (CITIUS). Universidad de Sevilla.*

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01. Summary

During 2019 Hombre y Territorio (HyT), in accordance with the agreement held with Project LIBERA, developed a procedure for collecting samples and identifying microplastics in rivers, with the aim that it may be used by different sectors [of society]. Thus, different levels of detailed studies are favoured and the implementation of initiatives from an educational, participatory, monitoring, management and / or research point of view is facilitated.

This document arises as the result of an exhaustive bibliographic study, consultations and meetings with experts and national and international projects, participation in methodology forums at both state and European levels, field and laboratory tests and an extensive campaign on various rivers and streams throughout Spain. All bibliographical data, personnel and projects consulted can be found in the section entitled 'Annexes'.

The sampling methodology is designed to be simple, feasible and logistically profitable; to this end, instructions and a field sheet have been prepared with the intention of providing a guide and checklist for data collection to obtain additional information on the sampling point or area. Thus, a reminder to carry out littering sampling ('littering':abandonment of litter in nature) is also included whenever a sample is taken for microplastics: this littering data is of great use in identifying possible sources of material entry into aquatic systems.

Structured in a similar way, the identification methodology is divided into two independent but complementary sections: both contain instructions and steps to follow, complete with data, images and links to facilitate their execution.

This document also includes instructions for handling, safety, storage and maintenance of samples, in addition to model field and laboratory sheets.

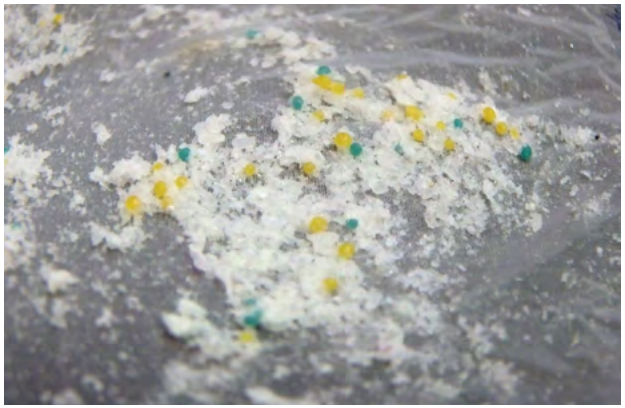
The end result isa methodological guide, protocol or work method structured in a series of complementary but not exclusive phases or steps, , so that it can be used up to the chosen level according to the objective, budget, time scale or level of detail required for each initiative.

This document is one of the results of the agreement HyT holds with Project LIBERA SEO / BirdLife in partnership with Ecoembes (2019).

02. Introduction, justification and objectives

Rubbish is a major problem on a global scale: thousands of tons of manufactured materials are used every day in various industrial, agricultural, production and domestic activities. A high percentage of these materials are single use, which together with those that have worn out or have been discarded, broken or replaced ,end up being considered waste.

An increasing quantity of this waste is recycled or reused and little by little some production models are are changing in favour of the reduction of waste products. The goal of the so-called circular economy is to drive this key paradigm shift.



Within this waste, we find that plastics and their derivatives are one of the main components, constituting around 10% of the weight of the total rubbish generated by a population (Seoánez Calvo, 2000); if the volume is taken into account, thisfigure shoots up further still. Plastic has an indisputable function in our society and is present in almost everything: clothing, electronic devices, tires, packaging, construction, transport... but it is evident that the high production rate, high consumption, its enormous versatility in addition to its poor recycling and reuse rate make it the most visible insignia of the state of chronic overconsumption in which the world finds itself today.

Plastics also have a long life after being discarded due to their resistance to external agents, rendering their degradation in the environment slow and progressive. Little by little, plastics disintegrate into smaller and smaller pieces until they become tiny particles commonly referred to as microplastics (fragments measuring under 5 millimetres).

It is estimated that microplastics exist in practically all habitats in the world (Zhang *et al*., 2019) and their study is increasingly relevant due to the harmful effects that they can have on human health and the environment. As they are minute particles, filter organisms assimilate them in large quantities. When these organisms are theningested by larger and larger animals in the trophic web, these particles accumulate until they reach food that is consumed on a daily basis (D. Cox *et al*. 2019). The effects that the ingestion of microplastics may have on human health, especially in high quantities, are still unknown. Their presence has been confirmed in certain foods and beverages and in addition, this intake may lead to the assimilation of additives in these materials, many of which are known carcinogens or endocrine disruptors (Reche *et al*. 2019).

The ocean/marine environment? is perhaps the most studied system in this sense and it is estimated that roughly 10 million tons of rubbish reach our oceans each year, with about 80% of this being of plastic origin (Citizen Decalogue of the Spanish Association of Marine Garbage, AEBAM 2014). Furthermore, the powerful disintegrating effect of seawater and the sun cause rapid decomposition into smaller elements, which easily enter the marine food web.

Terrestrial ecosystems are often less studied concerning this issue, yet it is evident they play a crucial role. It is estimated that about 80% of all rubbish that reaches the sea comes initially from the land (Citizen Decalogue of the Spanish Garbage Association Marinas, AEBAM 2014) and thus direct entry elements such as rivers are key to establishing the sources of rubbish entry into the sea, and therefore are also key to its reduction. In fact, it has been calculated that approximately 95% of all plastics that reach our oceans globally come from a limited number of rivers, located principally in Asia (Jambeck *et al*, 2015). Although none of these rivers are located in Spain, estimates place Spain as second in the quantity of plastics dumped into the Mediterranean, which, being a semi-enclosed sea, becomes a trap for pollution (Aquae Foundation report with data of the European Parliament Research Service, Greenpeace and WWF). The Mediterranean is considered the sea most polluted by plastics on the entire planet.

For this reason, the study and reduction of plastic pollution sources is of particular importance, taking advantage in the process to implement actions fomenting information, awareness and participation.

Currently, the Water Framework Directive (Directive 2000/60/CE) does not link the presence of this waste or their derived pollutants as quality indicators. However, this Directive and the related Directive on the Protection of the Marine Environment concerning the presence of rubbish and microplastics in riverine environments, as well as certain compounds related to their presence, are being reviewed and may eventually be included as indicators.

Taking into account the scenario outlined in the previous section, the aim of this document is to develop a methodological protocol for the sampling and identification of microplastics in rivers and other continental waterways, which can serve as a tool in scientific fields, for management and control in the public sphere, as well as in an educational contexts. Furthermore, it has been designed to generate greater awareness of the problem, its origin and possible solutions.

03. Potential users

RESEARCH

The methodology included in this document is based on and has been used to carry out the first extensive study of microplastics in rivers in Spain, carried out by Hombre y Territorio in collaboration with CSIC and SEO / BirdLife in 2019: It has thus been possible to define a methodology for sampling based on experience and continuous improvement, versatile and applicable in various scenarios, which complies with the principles of safety, asepsis, regulation and replicability.

This methodology may, therefore, be used in other similar studies that require sampling of microplastics in rivers, within the context of scientific research.

MANAGEMENT

The material included for sampling, in addition to the field sheet model and the laboratory analysis with a magnifying glass are designed to be implemented by a monitoring and regulatory network, for example in a catchment area or Hydrographic Confederation. Likewise, the process of filtering and laboratory observation under a magnifying glass is perfectly replicable by administrative entities (local, regional or national). The final phase of "polymer identification" is optional depending on the budget and objectives of the study.

CITIZEN SCIENCE

Citizen science is one of the most powerful tools in the field of research, conservation and awareness. New technologies and easy access to citizen information help global problems to become increasingly visible in wider society, cultivating in the process a sense of altruistic participation in supportive andcollaborative actions to help reduce certain threats. Global and climate change are a clear example of this, with the various effects in terms of pollution, destruction of habitats, disappearance of species and environmental changes.

On a global scale, the number of iniciatives striving to contribute information on the presence of microplastics in rivers is ever-increasing. Both the United Kingdom and United States have well-established networks (Plastic Aware Projecy, 100 Plastic Rivers) comprised of specialists and volunteers, collaborating in the development of citizen science to obtain information about the diverse sources of microplastics in our oceans.

EDUCATION

The contents of this document, its bibliographic annexes and the first steps for sampling and analysis are perfectly replicable in the secondary or high school education sector as part of field experiements in science departments, while also serving to raise awareness of the issue. In fact, workshops have previously been carried out with schoolchildren in order to offer the most appropriate methodology.

04. Microplastics: definition and origin

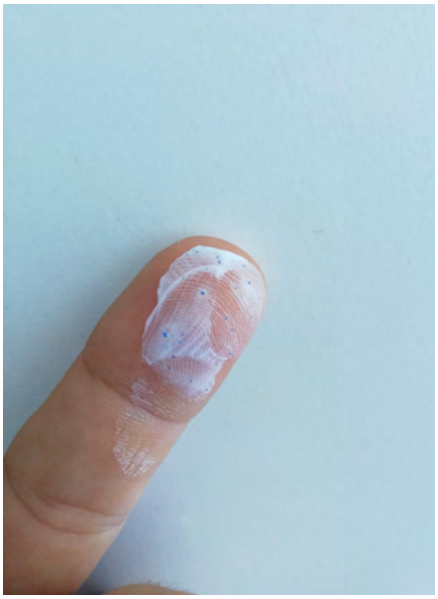
The materials we commonly refer to as plastics are made up of organic or semi-synthetic compounds that may come from renewable or fossil raw materials. They are normally manufactured in industrial processes and constitute an extensive family of polymers, with a wide variety of formulas, compoundsand additives. They have been employed for many years now in an increasing number of uses. Their low production cost and versatility have made them essential in many areas. However, there has been an excess of all types of plastic production relating to everyday life, from livestock, agriculture and fishing to industry, which together with a high disposal rate and low percentage of reuse and recycling, generates a surplus that, in many cases, ends up in the natural environment.

Although there are hundreds of formulations of polymers and other compounds, [in general and] for the purpose of this study we will focus on the following: PU (polyurethane), HDPE (high density polyethylene), LDPE (low density polyethylene), PP (polypropylene) , PS (Polystyrene), EVA (Ethylvinylacetate) and OTHERS (including other compounds such as rubbers, etc.). The reason for focusing on these materials is their average buoyancy, determining whether they sink or remain in suspension upon reaching liquid media (Table 1). These groups of polymers, alone or in combination, form the groups of elements with which we will work in our analysis.

> Microplastics are plastic fragments of up to 5 millimetres that may come from products of this size in origin (primary) or from the fragmentation of larger plastic elements (secondary).

Although **primary microplastics** may come from the loss or abandonment of agricultural or industrial materials (such as "nurdles", pellets or plastic beads used in industries as a primary element for the manufacture of objects), the main origin is urban environments. Even today, many of the hygiene products that we use include minute spheres (microbeads) or small plastic fragments

to generate a colour, an appearance, or to enhance exfoliation or brushing effects.



Secondary microplastics, on the other hand, therefore come from the breakdown, degradation or disintegration of larger elements. Abandonment of materials in the natural environment means they are subjected to extreme environmental conditions (variations in temperature, ultraviolet radiation, wet/dry conditions), accelerating their degradation and disintegration. Similarly, if not properly managed the remains of agricultural or industrial materials, many of them derived from plastics, end up abandoned and scattered in natural environments. Wind and rain are vectors of displacement for many materials that end up in rivers and eventually, the sea. This type of waste in natural environments becomes ***basuraleza*** (combination of the Spanish words for 'rubbish' and 'nature'), a term coined by Project LIBERA to refer to rubbish that ends up in nature.

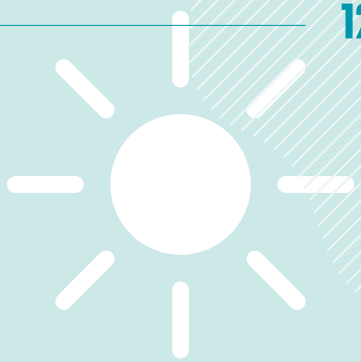
> Purification systems, if well managed, can reduce the percentage of fibres and other micro-elements by around 95%.

An intermediate case would be that of synthetic textile fibres that become detached from clothing during washing: hundreds of fibres can be shed by clothing in each wash, which are then directly incorporated into the urban hydraulic system (CEDEX Report for Marine Strategies in Spain). Nothing more than a small calculation is needed to estimate how many fibres a population with a high use of synthetic garments can generate.

