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Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination

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ABSTRACT

Research studies on the effects of microlitter on marine biota have become more and more frequent the last few years. However, there is strong evidence that scientific results based on microlitter analyses can be biased by contamination from air transported fibres. This study demonstrates a low cost and easy to apply methodology to minimize the background contamination and thus to increase results validity. The contamination during the gastrointestinal content analysis of 400 fishes was tested for several sample processing steps of high risk airborne contamination (e.g. dissection, stereomicroscopic analysis, and chemical digestion treatment for microlitter extraction). It was demonstrated that, using our methodology based on hermetic enclosure devices, isolating the working areas during the various processing steps, airborne contamination reduced by 95.3%. The simplicity and low cost of this methodology provide the benefit that it could be applied not only to laboratory but also to field or on board work.

1. Introduction

During the last years an increasing number of organisms have been documented to ingest marine litter (macro and micro litter items), alarming their potential input and accumulation in the trophic web and the potential risks on marine life and also human health. A growing number of studies demonstrated that marine litter can be harmful for a wide range of marine organisms either due to entanglement or due to ingestion (CBD and STAP-GEF, 2012; Kühn et al., 2015). Last years, concern is increasing about the threat of microplastics to marine life as they are considered to be responsible not only for physical harm by ingestion, but also for the transportation of harmful chemicals through the food web (UNEP, 2014; GESAMP, 2015).

Microlitter, defined as particles smaller than 5 mm (Arthur et al., 2009) can be ingested by marine organisms (Woodall et al., 2015 and references there in). Phuong et al. (2016), reviewing microplastics ingested by marine organisms found discrepant results possibly due to artefacts caused by background contamination. Fibres are one of the forms in which microplastics can be found (Dris et al., 2015) and contamination problems are related mainly to them. This is because microfibres are ubiquitous in the marine ecosystems (Phuong et al., 2016), in the atmospheric fallout (Dris et al., 2015), in the everyday human working and living environment and they can be easily transported by the air (airborne microfibres). Thus, the risk of artefacts by microfibre background contamination is a crucial concern that can affect the objectivity of the methodology used in microlitter analyses and the outcoming results. The high probability of background contamination by airborne microfibres during sample collection and laboratory analysis has been well documented by several authors (Roux et al., 2001; Fries et al., 2013; Woodall et al., 2015; Duis and Coors, 2016).

Samples can always become contaminated by fibres (natural or synthetic) present in the air of the laboratory, on the clothes of workers or in poorly cleaned instruments (Hidalgo-Ruz et al., 2012). Studies on microlitter in marine biota have adopted two different methodologies in order to overcome background contamination by microfibres: 1) contamination mitigation techniques and inclusion of microfibres in the analysis 2) total exclusion of microfibres to avoid risks of artefacts. The first methodology is based on controlled clean laboratory procedures and background contamination control by using blank filters (Cauwenberghe et al., 2013; Nuelle et al., 2014; Witte et al., 2014; Woodall et al., 2014; Collard et al., 2015; Devriese et al., 2015; Lusher et al., 2013; Lusher et al., 2015; Woodall et al., 2015). The second methodology has also been found in several works (Davison and Asch, 2011; Fries et al., 2013; Foekema et al., 2013; Besseling et al., 2015). However, excluding microfibres may bias the quantification and interpretation of the effects of marine microlitter pollution (Woodall et al., 2015). Moreover, clean lab protocols (Woodall et al., 2015) could be considered expensive or difficult to be applied since the same installations are also used for various purposes.

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Fig. 1. Stereomicroscope visual area isolated by plastic cover.

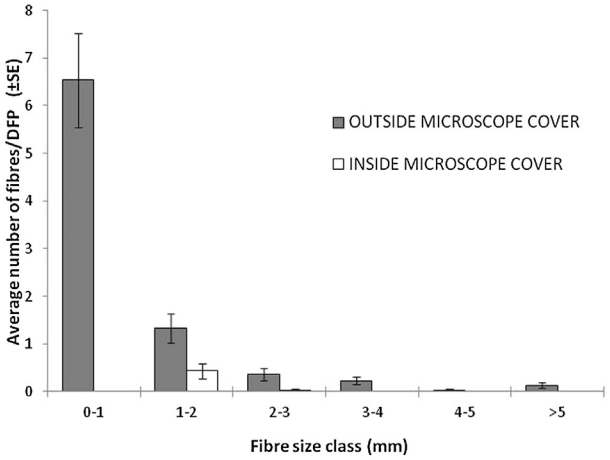

Fig. 2. Average number of fibres (±standard error) recovered from Dampened Filter Papers (DFPs) inside and outside the microscope cover grouped by size classes.

The aim of this study is 1) to describe the extent of the airborne contamination by microfibres and 2) to propose a low cost and easy to apply methodology in order to minimize the external contamination by airborne microfibres during fish sample processing for gut content litter analysis.

2. Material and methods

The methodology applied in this study was based on the utilization of hermetic enclosure devices during the steps of high risk airborne contamination. The efficiency and feasibility of this methodology was tested during two critical steps of samples processing: gastrointestinal contents sorting under stereomicroscope and microplastic extraction (chemical digestion and filtration) from gastrointestinal contents.

Some general precaution measures were adopted to minimize the risk of airborne contamination. All equipment was thoroughly washed before use with purified water (Milli-Q) and surfaces were deeply cleaned. All personnel wore natural fibre clothing and doors and windows were kept shut. All liquids used in our experiments were tested for contamination (purified H_2O and H_2O_2) by filtering twice.

For the gastrointestinal content analysis, samples of 3 demersal fish *Mullus barbatus* (Linnaeus, 1758), *Citharus linguatula* (Linnaeus, 1758), *Pagellus erithrinus* (Linnaeus, 1758) and a pelagic one *Sardina pilchardus* (Walbaum, 1792) were collected during experimental trawl surveys carried out off Corfu Island (Eastern Ionian Sea) during September 2014 and April 2015. Fish were frozen soon after hauling and stored at −15 °C. In the laboratory, the samples were defrosted at 5 °C prior examination. Fish were dissected and stomach and intestine removed quickly and transferred in plastic vials immediately until the next processing steps to avoid contamination.

2.1. Description and testing of the methodology during stereomicroscope analysis

A plastic cover, isolating the stereomicroscopic observation area was used in order to prevent the flow-in of airborne transported contaminants and minimize the microfibres background contamination (Fig. 1). Sample manipulation instruments were inserted into the observation area through holes in the plastic cover wall, and handled externally. Similarly, small holes adjusted to the ocular lenses and focus knobs permitted their use.

Dampened Filter Papers (DFPs) (7 cm diameter) were placed into clean plastic petri dishes inside and outside the microscope cover in order to collect any air-borne microfibre contamination inside and outside the microscope cover. A total of 30 couple replicates (inside and outside the cover) were used in order to test the repeatability of the trends observed. The time of exposure for each replicate was analogous to the mean time needed for a gut content analysis. DFPs were screened under the binocular stereomicroscope cover at 20× to 32× magnification and all fibres were counted. Their morphological characteristics

Table 1
Microfibres recorded in Dampened Filter Papers (DFPs) inside and outside the microscope cover, grouped by colours. The values represent the total number (N), the percentage, the average number (N) per DFPs and the occurrence (Occ.) of fibres recovered. Values in parentheses represent standard deviation.

Fibre colours	Outside microscope cover (30 replicates)				Inside microscope cover (30 replicates)			
	Fibres N	Fibres %	Average N	Occ. (%)	Fibres N	Fibres %	Average N	Occ. (%)
Black	117	45.17	3.90 (3.16)	93.33	9	64.29	0.30 (0.60)	23.33
Blue	90	34.75	3.00 (2.78)	83.33	3	21.43	0.10 (0.40)	6.67
Red	33	12.74	1.10 (1.83)	50.00	2	14.29	0.07 (0.25)	6.67
White	14	5.41	0.47 (0.97)	26.67				
Green	3	1.16	0.10 (0.40)	6.67				
Pink	1	0.39	0.03 (0.18)	3.33				
Yellow	1	0.39	0.03 (0.18)	3.33				
Total	259	100.00	8.63 (6.77)	100.00	14	100.00	0.47 (0.90)	26.67

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