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Marine Biodegradability and Ecotoxicity of MWool® Recycled Wool Fibers: A Circular-Economy-Based Material

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Abstract: Pollution of the marine environment by microfibers is considered a problem for ecosystem conservation. The amount of microplastic, localization of sources, and associated ecotoxicity are well known in the literature. Wastewater from washing machines is the main source of microplastic fibers in the aquatic environment, and fabrics made from recycled plastic are widely reused. The circular economy also promotes recycling of dyed natural wool materials as a basis for making new clothing, but in this case, less research has been conducted on the behaviour and effects of recycled wool microfibers in marine ecosystems. MWool® (MW) and MWool® carded (MWc) products made from recycled wool fibers were tested in mesocosms to investigate the biodegradation of wool fibers over a 260-day period and the effects of this process on marine ecosystems in terms of microfiber inputs and the ecotoxicological effects of by-products and chemicals released during degradation. The early degradation process was associated with the loss of artificial pigments from the dyed wool, particularly pink and red, which occurred within 30–90 days of exposure. Mean release of microparticles into contact water is significantly different from control (T0, $p < 0.01$) at 90 days MWc (36.6 mg/L) and 180 days MW (42.9 mg/L). The biodegradation process is accompanied by swelling of wool fibers, which is associated with a significant increase in mean wool thickness ($p < 0.05$, 18.8 ± 2.1 µm at T0 vs. 24.0 ± 7.1 µm). In both cases, the contact water was not associated with signs of ecotoxicity for the marine species tested in this study (*Phaeodactylum tricornutum*, *Brachionus plicatilis*, and *Paracentrotus lividus*).

Keywords: biodegradation; marine environment; mesocosm study; ecotoxicological assays; recycled wool natural fibers products; waste valorisation by circular economy

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1. Introduction

In the last decade, pollution by microparticles (fibers and fragments) caused by human activities, both in the environment [1] and in consumer products [2,3], has gained worldwide importance. Recent research addressing the sources of microparticles has shown that textile release is a source of microplastic pollution in the marine environment. Recent studies have shown that an amount of 124–308 mg/kg (approximately 640,000 to 1,500,000 pieces) of fibers are released from washing machines, depending on the type and nature of the garments washed [4]. A global characterization of microfibers in ocean surface waters revealed that most fibers floating in the world's oceans are made of cellulose and that the amount of microplastics is less than that of natural fibers. Although they represent less than 2% of global production, 12.3% of microfibers in the samples studied are classified as fibers of animal origin, while cellulose accounts for the majority (79.5%) [5]. It has been shown that textiles made of cotton, wool, and viscose fibers release more microparticles during washing than textiles made of polyester [6–8]. It has been calculated that household

washing machines still release approximately 15 thousand tons of cotton and synthetic fibers per year [6].

Microfibers of cellulosic origin were also released from washing machines when washing textiles made of polyester–cellulose blends [4]. A normal washing machine filter is not capable of retaining 40% to 75% of the total mass of fibers [6]. Recent studies in Finland (5.5×10^6 population) found that annual emissions of polyester and cotton microfibers from household washing machines ranged from 154,000 (1.0×10^{14}) to 411,000 kg (4.9×10^{14}) [7]. In addition, detergent use has a significant impact on the release of microplastics from synthetic materials in washing machine water, but is ineffective for cotton, which causes the highest emissions in wastewater [6]. Increasing water temperature can affect the amount of microfibers released for both polyester and cellulosic fabrics; however, cellulosic fabrics release more microfibers (0.2–4.0 mg/g fabric) than polyester (0.1–1.0 mg/g fabric) during accelerated washing [8].

Recent studies have shown that the direct release of microfibers from garments into the environment due to wear is as important as their release into water [9] increasing the importance to study their fate in the environment. Although cellulose-based fibers, such as cotton and wool, are considered natural and biodegradable [10], there is not much literature on their behaviour in the marine environment [11].

