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On the horns of a dilemma: Evaluation of synthetic and natural textile microfibre effects on the physiology of the pacific oyster *Crassostrea gigas*

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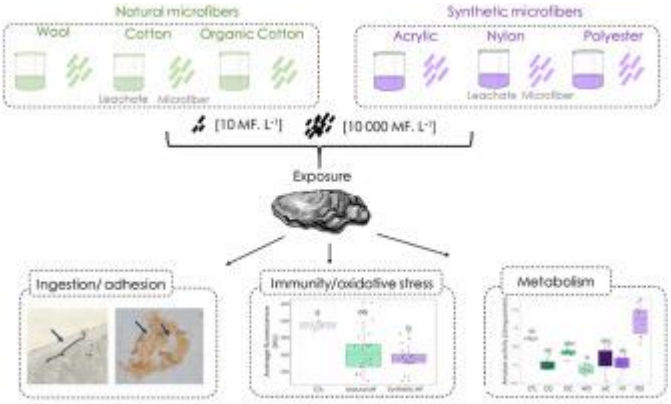
Abstract :

Fast fashion and our daily use of fibrous materials cause a massive release of microfibres (MF) into the oceans. Although MF pollution is commonly linked to plastics, the vast majority of collected MF are made from natural materials (e.g. cellulose). We investigated the effects of 96-h exposure to natural (wool, cotton, organic cotton) and synthetic (acrylic, nylon, polyester) textile MF and their associated chemical additives on the capacity of Pacific oysters Crassostrea gigas to ingest MF and the effects of MF and their leachates on key molecular and cellular endpoints. Digestive and glycolytic enzyme activities and immune and detoxification responses were determined at cellular (haemocyte viability, ROS production, ABC pump activity) and molecular (Ikb1, Ikb2, caspase 1 and EcSOD expression) levels, considering environmentally relevant (10 MF L−1) and worst-case scenarios (10 000 MF L−1). Ingestion of natural MF perturbed oyster digestive and immune functions, but synthetic MF had few effects, supposedly related with fibers weaving rather than the material itself. No concentration effects were found, suggesting that an environmental dose of MF is sufficient to trigger these responses. Leachate exposure had minimal effects on oyster physiology. These results suggest that the manufacture of the fibres and their characteristics could be the major factors of MF toxicity and stress the need to consider both natural and synthetic particles and their leachates to thoroughly evaluate the impact of anthropogenic debris.

Environmental Implication.

Microfibres (MF) are omnipresent in the world oceans with around 2 million tons released every year, resulting in their ingestion by a wide array of marine organisms. In the ocean, a domination of natural MF-representing more than 80% of collected fibres-over synthetic ones was observed. Despite MF pervasiveness, research on their impact on marine organisms, is still in its infancy. The current study aims to investigate the effects of environmental concentrations of both synthetic and natural textile MF and their associated leachates on a model filter feeder.

Graphical abstract



Highlights

► Environmental concentration of MF is sufficient to perturb oyster physiology. ► Tested natural MF elicit higher digestive and inflammatory responses than synthetic. ► The physical properties of natural MF might explain the observed perturbation. ► In general MF toxicity depends on their properties and intended use.

Keywords : fast fashion, textile microfibers, exposure, leachates, oysters, endpoint

1. Introduction

The rapid growth of the textile industry and consumption patterns (e.g. fast-fashion) have led to a 30% increase in fibre production worldwide over the past ten years, reaching 120 million tons in 2019 (The Fiber Year 2020). Although fibre production is dominated by synthetic materials (63%), among which polyester (56%) and acrylic (23%) are the most common (Barrows et al., 2018; Henry et al., 2019), natural fibres represent 31% of global production and cotton is the second most produced worldwide (25%) after polyester. This intensive production of fibres and our daily use and maintenance of fibrous materials over many years (e.g. household, agricultural or industrial textiles) have led to a massive release of microfibres into the environment (De Falco et al., 2020).

Microfibres (MF) are defined as any fibrous materials of natural or artificial origin that are less than 50 μm in diameter and 5 mm in length (Liu et al., 2019). They are present in the atmosphere (Dris et al., 2016), soils (Zhang et al., 2019; Zubris and Richards, 2005), lakes (Negrete Velasco et al., 2020; Whitaker et al., 2019) and oceans (Barrows et al., 2018; Suaria et al., 2020) and are considered the most common anthropogenic particles in the world (Gago et al., 2018). As concerns the oceans, it has been estimated that around 2 million tons of MF are released every year through domestic washing, and the textile and tire industries, fishing nets and ropes (Mishra et al., 2019) or wet wipes and sanitary towels (Ó Briain et al., 2020). Although disentangling MF sources remains challenging (Salvador Cesa et al., 2017), household washing machine effluent is considered as one of the major sources of MF pollution in the oceans, corresponding to 35% of the sampled MF (Boucher and Friot, 2017).

Microfibres are omnipresent in the world's oceans (Barrows et al., 2018), with sub-surface concentrations ranging from 0.02 MF L^{-1} to 25.8 MF L^{-1} (Suaria et al., 2020). Research on the impact of MF on aquatic biota is still in its infancy due to the difficulties of characterizing and manipulating MF (Ryan et al., 2020). Microfibres are traditionally linked to plastic

pollution, as it is assumed that they are mostly derived from synthetic textiles. However, a recent study where MF were collected from six ocean basins showed a domination of natural fibres (91.8%) over synthetic ones, with most of the natural fibres being cellulosic (79.5%) (e.g. cotton) or of animal origin (12.3%, e.g. wool) (Suaria et al., 2020). The biodegradation of cotton yarn in the marine environment takes less than a year (240 days for 75% biodegradation), which is much slower than the 4% estimated for synthetic MF over the same period of time (Zambrano et al., 2021, 2019). The ubiquity and abundance of cotton MF on the one hand, and the low biodegradability of synthetic MF on the other hand suggest a high availability of both types of MF to marine filter feeders.

The incorporation of chemical compounds to improve textile properties, as well as dyeing and pesticide treatments (up to 8000 different chemical compounds are used in the textile industry; Kant, 2012) during textile production (Carney Almroth et al., 2021), also calls into question the alleged harmlessness of natural MF. Despite a recent study showing the release of MF-associated chemical additives under ultraviolet light (Sørensen et al., 2021), toxicological studies seldom investigate the impact of fibre leachates (i.e. associated chemical additives) on aquatic organisms. Similarly, to our knowledge, few studies have addressed the toxicological effects of both natural (lyocell made from cellulose) and synthetic MF (Polypropylene, PP and polyethylene terephthalate, PET) on aquatic organisms, highlighting gut damage in shrimps (*Artemia franciscana*) and water flea (*Daphnia magna*) exposed to high concentrations (500–2000 mg L^{-1}) of both types (Kim et al., 2021a, b).

The effects of synthetic MF on aquatic organisms have, nevertheless, received more attention. In marine organisms, successful ingestion of synthetic MF has been observed experimentally in bivalves (Woods et al., 2018), crustaceans (Coppock et al., 2019) and cnidarians (Romanó de Orte et al., 2019). In most cases, ingested synthetic MF were found in the digestive tracts, with impacts ranging from a disturbance of their energy metabolism and

alteration of feeding behaviour (Watts et al., 2015; Welden and Cowie, 2016) to an increase in mortality rate (Horn et al., 2020). In bivalves such as mussels, other effects include tissue and DNA damage after a 7-day exposure to lint recovered from a tumble dryer (1 $x10^5$ and 1.8 $x10^5$ mg L^{-1}) (Alnajar et al., 2021) or an increase in necrosis, ROS production and acetylcholinesterase activity following a 96-h exposure to PET MF (100 mg L^{-1}) (Choi et al., 2021).

By investigating the impact of different textile MF of natural (wool, cotton, organic cotton) and synthetic (acrylic, nylon, polyester) origins and their associated chemical additives on the physiology of a sessile marine bivalve, the current study will examine the capacity of oysters to ingest both natural and synthetic textile MF and explore the effects of both MF and their associated chemical additives on key physiological functions (e.g. metabolism, digestion, immune response and detoxification processes) using molecular and cellular endpoints. The Pacific oyster *Crassostrea gigas* makes a relevant model species for this study as it provides important ecosystem goods and services and has the ability to filter a large amount of water (Troost, 2010). To provide support for decision-making processes regarding MF pollution, two scenarios will be considered: an environmentally relevant scenario (average MF concentration in the world oceans; 10 MF L^{-1}) (Ryan et al., 2020; Suaria et al., 2020) and a worst-case scenario, useful for understanding organisms' response mechanisms (an MF concentration 1000 times higher; 10 000 MF L^{-1}).

2. Material and methods

2.1. Precautions and procedural blanks

During the preparation of MF and the whole course of the experiment, special care was taken to avoid fibre contamination (Prata et al., 2020). These precautions included wearing a unique cotton-blend white lab coat, successively washing laboratory equipment with filtered solutions of distilled water and ethanol, preliminary filtration of all solutions on glass filters (GF/F,

porosity 1.2 µm), burning glass vessels before use (450°C, 6 h) and covering them with aluminium foil. Multiple procedural blanks were used throughout the preparation and experiment, during fibre and leachate preparation, oyster acclimation and exposure, tissue collection, histological Raman imagery and digestion of oyster tissues. These blanks consisted of pre-washed and pre-burned glass beakers (200 mL) half-filled (100 mL) with ultra-purified filtered water. One or several beakers (depending on the size of the room) were put out at the beginning of each step of the experiment listed above, retrieved at the end, and filtered on glass filters (GF/F, porosity 1.2 µm). For each step, the filters were examined visually under a binocular magnifier for the presence of MF (EVOS™ XL Core Imaging System; ×10–20 magnification), and MF composition was examined using a FTIR (JASCO IRT-5200) when needed.

2.2. Organisms and husbandry

800 oysters (age 12 months, average size 39.9 ± 5.9 mm, average weight 9.4 ± 3.5 g; produced as described in Petton et al., 2015) was transferred from the Ifremer nursery in Bouin (France) to the experimental facility in Plouzané (France) in February-March 2021. The oysters were acclimatized for 7 days in a 400-L tank supplied with UV-treated 10-µm filtered seawater and fed with a basal ration of a mix of diatoms (*Tisochrysis lutea* and *Chaetoceros gracilis*, $3x10^4$ cell mL^{-1}) (Brown *et al*., 2002). Seawater temperature was slowly incremented (1.5°C day) from the *in situ* temperature of 13°C to an optimum temperature of 20°C, at which oyster dietary intake and metabolism are promoted (Le Moullac et al., 2007).

2.3. Microfibre and leachate preparation

Three natural and three synthetic fibres commonly used in a wide range of clothing products were commercially purchased. Natural yarns were wool (WO, Phildar; 154364), cotton (CO, Phildar; 200442) and organic cotton (OC, Phildar; 226494), and synthetic threads were

polyester (PES, Gütermann; 23324), acrylic (AC, MT; 232993) and polyamide nylon (NY, Goodfellow; AM325705). The composition was examined on each type of fiber using an FTIR (JASCO IRT-5200) equipped with a DC-500 diamond cell for micro IR sampling. Apart from the nylon (transparent), the fibres were grey or blue in colour to facilitate their visual identification. Microfibres were prepared by sectioning filaments (60 µm length) following the microtome method of Cole (2016) (Histological platform H2P2, Rennes, France). They were then filtered on polycarbonate membrane filters (8 µm; Nucleopore), rinsed with ultrapure water, resuspended in ultrapure water and Tween-20 (0.01%), quantified using a Malassez haemocytometer and kept in dark conditions at 4°C. Microfibre length and diameter were assessed microscopically on 440 randomly selected individual MF per fibre type (Zeiss, 10×). Surface features of MF were observed by scanning electron microscopy (Quanta 200 MK2; FEI, Hillsboro, OR, United States) with xT microscope software (FEI). Fibre roughness was analysed using an optical profilometer (Sensofar metrology) with confocal in fusion mode (x 150). Measurements were taken on three individual MF per fibre type. On each individual MF, an average of ten measurements were made on a region of 2 × 10 µm.

For leachate preparation (Ci = 1x 10^5 MF L^{-1}), each microfibre studied (n = 6) was added to a separate laboratory glass bottle filled with 200 mL of filtered sterile seawater and incubated at room temperature in the dark on an orbital shaker (250 rpm) for nine days (Carney Almroth et al., 2021). Afterwards, the microfibres were filtered off on glass microfibre filters (1.6 µm, Dutscher) and leachate solutions were kept at 4°C. The absence of microfibres from leachate solutions was checked by microscope on 5 samples of 100 µL (Zeiss, 10×).

