Water Emerging Contaminants & Nanoplastics

Impact of face masks weathering on the mussels Mytilus galloprovincialis

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**Impact of face masks weathering on the mussels Mytilus galloprovincialis**

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**Abstract**

The COVID-19 pandemic created an unprecedented need for single-use face masks, leading to an alarming increase in plastic waste globally. Hence, face masks littering and improper waste management contributed to an additional burden to the current plastic pollution in the ocean, but, the environmental and marine ecotoxicological impact in the ocean is not fully understood. This study aims to investigate the ecotoxicological impact caused by the weathering of disposable face masks, once they reach the marine environment, on mussels *Mytilus galloprovincialis*, by assessing biochemical, cytotoxic, and genotoxic effects.

The presence of nano and microplastics was analysed in the mask leachate. The leachate was also used for *in vivo* and *in vitro* toxicity bioassays to assess its effects in *Mytilus galloprovincialis*. The *in vivo* exposure to face mask leachate for 14 days of *M. galloprovincialis* induced a significant increase in CAT activity in mussel gills, although not enough to prevent oxidative damage to cell membranes. DNA damage was also registered in mussels’ haemocytes after *in vivo* exposure to mask leachate. The *in vitro* Neutral Red cytotoxicity assay indicated that leachate concentrations ≤ 0.5 g/L⁻¹ pose a significant risk to the health of mussels' haemocytes, which seems a reliable tool for the cytotoxicity impact assessment of face masks in the marine environment. Therefore, the leachate obtained from face masks in seawater causes oxidative stress, oxidative damage, cytotoxicity, and genotoxicity in *M. galloprovincialis*, indicating that the plastic burden generated by disposable face masks in the ocean and its subsequent weathering represents a ubiquitous and invisible threat to the marine biota.

**Keywords**: Face masks, leachate, microplastics, toxicity, marine mussels.

**Highlights:**
Weathering of face masks in the ocean leach MP, NP and hazardous chemicals;
Face mask leached a significant number of MPs and NPs particles, mainly fibres (97%);
Leachate from face mask weathering cause oxidative stress and damage, cytotoxicity, and genotoxicity in M. galloprovincialis;
Mussels’ haemocytes are a sensitive tissue for cytotoxic assessment from face mask leachate.

1. Introduction

The severe acute respiratory syndrome of coronavirus (SARS-Cov-2), first detected in 2019, gave rise to the COVID-19 pandemic\(^1\). Social distance, travel restrictions, lockdowns, and sanitary measures were globally adopted to avoid airborne virus transmission and reduce its spreading. One of the most widely accepted actions was the usage of single-use plastics (SUPs)\(^2\), and personal protective equipment (PPEs), including protection suits, surgical face masks, examination gloves, and face shields, employed by frontline health professionals and the general population\(^3\). As a result of the remarkable shift in the demand for disposable items, a new plastic waste boom emerged, scaling up the already existent plastic pollution crisis\(^4,5\).

In this context, with the mandatory use of disposable face masks (DFMs) worldwide, an explosive demand for its supply at exceptional levels occurred. On the rise of the coronavirus outbreak, projections estimate that 129 billion face masks were used monthly worldwide, amounting to over 1.24 trillion discarded globally since the start of the pandemic\(^5,6\), Yang et al., 2023) and in the case of Portugal, PPE usage represents, an additional contribution of 4.97% to the municipal solid waste\(^7\). Global improper disposal of these face masks led to their ubiquitous presence in urbanised areas, lakes, beaches, and mountains worldwide\(^8-11\). In addition, face masks that end up in landfills or open dumps may easily leak into the surrounding environment and be flushed into rivers and coastlines by rainfall or wind\(^12,13\), ultimately reaching the ocean. Considering the global production, it is estimated that about 1.56 billion face masks entered the marine environment in 2020\(^14\).

Once in the marine realm, DFM\(s\) pose a physical threat to marine life through entanglement or ingestion\(^15\). Moreover, face masks undergo weathering (also known as ageing) by sunlight, mechanical abrasion, oxidation, and biodegradation, breaking down the textile material into microplastics (MPs) and nanoplastics (NPs) (plastic fragments less than 5 mm and 1 μm, respectively), whereby fibres are dominant (70%)\(^16,17\). Research revealed that a single face mask might release from
3,600 to $1.6 \times 10^7$ microfibres in water, according to the time to which they undergo physical and chemical disturbances\cite{17–19}. A myriad of synthetic microfibres is dispersed in the marine environment\cite{20,21} and disposable face masks were pointed out as a critical secondary source of plastic burden in the ocean\cite{7,16}.

Polypropylene is the most used plastic polymer assembled in DFMs, but other polymers like polyurethane, polyester or polyacrylonitrile can also be incorporated into its structure\cite{1}. The release of plastic polymers may act as vectors of hazardous substances, such as persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, or emerging contaminants. Moreover, chemical additives from face masks’ matrices may also be released into the marine environment, including plasticisers, pigments, dyes, metals, antioxidants, stabilisers, and lubricants\cite{13}. The plastic burden from MPs and NPs released from DFMs may enter marine biological systems through ingestion, dermal contact or filtration\cite{16}, particularly in the case of filter-feeding bivalves.

Microfibres are known to be ingested by crustaceans, molluscs, fishes, birds, and seals \cite{22–24}. An extensive body of evidence demonstrated that marine organisms undergo MPs and NPs ingestion, leading to inflammatory responses, oxidative stress, membrane damage, cytotoxicity, genotoxicity, cell death and reproductive impairments, detected either through in vivo or ex vivo exposures\cite{25–28}. Likewise, ecotoxicological assessments also indicate the extent of the biological effects following the exposure to microplastic fibres (nylon, polyester, polypropylene polymers) on marine zooplankton representatives\cite{29,30} and mussels\cite{31–33}.

However, to the best of the authors’ knowledge, the present study is the first marine ecotoxicological assessment conducted on the weathering of DFMs. Considering the high representation of disposable face masks in the current marine litter composition and the biological disturbances associated with MPs and NPs across several biological levels in the marine biota, there is a pressing need to investigate whether the weathering and fragmentation of DFMs in the ocean pose an additional ecotoxicological threat to the marine environment. The present study hypothesizes that DFMs ageing release plastic particles and further cause biochemical, cytotoxic, and genotoxic injuries to the marine mussel *Mytilus galloprovincialis*. The main objective is to unravel the biological responses posed by DFMs on the mussels through in vivo and in vitro assays. The use of a cell-based in vitro approach conducted with mussels’ *M. galloprovincialis* haemocytes under DFMs leachate exposure revealed a notorious advantage in reducing the considerable number of mussels needed to carry out experiments, allowing the screening of a broader spectrum of exposure conditions and rapid generation of consistent data\cite{34}, which is line with demanding regulatory needs of the European Union. The findings herein will shed
light on the biological effects produced by the exposure of this ubiquitous and unprecedented type of plastic litter in marine mussels.