Fortunately, many studies suggest that purification systems, if well managed, can reduce the percentage of fibres and other micro-elements by around 95% (CEDEX Technical Report for MITECO 2018). However, this fact does not resolve the issue as it does not eliminate theproblemat its source. In many areasthere is little or no purification activity, a strong indication that a huge quantity of these elements still end up in rivers, and from there, flowout into the sea.

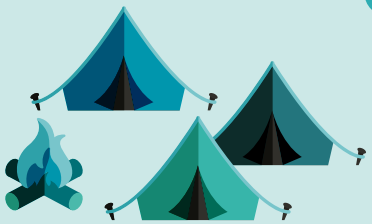
SOURCES OF DEGRADATION

Wind
UV Radiation
Contrasts in tempertature
Rain



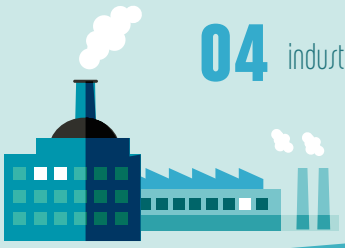
01 settlements

02 recreational areas

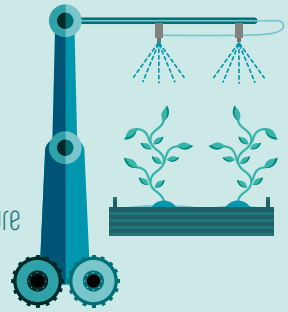


03 landfills

04 industry

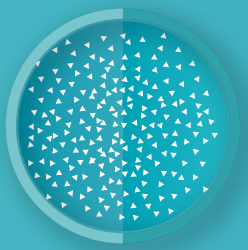


05 agriculture

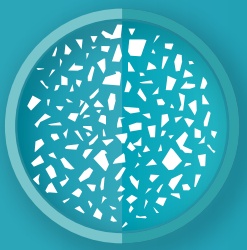


Synthetic clothing
Cosmetics
Slag heaps
Industry
Littering
Landfills
Treatment plants

primary microplastics



secondary microplastics



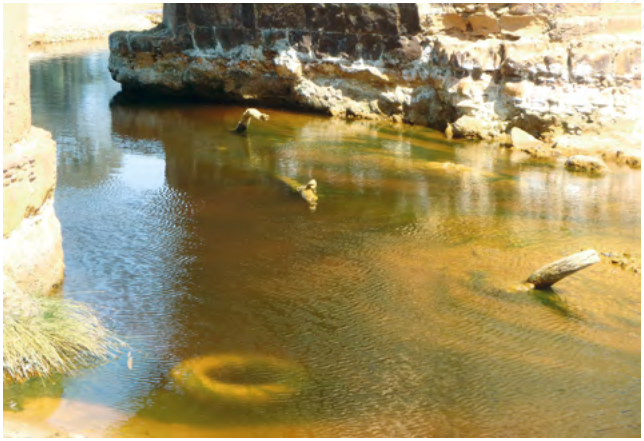
05. Working phases

This document presents a series of steps or phases to launch a microplastics analysis study.

These steps or phases have been established in a sequence in order to be as adaptable as possible to the needs of each project, initiative, entity or study.

5.1. Scope and objectives

This is possibly the most important part of any study, project or activity that we intend to set in motion: it is necessary to pause for a moment to consider what we want to achieve, for whom our activity is intended, the scope of the project and our budget, taking into account the costs incurred and the time that we (or others) have set aside for its completion.



» Rubbish, or *basuraleza*, in mid-section of stream: abandoned tyre submerged in the Río Tinto (Huelva).

Although the field materials are not expensive, it must be taken into account that a large number of samples appreciably prolongs the field work as well as subsequent analysis in the laboratory: all this must be planned in advance so as not to break the balance between the objective and delivering results, on time and on budget.

This methodology is valid for all levels of experience: for example, for educational activities. Although in this document we refer to a binocular laboratory microscope, the use of certain desktop or even field microscopes —well-secured and following the identification sequence— is perfectly viable for the identification of fragments in the filter, although the minimum size visible may be limited. Another example could be the monitoring of a tributary stream,60 kilometres in length, crossing several settlements before joining the main river. In this instance, control points may be required at the headwaters, middle section and endl, or sampling before and after areas of potential discharge (municipal wastewater treatment plants, agricultural holdings, etc.).

5.2. Planning and selection of materials

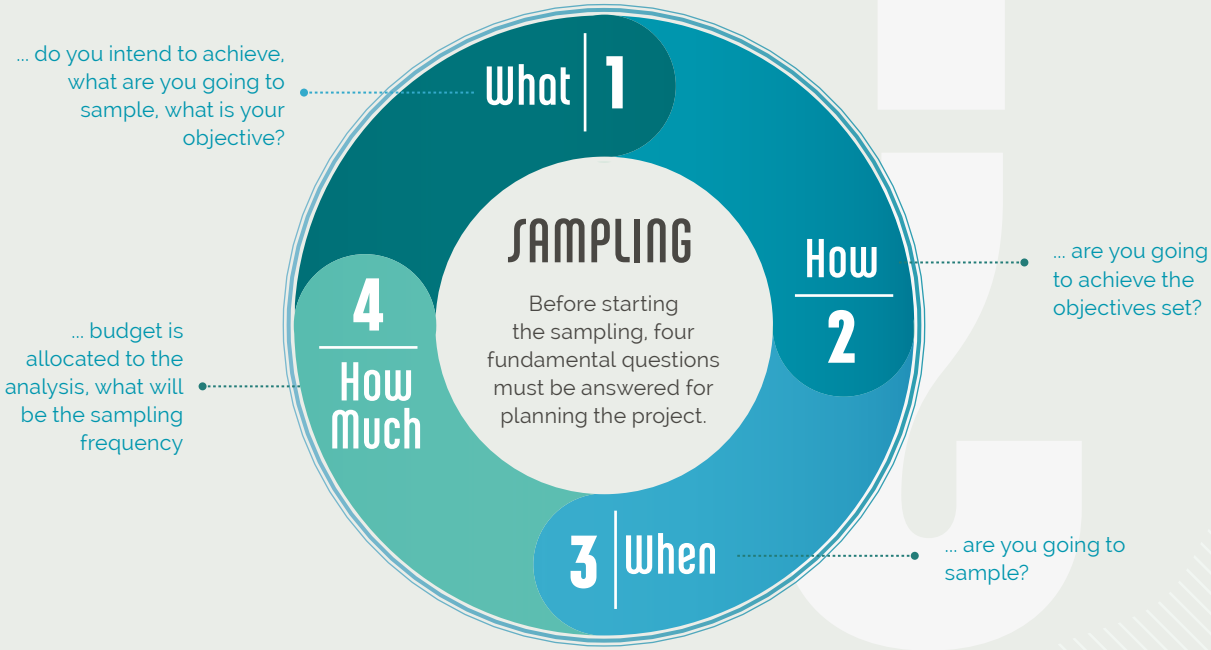
The importance of this section is that, once we have set our objective, we can now plan where, how often, in what area/ areasand when we are going to start sampling. Spanish rivers can be impressively varied, but in general, the vast majority are classified as medium or small, in contrast to other European rivers displaying greater flow, length and channel-width. In fact, only 3 Spanish rivers are among those with an average annual flow of more than 100 m^3/s, in comparison with aprrox. 50 m^3/s in the rest of Europe (report by marine litter Technical Group for the Framework Directive of Marine Strategy in Europe). It is therefore possible to apply this methodology in the vast majority of Spanish rivers and their different sections,

or at the very least it can be used to complement other more expensive and advanced methodologies. Following this step and following the guidelines in the previous section, the study must then be defined in greater detail.

It is important to bear in mind that the following sections are l recommended guidelines based on empirical knowledge, but in anybut nonetheless may be subject to modifications or adaptations as required by the sampling circumstances.

When to sample? It depends on our objective and the hydrological nature of the river / section / basin that we are studying.

» Every river is different.



5.2.1. WHAT DO WE SAMPLE?

The plastic fragments that reach rivers via different routes can behave very different ways depending on their size, state and, above all, composition. The basis for the sampling of microplastics presented here is based on those found in the water column with positive or neutral buoyancy. In the first few centimetres of the body of water, depending on their composition, a part of the polymers show greater or lesser floatability in water. Another group have a much higher density than water and settle immediately.

The characteristics of different polymers are displayed in the table below (adapted from BASEMAN, 2019):

↓ **Table 1.** *Buoyancy of different polymers (adapted from BASEMAN, 2019).*

Abbreviation	Polymer	Density* (g cm-3)	Buoyancy**	
PS	Polyestiren	0.01 – 1.06	▲	Positive
PP	Polypropilen	0.85 – 0.92	▲	Positive
LDPE	Low density polyethylene	0.89 – 0.93	▲	Positive
EVA	Ethylvinylacetate	0.93 - 0.95	▲	Positive
HPDE	High density polyethylene	0.94 – 0.98	▲	Positive
PU	Polyurethane	1.00 – 1.03	▲	Positive
PA 6,6	Nylon 6,6	1.13– 1.15	▼	Negative
PMMA	(Polymethyl) methacrylate	1.16 – 1.20	▼	Negative
PC	Polycarbonate	1.20 – 1.22	▼	Negative
PA	Polyamide	1.12 – 1.15	▼	Negative
PET	Polyethylene terephthalate	1.38 – 1.41	▼	Negative
PVC	Polyvinylchloride	1.38 – 1.41	▼	Negative
PTFE	Polytetrafluoroethylene (Teflon)	2.10 – 2.30	▼	Negative

*The density of the different materials can vary depending on the additives added in production.

**Density of fresh water 1; density of sea water 1.025.

This is why we are going to sample only the first few centimetres of our river or stream; sediment sampling would have to be carried out in order to investigate the remaining polymers, a technique not dealt with in this document.

» Only 3 Spanish rivers are among those with an average annual flow of more than 100 m³/s, in comparison with aprrox. 50 m³/s in the rest of Europe (report by marine litter Technical Group for the Framework Directive of Marine Strategy in Europe).

5.2.2. SAMPLING MATERIALS

Materials* required for fieldwork:

☑ Field sheet (model included in annexes)	☑ 5-8 litre bucket (preferably stainless steel)
☑ Sample inventory sheet (model included in annexes)	☑ 1-2 litre capacity container (preferably stainless steel)
☑ eLitter sampling sheet (included in annexes)	☑ Sampler with telescopic pole (optional)
☑ Camera or mobile phone	☑ Ropes and carabiners
☑ Thermometer (preferably electronic, to measure ambient and river/stream temperatures)	☑ Boots and/or waders
☑ Filtration equipment (preferably metal or PVC) with a screw-on cap and hollow at the base to fix the field filter and let the water pass through.	☑ Washing bottle with distilled water (or tap water if unavailable)
☑ Nytal-Nytex filters	☑ Measuring tape
☑ Tweezers (preferably metal)	☑ Permanent marker, pencil, eraser, pencil sharpener and pen
☑ Gloves (preferably cotton)	☑ GPS (optional)
☑ Wide-mouth jars (preferably glass)	☑ Current meter (optional)
☑ Peroxide	☑ Secchi disk (optional)

After various tests in the field, a series of **water intake and filtration equipment is proposed as sampling material that allows specified volumes of water to be dumped (for example, litre by litre), collected either bybucket or via the container. In addition, it is recommended that the filtration equipment be fitted with a **screw-on cap with hole** that allows the field filter to fit and also facilitate transfer to the wide-mouth container, having previously introduced the mouth of the filter equipment (see photos).*

*It is recommended that the sampling be carried out with **45-micron nytal-nytex filter mesh**. This material, which is sold in sheets and can be purchased in specialist entomology or laboratory stores, is highly resistant and flexible, allowing the fluid passage of large volumes of water while retaining materials within the microplastic range (up to 5 millimetres) and visible with the naked eye (100-200 microns).*

This equipment system permits the transportation of a significant number of materials in a reduced space; it also reduces the potential contamination between samples by using a single filter for each sampling point. Furthermore, being made of PVC (a material with negative buoyancy) we further ensure the absence of contamination with the potential to alter results.