2.4. Chemical profiling of microfibres

Organic micropollutants, e.g. organochlorine pesticides, organophosphorus pesticides, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and chlorobenzenes, as well as additives (non-target approach) were

targeted based on data available in the literature (Sait et al., 2021) and assayed on the prepared microfibres through a multi-residue method. Although not certified for the fibre matrix, the procedure is in line with the standardized guidelines of ISO17025 and adapted from the one used for the analysis of compounds in solid matrices (LABOCEA laboratory, Plouzané, France). After checking that the extraction solvents do not impair the fibres, molecules present on the fibres were extracted using methanol (24h contact for organic micropollutants; 30 sec contact for additives) followed by a dichloromethane extraction, before being targeted by gas chromatography coupled with tandem mass spectrometry (GC-MSMS, Agilent 7890 GC system linked to an Agilent 7010 triple quadrupole MS) and identified by multiple reaction monitoring (MRM) spectral mode (organic micropollutants) or scan mode (non-targeted research for additives). This mass spectrometry technique allows the selective and sensitive quantification of compounds in complex matrices.

2.5. Experimental setup and sampling

Two complimentary exposures were conducted. In February 2021, oysters were exposed for 96 h (based on Choi et al., 2021; Magara et al., 2019) to 0 (control), 10 MF L^{-1} and 10 000 MF L^{-1} of previously prepared natural (CO, OC, WO) or synthetic (AC, PES, NY) MF. This led to 13 treatments performed in triplicate tanks randomly rotated within the experimental room to avoid tank position effects.

Oysters of similar weight (two-way ANOVA, P = 0.43) were randomly placed in 6-L tanks (n = 11 oysters/ tank) containing 1 µm-filtered seawater (20.4°C ± 0.4°C) equipped with a pressurized aeration system. MF added to a solution of diatoms (12 x 10^4 cells mL) and Tween-20 (0.0002%) was directly poured into the experimental tanks every day at 9 am for 3 days. For control tanks, only Tween-20 (0.0002%) was added to the diatom mix. This was done to avoid MF clumping or sticking to the tank walls (e.g. Sussarellu et al., 2016). The second exposure was conducted according to the same experimental design, except that the oysters

were exposed to the leachates obtained from the MF. The same concentrations were used and Tween-20 (0.0002%) was added to allow comparison with MF exposure. Similarly, to the first exposure, there was no differences in oyster size across treatments (two-way ANOVA, P = 0.28).

At the end of each exposure, 3 oysters per tank were randomly collected (n = 9/ condition) and their haemolymph (50–450 µL) was extracted from the posterior adductor muscle using a hypodermic syringe (23-gauge needle). Sample quality and purity were checked microscopically and the haemolymph of three individuals per treatment was pooled (n = 3 pools per treatment). The digestive gland and gills of the same individuals were excised using stainless-steel scissors and immersed in a nitrogen-frozen solution of isopentane for later histological Raman imagery analysis. The digestive gland and gills of the remaining individuals (n = 24 per treatment) were excised, frozen in liquid nitrogen and kept at -80°C for later biochemical and molecular analysis.

2.6. Uptake of MF and presence in tissues

These analyses were only performed on oysters exposed to the highest concentration of MF and unexposed individuals (controls for putative contamination) to facilitate the visualisation and analysis (first experiment).

2.6.1. Histological Raman imagery

The digestive glands and gills maintained in frozen isopentane were sectioned (10 and 30 µm) on a cryomicrotome (Leica), collected on glass slides and frozen at -20°C. The sections were then imaged and analysed by Raman mapping using a confocal Raman imaging system (Alpha 300R, Witec, Ulm, Germany). First, a picture of the whole tissue section was taken using a 20× objective (NA = 0.4, Zeiss) to localize the MF and morphological characterization was then made through HD picture (NA = 0.9, Zeiss). Raman spectra were then recorded on each detected MF, using a laser wavelength set at 785 nm. Experimental parameters for Raman

224 analyses (power: 10 to 40 mW, acquisition time: 10 to 30 s, accumulations: 2) were set to limit
225 fluorescence and increase the spectral quality of the analysed fibres. Fibres were identified by
226 comparing the acquired spectra with a homemade database previously created using the MF of
227 the current study.

228 *2.6.2. Digestion of oyster tissues*

229 The digestive glands of oysters (n = 3/ MF type) were digested using a protocol adapted to the
230 identification of both synthetic and natural MF (Treilles et al., 2020). A negative control
231 (without digestive gland) was used to assess putative air contamination. Briefly, digestive
232 glands were rinsed in filtered distilled water to avoid contamination and were incubated in a
233 10% filtered KOH (100 mL) solution at 40°C under constant shaking (300 rpm) for 24 h. The
234 obtained solutions were then filtered through cellulose nitrate membrane (0.45 µm, Thermo
235 Fisher) and examined under a binocular magnifier (EVOS™ XL Core Imaging System; ×10–
236 20 magnification). Microfibre composition was analysed and compared to the one obtained
237 previously on commercially purchased fibers (section 2.3) using the same FTIR (JASCO IRT-
238 5200).

239 *2.6.3. Analysis of faeces*

240 Oyster valve opening and production of faeces were recorded every morning during the 3 h
241 following feeding and introduction of MF, using several GoPro®. These recordings allowed us
242 to discriminate between the production of faeces and pseudo-faeces and to ensure that each of
243 the oysters filtered water in the aquaria during the first hours of exposure. Faeces were collected
244 from the aquaria every morning prior to the water change and MFs were quantified using a
245 Malassez haemocytometer under a microscope (Zeiss, 4×). Fibre type was identified visually
246 based on their characteristics (shape, colour, cross-section heterogeneity).

247 *2.7. Molecular, biochemical and cellular endpoints*

248 *2.7.1 Enzymatic activity of digestive and metabolic enzymes*

Total protein extraction was performed on three pools of digestive gland (n = 3 digestive glands per pool) per treatment, according to the protocol of Guévélou et al. (2013). Total protein content was measured using the DC protein assay (Bio-Rad) in 96-well microplates, using a Synergy HT microplate reader (Bio-Tek). Concentrations were obtained using Gen5 software version 2.03 (Bio-Tek). Then, amylase activity was measured using the method developed by Samain et al. (1977), adapted for oyster tissues by Huvet et al. (2012). Amylase activity was expressed in U/mg soluble proteins and corresponds to the amount of enzyme that degrades 1mg/ min starch at 45°C. Finally, enzymatic activities of hexokinase (HK; EC 2.7.1.1) and pyruvate kinase (PK; EC 2.7.1.40) were measured according to Greenway and Storey (1999). After the addition of assay buffer to protein lysate, the increase in NADPH or decrease in NADH was monitored for HK and PK, respectively, by measuring the absorbance at 340 nm for 10 min at 25°C using a Synergy® HT microplate reader (BioTek). Enzymatic activities were related to the total protein concentration of each sample.

2.7.2. Cellular oxidative and immune response

Pooled haemolymph samples (see section 2.5 for details of the extraction) were filtered on an 80-µm mesh to eliminate aggregates or large pieces of debris and held on ice. Analysis of haemocyte viability, ROS production and ABC transporter activity were performed using an EasyCyte Plus cytometer (Guava Technologies, Millipore, Billerica, MA). Acquisition was performed for 30 s at a flow rate of 0.59 mL s^{-1}.

Hemocyte viability was measured using both SYBR Green I (Molecular Probes) and propidium iodide (PI, Sigma)(Delaporte et al., 2003). SYBR Green stains cells containing DNA (both dead and living) whereas PI only stains dead cells. Although this double staining allows the measurements of both live and dead haemocytes, results were expressed as the percentage of dead cells.

ROS production was assessed using 2',7'-dichlorofluorescein diacetate DCFH-DA (Sigma), a cell-permeable, non-fluorescent compound according to Hégaret et al. (2003). When entering active cells, DCFH-DA is deacetylated by cellular esterases to a non-fluorescent compound, DCFH, which emits quantifiable green fluorescence upon oxidation by intracellular ROS. ROS production was expressed in arbitrary units (AU) corresponding to the average level of green fluorescence of each sample.

The ABC transporter activity of haemocytes was assessed using calcein-AM (Sigma), which is a non-fluorescent ABC transporter substrate. Calcein-AM crosses the cell membrane and, after undergoing the action of esterases, accumulates as fluorescent calcein in the cytoplasm. The accumulation of fluorescent calcein is, therefore, inversely proportional to the activity of the ABC transporters (Marques-Santos et al., 2017). A preliminary experiment using transporter inhibitors (MK-571,10mM, Sigma) was performed to check ABC transporter activity (Marques-Santos et al., 2017). The obtained results were expressed in arbitrary units (AU) of fluorescence.

2.7.3. Gene expression of immune and detoxification genes

Total RNA extraction was performed on three pools of gills (n = 3 gills per pool) per treatment, on the same individuals used for the biochemical analyses. Briefly, the gills were ground in liquid nitrogen and total RNA was extracted in Extract-all reagent (Eurobio), at a concentration of 1 mL/ 30 mg powder, and treated with DNAse (MoBio, 1 U μg^{-1} total RNA). Total RNA concentrations were assessed using a ND-1000 spectrophotometer (Thermo Fisher Scientific) and RNA integrity was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies). 2-µg of total RNA was reverse transcripted using the iScript cDNA Synthesis kit (Bio-Rad). After RNA denaturation (10 min, 70°C), reverse transcription was realised for 5 min at 25°C, 20 min at 46 °C and 1 min at 95°C and then immediately stopped on ice.

Four genes involved in the oyster inflammation response (NF-κB inhibitors 1 and 2), the detoxification process (extracellular superoxide dismutase) and apoptosis (caspase 1) were targeted (Table S1). Primer pairs of each gene were tested for efficiency across a dilution series of reference cDNA corresponding to a pool of cDNA from all the treatments. Dynamic range analysis was performed in a CFX Connect Thermocycler (Bio-Rad) in a final volume of 15 µL using the 2X SSO Advanced SYBR Mastermix (Bio-Rad). Amplification settings were 95°C for 3 min, 40 cycles at 95°C for 10 s, 20 s at 60°C and a final 60°C for 20 s. Both actin and elongation factor 1 were used as internal controls for gene expression. The calculation of relative mRNA levels of selected genes was normalized to both actin and elongation factor 1 as no significant differences in Cq values were observed between treatments for these two genes (Kruskal–Wallis test, $P = 0.51$, coefficient of variation (CV) = 3.79 for actin and $P = 0.096$, CV = 3.81 for elongation factor 1). Relative expression analyses were performed with the comparative ΔΔCt method (Livak and Schmittgen, 2001) as follows: Relative expression = $2^{-\Delta\Delta Cq}$ with $\Delta Cq = [Cq\ (cDNA\ sample) - Cq\ (cDNA\ reference)]$ and $\Delta\Delta Cq = \Delta Cq - \Delta Cq$ (average of internal controls).

2.8. Statistical analysis

Normality and variance homogeneity were screened using the Shapiro and Levene tests, respectively. When the assumptions of normality and homogeneity of variance were not met, a non-parametric Kruskal-Wallis test was used. Non-parametric Kruskal–Wallis (KW) test followed by a Dunn post-hoc test was performed to compare the effects of overall fibre origin (i.e. natural MF, synthetic MF or control without MF). Kruskal–Wallis test followed by a Dunn post-hoc test was then performed to compare the effects of fibre type (i.e. of the six different selected fibres: acrylic, cotton, nylon, organic cotton, polyester and wool), regardless of the concentration. Finally, to assess a possible dose-dependent effect, a Student test was performed, considering the two concentrations tested for each fibre. The exact same tests were performed

322 for oysters exposed to MF leachates. Differences were considered significant when $P < 0.05$.

323 All statistical analyses were performed using R Studio.

324

325

3. Results

3.1. Microfibre characterization, uptake and effects

3.1.1. Microfibre characterization

The average length and diameter of the microfibres produced in this study were 100 ± 55 µm and 16 ± 5µm, respectively, and displayed an aspect ratio (average length / average diameter) between 5.2 and 7.3 (Table 1). Specifically, 100% of nylon MF were between 20 and 100 µm while this percentage was lower for the other synthetic MF (70% and 90% for PES and acrylic respectively) and natural MF (77% for organic cotton and wool and 92% for cotton) (Fig. S1). Nylon MF displays a more homogenate diameter (100% between 6 and 15 µm) than other fibres with greater standard deviation, notably for wool MF, whose diameter ranges from 4 to 40 µm (Fig. S1). In general, the roughness of synthetic MF (15–40 nm) was at least half that of natural MF (80–200 nm), the two extremes being nylon (15 nm) and organic cotton (200 nm) (Fig. 1, Table 1).

