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Products released from surgical face masks can provoke cytotoxicity in the marine diatom *Phaeodactylum tricornutum*

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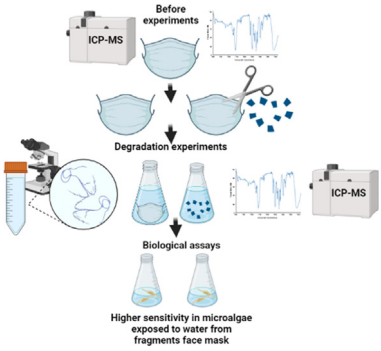
HIGHLIGHTS

- Surgical face masks (FMs) can be a source of Mn, Zn and Ni in marine environments.
- The release of chemicals and microfibres from FMs to water depends on the stage of disintegration.
- Water from face masks provoke cellular changes in the diatom *P. tricornutum*.
- Water from fragments showed a higher toxicity than water from a whole face mask.
- Photosynthetic apparatus was the most sensitive response tested in the microalgae.

GRAPHICAL ABSTRACT



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ABSTRACT

Surgical face masks are more present than ever as personal protective equipment due to the COVID-19 pandemic. In this work, we show that the contents of regular surgical masks: i) polypropylene microfibres and ii) some added metals such as: Al, Fe, Cu, Mn, Zn and Ba, may be toxic to some marine life. This work has got two objectives: i) to study the release rate of the products from face masks in marine water and ii) to assess the toxicity in *Phaeodactylum tricornutum* of these by-products. To achieve these two objectives, we performed release kinetic experiments by adding masks in different stages of fragmentation to marine water (i.e. whole face masks and fragments of them 1.52 ± 0.86 mm). Released microfibres were found after one month in shaking marine water; 0.33 ± 0.24 and 21.13 ± 13.19 fibres·mL^{-1} were collected from the whole and fragmented face masks, respectively. Significant amounts of dissolved metals such as Mn, Zn and Ni, as well as functional groups only in the water containing the face mask fragments were detected. Water from both treatments was employed to study its toxicity on the marine diatom. Only the water from the face mask fragments showed a significant, dose-dependent, decrease in cell density in *P. tricornutum*; 53.09 % lower than in the controls. Although the water from the face mask fragments showed greater effects on the microalgae population than the water from the whole face mask, the latter treatment did show significant changes in the photosynthetic apparatus and intrinsic properties of the cells. These results indicate that during fragmentation and degradation face masks a significant chemical print can be observed in the marine environment.

1. Introduction

During the COVID-19 pandemic, the use of personal protective equipment (PPE), such as face masks, has contributed to significant advances in controlling the spread of the SARS-CoV-2 virus (Howard, 2020; Worby

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and Chang, 2020). Estimates of the annual demand for PPE has increased 207 times between 2019 and 2020 with a value of about US $166 billion on the global market (Roberts et al., 2021).

Currently, face masks are considered one of the main measures to avoid the progression of the virus and their use is a legal requirement both indoors and outdoors in public spaces in many countries. Data indicate a consumption of approximately 129 billion face masks for 7.8 billion people across the globe per month during the year 2020 (Kalina et al., 2020). To deal with the unexpected and enormous amount of waste generated, an appropriate strategy to manage this threat is mandatory to avoid potential environmental impacts. The situation has led to illegal plastic waste disposals (increasing by 280 % worldwide), these inherently alter the adjacent natural habitats (Reportlinker, 2021). From the beginning of the pandemic, a high number of face masks were already found in terrestrial and aquatic systems (Ammendolia et al., 2021). Chowdhury et al. (2021) predicted that an amount of between 0.15 and 0.39 million tonnes of PPE waste entered the ocean during the first year of the pandemic. This was observed in different places; for example, >105,000 t of face masks per month are dumped into the environment by Africans (Benson et al., 2021a, 2021b), compiled monitoring studies on COVID-19 PPE plastic waste in urban and natural areas, and found a density of PPE from 0.001 items·m^{-2} up to 0.1 items·m^{-2} in beach transects. Face masks have already been found in natural compartments, reaching >70 surgical face masks along 100 m of shoreline, as reported by the Oceans Asia Organization in Hong Kong. For example, 54 items·m^{-2} were found in 40 days on sandy and rocky beaches in Bushehr (Iran) (Akhbarizadeh et al., 2021).

Plastic polymers are the main composition of surgical face masks. The daily use of face masks and an improper management of their disposal make the policies promoted before the COVID-19 pandemic concerning plastics more complicated to carry out (such as the Basel Convention, United Nations Convention on the Law of the Sea, International Convention for the Prevention of Pollution from Ships, Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection and the United Nations Global Partnership on Marine Litter). Due to their composition, surgical face masks are a large source of plastic pollution and the additives associated to their manufacturing processes such as: stabilisers, antioxidants and UV stabilisers, plasticisers, curing agents, brominated flame retardants, biocides, blowing agents, fillers, inorganic pigments, organic pigments, heat stabilisers, soluble colorants, reinforcements and slip agents (Sendra et al., 2021a, 2021b) must be taken into consideration too. It has been demonstrated in a recent work by Liu and Mabury (2021) that the three layers of a surgical face mask are composed of chemical additives, used to maintain their high quality, that are plastic-containing products such as synthetic antioxidants, synthetic phenolic antioxidants and organophosphate antioxidants. In a recent work by Sendra et al. (2022) it was revealed that surgical face mask fragments in freshwater and under conditions that included UV-C for 7 days are able to leach: i) inorganic (Fe, Cu and Zn) and ii) organic by-compounds (pyridine 2,4,6-trimethyl, 1-dodecanamine *N*, *N*-dimethyl and alanine *N*-methyl-N-methoxycarbonyl-undecyl ester) into water. These soluble compounds leave a chemical footprint in the water that could trigger unwanted effects within the aquatic ecosystem.

Moreover, when face masks reach the natural environment, environmental agents initiate their degradation and fragmentation processes that reduce the mask into smaller pieces called microplastics (MPs < 5 mm; (Thompson et al., 2004)) and nanoplastics (NPs < 100 nm; (Jambeck et al., 2015)) in the form of fibre fragments. In a recent work by Ma et al. (2021) it was reported that more than one billion NPs and MPs were released from each surgical or N95 face mask in 1 L of ultrapure water over 3 min of shaking. Irregularly-shaped particles were found in the water collected, ranging from 5 nm to 600 μm, but most of them were nano scale sized <1 μm. These authors also found differences in the fibres released among the layers. The middle layers of the masks released more particles than the outer and inner layers. Furthermore, De Felice et al. (2022) have also shown that a during just one wash, face masks released an average of 284.94 ± 73.66 microfibres, independently of the fabrics.

These by-products (inorganic, organic and fibres) released and/or formed from face mask degradation in water could affect aquatic organisms. Therefore, a study concerning the effects of face-mask products should be performed to protect the environment and aquatic ecosystems. However, very few data are available about the toxicity and effects of face masks on aquatic organisms. The work by Sendra et al. (2022) has revealed that face mask products can up- and down-modulate several biological processes in zebrafish larvae by modulating differentiated expressed genes found in the results of an RNA-seq analysis. Among the most modulated biological functions, reproductive processes were the most affected. The study of the potential and real effects of face masks is relevant for the most representative groups of organisms. Nevertheless, ecotoxicological studies concerning primary producers are also very important due to their ecological relevance in the food web. Some changes in microalgae communities could provoke unwanted effects in higher organisms. It was demonstrated in the work by Ma et al. (2021), that the microfibres released from face masks may be adsorbed by diatoms. This could indicate that the interactive force between the cells and micro and nanofibres could be similar to nanoscale interaction (Nel et al., 2009). The literature available about primary producers has revealed that they can ad- and absorb nanoplastics thus provoking unwanted effects at cellular and molecular levels, especially in the photosynthetic apparatus (Déniel et al., 2020; Hazeem et al., 2020; Natarajan et al., 2020; Sendra et al., 2019; Venâncio et al., 2019; Yang et al., 2021).