2. Material and Methods

2.1. Weathering procedure

Due to the broad use during the COVID-19 pandemic and its wide disposal in urban and natural spots, DFM were selected to assess the release of plastic particles to seawater, simulating natural weathering conditions. Instead of applying virgin DFM, timeworn face masks were collected to mimic realistic conditions of the masks ending up in the marine environment. After their collection, the elastic ear loops were removed from the surgical masks.

Twelve DFM were immersed (10 g of masks) were immersed in three litres of natural seawater (salinity 35) from the Ria Formosa lagoon, previously UV-sterilized and filtered (FSW) through 0.8 µm glass microfibre filters (Whatmann) and the leachate prepared according to the method proposed by Almeda et al.[35]. For this purpose, the container was vigorously agitated over 72 h, simulating wave abrasion. After that period, masks were removed and dried in an oven at 60°C for three days to identify the polymer composition. The mask leachate was then frozen at -20°C until further use. To limit the overestimation of MPs present in the leachate, glassware and cotton clothing were adopted and applied during the whole assay to avoid plastic contamination and a blank was run in parallel to assess possible MP and NP contamination.

2.2 Plastic polymer composition of face masks

Wave-weathered disposable face masks were analysed using Fourier Transform Infrared Spectroscopy (ATR-FTIR, Thermo Fisher Nicolet iS10) to identify the polymers present in the masks’ structure. The spectra were acquired using a resolution of 4 cm⁻¹, 16 scans, 4000-650 cm⁻¹ spectral region, and transmittance mode. The obtained spectra were compared with existing databases with the OMNIC software. This software uses Pearson correlation to give a coincidence value. After the analysis, only the results above 70% of coincidence were considered positive. The different layers from all the masks used to generate the leachate were separated and tested individually, namely the outer lyophobic non-woven layer (O layer), a middle melt-blown layer (M layer) and the inner hydrophilic nonwoven layer (I layer).
2.3 Analysis of plastic particles in mask leachate

Another leachate from used masks was prepared, following the same procedures described in section 2.1 to analyse the presence of MPs in the extract. The liquid was filtered through 0.8 µm glass microfibre filters (Whatman) and the filters were dried in an oven at 60°C to ensure that chemical composition of the MPs and NPs were not altered. Filters were evaluated under an Edublue stereomicroscope (Euromex) at ×4 magnification. The present particles were counted, and their colour was assessed. In addition, the particles were measured in length and width, and their equivalent diameter was calculated.

To avoid interference from other submicron materials in seawater, NPs released in mask leachate were determined using artificial seawater (ASW), salinity 35, prepared according to ASTM D1141-98 standard.

To analyse the NPs, total organic carbon (TOC) measurements were performed in a Shimadzu TOC-VCSH equipped with an autosampler. Samples were filtered through Puradisc 25 TF filters (1 µm pore size) and analysed in Non-Purgeable Organic Carbon (NPOC) mode. Moreover, the size of the NPs in the leachate was confirmed with a Malvern Zetasizer Nano ZS apparatus. This equipment uses Dynamic Light Scattering (DLS) technology to analyse the particles present in the submicron portion of the leachates. With this aim, samples were filtered through 1 µm with Puradisc 25 TF filters. Comparison between fragments and fibres was allowed by calculating an equivalent diameter, converting the two flat dimensions into comparable diameters as done elsewhere[36,37].

2.4 M. galloprovincialis in vivo bioassay

Mussels M. galloprovincialis (n = 90; 6.0 ± 0.34 cm shell length) were handpicked during low tide in the Ria Formosa lagoon (Faro, Southern Coast, Portugal) and transported alive to the laboratory, scrap-cleaned and distributed over six glass aquaria containing 7 L of natural seawater. Mussels were acclimated over 5 days, at 16 ± 1°C, salinity 35 ± 1.0 and pH 8.0 ± 0.2 with continuous aeration during a 12 h light:12 h dark photoperiod. Seawater was renewed every 48 h during the acclimation period, and organisms were fed with marine microalgae Tetraselmis chuii.

After the acclimation period, ninety mussels were randomly selected and exposed for 14 days to each treatment (CT and 100 mg.L⁻¹ of DFM leachate) in a triplicate design in 10-L glass aquaria filled with 7 L of seawater (15 animals per aquaria, density = 2 mussels L⁻¹. The seawater was changed every 48 h, and the leachate was concentration re-established. Animals were fed with the only food present in the seawater. Throughout the 14 days of the bioassay, the system was kept under constant aeration, controlled photoperiod, salinity (36), pH (8.0 ± 0.2), temperature (16°C), and oxygen saturation (96 ±
4 %). On the 14th day of the bioassay, mussels (n=6 per treatment) were collected for the determination of the individual biometric parameters (length, height, and width) and for the calculation of the condition index (CI). For that purpose, the soft and drained body tissues were weighted, and the CI for each organism was calculated according to the equation:

\[
CI(\%) = \frac{\text{whole soft tissue (wet weight)}}{\text{whole drained body tissue}} \times 100
\]

On the last day of the experiment, mussels from each treatment (n=6) were collected and dissected into gills and digestive glands, which were subsequently flash-frozen in liquid nitrogen and stored at -80°C until further biochemical analysis to assess antioxidant and oxidative damage effects by measuring the activity of antioxidant enzymes (SOD, CAT and G6PDH), biotransformation metabolism (GST), lipid peroxidation (LPO) and respective total protein content.