3.1.2. Microfibre uptake

Valve opening and active filtration were observed for every treatment and each aquarium following feeding and exposure to MF. Analyses combining HD images and Raman spectroscopy allowed the identification of MF in the gills of oysters exposed to cotton, organic cotton and acrylic (Fig. 2), while no MF was detected in the gills of oysters exposed to polyester or nylon or in unexposed controls. The wool Raman spectrum was not identifiable (non-exploitable spectrum). No MF was able to be detected in oyster digestive glands using histological Raman imagery. In contrast, the KOH digestion protocol enabled the identification of MF in the digestive gland of oysters exposed to all MF except wool (Fig. S2). The fibre types were confirmed by FTIR analysis and systematically matched with the types of the corresponding fibres used for exposure, except for the digestive gland of oysters exposed to

wool and those left unexposed, for which cotton fibres (1 to 2 MF) were observed. As these fibres were also present in the negative control (1 to 2 MF), they are likely to have come from air contamination (Fig. S3). Finally, MFs were observed in the faeces of oysters exposed to every type of MF at both environmental and high concentrations (Fig. S4, Table S2). No MF was detected in the faeces of oysters from the control treatment and no production of pseudo-faeces was detected (Fig S5).

3.1.3. Additive chemical profiling

In total, 15 contaminants (10/57 PAH and 5/10 PCB) were detected in at least one MF type. In terms of chemical occurrence, the most contaminated fibres were wool (10 PAHs, 5 PCBs), followed by polyester (7 PAHs, 3 PCBs), cotton (7 PAHs) and nylon (6 PAHs, 1 PCB), acrylic (4 PAHs, 1PCB) and organic cotton (4 PAHs) (Table 2). In terms of chemical loads, the most contaminated fibres were nylon and polyester; this was notably due to the high concentration of phenanthrene (186.7 and 80.7 µg kg^{-1}, respectively). Regarding PAHs, it is noteworthy that all fibres were contaminated with at least 4 of these compounds (OC) and up to 10 compounds (Wool). Anthraquinone, fluoranthene and phenanthrene were detected in all fibres. Anthraquinone concentration ranged from 0.9 µg kg^{-1} in acrylic to 17.0 µg kg^{-1} in wool fibres and concentrations for cotton, nylon, organic cotton and polyester varied between 2.4 and 4.7 µg kg^{-1} (average = 3.0 ± 1.1 µg kg^{-1}). Concentrations of fluoranthene were similar for every type of fibre (average = 4.0 ± 1 µg kg^{-1}) apart from nylon (26.9 µg kg^{-1}). Concentrations of phenanthrene were highest in samples of nylon and polyester (186.7 and 86.9 µg kg^{-1} respectively), ranged from 7 to 14 µg kg^{-1} in natural fibres, and were close to the detection limit for acrylic (2.9 µg kg^{-1}). Pyrene was detected in all fibres except acrylic, with relatively low concentrations for cotton, polyester and organic cotton (average = 2.4 ± 0.7 µg kg^{-1}), and concentrations ranging from six to thirteen times higher than the detection limit for wool (8.4

374 µg kg^{-1}), and nylon (16.5 µg kg^{-1}), respectively. Benz(a)anthracene was only detected in wool
375 and at a low concentration (4.0 µg kg^{-1}). Indenopyrene was only detected in natural MF (wool
376 and cotton), while lindane was detected in both natural and synthetic MF (wool and polyester).
377 For both indenopyrene and lindane, concentrations were of the same order of magnitude as the
378 detection limit (1.2–1.7 µg kg^{-1}). Fluorene and naphthalene were detected in nylon, polyester,
379 cotton and wool, with the highest concentration in nylon (29.2 and 36.0 µg kg^{-1} for fluorene and
380 naphthalene, respectively). Concentrations of naphthalene in polyester, cotton and wool were
381 comparable (average = 11.3 ± 3.2 µg kg^{-1}) as were the concentrations of fluorene in natural
382 fibres (average = 4.5 ± 1.8 µg kg^{-1}).

383 Regarding PCB, no compounds were detected in cotton and organic cotton and only one
384 was detected in nylon (PCB28) and acrylic (PCB101) fibres. Polyester and wool showed higher
385 occurrences of PCB, but their overall concentrations remained low regardless of fibre type
386 (average = 1.5 ± 0.9 µg kg^{-1}) (Table 2).

387 The scan mode analysis led to the identification of 13 different plastic additives, among
388 which 8 were detected in acrylic, 7 in organic cotton, 5 in nylon, 4 in wool and polyester and 3
389 in cotton (Table 3).

390 *3.1.4. Effects of MF exposure*

391 In general, there were no significant dose-dependent effects of exposure to natural or synthetic
392 MF.

393 *3.1.4.1. Enzymatic activity of digestive and metabolic enzymes*

394 A significant decrease in amylase activity (KW test, P = 0.03) was observed in oysters exposed
395 to natural MF (1.05 ± 0.11 UI/mg) compared with the control oysters (1.03 ± 0.03 UI/mg) (Fig.
396 3A). Analyses by fibre type showed that this decrease in amylase activity was only significant
397 for individuals exposed to wool MF (0.96 ± 0.07 UI/mg; Dunn post-test, P = 0.049) compared
398 with unexposed oysters (1.03 ± 0.03 UI/mg) (Fig. 3B). On the contrary, exposure to PES MF

399 (1.61 ± 0.22 UI/mg) led to a significant increase in amylase activity compared with organisms
400 exposed to other MF such as cotton (1.03 ± 0.08 UI/mg; Dunn post-test, P = 0.014), wool (0.96
401 ± 0.07 UI/mg; Dunn test, P = 0.0004) or nylon (1.02 ± 0;12 UI/mg; Dunn post-test, P = 0.013)
402 (Fig. 3B).

403 Regarding glycolytic enzymes, no effects of tested treatments (synthetic or natural MF) were
404 observed on the activity of the hexokinase (Fig. 3C) (KW test, P = 0. 767) or pyruvate kinase
405 (KW test, P = 0.074) (Fig. 3D).

406 *3.1.4.2.Cellular oxidative and immune response*

407 There were no significant differences in haemocyte viability and ROS production among
408 treatments (KW test, P = 0.507 and P = 0.077, respectively) (Fig. 3 E&F). On the contrary, a
409 significant increase in ABC transporter activity was observed in oysters exposed to both
410 synthetic (2823 ± 689 AU; Dunn test, P = 0.031) and natural MF (3141 ± 1218 AU; Dunn test,
411 P = 0.042) compared with unexposed oysters (4750 ± 290 AU) (Fig. 3 G). In particular,
412 comparison between fibre types showed a significant decrease (KW test, P = 0.006) in
413 fluorescent calcein in organisms exposed to organic cotton MF (2199 ± 481 AU) especially at
414 environmental concentration compared with unexposed organisms (4750 ± 290 AU) or
415 organisms exposed to wool MF (4068 ± 1447 AU). However, no differences were observed
416 among the different synthetic fibres (Fig. 3 H).

417 *3.1.4.3.Gene expression analysis of immune and detoxification genes*

418 Significant increases in mRNA content of Ikb1 (KW test, P = 0.042), Ikb2 (KW test, P = 0.006)
419 and caspase 1 (KW test, P = 0.004) (Fig. 4) was observed in oysters exposed to natural MF
420 compared with unexposed oysters (in the case of Ikb2) or oysters exposed to synthetic MF (for
421 both Ikb1 and caspase 1). No effects of exposed treatments were observed on the mRNA content
422 of EcSOD (test KW, P = 0.980) (Fig.4 D). When investigating the effects of fibre type on the

mRNA content of these four selected genes, very few variations were observed, but a significant decrease in caspase 1 content (test KW, P = 0.022) was observed in organisms exposed to PES MF compared with oysters exposed to wool MF (Fig. S6).

3.2. Leachate effects

Similarly to MF exposure, there were no dose-response effects of leachate exposure on biomarker activity or expression.

3.2.1 Enzymatic activity of digestive and metabolic enzymes

There were no effects of leachates from either natural or synthetic MF on the amylase activity (KW test, P = 0.087) (Fig. 5A). A significant diminution in HK activity (KW test, P = 0.0005) was observed in oysters exposed to the leachates of synthetic MF (0.80 ± 0.76 mUI/mL/mg) compared with those exposed to the leachates of natural MF (1.75 ± 0.45 mUI/mL/mg) (Fig. 5 B). This decrease in HK activity was substantial in organisms exposed to PES leachate, which was significantly different from wool, organic cotton and nylon leachates (KW test, P = 0.00004) (Fig. 5 C). Regarding PK, a significant increase (KW test, P = 0.035) in its activity was observed in oysters exposed to natural MF leachates (12.22 ± 2.14 mUI/mL/mg) compared with controls (9.07 ± 0.27 mUI/mL/mg) (Fig. 5 D). However, no effects of fibre type were detected.

3.2.2. Cellular oxidative and immune response

Exposure to the leachates of natural or synthetic MF had no effects on haemocyte viability (KW test, P = 0.22), ROS production (KW test, P = 0.133) or ABC transporter activity (KW test, P = 0.968) (Fig. S 7).

3.2.3. Gene expression analysis of immune and detoxification genes

445 A significant increase in the Ikb2 mRNA content (KW test, P = 0.05) was observed in oysters
446 exposed to the leachates of natural MF compared with controls (Fig. 5 E). Specifically,
447 exposure to the high concentration of cotton leachates induced a two-fold increase of Ikb2
448 mRNA content (KW test, P = 0.001), while exposure treatments had no effect on the mRNA
449 content of Ikb1 or caspase 1. A significant increase in EcSOD mRNA content (KW test, P =
450 0.046) was observed in oysters exposed to leachates from synthetic MF, compared with controls
451 (Fig. 5 H).

452

4. Discussion

4.1. Environmental relevance and major findings

Microfibres are omnipresent in the world's oceans, but only a few studies have explored their impact on marine organisms (Kutralam-Muniasamy et al., 2020), mainly focusing on synthetic MF and disregarding the risks posed by natural MF or associated compounds. In the current study, MFs were selected based on their environmental relevance: polyester and cellulose (cotton) are the most abundantly produced fibres (Henry et al., 2019) and are also the most commonly reported in the oceans (Mateos-Cárdenas et al., 2021), while acrylic and wool are the second most produced synthetic and natural fibres, respectively. As for polyamide (nylon), it is frequently used in the production of nets and ropes used in fishing or aquaculture industries and could, therefore, constitute a significant proportion of MF pollution in the oceans (Welden and Cowie, 2017). Overall, our study reveals that exposure to textile natural MF and associated leachate elicit higher digestive and inflammatory responses in a keystone filter feeder than their synthetic counterparts. Such differences might be related to both physical and chemical characteristics: natural MF are rougher and less even in diameter and length than synthetic MF and exhibit (in the case of the wool used in this study) higher PCB and PAH loads. In more general terms, physical (e.g. roughness causing inflammation through organ contact) and chemical (occurrence and loads of contaminants) toxicity of a fibre is highly dependent on its properties and hence on its intended use. Regardless of the contaminant (MF or leachates), there were no dose-dependent effects, suggesting that short-term exposure to environmental concentrations of MF (10 MF L^{-1}) is sufficient to modulate oysters' digestive functions and inflammatory responses, which could have potential knock-on effects on oyster health over long term exposures.

