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Review

Cross-Contamination as a Problem in Collection and Analysis of Environmental Samples Containing Microplastics—A Review

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Abstract: Research conducted so far on the presence of microplastics in the environment shows that these items are ubiquitous pollutants and therefore constitute an inherent part of our lives. This constitutes a significant problem in many aspects, and one of them is the correct identification of microplastics in environmental samples. Environmental samples can be easily contaminated by plastic microparticles from other sources if proper precautions are not taken during sampling and analysis. The consequence of not taking this cross-contamination into account when analysing the results may be their significant overestimation. This review aims to draw attention to the problem of cross-contamination that accompanies the collection and analysis of samples for the presence of microplastics, and to discuss this issue in a comprehensive manner. The article indicates potential sources of cross-contamination, lists the mitigation methods, and describes the possibilities of assessing this type of contamination. Moreover, the review examines how cross-contamination control appears in practice, based on the available literature data.

Keywords: microplastics; microplastic pollution; cross-contamination; background contamination; contamination control

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1. Introduction

Microplastics (MP) are small fragments of plastics (1 µm–5 mm) that may be purposely produced in such a form (primary MP), or originate from degradation of larger plastic objects (secondary MP) under the influence of different environmental factors such as UV radiation, temperature, or waves [1]. It is questionable whether one of these categories can be unambiguously ascribed to particles that originated from the abrasion of large plastic items during their production, use, or maintenance (e.g., as a result of the use of tires). In this paper, however, such particles are treated as primary MP in accordance with the division presented by Boucher and Friot [2].

One of the main sources of primary MP are synthetic textiles that are subject to abrasion or crumbling during washing, consequently leading to the generation of vast amounts of microfibres that are discharged with wastewater [2,3]. Research by De Falco et al. [3] evidenced that the amount of released MP depends, among others, on the type of applied detergent, temperature, and water hardness. Therefore, industrial laundries may generate considerably more pollution with microplastics. The use of much more aggressive conditions of the washing process on an industrial scale contributes to more significant mechanical and chemical stresses favouring fabric disintegration. Moreover, the authors [3] estimated that the average amount of MP released during washing polyester textiles (for a load of 5 kg) exceeds 6 million particles in the form of microfibres.

Car tires are another equally important source of primary MP. As a result of contact with the road surface during the vehicle's movement, they are subject to wear. As a result, microscopic particles are released to the environment, built of a mixture of polymers, both synthetic (primarily styrene–butadiene rubber) and natural, as well as various admixtures [2,4].

The remaining sources of primary MP include plastic pellets, used for the production of plastic goods, different types of coatings (e.g., applied in the shipbuilding and construction industry), personal care products and cosmetics, artificial grass, and many others [2].

As shown in these examples, in most cases, the release of MP to the environment is of incidental character, i.e., it is a natural consequence of the use and aging of items made entirely or partially of plastics. The exception is personal care products containing plastic microbeads that consumers consciously purchase. In this case, they are produced purposely to improve the value of cosmetic products, which are eventually discharged to the environment, causing the pollution [2].

The other category, namely secondary MP, refers to plastic particles originating from the degradation of larger items, such as plastic bags, bottles, or fishing nets [1,2].

Pollution with microplastics is an especially valid issue attracting much interest. It results from their common occurrence confirmed by numerous studies. The presence of MP was confirmed in various environmental matrices (among others, in the water, soil, and air), including drinking water and food products [1,5]. Considering the latter two cases, this is particularly important from the point of view of its potential impact on human health.

A starting point for implementing any legal regulations regarding the acceptable content of MP (e.g., in treated water or treated sewage) is evidencing their negative effect on the environment, and particularly on living organisms, including humans. This in turn requires knowledge regarding the actual MP concentrations in the environment, and therefore conducting monitoring of such pollution. Measurements permitting identification of the presence and determination of concentrations of MP currently require highly advanced analytical techniques. It constitutes a severe obstacle in the popularisation of research on the ecotoxicology of microplastics.

Many studies have been conducted so far in the scope of MP toxicology. Adverse effects caused by these micropollutants, however, still require confirmation [5]. MP measurements in the water environment are also conducted, although they mainly concern marine waters, whereas information concerning freshwater environments is limited [6]. The greatest issue is the lack of standardisation of sampling, separation, and identification of MP. It makes comparisons between the available data difficult or even impossible [5,7]. The issue of cross-contamination plays a very important role in the problem. It can considerably affect the quality of the results obtained, leading to their overestimation (Section 5). Therefore, conscious control of this phenomenon is of utmost importance.

This publication is the first review paper entirely devoted to the issue of cross-contamination related to the analysis of MP from environmental samples, and particularly water samples. However, there are several review papers on methodology of sample collection and analysis that address this issue, mainly concerning biota samples [8,9]. This article discusses the most important issues related to cross-contamination, such as sources of the contamination, possible methods of reducing the risk of sample contamination during research, and measurement methods of cross-contamination. In this paper, cross-contamination is defined as microplastics released to environmental samples in an uncontrolled way during their collection and analysis. For the purposes of this article, based on publications from reviewed scientific journals from the last 20 years (i.e., from the beginning of 2000 to the end of 2019), an extensive and accurate database was developed, clearly showing the approaches towards cross-contamination in MP research during recent years. The database providing the basis for writing this review paper contains 552 research papers regarding research in the scope of analysis of the presence of MP in different ele-

ments of the environment, i.e., in water, sediments, soil, living organisms, and air. Among these, 180 papers refer to the water environment.