2.5 In vitro cytotoxic assessment

Adult specimens of *M. galloprovincialis* (n = 15) from the Ria Formosa coastal lagoon (5.0 ± 0.3 cm shell length) were kept in a 25 L tank filled with clean natural seawater (salinity 35 ± 1), pH 7.8, temperature 18.5°C and constant aeration, until haemolymph extraction. Mussels were fed every two days by incorporating ~ 10 ml microalgae mix of *T. chuii* into the aquaria. After the three days of mussel acclimation, individuals (n=10) were randomly chosen, and their haemolymph was retrieved under aseptic conditions\[38,39\]. Briefly, haemolymph was obtained from the posterior adductor muscle using a 2-mL sterile hypodermic syringe. Haemolymph collected from the ten specimens was pulled into one 15-mL Falcon tube and mixed with anti-aggregation solution (pH 6.7; 171 mM NaCl; 0.2 M Tris; 0.15% v/v HCl 1 N; 24 mM EDTA) in a 1:3 ratio, to prevent cell clumping and agglomeration\[39,40\]. Aliquots of this cell suspension were then used for cell counting in the Neubauer chamber (Hirschmaan, Eberstadt, Germany) through cell staining with the addition of Trypan blue dye (0.4% in physiological solution; v/v). Cell viability was determined by the percentage of live cells in cell suspension (100 cells counted). The following equation was used to calculate cell density:

\[
\text{Viable cells per mL} = \frac{\text{Viable cells}}{n^3 \text{ of squares counted}} \times \text{dilution} \times 10,000
\]

Subsequently, the cell suspension was seeded into 96-well flat microplates (2 x 10^5 cells.mL^-1; 50 µL per well) and exposed, over 24 h in the dark, to a range of leachate concentrations prepared from a
stock solution of mask leachate (10 g L\(^{-1}\)) sequentially diluted in Dulbecco's Modified Eagle Medium (hereafter DMEM, pH 7.4) to obtain the following tested concentrations of the leachate: 1, 2.5, 5 and 7.5 g L\(^{-1}\). These solutions were prepared on the day of the bioassay and maintained at 4°C, in the dark, until incubation of mussel haemocytes to the respective exposure conditions. Blanks containing only DMEM cell culture media and anti-aggregation solution, absent of cells, were prepared as a reference, jointly with a negative control group (CT-; cells jointly with an anti-aggregation solution and DMEM) and a positive control group (CT+) prepared with sodium dodecyl surfactant (5 mM SDS, in DMEM), known to cause cytotoxic effects in the endpoint measurement. Eight replicates were prepared per treatment and control conditions. After the 24-h incubation, centrifugation at 1200 rpm (10 min, at 4°C) was carried out to promote cell adhesion to the bottom. The supernatant (medium) was discarded, and cell viability assessed through the Neutral Red (NR) assay described in 2.5.1.

2.5.1 Neutral Red cytotoxicity assay

The Neutral Red (NR) assay was applied to reveal the viability of mussel haemocytes through the capacity of live cells to incorporate the dye in lysosomes via non-ionic passive\(^{[41]}\), according to the protocols of Katsumiti et al.\(^{[39]}\) and Fonseca et al.\(^{[42]}\), with slight adaptations. Subsequently, 50 µL of filtered (Sartorius, 0.22 µm cellulose acetate filters) NR working solution was added to each microplate well and left in the dark for incubation over 1 h. Afterwards, to remove the excess dye from the medium, microplates were again centrifuged and gently washed with PBS until complete removal of the dye from the blanks. An acetic acid and ethanol solution (1:100 v/v) was seeded into a microplate and left over 20 min in the dark at 18°C for dye extraction from viable cells. Afterwards, the cell suspension was transferred into a new V-bottom 96 well microplate and centrifuged. The supernatant was carefully placed into a new flat bottom microplate to measure the absorbance obtained from the neutral red extracts of the viable cells (550 nm, Infinite M200 Pro, TECAN®). Live and viable cells present higher absorbance, given the higher embodiment of dye into the lysosomes.

2.6 Cell viability and genotoxicity

Haemolymph was extracted as previously described (Section 2.5) and divided into two aliquots: one for the Trypan blue exclusion assay to measure cell viability, and the other was used for the Comet assay to assess genotoxicity.

Cell staining was performed with Trypan blue dye (0.4% in physiological solution; v/v) in a proportion of 1:1 (cell suspension: Trypan Blue 0.4%), whereby the percentage of live cells was determined. Cell viability was obtained through the relative number of viable and non-viable cells by counting blue-
stained cells as dead and the translucid ones as alive. Results were expressed as a percentage of viable cells over total cells.

DNA damage was estimated using the alkaline Comet assay, adapted for marine mussels by Gomes et al.\textsuperscript{[43]}. Microscope slides were pre-cleaned with ethanol and cast with normal melting point agarose (NMA) in Tris-acetate EDTA. Individual haemolymph aliquots were centrifuged at 3000 rpm over 3 min (4°C), and the pellets were suspended in 0.65% low melting point agarose (LMA, in Kenny's salt solution) and cast over the microscope slides. Cells in the slides were then submitted to a lysis step over 1 h, and electrophoresis was carried out for 5 min at 25 V and 300 mA, followed by immersion in a neutralising solution (0.4 mM Tris, pH 7.5) over 15 min.

For the evaluation of the DNA in the comet tail (tail DNA %), slides were stained with DAPI, and pictures were taken from 50 random cells from each slide under a magnification of ×400 in an optical fluorescence microscope (Axiovert S100) coupled with a camera (Sony). Scoring analysis was performed using Imaging Software Komet 7.1 (Kinetic Imaging Ltd). Results are expressed as mean tail DNA % ± STD.

2.7 Biochemical analysis in M. galloprovincialis

2.7.1 Antioxidant and biotransformation enzyme activities

Antioxidant (SOD, CAT, G6PDH) and biotransformation (GST) enzyme activities were determined in gills and digestive glands from unexposed and leachate-exposed mussels. For that purpose, tissues of organisms (n=6 per treatment) were individually homogenised in 5 mL of Tris-sucrose buffer (20 mM Tris, 0.5 M sucrose, 0.075 M KCl, 1 mM DTT, 1 mM EDTA, pH 7.6). The homogenate was centrifuged at 500 g, under 4°C, and the supernatant re-centrifuged at 12,000 g (45 min, 4°C). Cytosolic fraction was isolated and stored at -80°C to determine enzymatic activities and total protein content.

SOD activity was determined through the method described by McCord and Fridovich\textsuperscript{[44]}, whereby the decrease in the absorbance of the substrate cytochrome-\textit{c}, by competition with the xanthine oxidase/hypoxanthine system, is measured spectrophotometrically at 550 nm. Results are expressed as U mg\textsuperscript{-1} protein. To evaluate CAT activity, the decrease in the absorbance of the hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) was measured, revealing its consumption at 240 nm. CAT activity is herein presented in nmol min\textsuperscript{-1} mg\textsuperscript{-1} protein.