4.2. Monitoring MF uptake and fate requires complementary techniques

Ingestion and egestion of MF by *C. gigas* exposed to both natural and synthetic MF were demonstrated using both tissue digestion and cutting-edge histological Raman imagery (HRI). Adhesion of MF to the gills was observed by HRI in oysters exposed to natural (cotton and organic cotton) and synthetic (acrylic) MF, but not in oysters exposed to nylon, PES or wool. Adhesion of microplastics (synthetic MF) was already observed on mussel gills where it accounted for around 50% of the MP in the whole tissues of mussels (Kolandhasamy et al., 2018). However, the inconsistency in fibre detection suggests that the use of HRI in MF detection in oyster tissue was not effective and requires further technical development. Other points to be considered for future improvement include fibre contamination during histological section preparation, time-consuming naked-eyed localization of MF in multiple layers of tissues and non-quantitative assessment. Tissue digestion provided a broader picture of MF presence in oyster DG but the failure to identify wool MF suggests its dissolution after 40°C KOH digestion (Treilles et al., 2020) and indicates the need for new extraction procedures for animal fibres. Our MF ingestion results are consistent with *in situ* observations showing the presence of MF in the digestive gland of natural populations of the oyster *Crassostrea virginica* (Craig et al., 2022) and laboratory-based experiments highlighting the capacity for filter-feeding bivalves to ingest MF (Alnajar et al., 2021; Cole et al., 2020).

The length of MF used in the present study was not representative of those collected in the oceans: they were shorter on average (*in situ* peak abundance: 0.8–0.9 mm) (Suaria et al., 2020) and corresponded better to the size range of particles that can be ingested by oysters (optimal ingestion size 2–200 µm in pearly oyster, Pouvreau et al., 1999). While successful ingestion of small MF (average 100 µm) was observed in the present study, one would expect that, in the natural environment, oysters would reject MF that was too large through pseudofaeces. However, analyses of *C. virginica* biodeposits from their natural environment showed that around 90% of observed MP were synthetic fibres, with no differences in MF size between

faeces and pseudofaeces (0.5–20 mm) (Craig et al., 2022). Unlike those of most anthropogenic particles, MF cross-sections can have various shapes (e.g. circular, ribbon, L-shaped, trilobal) (Salvador Cesa et al., 2017), increasing their bioavailability to marine organisms and suggesting that, in the case of MF, size might not matter. The presence of large particles (600–900 µm) in oyster stomach content (Ward et al., 2019a) generalizes this hypothesis and confirms that regardless of particles shape, oysters' mouths can stretch considerably and ingest particles up to hundreds of micrometres in size (Peharda et al., 2012).

4.3. Exposure to natural MF elicits digestive and inflammatory regulation

Alpha-amylase is a key enzyme in carbohydrate digestion, maximizing food absorption and conversion (Huvet et al., 2015). It catalyses the hydrolysis of starch and glycogen and, therefore, constitutes one of the first steps in the production of glucose within an organism (Prudence et al., 2006). The reduction of amylase activity in oysters exposed to wool could be linked to a decrease in food intake through a reduction in filtration activities as previously observed in mussels exposed to synthetic MF (24–72 h, PET, 3–30 MF mL^{-1}) (Woods et al., 2018). An alternative hypothesis is that satiety is more rapidly achieved when animals are exposed to wool MF, the latter replacing food in the digestive tract (dilution effect; Scherer et al., 2020)). The significant effect of wool over the other types of natural and synthetic MF studied could be due to the intrinsic characteristics (e.g. higher roughness; heterogeneity in length and diameter) as well as higher PCB and PAH loads. However, the absence of effects observed upon exposure to the leachates of natural MF suggests rather that natural MF has a mechanical effect on the oyster's digestive system. Although disruption of digestive functions has already been observed in several organisms after exposure to nano- and microplastics of different shapes (Choi et al., 2022; Yin et al., 2021), this is the first report of digestive impairment in marine organisms upon exposure to natural microfibres. Due to the pivotal role of amylase in glucose production, variations in amylase activity are often thought to influence

variations in glycolytic enzymes (Niu et al., 2012), which was not the case here upon natural MF exposure. On the opposite, exposure to natural MF leachates induced an increase in PK activity, suggesting an increase of pyruvate production through glycolysis. These results suggest that glucose intake through food absorption and conversion might be reduced upon exposure to natural MF, especially wool, but could be counterbalanced by the use of other glucose sources in the organism (i.e. energy reserves) (Sokolova et al., 2012). A reduction of energy intake, albeit partial, could have repercussions for organism functioning including immune and inflammatory processes.

Expression of effectors of the NF-κB and apoptotic pathways were also modulated upon exposure to natural MF. Nuclear factor κB (NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells) are essentials components in the establishment of marine invertebrates' immune, apoptotic or inflammatory response (Vallabhapurapu and Karin, 2009). However, to avoid cell and tissue damage, the expression of these nuclear factors is controlled by inhibitors (IκB), which are represented by two homologous genes in *C. gigas* (IκB1 and IκB2) (Zhang et al., 2011). In the current study, these two genes were up-regulated in oysters exposed to natural MF, which suggests a runaway activation of the inflammatory system upon exposure to natural MF, followed by a regulation of the inflammatory reaction through the inhibition of NF-κB. This inflammatory response is congruent with the swelling and deciliation observed in mussel gills upon exposure to microfibrous lint (180 mg L^{-1}) (Alnajar et al., 2021), suggesting that attachment of natural MF to oysters' gills could be perceived as a non-self-component. However, the up-regulation of IκB2 in oysters exposed to both natural MF and their associated leachates suggested a mechanical and chemical modulation of the oyster's inflammatory response. Similarly, the up-regulation of caspase 1 (, a crucial enzyme in apoptosis, operating at the end of the reaction chain (Lu et al., 2017) suggests a deleterious effect of natural MF adhesion to gill cells. Although a 96-h exposure to environmental concentrations (0.5 µg L^{-1})

552 of synthetic MF (PET) increased necrosis in haemocytes of the mussels *Mytilus*
553 *galloprovincialis* (Choi et al., 2021), in our study there was no effect of synthetic MF on
554 inflammatory or apoptotic markers tested (Ikb1, Ikb2 and caspase 1).

555 *4.4. Activation of ABC transporter: a common impact of natural and synthetic MF*

556 ABC pumps are membrane transporters involved in the exclusion of xenobiotics from
557 organisms' cells and tissues and, as such, constitute another line of defence against
558 environmental pollutants in oysters (Minier et al., 2006). In the current study, increased activity
559 of haemocyte ABC transporters was observed in oysters exposed to environmental
560 concentrations of organic cotton and synthetic MF in general. Such activation of ABC pumps
561 following short-term exposure to synthetic MF as previously observed in mussels exposed to
562 polystyrene microbeads (Franzellitti et al., 2019) suggests the release of toxic compounds upon
563 particle ingestion. Indeed, the absence of ABC transporter activation in oyster haemocytes upon
564 leachate exposure suggested that the release of water-soluble content was negligible in the
565 leachates used in the oyster exposures. Instead, targeted chemical analyses performed on all
566 MF revealed a significant presence of hydrophobic organic compounds (PAH, PCB, and plastic
567 additives) that are unlikely to have leached in seawater but may have leached within the oyster's
568 digestive system (Bakir et al., 2014). Interestingly, the detection of a flame retardant (bibenzyl),
569 plasticizers (benzyl butyl phthalate, DEHP), a bactericidal agent (1-undecanol, Togashi et al.,
570 2007) or a chemical involved in textile dying and treatment products (dodecanoic acid, isooctyl
571 ester, Echa Europa) in organic cotton is consistent with the activation of ABC pumps upon
572 exposure to this specific MF but is counterintuitive with respect to the expected characteristic
573 of organic products, i.e. being additive-free. This confirms that untargeted chemical profiling
574 is needed to assess the risk associated with these materials, even for those that are sold as natural
575 and eco-friendly. This is especially true considering the tremendous diversity of additives

(>4000) used in the plastic industry, for which the chemical identity and toxicity are mostly unknown (Groh et al., 2019).

The textile manufacturing industry is one of the most chemical-intensive in the world with the use of up to 8000 different chemical compounds (Niinimäki et al., 2020). In the current study, PAHs and PCBs associated with selected fibres were detected at concentrations lower than the 'effects range low' (ERL) defined by OSPAR (oap OSPAR org). The ERL is a quality guideline that aims to assess the ecological implications of contaminant concentrations and, therefore, provides an indication of the concentration at which a contaminant potentially would have adverse biological effects on an organism. Consequently, leached chemicals had little effect on the biomarkers selected in the present study. Research investigating the toxicity of plastic leachates together with proper identification of their cohort of chemical additives is still in its infancy (Zimmermann et al., 2020) and contrasting results exist in the literature depending on the biological model, the tested material and the exposure parameters (Carney Almroth et al., 2021; Zimmermann et al., 2020). This suggests that greater transparency and knowledge about chemical formulations are needed to properly investigate the eco-safety of plastic *vs* natural materials (including fibres) which is also true for eco-designed materials (Anderson and Shenkar, 2021).

4.5. From fast fashion to slow fashion: toward a more sustainable fashion industry

The current business logic in the fashion industry is based on intensification and an escalation of the production and consumption of low-quality and low-price clothes and textiles. From the agriculture or petrochemical production of raw materials to the manufacturing and distribution, this business has a substantial environmental impact (Quantis, 2018; World Resources Institute, 2017). At the end of the supply chain, unsold items and waste frequently end up in landfill where fibres and chemical additives might have repercussions on organisms' health. It is, however, interesting to note that the environmental impact is different depending on the origin

601 of the fibre. On the one hand, it has been estimated that the production of natural fibres such as
602 cotton and wool had higher freshwater consumption and CO_2 emissions than synthetic fibres
603 (Niinimäki et al., 2020) and the current study shows that their presence in the oceans might
604 have detrimental effects on marine biota. On the other hand, the production of synthetic fibres
605 such as polyester or polyamide requires more energy than natural ones (Sandin et al., 2019) and
606 their ingestion also causes a risk for marine biota (Hope et al., 2020; Jemec et al., 2016).
607 Although counter-intuitive considering natural fibre biodegradation capacity, these results
608 encourage a modification of our consumption habits, shifting from fast to slow fashion rather
609 than promoting natural fibres over synthetic ones. Slow fashion promotes circularity by
610 extending product lifetime (Niinimäki, 2018). Such circular approaches have recently started
611 to emerge, including the leasing and renting of clothing (Zamani et al., 2017), second-hand
612 sales (Abtan et al., 2019), repair services and material recycling (RSA Action and Research,
613 2016).

614 *4.6. Conclusions*

615 This innovative approach coupling the exposure with natural and synthetic fibres with
616 biomarkers endpoints highlights the disruptive effects of selected MF and their associated
617 leached chemicals on oyster metabolism and response to stress (e.g. inflammation,
618 detoxification, immune system) regardless of the exposure concentration. These results indicate
619 that the concentrations of MF currently found in the oceans may already be responsible for
620 physiological disturbances in oysters even after short-term exposures. Within the framework of
621 this study, natural MF, the most abundant and ubiquitous MF in the oceans, had a higher impact
622 on oyster digestive and inflammatory processes than man-made synthetic MF, which caused
623 very moderate physiological disturbances in *C. gigas*. This counter-intuitive observation raises
624 the question of the safety of so-called natural compounds for which the perception of risk is
625 low. The intrinsic characteristics of natural MF (physical structure) or their manufacture

(chemical molecules associated with their treatment for particular properties) should not be neglected as they may cause toxicological effects. The current study urges the limitation of MF pollution.

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Tables, Figures and Supplementary figures and tables

Tables

Table 1. Average length, diameter, aspect ratio and roughness of the produced MF

Type of fibre	Average length (µm)	Average diameter (µm)	Average aspect ratio	Mean roughness (nm)
Acrylic	98 ± 46	18 ± 4	5.3	40
Nylon	68 ± 40	11 ± 2	6.3	15
Polyester	107 ± 50	15 ± 4	7.3	35
Cotton	103 ± 65	18 ± 8	5.9	80
Organic Cotton	113 ± 69	16 ± 6	7.1	200
Wool	104 ± 55	20 ±6	5.2	100

Table 2. Concentrations (µg dry kg^{-1}) of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs) detected in selected fibres. PES: polyester, nd: not detected.

