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# Marine Pollution Bulletin



journal homepage: www.elsevier.com/locate/marpolbul

# Cross-contamination by COVID-19 mask microfibers during microlitter analysis of marine biota



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microlitter analysis on marine biota.

ARTICLE INFO	A B S T R A C T
Keywords: Face masks Covid-19 Microfibers Cross-contamination Marine biota Microplastics	Face masks have been adopted as an essential measure to prevent transmission and spread of the virus infection during the pandemic of Covid-19. The present study evaluates the potential microfibers transfer from face masks to other recipients and the potential cross-contamination of samples by microfibers released from masks worn during the analysis of microlitter ingestion by fish. Results indicated that masks could easily transfer endogenous (originated from the mask tissue itself) and exogenous microfibers (with a different origin than the mask tissue itself) to other recipients (adhesive tape and air in our experiment). Exogenous fibers may be carried from everywhere and potentially released everywhere. Microfibers are also released into the air, driven by the airflow generated by breathing, and can be transferred to blanks and samples. Microfiber contamination by facial masks increases the risk of samples cross-contamination and raises concerns about the results reliability of the

# 1. Introduction

Microplastics are particles sized <5 mm (Arthur et al., 2009) accumulating in marine habitats to a global extent (Gall and Thompson, 2015; Thompson et al., 2004). Single microplastic particles manufactured for commercial use are defined as primary, whereas secondary microplastics are being released into marine environments due to the degradation of larger plastic litter (Cole et al., 2011). Each year, >42 million tons of synthetically originated fibers are being produced only in the clothing industry for textile manufacturing purposes (Kelly et al., 2019); most of them end up on marine environments and thus became available to marine organisms. Due to their size, microplastics are easily ingested by marine functional groups raising concern for human health by entering the food chain (Sana et al., 2020). Ingestion of microplastics and marine litter in general, reported in a variety of marine taxa (e.g.: Anastasopoulou and Fortibuoni, 2019; Anastasopoulou et al., 2013; Anastasopoulou et al., 2018; Bordbar et al., 2018; Cole et al., 2013; Kühn and Van Franeker, 2020; Rummel et al., 2016), resulted in harmful effects on the affected organisms (Anastasopoulou and Fortibuoni, 2019). Litter ingestion by marine biota constitutes one of the European Union's descriptors utilized to assess the Good Environmental Status (GES) of the European Seas as established by the Marine Strategy Framework Directive (JRC, 2013).

Since the declaration of the Covid-19 disease as a pandemic by the World Health Organization (WHO, 2020), disposable personal protective equipment (PPE) has been adopted as an essential measure to prevent the spread of the infection. Several authors (Abedin et al., 2022a; Abedin et al., 2022b; Akhbarizadeh et al., 2021b; De-la-Torre and Aragaw, 2021; De-la-Torre et al., 2022b; Rakib et al., 2021) have shown how the indiscriminate disposal of PPE wastes and their accumulation in beaches, coastlines, rivers, and littering cities can be a significant source of microplastics, although the magnitude of this contamination is still unknown. Face masks, the most abundant type of PPE, if exposed to environmental conditions, may be compromised in their fibrous structure, leading to rougher surfaces, cracks, ruptures, and releasing of microfibers. The released microfibers become easily transportable to soil and/or marine environment with deleterious consequences (Akhbarizadeh et al., 2021b; Aragaw, 2020; De-la-Torre et al., 2022a; De-la-Torre et al., 2022b; De-la-Torre and Aragaw, 2021; Fadare and Okoffo, 2020; Rathinamoorthy and Balasaraswathi, 2022; Saliu et al., 2021; Shen et al., 2021; Wang et al., 2021). Masks release both high-density microplastics that may sink and reach the bottom sediments and lowdensity neustonic microplastics (Abedin et al., 2022b). Microplastics spread throughout the marine ecosystem and can be easily ingested by

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https://doi.org/10.1016/j.marpolbul.2022.113883

Received 1 March 2022; Received in revised form 3 June 2022; Accepted 22 June 2022 Available online 4 July 2022 0025-326X/© 2022 Elsevier Ltd. All rights reserved. aquatic organisms and transferred through the trophic web to different trophic levels. Aquatic organisms contaminated with microplastics can potentially be consumed by humans (Akhbarizadeh et al., 2020).

People, during the SARS-CoV-2 pandemic, used facial masks that can be grouped into the following categories (Chua et al., 2020; Duncan et al., 2021): i) garments repurposed for masks such as scarfs, neck warmers, bandanas, etc.; ii) mask made from household materials such as cotton T-shirt, scarf, etc.; iii) simple fabric masks of one or two layers; iv) fabric masks with an included layer specifically to augment filtration efficiency; v) disposable procedure/surgical masks of the kind used in health care settings; vi) certified filtering face piece respirators (FFR), such as FFP2, KN95 and N95 masks.

Masks made from household materials typically include synthetic and natural fabrics: clothing, silk, tissue paper, kitchen towels, pillowcase, etc. (Chua et al., 2020). Similarly, simple fabric masks can be made of natural (such as cotton, silk, and wool) or synthetic (polyester, rayon, acrylic, etc.) material. Disposable surgical masks, widely used during the COVID-19 pandemics, consist of three different layers manufactured from plastic polymers, especially polypropylene (PP). The outer layer is a nonwoven layer of spunbond PP with hydrophobic repellent treatment. The middle layer is a nonwoven filtering layer of meltblown PP. The inner layer is a nonwoven layer of spun-bond PP with hydrophilic surfactant treatment (Aragaw, 2020). FFRs, such as N95 masks, are spun-bond PP fabric for the outer and inner layer and melt-blown PP fabric for the filter layer (Yim et al., 2020). Fadare and Okoffo (2020) analyzing 3-ply face masks, indiscriminately abandoned in the environment at different stages of degradation, demonstrated that the outer layers are made of PP, while the inner layers are made of polyethylene high density. The authors pointed out that the indiscriminate disposal of 3-ply face masks could increase the accumulation of microplastics in the environment in a short time.