Given everything that has been stated above, the main aims of this work are i) to identify and quantify the inorganic compounds released from a whole surgical face mask as well as fragments of a face mask that have been agitated in marine water for one month to mimic natural processes, and ii) to assess the cytotoxicity of the water from whole or fragmented face mask experiments for the marine diatom *Phaeodactylum tricornutum*.

2. Materials and methods

2.1. Fragmentation and degradation of surgical face masks

The experimental fragmentation and degradation of face masks is shown in Fig. 1. Commercial surgical face masks (LyncMed, Beijing, China) sold in a local pharmacy in Spain were used for this study. Two conditions simulated two different stages of face-mask disintegration; i) a whole surgical face mask submerged in marine water, and ii) fragments of a face mask floating on and/or submerged in marine water. Both conditions did not consider the fasteners of the face masks. The surface marine water used for the experiments was collected by a boat in a clean offshore area of Cadiz Bay (Spain; 36.499109 N, −6.253689 W) and filtered using two consecutive filters of 0.47 and 0.22 μm filter prior to the experiments. The physico-chemical characterisation of the marine water collected was pH: 8.1, the salinity recorded with a seawater refractometer HI 96822 (HANNA) was 36 psu and organic matter measured as TOC with a TOC analyser Shimadzu TOC-V CHS was 1.5 mg L^{-1}.

The fragmentation of the face mask was performed with a scissor previously cleaned with ethanol. Each face mask was cut into pieces of approximately 2 mm. Three replicates were established in each condition: controls (marine water), a whole single face mask and face mask fragments. A single face mask (whole or fragmented) was placed to an acid-cleaned Erlenmeyer with 2 L of filtered marine water. The whole face masks were dunked into the marine water with a glass rod. The Erlenmeyer flasks were connected to a tube of PVC (D.I. × D.E. = 3/16 in. × 5/16 in.) to make the sampling over time easier and covered with parafilm. The Erlenmeyer flasks were in agitation (50 rpm) and in a photoperiod of 12 h of light/12 h of dark at room temperature (20 ± 2 °C). A total of 10 samples of water, according to each treatment, were collected over 1 month, seven of them over the first week, and the other three once a week. 30 mL were collected for inorganic analysis and 15 mL for analysis of the fibres released.

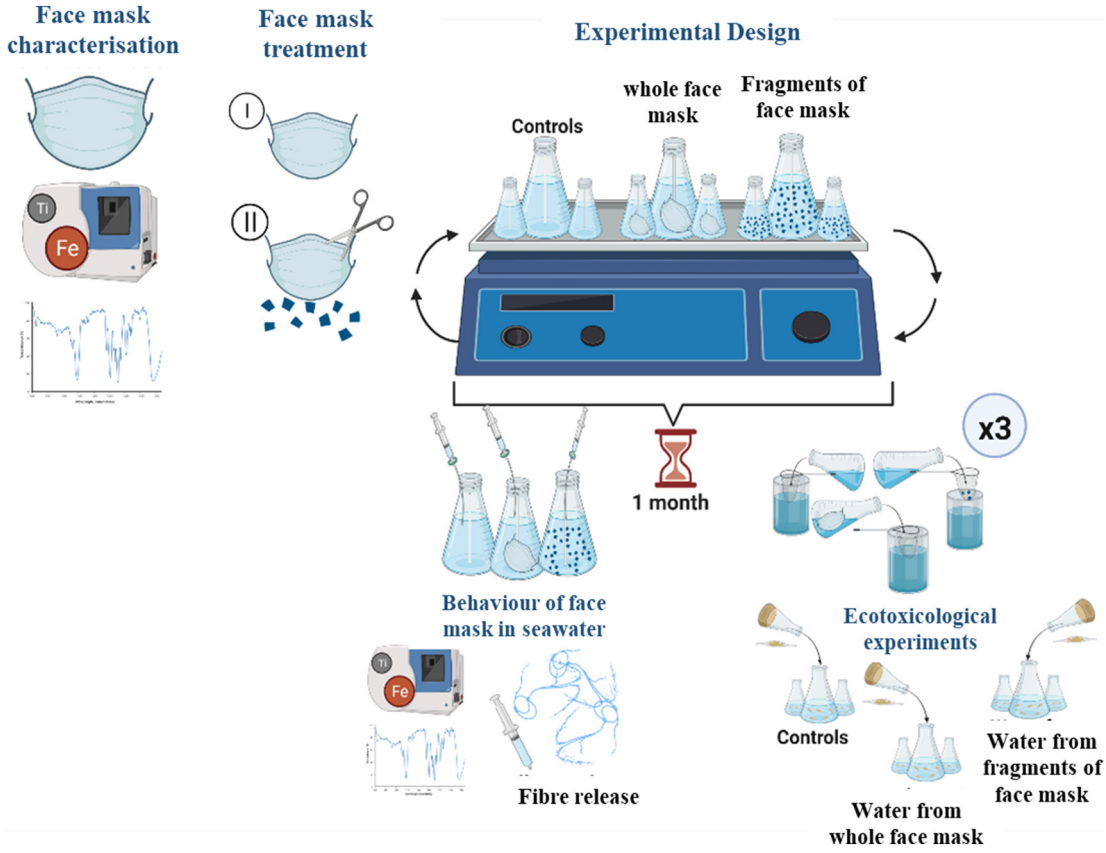

Fig. 1. Face mask characterisation and experimental design. Chemical and ecotoxicological experiments.

2.2. Analysis of surgical face masks and water collected during the experiments

The analysis and behaviour of the surgical face masks are explained in this section and the process is shown in Fig. 2. The different layers of the surgical face masks were analysed prior to the experiment. The materials of the different layers were analysed using Fourier transformed infrared spectroscopy (FTIR) through the Bruker Alpha System. The inorganic compounds of the blue and white layers of the face masks (n:5) were analysed by ICP-MS (ICAP-Q, Thermo-Fisher) after acid microwave digestion (CEM model Mars 5) according to the SW-846 EPA Method 3051A (USEPA, 1987). Furthermore, the nominal size of the face-mask fragments was measured by an optical microscope with an $n = 200$.

After the whole face mask or fragments had been submerged in marine water for one month the number of fibres, the inorganic elements and functional group released from the whole face mask and face mask fragments into the water were measured. 15 mL of water was collected in a flask for the analysis of the fibres and the fragments. The samples were filtered using a black polycarbonate filter of 0.47 μm. The black filter was measured in an optical microscope (5×) and all fragments and fibres were counted as well as the area and length of the fragments and fibres. Samples of the inorganic compounds were collected in a 30 mL metal free flask with a syringe and a 0.22 μm filter (both previously washed with HNO_3). The samples were kept at 4 °C until analysis. The concentrations of metals (i.e. V, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) were analysed using ICP-MS (ICAP-Q, Thermo-Fisher) after a pre-concentration using the seawater pre-concentration system (seaFAST). The accuracy of the analysis was established using Seawater Reference Material for trace metals (CASS-6, NRC-CNRC) (recoveries of 109 %, 88 %, 100 %, 90 %, 90 %, 96 %, 100 %, 99 %, and 121 % for V, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb, respectively were obtained). These last samples filtered by 0.22 μm were also analysed by Fourier-transformed infrared spectroscopy (FTIR) to study the functional groups released into the water from the whole masks and fragments of face masks after one month.