	Detection limit	**Acrylic**	**Nylon**	**PES**	**Cotton**	**Organic Cotton**	**Wool**
HAP							
Anthraquinone	0.63	**0.9**	**2.4**	**2.6**	**4.7**	**2.4**	**17.0**
Benz(a)anthracene	3.75	nd	nd	nd	nd	nd	**4.0**
Cypermethrin	2.50	**3.5**	nd	nd	nd	nd	**57.2**
Fluoranthene	1.25	**2.3**	**26.9**	**4.4**	**4.6**	**3.6**	**4.9**
Fluorene	2.50	nd	**29.2**	**10.0**	**3.2**	nd	**5.9**
Indenopyrene	1.25	nd	nd	nd	**1.3**	nd	**1.7**
Lindane	1.25	nd	nd	**1.7**	nd	nd	**1.2**
Naphthalene	6.25	nd	**36.0**	**14.6**	**8.1**	nd	**11.2**
Phenanthrene	2.50	**2.9**	**186.7**	**80.7**	**10.7**	**7.0**	**14.1**
Pyrene	1.25	nd	**16.5**	**1.7**	**3.1**	**2.6**	**8.4**
PCB							
PCB101	0.63	**0.7**	nd	**1.8**	nd	nd	**1.3**
PCB118	0.63	nd	nd	nd	nd	nd	**0.7**
PCB153	0.63	nd	nd	nd	nd	nd	**0.8**
PCB28	0.63	nd	**3.6**	**1.2**	nd	nd	**1.1**
PCB52	0.63	nd	nd	**1.7**	nd	nd	**1.8**

Table 3. GC-MSMS scan mode analysis of plastic additives detected in selected fibres. Crosses indicate the presence of the additive. PES: polyester

Plastic additive	**Acrylic**	**Nylon**	**PES**	**Cotton**	**Organic Cotton**	**Wool**
Hexadecanamide	X		X	X		
1,8-Diazacyclotetradecane-2,7-dione		X				
1-Undecanol					X	
2-((But-3-enyloxy) carbonyl) benzoic acid						X

Bibenzyl	X	X			X	X
Bis (2-ethylhexyl) terephthalate	X					X
Butyl benzyl phthalate	X	X	X		X	
DEHP	X	X	X	X	X	
Dodecanoic acid, isooctyl ester					X	
Hexadecanoic acid, ethyl ester	X					
Methyl stearate					X	
Octadecanamide	X			X		
Tris (2,4-di-tert-butylphenyl) phosphate	X	X	X		X	X
Dodecanoic acid, isooctyl ester	X	X	X		X	

Figures

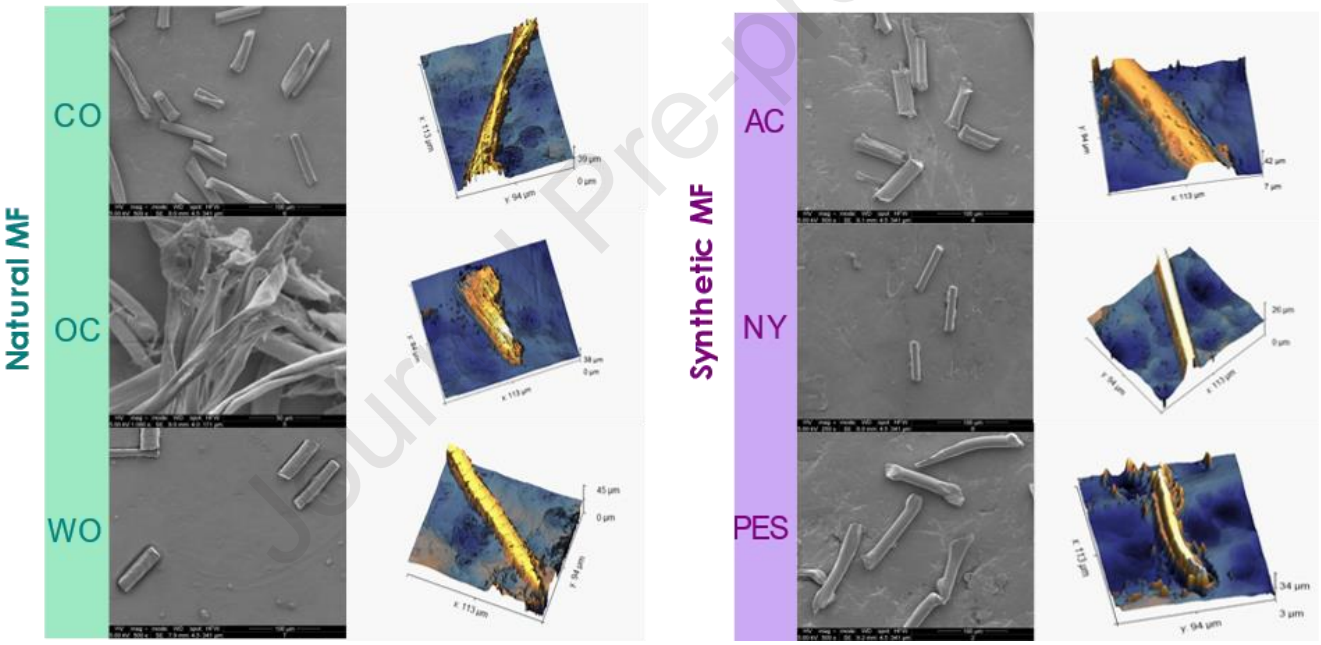
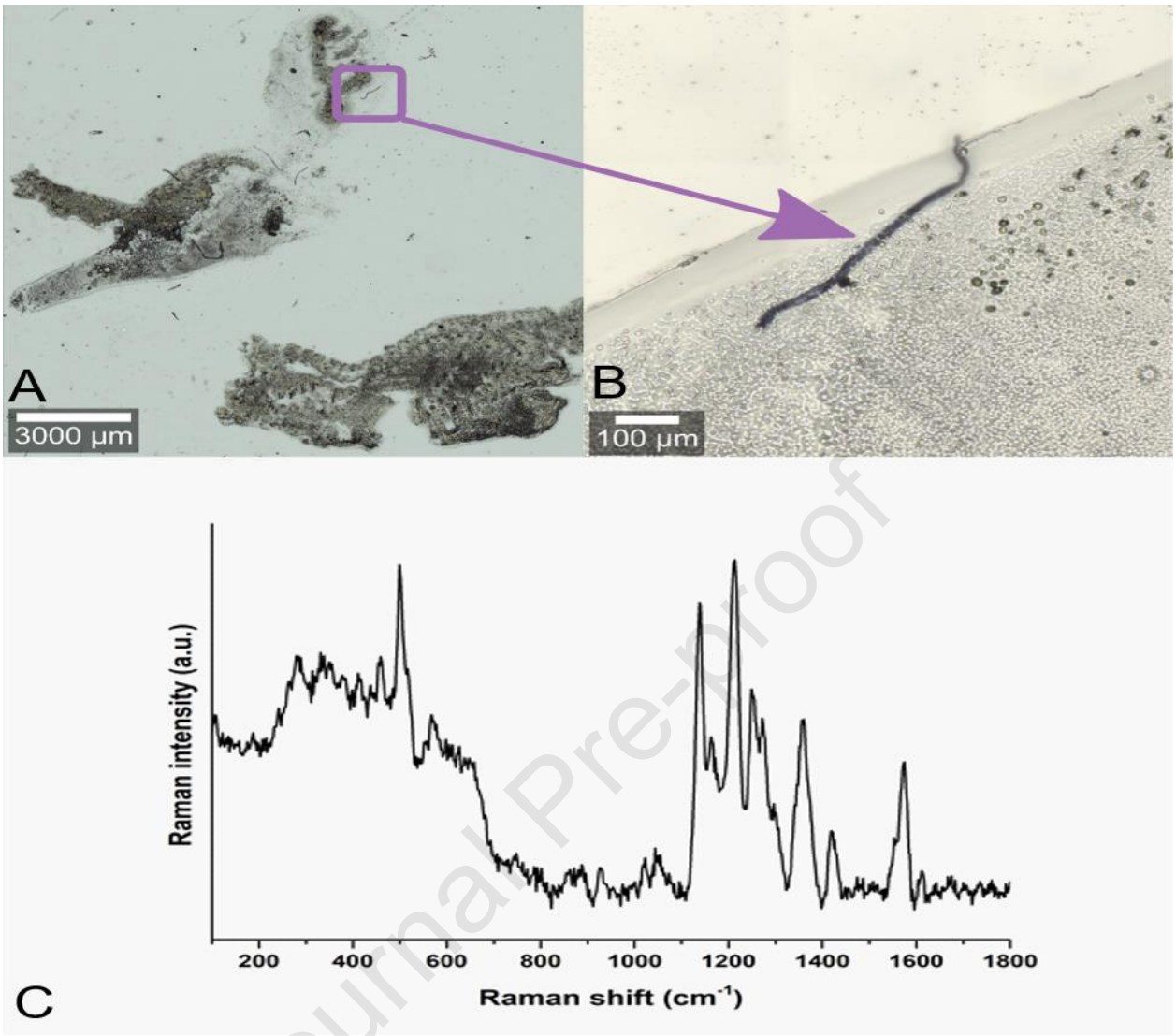


Figure 1. Microfibre characterization. Scanning electron microscope and optical profilometer images of selected synthetic and natural microfibres (MF). CO: cotton, OC: organic cotton, WO: wool, AC: acrylic, NY: nylon, PES: polyester.



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950 **Figure 2. Example of microfibre (acrylic) adhesion to oyster gills. A.** Mapping and imaging
951 of several layers of the sample. **B**. Localization and morphological characterization of an acrylic
952 microfibres (MF) within the gills. **C.** Characterization of the identified MF (treated Raman
953 spectra, power: 10 mW, acquisition: 20sx2, λ_{exc}= 785 nm).