As of March 2020, in Greece, it became mandatory to wear facecovering masks in all enclosed places of work to protect people and prevent airborne transmission of the virus. Therefore, any laboratory process and sampling effort require the use of a personal face mask. The potential release of microfibers from worn masks poses significant issues related to the risk of external contamination during microlitter analysis of marine biota and the risk of artifacts that can affect the objectivity of the methodology used and the outcoming results. There are no data regarding the kinetic of microfibers from the personal face masks worn during lab analysis to date and our knowledge.

The objectives of the present study are to evaluate 1) the potential microfibers transfer from worn face masks to other recipients and 2) the potential contamination of samples by microfibers released from masks during samples processing for microlitter detection in fish gut contents.

# 2. Material and methods

# 2.1. Quality control analysis during samples processing

This study was carried out during the examination of microlitters in the gut contents of fish collected in the Eastern Ionian and Aegean Seas in 2020 within the frame of the MEDITS (Mediterranean Trawl Survey) survey (Fig. 1). During the laboratory analysis, the use of COVID-19 face masks was already mandatory.

Researchers adopted strict contamination control measures to guarantee good quality of the results. During sampling processing, staff wore gloves and lab coats, cleaned thoroughly equipment and

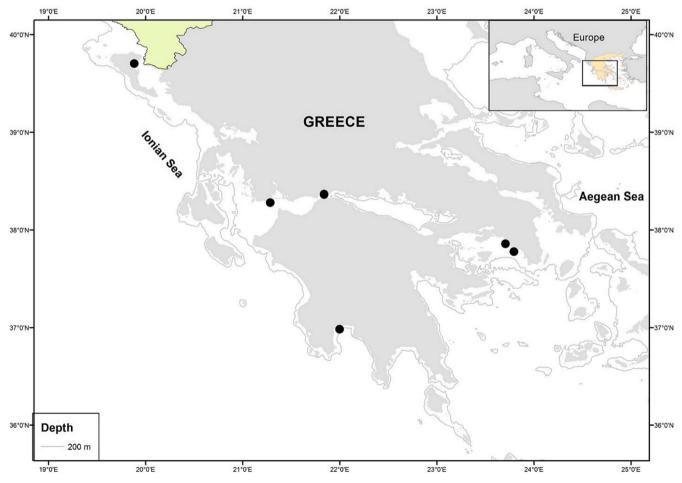


Fig. 1. Map of sampling locations in the Aegean and Eastern Ionian Seas in the Mediterranean.

workbench, turned off air conditioning, and implemented all processing and imaging steps under enclosed devices (Prata et al., 2021; Torre et al., 2016; Wesch et al., 2017).

For the microlitter analysis in gut contents, samples were defrosted and washed with 80  $\mu$ m filtered tap water to remove particles potentially retained on the fish surface during the onboard processing. Afterward, individuals were transferred under a glove box for the measurement of the biological parameters and dissection. For organic matter digestion, the extracted gut contents were weighed and transferred to glass vials in 10 % KOH solution (Honeywell Fluka<sup>TM</sup>, Germany). Vials were closed, removed from the glove box, and left on a hot plate adjusted at a constant temperature of 50° C until the digestion of organic matter was completed (Tsangaris et al., 2021). Digested stomach contents were filtered using two sieves of 250 and 80  $\mu$ m mesh size in sequence. Filtrates were rinsed with 80  $\mu$ m filtered water, transferred on a Petri dish, and analyzed under a covered stereomicroscope (Torre et al., 2016). Filtering was performed without enclosing devices.

During all the samples processing steps, control blanks, consisting of dumped papers in Petri dishes, were exposed to the air to detect airborne contamination by microfibers (Hermsen et al., 2017; Hermsen et al., 2018; Prata et al., 2021; Torre et al., 2016). Control blanks analysis was performed during samples processing before they were transferred under the enclosing devices (fish washing, transferring to the glove box and filtering of digested contents; External Control Blanks, ECB) and during samples processing under the enclosing devices (dissection and gut extraction; Control Blanks Cover, CBC) (Table 1). The time of blank exposure depended on the number of samples analyzed for each sampling event and ranged between 1 and 3 h.

Procedural blanks (Procedural Blank PB), constituted of a piece of muscle, liver, or gonad, clear of anthropogenic particles (Su et al., 2019), extracted from the processed samples were performed and processed in the same way as the extracted gut contents (Table 1).

ECB, CBC, and PB were observed under a covered stereomicroscope. Fibers were extracted, mounted on a microscope slide, measured, and classified according to their colors and origin (natural or synthetic) following the classification criteria of Prata et al. (2020). Briefly, natural fibers have an irregular surface and appearance; vegetable fibers appear flat and twisted; wool fibers present surface scales; synthetic fibers present, generally, a regular smooth surface and appearance. Fibers analysis was carried out with a stereomicroscope and microscope connected with a video camera (Sony Exwave HAD, Digital color videocamera), and the images were stored and analyzed with the Image Pro Plus Analysis software (Image pro Plus Vs. 4.5.29, 98/NT/2000 for Windows).