2.3. Microalgae toxicological assay

After the analysis of the behaviour of the face masks (with blue and white layer) in marine water, 1350 mL was put in an Erlenmeyer flask to perform the toxicological analysis. This water was filtered using a 0.22 μm cellulose filter and 4 dilutions (with a dilution factor of two) of the marine water that had been in contact with the face masks was performed. Therefore, a total of 5 treatments and the controls were considered.

The marine diatom *Phaeodactylum tricornutum* (Bohlin, 1987) was obtained from the ICMAN Marine Microalgae Culture Collection (IMMCC). The microalgae selected was chosen because it is a standard species used in toxicology tests (ISO 10253:2006). Cells were grown in a filtered (0.2 μm) marine culture medium (pH: 8.2) and F/2 marine medium without EDTA for two weeks prior to the experiment (Fábregas et al., 2000).

The experiments were performed in 100 mL Erlenmeyer flasks (total volume of the experiment 30 mL) under continuous visible light (300 $\mu E^{-2} s^{-1}$). Growth inhibition bioassays were developed following the OECD procedure (OECD, 1994), in order to determine the microalgae density after 72 h. The initial cell density of microalgae was 10^4 cells mL^{-1}. All treatments and controls were carried out in triplicate.

2.4. Flow cytometry analysis

The flow cytometer used in this work was a BD Accuri C6 equipped with an emission laser at 488 nm and different detectors: side scatter (SSC), forward scatter (FSC), detector FL1 [band pass (BP) 533/30 nm], FL2 [band pass (BP) 585/40 nm] and FL3 FL3 < 670 nm.

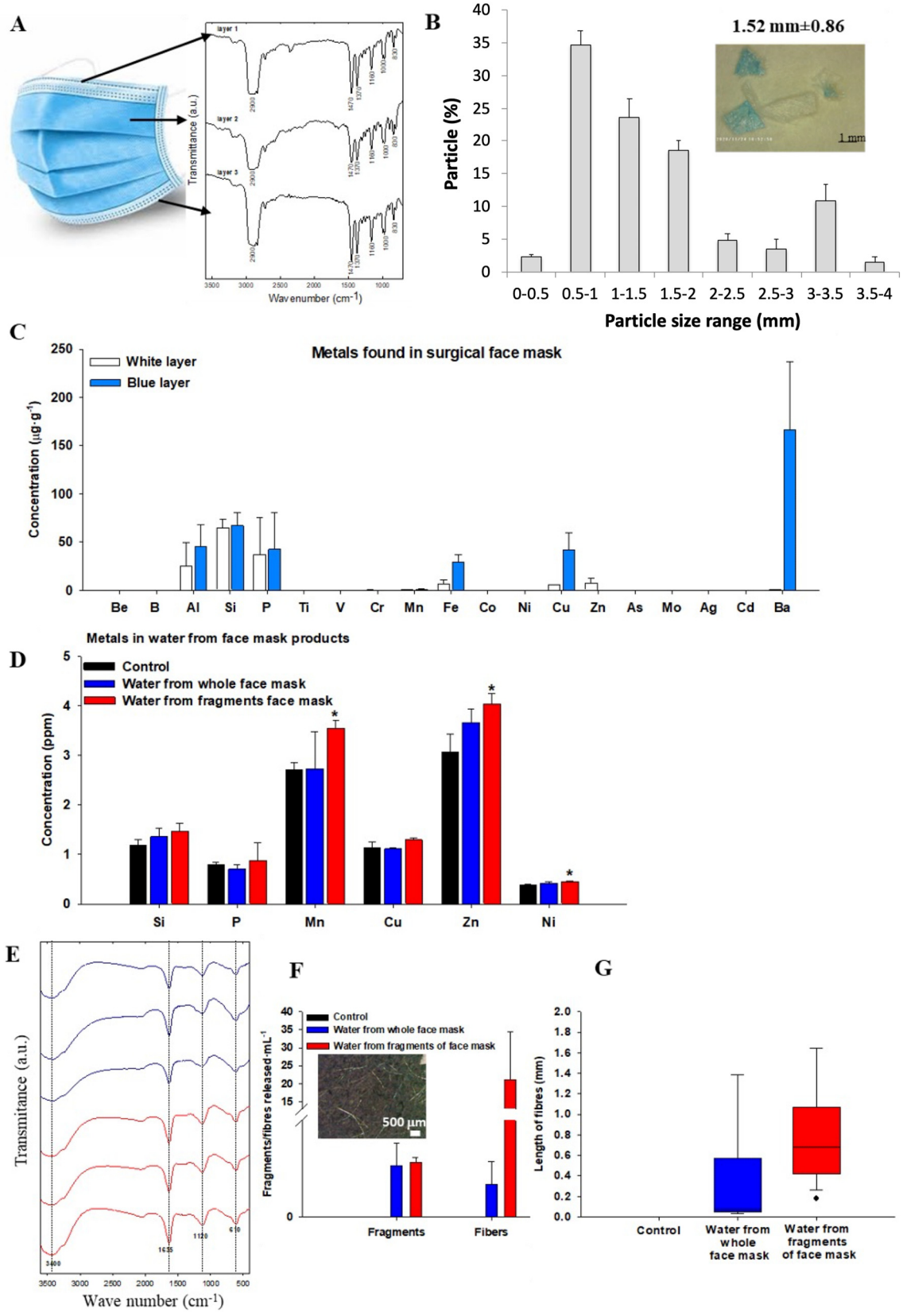

Fig. 2. Chemical characterisation and behaviour of face mask products after 1 month in natural marine water. Panel A shows FTIR of the three layers of surgical face masks. Panel B shows the nominal size of the face mask fragments used in this work. Panel C and D show the inorganic elements of the face masks and released into the marine water after 1 month (asterisks represent significant differences in respect to the controls; ANOVA post hoc Dunnett´s test $p < 0.05$). Panel E shows the functional groups found in the marine water exposed to the face masks over 1 month by FTIR. Panel F shows the total items (fragments or fibres) released into the marine water in a mL and Panel G shows the length of microfibres released into the marine water after one month of exposure.

All the responses measured were collected after 24, 48 and 72 h. The cell density of the microalgae population was determined using SSC related to cell complexity and the long pass filter of 670 nm (FL3) related to auto-fluorescence of chlorophyll *a* (Shapiro, 2005). Furthermore, the volume of microalgae was determined using a FSC detector related to microalgae volume.

The data for cell density was provided by the flow cytometer as cells·μL^{-1}. The data for cell complexity, cell volume and Chl a is given as the median of each response in accordance with the population of microalgae selected.

Production of intracellular ROS (reactive oxygen species: superoxide, hydroxyl and hydrogen peroxide) was quantified using the 2′-7′-dichlorofluorescein diacetate (DCFH-DA), following the procedures of Stachowski-Haberkorn et al. (2013) and Sendra et al. (2017a, 2017b). In brief, the samples were incubated with the fluorophore at a final concentration of 2 mM and in darkness at room temperature for over 20 min. The ROS present in the cells was measured using an FL1 detector [band pass (BP) 533/30 nm] as the positive percentage of microalgae with ROS in relation to the total microalgae population.