The activity of the housekeeping enzyme G6PDH was indirectly determined through the method described by Glock and McLean\textsuperscript{[45]}, adapted by Almeida et al.\textsuperscript{[46]}, through which the reduction of
NADP to NADPH is measured spectrophotometrically at 340 nm. Results are expressed as U mg$^{-1}$ protein. The metabolism of biotransformation mediated by GST activity was quantified according to the method of Habig et al.[47], adapted for microplate reader, by the conjugation of 0.2 mM reduced glutathione (GSH) with 0.2 mM 1-chloro 2,4 dinitrobenzene (CDNB), in a reaction mixture of 0.2 M KH$_2$PO$_4$/K$_2$HPO$_4$ buffer (pH 7.9), at 340 nm. The respective enzymatic results are expressed as CDNB nmol min$^{-1}$ mg$^{-1}$ protein.

**2.7.2 Lipid peroxidation**

Gills (n = 6) and digestive glands (n = 6) of *M. galloprovincialis* were individually homogenised in Tris-HCl buffer (20 mM, pH 8.6) with butylated hydroxytoluene (BHT) and centrifuged over 45 min (30,000 g, at 4°C). The resulting supernatant was stored under -80°C for further measurement of total protein content[48] and the determination of lipid peroxidation by-products, namely malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE), both products of the peroxidation of polyunsaturated fatty acids. The levels of MDA+4-HNE were determined according to the method described by Erdelmeier et al.[49]. For that purpose, malondialdehyde bis-(dimethyl acetal) (Sigma-Aldrich) was used as standard, and the absorbance of the samples was measured at 586 nm in a microplate reader (Infinite M200Pro, TECAN®). Results are expressed as nmol MDA + 4-HNE mg$^{-1}$ protein.

**2.7.3 Determination of total protein content**

Total protein concentration was determined in the cytosolic fraction of the aliquots regarding the analysis of antioxidant enzyme activity, GST activity and LPO levels, using Bovine Serum Albumin (BSA) as a standard[48]. Absorbance was read at 595 nm, and total protein concentrations were expressed as mg protein g$^{-1}$ wet-weight tissue.

**2.8 Statistical Analysis**

Results regarding biomarker responses were first checked for normality and homogeneity by the Kolmogorov-Smirnov and Bartlett's tests using GraphPrism 9 (GraphPad Software, Inc.). Student's t-test was applied to determine significant statistical differences between the effects addressed in paired samples from mask-leachate and control treatments. The critical value for statistical significance was $p < 0.05$. 
3. Results

3.1 FTIR analysis

The FTIR analysis of the surgical masks confirmed that the composition of the three protective layers was polypropylene. All the layers showed the same typical bands of this polymer with the CH₃ and CH₂ stretches (asymmetric and symmetric) in the region 3000-2850 cm⁻¹, as well as the methyl group present near 1380 cm⁻¹ and the aromatic ring in 1450 cm⁻¹ (Figure 1).

3.2 Particle size distribution in the mask leachate

Face mask leachate revealed the presence of 126 microparticles.m⁻³. The main morphology was fibres (97%) of different colours, except for some coloured fragments, at a density of 3.4 fragments.m⁻³ (Figure 2). The average size for fibres was 66.5 ± 24.4 µm and 34.6 ± 15.9 µm for fragments, showing the bigger size of the fibres in these samples.

The TOC calculation determined the presence of NPs (below 1 µm). In all cases, the amount of carbon in leachate was higher than in negative controls. The concentration of carbon after 72 h was 3.21 mg.m⁻³ in leachate, in contrast to the 0.72 mg.m⁻³ found in the ASW control, indicating that NPs have been leached from the facial masks. DLS analysis confirmed the presence of submicron particles in the leachate in the 195.6 ± 96.6 nm range (Figure 3) that were not present in the controls. In addition, few NPs in the 10-100 nm size range were present.

3.3 Antioxidant and biotransformation enzyme activities

After 14 days of exposure to the mask leachate, mussels revealed an increasing trend in SOD activity in gills, although not significant when compared to controls (p > 0.05; Figure 4A), whereas digestive glands experienced a significant increase in SOD activity compared to unexposed mussels (p < 0.05) (Figure 4B). In contrast, the mechanism that H₂O₂ scavenging exerted by CAT activity increased significantly in leachate-exposed mussels’ gills (Figure 4C), whilst in digestive glands, levels were comparable to the controls (p > 0.05) (Figure 4D). Regarding G6PDH activity, although there was a decreasing trend in the gills exposed to the mask leachate, this decrease was not significant when compared to unexposed mussels (p > 0.05) (Figure 4E) while in digestive glands, G6PDH activity from leachate exposed mussels significantly decreased in comparison to control levels (p < 0.05) (Fig. 4F). The results of the biotransformation metabolism showed that GST activity decreased in the gills of mussels exposed to mask leachate, while there was a slight increase in GST activity in the digestive glands. However, this trend was not significant in either tissue when compared to the control group (p > 0.05; Figure 5).
3.4 Lipid peroxidation

Levels of LPO by-products detected in gills from mussels exposed to mask leachate were significantly higher (2.7-fold) than those from the controls \((p < 0.05; \text{Figure 6A})\), whereas in digestive glands, no significant differences were detected \((p > 0.05; \text{Figure 6B})\).

3.5 Genotoxicity

Haemocytes retrieved from mussels exposed over 14 days to mask leachate experienced a significant increase of 150% of DNA tail compared to the control treatment \((p < 0.05; \text{Figure 7})\).

3.6 In vitro cell viability

As observed in Figure 8, haemocytes revealed a significant and monotonic dose-response relationship with a decrease in cell viability from the concentration of 0.5 g.L\(^{-1}\) and onwards \((p < 0.0001)\). This significant change in cell viability indicates that concentrations of the leachate > 0.25 g L\(^{-1}\) led to the mussel’s haemolymph cell death.

4. Discussion

Findings from the present study are the first data unravelling the biochemical, cytotoxic, and genotoxic disturbances caused by weathering of DFM that release of MPs and NPs to seawater in the relevant marine sentinel species *M. galloprovincialis*,.

Simulation of face mask weathering carried out in the present investigation was accountable for generating a total of 126.4 microparticles.m\(^{-3}\) in the aquatic system, most of which are fibres (95%).

The number of fibres released depicts a high disparity other weathering assessments with tri-layer masks[17]. Variations in the number of fibres released by non-woven face masks can be noted based on the weathering duration and exposure conditions to which they are submitted[17]. Current challenges were enumerated and emphasised regarding realistic simulation of fibres pollution due to the lack of harmonisation of techniques applied across studies for analytical detection and quantification of fibres.[50,51].

In previous studies, face masks were submitted to mechanical and chemical external forces under laboratory conditions (rotating blender, treatment with alcohol/detergents) that are not similar to those experienced in the open environment[51,52]. Methodologies based on unrealistic simulations of shear stress forces are prone to generate a substantially higher amount of submicron fragments and particles, and the calculation of MPs and NPs generated from mask leachates may be overestimated[6]. The impact of face masks in the marine environment is in its infancy, and therefore, there are currently no
standardised methods for analytical procedures and ecotoxicological assessment on this topic. As a consequence, there is a lack of calibrated procedures to ensure the realistic estimation of the impact and weathering of face masks under marine environmental conditions \[51,52\]. The vibration of functional groups (Figure 2) confirmed the polypropylene composition of the disposable surgical face masks, whose breakdown mainly occurs in the marine environment through photo- and thermo-oxidative degradation \[53\]. It is hypothesised that the application of used face masks in the present assessment, with different times of utilisation, contributed to contamination of the mask leachate with coloured fibres other than blue and transparent-white \[54\], potentially from the entrapment of MPs and NPs suspended in the air, or from tissues or clothing which may be accountable for the broader burden of MPs and NPs amount released in the leachate \[13,16\]. Furthermore, when MPs and NPs are present in seawater, they tend to aggregate \[55\]. This aggregation might significantly affect the bioavailability of the mixtures of these particles in the marine environment \[55,56\].

To date, scarce ecotoxicological investigation regarding face mask weathering has been conducted using aquatic species, with few approaches focused on marine biological models \[57\]. In the present ecotoxicological assessment, gills of mussels submitted to the mask leachate presented a SOD activity similar to unexposed mussels. However, a significant increase in CAT activity was addressed as a hydrogen peroxide scavenging mechanism (Figure 4C) to counteract the harm generated by the physical stress promoted by MPs and NPs ingested. Such a trend was also reported in marine mussels \[M. galloprovincialis\] under exposure to NPs and to emerging chemical contaminants \[33,40,55,58\], revealing that sources of hydrogen peroxide generation other than upon superoxide anion dismutated by SOD could be operating for CAT activation \[59,60\]. In contrast, SOD, the first line of defence in protecting tissues against oxidative stress \[61\], demonstrated to be an efficient response in digestive glands of \[M. galloprovincialis\] to overcome the harm generated by the accumulation in this tissue of the micro and nanoparticles ingested by the mussels \[31\], potentially jointly with the accumulation of the other chemical additives released from the face masks \[16,62,63\] that were not analysed in the present work. Although CAT activity works in coordination with the activity of SOD, catalysing the reduction of hydrogen peroxide into water, the activity of such enzyme was not significantly altered in digestive glands, possibly due to the H\(_2\)O\(_2\) clearance carried by peroxidases present in various subcellular compartments, such as glutathione peroxidases (GPx), which have a critical role in protecting cells against oxidative stress \[60,61,64\]. The decrease in the activity of G6PDH in gills, although significant only in the digestive gland, hypothesises the interference of the mask leachate on the activity of the glutathione-dependent system. Such an enzyme consists of an additional component of the antioxidant system accountable for catalysing the regeneration of the reduced nicotinamide adenine dinucleotide
phosphate (NADPH). This essential cofactor operates jointly with glutathione reductase (GR) in the regulation of the intracellular supplies of the reduced form of glutathione (GSH), a potent *in vivo* antioxidant agent against the oxidative damage caused by reactive oxygen species. G6PDH-deficient cells experience a decrease in the GSH recycling mechanism that promptly compromises the ability of the antioxidant systems to detoxify hydrogen peroxide, thus unable to withstand oxidative stress.

GST activity is associated with the biotransformation metabolism of organic compounds, using catalysing the conjugation of the reduced form of glutathione (GSH) to non-polar compounds that contain an electrophilic carbon, nitrogen or sulphur atom, leading to the generation of less reactive products, with an ultimate protective role against oxidative stress. The present findings revealed the absence of a biotransformation mechanism carried out by GST activity after mussels’ exposure to mask leachate. This denotes low levels of organic chemicals taken up by mussels possibly due to the low levels of organic chemicals present in the leachate or on the masks. Beyond the physical stress carried out by micro and nano-sized fibres and particles, weathering and deterioration of the face masks are also accountable for contributing to the input of chemical additives in the environment, such as dye compounds, fragrances, antiviral and antibacterial agents. Sullivan et al. addressed the release of leachable inorganic and organic substances from the blue disposable face masks (like those used herein), namely metals, plastic additives, surfactant molecules, polyethylene glycol, polyamide-66 monomer and oligomers (nylon-66 synthesis), surfactant molecules, dye-like molecules and polyethylene glycol. These chemicals could have been released and then taken up by mussels in the present case, although further chemical confirmation is required. No significant alterations in GST activity were also registered in the digestive glands of mussels *M. galloprovincialis* exposed to 50 nm NPs (10 μg.L⁻¹) compared to unexposed individuals, in contrast to a significant suppression in GST activity in the gills of respective animals. Paul-Pont et al. also addressed that the biotransformation mechanism was not altered in the digestive glands of *Mytilus* spp., submitted to polystyrene (PS) MPs (2-6 μm), at 32 mg.L⁻¹, over seven days. However, at the end of the depuration period of seven days, following PS-MPs exposure, GST activity significantly increased compared to unexposed individuals. Accordingly, Li et al. emphasised that wide variability in GST activity response was also reported in bivalves exposed to MPs and NPs. Likewise, as addressed by Prokic et al., the overall antioxidant system of organisms under MPs and NPs exposure exhibited diverse responses, from the absence of significant changes to a decrease and induction of enzymatic activities. Indeed, there is a massive variability between features and parameters to which organisms are exposed in ecotoxicological approaches, namely the form of the
plastic material (e.g. fibres, particle, bead, powder), polymer composition, size of the particle, time of exposure, acclimation conditions, thus altogether accountable for generating scattered ecotoxicological profiles of responses, that also changes across species and tissues analysed. Although the reactive oxygen species (ROS)-scavenging antioxidant system was herein activated due to exposure to the mask leachate, the high levels of LPO by-products in the mussels’ gills evidenced that the protective mechanisms could not efficiently neutralise ROS to prevent cellular lipids from oxidative damage in this tissue\textsuperscript{75,76} and that micro and nanoparticles induce oxidative damage in the gills. Results from the meta-analysis conducted by Li et al.\textsuperscript{13}, aiming to elucidate the role of oxidative stress in toxicity elicited by MPs and NPs in marine species, evidenced that end products of LPO are a reliable index of membrane damage when the ability of the cells to maintain redox balance declines, and the antioxidant system is suppressed, leading to cellular damage of the tissues and potentially fitness costs\cite{Goncalves2022}. Accordingly, it is noteworthy that DNA damage was registered in haemocytes under \textit{in vivo} exposure to the mask leachate, indicating genotoxicity, which may be mainly linked to oxidative damage. These findings collectively corroborate with a vast body of research revealing significant disturbances caused by polypropylene microfibres and fragments and NPs on antioxidant systems from \textit{Mytilus} spp.\cite{26,34,77,Goncalves2022}. Considering the stress depicted by microfibres, Choi et al.\textsuperscript{33} observed a disruption in SOD and CAT activities, both in gills and digestive glands of \textit{M. galloprovincialis} exposed to 1 mg.L\textsuperscript{-1} of PET micro fibre. In addition, a monotonic dose-response pattern was verified for apoptotic mechanisms and the induction of DNA damage in haemocytes, registered from the concentration of 0.1 mg.L\textsuperscript{-1} \textsuperscript{33}. Herein, mussels’ haemocytes in the \textit{in vitro} Neutral Red assay confirmed its sensitivity and reliability in assessing the cytotoxicity posed by weathering and degradation of disposable face masks in the marine environment. A concentration of 0.5 g.L\textsuperscript{-1} of weathered face masks showed cellular disturbances leading to cytotoxicity and cell death. Sendra et al.\textsuperscript{78} addressed that after 3 h of \textit{M. galloprovincialis} haemocytes exposure to PS MPs (1 µm – 10 mg L\textsuperscript{-1}), a subpopulation of large granular cells exhibited significant cytotoxicity compared to the control group. Additionally, these cells reached values higher than 58% of apoptotic cells when individually exposed to PS NPs of 50 and 100 nm at 10 mg.L\textsuperscript{-1}. Chang and Wang\textsuperscript{79} assessed the cytotoxicity of filtered face masks’ leachate (300 g.mL\textsuperscript{-1}) on human alveolar basal epithelial cells (A549), revealing significant inhibition in cell proliferation and induction of DNA damage, ultimately leading to cell death with enhanced exposure time, as a result of exposure to multiple phthalate acid esters. However, to date, no studies have been
conducted on the cellular disturbances caused by face masks in the innate immune system of marine mussels.