# 2.2. Surface face mask analysis

Fiber type composition of the surface of three different face masks (blue cotton mask: BCM; blue synthetic mask: BSM; green synthetic mask: GSM) worn for Covid-19 personal protection during the samples processing was analyzed. Fibers were sampled using the tape lifting technique, which is widely accepted in forensic science for crime scene analysis and allows the recovery of fibers able to be detached from a given surface by the adhesive tape strength (De Wael et al., 2008). This methodology will enable us to estimate the kinetics of the fibers released from the mask's surface to another recipient. Samples of  $20 \times 60 \text{ mm}^2 \text{ M}$  Scotch® adhesive tape were placed on the external surface of the mask, pressed, removed, and placed on a clean microscope coverslip of  $24 \times 60$  mm. Coverslips with the adhesive tape were mounted on a microscope slide for microscope examination. Sampling was repeated three times for each face mask. During microscope analysis, fibers were measured and classified in the same way as the fibers detected in blanks. The number of fibers detected was standardized in number of fibers/cm<sup>2</sup> of mask surface area.

# 2.3. Fiber releasing from masks to air

We estimated the actual microfibers releasing capacity from the face mask surfaces to the air by filtering the air exhaled through the mask. We utilized a sampling device consisting of a 6 cm length x 4.4 cm diameter PVC cylinder equipped with a filter sieve of 250  $\mu m$  mesh size to one end. The open end of the air filter device was positioned on the worn mask surface in correspondence with the oral fissure. Air was exhaled three times through the mask and filtering device from 3 different face mask areas. The exhaling strength during the experiment mimed the exhaling strength during the lab activities, and the filtered air for each exhaling act was standardized at 0.5 lt. Analysis and classification of fibers followed the same criteria for fibers in blanks and mask surfaces.

# 2.4. Endogenous and exogenous fibers

In this study, we considered as exogenous fibers, all those fibers that appeared under the microscope clearly different in regard to the synthesis (natural or synthetic) or color from the fibers that the mask tissue itself might produce (endogenous fibers). Examples: natural fibers on a synthetic mask, synthetic fibers on a cotton mask, red synthetic fibers on a blue synthetic mask, and red synthetic fibers on a green synthetic mask.

# 2.5. Data analysis

The fiber types of different origin and color found in the blanks (ECB, PB, CBC), the face mask surfaces, and fibers released by the air exhaled through the masks were compared using multivariate analysis (Cluster Analysis). Data were standardized and square root transformed to reduce the weighting of abundant fiber categories before calculating similarity matrices based on the Bray Curtis similarity index. In addition, similarity percentage analysis (SIMPER) was used to identify the fiber type contributing to the dissimilarity between the groups.

# Table 1

Total Number (N) and mean number ( $\pm$  standard deviation) of fibers detected in control blanks outside enclosing devices (ECB), control blanks inside enclosing devices (CBC), Procedural blanks (PB) and all blanks (TOT) according to their colors and origin (natural or synthetic). NC (Not Classified).

Fibers		ECB		PB		CBC		TOT	
Origin	Color	N	Mean N/Blank	N	Mean N/Blank	N	Mean N/Blank	N	Mean N/Blank
Natural	Black	12	0.14 (0.44)	3	0.03 (0.17)	1	0.04 (0.19)	16	0.07 (0.31)
	Blue	24	0.28 (0.98)	2	0.02 (0.14)	1	0.04 (0.19)	27	0.12 (0.63)
	Total Natural	36	0.42 (1.2)	5	0.05 (0.21)	2	0.07 (0.26)	43	0.2 (0.78)
Synthetic	Black	21	0.25 (0.74)	1	0.01 (0.1)	3	0.11 (0.31)	25	0.11 (0.49)
	Blue	10	0.12 (0.42)	2	0.02 (0.14)			12	0.05 (0.28)
	Red	5	0.06 (0.32)					5	0.02 (0.2)
	White-Transparent			1	0.01 (0.1)			1	0 (0.07)
	Total Synthetic	36	0.42 (0.98)	4	0.04 (0.19)	3	0.11 (0.31)	43	0.2 (0.66)
NC	-	24	0.28 (1.39)	12	0.11 (0.37)	0		36	0.16 (0.9)
ALL TOT		96	1.13 (2.28)	21	0.2 (0.48)	5	0.18 (0.39)	122	0.55 (1.53)

For the statistical analyses, the softwares STATGRAPHIC Centurion XVI (Version 16.1.11) and PRIMER 6 package (Clarke and Warwick, 2001) were used.

# 3. Results

# 3.1. Blank analysis

A total of 220 blanks (85 outside the glove box, ECB; 28 inside the glove box, CBC; 107 procedural blanks, PB) were examined during the analysis of microlitter in the gut contents of 236 fish. Microfibers were detected only in the 22 % of the 220 blanks examined. Overall, 122 microfibers were counted. The occurrence of microfibers in the blanks was less in PB (16 %) and CBC (18 %) than that in the ECB (32 %). Similarly, the mean number ( $\pm$  s.d.) of fibers per blank, was less in PBs (0.20  $\pm$  0.48) and in the CBCs (0.18  $\pm$  2.02) than that in the ECB (1.13  $\pm$  2.28) (Table 1, Fig. 2). Microfiber analysis demonstrated that synthetic and natural fibers were almost equally distributed in all blank types examined (Fig. 3). Black and blue were the dominant colors (Fig. 4). Of the 122 microfibers counted, 104 were measured and their size was ranged between 0.014 mm and 11.209 mm (mean 1.88 mm  $\pm$ 1.66). Most of them (96 %) were <5 mm. The mean size of the synthetic (n = 43) and natural (n = 42) microfibers were not differed (ANOVA; F = 0.52; p = 0.47).