2.5. Photosystem II analysis

The effective quantum yield of photosynthetic energy conversion in PSII (EQY), total chlorophyll *a* concentration (μg·L^{-1}) and apparent electron transport rate (ETR) were measured fluorometrically using a Phyto-PAM instrument (Heinz Walz GmbH) equipped with an ED-101 US/MP Optical Unit (Schreiber et al., 1995). The samples were acclimated in darkness for 15 min before the analysis.

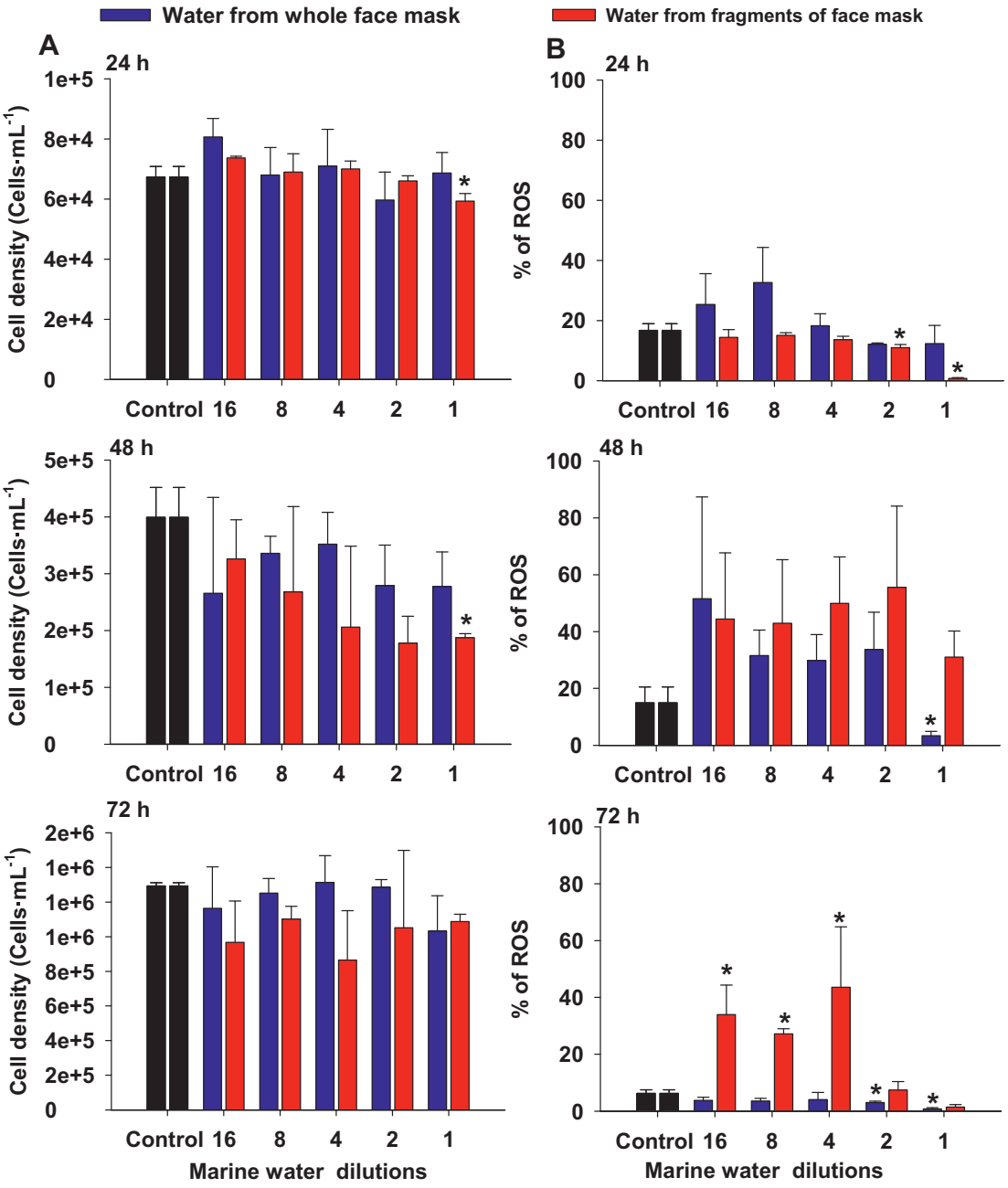

Fig. 3. Cell density and percentage of intracellular ROS of *Phaeodactylum tricornutum* after 24, 48 and 72 h of exposure to the water from a whole face mask and water from fragments of a face mask; Panels A and B respectively. Asterisks (*) show significant differences with respect to the controls (ANOVA test with Dunnett's post hoc; $p < 0.05$).

3. Results

3.1. Face mask characterisation and behaviour in marine water

Fig. 2 shows the chemical composition of the surgical face masks employed in this work, as well as their behaviour in natural marine water over time.

The three layers of the surgical face mask are formed by polypropylene as a plastic polymer (Fig. 2.A). The face masks were studied either whole or in fragments in this work, the fragments had a nominal size of 1.52 ± 0.86 mm (Fig. 2.B). Further chemical characterisation was performed to identify the inorganic elements in the white and blue layers of the face masks. Some elements such as Fe, Cu and Ba showed a higher concentration in the blue layer in respect to the white layer with values of 29.22 ± 7.90 ppm for Fe, 41.61 ± 18.16 ppm for Cu and 166.00 ± 70.85 ppm for Ba in the blue layer (Fig. 2.C). On the other hand, Zn (7.37 ± 5.36 ppm) was only detected in the white layer. Focusing on these elements present in the surgical face masks, the water from whole and the fragments of face masks was analysed after 1 month in natural seawater and constant shaking to study the leaching processes from the facemasks to the water (Fig. 2.D and Table S1). No significant differences were found between the controls and both treatments with face masks in the marine water. However, there were significant differences with regard to three elements (Mn, Zn and Ni) between the water from the controls (2.7 ± 0.15, 3.07 ± 0.35 and 0.38 ± 0.01 ppm for Mn, Zn and Ni respectively) with respect to the water that had contained the fragments of the face masks (3.55 ± 0.15, 4.03 ± 0.21 and 0.45 ± 0.02 ppm for Mn, Zn and Ni respectively) after 28 days ($p < 0.05$).

The released kinetic of the three metals (Mn Zn and Ni) was studied over time (four weeks; 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days). The metals released were measured in water from the whole and the fragments of the face masks and compared to the control concentrations, which are considered as the background level (Fig. S1). The three elements were only released from the face mask fragments. Mn showed higher concentrations in the water from day 3, whereas an increase of Zn was reported in the third and fourth weeks. Finally, Ni showed an increase (1.18 fold change in relation to controls; with a concentration of 0.45 ± 0.02 ppm) in relation to the background levels at 21 and 28 days.

Fig. 2.E shows the FTIR spectra of the marine water controls, and water from the whole and the fragments of face masks. The marine water controls did not show any peaks in the spectrum when the samples were measured. However, both treatments (water from the whole face mask and water from the fragments of the face masks) indicated the presence of alcohols. The characteristic bands are at 3400 cm^{-1} (O—H stretching), 1120 cm^{-1} (C-OH stretching) and 610 cm^{-1} (O—H bending). In addition, amine compounds may also be contained in the masks. The characteristic bands are at 1635 cm^{-1} (N—H bending) and 1120 cm^{-1} (C—N bending).