Considering MPs and NPs global plastic pollution issue and the biological disturbances addressed across several biological levels in representative marine species, it becomes crucial to include face masks in plastic pollution research for a more accurate projection of the global plastic budget\cite{51}. In addition, research evidence has been built revealing that exposure to MPs and NPs can trigger higher ecotoxicological effects than microbeads or powdered plastic\cite{80}. Bearing in mind the uncertainties regarding future sanitary crises, it becomes urgent to reduce the environmental impact of the face mask legacy, restricting the amount of these items and taking all measures to avoid reaching coastal and marine ecosystems. In this sense, the manufacturing application of plasticisers in disposable masks should be strictly controlled and regulated to reduce environmental and public health issues from exposure also to phthalates, metals, MPs and NPs\cite{79,81}. Strong cooperation among scientists, healthcare professionals, industries, and policymakers should be held to join efforts to transition towards a circular economy that may repurpose products at the end of their life cycle to be reused or become raw materials\cite{82}.

**Conclusions**

Despite the growing scientific evidence on the mechanistic ageing and weathering of single-use face masks and subsequent interactions with aquatic biota, there are still many uncertainties regarding the overall toxicity caused by the multitude of chemical compounds and types of particles that leaches from single-use face masks into the marine environment. The present study brings novel findings of the harmful legacy of global face masking in the marine environment by unravelling the oxidative, cytotoxic, and genotoxic disturbances caused by disposable face mask weathering in the mussel *M. galloprovincialis*. Herein, mussel haemocytes arise as a reliable tool for the *in vitro* cytotoxicity assessment regarding the impact of face masks in the marine environment.

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Author’s contribution

Study conception and design: Fonseca, T., Edo, C., Vilke, J. M., and Bebianno, M. J.

Experimental work and biomarkers analysis: Fonseca, T., Edo, C., Vilke, J. M., and Astudillo-Pascual, M.


Draft manuscript preparation: Fonseca, T., Edo, C., Vilke, J. M., Astudillo-Pascual, Gonçalves, J.M, and Bebianno, M. J.

Availability of data and material – Data will be available at the website of RESPONSE the project

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Conflicts of interest - “All authors declared that there are no conflicts of interest.”

Ethical approval and consent to participate - Not applicable

Consent for publication – “Not applicable”

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ambient environmental conditions. Environ Res 2023;217(September 2022).


Response to Reviewers

Impact of face masks weathering on the mussels *Mytilus galloprovincialis*

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Comments from reviewer(s):

**Reviewer 1:**

The article aims to investigate the ecotoxicological impact caused by the weathering of disposable face masks, once reaching the marine environment, on mussels *Mytilus galloprovincialis*, by assessing biochemical, cytotoxic, and genotoxic effects. And the article is very creative and is the first marine ecotoxicological assessment conducted with face mask weathering. However, the paper also has many shortcomings, such as various format problems, and some parameters of the experiment are also questioned. On top of that, the author does not seem to provide a corresponding any chart. Here are some detailed suggestions:

1. The manuscript content is missing and no pictures are provided.
   **R:** The document was reviewed and ensured all figures are now appropriately included.

2. There are many formatting problems in the article, such as line 121 "……on a 5-L glass container……", "5-L" should be changed to "5 L", and the space between numbers and degrees Celsius should also be removed. There are also many similar format problems in the following content, which will not be pointed out here, and I hope the author can carefully check and modify it.
   **R:** The text was revised to ensure consistent and correct formatting throughout the manuscript, including removing the space between the number and °C for expressing temperature.

3. It is suggested that the unit format of the full text should be unified, such as "10 g/L" in line 203 and "0.5 g L⁻¹" in line 345.
   **R:** The style of the units throughout the text were unified, choosing the format of a symbol for a variable followed by a superscript (e.g. g.L⁻¹), according to SI Unit rules.