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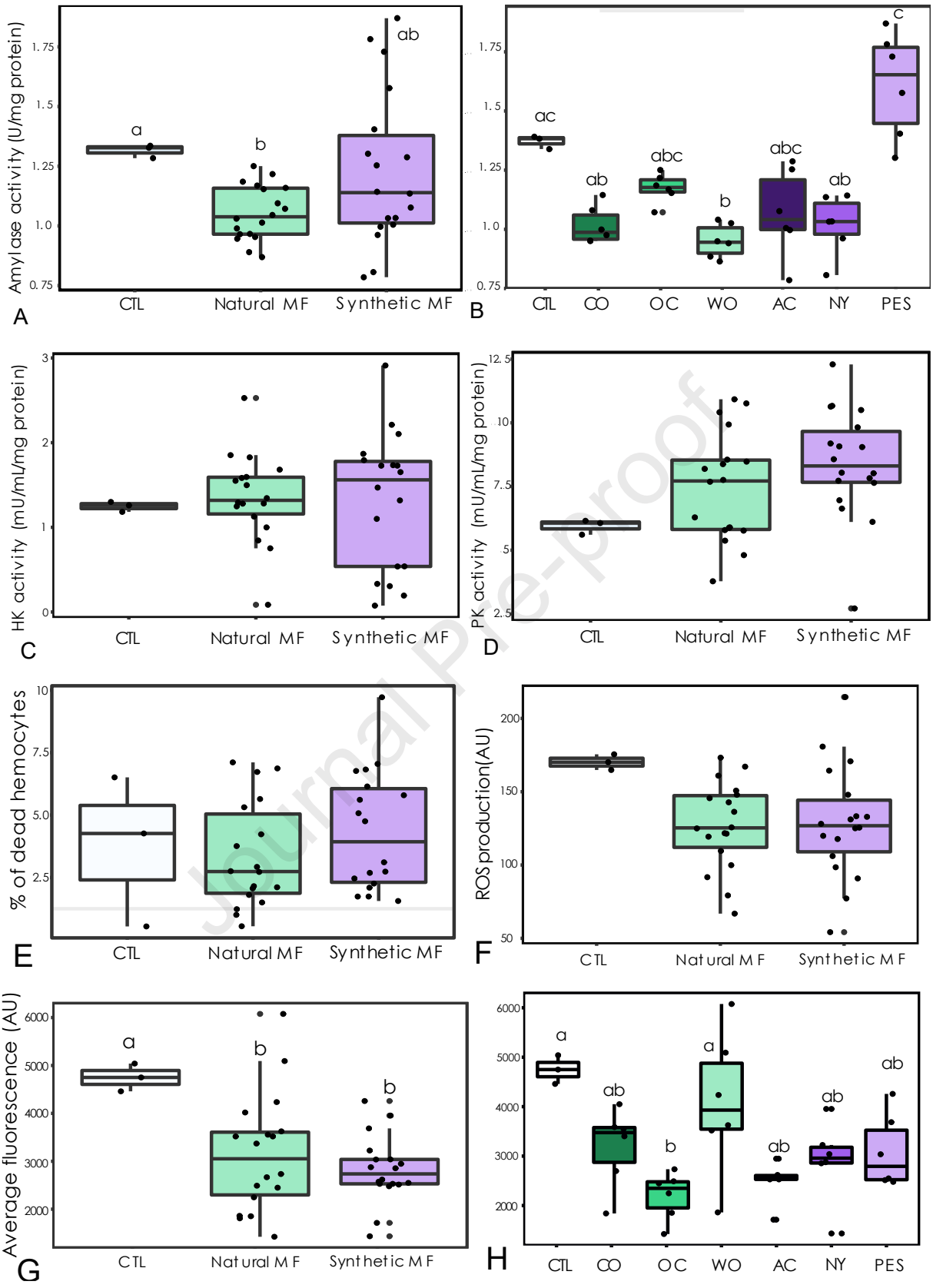
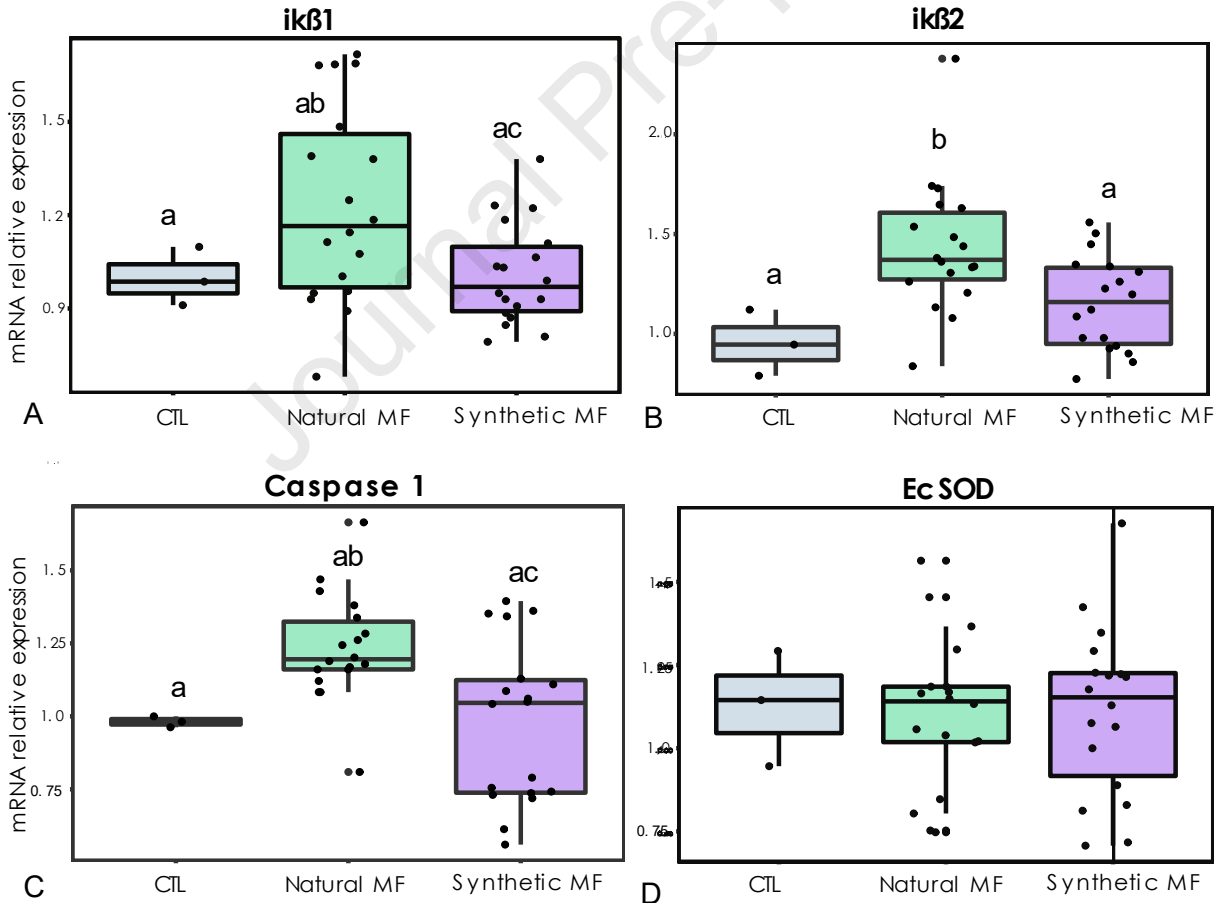


Figure 3. Activity of digestive and metabolic enzymes and haemocyte parameters in oysters exposed to natural or synthetic microfibres or unexposed. A. Amylase activity of

960 oysters exposed to natural or synthetic MF or unexposed. **B.** Amylase activity of oysters
961 exposed to the six selected types of MF (CO: cotton, OC: organic cotton, WO: wool, AC:
962 acrylic, NY: nylon, PES: polyester). **C.** Hexokinase (HK) activity of oysters exposed to natural
963 or synthetic MF or unexposed. **D.** Pyruvate kinase (PK) activity of oysters exposed to natural
964 or synthetic MF or unexposed**. E.** Percentage of dead haemocytes. **F.** ROS production. **G.**
965 Average fluorescence of calcein, which is inversely proportionate to ABC transporter activity.
966 **H**. Details of average fluorescence of calcein for each selected fibre. Different letters above the
967 boxes indicate significant differences between treatments (Kruskal–Wallis test). CTL: control,
968 MF: microfibres.

969



970

971 **Figure 4. mRNA relative expression of four genes in oysters exposed to natural or**
972 **synthetic MF and unexposed (CTL).** Different letters above the boxes, indicate significant
973 differences between treatments (Kruskal–Wallis test). CTL: control, MF: microfibres

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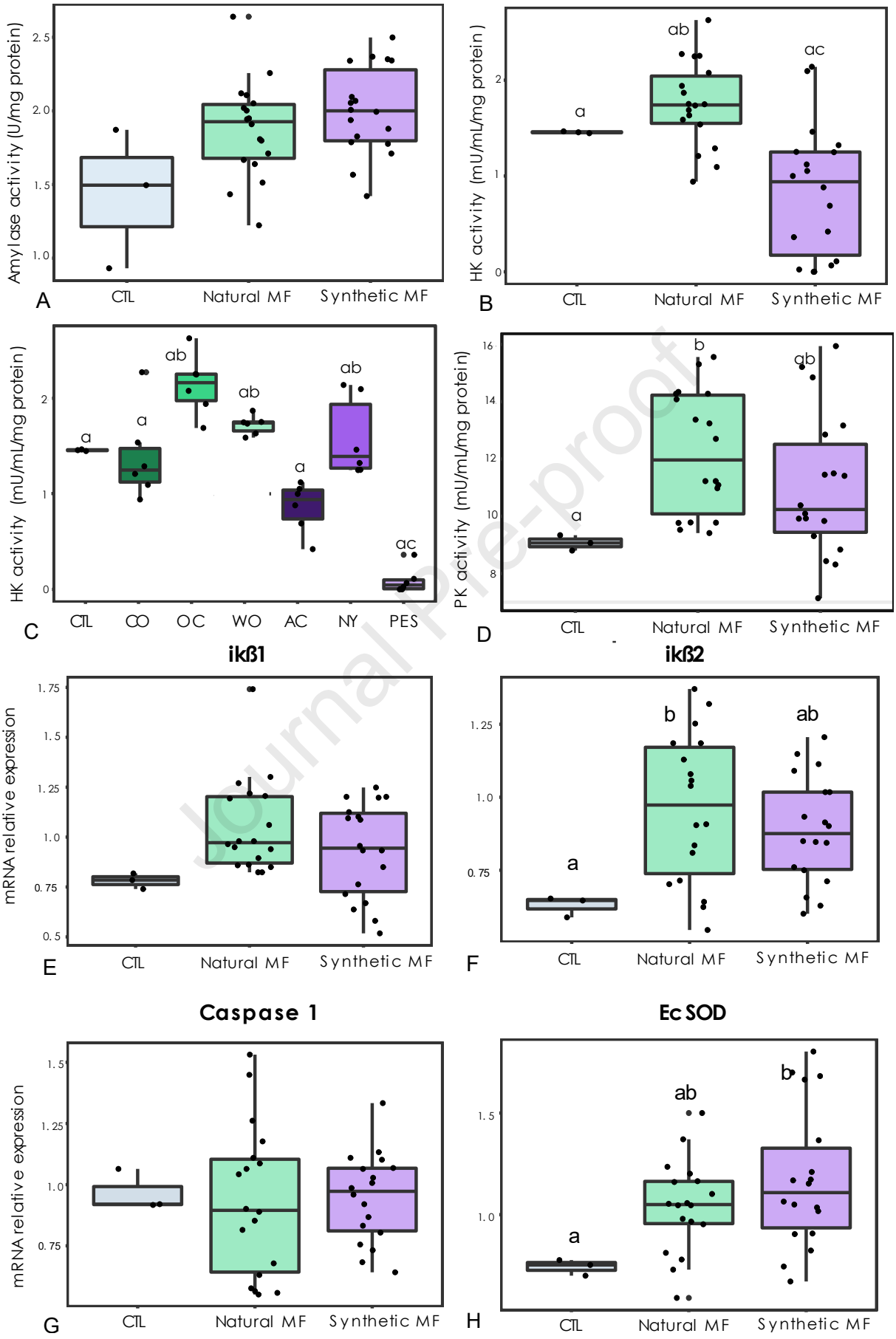
Amylase activity (U/mg protein)
2.5
2.0
1.5
1.0
A
CTL
Natural MF
Synthetic MF
HK activity (mU/mL/mg protein)
ab
ac
a
2
1
0
B
CTL
Natural MF
Synthetic MF
HK activity (mU/mL/mg protein)
a
a
ab
ab
a
ab
ac
2
1
0
C
CTL
CO
OC
WO
AC
NY
PES
PK activity (mU/mL/mg protein)
16
14
12
10
8
a
b
ab
D
CTL
Natural MF
Synthetic MF
ikß1
mRNA relative expression
1.75
1.5
1.25
1.0
0.75
0.5
E
CTL
Natural MF
Synthetic MF
ikß2
1.25
1.0
0.75
a
b
ab
F
CTL
Natural MF
Synthetic MF
Caspase 1
mRNA relative expression
1.5
1.25
1.0
0.75
G
CTL
Natural MF
Synthetic MF
Ec SOD
1.5
1.0
a
ab
b
H
CTL
Natural MF
Synthetic MF

975

976 **Figure 5. Enzymatic activity and mRNA relative expression of four genes in oysters**
977 **exposed to the leaching chemicals of natural or synthetic MF and unexposed. A.** Amylase
978 activity, **B**. Hexokinase (HK) activity, **C**. Details of hexokinase activity for each selected type
979 of fibre; CO: cotton, OC: organic cotton, WO: wool, AC: acrylic, NY: nylon, PES: polyester.
980 **D**. Pyruvate kinase activity. Different letters above the boxes, indicate significant differences
981 between treatments (Kruskal Wallis test). CTL: control, MF: microfibres

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985 **Supplementary tables**

986 **Table S1**: List of selected and internal control genes used in this study, with primer sequences,
987 GenBank accession numbers and references.

Gene		Sequence	GenBank Accession number	Reference
		Selected genes		
IKb1	Fwd	5'-GAAAAAGTGGCAAGAGTGTC-3'	DQ250326	Cao et al., 2018
	Rev	5'-GAAGAGTCATCGAAAGCAAC-3'		
IKb2	Fwd	5'-CAGCATTCACTGACGACGAT-3'	NM_001308876.1	Pauletto et al., 2017
	Rev	5'-TCTGCCTCAGTTTGTCGTTG-3'		
Caspase 1	Fwd	5'-AAGCGATGAGCCCAGTGTGTTTCT-3'	HQ425704.1	Bebianno et al., 2017
	Rev	5'-CGCTGTCTGTATTGTAGTGGCAACTGGT-3'		
EcSOD	Fwd	5'-CTTCATGCCAGGCAACCT -3'	CU6811762.1	Gonzalez, 2005
	Rev	5'-TGACGTTGAATCCGGTCA -3'		
		Internal control		
EF1-α	Fwd	5'-ACGACGATCGCATTTCTCTT -3'	XM_034472995	Fabioux et al., 2004
	Rev	5'-ACCACCCTGGTGAGATCAAG -3'		
Actin	Fwd	5'-GCCCTGGACTTCGAACAA -3'	CP048844.1	Rodet et al., 2005
	Rev	5'-CGTTGCCAATGGTGATGA -3'		

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989

990 **Table S2**: Average number of microfibres (MF) collected in the faeces of oysters exposed to
991 six different types of MF at two concentrations.