# 3.2. Mask surface analysis

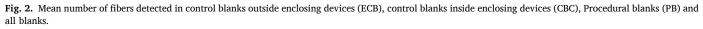
A total of 226 fibers were collected from the 3 adhesive tapes applied on the Blue Cotton Mask (BCM) surface. This corresponds to 6.28 fibers/ cm<sup>2</sup> of adhesive tape, a value higher than those released by the other two types of face masks (Table 2). Seven fiber categories were identified in regard to their origin and color: synthetic black, synthetic blue, synthetic red, synthetic white transparent, natural black, natural blue and natural red (Table 2, Figs. 4, 5).On the BCM surface, 76 % of the fibers analyzed were natural and 24 % were synthetic. Natural fibers were mainly of blue color (85 %), followed by red (8 %) and black (7 %) ones, while the synthetic fibers were mainly black (46 %) and blue (44 %), followed by red (6 %) and white transparent (4 %) (Fig. 4). The size of the fibers was ranged between 0.06 and 8.6 mm (mean 1.68 mm  $\pm$  1.59, Fig. 6) and most of them (94 %) were <5 mm.

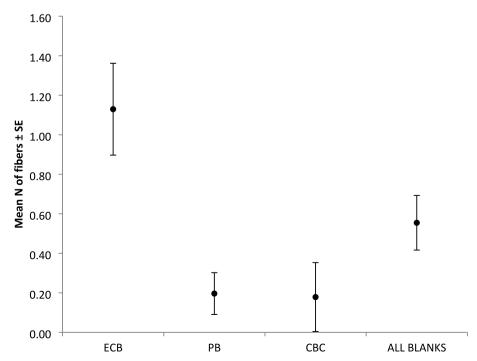
The same fiber categories found in BCM surface were also found on the Blue Synthetic Masks (BSM) surface (Table 2, Figs. 4, 5). Of the 100 fibers identified, the 34 % were natural and the 66 % were synthetic. Fibers of blue color outnumbered black ones in both of natural (blue fibers: 62 %; black: 29 %) and synthetic (blue: 68 %; black: 30 %) origin. A small proportion of natural fibers was red (9 %), whereas the rest of the synthetic fibers was white transparent (6 %) and red (2 %). Their size ranged from 0.03 to 11.01 mm (3.04 mm  $\pm$  2.66, Fig. 6) and the 78 % of them were <5 mm.

The same fiber categories detected on the above other two types of face masks examined (BCM and BSM) were also identified on the Green Synthetic Masks (GSM) surface (Table 2, Figs. 4, 5). It is interesting to point out that, even though the color of the GSM mask was green, most of the fibers examined on the microscope appeared to be blue. A total of 129 fibers were collected and similarly to the case of BSM, 31 % of them were natural and 69 % were synthetic. Natural and synthetic fibers were mainly blue (68 % and 79 % respectively). Natural fibers were also black (30 %) and red (3 %), whereas the synthetic ones were black (13 %), red (7 %) and white transparent (1 %). Their size was ranged between 0.17 and 14.4 mm (3.66 mm  $\pm$  3.12, Fig. 6) and the 74 % of them were <5 mm.

# 3.3. Analysis of fiber releasing from mask to air

Overall 44 fibers were collected from the exhaled air through the three type of masks tested (Table 3, Figs. 5, 6, 7). BCM released at least 4 times more fibers (8.22 fiber/lt exhaled air) than BSM (1.59 fiber/lt exhaled air) and GSM (2 fiber/lt exhaled air). Most of the fibers collected from the BCM (fibers Released from BCM: RBCM) were natural fibers (89. 2 %), while those from the BSM (fibers Released from BSM: RBSM) the 57.1 % of them were synthetic and all fibers from GSM (fibers Released from BSM: RBSM) were synthetic (100 %). BCM released principally blue natural fibers (62.2 %), while GSM released blue and black natural and synthetic fibers almost equally. GSM masks released





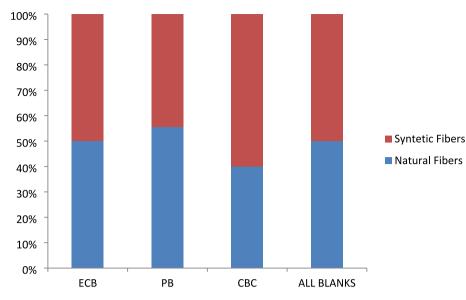


Fig. 3. Percentage proportion (%) of natural and synthetic fibers detected in control blanks outside enclosing devices (CB), control blanks inside enclosing devices (CBC), procedural blanks (PB) and all blanks.

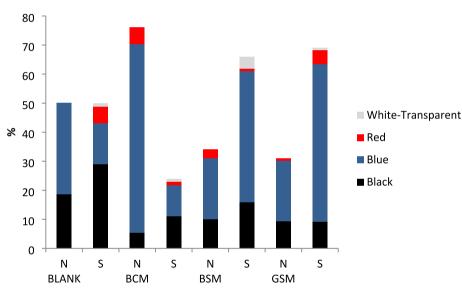


Fig. 4. Percentage proportion (%) of fibers found in the Blanks, Blue Cotton Masks (BCM), Blue Synthetic Masks (BSM) and Green Synthetic Masks (GSM) by color and origin (N=Natural; S=Synthetic). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

Total number (N) and mean number (N)/ $cm^2$  ( $\pm$  standard deviation) of fibers collected on the adhesive tapes from the masks surface. Where: BCM, blue cotton mask; BSM, blue synthetic mask; GSM, green synthetic mask; ALL, all the masks together.

Fibers		BCM		BSM		GSM		TOT	
Origin	Color	N	N/cm <sup>2</sup>						
Natural	Black	12	0.33 (0.22)	10	0.27 (0.1)	12	0.33 (0.14)	34	0.31 (0.14)
	Blue	147	4.08 (0.79)	21	0.58 (0.3)	27	0.75 (0.17)	195	1.81 (1.76)
	Red	13	0.36 (0.29)	3	0.08 (0)	1	0.03 (0.05)	17	0.16 (0.21)
	Total Natural	172	4.77 (1.16)	34	0.94 (0.39)	40	1.11 (0.34)	246	2.28 (1.98)
Syntetic	Black	25	0.69 (0.32)	16	0.44 (0.17)	12	0.33 (0.14)	53	0.49 (0.25)
-	Blue	24	0.67 (0.29)	45	1.25 (1.12)	70	1.94 (2.35)	139	1.29 (1.42)
	Red	3	0.08 (0)	1	0.03 (0.05)	6	0.17 (0.22)	10	0.09 (0.13)
	White-Transparent	2	0.06 (0.05)	4	0.11 (0.19)	1	0.03 (0.05)	7	0.06 (0.11)
	Total Synthetic	54	1.5 (0.3)	66	1.83 (0.82)	89	2.47 (2.42)	209	1.94 (1.35)
ALL TOT	•	226	6.28 (1.43)	100	2.78 (0.43)	129	3.58 (2.63)	455	4.21 (2.19)

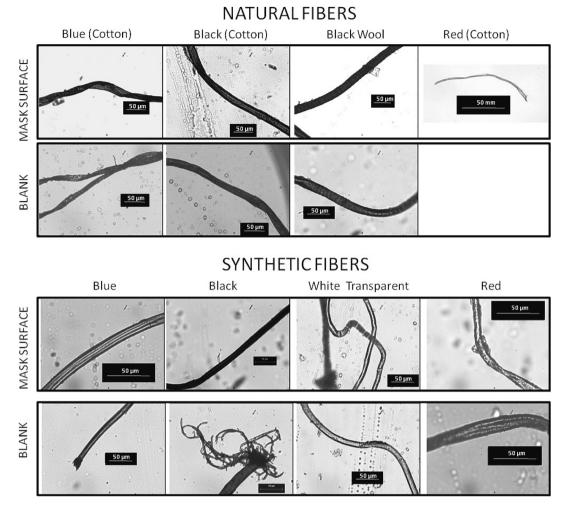


Fig. 5. Different fiber types found in Blank and Mask Surfaces.

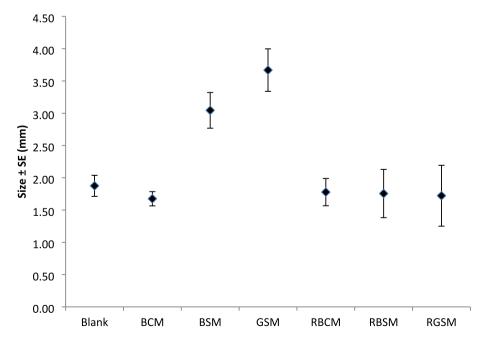


Fig. 6. Mean size  $\pm$  SE of fibers detected in Blanks (Blank) in Blue Cotton Mask (BCM) surface, Blue Synthetic Mask (BSM) surface, Green Synthetic Mask (GSM) surfaces, air exhaled from Blue Cotton Mask (RBCM), Blue Synthetic Mask (RBSM) and Green Synthetic Mask (RGSM). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

# Table 3

Total Number and Number (N)/ lt of fibers detected on the filtered exhaled air through Blue Cotton Mask (fibers Released from BCM: RBCM), Blue Synthetic Mask (fibers Released from BSM: RBSM), Green Synthetic Mask (fibers Released from GSM: RGSM) and all the masks considered (ALL).

Fibers		RBCM		RBSM		RGSM		ALL	
Origin	Color	N	N/1	N	N/1	N	N/1	N	N/1
Natural	Black	4	0.89	1	0.22			5	1.11
	Blue	23	5.11	2	0.44			25	5.56
	Green								
	White Transparent	6	1.33					6	1.33
	Total Natural	33	7.33	3	0.67			36	8.00
Synthetic	Black	1	0.22	1	0.22			2	0.44
	Blue			1	0.22			1	0.22
	Green					2	0.44		
	White Transparent	3	0.67	2	0.44	7	1.56	5	1.11
	Total Synthetic	4	0.89	4	0.89	9	2.00	8	1.78
ALL TOT		37	8.22	7	1.56	9	2.00	44	3.26

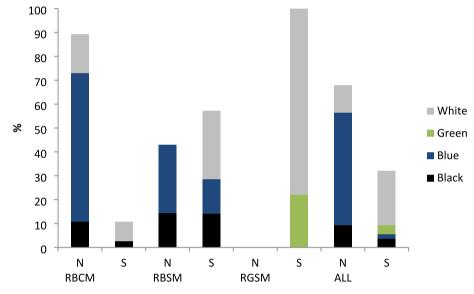


Fig. 7. Percentage proportion (%) of fibers found in the filtered exhaled air through Blue Cotton Masks (RBCM), Blue Synthetic Masks (RBSM) and Green Synthetic Masks (RGSM) by color and origin (N=Natural; S=Synthetic). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mostly white-transparent fibers (77.8 %).