Fig. 2.F shows the total fragments and fibres released into the water for both face mask treatments. Total fragments showed a similar number of fragments released by mL; 0.53 ± 0.23 and 0.56 ± 0.04 fragments·mL^{-1} respectively. However, the number of fibres was significantly higher in the water from the fragments of the face masks (21.13 ± 13.19 fibres·mL^{-1}) than the water from the whole face masks (0.33 ± 0.24 fibres·mL^{-1}). With respect to the length of the fibres found in both treatments (Fig. 2.G), the water from the whole face mask had fibres of 0.22 ± 0.3 mm, while the length of fibres from the water from the fragments of the face masks was 0.93 ± 0.28 mm.

3.2. Cytotoxic responses of the microalgae exposed to face mask products

Fig. 3.A shows the cell density of a *Phaeodactylum tricornutum* population exposed to water from a whole face mask and water from fragments of a face mask over 24, 48 and 72 h. The microalgae population exposed to water from the whole face mask did not show any significant decrease in cell density with respect to the controls over the time of the experiments ($p > 0.05$). However, the microalgae population exposed to the water from the fragments of a face mask showed a dose-dependent response when the water was less diluted. A significant decrease in cell density was observed after 24 and 48 h of exposure to this water with the face mask fragments ($p < 0.05$). After 48 h of exposure, the microalgae population showed a cell density 7.11 times lower than the controls. However, this trend was not observed after 72 h under face mask fragments treatment. This indicates a recovery in the cell population after a chronic exposure (72 h) to fragments of a face mask; (Fig. 3.A). In some bars of the graphs, we can find high standard deviation. This is due to the biological variability among biological samples. These differences are higher when the effects of the treatments are not quite severe. However, when a high toxicological response is found in microalgae this variability is lower due to the biological response in microalgae population is similar.

Fig. 3.B shows the percentage of intracellular ROS in *Phaeodactylum tricornutum* populations exposed to water from the whole face mask and water from the fragments of a face mask over 24, 48 and 72 h. An increasing trend in the percentage of ROS was observed after 24 and 48 h after exposure to 16 to 4 dilutions of water from a whole face mask and 48 and 72 h after exposure of water from the fragments of a face mask. However, this increasing trend was significant only after 72 h of exposure to water from the fragments of a face mask, it was 6.98 times higher than the controls. The percentage of ROS decreased significantly when the microalgae were exposed to treatments without dilutions, such as after 24 h of the microalgae being exposed to the water from the fragments of a face mask (21.41 times lower than controls) and 48 and 72 h of exposure to water from a whole face mask (Fig. 3.B).

Fig. 4 shows the intrinsic properties of the microalgae such as cell complexity (SSC, Fig. 4.A) and cell size (FSC, Fig. 4.B). Concerning cell complexity, the microalgae exposed to the water from a whole face mask showed a trend to increase at higher dilutions of this treatment (16 and 8 factor dilutions increased significantly respect to the controls; ($p < 0.05$). After 48 and 72 h the trend under this treatment was different showing a decrease in SSC at all factor dilutions after 48 h ($p < 0.05$) and significant differences under 2 and 1 factor dilutions after 72 h. In relation to the changes in cell complexity of the microalgae exposed to water from the fragments of a face mask, a significant increase was found after 48 h at 8 dilution factors ($p < 0.05$). However, a significant decrease in cell complexity was found in the microalgae exposed to no dilution of water from the fragments of a face mask ($p < 0.05$) and 2 and 1 dilution factors after 72 h of exposure to this treatment ($p < 0.05$).

In relation to the cell size of the microalgae (Fig. 4.B), it was observed that after 24 h the microalgae decreased their cell size when they were exposed to all dilution tested of water from a whole face mask ($p < 0.05$) and from 4 to 1 dilutions tested from the fragments of a face mask ($p < 0.05$). A trend to increase cell complexity at higher dilutions after 48 and 72 h of exposure to both treatments was observed; however, the cell complexity decreased when the dilution factors were lower ($p < 0.05$).

Fig. 5 shows the autofluorescence of chlorophyll *a* (Fig. 5.A) and the total chlorophyll of the microalgae population (Fig. 5.B) exposed to both treatments of water from the face masks over time.

We can observe that, in general, FL3 was a more sensitive response than total chlorophyll. After 24 h of exposure, the autofluorescence of the microalgae decreased when the microalgae were exposed to all dilutions of the water from a whole face mask ($p < 0.05$) and from 4 to 1 dilutions of the water from the fragments of a face mask ($p < 0.05$). This trend was observed when measuring total chlorophyll but it was only significant in the microalgae exposed to non-diluted water from the fragments of a face mask ($p < 0.05$).

After 48 and 72 h an increasing trend in both responses was observed in the microalgae exposed to higher dilutions (16, 8 and 4) in the case of the microalgae exposed to water from a whole face mask after 48 and 72 h of exposure ($p < 0.05$), and the microalgae exposed to 16, 8, 4 and 2 dilution factors of water from the fragments of a face mask after 48 and 72 h of exposure ($p < 0.05$). However, a different trend was observed for these two responses when the microalgae were exposed to both treatments without any dilution. A significant decrease in autofluorescence and total chlorophyll was observed after 24, 48 and 72 h of exposure (Fig. 5, $p < 0.05$).

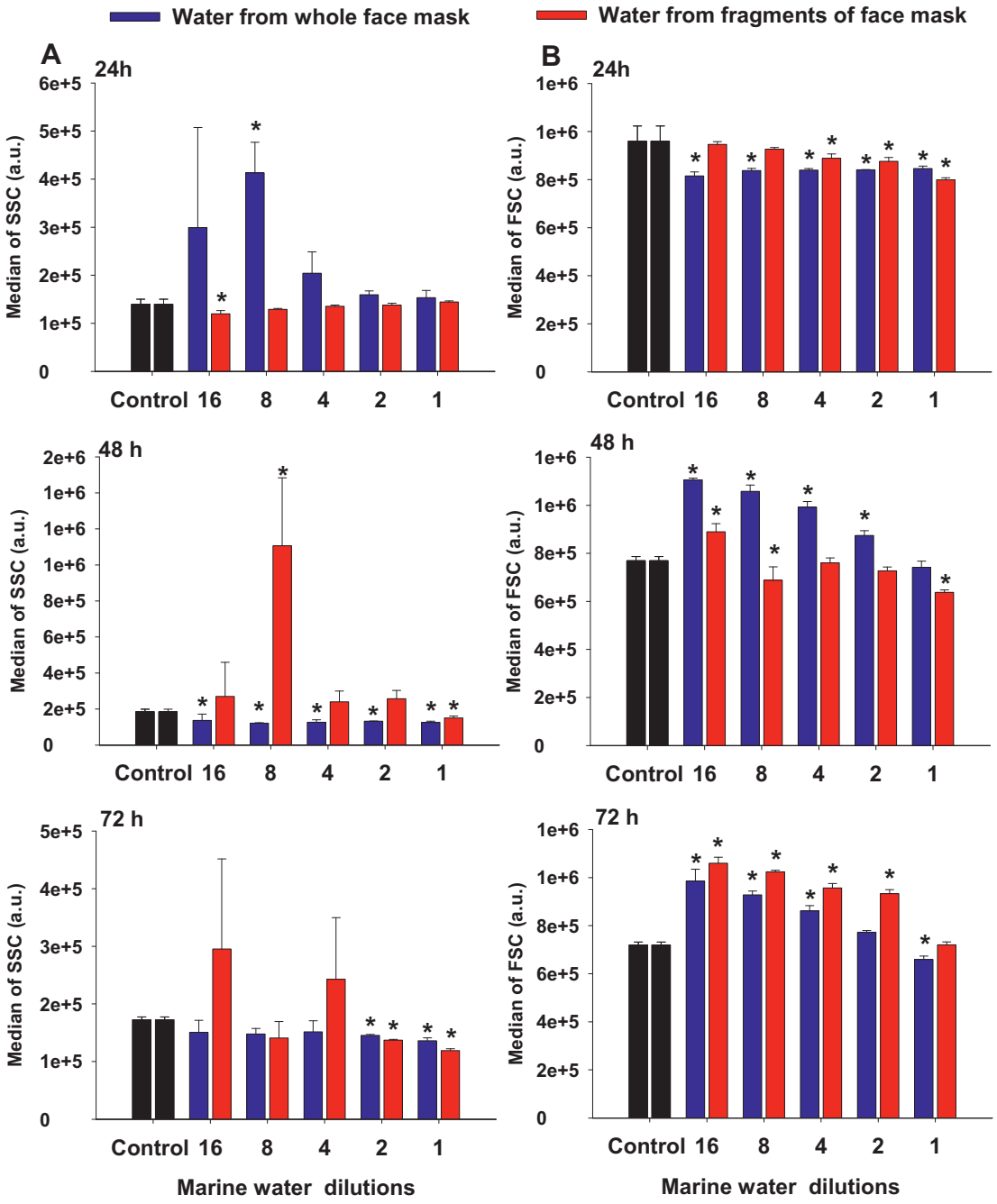

Fig. 4. Intrinsic properties of *Phaeodactylum tricornutum* after 24, 48 and 72 h of exposure to water from a whole face mask and water from fragments of a face mask. Cell complexity (Panel A) and cell size (Panel B). Asterisks (*) show significant differences with respect to the controls (ANOVA test with Dunnett's post hoc; $p < 0.05$).

Fig. 6 shows the effects on the photosynthetic apparatus in microalgae exposed to water from face mask products through of effective quantum yield of photosystem II (EQY, Fig. 6.A) and apparent electron transport of photosystem II (ETR, Fig. 6.B). The EQY showed a significant decrease in the microalgae, which had been exposed to water from the fragments of the face mask after 24, 48, 72 h ($p < 0.05$). For the microalgae exposed to water from a whole face mask, significant differences for this treatment without any dilution were found after 24 and 72 h (Fig. 6. A; $p < 0.05$).

With respect to the ETR responses, there were significant changes in comparison with the controls for both treatments and all dilutions after 48 h (Fig. 6.B; $p < 0.05$). The microalgae populations recovered their ETR at all dilutions of the water from a whole face mask except for the treatment without dilution ($p < 0.05$) after 72 h of exposure. The microalgae exposed to the water from the fragments of a face mask showed a significant decrease at dilutions 16, 8, 4 and 1 dilutions of this treatment ($p < 0.05$).

4. Discussion

Surgical face masks have become the personal protective equipment for medical staff and the general population during the COVID-19 pandemic situation and, like most other "rubbish", they can end up in natural aquatic compartments, which affects their natural populations (Sangkham, 2020).

The surgical face masks used in this study are fabricated of mainly plastic polymer such as polypropylene and inorganic compounds. It is not easy for most of the inorganic compounds to be released into water from the treatments involving whole face masks. However, significant amounts of

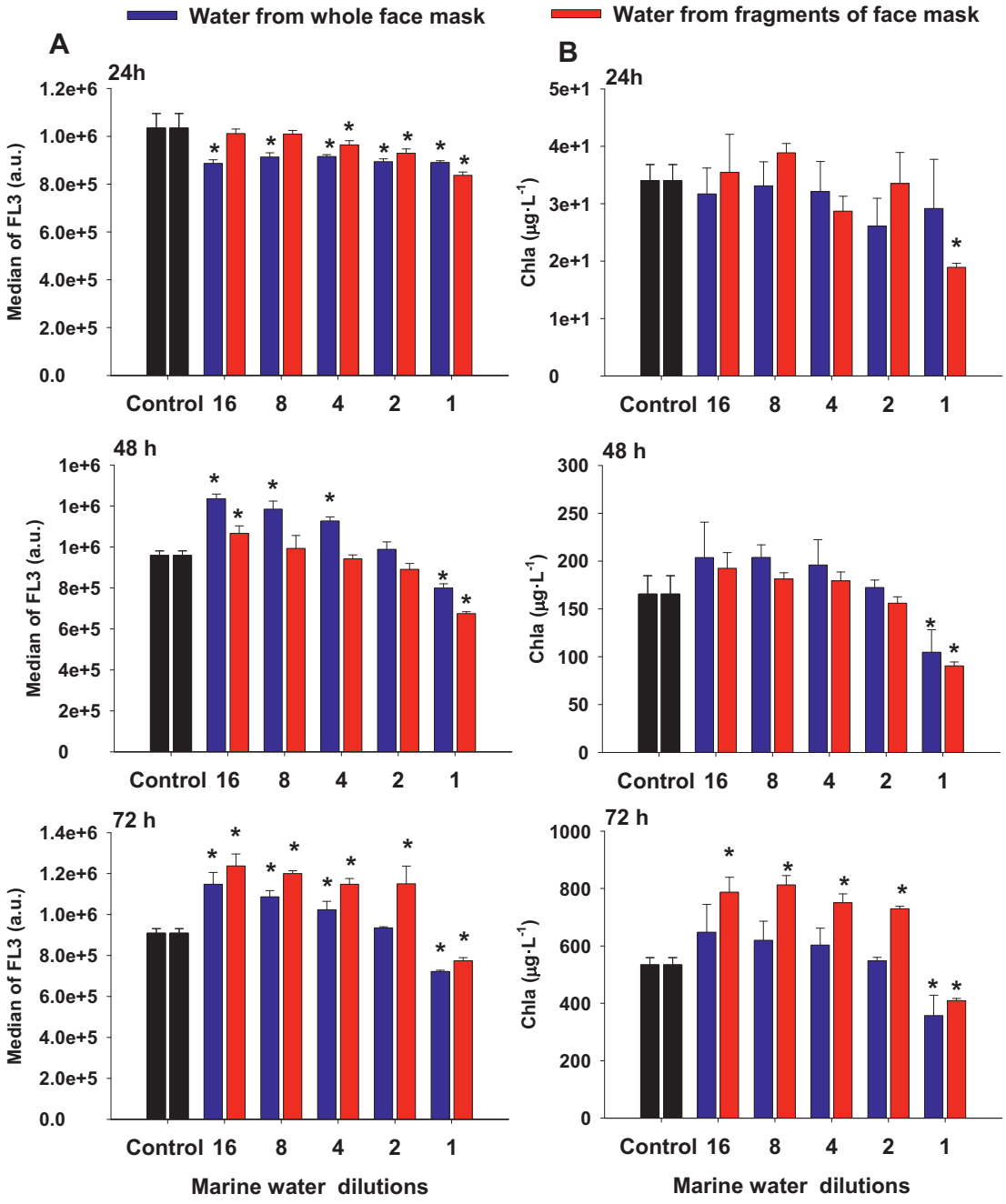

Fig. 5. Autofluorescence of chlorophyll *a* (Panel A) and total chlorophyll content (Panel B) in *Phaeodactylum tricornutum* after 24, 48 and 72 h of exposure to the water from a whole face mask and water from the fragments of a face mask. Asterisks (*) show significant differences with respect to the controls (ANOVA test with Dunnett's post hoc; $p < 0.05$).