992

MF type/Concentrations	Average MF counted in faeces / aquarium / day of experiment [10 MF L^{-1}]	[10 000 MF L^{-1}]
Unexposed (control)	0	0
	Natural MF	
Cotton	2 ±1	58±35
Organic cotton	2 ±2	4 ±2
Wool	2±1	85±38
	Synthetic MF	
Acrylic	1±1	94±50
Nylon	2±1	10±7
Polyester	1±1	4 ±3

993

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995

996 **Supplementary figures**

997

998 **Fig S1. Length (left) and diameter (right) frequency of prepared microfibres.** CO: cotton,

999 OC: organic cotton, WO: wool, AC: acrylic, NY: nylon, PES: polyester.

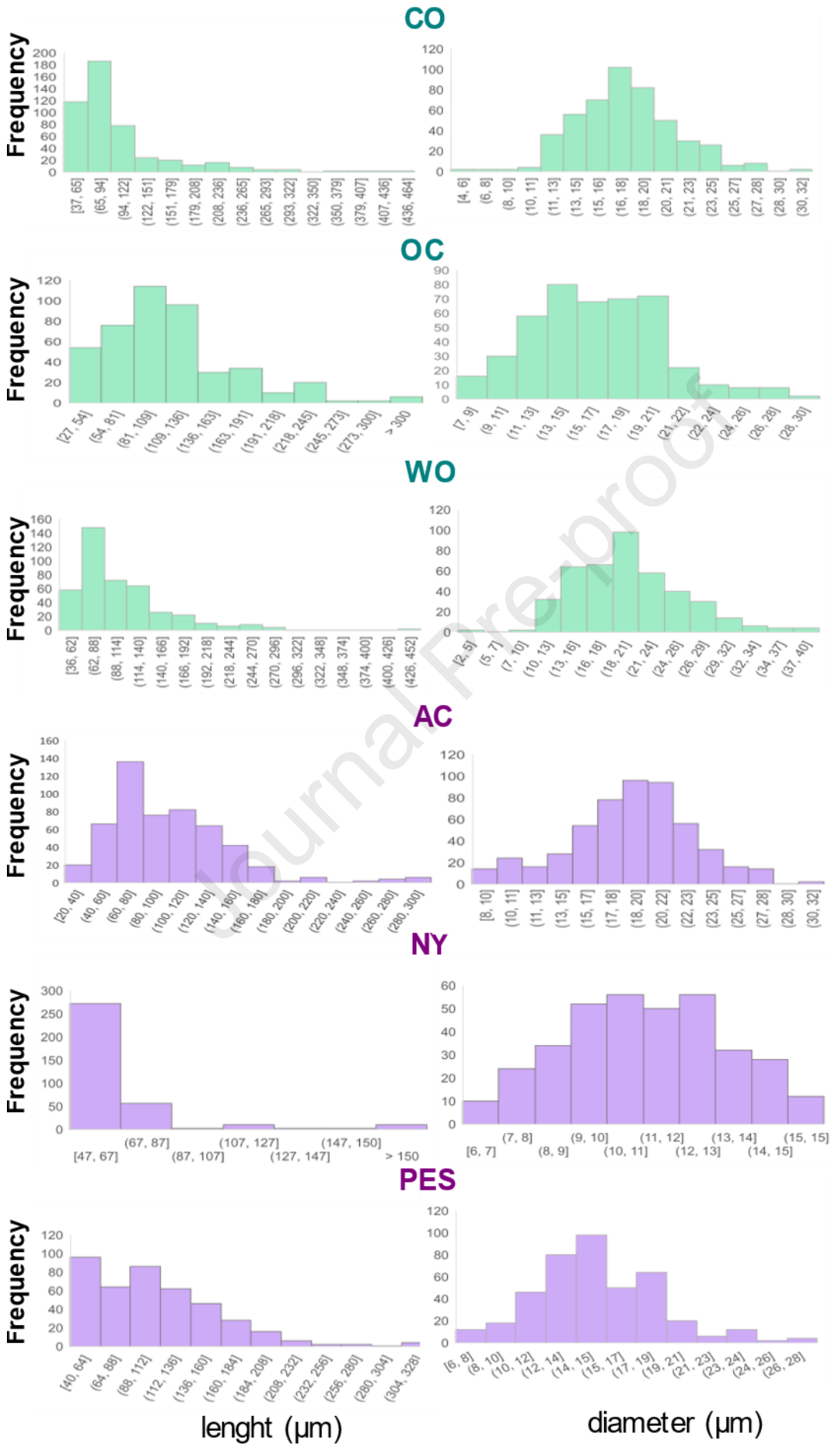

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Figure S2. Optical microscope images of microfibres and their corresponding spectra identified after KOH digestion of oyster digestive gland.

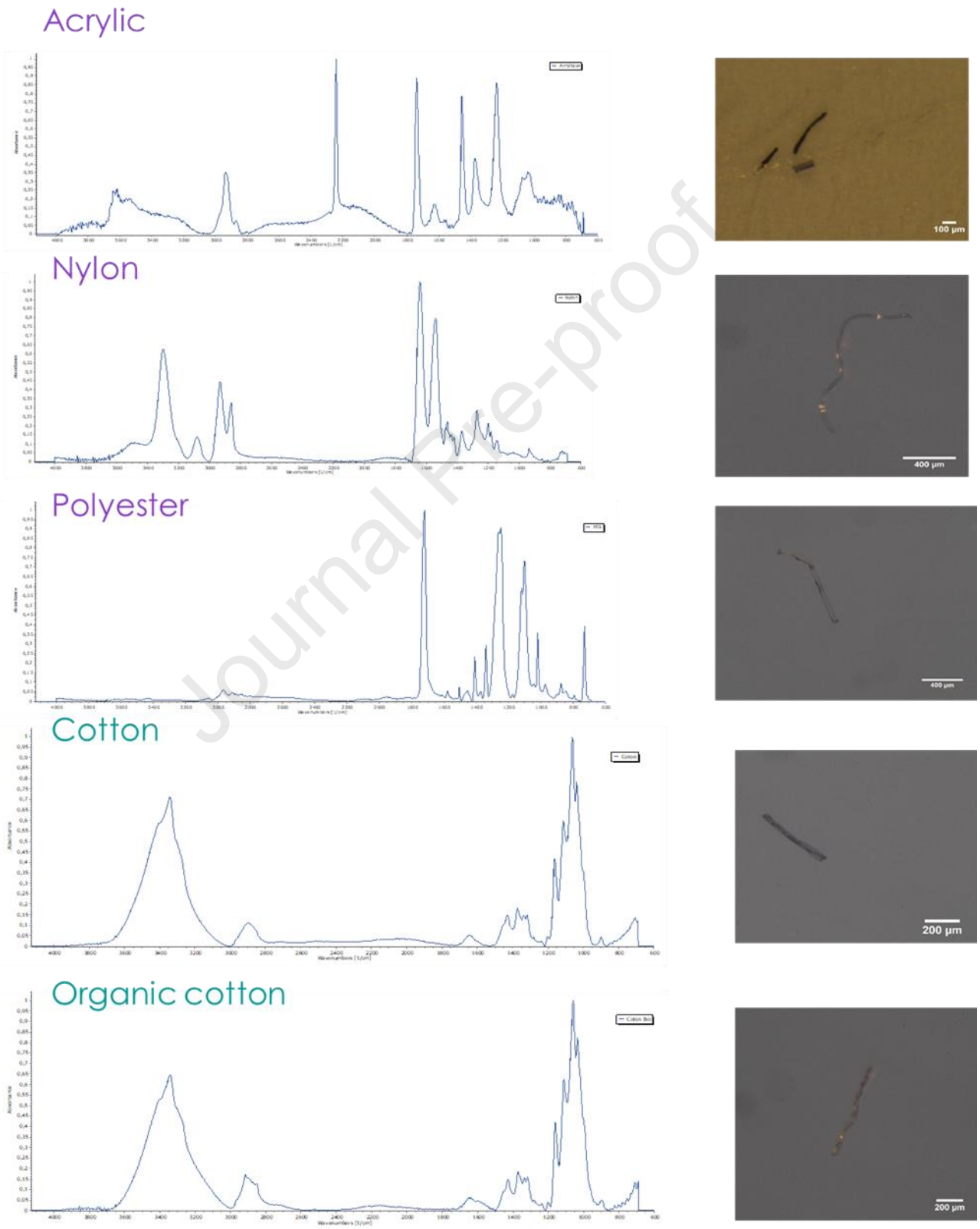


Fig S3. Optical microscope images and spectrum of cotton contamination observed in the digestive gland of oysters exposed to wool, unexposed or in the negative control of the digestion protocol.

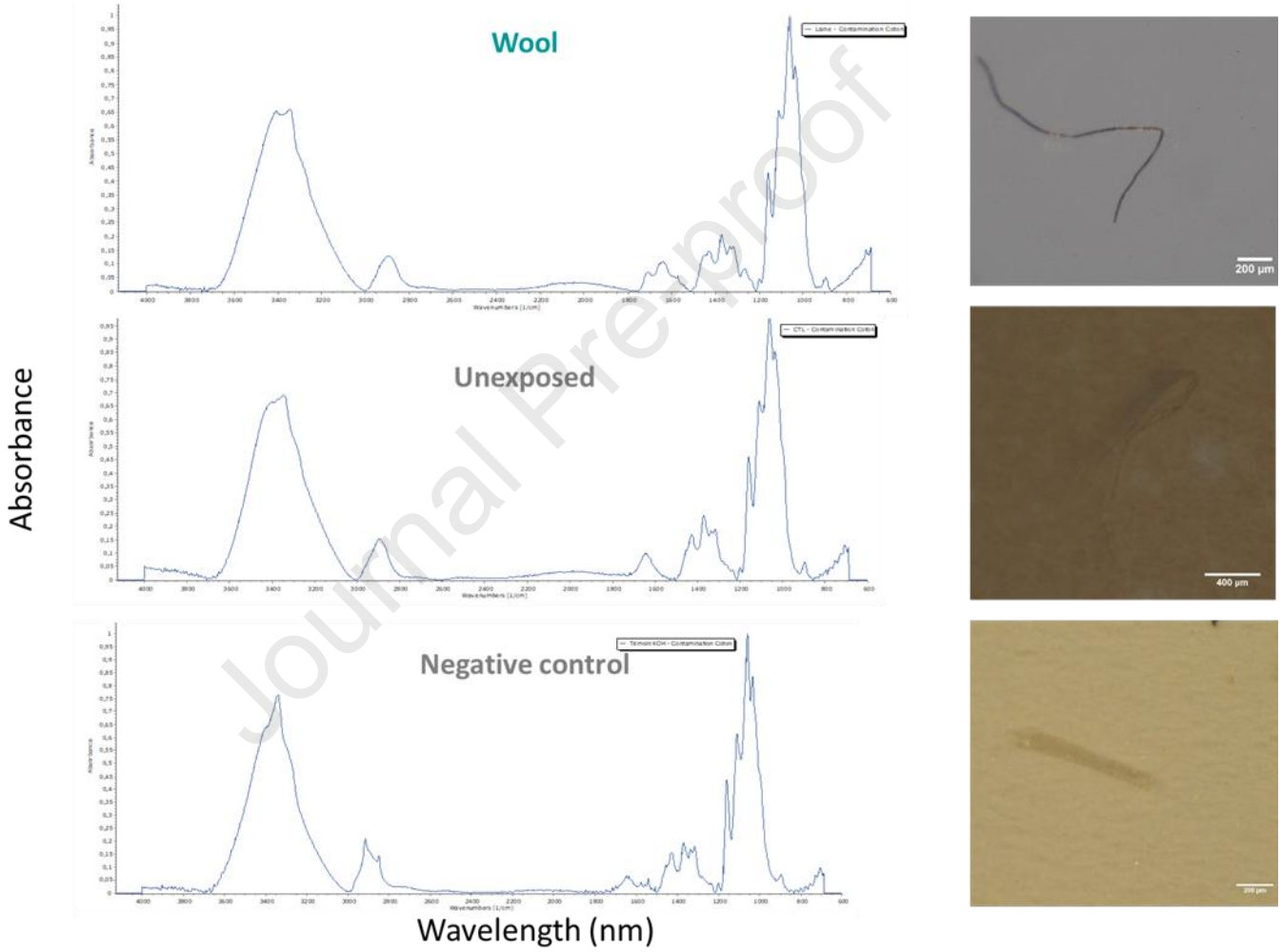


Figure S4. Microfibres in faeces. Optical microscope images of MF presence in the faeces of oysters exposed to the six types of microfibres. CO: cotton, OC: organic cotton, WO: wool, AC: acrylic, NY: nylon, PES: polyester, MF: microfibres.