2. Sources of Cross-Contamination

As mentioned above, a source of MP can be all items made of plastics, which undergo ageing or wear when exposed to mechanical stresses. Plastics, particularly those with dimensions at a micrometre level, are characterised by small inertia, i.e., they can be easily carried and suspended in the air. The smallest of them develop stable aerosol systems, and larger particles show a higher sedimentation rate, resulting in their short presence in the atmosphere [4]. Due to this, the most serious problem of cross-contamination is MP present in the air that can be subject to air deposition. Emission of micropollutants is caused by, among others, synthetic textiles, easily releasing MP in the form of fibres as a result of abrasion, together with shoes, laboratory equipment made of plastics (e.g., bottles, trays), and furniture [10,11].

Example equipment that can be responsible for sample contamination during MP research are nets (such as Neuston nets, Bongo nets, or Manta trawls) used to collect volume-reduced samples [7,11,12]. The nets, or more precisely their central part—the mesh—are made of plastics. Therefore, it is possible that synthetic fibres can be released to the collected material. Moreover, an inaccurately rinsed sample collection container located at the end of the net may also be a source of cross-contamination. Mu et al. [12] found several dozen fibres in field blank samples that were identified to originate from nets used for sampling.

Research in the scope of air contamination with plastic microfibres, both indoors and outdoors, was conducted by Dris et al. [10]. The analysis covered four sites: one outdoors and three representing a closed space (two private apartments and one office). The results showed that the mean concentration of contaminants indoors, reaching a level of 5.4 fibres/m^3, was much higher than outdoors, where a value of 0.9 fibres/m^3 was recorded. Moreover, it was calculated that the rate of deposition of fibres in the indoor environment varied from 1586 to 11,130 fibres/day/m^2. This suggests considerable contamination with microplastics of indoor spaces, irrespective of their purpose, and high probability of their transfer from the air to nearby surfaces. The conclusion drawn from these findings is that the thorough control of contamination during the analysis of MP samples is a highly important element of laboratory research, and should not be disregarded (Section 4).

3. Methods of Reducing Cross-Contamination

Considering the knowledge that emission of microplastic pollutants is caused by items made of plastics, the obvious solution is their elimination to limit the possibility of cross-contamination [7,11]. When elimination of such items is not possible, special caution measures should be taken, and procedures for activities should be developed to minimise the risk of undesirable migration of pollutants.

Many different methods of mitigation of cross-contamination exist. A large majority are universal and can be applied irrespective of the type of analysed samples. The most popular methods include, among others: wearing clothing containing no plastic fibres, cleaning laboratory surfaces and entire equipment before use, covering samples and laboratory equipment, work in conditions of controlled air flow, and the use of exclusively glass or metal laboratory dishes (Appendix A).

The release of synthetic microfibres from clothing can be avoided by avoiding synthetic textiles. Moreover, during work conducted at a laboratory, it is recommended to wear cotton coats or overalls [13–23], which is a commonly implemented practice. Such a plastic-free outfit is supplemented with gloves, usually made of nitrile [21–26] or latex [20,27,28]. Several publications also mention headgear, e.g., in the form of a shower cap [22]. Guidelines developed by Gago et al. [7] also suggest taking notes regarding the type and colour of clothing worn by each person involved in the research. This can considerably aid the identification of microfibres in samples. Moreover, a larger number of

people obviously generate more contamination. Therefore, it is recommended to assign a possibly low number of persons to each task [29].

Analogically, as in the case of the aforementioned notes regarding the type and colour of clothing, a register of selected information on the surroundings (e.g., colour of ship deck) should also be run [30]. They may become a potential source of additional contamination.

Covering samples or laboratory equipment usually involves aluminium foil [17,21,25–27,31–34] which is inexpensive and easily available. However, it is a delicate material, and can be easily torn, ceasing to fulfil its protective function. An alternative solution is a protection made of a more durable material, e.g., a glass lid [32,35] or a wooden cover, as used to cover a metal sieve described in one of the publications [36]. An ingenious solution for protection against cross-contamination was also presented by Torre et al. [37] by placing the stereoscopic microscope in a properly fitted plastic cover, thereby isolating the sample from external contamination during its visual examination. The authors showed that the microscope cover reduced airborne contamination by 95.3% compared to the analysis without any protection. Due to the high potential of this method to eliminate cross-contamination, it has already been implemented in several other studies [14,38].

Work in conditions of controlled air flow can limit the deposition of contaminants. These conditions can be provided by means of a fume hood or laminar flow cabinet equipped with an air filter [14,18,19,22,23,38–41]. Study results by Wesch et al. [42] showed that processing samples inside a laboratory fume hood contributed to a reduction of sample cross-contamination by almost 50%. Limiting access of air from the outside is equally important. This involves keeping windows and doors closed during the analysis [13,27,43].

As mentioned above, particles contained in the air, particularly microfibres, have a tendency for deposition. Therefore, it is highly important to clean different types of work surfaces and laboratory equipment before their use, because contaminants from the air might have accumulated on them in the meantime. Substances used for this purpose most commonly include: different types of alcohols, e.g., ethanol [13,17,24,28,44,45]; acetone [40,46]; distilled water [21,27,36,41]; deionised water [13,25,38]; and ultrapure water [17,18,25,47–49]. Review of literature also reveals the use of other substances such as double-distilled deionised water [34]; nitric acid [26]; or "Decon 90", i.e., an agent typically made for cleaning surfaces [35]. Washing surfaces is frequently performed in several repetitions, usually three times [13,16,21,50], although several different substances are sometimes used in a sequence [35,44]. In some studies, cleaning laboratory glassware with liquids is additionally followed by heating in an oven or autoclave at high temperatures for several hours to minimise the adhesion of microfibres to their surface and combust any organic matter [16,49]. Due to the tendency of micropollutants to deposition, apart from cleaning laboratory equipment and surfaces, it also seems reasonable to thoroughly scrub hands and forearms by sample operators before starting laboratory analyses [23,25,50].

An interesting solution that appeared in several publications is increasing air humidity in the laboratory [15,19,46]. It results in a decrease in the amount of contaminants in the air, although on the other hand it contributes to their permanent deposition on surfaces. In this case, the aforementioned careful cleaning of surfaces before their use is essential.

Less popular methods of reducing cross-contamination include, among others, filtering all reagents used in the analyses (through a membrane filter with medium pore size up to several μm) [27,32,41,45,49].

Irrespective of whether and what measures of caution are applied for reduction of cross-contamination, many authors particularly recommend simultaneous analysis of control samples (Section 4) [17,19,21].

4. Assessment of Cross-Contamination

Obtaining the most reliable study results requires taking relevant steps aimed at quality assurance and quality control (QA/QC) of the conducted research [51]. One of such steps is the measurement of cross-contamination through analysis of control samples, including, among others, blanks.

Blanks are a necessary element of the process of validation of new or existing laboratory procedures, but they are also very frequently included in routine analytical activities related to sample analysis aimed at the assessment of contamination accompanying such activities [52].

In the analysis of MP samples from the water environment, analysis of blanks concerns water free from contamination, such as distilled water or prefiltered water that is then subject to procedures identical to those for the analysed environmental samples [17,19,21,49].

Another type of control samples are samples providing information on the amount of contaminants present in the air that is subject to air deposition in the vicinity of the conducted activities (background contamination), and therefore probably also permeate into the analysed samples [13,24,31,35,50].

In the scope of control of background contamination, empty Petri dishes [39,48,53] or single filter papers [18,33] are usually used. Several publications describe combining these two solutions, where filter papers are placed in Petri dishes [43,50]. Another method for conducting this type of control analyses is filling Petri dishes with water [24,25,54], or moistening filter papers [35] to provide a substrate with strong adhesion, contributing to capturing contaminants from the air. Next, the resulting samples are distributed at selected sites for a specified period of time, usually the duration of particular stages of the analysis, or duration of the entire analysis, and then they are analysed. Control of cross-contamination is sometimes also conducted in conditions of forced air flow, i.e., through sucking air through a filter paper in a vacuum filtration system for a specified period of time. Such a technique was employed in research by Wang et al. [21], using a glass fibre filter paper with a pore diameter of 0.45 µm, and adopting the filtration time of one hour.

The literature review showed a largely arbitrary approach to nomenclature of control samples. Example descriptions of control samples encountered in publications include: "procedural laboratory blank" [19], "method blank" [36], "air contamination control" [35], "blind control" [45], or "blank control" [16]. There is also a division into positive and negative controls [55], although these terms are rarely used. The use of many different names, often in reference to the same activity, can be misleading. Therefore, standardising the terminology is advisable.

Control samples require more labour due to the necessity of their preparation and analysis. Depending on the equipment used, it can be a tedious process, and therefore a reason why it is an element neglected in part of studies. The review and analysis of the literature show that 80 out of 180 research papers focusing on MP analysis in water samples do not mention control samples. This suggests that they were not considered in the research. Although, as mentioned above, analysing control samples is an additional more labour and time-consuming task, it provides much valuable information that considerably improved the quality of the study results obtained. Most importantly, the analysis of the discussed samples provides information on the quantity of micropollutants to which primary samples were exposed during particular stages of research. Due to this, the values obtained can be preliminarily considered in processing results while avoiding their overestimation. Moreover, quantitative analysis of control samples can facilitate the identification of potential sources of MP emission, which can then be eliminated from future analyses, thus reducing the current level of cross-contamination.

5. Control of Cross-Contamination in Practice

A large portion of articles includes no information on the control of cross-contamination during research [38,56]. These papers sometimes mention using "clean" tools, but this type of information is very imprecise, and does not evidence taking any steps towards mitigation of cross-contamination.

Part of the publications includes only a short note concerning taking relevant steps in order to eliminate the possibility of contamination of samples during research. However,

they do not specify such steps, and therefore provide no details of the applied procedure preventing contamination.

In many papers, methods of reduction of cross-contamination are combined with several sentences of the methodology description. There are also publications including a separate chapter regarding the provision of quality assurance and quality control (QA/QC) during research. Such chapters have many different titles, such as "Background contamination" [15,44,46], "Quality assurance (QA) and quality control (QC)" [47], "Contamination prevention" [31,32,49], "Reducing and measuring contamination" [50], "Laboratory QA/QC" [13], "Contamination avoidance" [20,40], and "Anti-contamination measures for microplastic analysis" [19].

The number of research papers in the scope of MP analysis in the water environment that include information regarding taking any conscious steps aimed at reducing cross-contamination in conducted research is increasing yearly (Figure 1). An analogical increasing trend in terms of the publications number is also observed in reference to articles covering control samples (Figure 2). Furthermore, it can be seen that most of the methods of mitigating cross-contamination, such as wearing protective clothing (gloves or cotton laboratory coats) or filtering chemical reagents before use, show a similar upward tendency as demonstrated in the Supplementary Materials (Figures S1–S10). However, this increase is not always proportional to the number of new research papers on microplastics, but it is nevertheless indicative of a growing awareness of the issue of cross-contamination occurring during the MP research. Interestingly, as shown in the diagrams (Figures 1 and 2), before 2013, no research papers regarding the presence of MP in the water environment mentioned either the implementation of methods for control of cross-contamination, or control samples. These are, therefore, practices implemented in recent years only. Moreover, in 2013, control samples were analysed in only one study, and in 2019 the number increased to 45.

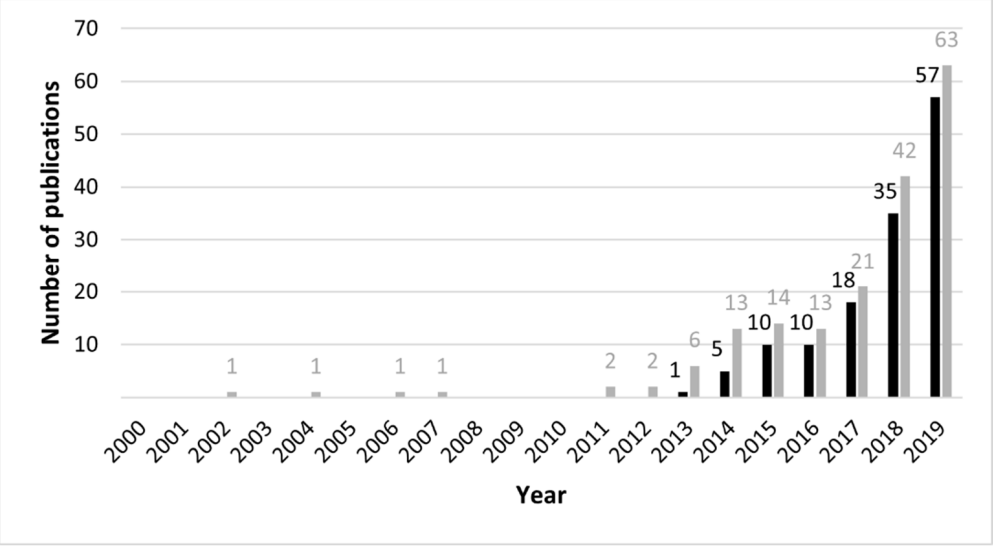


Figure 1. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing any solutions aimed at reduction of cross-contamination (black).

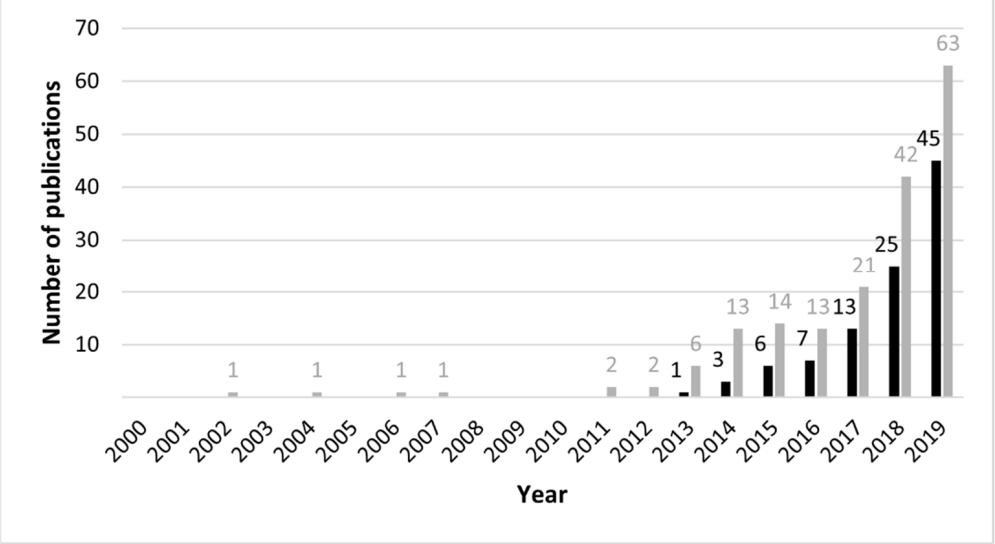


Figure 2. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing control samples (black).