Mn, Zn and Ni were released into the water from fragments of the face masks. These elements showed significant differences ($p < 0.05$) in their concentrations between the water from the controls and from the water that had included fragments of face masks; indicating that these elements are released from the beginning of the experiments in the case of Mn, and from 21 to 28 days of the experiments in the case of Zn and Ni ($p < 0.05$). In a recent study by Sendra et al. (2022) it was shown that a highly degraded face mask placed in freshwater released inorganic compounds such as B, Al, Ti, Fe, Cu and Sr over a 7-days period of degradation which included UVC. Although some of these compounds are also present in the face masks used in the present study, these compounds were not released into natural marine water under our specific conditions. Although Sullivan et al. (2021) have reported that the leaching of chemical compounds is a characteristic of this type of face mask, the period of time, the fragments of the face mask, the matrix and the degradation process are essential factors to study the leaching of chemical compounds from face masks into aquatic systems. In addition to the inorganic compounds, functional groups such as alcohols (O—H stretching, C-OH stretching, O—H bending) and amines (N—H bending and C—N bending) were identified as the functional groups in a soluble form in our study. In the study by Sendra et al. (2022) it was shown that organic compounds did not leach from face masks into freshwater; however, three organic by-products were recognised (pyridine 2,4,6-trimethyl, 1-dodecanamine *N*, *N*-dimethyl and alanine *N*-methyl-N-methoxycarbonyl-undecyl ester). The infrared spectrum of these compounds was downloaded from the NIST chemistry webbook site. Although, organic compounds were measured by

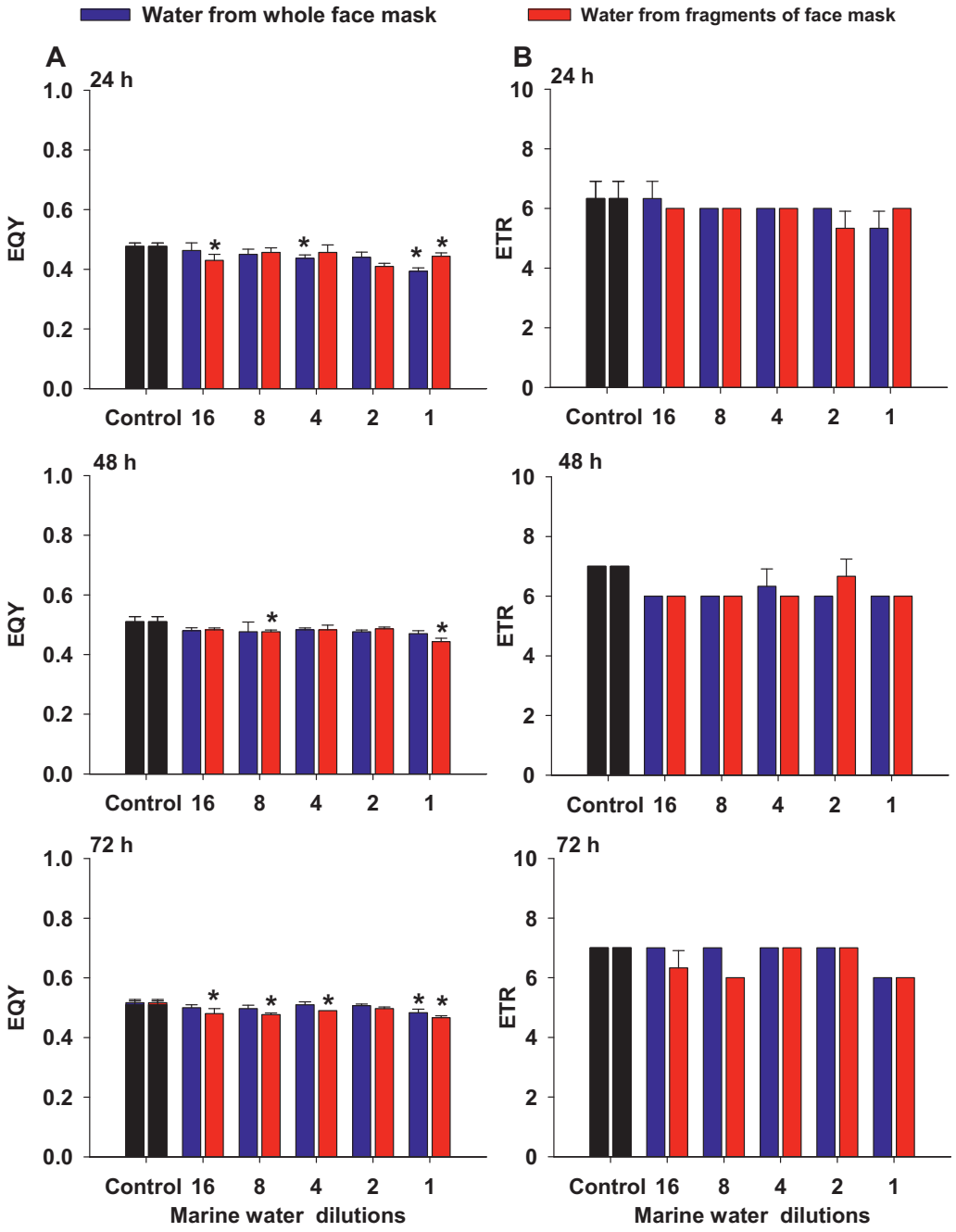

Fig. 6. Changes in the photosynthetic apparatus of *Phaeodactylum tricornutum* after 24, 48 and 72 h of exposure to the water from a whole face mask and water from the fragments of a face mask. Effective quantum yield of photosystem II (Panel A) and apparent electron transport of photosystem II (Panel B). Asterisks (*) show significant differences with respect to the controls (ANOVA test with Dunnett's post hoc; $p < 0.05$).

IR but they not measured by chromatography the IR spectrums with amine groups identified in the present work could coincide with the organic compounds measured by chromatography identified in freshwater by Sendra et al. (2022).

Independently of the inorganic and organic compounds released into the water from the face masks, these PPEs are a source of microplastic fibres. A recent study by Saliu et al. (2021) estimated the release of microfibres from a surgical mask dumped into a marine environment. An experimental analysis was carried out under weathering experiments with artificial seawater. Data indicated that under 180 h UV-light irradiation and vigorous stirring a whole surgical face mask released about 173,000 fibres·day^{-1}. Furthermore, microscope and FTIR analyses carried out using face masks found on beaches have indicated similar morphological and chemical degradation as that observed in relation to the face masks used in the laboratory experiments (Saliu et al., 2021). In this study by Saliu et al. (2021) the degradation of a whole face mask was analysed; however, we studied both whole, and fragments of face masks over a long-term experiment (four weeks). In the marine environment, degradation can be enhanced by abrasion due to sediments and waves, UV radiation and microorganisms (Chubarenko et al., 2018). Therefore, the quantity of microfibres released from face masks could be underestimated under laboratory conditions in relation to natural conditions. In the present study, the microfibres were measured and observed with an optical microscope (5×); however, smaller fragments and fibres at nanoscale level may be present in the marine water employed for the experiments, and they were not identified. Once the microfibres are released into natural environments, they could suffer

secondary degradation processes and they could reach to smaller and smaller sizes (nanofragments and nanofibers), able to release chemical compounds and adsorb organic pollutants and metals such as other authors have revealed with microfibres from other sources of pollution (Mishra et al., 2019). Therefore, the microfibres released could be recognised as a footprint of face mask pollution more than the release of inorganic and/or organic compounds from them. Currently in aquatic systems, microfibres are the main source of microplastic pollution with 13 million tonnes of coastal synthetic fabric waste entering the oceans each year (Mishra et al., 2019). The concentration of microfibres (from ropes, fishing nets, and textiles (Liu et al., 2019) is higher than the microplastics with fragments shape found in the ocean, further, face masks may be considered as a new source of microfibres, which is not contemplated by previous models.

Zebrafish were exposed to filtered (0.47 μm) freshwater previously in contact with face mask products, therefore the effects found in the zebrafish were provoked by these inorganics and/or organic soluble compounds and/or fibres <0.47 μm; (Sendra et al., 2022). In the present study, the effects of the fibres released independently of their size are considered in the reactions triggered in the marine diatom. Micro and nanofibres "per se" have been widely studied and they can provoke unwanted effects in marine ecosystems. Several studies have revealed that microfibres from different types of polymers can cause damage in: microphytobenthos (Hope et al., 2020), echinoderms (Mohsen et al., 2020), actiniarian (Romanó de Orte et al., 2019), crustaceous (Botterell et al., 2020; Horn et al., 2020; Woods et al., 2020), bivalves (Sendra et al., 2021a, 2021b) and fish (Acharya et al., 2021; Li et al., 2021). However, to the best of our knowledge, the effects of surgical face mask fibres, as well as the degradation of their products in water, have not been assessed on marine organisms until now. Only a recent work by Sendra et al. (2022) in freshwater has revealed that the products released by face mask degradation can provoke significant changes in the zebrafish transcriptome with an important down-modulation of genes related to reproductive processes.

Cellular changes in the primary producers, marine microalgae are analysed in this study. They are food producers for >70 % of the world's biomass (Lembi and Waaland, 1988), and any processes and substances that affect their population can cause consequences at higher trophic levels. Diatoms represent a species-rich lineage of unicellular eukaryotic microalgae that play crucial roles in the global cycling of carbon and oxygen and they play a relevant importance in the diet of many aquatic food webs (Armbrust, 2009). A significant decrease was observed in the *P. tricornutum* population exposed to water from fragments of a face mask after 24 and 48 h (short-term experiments). However, the population of microalgae recovered cell density after 72 h (long-term experiments). This may indicate that in a monococulture a *P. tricornutum* population has the ability to recover cell density. The recovery and detoxification of *Chlorella pyrenoidosa* exposed to nanoplastics was also observed in the work by Yang et al. (2021). In this work, the detoxification phenomenon was attributed to the promotion of cell proliferation indicated by the transcriptomic study, due to the acceleration of the degradation of damaged proteins and the regulation of intracellular osmotic pressure in microalgae. However, toxicity does not occur in monoculture in nature, therefore the competence among phytoplankton taxa is continuous in nature under different scenarios of contamination. The taxon of diatoms is really used in ecotoxicology studies due to its sensitivity in respect to other taxa (Sendra et al., 2017b, 2018). When microalgae are in a community and a taxon is more sensitive than others, the most sensitive taxon is at a disadvantage in comparison with the rest of the community and probably under these circumstances it is not possible for the microalga population to recover. Although cell density is an excellent endpoint to assess the toxicity of chemical compounds, some other toxicological responses are even more sensitive, such as intracellular ROS and changes in the photosynthetic apparatus. The generation of oxidative stress is modulated by endogenous processes and exogenous conditions. The defence mechanisms for microalgae include antioxidant enzymes, as well as non-enzymatic antioxidant molecules such as phytochelatins, pigments, polysaccharides, and polyphenols (Cirulis et al., 2013). Therefore, a reduction in the concentration of chlorophyll *a*, when microalgae are under increased oxidative stress, enables the cells to reduce basal ROS (Nymark et al., 2009). This relation between these two blocks: i) chlorophyll *a* and damage to the photosynthetic apparatus with respect to ii) ROS production was revealed in this work.

Cell complexity, autofluorescence of chlorophyll *a*, effective quantum yield and electron transport of photosystem II proved to be more sensitive in order to assess toxicity than cell density and intracellular ROS. Further, the microalgae *Phaeodactylum tricornutum* exposed to water from the fragments of the face masks revealed higher undesirable effects than the microalgae exposed to the water from the whole face masks.

Although surgical face masks are still not considered an emergent pollutant due to their reduced use and presence in our lives before COVID-19, the pandemic has increased their presence in natural environments. Our knowledge about disintegration and degradation processes and their behaviour in natural matrices has to be reassessed since there is now a new source of microfibres in the environment. The extrapolation of micro and nanofibres from face masks in respect to common sources of microfibres is not possible due to the scarce information about the life cycle of the surgical face mask; such as disintegration time, variables dependent of its degradation, toxicity and interaction with the surrounding environments and behaviour and effects in the inhabitant organisms. In this work has been revealed the effects of released compounds of face mask degradation in a model species of microalgae. The unwanted effects in phytoplankton could provoke an impact in food web. In this way, the importance to repeat these toxicological assays with further groups of aquatic organisms is mandatory. As well as to study the effects of the most common face mask such as N95.

5. Conclusions

Surgical face masks are more present than ever in our lives at a planetary scale due to the COVID-19 pandemic. However, they are also present in natural ecosystems, mainly in aquatic environments, with a high number of sightings reported by governmental and non-governmental organisations.

Just like any other litter, face masks start the end of their life cycle thorough fragmentation and disintegration processes. We have shown that surgical face masks are mainly composed by polypropylene microfibres and some inorganic compounds, among them; metals such as Al, Fe, Cu, Zn and Ba, in this work. However, only the fragmented form (1.52 mm) used in this work released significant concentrations of Mn, Zn and Ni with different release kinetic profiles into marine water; Mn was released within a short time (at 3 days of exposure) and Zn and Ni were released over a longer time (at 21 days of exposure). The infrared profile also indicated the presence of alcohols and amine groups in water that had previously been in contact with both treatments of face masks, probably due to by-products of the degradation processes. Furthermore, microfibres from both treatments were released into the surrounding media, the fragments of the face masks released more fibres than the whole face masks. *Phaeodactylum tricornutum* showed several changes in the responses evaluated when it was exposed to both face mask treatments. Although there were changes in the photosynthetic apparatus and intrinsic properties of *P. tricornutum* under both treatments, the diatoms exposed to the water from the fragments of the face masks indicated the highest toxicity. Therefore, this work reveals, for the first time, the impact of face masks on primary producers, and the importance of the by-products released during their degradation phases. Only by avoiding the mismanagement of surgical face mask disposal, can we control their contribution to the litter in the natural environment and protect ecosystems.

CRediT authorship contribution statement.

M. Sendra: Investigation, Writing - original draft. **A. Rodríguez-Romero:** Investigation. **M.P. Yeste:** Investigation. **J. Blasco:** Methodology, Supervision-review & editing. **A. Tovar-Sánchez:** Writing - review & editing, Conceptualization, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156611.

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