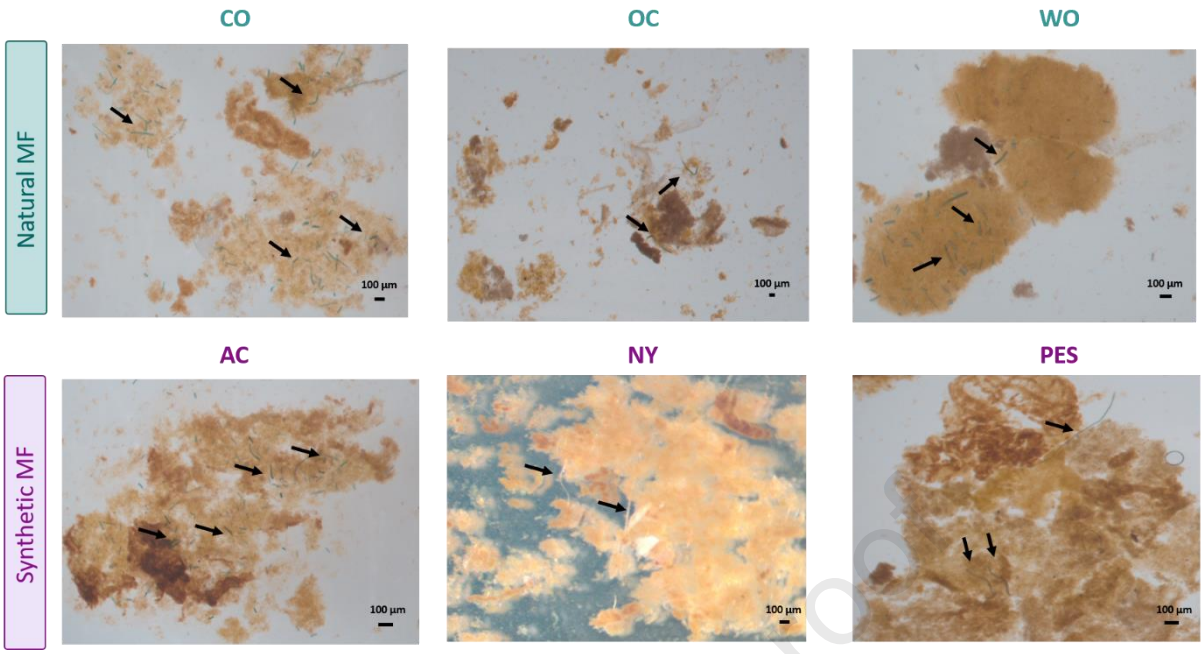


Fig S5. Illustration of faeces production in oysters exposed to polyester (A) and wool (B).

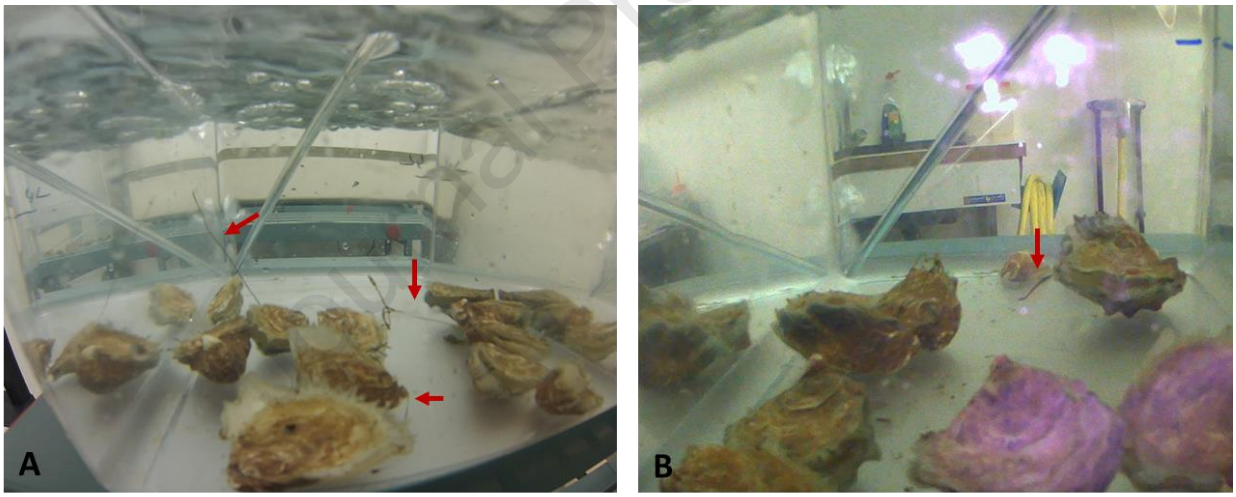


Fig S6. mRNA content of caspase 1 in oysters exposed to six different microfibres. CTL: control, CO: cotton, OC: organic cotton, WO: wool, AC: acrylic, NY: nylon, PES: polyester. Different letters above the boxes indicate significant differences between treatments (Kruskal–Wallis test).

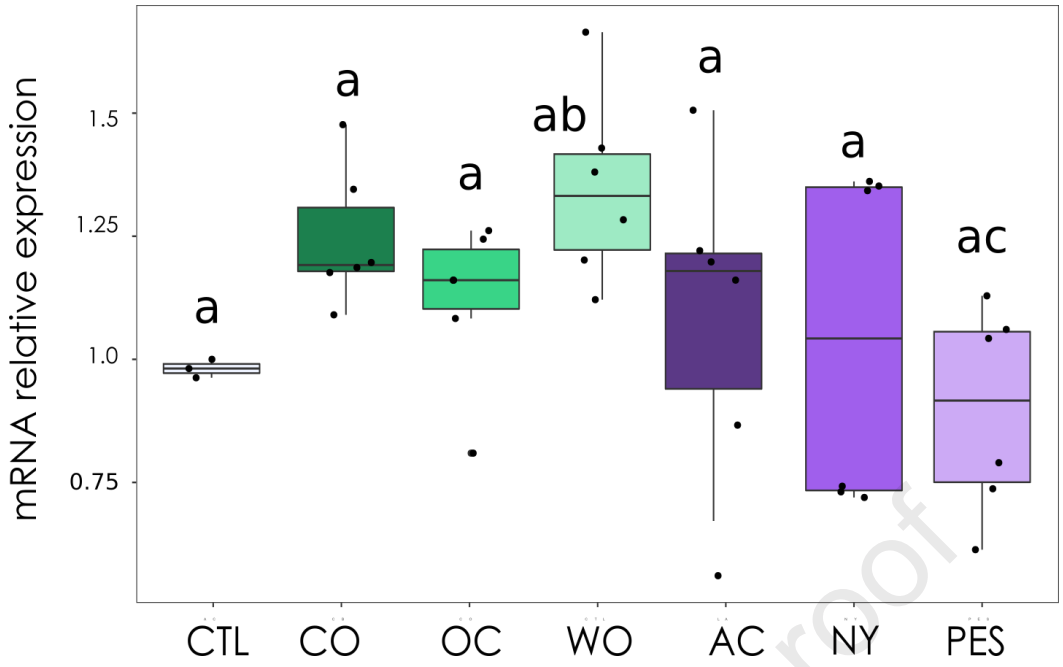
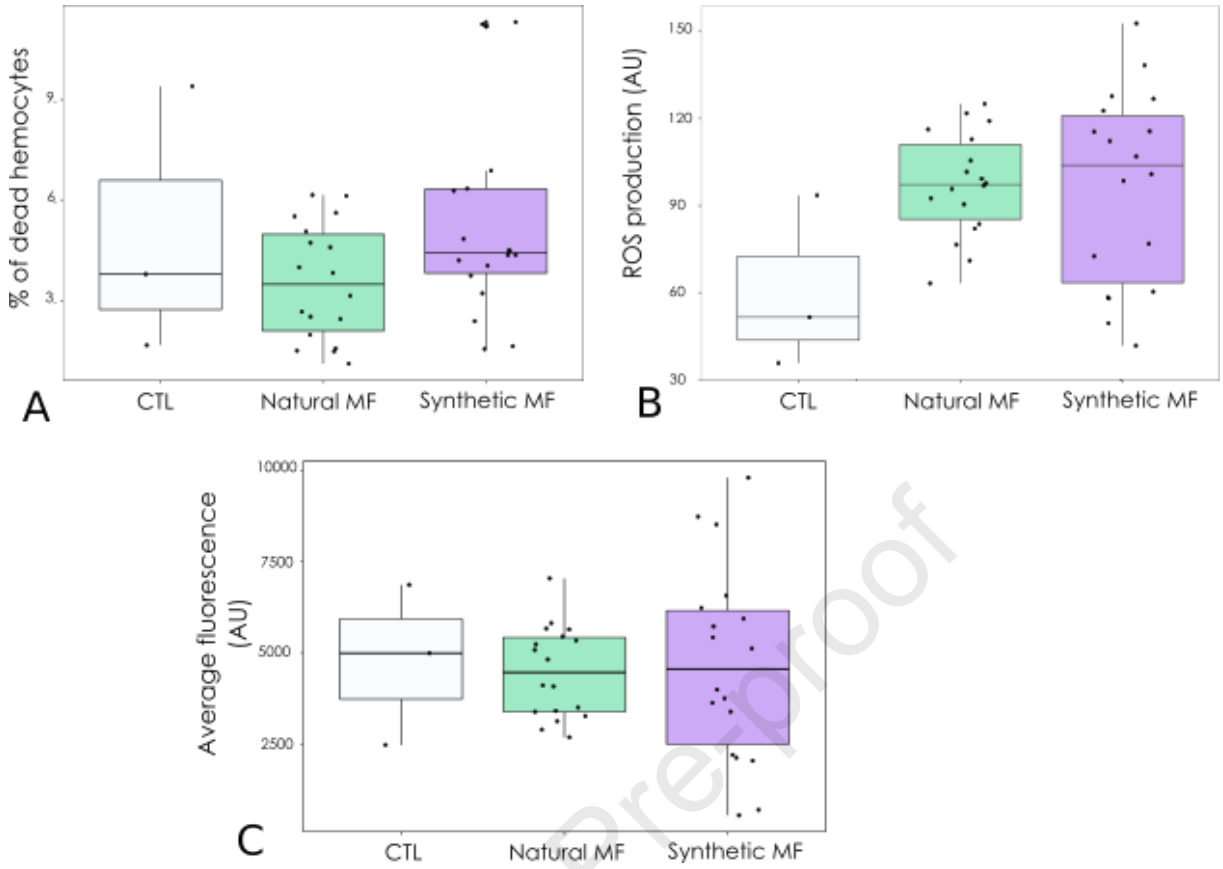


Figure S7. Haemocyte parameters in oysters exposed to the leaching chemicals of natural or synthetic MF or unexposed. A. Percentage of dead haemocytes. **B.** ROS production. **C.** Average fluorescence of calcein which is inversely proportionate to ABC transporter activity. CTL: control, MF: microfibres.



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Highlights (max 85 characters/ bullet point)

- Environmental concentration of MF is sufficient to perturb oyster physiology
- Tested natural MF elicit higher digestive and inflammatory responses than synthetic
- The physical properties of natural MF might explain the observed perturbation
- In general MF toxicity depends on their properties and intended use

Authors contribution

Détrée Camille: Conceptualization, Data curation, Formal analysis, Funding acquisition; Investigation; Methodology, Writing - original draft. **Clementine Labbé**: Data curation, Formal analysis, Investigation, Writing - review & editing. **Ika Paul-Pont:** Conceptualization, Investigation, Methodology, Funding acquisition, Resources, Writing - review & editing. **Enora Prado:** Investigation, Formal analysis, Writing - review & editing. **Maria El Rawke:** Investigation, Formal analysis, Writing - review & editing. **Lena Thomas:** Investigation, Writing - review & editing. **Nicolas Delorme:** Investigation, Writing - review & editing. **Nelly Le Goic:** Investigation, Writing - review & editing. **Arnaud Huvet**: Conceptualization, Investigation, Methodology, Funding acquisition, Resources, Writing - review & editing

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: