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REVIEW / REVISIÓN

MEASURES TO PREVENT CROSS-CONTAMINATION IN THE ANALYSIS OF MICROPLASTICS: A SHORT LITERATURE REVIEW

Medidas para prevenir la contaminación cruzada en el análisis de microplásticos: una breve revisión de la literatura

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Key words: quality assurance, blanks, reliability, background contamination, airborne fibers.

ABSTRACT

Marine environments are the most studied habitats when addressing microplastic pollution. However, there are no standardized methodologies for this analysis, so methodologies are often adapted by researchers. This situation has raised doubts concerning the reliability and reproducibility of results that are related to the null or little use of measures to avoid cross-contamination. The objective of this work was to carry out a short review and analyze the different measures that have been reported in research articles for different marine habitats, published in the ScienceDirect database in 2020, to avoid cross-contamination during fieldwork and laboratory work. From the 115 analyzed articles, eight did not report measures at any stage, 61 took measures during sampling, and 98 did it in the processing stage. Even though most studies take steps to prevent cross-contamination, they do not specify the percentage of contamination avoided. However, from the concentrations of microplastics in the blanks and the total microplastic concentrations in the samples, we estimate that between 4.8 and 69 % of contamination is avoided in sampling and between 0.1 and 48.8 % in the laboratory. This shows the need to establish standards for sampling and sample processing, which must include measures regarding the marine environment studied and the stage addressed, as well as the minimum percentages that should be met for the data to be considered valid and reliable.

Palabras clave: control de calidad, blancos, confiabilidad, contaminación de fondo, fibras en el aire.

RESUMEN

Los ambientes marinos destacan entre los hábitats estudiados para la contaminación por microplásticos. Sin embargo, no existen metodologías estandarizadas para su análisis

por lo que a menudo se adaptan métodos propuestos por los investigadores. Esto ha generado dudas sobre la confiabilidad y reproducibilidad de los resultados, relacionadas con la nula o limitada utilización de medidas para evitar contaminación cruzada. El objetivo de este trabajo fue realizar una revisión corta y analizar las diferentes medidas que se han reportado en artículos de investigación de diferentes hábitats marinos, publicados en ScienceDirect en 2020, para evitar la contaminación cruzada durante los trabajos de campo y laboratorio. De un total de 115 trabajos, ocho no reportaron medidas, 61 tomaron medidas en los muestreos y 98 lo hicieron en el procesamiento de las muestras. Aun cuando la mayoría de los estudios adoptan medidas para evitar la contaminación cruzada, éstos no especifican el porcentaje de contaminación evitada. Sin embargo, a partir de las concentraciones de microplásticos en los blancos y las concentraciones totales de microplásticos en las muestras, estimamos que para el muestro se evita entre 4.8 y 69 % de contaminación y en el laboratorio de 0.1 a 48.8 %. Lo anterior muestra que es necesario establecer estándares para el muestreo y procesamiento de muestras, los cuales deben incluir las medidas que deben tomarse por ambiente estudiado y etapa abordada, así como los porcentajes mínimos que deben cumplirse para que los datos se consideren válidos y confiables.

INTRODUCTION

Plastic particles ≤ 5 mm, in any of their dimensions, are called microplastics (MP) (UNEP 2014) and can be classified as primary or secondary. Primary MP are made in those sizes for industrial purposes; e.g., pellets are the raw material for larger plastics (GESAMP 2016), and microbeads are ingredients of a wide range of personal care products (Sun et al. 2020). Secondary MP are originated when larger plastics are fragmented, either by biotic or abiotic processes (GESAMP 2016). Sources of MP include plastics, agriculture, construction, textile, and tourism industries; fishing and aquaculture; land and sea transportation; personal care products; solid waste management, and wastewater treatment plants, among others (GESAMP 2016). MP originating from these sources is transported by rain, wind, and runoff to several parts of the environment. Today, MP are ubiquitous in the atmosphere (dust, air, and snow; Zhang et al. [2020a]), terrestrial environments (He and Luo 2020), freshwater bodies (Hengstmann et al. 2021, Lu et al. 2021), food products (Mortensen et al. 2021), and marine environments (Alimba and Faggio 2019).

Of all the environments where the presence of MP has been evaluated, marine and coastal habitats have been the most studied. For example, MP investigations have been conducted on beaches, seawater, seabed, estuaries (Harris 2020), mangroves (Deng et al. 2021), marine fauna (Wang et al. 2021, Ferrante et al. 2022), and coral reefs (Huang et al. 2021), to name a few. So, even though research on the presence of MP in the environment has increased exponentially in recent years, there are no standardized methodologies for MP sampling in the field and for the analysis of samples in the laboratory (Tirkey and Upadhyay 2021). It is common for researchers to rely on methods that have been proposed by different working groups (Masura et al. 2015, Viršek et al. 2016, Besley et al. 2017, Álvarez-Zeferino et al. 2020, Lin et al. 2021), which often adapt based on the needs of their approaches (Bridson et al. 2020, Chen and Chen 2020, Godoy et al. 2020, Maynard et al. 2021).

The lack of standardized methodologies for the sampling and analysis of MP implies that, in most cases, results are not comparable due to variations in the number of replicates, the size of the studied MP (Wang and Wang 2018), the concentration units reported and the sampling depth (Stock et al. 2019). In addition, the data reliability problem is related either to underestimating or overestimating MP concentrations. The underestimation can occur by adherence of the MP to the used materials and equipment, the destruction of the MP during the sample digestion (Wang and Wang, 2018), or the loss of samples, while the overestimation occurs when there is cross-contamination during sample processing (Tirkey and Upadhyay 2021) and when false positives are considered as MP, such as shells or natural fibers (Álvarez-Zeferino et al. 2020). Cross-contamination is a critical issue as MP are found everywhere, including indoor air (Yao et al. 2022), therefore samples to be studied can easily be contaminated if steps are not taken to reduce the error. Cross-contamination alters the levels of MP concentration in the studied environment, hence the reliability of data (Prata et al. 2021).

It is essential to consider each stage involved in the MP study because microfibers are the most common type of particles in cross-contamination. They are present in the ambient air of the sampling site or workplace and can adhere to equipment and materials used altering the results, especially when analyzing microscopic MP (Torre et al. 2016). Currently, there are no guidelines that indicate which steps should be followed to avoid cross-contamination, although some researchers, based on their experience, have established specific measures (Kazour and Amara 2020, Patterson et al. 2020, Robin et al. 2020, and He et al. 2021) mostly focused on the analysis of samples in the laboratory. Therefore, this work aimed to carry out a short review and analyze the different measures used to avoid cross-contamination during field and laboratory work, in order to identify good practices, deficiencies, or knowledge gaps, as well as to propose some general recommendations. It includes different research articles published in 2020 that report the presence of microplastics in various marine ecosystems such as mangroves, marine vegetation, surface water, marine sediments, beaches, water column, and marine fauna.

METHODOLOGY

Research articles and short communications were obtained from the ScienceDirect database in January 2021, including only works published in English during 2020. The keywords used were "microplastics" and the name of some of the marine environments "surface seawater", "seawater column", "sea bottom sediment", "mangroves", "beaches", "marine fauna", "marine vegetation", and "phytoplankton", joint by the Boolean operator "+".

The articles identified by applying the abovementioned identifiers were reviewed (only the abstract and when necessary, the complete article) to select those that met the inclusion criteria: articles from indexed journals; works developed in the marine environments described above, excluding rivers, estuaries, lagoons, air, drinking water, to name a few; MP as the main topic, excluding nanoplastics; and not in vivo intake on the marine fauna studies. Subsequently, the repeated articles were eliminated, resulting in a total of 115 articles (**Fig. 1**).

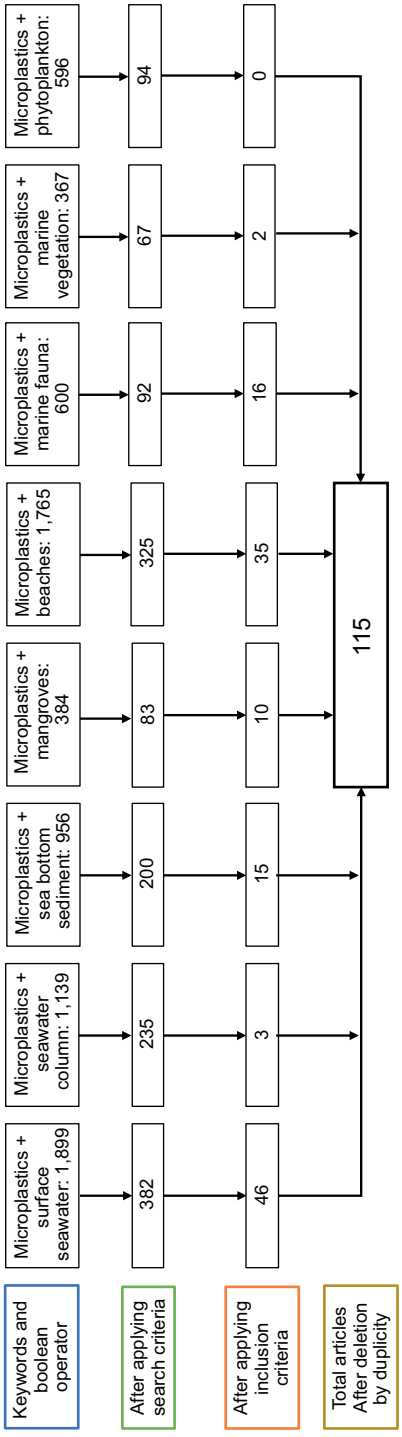

Fig. 1. Steps of the search and selection of articles.

In each article, the measures taken to avoid cross-contamination were identified in field and laboratory work. The methodology described in each article was reviewed; first, it was sought if there was a specific section in the methodology where the different measures were described, e.g., "quality control" (QC)", "contamination control" or "quality assurance" (QA). If there was no such section, the complete methodology was read to identify the different measures taken. For each article, a separate list was

made of the measures taken in the sampling and processing of the samples (in the laboratory), which were grouped in separate spreadsheets. Subsequently, a count was made in the spreadsheets of how many measures each article applied in each stage and how many articles had applied a specific measure in each stage.

In addition to the above, each article was reviewed to verify if there was a report of samples' analysis comparison without measures to avoid cross-contamination to estimate the efficiency percentages. For the blank's cases, in each article the percentage of contamination level avoided by implementing this measure was calculated. The calculation consisted of dividing the concentration of microplastics found in the blanks by the total concentration of microplastics in the samples, multiplied by 100. As shown in equation 1:

$$\% \text{ contamination} = \frac{100 \times \text{concentration of MP in blanks}}{\text{Total concentration of MP in sample}} \quad (1)$$

RESULTS

Eight of the 115 articles analyzed did not report any measure to prevent cross-contamination in the study of MP in marine environments (de-la-Torre et al. 2020, Deng et al. 2020, Katsumi et al. 2020, Khoironi et al. 2020, Ory et al. 2020, Portz et al. 2020, Vilakati et al. 2020, Schmidt et al. 2021). One of these studies expressly states that rigorous contamination control measures were not needed because its objective was to evaluate the presence of MP with a size of 1 to 4.75 mm (de-la-Torre et al. 2020), and two studies specify that only microfibers were excluded from the MP count due to the risk of contamination for their presence in air (Ory et al. 2020, Schmidt et al. 2021).

In the rest of the articles (104 works), measures were taken during the sampling (Cutroneo et al. 2020), the treatment of the samples in the laboratory (Keisling et al. 2020), or both processes (Athapaththu et al. 2020). Some of these studies specified that such measures were taken to control (Garcés-Ordóñez et al. 2020), prevent (Godoy et al. 2020), or minimize (Cozzolino et al. 2020, Jeyasanta et al. 2020) contamination from airborne textile fibers. Articles that considered measures to avoid cross-contamination included these in a specific section of the methodology (Bakir et al. 2020, Liu et al. 2020, Castro et al. 2020) or mentioned them between in its different stages (Godoy et al. 2020, Ripken et al. 2021). These differences highlighted the lack of standardization on the inclusion of measures to prevent cross-contamination in the reviewed MP studies.

Measures taken in microplastics sampling

Of the 115 investigations, 61 included measures to control cross-contamination during MP samplings within their methodological section, which represents 53.04 % of all the analyzed articles. While some articles implemented up to five different measures (Novillo et al. 2020), in most studies only one specific action was included (Frías et al. 2020, Lechthaler et al. 2020), as shown in **figure 2**. The prevention actions are shown in **figure 3**. The three most common are the use of non-plastic containers for sample storage (25 studies), non-plastic utensils for sample collection (24 studies), and non-plastic samplers (21 studies).

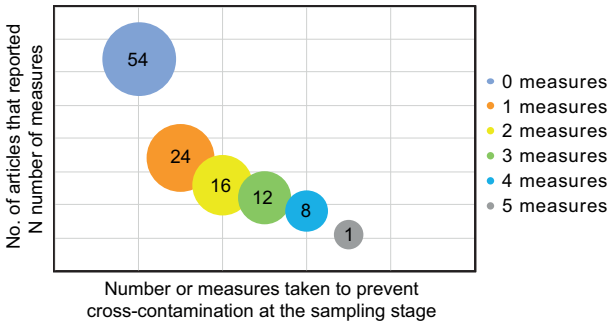

Fig. 2. Frequency of items by number of measures taken during microplastics sampling.

In sample storage, the alternatives to plastic containers are glass bottles (Chen and Chen 2020, Li et al. 2020, Liu et al. 2020), aluminum containers (Saeed et al. 2020, Zuo et al. 2020), and aluminum foil (Álvarez-Zeferino et al. 2020, Deng et al. 2020, Teng et al. 2020), depending on the type of sample in question, while the collection utensils are metal spoons, spatulas, or shovels used for sediment samples (Li et al. 2020, Urban-Malinga et al. 2020).

On the other hand, non-plastic samplers vary depending on the type of sample: in seabed sediments, metallic ones are generally used, such as the Van Veen grab, the gravity corer (Lechthaler et al. 2020), and the Ekman dredge (Tsang et al. 2020). In beach sand samples, in addition to those mentioned above, wood (Godoy et al. 2020) and metal (Urban-Malinga

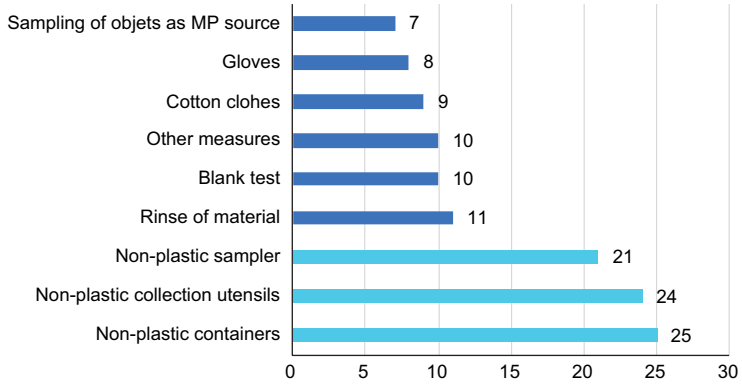

Fig. 3. Frequency of application of measures to control cross-contamination in microplastics sampling. Other measures included protecting, covering, or rinsing equipment between samples, and wearing of cotton coats.

et al. 2020) square quadrats, as well as metal cylinders (Bucol et al. 2020) are also used. In water samples, the alternative is glass (Narmatha et al. 2020) and metal (Ryan et al. 2020) buckets.

The use of non-plastic materials during MP sampling is a good strategy. However, before use, they must be properly washed. In that sense, in 11 studies materials were rinsed before use (**Fig. 3**) with distilled (Jones et al. 2020), deionized (Tata et al. 2020), sea (Ryan et al. 2020) or Milli-Q (Wang et al. 2020a) water, and two studies specify that they filter these media before use (Urban-Malinga et al. 2020, Wang et al. 2020b). Filtering water is an essential step to avoid contamination by the particles it may contain.

In addition, it is recommended that the washing and cleaning of materials be carried out in a closed room and dried under an extraction hood or on a stove, so that plastic particles do not adhere to the material's surfaces, especially airborne fibers. At the same time, all clean materials must be wrapped (preferably in aluminum or cotton cloth) until use. This measure, grouped in the category of "others", was only reported in five studies (Athapaththu et al. 2020, Jones et al. 2020, Narmatha et al. 2020. Tata et al. 2020, Zheng et al. 2020).

Another recommendation, but only for studies where it is not possible to replace the sampling tools with non-plastic utensils, is that, before the fieldwork, these are carefully checked to verify their condition and ensure that there is no presence of burrs that promote plastic particles detachment. This measure will help to prevent the overestimation of final MP concentrations. A complementary measure when there are burrs on the utensils and these cannot be replaced, is that samples of the burrs are taken, and if similar particles appear in the final MP quantification, they are discarded. The latter has already been reported in some studies analyzed here (Courtene-Jones et al. 2020, Jones-Williams et al. 2020, Tošić et al. 2020), in which the sampling of objects or materials as possible sources of microplastics was carried out (**Fig. 3**).

Among the least considered measures, which are grouped under the category of "others", is the rinsing of the sampler between each taken sample (Ferreira et al. 2020, Jones et al. 2020) and wearing a cotton lab coat (Prata et al. 2020). Of these two, only the first is considered essential to avoid cross-contamination between samples. In the case of lab coats, it is recommended they are only used if the clothing worn during the samplings is not made of cotton, since otherwise they could obstruct and hinder fieldwork.

Measures taken during sample processing in the laboratory

Of the 115 articles analyzed, 98 considered different strategies to avoid MP cross-contamination during sample processing in the laboratory (**Fig. 4**). In addition, the number of measurements taken in this stage is more significant than for field samples, since they range from one (Huang et al. 2020) to 12 (Frías et al. 2020), which indicates that sampling in marine habitats is where the highest cross-contamination most likely occurs because fewer measures are taken to prevent it. The lack of implemented measures in fieldwork could be due to the complexity of the samplings or the difficulty in controlling external agents, in contrast to laboratory setups, or because the time to carry out the work should be only the necessary or essential time, due to the presence of activities like tourism, fishing, meteorological conditions or the logistics associated with sampling.

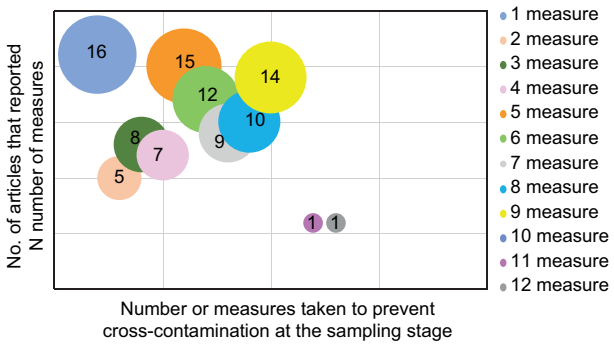

Fig. 4. Number of measures taken in laboratory sample processing by number of articles in which they are mentioned.

Although more measurements are considered in the laboratory than in fieldwork, both stages follow the same trend. In both, the most usual is to make the least number of measures (one), and the less typical is to implement the largest number of measurements (five measures in samplings, and 11-12 measures in laboratory work). This trend might be explained by (1) the lack of knowledge about the existence of cross-contamination during the sampling or samples treatment; (2) the limited availability of resources to implement it; (3) the amount of time and effort involved in carrying them out at each stage, which lengthens the work period, and (4) the lack of standardized protocols that address measures to minimize or avoid cross-contamination.

The set of measures that the different articles have implemented for sample processing is shown in **figure 5**. The three most common measures are the evaluation of blank samples (72 studies), the rinsing of the material (56 studies), and the wear of lab cotton coats (56 studies). In addition to being used during the processing of the samples, the evaluation of blank samples is also carried out in field samplings, except that, fewer studies practice it at this stage (**Fig. 3**). Blank samples in fieldwork are mostly used to evaluate contamination by deposition of MP present in the air (Jiang et al. 2020a).

The evaluation of blanks in laboratory work is always carried out in Petri dishes placed close to the samples to be analyzed. The implementation might vary, but common practices include: (1) previously filtered water (Laptenok et al. 2020), (2) unfiltered water (Bucol et al. 2020), (3) new wet filters (Jones-Williams et al. 2020), or (4) new dry filters (Zheng et al. 2020). Some studies perform this measurement in all stages of the laboratory work (Jiang et al. 2020, Teng et al. 2020), while others do it only in some stages, such as sample digestion (Zhang et al. 2020b), density separation test (Fred-Ahmadu et al. 2020) or MP count (Mak et al. 2020). Other studies do not specify the stage (Lechthaler et al. 2020, Narmatha et al. 2020). The number of replications on blanks also varies. It has been seen that three (Silvestrova and Stepanova 2021), four (Lechthaler et al. 2020), six, and 14 replications are used (Lindeque et al. 2020). However, the most common are three replications.

On this basis, it is suggested to place moistened filters (with previously filtered distilled water) in Petri dishes as close as possible to the samples during all the laboratory work (not only in some stages) and with at least three replications. This will allow reliable data on the count of MP deposited by air and, therefore, correct the final MP count, for which the mean number of MP found in whites must be subtracted from the total number of particles of each analyzed sample (Lindeque et al. 2020).

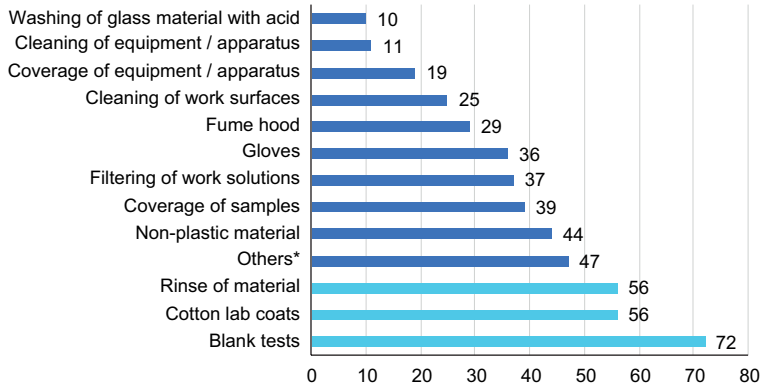

Fig. 5. Frequency of application of measures to control cross-contamination in sample rocessing. Other measures included keeping track of researchers' clothes colors, cleaning materials and hands with ethanol, and checking for particles by using microscopes in utensils prior to handling samples.

Blanks in the laboratory help estimate the percentage of reduced or avoided contamination. As mentioned in the methodology, in this research we tried to make that estimate for the laboratory work of each study. However, this was not possible in the vast majority of studies because they did not specify MP concentrations in blanks (41.7 % of studies), reported the absence of microplastics in blanks (26.4 % of studies), did not report concentrations in the same units (12.5 % of studies), or combined field and laboratory information (1.4 % of studies).

For articles where these data were obtained, it was found that reductions in contamination percentages ranged from 0.1 % (Zhang et al. 2020b) to 48.8 % (Jones-Williams et al. 2020). This information was already included in two studies, such as in Dodson et al. (2020), with a percentage of < 5.3 %, and Jang et al. (2020), who reported a general percentage of 9.4 % (6.4 % in bivalves and 3.0 % in seawater).

On the other hand, for rinsing the materials (as in the sampling stage) it is common to use distilled (Laptenok et al. 2020), deionized (Tata et al. 2020), or Milli-Q water (Robin et al. 2020), which is sometimes filtered before use (Pérez-Venegas et al. 2020, Rist et al. 2020). The filtering of water prior to the rinsing of materials is essential since it is the filtering of working solutions (a measure contemplated by 37 studies, see **Fig. 5**), for example the density separation solutions (Bridson et al. 2020, Jiang et al. 2020b) or the digestion of the samples (Narmatha et al. 2020, Wang et al. 2020b), because either water, solid or reagent liquids may contain MP, which in turn could contribute to an overestimation of the final results.

Finally, using cotton lab coats in the laboratory is essential to avoid the contamination of samples by the detachment of synthetic fibers. Also, under the cotton lab coat researchers always wear cotton clothing to minimize leakage of synthetic microfibers. Only eight studies implemented this measure, which was included in the category "others" (Athapaththu et al. 2020, Ghayebzadeh et al. 2020, Jones et al. 2020, Patchaiyappan et al. 2020, Rahman et al. 2020, Tata et al. 2020, Zhou et al. 2020, Ripken et al. 2021).

Additionally, it was found that two studies that did not contemplate the use of cotton clothing during the experimentation, reported that the color of the synthetic fiber of the researchers' clothes was registered in order to correct the results if fibers resembling the clothes fibers were found in the samples (Castro et al. 2020, Frías et al. 2020). However, registration could become complicated if a piece of clothing has multiple colors.

In addition to the use of cotton clothing and the registration of synthetic clothing, another measure described in the "others" category was to minimize the number of people working in the laboratory (Jones-Williams et al. 2020) by conducting the experiments in a closed room (Jiang et al. 2020a), turning off fans (Tran-Nguyen et al. 2020), wearing face masks (Athapaththu et al. 2020), drying the glassware in a muffle at 400-500 ºC (Ramírez-Álvarez et al. 2020, Rist et al. 2020) for 3-5 h before use (Peng et al. 2020, Robin et al. 2020), rinsing the outside of the fauna samples (for example, the scales and fins of the fish, and the shells of the oysters) with Milli-Q water (Mak et al. 2020) or with previously filtered seawater (Plee and Pomory 2020), to mention a few. Although these measures are not very common, they are considered adequate to minimize cross-contamination. The more measures are taken to avoid cross-contamination, external MP will decrease and results will be more reliable.

Shared measures for sampling and laboratory processing

A summary highlighting shared and non-shared measures in sampling and laboratory processing can be seen in **figure 6**, which shows that measures shared in both stages are blank samples, wear of cotton clothes, rinse of materials, and non-plastic materials. It can also be seen that the most significant number of non-shared measures correspond precisely to activities in the laboratory.

DISCUSSION

Evidence about the efficiency of measures to reduce cross-contamination is very scarce; so far, only two studies were identified that have addressed this issue. Wesch et al. (2017) evaluated airborne contamination in the analysis of microplastics in fish by processing the samples in different workplaces. Their results showed that performing the analysis in a clean beach reduced contamination by 96.5 %, compared to an indoor laboratory (5.0 %), a mobile laboratory (11.1 %) and a fume hood (50.0 %). However, it is essential to mention that these findings are limited to that specific laboratory and the type of equipment used.

On the other hand, Bosshart et al. (2020) tested the processing of fish samples to analyze the presence of microplastics in the laboratory in three different environments: without protection, under a mosquito net, and with the horizontal flow hood under the mosquito

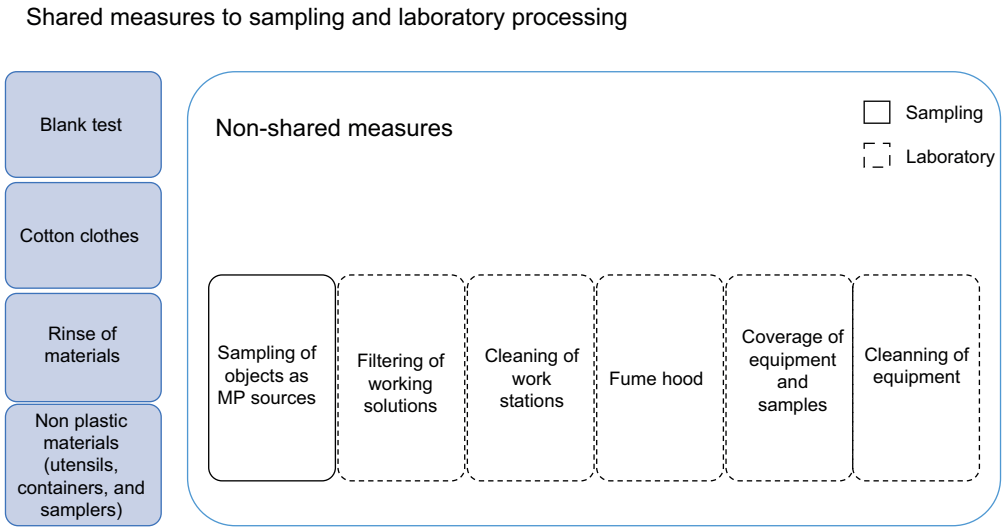

Fig. 6. Shared and non-shared measures between sampling of microplastics in fieldwork and processing microplastic samples in laboratory.

net. Three blanks were run in each environment and treated similarly to the samples (including vacuum filtration). Covering the horizontal flow hood with a mosquito net reduced airborne contamination by approximately 84.0 % compared to the laboratory without protection (0.0 %) and the laboratory under a mosquito net (48.39 %). Additionally, it was found that vacuum filtration sucked in an additional 2-4 fibers, even under the cotton midge, which increased contamination by 26.0 %.

In addition, as mentioned in the methodology and in previous sections, we calculated the percentage of contamination reduction from the concentrations of microplastics found in the blanks and the total concentrations of microplastics found in the study samples. Regarding the laboratory environment, percentages of contamination reduction were found in a range from 0.1 % (Zhang et al. 2020b) to 48.8 % (Jones-Williams et al. 2020), while percentages in the field environment were found from 4.8 % (Rist et al. 2020) to 69.0 % (Ryan et al. 2020).

Since information on the efficiency of measures is scarce, it is difficult to establish the percentage of pollution reduction provided by each applied measure and, therefore, an average percentage of a set of measures. However, it is essential to highlight that there are guidelines for the monitoring of microplastics in marine environments (Calcutt et al. 2018, GESAMP 2019, Setälä et al. 2019, Michida et al. 2020) that recommend taking specific measures to avoid cross-contamination both in fieldwork (**Table I**) and in the laboratory (**Table II**).

Among the measures recommended by the guidelines for sampling and laboratory processing, it is observed that some are similar (highlighted in bold type in both tables) to those reported in the articles reviewed here, but there are other measures not included that could also be of great help to reduce cross-contamination.

It is important to highlight that each guide focuses on something specific; for example, the work of Calcutt et al. (2018) is a guide for sandy beaches, while Michida et al. (2020) and GESAMP (2019) are guides for studies of microplastics in the ocean and Setälä et al. (2019) focus on studies in all types of marine environments, which highlights that sometimes there may be specific measures for a particular environment, while in other cases the same measure can be applied to different environments; that is, there is no consensus as to what measures should be carried out in each study setting.

In addition to the lack of specific measures for each environment, there are other issues that must be addressed: (1) design of standardized protocols for sampling and sample processing; (2) inclusion of specific measures in standards for different marine environments (such as beach, surface water, bottom sediments, fauna and flora) and stage (for example, sampling, flotation tests, digestion, extraction, filtration and microscopic identification); (3) establishment of minimum percentages by measurement to obtain valid and reliable results (e.g., Galgani et al. [2013] state that procedural contamination should be less than 10 % of the average values determined from the samples themselves), and (4) consistency between

TABLE I. MEASURES RECOMMENDED BY DIFFERENT GUIDES TO AVOID CROSS-CONTAMINATION IN THE SAMPLING OF MICROPLASTICS.

Measures taken during sampling	Type of guide	Reference
• **Avoid the use of plastic tools; instead use metal buckets, metal spoons or glass jars.** These tools should be kept in bags before use • Use filtered seawater for washing the sieves and cover the container holding the filtered water • **Keep buckets and jars covered with aluminum or other non-plastic lids to prevent plastic fibers from entering the containers** • Keep sampling equipment free of sand by using a towel or box to place it in while not in use • **The whole team should wear natural fiber clothing** • The person collecting the sand should be positioned downwind • Avoid transferring samples between containers as little as possible as contamination may occur at the time of transfer	Focused on sandy beaches	(Calcutt et al. 2018)
• **Sampling containers should be washed with distilled filtered water before use** • Wear clothing with distinguishable or unique colors so that in case the sample is contaminated these fibers can be differentiated • To avoid bow wave effects, fittings (tubes) should be placed at a suitable length to keep nets away from the hull. The fittings should also be installed on the sides and as far forward of the vessel as possible to avoid wake effect • For water sampling with nets, the nets should be washed thoroughly before each sampling from the outside in, and **care should be taken when storing the nets. Perform a field blank test for at least one of the nets to confirm whether the rinses were effective. If several samples are taken with the net, perform control tests periodically** • Avoid plastic products from the boat and consider the plastic parts of the boat or vehicle	Focused on ocean surface	(Michida et al. 2020)

Measures highlighted in bold are similar to those reported in the articles included in this revision.

TABLE II. MEASURES RECOMMENDED BY DIFFERENT GUIDES TO AVOID CROSS-CONTAMINATION IN LABORATORY PROCESSING.

Measures taken during laboratory processing	Type of guide	Reference
• Decrease steps during sample processing, as it will reduce the possibility of cross-contamination • **Use blanks during the whole process with filtered water**	Focused on marine environments	(Setälä et al. 2019)
• Use negative controls to identify air contamination • **Use controls in triplicate for each batch of samples** and give them parallel treatment to the samples • **Use filtered water** and plastic-free organism tissue for controls • Repeatedly rinse the material and keep it in a clean space • **All materials as well as solutions should be covered and checked before and covered after use** • Samples of fauna should be rinsed and checked for external contamination if possible • **Use 100 % cotton lab coat** • When samples are moved from clean air areas, use blanks such as Petri dishes by placing them next to the sample	Focused on ocean	(GESAMP 2019)
• **Perform analysis in clean rooms and benches, clean real estate** • Equipment and material used should be checked under a microscope to observe any adhering microplastic. The filtration vacuum equipment should be washed with filtered water between each sample • **All reagents should be vacuum filtered through Whatman GF/D filter papers immediately prior to use**	Focused on ocean surface	(Michida et al. 2020)

Measures highlighted in bold are similar to those reported in the articles included in this revision.

the size and characteristics of the MP considered in the blanks and the MP found in the study samples.

Summary of measures for sources of microplastics contamination

According to the results section, MP that contribute to contamination of samples (either during their collection or processing) may have different sources, such as deposits from the air-environment or interiors; wear of containers, samplers, utensils, and plastic materials in general; microfibers detached from clothing and other synthetic textiles (e.g., flannels and curtains); those present in reagents and water, and, finally, cross-contamination between samples. That is why various measures (detailed in **Table III)** must be taken to avoid cross-contamination and thus guarantee more reliable results.

CONCLUSIONS

Research on MP has grown in quantity and complexity since its presence in the environment was reported. As studies have increased, their objectives have diversified and become more complex. This increase in the number and depth of research implies the need of standardized protocols for sampling methods and analysis of samples in the laboratory, so that the results are comparable, reliable, and, desirably, reproducible.

As observed in this work, even if many of the analyzed articles include different measures to avoid cross-contamination in sampling and sample processing, they do not specify the percentage of cross-contamination avoided by applying specific measures. In some cases, it was possible to calculate the percentage of contamination avoided from the concentrations found in the blanks and the total MP concentrations in the samples. In general, it was found that these percentages range from 0.1 to 48.8 % for the laboratory, while in the sampling they range from 4.8 to 69.0 %.

Therefore, it is considered essential that the standards of sampling and processing of samples must include various measures to avoid cross-contamination by stage and by marine environment, so that

TABLE III. MEASURES RECOMMENDED BY SOURCE OF MICROPLASTICS CONTAMINATION.

Air	Wear of plastic materials	Synthetic clothes and textiles	Reagents, solutions, and water	Cross-contamination between samples
Evaluate blank samples during sampling and at each stage of laboratory sample processing. Subtract, from the total number of MP, the particles found in the blank samples. Rinse the samplers, containers, utensils, and general material before using and dry them in an oven. Cover samplers, containers, utensils, apparatus, and work surfaces with aluminum or cotton cloth until use. Process samples in a closed room, with fans turned off. Process the samples in a fume hood.	Thoroughly inspect samplers, containers, and plastic utensils before using in the field and laboratory. Take samples of burrs present in plastic materials. Discard from the final MP quantification particles similar to the burrs of plastic materials. Use glass or stainless-steel samplers, containers, and utensils.	Wear cotton clothes during laboratory sample sampling and processing. Wear a cotton gown during sampling, only when wearing synthetic textile clothing, and at all stages of sample processing in the laboratory. In case of wearing synthetic clothing and using other synthetic textiles, carry out a record of these. Use cotton flannels to clean samplers, containers, utensils, apparatus, and work surfaces.	Filter water (distilled, deionized, marine, Milli-Q, or potable) before using. Filter solutions for sample digestion or density separation before using.	Rinse the samplers, containers, and material in general between each sample taken in the field. Rinse laboratory supplies and utensils before processing each sample in the laboratory.

Source: own elaboration.

the sampling and processing (and even the reading of MP under a microscope) are covered. These standards must also establish the minimum percentages of avoided contamination that must be met for the results to be considered valid.

Standardization is a cornerstone of scientific advancement because it allows validated and replicated results which in turn allow science to advance and deepen our collective knowledge on a specific topic. Quality control to avoid cross-contamination can contribute significantly to better identify MP sources, to understand their distribution and transport, to study the interaction between ecosystems, and the possible risks in marine species. In the medium and long term, it will help to establish measures to prevent MP generation and entry into different environmental compartments.

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