Moreover, the release of pollutants from such microfibers could affect the fate and transport of chemical pollutants in the trophic network of the aquatic environment due to their high emission levels in water and chemical adsorption properties [7]. For these reasons and given the worldwide release of microfibers into the aquatic environment, research on their biodegradability and associated ecotoxicological effects on trophic networks is crucial to ensure environmental protection.

A particularly important aspect is the development by the European Union of an action plan for the circular economy, which promotes the recycling of disposed materials to produce new commercial products to improve the global sustainability of human activities. To cite just a few examples: dredged sediments, if not polluted, are promoted by Italian law (M.D. 173/2016) [12] for beach replenishment, and several applications have been reported in the literature [13,14]. Recent experiences with circular economy have also been reported for reducing the disposal of *Posidonia oceanica* leaves harvested from the seabed [15] and shellfish claims [16].

Another important experience with the application of circular economy to efficiently transform waste into a resource is Manteco SpA, an Italian company that produces wool fabrics from recycled wool. The fabrics, and therefore the garments, produced by Manteco SpA with MWool® are usually labelled with the indication that they can only be dry cleaned, but it has been shown that the release of microfibers from the garments into the air when worn is very important [9]. The life cycle assessment of MWool®, a mechanically recycled wool, has shown that this product reduces the impact on the environment [11]. Due to the interest in this recycled product and the limited knowledge of microfiber degradation and its impact on the aquatic environment, this study focused on specific aspects of MWool® degradation in the aquatic environment.

The objective of this study was to use a mesocosm experiment to determine whether degradation of mechanically recycled and dyed wool fibers occurs in the marine environment and whether the results of the degradation have an impact on the environment. The occurrence of wool fiber degradation in the marine environment was determined during a 260-day exposure experiment in mesocosms under rather conservative environmental conditions. Analyses were performed to determine fiber degradation (μFT- IR spectra, FESEM microscopy, and release of total suspended solids) at different time periods. In addition, the ecotoxicity of degradation products and chemicals released over time in seawater was evaluated in three species that are representatives of marine trophic webs (*Phaeodactylum tricornutum*, *Brachionus plicatilis*, and *Paracentrotus lividus*).

2. Materials and Methods

2.1. Logic Model of Experiments

The logic model applied to the experiment was sized to reduce the statistical errors (Type I, mistaken rejection of a true null hypothesis; and Type II, the failure to reject a null hypothesis that is actually false) according to the literature [17]. Two different commodities (Figure 1) were tested: MWool® (MW) and MWool® carded (MWc). The MWool® samples were examined before the start of the experiment to determine the original structure of the fibers using Field Emission Scanning Electron Microscopy (hereafter, FESEM), the average FT-IR spectra, the average size of the fibers, and the range of variation. These results were collected for both MW and MWc materials and were considered representative of the initial state of the wool (T0, start time) in order to make comparisons with the different degradation times tested (T1, T2, T3, T4, T5, T6, and T7), where T1–T7 represent 30, 60, 90, 120, 180, 200, and 260 days from T0, respectively.