# 3.4. Comparison between fiber composition of masks surfaces, blanks and exhaled air trough masks

The Cluster Analysis was performed on the fiber types of different origin and color found in the blanks (ECB, PB, CBC), the face mask surfaces (BCM, BSM, GSM) and fibers released by the air exhaled though the masks (RBCM, RBSM, RGSM). The analysis showed that all fibers were grouped together except the fibers filtered from the air exhaled through the GSM (RGSM) (Fig. 8). SIMPER analysis indicated a dissimilarity of 88 % between these two groups. This dissimilarity between fibers released from RGSM and all the other samples was mostly caused by the high percentage of white transparent fibers in RGSM (21. 44 % contribution) and the absence of natural blue natural fibers in RGSM (19.06 % contribution). Moreover, the natural blue and natural black fibers mainly contributed to the high similarity (69.89 % average similarity) of the fiber types found in the blanks, mask surfaces and fibers filtered from the BCM and BSM(SIMPER analysis; results not shown).

# 3.5. Fiber size analysis

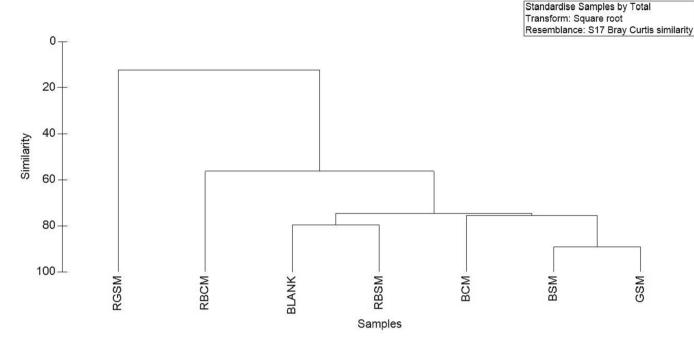
The mean size of fibers collected in blanks (ECB, PB, CBC), BCM

surface and released from BCM, BSM and GSM were not statistically different (ANOVA: F = 0.28, p = 0.88, Fig. 6). Similarly, the mean size of fibers collected from BSM and GSM surface was not differed (ANOVA: F = 2.11, p = 0.14). Moreover, the fibers sampled from the synthetic masks (BSM and GSM) were significantly larger than fibers in blanks and BCM together (ANOVA: F = 70.54, p < 0.01).

# 4. Discussion

The analyses of blanks performed during the samples processing for microlitter detection in fish gut contents confirmed that airborne microfibers (<5 mm in length) are the most relevant external contaminants (e.g.: Torre et al., 2016; Wesch et al., 2017; Woodall et al., 2015). Even though control conditions were applied in every samples processing step to minimize any contamination, we should not underestimate the external contamination by microfibers (Woodall et al., 2015; Torre et al., 2016; Wesch et al., 2017; Prata et al., 2020; Prata et al., 2021; Belontz and Corcoran, 2021).

To estimate the risk of the sample microfiber cross-contamination resulting from the worn face masks, we estimated the type of fibers that may potentially be transferred from masks to blanks (or samples). The tape lifting technique used in this work, it's a robust method widely used in forensic science for crime scene analysis, which allows the recovering of fibers able to be detached from a given surface by the



**Fig. 8.** Cluster analysis of standardized and square root transformed data of fiber types distribution grouped by origin (syntetic and natural) and color of Blanks (BLANK), Blue Cotton Mask (BCM) surface, Blue Synthetic Mask (BSM) surface, Green Synthetic Mask (GSM) surfaces, air exhaled from Blue Cotton Mask (RBCM), Blue Synthetic Mask (RBSM) and Green Synthetic Mask (RGSM). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

adhesive tape strength (De Wael et al., 2008). Our results showed that the fibers that can be transferred from masks to tape were both endogenous (fibers originated from the mask tissue itself) and exogenous microfibers (fibers with different origin than the mask tissue itself). The presence of exogenous microfibers indicated that masks can operated as potential airborne microfibers collectors and these microfibers can be potentially transported and released everywhere. Several mechanisms have been proposed for adhesion of airborne particles of natural origin and man-made by the face masks according to their size (e.g. gravity sedimentation, inertial impaction, interception, diffusion and electrostatic attraction (Tcharkhtchi et al., 2021 and references therein). Considering the size of the microfibers in our study (larger than 500 µm) and their ability of charging electricity, inertial impaction and electrostatic attraction are the mechanisms, more likely, responsible of fibers capture on the mask surfaces (Tcharkhtchi et al., 2021). Inertial impaction occurs when the particles' inertia is too large to induce changes in particles movement direction in the airflow close to the mask surface. Microfibers collide and adhere to the mask tissue, driven by their inertia. Electrostatic attraction happens when fibers or granules, electrically charged, are attracted by the oppositely charged mask surface.

The high similarity between the fiber types detected in blanks and masks' surfaces suggested that the microfibers detected on masks by tape lifting can be transferred from masks to blanks and likely to the processed samples. This implies that most of the microfibers recorded on the surface of the mask can be transferred to the air, blanks, and samples. This hypothesis was demonstrated by analyzing the airborne contaminants in the air exhaled through the masks. Our results showed that the airflow generated by breathing can drive microfibers from the masks to the air confirming that the critical airflow velocities, able to detach fibers of sizes similar to those found in our blanks, are comparable to the exhaled human air velocity (2.2 m/s to 9.9 m/s) (Fan et al., 1997; Mhetre and Abhyankar, 2017). The microfibers released from BCM and BSM and those detected in the blanks were highly similar, indicating that these masks may be responsible for external contamination.

However, the fibers identified from the GSM surfaces by tape lifting

were more similar to those found on blanks than those detected by filtering the exhaled air through the same mask. This evidence indicated that, during the experiment, in fact only a part of the fibers that can be potentially released by the green synthetic masks was transferred to the air. This may suggest that the air volume filtered (1.5 It for each of the three replicates per mask) during the analysis of the exhaled air was not enough to detect all these fibers that can be transferred from mask to air during a sample processing session (2–4 h). Nevertheless, this evidence may indicate that the similarity between the fibers type detected on blanks and masks' surfaces cannot be explained exclusively by the direct transfer of microfibers from the masks to blanks.

The similarity between the fiber types detected on blanks and masks surface may also depend on the microfiber airborne contamination already existing in the lab because masks, acting as filter during inhalation, may capture the same fibers that can be detected on blanks. In this case, the mask, carrying on airborne particles, may become a collector of contaminants that can be potentially released everywhere. The preexistence of microfiber contaminants in the processing area is plausible even if all precautions have been taken (Belontz and Corcoran, 2021; Prata et al., 2021; Torre et al., 2016; Wesch et al., 2017). This preexisting contamination may depend on various factors e.g. not proper cleaning condition, free circulation of personnel in the lab, contaminated microfibers airflows generated by air convectors. Source of microfiber contamination could also occur from open windows or doors in order to aerate the air into the room. Microfibers including microplastics can be easily found in the urban atmospheric fallout and urban suspended particulate matter and deposit on several surfaces (Akhbarizadeh et al., 2021a; Dris et al., 2017). The control of exogenous fibers abundance on mask's surface by tape lifting can be an easy way to evaluate the actual level of microfiber airborne contamination in the laboratory area.

Our results showed that the microfibers collected by tape lifting on synthetic mask surfaces were significantly larger than the microfibers detected on cotton mask's surface, blanks and air exhaled trough mask's surfaces. These differences indicated that the adhesive strength of the tape is likely able to detach larger endogenous synthetic microfibers than the kinetic force generated by breathing.

The number of microfibers/cm<sup>2</sup> detected on the cotton mask's surface by adhesive tape was significantly higher than that of the synthetic masks, confirming that cotton fibers are more favorably transferred compared to the synthetic ones (Skokan et al., 2020, De Wael et al., 2008). These differential transfer mechanisms may be explained by a combination of parameters such as the length of the cotton fibers and their availability on the surface tissue, as also stated by Skokan et al. (2020). Fig. 9 presents a large number of cotton fibers that are joined loosely on the mask's tissue surface and become, in this way, available for detaching and transferring to other recipients.

Recent studies on the microfiber release capacity from disposable surgical masks (De-la-Torre et al., 2022a; Morgana et al., 2021; Rathinamoorthy and Balasaraswathi, 2022; Saliu et al., 2021; Shen et al., 2021; Wang et al., 2021) demonstrated that a single mask could release thousands of microplastic fibers in the environment and that the amount of microfibers released depends on the level of fabric deterioration. Fabric deterioration may depend on naturally weathering, exposition to different conditions states (dry, wet, freshwater, and seawater), different mechanical stress forces, UV and artificial seawater, water detergent, and alcohol. Rathinamoorthy and Balasaraswathi (2022) demonstrated that the microfiber release at dry state was 22,053.84  $\pm$  647.84 fibers/ mask, in case of a new mask and 100,780.17  $\pm$  35,538.62 fibers/mask in case of a weathered mask. Morgana et al. (2021) confirmed that even at a low level of fabric deterioration, a single mask could release thousands of microplastic fibers in water. Shen et al. (2021) concluded that a fully aged mask could release several billions of microplastics into the environment. Similarly, Wang et al. (2021) demonstrated that a single weathered mask could release >1.5 million microplastics into the aqueous environment. Saliu et al. (2021) confirmed that a single surgical mask exposed to artificial weathering (180 h UV-light irradiation and vigorous stirring in artificial seawater) might release up to 173,000 fibers/ day. Our study showed that a worn face mask could release microfibers even by breathing (between 1.59, 2, and 8.22 fibers/lt of exhaled air in GSM, BSM, and BCM, respectively).

Our results suggest that the use of face masks can increase the level of microfibers not only in the laboratory but in any indoor environment. In the pro-COVID-19 era, Soltani et al. (2021) and Kashfi et al. (2022) have observed high levels of microplastics in indoor dust and have warned that the young children are more exposed. In the COVID-19 context, inhalation of microplastics due to face masks increases concerns about possible negative impacts on human health (Torres-Agullo et al., 2021).

The fact that the face masks worn during laboratory analyses are undoubtedly an additional source of microfibers contamination, as has been supported by several scientists, confirms that one of the major limitations of microplastic pollution studies is the risk of external contamination during laboratory analyses. One of the researcher's main goals is to provide objective and repeatable results. In cases where a methodological but predictable flaw may compromise results, scientists are expected to point out the possibility of unrealistic and misleading results. The impact of contamination by the face mask will be more evident in the future, and it would be interesting if future works try to quantify this impact on microplastics research worldwide.

The unprecedented rise of the face masks global production during the COVID-19 pandemic has also increased concern regarding the addition of a significant new source of microplastics that can be released

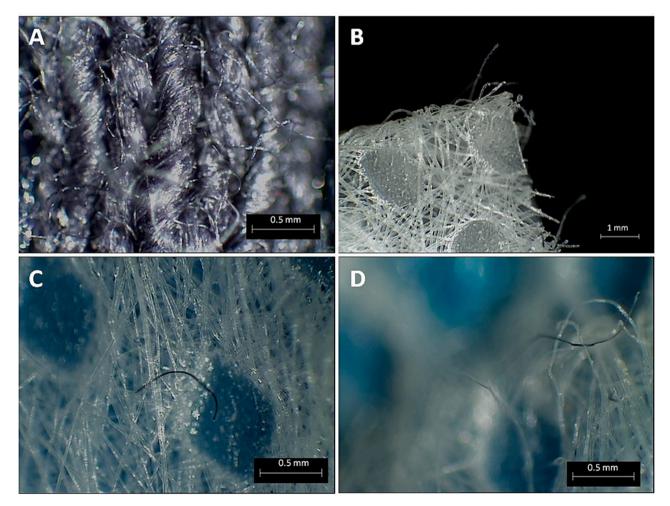


Fig. 9. Cotton (A) and synthetic (B) mask surfaces. Exogenous fibers (B, C) captured on synthetic mask surfaces.

into the environment. The indiscriminate abandonment of PPE wastes during the COVID-19 pandemic, well documented in recent articles (Abedin et al., 2022a; Abedin et al., 2022b; Akhbarizadeh et al., 2021b; De-la-Torre and Aragaw, 2021; De-la-Torre et al., 2022b; Rakib et al., 2021), resulted in their accumulation in beaches, coastlines, rivers, and littering cities. The transformation of the accumulated masks into microfibers including microplastics can threaten the aquatic and terrestrial ecosystems showing that the global pandemic has not reduced the challenge of increasing environmental plastic pollution (Aragaw, 2020).

Our study demonstrated that face masks worn during samples processing in laboratories could be significant source of external contaminants. The contamination of microfibers in fish gut content analysis raises concerns because it potentially overestimates the amount of microfibers ingested by fish, inducing biases at the results (Belontz and Corcoran, 2021; Prata et al., 2021). Microfiber detection in gut contents is usually confined to a few (often 1 item in all the gut content) and low weight (often <0.001 g) items that correspond, typically, to a very small proportion of the whole gastrointestinal contents (Torre, personal communication). For all the above reasons, it is crucial to analyze and estimate the airborne microfiber external contamination during the samples processing. According to our research, the face masks worn during the COVID-19 pandemic should be considered a major source of microfiber contaminants. We strongly recommend the evaluation of the fibers that can be potentially released from the face masks before any lab analysis.

#### 5. Conclusions

Accurate analyses on the detection of microplastics on biota require avoidance of microfiber contamination. The density of microplastics originating from several sources has been reported widely. Furthermore, the hypothesis that face masks are a potential source of microlitter contamination has arisen in the last years among scientists, although the magnitude of this contamination is still unknown.

This study focused on the potential risk of the sample microfiber cross-contamination resulting from the face masks worn during microlitter analysis on marine biota due to the COVID 19 pandemic. Our results showed that the face masks could be a significant source of microfibers contamination. The presence of exogenous microfibers on the mask's surface indicated that masks can operate as potential airborne microfiber collectors and that these microfibers can be potentially transported and released everywhere. Moreover, masks could act as an additional source of microfibers pollution through their degradation, as has also been reported by other authors (e.g., Saliu et al., 2021). The transferring capacity of microfibers to air/water likely depends on the degradation rate of a specific mask. The analysis of the airborne contaminants detected by filtering the air exhaled through the masks showed that fibers could be transferred from the masks to the air, driven by the airflow generated by breathing. These microfibers can consequently be transferred to the blanks and samples. This issue makes the problem of contamination more complicated and confirms how external contaminants may easily bias analytical studies. Furthermore, it has been stressed that face masks can increase the level of microfibers not only in the laboratory but in any indoor environment increasing concerns about possible negative impacts on human health.

Contamination by microfibers in fish gut content analysis raises concerns because it potentially overestimates the actual amount of microfibers ingested by fish. Working in clean-room conditions and using clean-air devices is essential for reliable results in microlitter analysis detection on biota. However, quality control analysis is required to minimize the overestimation of microfibers resulting from masks or other sources.

# CRediT authorship contribution statement

Michele Torre: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Maria Eleni Kafritsa: Conceptualization, Methodology, Writing – review & editing. Aikaterini Anastasopoulou: Supervision, Conceptualization, Investigation, Writing – review & editing.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Data availability

Data will be made available on request.

# Acknowledgments

The present work was carried out based on samples collected during the MEDITS surveys of 2020 conducted by the Hellenic Centre for Marine Research in the framework of the national DCF program funded by the Ministry of Rural Development and Food of Greece. Authors would like to thank Mr. Dokos for contributing to the graphical editing. Special thanks to the architect Camilla Torre for her contribution to the graphical abstract. We would like also to thank the anonymous reviewers for their time and effort in improving the quality of the manuscript by providing constructive comments.

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