The purpose of the **field sheet** is to collect as much information as possible about the area where the water sample is taken; it is essential to have this record and information about the site for after sampling has been completed. During a campaign lasting weeks or months in which several zones are visited, failure to rigorously record data can easily lead to confusion. In light of this, it is important to record the location with as much precision as possible: if the area has been previously located, it can be indicated using a georeferencing service (such as Google Earth or Pixelis). It is also possible (as long as there is coverage) to send a **WhatsApp** message with the location and then pass this information to the field sheet or the digital summary document.

Taking a **photograph** of the area is also recommended, in addition to filling in all information possibleon the field sheet and noting down any comments that could later clarify specific aspects of the site, day, situation, etc..

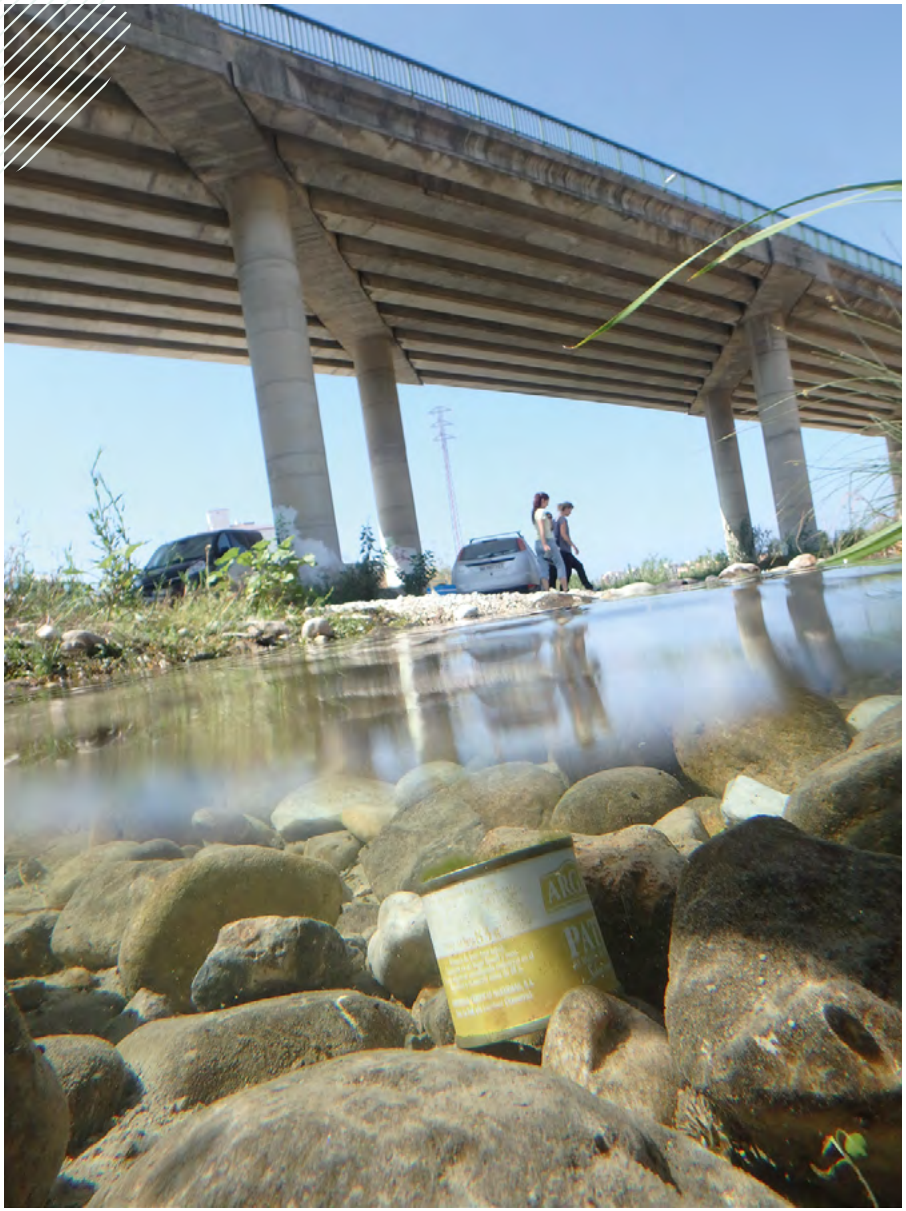
The purpose of the field sheet is to collect as much information as possible about the area where the water sample is taken.

5.2.3. SELECTION OF SAMPLING POINT

When selecting the sampling point we must take into account several considerations, for example, whther the river, stream or stretch to be studied is fordable or not. If the zone is fordable, and always evaluating theconditions to ensure safety and accessibility, we will collect the water directly using the container or bucket (with or without the help of the extendable pole or ropes and carabiners). We will always initiate the transect against the current, to avoid contaminating the sample with sediment that might be disturbed when walking along the riverbed. In the case of sections that cannot be waded or with strong current, we will collect the water indirectly from a perimeter (bridge, dam) or floating (boat) structure using the bucket and ropes.

A model field sheet can be found in the section 'Annexes'.

» Depending on the section, we will choose to sample indirectly (bridge) or directly (from the riverbed).



When selecting the sampling point and possible replications (depending on the initial objectives and selection), samples by section (high-medium-low), control samples and before-re-after possible effluents or other factors with potential to influence results can be taken into account (WWTP, agricultural zone, industrial zone, etc.). These considerations will depend, as previously mentioned, on each study and its corresponding objective, team and budget.

When selecting the sampling point, samples by section, control samples and before-after possible effluents or other factors with potential to influence results can be taken into account.

5.3. Sampling

5.3.1. HOW DO WE SAMPLE?

The best section, whether fordable or not, from which to take a sample should be chosen based on the following conditions:

Safety (personal and of the materials).

We **never sample alone**, we always **plan** where we are going, how we will access the site and pre-check the area before starting.

Accessibility (of particular importance in muddy areas or where there are e riverbanks with dense vegetation).

If a site does not meet the requirements for safety and comfort while sampling, it is better to look for another.

Potentiality.

Backwater areas are preferable or, failing that, close to the riverbank or in the internal curve of a meander: this is to ensure that we collect water in areas where microplastics are likely to accumulate.

Orientation.

It is important to take the current and wind direction into account, always selecting the sampling area in the **leeward section** (where the wind is heading). As we have previously mentioned, many plastics have positive buoyancy and are carried not only by the current but also by the wind.

Blockage and intrusion.

Avoid, as far as possible, the "fishing" of that could potentially block the filter (leaves, floating algae...).

Asepsis.

To avoid possible contamination of the samples, we will avoid, where possible, wearing **synthetic garments** during the sampling process.

Water is collected from the first 20-30 centimetres of depth, which is the depth to which the 5-8 litre bucket sinks or to which we should introduce the container. In the case of wading, we will continue upstream from the starting point so as not to "fish" sediments or turbulence that we may generate, keeping an eye out for possible accumulation areas (for example, on the banks or in riparian vegetation). In the case of sampling in a non-fordable section, we will lower the bucket in different zones of the selected area.

With the intention of setting a standard methodology for follow-up studies, periodic controls, an analysis of presence/absence or for intercalibration studies between working groups, we have defined a **sampling effort** or predetermined unit for use when sampling in the field. **After having carried out various tests in different locations, studying the average time of an extensive field campaign, and depending on the kind of filter chosen, for the purposes of this methodology (LIBERA) we have opted for a type of sampling effort that relatesthe litres filtered with the sampling time at each point/replica**. Not all water is the same and not all sampling points have the same characteristics, it is therefore necessary to specify a maximum number of litres to be filtered (in some cases the filter never reaches its limit) or set a time limit (sometimes the type of water quickly blocks the filter and we can spend too much time filtering a certain amount).

This means that the minimum sampling unit will be established at each point, and that at least one sample will be collected in each area, given that for follow-up planning in a specific space of time or for a scientific quantification study, the protocol can be extended and a series of replicas indicated, in the case of areas with a channel wider than 20 metres, for example.

It is useful to define a sampling effort, dependent for example on time or litres filtered.

In order to facilitate its application in a variety of situations, the suggested sampling effort is based on experience obtained in the comissioning of this manual, from extensive sampling campaigns and a project for the monitoring ofa hydrological basin control network carried out by technical personnel.

5.3.2. SAMPLING SEQUENCE

Upon arrival at the chosen area, and after filling in the first sections on the field sheet,

01 We will take one of the containers and mark it with an **indelible marker** according to our chosen **code***, in addition we can also use adhesive labels.

(Example NOM_ XYZ_DATE): GUA_11b_31012019 (River Guadalquivir, upper section, replica 1, after effluent)

- **NOM**: three capital letters or three numbers referring to the river. You can take the first three or the first, middle and last letters of the name (fully indicated on the sheet) or assign a code based on a predetermined methodology (Community-Province-Municipality)
- **X**: indicates the approximate section of the river (1-upper, 2-mid 3-lower)
- **Y**: indicates, if any, the cross-section replica (X1Z, X2Z, X3Z...)
- **Z**: indicates the location, if any, to relation to an effluent point (a-before, d-after).
- **DATE**: date of sampling in the form of day_month_year.

After labeling the sample should be washed with water from the sampling area at least three times.

02 The **filtration equipment** should be washed with water from the sampling area at least three times. Afterwards, place one of the **filters** in the opening of the container, making sure that the cap fully blocks the filter without leaving any holes through which the water could pass without being filtered. We can check the seal by tapping the outside of the filter.

» Checking the filter seal.

03 After starting to **count the time**, begin to **pour water into the filtration equipment**, first a small amount to see how it is filtered, subsequently adapting the rhythm to the flow of water through the filter. The outside of the filter should be tapped regularly using the fingertips in order to resuspend the materials that could be blocking the mesh. To carry out this movement safely it is necessary to first ensure that the filter is correctly fitted (step 2).

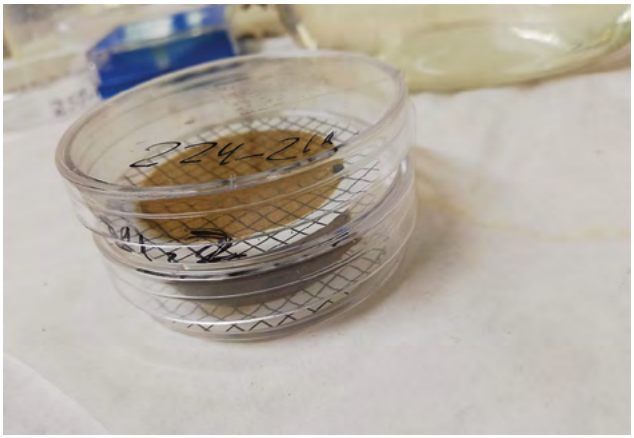
04 On finishing the sampling effort (measured inlitres or time, depending on the water conditions) tip a quantity of water from the area (preferably filtered, obtained water that falls from the filter) into the **sample receiving** container until reaching aprrox. one centimeter from the top edge.

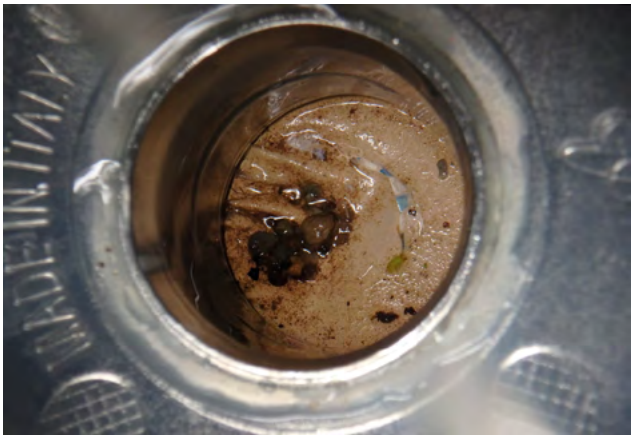
05 Upon finishing the sampling effort, **unscrew the cap** and carefully separate the filtration equipment from the cap and filter (which will normally come out together). Immediately insert the end of tube of the filtration equipment into the container with water to collect possible fragments adhered to the sides or lip of the equipment. Then proceed to carefully place the cap with the filter on top of the container and push the filter inside.

06 After placing the filter inside the previously labeled container, add a small amount of hydrogen peroxide to oxidize the organic matter and hermetically sealing the container, gently shaking it to distribute the oxidant.

07 Preferably store the container in a dry, dark place and we finish filling in the field and eLitter sheets.

08 When putting the container away or at the end of the day (if we have sampled in several different zones), the sample inventory sheet should be **completed and reviewed** with the data collected.



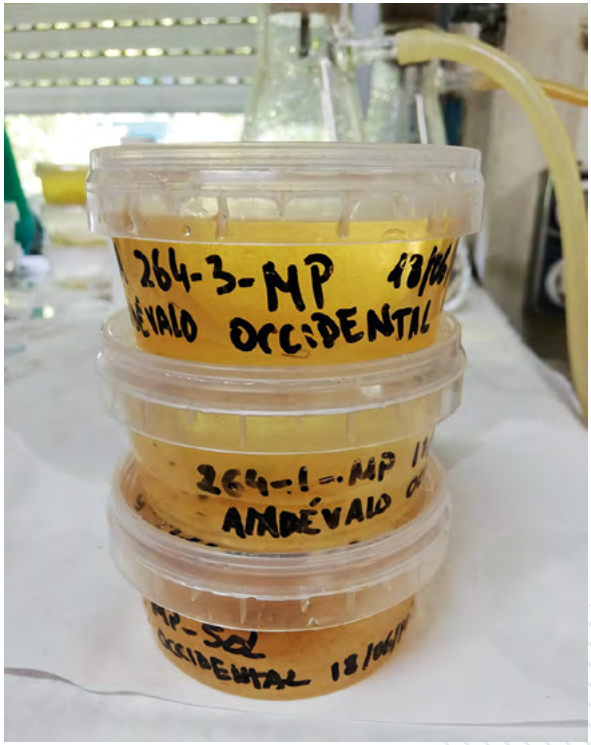


5.4. Storage and pre-treatment

One of the advantages of this sampling system,provided that the steps indicated above have been followed, is that the samples require little care until laboratory analysis is carried out. **We ensure that we have collected the sample correctly in the field**, that we have added an amount of oxidant equalling approximately 10% of the total volume of the sample (eg. if the sample is 90ml, 9ml of oxidant should be added) and balanced in relation to the apparent organic matter content of the sample, of **having closed the container well and kept it in a cool, dry and preferably dark place** (to avoid the proliferation of algae). If using plastic containers, we must be careful to prevent the lid from opening as a result of an increase in temperature or the metabolism of the sample.

When the samples arrive at their destination, a preliminary treatment is carried out in order to reduce the total space that our samples occupy and will ensure correct and lasting storage. Our aim is to maintain an orderly and clean workspace, protected from potential breezes or possible re-suspension of dirt and avoiding, where possible, the wearing of synthetic clothing during filtration operations.

> One of the advantages of this sampling system is that the samples require little care until laboratory analysis is carried out.



5.4.1. MATERIALS

This and the following phases (Analysis-1: separation and identification under magnifying glass) are the most important as they mark the limit between a general and more specific study. For these phases, a series of relatively accessible materials are proposed and even readily available in many institutions (including primary and high schools) and projects. Furthermore, all materials are partly "adaptable" according to the type of Project, nature of the study, objectives and budget.

☑ Sample inventory sheet, filled out (*model included in annexes*)	☑ Cellulose nitrate filters (for this methodology we recommend filters with a 0.45 or 0.8micron pore size and 47 mm diameter)
☑ Laboratory and Analysis Record Sheet-1 (*included in annexes*)	
☑ Fridge	☑ Indelible marker
☑ Filtration equipment and vacuum pump	☑ Pen, pencil, eraser and pencil sharpener
☑ Drying chamber*	☑ Camera or mobile phone
☑ Entomological forceps and laboratory forceps (stainless steel)	☑ Transparent or translucent tape
☑ Petri dishes, min. 50mm diameter	☑ Hydrogen peroxide and distilled water

* A drying chamber is an airtight space (a box or dark container) where samples can be placed without risk of contamination while they dry completely. For this we purpose, several packets of silica gel should be placed inside.

5.4.2. SEQUENCE

At the destination a second filtering is carried out, now using a vacuum pump, on each of the samples obtained in the field in order to concentrate the sample and eliminate the water. Each sample is filtered through a filter/disc that is subsequently placed in a petri dish along with the sample identification, according to the chosen system.

For this methodology, square and sterilized cellulose nitrate filters with a pore size of 0.45 and 0.8 microns have been selected.

01 Before beginning, **wash** the container funnel of the filtration equipment with **distilled water** and place, with the help of tweezers, a new filter in the cone.

02 After inserting the filter, **pour a small amount of water** from the sample into the funnel then **activate the switch**. By doing this we will be able to observe the filtration rate of the sample, and we can then proceed to pour a larger quantity. Depending on the content in suspension in the water (algae, bacteria, solids) it will have a higher a lower speed of filtration: if we pour in too much water and the filter collapses, we will have to re-collect all the water and use another filter, greatly complicating the operation.

03 If all the water in the container passes through the filter, a **single filter** can be used for our sample. If we observe that the filter becomes blocked and the water takes too long to pass through, cease adding water and wait for the volume that we have added to finish filtering before changing the filter and continuing filtration of the sample.

At the destination a second filtering is carried out, now using a vacuum pump.

04 In either of the previous two cases, at the end of filtering the pump should be left in operation for a few more seconds so that all moisture is eliminated. Next, turn off the switch and pour a small amount of **hydrogen peroxide** on the sides of the funnel: this water will carry away any fragments that have stuck to the sides and will partially oxidize the matter stuck to the filter. After letting it act for a few seconds, activate the switch again and wait until the filter is dry.

05 When removing the funnel we will observe that the filter displays a **circular markthe** characteristic colour of water and its suspended particles (green, yellow, brown). Carefully examine the end of the funnel in case there are any adhered particles and, if necessary, remove them with precision tweezers and carefully place them on the edge of the uncoloured filter.

06 Pick up the filter using tweezers and place it in the corresponding **petri dish**. In this moment, as an alternative, a few drops of hydrogen peroxide may be placed on the central part of the filter and left to act. Add just enough to soak the filter without spilling liquid on the dish.

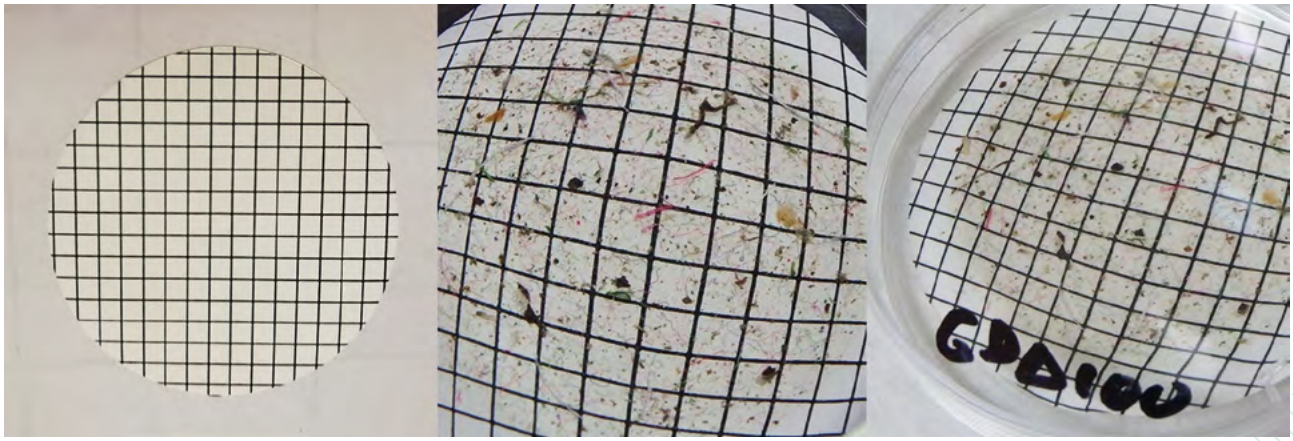
07 Immediately place the open and labeled petri dish with its filter inside the **drying chamber**. The sample identification on the dish should be written in indelible marker both on the top (to facilitate reading and identification) and bottom (to reduce the risk of confusion when handling the filters later) of the petri dish.

08 After **48 hours in the drying chamber** (which must be kept in a cool, dry place), we will proceed to close the dish until it is identified under the microscope. To avoid possible losses or confusion, the two parts of the dish are fixed using transparent adhesive tape on each side. Thus, when identification is carried out under a magnifying glass, only one piece will be removed, leaving the other as a hinge. The filtering and drying process and the closure of labeled petri dish allows the samples to be stored for a longer period.

After filtering, therefore, a filter is obtained with the content "partially" embedded (cellulose nitrate) as a result of the pressure to which it has been subjected, allowing for safer transport and handling.

Even so, it is important to treat the sample with caution so as not to separate the different fragments. If this were to occur, they would be trapped in the petri dish containing the filter and could be easily recovered.

After filtering, therefore, a filter is obtained with the content "partially" embedded.



» Filter appearance before and after filtration, and after labeling.

5.5. ANALYSIS-1: separation and identification under microscope

To carry out the initial identification of microplastics, a binocular stereo microscope should preferably be used, as well as an optical microscope and other direct and indirect means of verification. All information obtained should be transferred to a record sheet, following the model included in the annexes, which will serve as a record sheet for possible submission to Analysis-2.

In order to identify microplastics under the microscope, we must first be aware of the appearance of different elements that we might find and also what other things may appear in the filter which are obviously not microplastics (small rocks, algae, remains of vegetation, invertebrates or their remains). The person who performing the analysis must be well trained and/or have a reference catalogue to aid identification. For this reason, an identification aid and elimination guide is included as an annex to this document, which also includes some of the verification tests that can be carrified out if we are still in doubt.

When identifying, try to maintain an orderly and clean workspace, away from areas with a breeze or possible re-suspension of dirt, and avoid wearing synthetic clothing during filtration operations as much as possible. Always remember to wear a lab coat.

5.5.1. MATERIALS

☑ Binocular loupe with 10x-20x magnification	☑ Entomological point punch**
☑ Filter holder* (*recommended*)	☑ Lighter
☑ Laboratory and Analysis Record Sheet-1 (*included in annexes*)	☑ Camera or mobile phone
☑ Identification aid and elimination guide (*included in annexes*)	☑ Ballpoint pen, pencil, eraser and sharpener
☑ Entomological forceps and laboratory forceps (stainless steel)	☑ Optical microscope (*optional*)

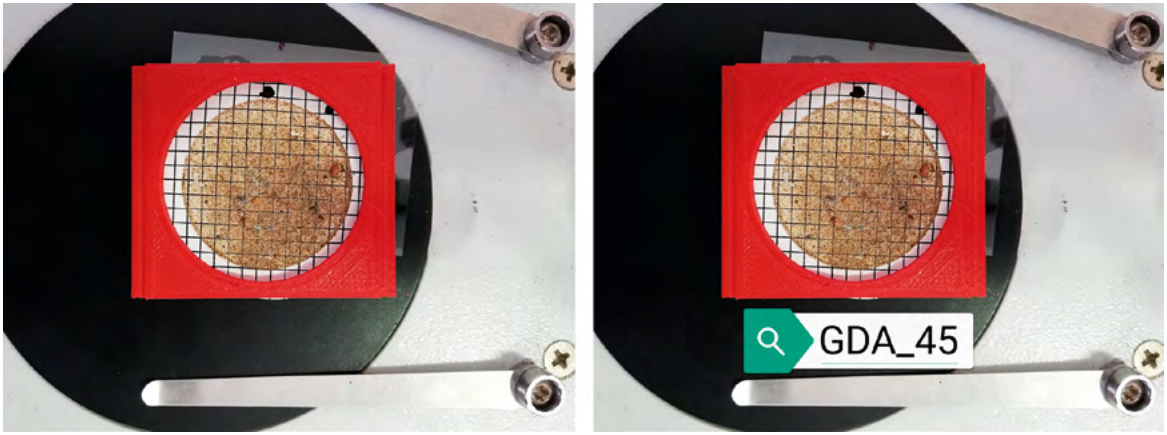
* This can be homemade (using a square cover or laminated glass) or made using a 3D printer, for example, allowing us to "move" the filter under the microscope in order to check the sample.

** An entomological point punch is made up of an entomological needle and a handle that can be made by hand with a pipette or an eppendorf: its function is to handle small elements and, in our case, access the elements to perform a heat resistance test.

5.5.2. SEQUENCE

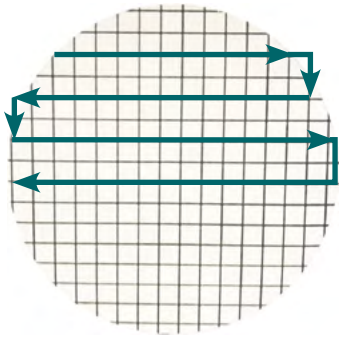
01 Place the filter in the support **under the microscope** and before fixing it, make a mark using a black marker that will indicate north (0 ° -360 °) to help guide us in the identification process and, in the event of the sample being sent to thelaboratory for Analysis-2. This point will mark the reference origin for the identification of elements to be analysed, corresponding to zero-360 on the laboratory sheet.

02 Before starting, a **general photograph** of the filter should be taken and labeled digitally: this serves to create the record of the sample and to facilitate the location of the selected fragments for identification using infrared.



» The filter before and after digital labeling, fixed in place and with its mark of origin.

03 The **search for and counting of fragments** is carried in the entire filter: usingthe marked initial point of reference as a guide and, following the grid, we scan from left to right. Upon reaching the other side, descend and continue the analysis from right to left: this parallel or 'zig-zag' search method ensures a thorough sample review and an effective way of maintaining orientation.

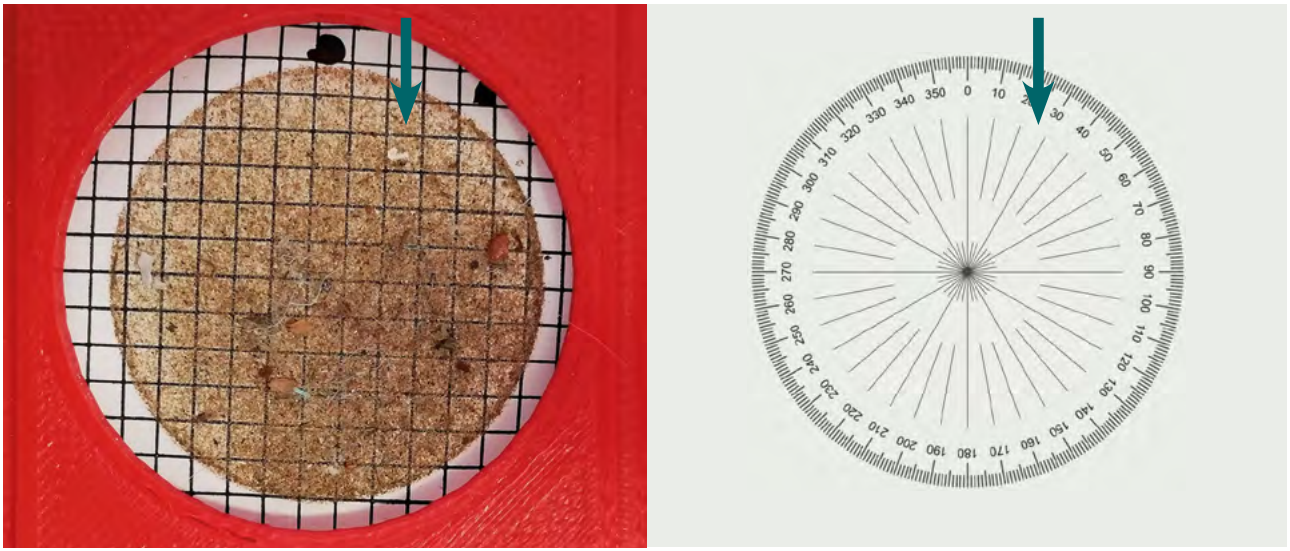


» Zigzag filter analysis.

04 When an element is located, it should be identified according to the **6 general types** that appear on the sheet (fiber, fragment, sphere, film, sponge, rubber). With the help of the identification aid and elimination guide (included in annexes), it should first be ruled out thatthe element is not a rock, animal remains (whole or part of rotifer, mollusk, crustacean, fish scale) or plant (individual or colony of algae, fragment of upper plant).

05 Picking up the item on the sheet, it should then be placed on the corresponding cell in order to identify its **dominant colour** (Green-v-, White-b-, Red-r-, Blue-a- ...): this allows for later classification, if needed, if they this type or another (possible origin), thus obtaining a quantitative evaluation by typology.

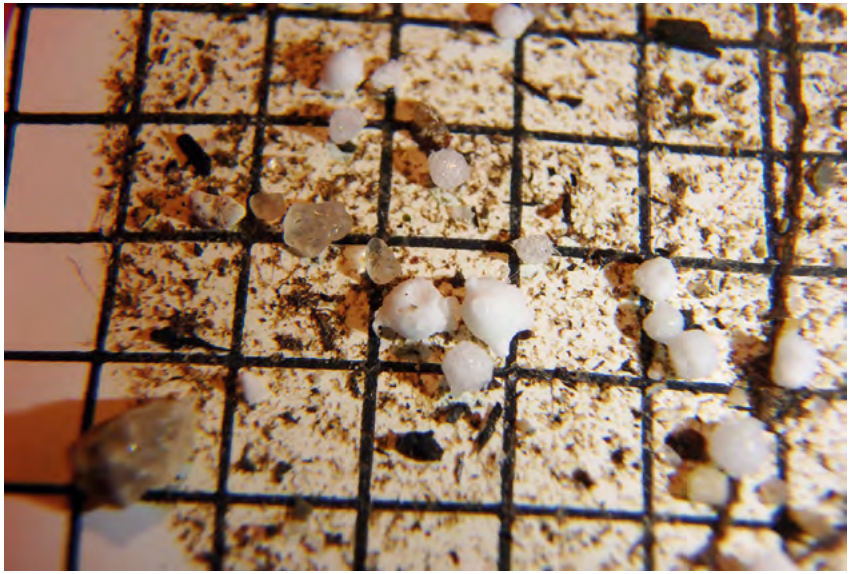
06 If the element is undoubtedly a **plastic derivative** and is of a sufficient size for infrared analysis (150-200 microns in diameter), this should be indicated in the last cell on the sheet, along with its location. For this we use the sexagesimal system (degrees), which allows us, having marked north as our reference point (origin marked with marker), to locate any element by taking into account its orientation and distance from the center, via theoretical bearings leading outwards from the center. The fragment can also be located approximately on the diagram in the final cell.



» Filter, sexagesimal system marked on sheet, showing location of white fragment (Fb) selected for IR (in the example, the selected fragment would be located approximately at the end of the 30 ° bearing, facilitating the identification of its location by external technicians by placing it on the record Analysis-1 sheets).

07 If the element is too small for analysis, it should be registered on the sheet but not indicated as a possible element for identification. In this case, **several elimination tests** can be carried out to verify its nature and reduce uncertainty regarding its origin (see identification aid and elimination guide, included in annexes). In the event that a significant quantity is detected of any element that requires one or more of these tests, the result being a high probability, it should therefore be identified according to protocol and indicated on the sheet for infrared analysis.

08 After finishing the count, **place the filter in its dish** and, depending on the final decision, it can now be sent for Analysis-2 in the laboratory or be stored in a cool, dryplace.



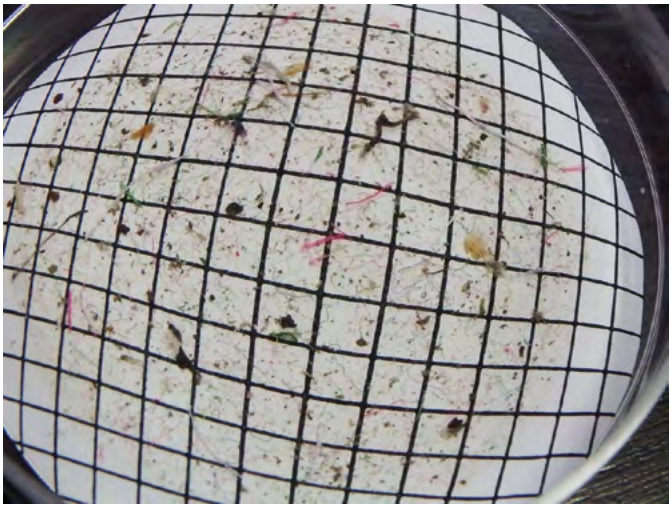
5.6. ANALYSIS-2: identification of polymer composition

Fourier-transform infrared transmission spectroscopy (FTIR) is used in the characterisation of different compounds to identify functional groups present in samples. This technique is sensitive to the structure of the element as it reflects the vibrations of the groups of atoms, hence their use in the identification of organic groups in particular.

The technique selected in this manual for the identification of the nature of the polymer is Fourier-transform infrared transmission spectroscopy (FTIR) using attenuated total reflectance (ATR). With this technique, the characteristic spectrum of each element is quickly and reliably obtained. This spectrum, when used in conjunction with a library of materials incorportated in the equipment, indicates the nature of the element with a percentage probability. **It is the fastest and most reliable technique currently used in the identification of polymers worldwide**.

This technique works directly on the filter, having identified the element(s) selected as the objective (see Analysis-1: separation and identification under microscope), although the fragment(s) can also be isolated and sent for analysis. The element in question is identified and its transmission spectrum is obtained via a procedure in the laboratory in which infrared light is shone through the material.

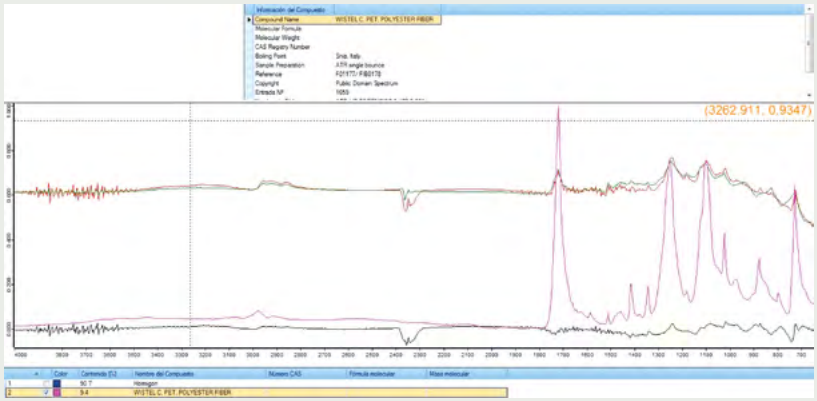
Fourier transform infrared transmission spectroscopy (FTIR) using attenuated total reflectance (ATR) makes it possible to quickly and reliably obtain the characteristic spectrum of each element.



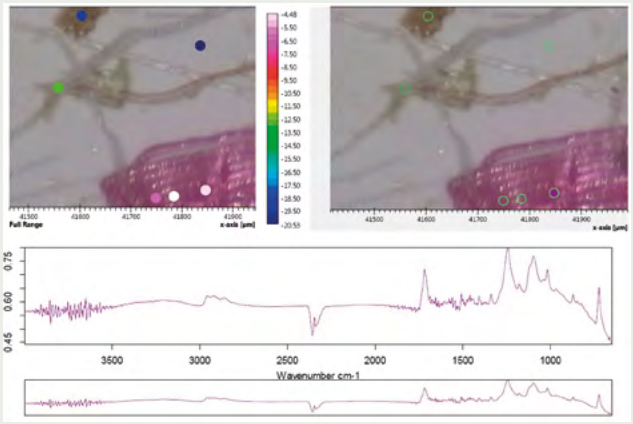
» The sample is identified (in this case, pink fiber in the centre of the filter), marked on the laboratory sheet and sent for IR analysis.

Other techniques exist such as detection under fluorescent light (blue light 450-510 nm2) after treatment with dye (Nile red), gas chromatography coupled with mass spectrometry or the use of an electron microscope, allowing analysis of the material's microstructure in addition to the detection of its chemical composition.

In Spain there are a growing number of public and private laboratories that offer these services. For the development of this manual, tests and trials have been carried out using the microanalysis and microscopy services of CITIUS (General Research Services) at the University of Seville.



» The potential sample (in this case pink fiber) is located and its reflectance spectrum is obtained. Image: CITIUS.



» The spectrum is compared with the library to obtain, in this case, the nature of the material: polyester. Image: CITIUS.

5.6.1. SEQUENCE FOR STORAGE AND SENDING

It is important to create a protocol for the storage and traceability of the samples when they are sent from one place to another: in this case, it could be from the field to the office, from the field to the laboratory, from the office to the laboratory or from the laboratory to another external laboratory.

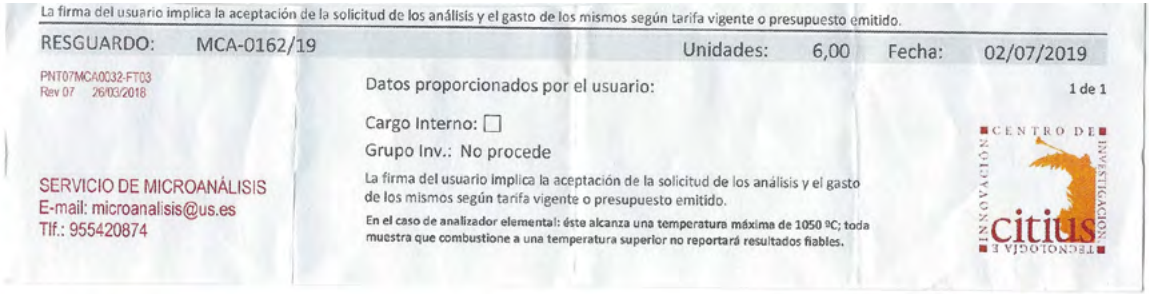
We must bear in mind the quantity and type of samples sent, treatments carried out, when they are sent, who sends them and who receives them, the analysis that we are going to request, deadlines for obtaining results and by what means. Through **record sheets** (such as those included in the annexes) we can track the "steps" through which each of the samples passes (collection in the field, sending to laboratory, filtering, identification, etc.).

In our case, and since we have the equipment for filtering and analysis using a stereo microscope, the example taken from shipments based on results obtained in laboratory Analysis-1. Batches of **well-labeled** filter samples should be sent together with laboratory sheet_1, on which the elements required for each analysis are indicated.

It is important to thoroughly assess which elements to send for analysis, given that the success of the analysis depends on our choice (budget and objectives permitting).

The labeling of the samples is therefore very important, as the ease of understanding, identification and analysis by different technicians or laboratories will depend on their correct selection and implementation. In this case, we have used three numbers or uppercase letters (depending on whether the sample is associated with an area already inventoried using a code or with a specific area using a name) followed by an underscore and 1 or 2 numbers depending on its location/replica/zone.

Identification examples
123_1 (*sample study of inventoried locations*)
ABC_2 (*sample study area/s with personal nomenclature or toponym*)


La firma del usuario implica la aceptación de la solicitud de los análisis y el gasto de los mismos según tarifa vigente o presupuesto emitido.

RESGUARDO: MCA-0162/19 Unidades: 6,00 Fecha: 02/07/2019

PNT07MCA0032-FT03
Rev 07 26/03/2018

Datos proporcionados por el usuario:

1 de 1

Cargo Interno: ☐

Grupo Inv.: No procede

La firma del usuario implica la aceptación de la solicitud de los análisis y el gasto de los mismos según tarifa vigente o presupuesto emitido.

En el caso de analizador elemental: éste alcanza una temperatura máxima de 1050 ºC; toda muestra que combustione a una temperatura superior no reportará resultados fiables.

SERVICIO DE MICROANÁLISIS
E-mail: microanalisis@us.es
Tlf.: 955420874

CENTRO DE INVESTIGACIÓN TECNOLOGÍA E INNOVACIÓN citius

» Proof of receipt of samples by the external entity in charge of carrying out infrared analysis.

5.7. Data loading and analysis

As with any group of data, we will work in the simplest and most effective way to achieve the objectives set for the study. We can do multiple analyses, both descriptive and hypothetical, taking into account that we are not working with living organisms (so in principle we will not be able to use community analysis).

Depending on whether the data is in units per litre, total units, type of elements, size or even volume, we can infer a series of results.

The most important thing is to design the table to which we are going to add the information: this design will determine the ease with which we can later extract the information we need, select only what we want or transfer it to different formats or software to perform different analyses.

One option is to make **two complementary tables**, each one with the samples in the same order but on different axes; thus in one of the tables we will have the samples in different rows while in the columns we can include all the information that we consider relevant (both numbers and text) to each one:

Sample	Name	Habitat	Date	Province	Strecth	Coordinates	Other environmental variables		
AAA									
BBB									
CCC									

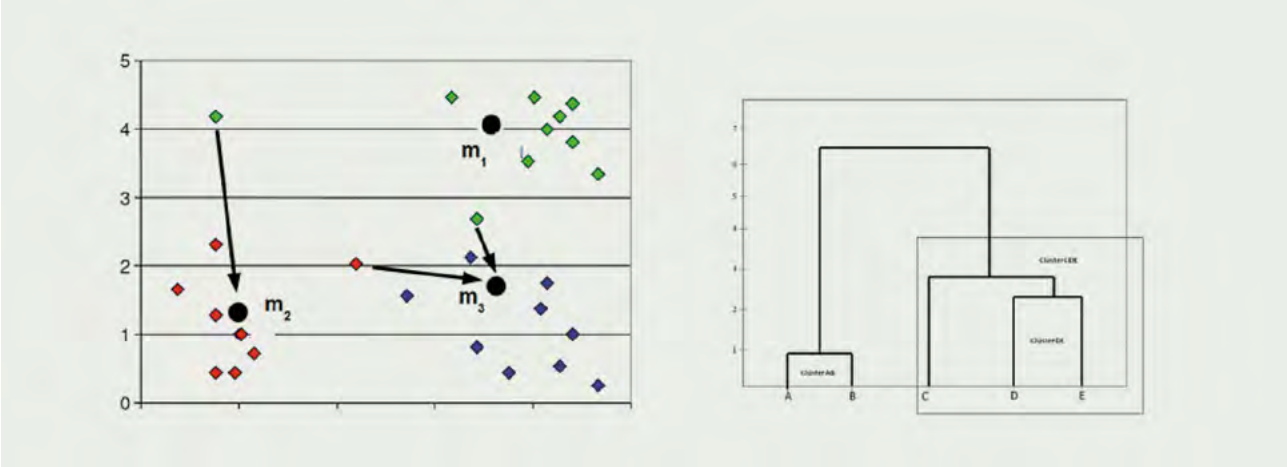
Sample	Width	Depth	Current	Filtered volume	Flow	Transparency	Other environmental variables		
AAA									
BBB									
CCC									

In **another table**, **following the order of the samples** (but this time transposed to the columns) we can review the data from the analyses, for example by categories:

	AAA	BBB	CCC	DDD	EEE	FFF	GGG	HHH	III
FIBERS									
FRAGMENTS									
SPHERES									
FILMS									
SPONGES									
OTHERS									

We therefore have two tables with the same basic structure (samples) but with different information, a one corresponding to numerical environmental variables or not and another that is purely quantitative. Some statistical analyses such as similarity require the grouping of samples into categories, many of the columns created in the first table can serve as categorisation elements to separate samples from different Autonomous Communities.

If we want to directly obtain the total or partial data of elements per sample, we can go directly to table 2 and calculate by type, volume filtered, etc.



» Examples of how an analysis of the distribution of samples by section could look in different formats.

06. Programmes and Networking. Bibliography and projects / experts consulted

For the purpose of this project, 16 national and international projects related to microplastics in rivers have been contacted, obtaining in the process a series of reports, protocols and answers to queries relating to the different phases of the methodology. The information sources consulted for the elaboration of this document can also be found below.

Projects, centres or studies consulted/contacted:

- Aalborg University, Environmental Engineering Dpt.: *Dr. Jes Vollertsen*.
- Microplastics Initiatives of Adventure Scientists: *Dra. Abigail Barrows*.
- Global Microplastics Initiative
- Florida Microplastic Awareness Project
- Marine and Environmental Research Institute
- St. Lawrence University, Biology Department: *Dra. Samantha Haab*.
- Coalition Clean Baltic: *Dr. Mikhail Durkin*.
- NOAA Marine Debris Program,
- University of Cádiz, Biology Department, Ecology Area. *Dr. Andrés Cozar Cabañas*,
- Fredonia University: *Dr. "Sam" Sherri A. Mason*,
- University of Applied Sciences: *Dr. Sven Huppertsberg*.
- Center for Studies and Experimentation of OP of the Ministry of Development (CEDEX).
- Zero Waste Association.
- Clean Landscape Association.
- University of Seville, Department of Plant Biology and Ecology: *Laura Serrano Martín*.
- Marine and Environmental Sciences Center (MARE). Faculty of Science and Technology, University of Coimbra, Portugal. *Filipa Bessa*.

During the course of the Project, we have **participated or presented results** in:

- Center for Studies and Experimentation of Hydraulic Works (CEDEX, Madrid), May 2019
- Workshop on Riverine Litter (OSPAR COMISSION, Paris), June 2019
- Seminar "Marine litter problem", Seville, October 2019.

Reports, articles and/or manuals consulted*:

» A Methodology for Measuring Microplastic Transport in Large or Medium Rivers, 2018. Marcel Liedermann *et al. Water* 2018, 10, 414.

» A watershed-scale, citizen science approach to quantifying microplastic concentration in a mixed land-use river, 2018. Abigail P.W.Barrows *et al. Water Research*.

» Advanced Method for the Treatment of Organic Aqueous Wastes: Wet Peroxide Oxidation, 1997. Hubert Debellefontaine *et al. Environmental Technologies and Trends*.

» Advanced Oxidation Processes for Waste Water Treatment, Chapter 12- Catalytic Wet Peroxide Oxidation. *Emerging Green Chemical Technology* 2018.

» Analysis of Microplastics using FTIR Imaging. Agilent report.

» Automatic Counting and Classification of Microplastic Particles, 2018. Javier Lorenzo-Navarro *et al.* (ICPRAM 2018).

» Determinación de la presencia de microplásticos en playas de Tenerife. Trabajo de Fin de Grado Daniel Cabrera Dorta (curso 2017-2018). Universidad de La Laguna.

» Florida microplastric awareness project volunteer manual, 2017.

» Grab vs. neuston tow net: a microplastic sampling performance comparison and possible advances in the field, 2017. Abigail *et al. Analytical methods*, 9.

» Guide to microplastic identification, 2017. MERI

» Identification and Assessment of Riverine Input of (Marine) Litter (Report for the EC DG Environment).

» Marine Anthropogenic Litter, Ch. 8 Methodology for the Detection and Identification of Microplastic.

» Microplastic scholar acdtivities, Sea Grant Oregon.

» Microplastics in aquatic environments: implication for Canadian ecosystems, 2016. Julie C. Anderson *et al. Environmental Pollution*.

» Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities, 2017. Alice A.Hortona *et al. Science of The Total Environment*, Volume 586, 15.

- Microplastics in freshwater ecosystems: what we know and what we need to know, 2014. Martin Wagner *et al. Environmental Sciences Europe* 2014, 26:12.
- Microplastics in freswater ecosystems: A review on occurence, environmental effects and mehods for mp detectection, 2018. JingYi Li *et al. Water Research* 137.
- Microplastics in irish freshwaters: a preliminary study, 2015. Cedro A. and Cleary J. International Conference on Environmental Science and Technology.
- Microplastics in the Freshwater Environment. Nanna Brande-Lavridsen, U. of Tennessee.
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Some potential projects for networking:

- Global Microplastic Initiative
- Global Microplastic Project
- Florida Microplastics Awareness Project
- Plastic aware project. Universidad de Florida
- 100 plastic rivers, Universidad de Birmingham
- Beaches, Rivers, volunteering and Territory Custody Program
- Andarríos Program (Andalusia)
- Volunteer Program in rivers (Murcia)
- Volunteer programs (State by CCAA)

07. Annexes

Annex 1:

Field sheet model

FIELD SHEET AND SAMPLING AREA DESCRIPTION

Sampling date: __ /__ /____ Start time: ___:___

GENERAL DATA

Province: _____ Municipality: _____

IBA: NO/YES (code _____) Protected area: NO/YES (_____)

River: _____ Section*: _____ Code: _____

Section width (m): _____ Section proof* (m): _____

Running water: YES/NO Speed* (m/s): _____ Flow* (m^3/s): _____

Water colour: _____ Water odour: _____ Transparency*: _____

Coordinates (*or send location to a phone number via whatssapp or another application*): _____

Photos:

Meteorology: ☐ Sunny ☐ Cloudy ☐ Rainy ☐ Other

Wind: ☐ Calm ☐ Breeze ☐ Medium ☐ Strong

Temperature: Ambient* (ºC): _____ Water* (ºC): _____

*Section: mid, upper or lower;

*Depth: Estimate the depth of the section.

*Speed: Calculate the time it takes for a floating object (leaf, branch) to travel a certain distance.

*Flow: Approximate calculation according to average width (m) x average depth (m) x water speed (m/sg.).

*Transparency. Visually estimate how many cm you can see down into the water (as far as the light reaches).

*Room temperature: Place a thermometer (always in the shade) in an area close to the sampling point.

*Water temperature: Place the thermometer into the river water or collect a sample with the bucket and measure the temperature, always in the shade.

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DESCRIPTION OF ENVIRONMENT

SECTION

Estate: ☐ Permanen ☐ Temporal ☐ Ephemeral

Surround: ☐ Natural ☐ Agricultural ☐ Urban ☐ Industrial

☐ Other (specify) _____

Aspect: ☐ Artificial ☐ Natural ☐ Naturalised

Open:
☐ without vegetation
☐ with sparse vegetation
☐ with dense vegetation (reeds), only on the riverbank

Dense:
☐ with tree-like vegetation but artificially planted
☐ with natural-looking riverside vegetation

VISIBLE IMPACTS

Waste flow (specify): _____

Littering (specify): _____

Waste (specify): _____

Works or infrastructure (specify): _____

Invasive or alien species (specify): _____

Photos: _____

SAMPLING CHECK LIST

Water samples: Time-type effort: _____ Effort type volume: _____

Littering sheet: _____

Annex 2:

E-Litter sampling sheet

eLitter

Littering FORM FOR LAND-BASED SAMPLING

TYPE OF ENVIRONMENT (river, path, watercourse, wood…): _____

NAME OF ZONE: _____

START COORDINATES: _____ FINAL COORDINATES: _____

Autonomous community:	**Province:**	**Municipality:**

AREA	Extension of area to be cleaned (length x width in metres):	
	Date of cleaning:	
	Date of last cleaning:	
WEATHER	Direction and strength of wind:	
	Rain, mist/fog, ice, snow or sand storms:	

Specify any circumstance that may have resulted in a recent increase in rubbish (cultural events, storms, drain overflow etc.):

FAUNA	Dead animals:	☐ YES ☐ NO	Nº:	
	Species:			
	Tangled in waste:	☐ YES ☐ NO	Type of waste:	

Part	PLÁSTIC	Total	Units
	Bags (shopping, food, freezer)		
	Drink bottles		
	Tops or lids		
	Bags, packaging, sticks… for sweets		
	Straws, cutlery, cups, mugs		
	Food packaging		
	Cosmetic packaging		
	Cords, laces		
	Packaging tape (straps, ties…)		
	Industrial packaging		
	Motor oil, glue, silicone containers (with applicator)		
	Cleaning product packaging		
	Ring packaging for cans		
	Agricultural product containers (fertilisers, pesticides…)		
	Large cans/drums (> 25 litres)		
	Pipes		
	Pieces of plastic 0-2.5cm		
	Pieces of plastic 2.5-50cm		
	Pieces of plastic > 50cm		
	Other unidentifiable plastic objects (specify in observations)		
Part	PAPER/CARDBOARD	Total	Units
	Paper napkins, tablecloths		
	Cartons (milk, juice…)		
	Cardboard boxes, or pieces of		
	Cigarette packets		
	Paper bags		
	Newspapers and magazines		
	Pieces of paper or cardboard		
	Other paper/cardboard (specify in observations)		

Part	WOOD (worked)	Total	Units
	Corks		
	Ice-cream sticks, cutlery…		
	Pallets		
	Boxes		
	Other pieces of wood < 50cm		
	Other pieces of wood > 50cm		
Part	**METAL**	**Total**	**Units**
	Drinks cans		
	Tops and lids, caps, ring-pulls		
	Tin foil		
	Food packaging, tins, trays…		
	Spray cans		
	Oil drums		
	Paint cans		
	Other pieces of metal < 50cm		
	Other pieces of metal > 50cm		
Part	**GLASS**	**Total**	**Units**
	Glass bottles and jars		
	Pieces of glass		
Part	**ELECTRICAL DEVICES AND BATTERIES**	**Total**	**Units**
	Batteries		
	Cables		
	Lightbulbs, fluorescent lights		
	Electrical devices (computers, fridges, telephones…)		
Part	**SANITARY WASTE**	**Total**	**Units**
	Condoms		
	Cotton buds		
	Sanitary towels, panty liners		
	Wet wipes		
	Tampons (with applicator)		

Part	MEDICAL WASTE	Total	Units
	Packaging and tubes for medication		
	Syringes		
	Other (cotton, bandages…)		
Part	**OTHER**	**Total**	**Units**
	Cigarette butts		
	Chewing gum		
	Remains of food		
	Rubber (balloons, balls, tape, valves…)		
	Tyres		
	Clothes and shoes (leather)		
	Other textiles		
	Construction materials		
	Other ceramic pieces		
	Domestic animal faeces		
	Other (specify in observations)		
	Observations:		

Key for waste categories:

- Packaging
- Paper/cardboard
- Glass
- Other
- Recycling point
- Organic
- Pharmaceutical
- Local Entity

WEIGHT OF WASTE CATEGORIES	Kg.
Yellow bin	
Blue bin	
Green bin	

WEIGHT OF WASTE CATEGORIES	Kg.
'Other' bin	
Organic matter bin	
Other parts (specify):	

Annex 3:

Model sampling inventory sheet

MODEL SAMPLING INVENTORY SHEET

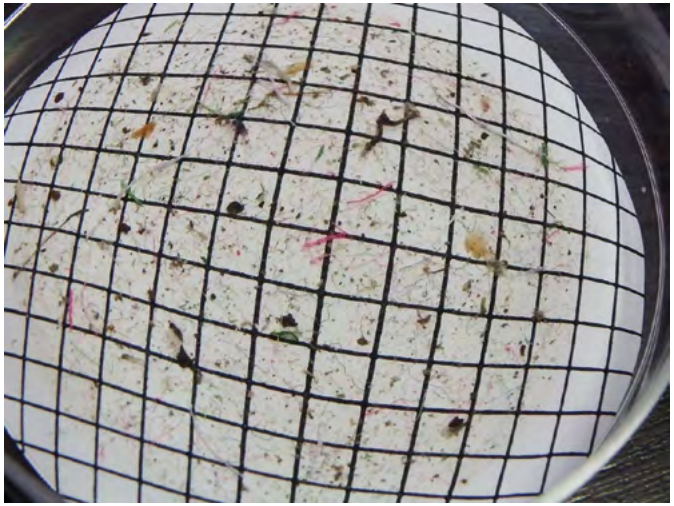
Sample	IBA	Date	Sample effort (l/t)	Field sheet	E-Litter	Comments

Annex 4:

Identification aid and elimination guide

IDENTIFICATION AID AND ELIMINATION GUIDE

The aim of this annex is to help identify with the greatest possible certainty the polymer of any fragment that we find during analysis in section "Analysis_1: separation and identification under microscope" of the manual. It is important to bear in mind that we will always rely on direct and indirect tests but without an analysis of molecular composition that certifies the nature of the element (for this we would have to send the sample to a microanalysis service). If we do not have the appropriate equipment, we therefore proceed to the step "Analysis_2: identification of the nature of the polymer".



In general, when locating an element, we should first observe it carefully under a stereo microscope. From this first visual analysis we can obtain a great deal of information, given that there are often certain elements that, although they seem plastic at first glance, are not.

The first step in the analysis is to rule out the possibility that the element may be rock, animal or plant remains. To ensure accurate identification/elimination, the person who performs this task should be well trained and / or use an elimination guide for reference, such as that presented below. After the initial process of elimination, we then proceed to the pre-identification process in order to associate the material with one of the 6 general types that appear on the sheet (fiber, fragment, sphere, film, sponge, others).

First of all, when locating an element, we will observe it under a magnifying glass. If we pass the discard, we will pre-identify it and associate it with one of the 6 general types.

Various different methods exist to assess the nature of the material, one or more of the following may be employed:

- Buoyancy: as we have indicated in the methodology, the potential elements that we may find in our filter were "floating" or in semi-suspension in the water column. If we place the fragment or element in question in a volume of water and it floats, there is a possibility that it might be a polymer.

» Tras localizar el elemento pasamos a realizar su valoración.

- Rehydration: given that the treatments performed on the sample include oxidation and dehydration by filtering, the appearance of a plastic element will not change when it is put back into water, while other elements will hydrate (swell) or change shape or volume.
- Symmetry: while some natural elements are symmetrical or display patterns that repeat, plastic elements are usually irregular.
- Flexibility: unlike many natural elements (e.g. rocks, minerals), plastic is more or less flexible when manipulated (in this case, using tweezers)
- Elasticity: many elements that may appear in the filter break easily when manipulated or bent whereas plastic has displays a characteristic elasticity that makes it so popular.
- Reaction to oxidant: even if treatment has already been carried out, the elements trapped in the filter can continue to react to an oxidizing environment: a plastic fragment will not react (creating bubbles or dissolving) to this treatment.
- Temperature reaction: unlike many inorganic and organic elements, plastics tend to deform when heat is applied: a lighter and entomological point punch can be used to heat and check the reaction of any element. Caution must be taken with the filter and the temperature induced at the tip of the punch.
- Colour and appearance: although natural materials come in an array of colours and shapes, certain elements display characteristics that make them practically unmistakable: experience considerably improves the accuracy of this evaluation.
- Identification under ultraviolet light after staining with Nile red dye: a somewhat more expensive and laborious technique but produces results of great use for the elimination process.
- Observation under an optical microscope: with help of a microscope, we can appreciate in the contour, appearance, texture and opacity of the samples in greater detail. It is highly recommended that we have one readily accessible in our workspace.

Some elements that we may find in the filters that **could create confusion** include:

- Rocks/minerals. Some elements can be carried by the current or resuspended at the time of sampling. Although they will normally be very hard and display a clearly negative buoyancy, they have the potential to be confused with elements such as FRAGMENT, RUBBER or FILM.

- Fish or reptile scales: reptiles and boned fish (also birds' legs and some mammals) have scales constituting predominantly rigid plates that grow from the skin of these animals and serve, among other purposes, as insulation and protection. In our case, we are likely to find mainly those of fish (or reptiles), which can display a variety of shapes. They can be confused with FILM-like elements but will normally have a series of parallel bands that correspond to the growth lines of the animal. Also, they will not display an immediate reaction to heat.

- Filamentous algae: present in many riverine habitats, these algae may be confused with elements such as FIBER or FILM. In addition to their characteristic green colour, we will observe that they are usually of equal width in their entire extension, they will have marked divisions corresponding to the cell walls and they will react in a particular way to several of the tests or methods previously explained (see table below). Very large algae with a globular appearance may also appear in the filter: they will display a highly dehydrated and deformed appearance, helping us to differentiate them from a FILM-type element.

- Remains of crustaceans or molluscs: these invertebrates are characterised by having predominantly hard parts made up of materials such as chitin or keratin, which may sometimes look very similar to a FILM or FRAGMENT type element. Remains of the exoskeleton or valve will have symmetrical or recognisable patterns on some sides, small hairs (bristles) or marks that can serve to indicate its origin. Likewise, small invertebrates such as copepods, ostracods, rotifers, or the juvenile stages of crustaceans (nauplii) will also present an appearance with characteristic features for their identification.

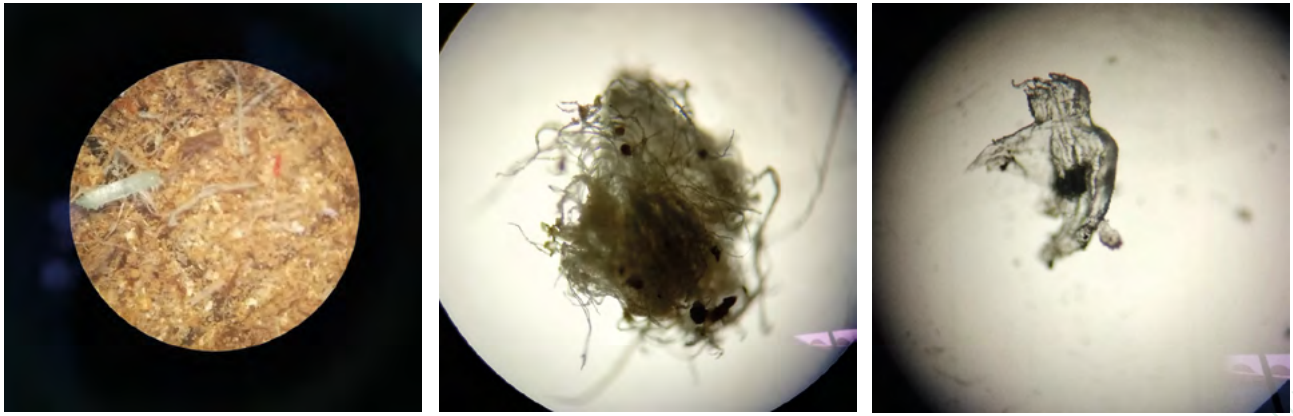
- Cotton: this element, both in its natural state and dyed, tends to be very common in streams and rivers due to their proximity to cultivation in addition to arrival through sewers, runoff, etc. Here there is a significant risk of confusion with FIBER type elements, the element's colour and reaction to different identification methods can help us decide whether the material is plastic in nature or not.

- Fragment of plant: a very common occurrence in samples, these elements usually display a highly characteristic appearance and colour (due to their fibrous vegetal nature), in addition to being brittle or especially flexible depending on their origin.

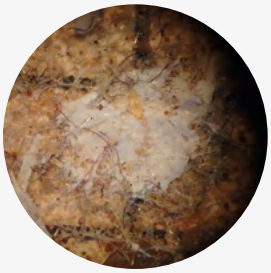
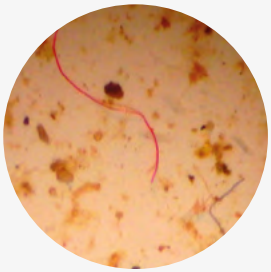
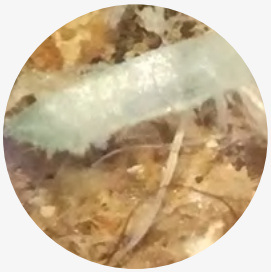
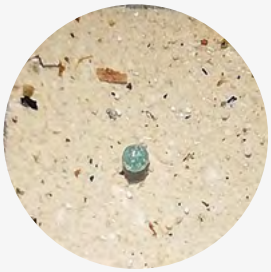
- Others: surely you will find other elements such as very shiny metals (flakes or fine fragments), foams or aggregates (in an area with high salinity) or of another type or nature (seeds or remains of seeds, viscous materials) that can confuse you: The resolution of these conflicts will depend on the various disposal methods, the skill and experience of the identification team and the composition analyzes.

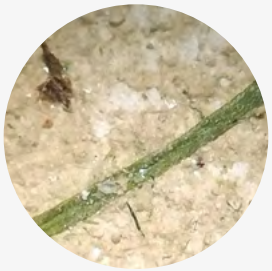
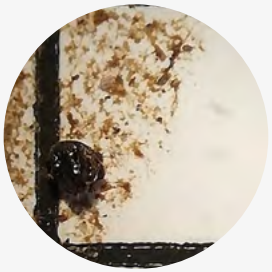
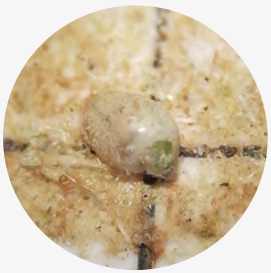
↓ **Table 2.** *Summary of indirect analyzes in relation to the nature of the element.*

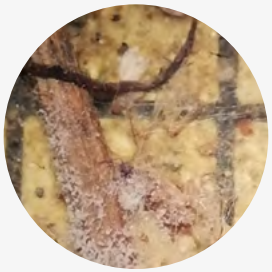
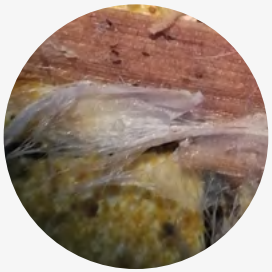
	ROCKS/ MINERALS	SCALES	ALGAE	CRUSTA-CEANS/ MOLUSCS	COTTON	PLANT	PLASTIC*	OTHERS
☑ **Buoyancy**	-	0	0, +	0,+	+	+	+	Variable and dependent on the composition of each element
☑ **Rehydratation**	0	0	+	0, +	+	0, +	-	
☑ **Symmetry**	0,+	-	+	0,+	-	0,+	-	
☑ **Flexibilility**	-	0,+	0,+	0,+	+	0,+	+	
☑ **Elasticity**	-	-	0,-	0,+	-	0,+	+	
☑ **Reaction to oxidant**	-	-	0,+	0,+	0,-	0,+	-	
☑ **Reaction to temperature**	-	-	-	-	-	-	+	
☑ **UV light**	-	-	-	-	-	-	+	
☑ **Colour and aspect**	Variable and dependent on the composition of each element							
☑ **Optic microscope**	Variable and dependent on the composition of each element							

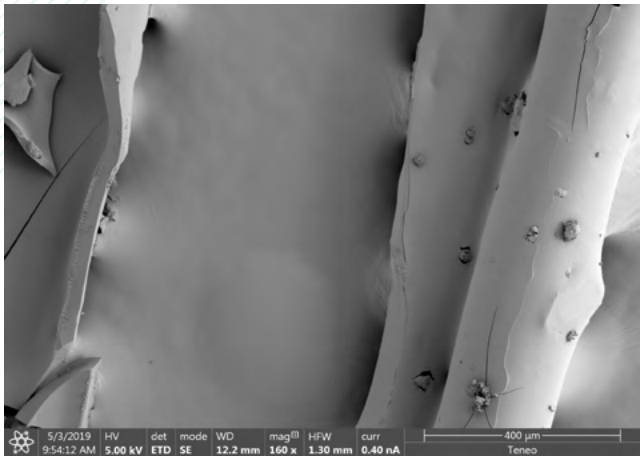


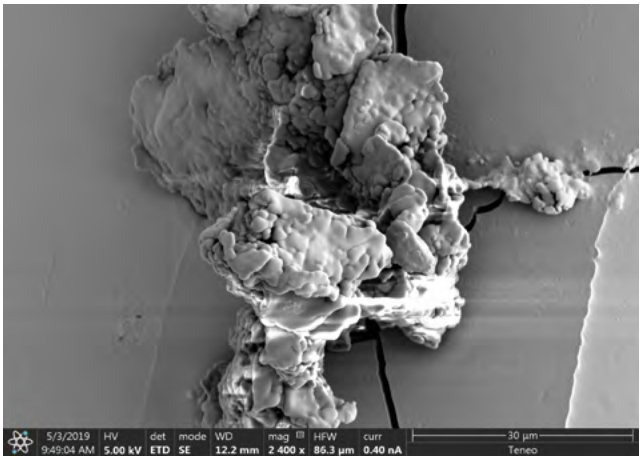
» Positive result of an element under microscope (1), the element is exposed to a heat source and observed under an optical microscope (2 and 3). The element is now approved and its location recorded for infrared analysis (Photos: HyT).

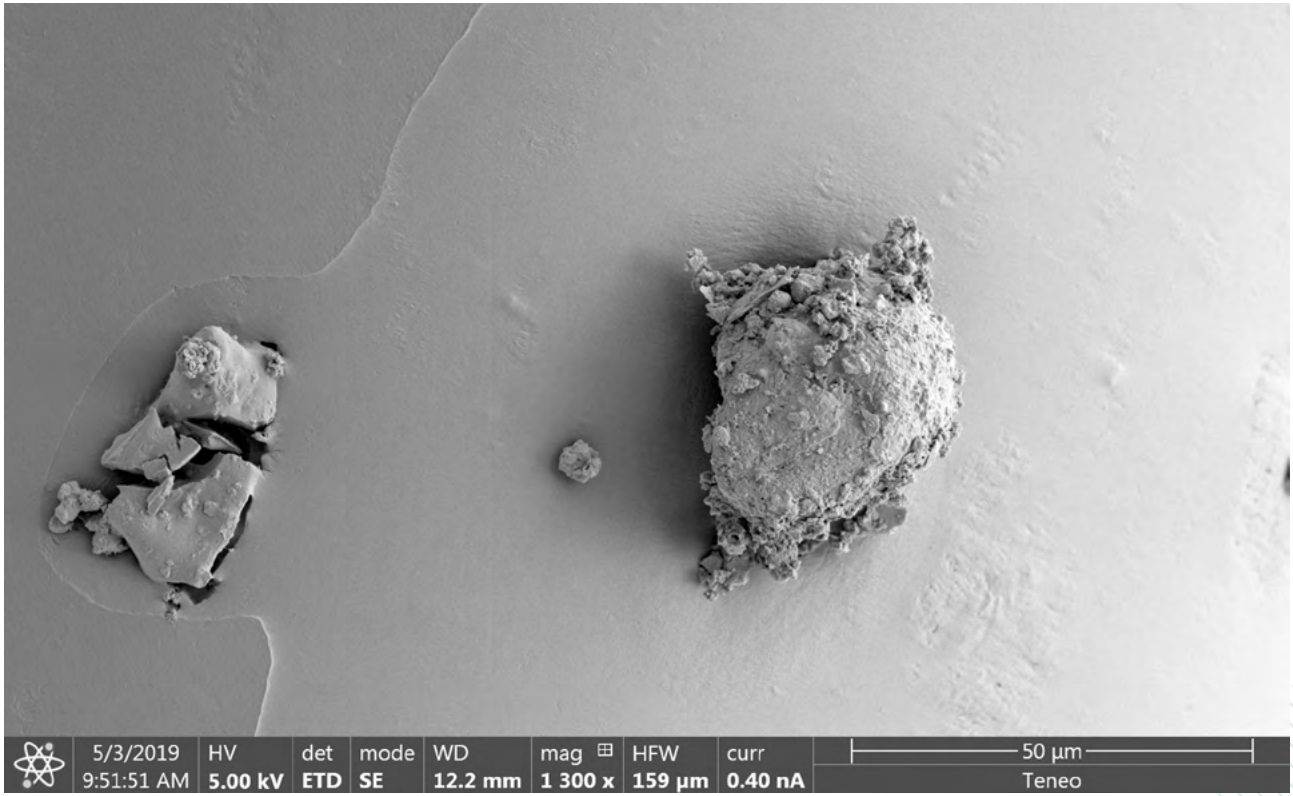
FIBER	FRAGMENT	FILM	SPHERE
			
			
			
			

SPONGE	ALGAE	INVERTEBRATES	AGGREGATE/SCALES
			
			
			
			

ROCKS	VEGETAL FIBERS	OTHERS
		
		
		





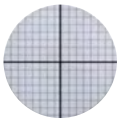
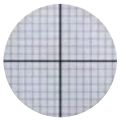
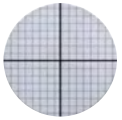
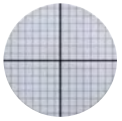
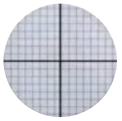
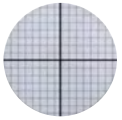


» Images of fibers seen through an electron microscope.

Annex 5:

Lab Record Sheet

PRE-ANALYSIS INVENTORY FOR MICROPLASTICS

Sample	IBA	Field	Filtration	Pre-ID	Fibers (FB)	Fragments (FR)	Spheres (EF)	Films (FL)	Sponges (EP)	Rubber (GO)	Comments and selection
											
											
											
											
											
											

A METHODOLOGY FOR SAMPLING,
ANALYSIS AND IDENTIFICATION OF
MICROPLASTICS IN RIVERS

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