Based on the developed database, the literature review showed that over the last 20 years, control samples appeared in 242 out of 552 articles (44%) referring to MP analysis in environmental samples. In the case of publications referring to water samples, the number was 100 out of 180 papers (56%). Although the presented proportions appear relatively low, the observed trend (Figures 1 and 2) suggests that the proportions will increase with time.

Information regarding control samples provided by authors is sometimes very limited, i.e., focused only on the analysis of control samples, and not including results of analysis of such samples. This may mean that no cross-contamination occurred in a given study, or it was sufficiently low to be considered insignificant. Nonetheless, such information should be included in publications, because they may prove useful from the point of view of discussion regarding cross-contamination.

Hermsen et al. [9] performed a quality assessment of selected research papers on MP analysis in biota samples. The evaluation criteria included, inter alia, methodology of sampling and MP identification, sample size, and control samples. Based on the results obtained, the authors [9] concluded that none of the assessed studies can be considered fully reliable due to the lack of certain information, e.g., in the field of analytical procedures used. As indicated by the authors of the abovementioned reviews [8,9], cross-contamination may occur at any stage of sample handling, i.e., during sampling, transport to the laboratory, and laboratory analysis. Therefore, it is extremely important to identify potential sources of secondary contamination during research in order to take appropriate actions towards reduction and to estimate the impact of cross-contamination on the results. Control and assessment of cross-contamination are particularly important in the case of samples where the occurrence of particles with small sizes (at a level of several micrometres) and in small quantities is expected, as confirmed in the study by Mintenig et al. [17]. The study involved the analysis of MP presence in raw (underground) water and treated water. In the scope of assessment of cross-contamination, a series of blanks was performed, analysing prefiltered consumable water in the same way as that used for collected samples of water. In all water samples, between three and 46 particles in the form of microfibres were detected in 1 m^3, and the analysis of control samples showed their average content at a level of 45 microfibres. The results provided the basis for the conclusion that water sample contamination with microplastics, both for raw and treated water, was in its entirety incidentally introduced to the samples during activities involved in their collection and analysis. This demonstrates the importance of proper methodology of sample collection and preparation in research, aimed at minimising cross-contamination of the analysed water.

6. Conclusions

This article discusses the existence of many potential sources of emission of contaminants in the form of MP. Cross-contamination of samples during research appears unavoidable. Due to this, the implementation of research focusing on the analysis of the presence of MP in the environment should be accompanied by undertaking conscious activities aimed at reduction of cross-contamination. However, applying many strict guidelines in the scope does not guarantee complete elimination of secondary contamination of the analysed samples, which points to the need for determining the share of cross-contamination with microplastics in sampled water. Although in reality, in reference to research conducted and described in the literature so far, the application of control samples is not a common practice. An increasing tendency has been observed in recent years regarding the number of studies, in which the control samples were implemented. It should also be noted that the effectiveness of particular methods of reducing cross-contamination is unknown and difficult to determine quantitatively. Therefore, appropriate research should be carried out to validate each of these approaches. As a result, it would be possible to develop a protocol detailing the mitigation and assessment measures for cross-contamination. To summarise, MP constitute an omnipresent pollutant, and failure to consider this fact in research can lead to considerable overestimation in the results obtained. In the situation of expectation of a trace amount of MP, it can constitute a serious problem. In order to guarantee the highest possible quality of study results in the scope of MP analysis in environmental samples, it is necessary to standardise the methodology of sampling, separation, and identification of MP, with consideration of procedures aimed at reduction and measurement of cross-contamination.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su132112123/s1, Figure S1: Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing latex or nitrile gloves (black), Figure S2: Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing laboratory coats (black), Figure S3. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing fume hoods or laminar flow cabinets (black), Figure S4. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing covering samples or laboratory equipment with aluminium foil or other nonplastic covers (black), Figure S5. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing polymer-free clothing (black), Figure S6. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing washing glassware or laboratory equipment several times in a row prior to use (black), Figure S7. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing filtration of chemical reagents (e.g., H_2O_2) before use (black), Figure S8. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing sterilisation of glassware or laboratory tools before use (black), Figure S9. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing washing glassware or laboratory equipment with agents other than water, such as alcohol, acids, or special detergents (black), Figure S10. Number of publications (annually) concerning MP analysis in the water environment in total (grey) compared to publications employing the use of equipment made of materials other than plastic, e.g., glass, whenever possible (black).

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Appendix A

Table A1. Summary of cross-contamination mitigation methods in selected research works on the presence of microplastics in various environmental matrices ("item" refers to a single microplastic particle, SD means standard deviation).

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water, biota	North Atlantic Ocean, Rockall Trough	Yes	70.8 items/m^3	Whenever possible, equipment made of metal or glass was used during sampling and sample analysis. All equipment was wiped three times with 70% ethanol prior to use. Deck was flushed with water from the ship's fire hose prior to work. The number of people working with the samples was reduced to a minimum. Personal protective equipment was worn during sampling. Samples of fibres from clothing, ropes from the ship, or other potential sources of contamination were collected for comparative analysis with water samples. All water filters and hoses on the deck were thoroughly checked for contamination. The spigot of each Niskin bottle was rinsed with deionised water prior to sampling. The door was kept closed throughout the experiment and the air ventilation was sealed. Work surfaces were cleaned three times with 70% ethanol prior to work. All equipment was inspected under a microscope before use. Samples were kept covered. Laboratory coats made of cotton and clothes made of natural fibres were worn. Control samples were run.	[13]
water	Canada, Vancouver Island, Baynes Sound	Yes	5.28 (±4.17) items/L (1 L jar samples) 0.91 (±0.76) items/L (10 L bucket samples)	Sample analysis was carried out in a laminar flow hood. Coveralls made of cotton and headscarves were worn. The microscope was enclosed in a clear plastic cover during visual identification. Procedural blanks were run.	[14]
sediment	Baltic Sea, Isle of Rügen	Yes	88.10 items/kg dry weight or 2862.56 items/m^2	Samples were covered with watch glasses between all processing steps. The humidity in the laboratory was increased. Laboratory coats made of cotton were worn. Blank tests were run.	[15]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water	South China Sea, Zhubi Reef	Yes	from 1400 to 8100 items/m^3	All tools used for sampling were washed with purified water before use. After sampling, the samples were sealed. Laboratory coats made of cotton were worn. Containers made of glass were used instead of plastic ones. Containers were rinsed three times with clean water and dried (120 °C, 4 h) before use. Blank controls were run.	[16]
water	Germany, Oldenburg–East Frisian water board	Yes	from 0 to 7 items/m^3	Filter units were rinsed with Milli-Q water prior to use. Laboratory coats made of cotton and clothes made of natural fibres were worn. Work surfaces were cleaned with 30% ethanol. All equipment was rinsed with Milli-Q water and ethanol before use, and then covered with aluminium foil. Samples were covered with aluminium foil. Blank samples were run.	[17]
water, sediment	Spain, Ebro Delta	Yes	3.5 (±1.4) items/m^3 (surface waters) 422 (±119) items/kg dry weight (sandy beaches) 2052 (±746) items/kg dry weight (estuarine benthic sediments)	Sample analysis was carried out in a laminar flow cabinet. Laboratory coats made of cotton were worn. Glass materials or stainless-steel materials were used when possible and all equipment was rinsed two times with Milli-Q water before use. Blanks were run.	[18]
water	Baltic Sea, Denmark, South Funen Archipelago	Yes	0.07 (±0.02) items/m^3	Laboratory coats made of cotton were worn. Work surfaces were moistened prior to sample handling. The humidity in the laboratory was increased. Sample analysis was carried out in a fume hood. Samples were kept covered, e.g., with watch glasses. Procedural laboratory blanks were run.	[19]
sediment	United Kingdom, Birmingham, Edgbaston Pool	Yes	up to 25–30 items/100 g dry weight	Laboratory coats made of cotton were worn and clothes made of synthetic fibres were avoided. Samples were kept covered. All samples were processed by one person who used latex gloves. Whenever possible, nonplastic equipment was used. Plastic equipment was checked for its optical properties under the microscope. Procedural blanks were run.	[20]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water	China, Wuhan, Hanjiang River and Yangtze River	Yes	from 1660.0 (±639.1) to 8925 (±1591) items/m^3	Laboratory glassware was rinsed three times with distilled water before use and covered with tin foil after each step. Laboratory coats made of cotton and nitrile gloves were worn. Workplace for stereomicroscopic inspection was thoroughly cleaned prior to sample analysis. Field blank tests and method blank tests were run.	[21]
water, sediment, biota	China, Qinghai Lake	Yes	from $0.05 \cdot 10^5$ to $7.58 \cdot 10^5$ items/km^2 (lake surface water) from $0.03 \cdot 10^5$ to $0.31 \cdot 10^5$ items/km^2 (inflowing rivers) from 50 to 1292 items/m^2 (lakeshore sediment) from 2 to 15 items/individual (fish samples)	Trawl was triple rinsed three before each sampling. Laboratory coats made of cotton, nitrile gloves, and shower caps were worn. All containers were covered with aluminium foil. Sample pretreatment was carried out in a fume hood. Sticky roller was used to clean the desktop, hands, and clothes. Blank controls were run.	[22]
water	China, urban estuaries	Yes	from 100.0 to 4100.0 items/m^3	All equipment was triple rinsed with filtered Milli-Q water and then covered with tin foil. Sample preparation was carried out in a clean laminar flow cabinet. Laboratory coats made of cotton and nitrile gloves were worn. Work surfaces in the laminar flow cabinet were cleaned with 70% alcohol. Laboratory operators cleaned their hands and forearms before analysis. The microscopic inspection stand was cleaned prior to sample analysis. Control samples were run.	[23]
biota	Red Sea, Saudi Arabian coast	Yes	26 items in 178 examined individuals	Fish samples (the gastrointestinal tract of fish) were kept capped in Falcon tubes until analysis. Dissection tools were cleaned with 70% ethanol prior to use. Nitrile gloves, laboratory coats and clothing made of cotton were worn. Blank samples were run.	[24]
water	Atlantic Ocean, transect from the European Coast to the North Atlantic Subtropical Gyre	Yes	from 13 to 501 items/m^3	Sampling devices and filter meshes were cleaned with deionised and Milli-Q water prior to use. After sampling, samples were transferred to a Petri dish and sealed. Laboratory coats, head covers, and nitrile gloves were worn. Laboratory operators washed their hands and forearms before analysis. Laboratory work surfaces were cleaned before use. All instruments and vials were washed with Milli-Q water before use. Analysis devices were protected with aluminium foil when not in use. Control samples were run.	[25]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water	Jamaica, Kingston Harbour	Yes	from 0 to 5.73 items/m^3	Glass jars used to store the samples were cleaned with dilute nitric acid and rinsed with deionised water. Laboratory glassware and stainless steel tools were used during sample processing. Aluminium foil was used to cover samples. Laboratory coats and nitrile gloves were worn. The windows in the laboratory where the microscopic analysis was carried out were sealed and access to the laboratory was restricted. Procedural blanks were run.	[26]
air	Iran, Asaluyeh County	Yes	900 items per 15 g of sample	All reagents and distilled water were filtered (through blue band filters) before use. Working surfaces were washed with ethanol. Laboratory glassware was cleaned with distilled water. Windows and doors were closed. Cotton laboratory coats, latex gloves, and face masks were worn. Samples and containers were covered with aluminium foil. Control samples were run.	[27]
water	Antarctica, Ross Sea	Yes	0.17 (±0.34) items/m^3	Clothing made of cotton was worn. Latex gloves were worn when sorting and counting microplastics. Whenever possible, glassware was used instead of plastic. In cases where plastic equipment had to be used, it was washed with ethanol before use. Filters were stored in glass Petri dishes sealed with Parafilm. Blank samples were run.	[28]
water	Baltic Sea	No	from 0.07 to 2.6 items/L	All equipment, including filters, was rinsed with tap water prior to sampling. Each filter was sealed in a separate ziplock PE bag until analysis in the laboratory. Samples were examined under the microscope immediately after removal from the PE bags. Notes were made on the colour of the ship deck, colours and material of the crew clothing, etc., as possible sources of contamination.	[30]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water	Northeast Atlantic Ocean	Yes	2.46 items/m^3	Collected samples were wrapped in aluminium foil. Laboratory coats, gloves and clothing made of cotton were worn. Laboratory glassware were cleaned with filtered water prior to use and covered after each step. Work surfaces were wiped with alcohol prior to sample analysis. All equipment were cleaned before use and examined under a microscope for airborne contamination. Control samples were run.	[31]
sediment	Italy, Po River	Yes	from 2.92 to 23.30 items/kg dry weight	PE bags in which the samples were stored for transport to the laboratory were checked for abrasion. Whenever possible, stainless steel and glass were used. Samples were covered with glass lids or aluminium foil. All reagents and water used for sample processing were filtered (over stainless steel mesh with pore size of 5 µm) before use. Cotton laboratory coats were worn during sample processing.	[32]
water, sediment	Portugal, Antuã River	Yes	from 58 to 193 items/m^3 (water sample from March) from 71 to 1265 items/m^3 (water sample from October) from 100 to 629 items/kg (sediment sample from March) from 18 to 514 items/kg (sediment sample from October)	Samples were covered and sealed with aluminium foil or Parafilm after each procedure. Equipment and work surfaces were cleaned before and during each step. Control samples were run.	[33]
water	Indonesia, Java, North Coast of Surabaya	Yes	from 0.38 to 0.61 items/L	Filtered samples were stored in a Petri dish closed with Parafilm sealing film. All instruments used for analysis were sterilised. Double-distilled deionised water was used in the laboratory procedures, e.g., to rinse laboratory equipment. Procedural blanks were run.	[34]
water	Pacific Ocean, Snake and Lower Columbia Rivers	Yes	from 0 to 5.405 items/L (grab samples) from 0 to 0.014 items/L (net samples)	Exposure time of the samples to air was limited. Samples were kept covered with jar lids, aluminium foil or glass eyewashes. Laboratory glassware and equipment were cleaned with Decon 90, triple rinsed with tap water, and then once rinsed with filtered water. Clothing made of cotton and white laboratory coats were worn. Samples were filtered in an air cabinet. After filtration, filters were kept in Petri dishes until visual identification. Control samples were run.	[35]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water	Atlantic Ocean, transect from the Bay of Biscay to Cape Town	Yes	1.15 items/m^3	Laboratory coats, gloves and clothing made of cotton were worn during sample handling. Stainless steel sieve was covered with a wooden cover. All containers were covered and cleaned with distilled water prior to use. Method blanks and controls were run.	[36]
water	Turkey, Mersin Bay	No	539,189 items/km^2 (before the flood) 7,699,716 items/km^2 (after the flood)	Laboratory equipment was rinsed with deionised water before and after each use. Procedures were carried out as quickly as possible. All used containers were kept closed. Sample analysis was carried out in a fume hood. Microscopic analysis of the samples was performed using a microscope in a plastic cover.	[38]
water	Italy, Tuscan coast	Yes	0.26 items/m^3	All materials used for sampling were cleaned before use. Glassware was used during laboratory procedures. Sample analysis was carried out in a clean air flow cabinet. Blank controls were run.	[39]
water, biota	Baltic Sea	Yes	0.21 (±0.15) items/m^3	All equipment was rinsed with acetone prior to use. Direct contact with filters and samples was limited. The use of plastic bottles was avoided. Sample processing, apart from microscopic observations, was carried out in a fume hood. Controls and blank samples were run.	[40]
water, sediment	China, Pearl River	Yes	0.57 ± 0.71 items/L (river water) 685 ± 342 items/kg dry weight (river bed sediment) 258 ± 133 items/kg dry weight (estuarine sediment)	Buckets and nets used for sampling were wrapped in aluminium foil and kept covered in stainless steel barrels. Laboratory coats and gloves were worn. Laboratory glassware was cleaned with distilled water and covered with aluminium foil prior to use. Sample pretreatment were carried out in a fume hood. Visual identification was performed on an ultraclean laboratory bench. All reagents and solutions were filtered (through 0.7 mm glass microfibre filters) before use. Procedural and laboratory blanks were run.	[41]
biota	Mediterranean Sea, Northern Sicilian coasts	Yes	2.7 items/specimens	Sample handling was performed in a closed room, access to which was strictly limited. Workspaces and equipment were cleaned prior to microplastic extraction. Samples were exposed to air for the shortest possible time. During microscopic analysis, microfibres were placed on corning slides and covered with a coverslip. Control blanks were run.	[43]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water, sediment	France, Brittany, Bay of Brest	Yes	0.24 (±0.35) items/m^3 (surface water) 0.97 (±2.08) items/kg dry weight (sediment)	Glass materials were used instead of plastic. All equipment was rinsed twice with distilled water, once with 70% ethanol, and then covered. Laboratory coats made of cotton were worn all the time. Work surfaces were wiped with 70% ethanol. Field and laboratory procedural blanks were run.	[44]
water	Germany, Berlin, urban watercourse	Yes	from 0.01 items/L to 95.8 items/L	All reagents were filtered (through 0.45 mm cellulose acetate filters) before use. Work surfaces were rinsed several times with pure water and 30% ethanol. Nonsynthetic clothing was worn. Blind controls were run.	[45]
air	Germany, Hamburg	Yes	275 items/m^2/day	Samples were covered and kept under fume hoods. Laboratory glassware was rinsed with acetone prior to use. The humidity in the laboratory was increased. An air filter was used. Procedural and laboratory blanks were run.	[46]
water, sediment	Bohai Sea	Yes	from 0.4 to 5.2 items/L	All tools and containers were cleaned with Milli-Q water prior to use. Devices used for sampling and analysis were covered with aluminium foil. Sample analysis (separation and identification) was conducted in a laboratory adapted to the analysis of microplastics. Nontextile robes, gloves, and head caps were worn. Procedural blanks were run.	[47]
water	United States, Western Lake Superior	Yes	from 0 to 110,000 items/km^2	Collection vessel of the Manta net was rinsed three times with Milli-Q water before use. Clothes made of cotton or wool were worn. Control samples were run.	[48]
water	Changjiang Estuary and East China Sea	Yes	157.2 (±75.8) items/m^3 (Changjiang Estuary) 112.8 (±51.1) items/m^3 (East China Sea)	In the field, all containers were rinsed with filtered in situ seawater prior to use. HDPE tanks and steel sieves were rinsed with Milli-Q water before use and covered with aluminium foil. All reagents were filtered (through 0.45 mm glass filters) before use. Laboratory glassware was rinsed with Milli-Q water and heated (450 °C, 8 h) before use. Clean glassware was covered with combusted aluminium foil. Steel tweezers were rinsed with Milli-Q water and sterilised before use. Sample processing was carried out in a clean laminar flow cabinet. Laboratory coats made of cotton and nitrile gloves were worn. Procedural blanks were run.	[49]

Table A1. *Cont.*

Type of Sample	Sampling Location	Control Samples (Yes/No)	Level of Microplastic Contamination (±SD)	Cross-Contamination Mitigation Methods	Ref.
water	UWA, New York City, Hudson River	Yes	0.985 items/L	All equipment was rinsed three times and covered before use and between sample processing. The sampler collector rinsed their hands and forearms three times and had bare wrists, i.e., without any accessories such as watches or bracelets. Air and water blanks were run.	[50]
water	Japan, rivers	Yes	1.6 items/m^3	Prior to use, sampling net was rinsed with river water collected and filtered in situ. Equipment made of plastic was avoided whenever possible. Laboratory robes made of cotton were worn. Blanks were run.	[53]
water	Western Mediterranean Sea	No	0.10 (±0.09) items/m^2	Manta trawl used for sampling was deployed 2 m from the ship's hull to avoid potential ship-based contamination. All materials used for sampling (plastic and nonplastic) were checked and rinsed with filtered seawater prior to use. Control samples were not run, but plastic fibres were excluded from further analysis to prevent potential bias in results caused by airborne contamination.	[56]

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