4. It is suggested that the writing of pH in the whole paper should be consistent, such as "6.7 pH" in line 192 and "pH 7.4" in line 204.
R: The necessary revisions were made to ensure uniformity in the representation of pH throughout the entire paper.

5. The subheading numbers on lines 230 and 253 of the article are the same. Please check and modify them.
R: The inconsistency in subheading numbers were rectified to ensure accurate and sequential order.

6. In this paper, blue surgical masks are used to assess the release of plastic particles. During the mask collection phase, how to ensure that the collection is medical-surgical masks and not disposable medical masks?
R: For clarification of the type of disposable mask selected for the study throughout the manuscript authors opted to use the following terminology: disposable surgical face mask and the following acronym (DFM).

7. The mask is vigorously stirred in seawater for 72 hours to simulate wave wear. Is there any reference for this stirring time?
R: This work was developed within the RESPONSE project framework, and the authors followed the standard project methodology proposed by Almeda et al. (2023) for the leaching of micronized plastics for toxicity testing in aquatic organisms, which indicates the use of 72 hours in the process of leaching.
Link to Almeda et al., 2023: https://doi.org/10.1016/j.chemosphere.2023.138894.

8. In this paper, the mask after simulating the wear of sea waves was dried in an oven at 60ºC for three days. Will the temperature of 60ºC not affect the mask?
R: It is worth noting that the drying of microplastic materials in an oven is a common practice in the literature, and it was demonstrated to have minimal impact on the identification of plastic materials (which is not recommended for deep chemometric analyses). This process was well-tested and widely accepted. Furthermore, regarding technical considerations, the melting temperature of polypropylene, commonly used in these masks, is 160ºC. It is at this temperature that noticeable changes may occur, as supported by findings in publications such as doi: 10.1016/j.matlet.2021.131270

9. In addition to using natural seawater, the article also uses artificial seawater. It is recommended to provide details on the preparation of artificial seawater.
R: The artificial seawater (ASW) prepared in the present ecotoxicological study was meticulously prepared following the guidelines outlined in the ASTM D1141-98 standard. Therefore, the text was revised, and the following sentence was added: ‘ASW (salinity 35) was reconstituted according to ASTM D1141-98 standard, ensuring a composition that adheres to the specified concentrations of sodium chloride, magnesium chloride, calcium chloride, sodium sulfate, potassium chloride, and sodium bicarbonate present in seawater (ASTM, 2021).’
10. The results and discussion part of the article only expounds the results and lacks the related reasons.

R: The discussion was changed to incorporate relevant explanations and reasons to offer a more thorough interpretation of the results.

Reviewer 2:
My comments are:

The authors should provide more detailed information regarding the sample size used for in vivo experiments and how it was calculated.

R: Detailed information about the sample size in the in vivo experiment section was included.

"In Figure 4, how do the authors explain the observed differences in results across various tissues?"

R: In both tissues, significant differences exist related to the responses of the antioxidant (SOD and CAT) and auxiliary (G6PDH) system enzymes. SOD and CAT significantly increased while G6PDH decreased. SOD response may be attributed to a need to reduce the superoxide anions generated by MPs, NPs, and possible chemicals present in leachate. The decreased G6PDH activity may indicate a deficiency in the glutathione metabolism (GSH) supply, considering that G6PDH acts by reducing NADPH, which is essential for the production of GSH. While in the gills, the increase in CAT activity is possibly related to the attempt to mitigate the stress produced by the MPs and NPs in the leachate.

Reviewer 3:
This paper describes the results of a number of scientific tests mostly on mussels probing the effects of plastic-related pollutants from face masks. The “product leachate” was generated by exposing a relatively heavy load of disposable face masks for 72 hours.

Overall, I suggest the author make some serious changes to many phrases and descriptions, grammar errors, loose organization, and a few questionable statements. Further, the authors need to more clearly describe their results in a way that is separate from the many cited studies.

R: The manuscript was thoroughly read and any grammar errors identified and corrected and clarity enhanced.

In the abstract, the second sentence states “However, most of these masks end up in the ocean adding another source of ocean plastic pollution.” Are there studies/data that verify this statement that most facemask waste ends up in the ocean and not in landfills?

R: According to the best of the authors’ knowledge, no scientific data precisely confirms that statement but several pictures showed the presence of the masks in the ocean.: However, the sentence was corrected as follows 'Hence, face mask littering and improper waste management have contributed to an additional burden to the current plastic pollution in the ocean’. This change aligns with scientific research conducted during the pandemic, emphasising the challenges posed by the closure of waste management systems related to health security [https://doi.org/10.1016/j.marpolbul.2020.111517](https://doi.org/10.1016/j.marpolbul.2020.111517) and the arrival
of litter, presumably containing disposable face masks, from open landfills into natural environments https://doi.org/10.1016/j.marpolbul.2021.111986

“After a 14-day in vivo exposure of M. galloprovincialis to face mask leachate (100 mg/L)” What does the 100 mg/L represent? Face mask microplastics? How much is dissolved vs particle leachate?
R: A leachate of 10 g of mask.L⁻¹ was prepared and the solution obtained diluted to represent 100 mg of original masks in 1 L.

Also, in the abstract, “After a 14-day in vivo exposure of M. galloprovincialis to face mask leachate (100 mg/L) the antioxidant system activated in the gills,” What antioxidant system?
R: The sentence was rephrased for the elucidation that antioxidant response involved: was an increase on CAT activity.

Incorrect grammar: “indicated that leachate concentrations ≤ 0.5 g L⁻¹ 25 poses” pose, not poses; and “Therefore, the leachate obtained from face masks in seawater cause oxidative damage” causes
R: the sentence was corrected.

How concentrated are the pollutants in the ocean? “that the plastic burden generated by disposable face masks in the ocean...”
R: The ocean has also been addressed as the final repository of face masks. Approximately 52 billion masks were produced worldwide in 2020, of which at least 1.56 billion were littered into the ocean, resulting in 4680 to 6240 tons of marine plastic waste (Oceans Asia, 2022). In addition, when these masks are released into the aquatic environment, they act as a potential source of microfibers.

Line 51: “One of the most worldwide accepted action” should be actions
R: The sentence was corrected.

Line 60. What is a semester? “In the first semester of 2020,”
R: The sentence was rephrased as follows: ‘On the rise of the coronavirus outbreak, projections estimate that 129 billion face masks were used monthly worldwide, amounting to over 1.24 trillion discarded globally from December 2019 to May 2021 since the start of the pandemic (Yang et al., 2023).’

Line 68 – no comma needed “entered THE marine environment, in 2020”. There are many additional grammar errors after this point in the manuscript.
R: The punctuation was removed.

While this is important to reduce external contamination (“To limit the overestimation of MPs counting in the leachate sample, glassware and cotton clothing were adopted and applied during the whole assay to avoid plastic contamination.), blank or control samples should be part of the procedure since microfibers are extremely ubiquitous.
R: Throughout the research, stringent controls were implemented, including the use of 1-micron fibreglass filters in the preparation of leachates from natural seawater and artificial seawater (ASW). Mussels’ tanks and leachate preparation remained sealed during the incubation to prevent airborne particle deposition. These measures were taken
to ensure the reliability of the results and minimise potential sources of contamination. These points were clarified in the manuscript.

How was this exposure determined, as it seems much higher than what would be encountered in ocean ecosystems? “mask leachate, at a concentration of 100 mg/L, jointly with a control group”
R: As mentioned earlier, a stock solution of mask leachate (10 g of face mask.L-1), was prepared and diluted in the test systems to reach the final concentration of 100 mg.L-1, to which mussels were exposed. However, apart from picture and media information about masks being collected in the ocean, there is no scientific evidence obtained from environmental measurements of face mask lin the ocean., Therefore, the selected concentration of 100 mg.L-1 reflects the potential leachate of the equivalent to 4 masks.L-1, which are prone to be encountered at this density in the natural environment, providing a more realistic perspective on the potential impact on marine environments.

Line 176: How are organisms weighed using a ratio? “each organism was weighted using the following ratio”. It seems that some of the details in the experimental section can be moved to a supplementary document.
R: Changes were made to clarify the sentence.

What is the difference between the presence and the density? “Face mask leachate revealed the presence of 126.4 microparticles/m3. The main morphology was fibres (97%), of different colours, except for some coloured fragments, at a density of 3.4 fragments/ m3 (Figure 2).
R: The sentence was changed to include the presence of 3.4 fragments/m3

The average size for fibres was 66.5 ± 24.42 µm and 34.6 ± 15.94 µm for fragments, showing the bigger size of the fibres in these samples. What was the size limitation based on the analytical tools used? Significant figures: “195.6 ± 96.59 nm range”
R: Regarding the size limitation of the Malvern Zetasizer Nano ZS apparatus, is the most common instrument used for particle size analysis and dynamic light scattering. For clarity, the typical size range for particles measured by this apparatus spans from 0.6 nanometers to 6 micrometers.

How were these levels determined? “Levels of LPO by-products detected in gills from mussels exposed to mask leachate”. The reliance on UV absorbance for many tests is concerning, given the lack of specificity of these types of measurements.
R: The spectrophotometric method for the quantitative determination of the lipid peroxidation-derived aldehydes MDA and 4-HNE has been widely applied in ecotoxicological assessments and is base on the method described by Erdelmeier et al (1998), showing to be sensitive and highly reproducible to infer oxidative damage in marine organisms (Gonçalves et al., 2023; Lopes et al., 2022; Queirós et al., 2021; Nardi et al., 2022).

Link for the manuscripts:
Nardi et al. (2022): https://doi.org/10.1016/j.envpol.2022.118970
The first paragraph of the discussion seems to repeat information presented in the Introduction.
R: The discussion section was revised, and the first paragraph was reduced to avoid unnecessary repetition.

Can the authors be more specific in this comparison (line 367)? “denotes a high quantitative disparity compared to other weathering assessments with tri-layer masks”. However, weather conditions vary considerably from place to place. “the realistic estimation of the impact and weathering of face masks under marine environmental conditions”
R: The text was rephrased to ensure clarity and coherence.

Lines 385-394: What are the points that the authors are trying to make in this part of the discussion?
R: The Fourier Transform Infrared (FTIR) analysis ensured that all layers of the surgical face masks comprise the same material, polypropylene. This confirmation is crucial for establishing the degradation characteristics across all layers, thereby reinforcing the homogeneity of the experimental setup and leachate generation. This information is vital for accurately assessing the environmental impact of polypropylene degradation, which remains uniform throughout the mask structure.

Were the authors also investigation the effects of leached chemicals? “potentially jointly with the accumulation of the chemical additives released from the face masks”
R: Despite plastic litter can leach a variety of hazardous chemical contaminants into the aquatic environment, producing harm to biota, as addressed by a growing body of evidence, data regarding the chemical screening carried out with the herein-produced face mask leachate could not be provided due to analytical constraints.

Line 445: “type” usually refers to the chemical content. “Form” would be a better term: “namely the type of the plastic material (e.g. fibres, particle, bead, powder)”
R: The sentence was corrected as suggested.

Reviewer 4:
Please address the following aspects:

Hypotheses under testing are needed
R: The hypothesis was added.

L117 Due to its broad use during the COVID-19 Maybe better: Due to FACE MASKS broad use during the COVID-19
R: The sentence was reformulated as suggested.

L512:LeacheS
R: The correction was made.
Reviewer 5:

The COVID-19 pandemic increased the need for single-use face masks, leading to an increase in its production and in a correct disposal policy. However, most of these masks end up in the ocean adding another source of ocean plastic pollution. This study aims to investigate the ecotoxicological impact caused by the weathering of disposable face masks, once reaching the marine environment, on mussels Mytilus galloprovincialis, by assessing biochemical, cytotoxic, and genotoxic effects.

Do authors have an idea about the percentage of single-use face masks among global plastic ocean pollution in Portugal?

R: To the best of the authors’ knowledge, no data is available to estimate Portugal’s contribution on disposable face mask pollution into the global ocean. To date, estimated data is available only on the acceptance of face mask usage and the number of masks used daily (90% acceptance of masks and 18.25 million masks/day, respectively) (Patrício Silva et al., 2021). Additionally, information is available on Portugal’s personal protective equipment (PPE) usage, represent an additional contribution of 4.97% to municipal solid waste (MSW) (Patrício Silva et al., 2021). Therefore, there is no data on the input of single-use masks into aquatic and marine environments.

COVID-19 and the global use of disposable face masks

Improper disposal and landfill mismanagement leading to face masks fate in aquatic ecosystems

Oxidative stress
Biotransformation
Oxidative damage
Genotoxicity
Cell viability

14-day bioassay exposing mask leachate to mussels

Face mask weathering in the environment: Release of plastic fibres

Uptake and effects in marine bivalves?

Mask leachate preparation

Mussels Mytilus galloprovincialis