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How to Control the Airborne Contamination in Laboratory Analyses of Microplastics?

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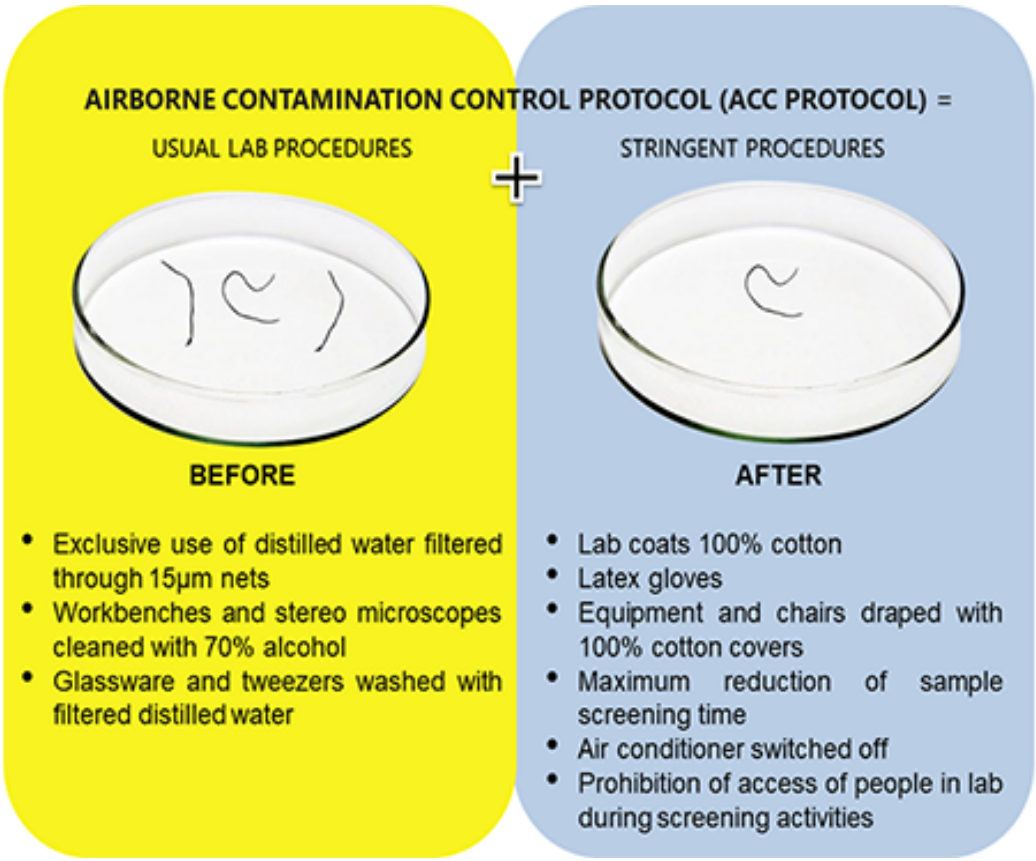
HIGHLIGHTS

- The contamination of blanks by airborne fibers in the lab was investigated.
- Microfibers could be overestimated in fish's gastrointestinal tract and surface water.
- We tested and approved an Airborne Contamination Control Protocol (ACC Protocol).

Abstract: Numerous studies have quantified microplastics in biological and environmental samples in recent years, but contamination by airborne microplastic particles during laboratory analysis remains an unsettling possibility. We designed and tested a protocol to minimize airborne contamination during the screening of samples in laboratory conditions in order to increase the level of certainty that microplastics counted really comes from samples. Despite the care and default measures in laboratory routine, some airborne contamination in blanks was found (3.8%) at the beginning of sample screenings. After introducing more stringent procedures on our airborne contamination control protocol (ACC Protocol), a highly significant ($p<0.0001$) reduction was registered (1.1%). Thus, we prove that the use of a more stringent protocol should be an essential part of future studies quantifying microplastics in any samples. This study concludes that a protocol with simple, low-cost, but stringent measures can reduce airborne microplastic contamination, being applicable to any laboratory setting.

Keywords: Microfibers; Dust; Quality laboratory control; Methodology; Strict protocol.

GRAPHICAL ABSTRACT



INTRODUCTION

Plastic pollution is ubiquitous in the marine environment and has become a major concern [1,2] due to the negative consequences to the composition of the marine habitat and the possibility of altering the functioning of ecosystems [3]. These consequences are the result of improper disposal, physical fragmentation and accidental loss from solid waste, which has led to an increase in the quantity of plastics and, consequently, microplastics in the environment [4].

Such debris poses a threat to the marine ecosystem [5], where pollutants are more common and persistent [6,7]. The regions adjacent to this environment also receive pollutants from industrial, domestic and agricultural effluents, which are often discharged directly into the water without adequate treatment. Moreover, industrial emissions, particle resuspension and other anthropogenic causes, such as traffic, construction and urban infrastructure, are potential sources of plastics in the atmosphere [8]. The resuspension of fractions of polymers and the release of microplastics (MPs) from synthetic fabrics, 3D printing, etc. are among the main sources of airborne contamination by MPs [9,10,11].

Microplastics are a growing problem, because their small dimensions [1,12,13,14] they are transported in large quantities by air [15] in dry or humid climates, covering long distances [16], especially the plastic particles lighter which may stay on airborne for longer periods [17], for up to 6.5 days average [18].

A large part of MPs transported through the air are found in closed environments [19,10]. This is a worrisome factor in screening performed in the laboratory setting, as the risk of airborne contamination by MPs is constant and results from inadequately cleaned instruments, clothing made from synthetic fabrics and household dust fallout [19,20], which can affect the methods and objectivity of laboratory analyses and interfere with the results [20], that most of the times do not consider the blanks [21].

Therefore, the Ichthyology Laboratory (LabIctio) at UEPB performed a detailed analysis of the incidence of airborne MPs in a controlled environment during screening related to studies conducted in the LabIctio. So it was developed a protocol of practical measures adopted to eliminate airborne contamination by MPs in the laboratory. Therefore, the aims of the present study were to 1) compare the incidence of MPs in blanks (defined as open Petri dishes left on the workbenches) related to samples screened in the LabIctio before

and after the improvement of a protocol for reduction in airborne contamination by MPs and 2) increase the reliability of the sampling method employed in the screening of MPs in the laboratory environment.

MATERIAL AND METHODS

Data sampling

This work was performed during the development of MP quantification studies in the LabIctio involving samples from three biological and environmental compartments: 1) gastrointestinal tract of small fishes sampled from shallow estuarine waters; 2) gastrointestinal tract of *Micropogonias furnieri* (Desmarest, 1822), a commercial fish (Whitemouth croaker) of the family Sciaenidae; and 3) surface water from an urban estuary with high anthropogenic impact, the Paraiba River Estuary [22]. During the analysis of the blanks from the three ongoing studies, standardization in the lab procedures was determined to reduce and eliminate airborne contamination during the screening for MPs.

Along the “before” period (first three months of screening), the usual lab procedures to avoid sample contamination included the use of 15µm screen to filter distilled water, all glassware and tweezers were washed with filtered distilled water, workbenches and stereoscopic microscope were cleaned with 70% alcohol, lab coats and gloves were worn during the screening procedures of the three kinds of samples mentioned. During these three months, the MPs recorded in the blanks revealed some airborne contamination, prompting the adoption of a set of stricter measures in addition to the usual lab procedures.

Airborne Contamination Control Protocol

The ACC Protocol consisted of such as even greater care in washing the 15µm net to prepare distilled water and 70% alcohol; all equipment and chairs in the lab were draped with 100% cotton covers; 100% cotton lab coats were worn and latex (rubber) disposable gloves were used rather than vinyl (polymer) gloves during the screening of the three materials used in the study. The air conditioner of the lab remained switched off; the samples were exposed to the lab environment for the least amount of time possible; and the door to the lab remained closed during the processing of the samples to avoid the entrance of people and minimize the occurrence of airborne contamination from the external environment.

The “after” period began at the fourth month of screening at the LabIctio, where each of the three Petri dishes used as blanks underwent three processes to improve the reliability of the procedures employed and for better control of the airborne contamination recorded earlier: 1) washing three times in filtered distilled water; 2) analysis under a stereoscopic microscope prior to exposure for determination of the presence of MP; and 3) after the absence of contamination by MPs was determined, the dishes containing filtered distilled water were placed alongside the stereoscopic microscope during the period in which the materials (gastrointestinal tracts and surface water) were being examined (Figure 1). This entire protocol was standardized for the determination of contamination by MP in the LabIctio before and after each sample being screened. In the same way as the MPs of the three materials analyzed, plastic fragments and MPs from the blanks were quantified and classified according to their physical characteristics into fibers, fragments and films as well as their original coloration.

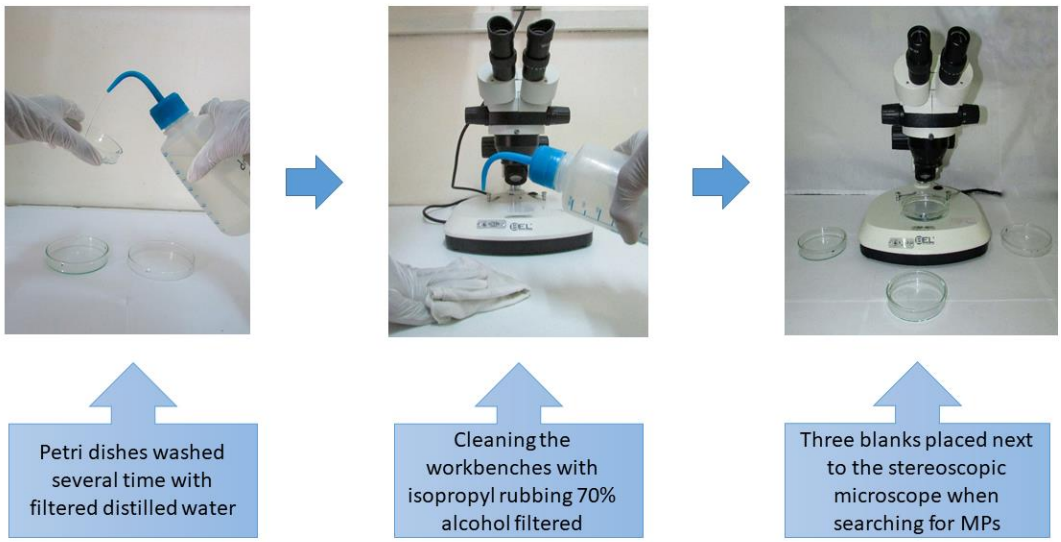


Figure 1. Airborne Contamination Control Protocol scheme.

Treatment of data

The Shapiro-Wilk test was used to assess the normality of the data. As non-normal distribution was demonstrated, the Mann-Whitney test was used for the comparison of means, with the level of significance set to 1% ($p < 0.01$). Two samples were compared. The first group corresponded to the number of MPs found in the blanks prior to the adoption of the ACC Protocol and the second group corresponded to the number of MPs found in the blanks after the more stringent ACC Protocol was adopted in the lab. The statistical analysis was performed with the R Studio software. March to May/19 was considered the “before” period (three months x three studies, n = 09) and June/19 to February/20 was considered the “after” period (nine months x three studies, n = 27). This difference in sample size was due to the measures required for the reduction of airborne contamination and the validation of the ACC Protocol adopted at the LabIctio.

RESULTS

A total of 5544 blanks were analyzed and 118 airborne MPs were found during the analyses of the three studies at the LabIctio (Table 1). In these blanks analyzed, all MPs detected were microfibers (Figure 2), most of which were either transparent (67) or blue (36), with a low incidence of black (13), pink (1) and red (1). Airborne MP contamination level on blanks was 3.8% (80) before adoption of ACC Protocol and it fell to 1.1% (38 MPs) after the adoption of the ACC Protocol for eliminate airborne contamination, considering three studies conducted in the last nine months in the LabIctio (Table 1). The comparison between the “before” and “after” periods revealed a highly significant difference ($p<0.0001$, Mann-Whitney test) in airborne MP contamination (Figure 3).

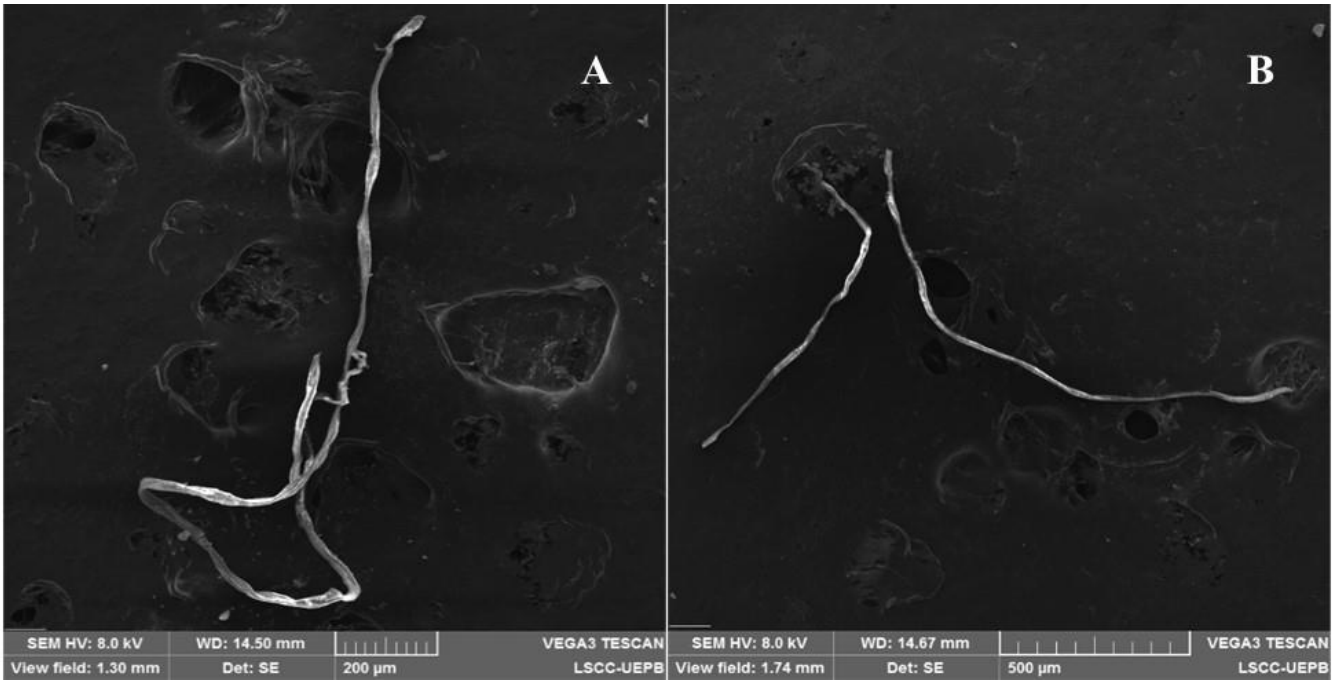


Figure 2. Photos of microfibers 200 µm (A) and 500 µm (B) taken in Scanning Electron Microscope (SEM).

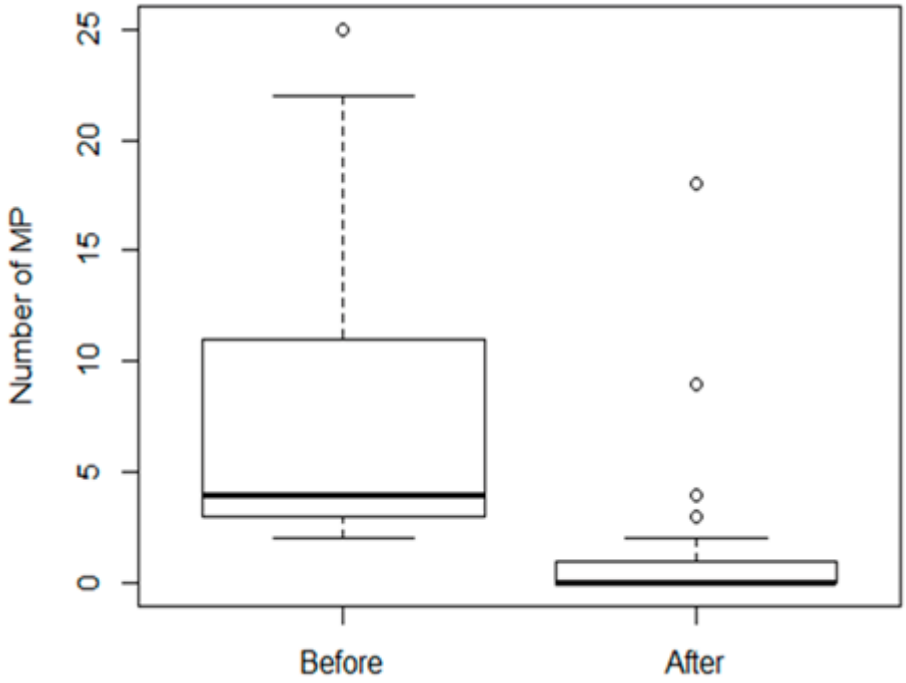


Figure 3. Number of MPs before (n=09) and after (n=27) adoption of ACC Protocol more stringent.

As expected, the largest number of MPs (39) in the blanks was recorded in March/19 (Table 1), followed by a significant reduction in subsequent months, especially beginning with June/19, when the ACC Protocol was adopted at the LabIctio. Between October/19 and February/2020, there were no further records of MPs in the blanks analyzed always in the same way and for the same time as the screening of the biological and environmental studied samples (gastrointestinal tract of estuarine fishes and Whitemouth croaker, and surface estuarine waters). In a similar way, the rate between MPs number and blanks show the effective fall before and after an ACC Protocol more stringent (Figure 4). The confirming that their adoption was adequate and that the simple procedures taken lead complete elimination of airborne contamination in the laboratory confirm that the objective of this study has been reached.

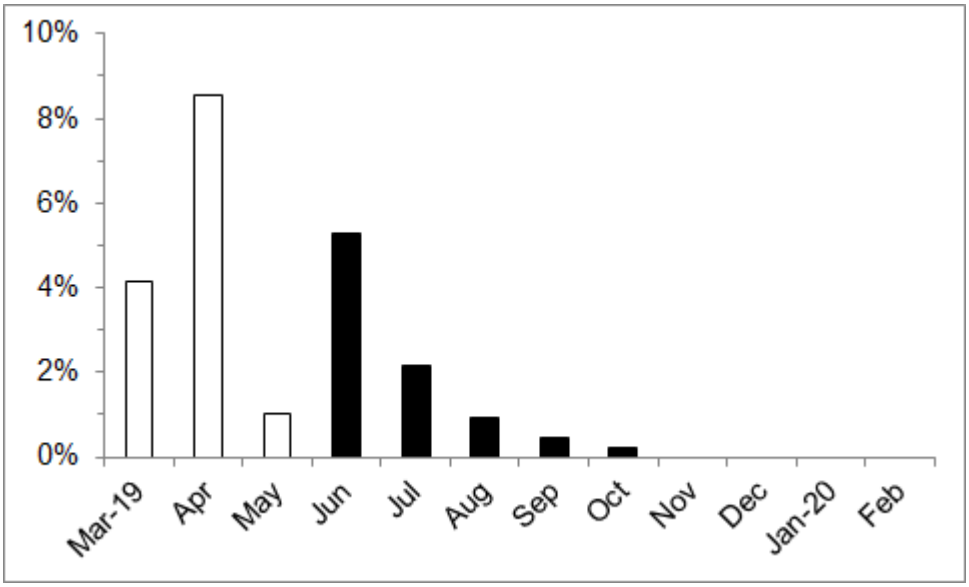


Figure 4. Percentage of MPs airborne contamination on blanks before (white bars) and after (black bars) adoption of ACC Protocol more stringent.

Table 1. Number of samples, blanks and MPs by month before (n=2106) and after (n=3438) adoption of ACC Protocol more stringent.

	Month	N° of samples	N° of blanks	N° of MPs
Before	March/19	312	936	39
	April	129	387	33
	May	261	783	8
	Total	**702**	**2106**	**80**
After	June	120	360	19
	July	170	510	11
	August	141	423	4
	September	233	699	3
	October	145	435	1
	November	59	177	0
	December	74	222	0
	January/20	104	312	0
	February	100	300	0
	TOTAL	**1146**	**3438**	**38**

DISCUSSION

The current production of most synthetic materials includes some type of fiber [23-24], which certainly leads to airborne contamination in closed environments, as reported by Dris and coauthors [25], Catarino and coauthors [10] and the present investigation. The predominant coloration of MP found in blanks in the present study was transparent, which is in agreement with data reported by Prata and coauthors [26] also on a laboratory setting. However, other colors from airborne contamination, such as blue, black, pink and red,

are commonly recorded [27], the likely origins of which are common garments, equipment and polymers in laboratories or even naturally present in the surrounding air [19,28-30].

The measures adopted in this study for the control of airborne contamination were effective, as evidenced by the reduction in airborne contamination during screening of different research material at the LabIctio (31). Thus, the desire to eliminate the occurrence of airborne contamination on workbenches and, consequently, in the samples being analyzed resulted in the development and use of a protocol that involves the prior preparation of laboratory equipment and the use of fabrics that do not release fibers or have synthetic polymers in their composition, such as the 100% cotton covers for equipment and chairs. According to Prata and coauthors [26], quality assurance measures should be more strictly applied when working with airborne fibers and microplastics, as particles from different sources of indoor and outdoor air pollutants can exert an influence on the quantification of MPs in samples [23].

Despite the care taken with the usual lab procedures at the onset of the screening, MPs in the blanks revealed contamination possibly related to small failures during the procedures, such as the movement of individuals within the laboratory during the processing of samples, as reported by Wesch and coauthors [30]. With the adoption of more stringent measures, we demonstrated that such a protocol control is essential when any material is being analyzed for the quantification of MPs in the laboratory, to ensure greater reliability the results of samples screened indoors.

The procedures performed with air circulation systems switched off, all materials duly cleaned and the maximum reduction of the exposure of the samples to the laboratory environment were of considerable importance to the development of a protocol that effectively eliminated most of airborne contamination during screening for MPs in the laboratory. Despite this evident reduction found in blanks over the months, the same did not happen with MP in our biological and environmental samples analyzed (personal communication). The search for avoid overestimation of MP incidence in laboratory screenings activities is highly recommended. Even with enhancement and commitment of the lab team, this goal requires the need to constant evaluate and restrict on the effects of airborne contamination. This also was described by Prata and coauthors [26] when no field blanks are conducted to evaluate the airborne fiber concentrations or as source of contamination indoor and by Torre and coauthors [20] when they demonstrate serious bias that may falsify the gut content analysis resulting in overestimation of the actual microfibers ingestion by marine biota.

CONCLUSION

The ACC Protocol effectively reduced airborne MP contamination, and hence increased the level of certainty that the MPs encountered were from the samples themselves and not from airborne contamination, underscoring the care that all researchers need to take in their laboratory routines. This study shows that simple, low-cost measures applicable to any workplace may be effective and important to future studies, which quantify MPs in biological and environmental samples ensuring greater reliability and quality in the final results of such samples.

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