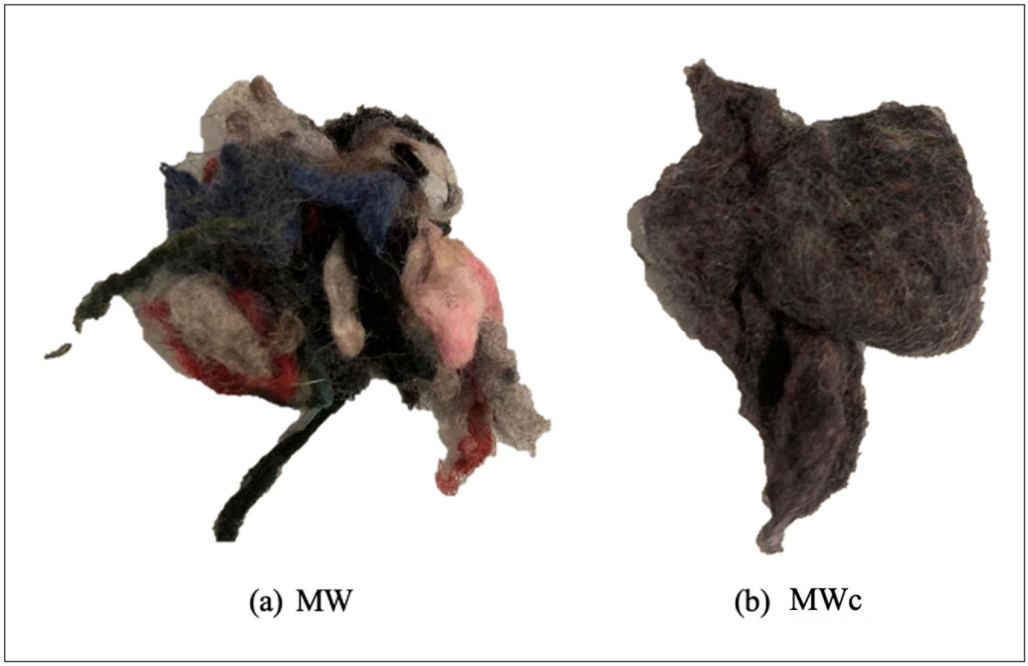

Figure 1. Examples of both raw material types tested. MWool® (MW, (**a**)) and MWool® carded (MWc, (**b**)).

Experiments were performed in a closed and controlled system to isolate variables that might affect experimental responses. Each sample was subjected to a treatment exposing it to seawater with a mass/volume ratio of 500 mg/L (Figure 2). The tests were performed in glass jar mesocosms stored in a temperature-controlled and monitored room that allowed regulation and control of the experimental conditions. The mesocosms were stored at 19 ± 1 °C under a standard light/dark cycle (16:8 h; white light; 5000 ± 500 lux) under natural oxygen conditions (moderate oxygen supply from the air).

Figure 2. Experimental activities: representative images. Mesocosms to test MWool® degradation in marine environments (**left** Image) and box-gloves (**right** image) used to manage samples under controlled conditions used for microfibers particles analyses.

Test variables (temperature, light exposure, oxygen, etc.) to perform exposure experiments were determined based on public outputs of ocean models (https://eurogoos.eu/models/; accessed on 18 March 2023).

The mesocosms were kept in a static condition without water changes to reduce the disturbance of the degradation process by water movement and water changes and to simulate the effects under the worst environmental conditions (more cautious environmental approach). The natural seawater used for this experiment was taken from a natural, non-polluted site (Talamone, Italy) and filtered at 7 µm to remove impurities but not the microbes responsible for the biodegradation activities. A natural inoculum of marine bacteria was added to the mesocosm at each exposure time (500 µL) to ensure degradation activity. The pH, oxygen level, and salinity were monitored twice per week to evaluate significant changes during exposure and make corrections as necessary. A decrease in pH was corrected by dropwise addition of NaOH (1 M) until an equilibrium of 8.0 ± 0.2 pH was reached, and any increase in salinity was compensated by addition of ultrapure water up to the original salinity. Mesocosms were prepared in statistical replicates (n = 3) for each condition tested and conveniently duplicated to allow interruption of the mesocosm at each exposure time of interest. Biodegradation in the ocean is a slow process [10]; therefore, longer exposure times were chosen in this study (30, 60, 90, 120, 180, 200, and 260 days from start time T0) than those tested in soils [10].

To allow a simpler presentation of the results, the exposure times in this document are given as follows: T0 is the start time; T1 (30 days); T2 (60 days); T3 (90 days); T4 (120 days); T5 (180 days); T6 (200 days); and T7 (260 days).

Negative controls of mesocosms were performed by preparing mesocosms with the same filtered seawater without adding MWool® to evaluate any changes in the system due to the controlled conditions. The bacterial inoculum was added to the negative controls at the same time as the tested samples. At each time point, the mesocosm replicates were destroyed, and degradation was determined by comparing the µFT- IR spectra and microstructural changes revealed by FESEM with those of T0.

In addition, the suspended solids (TSS) generated by the degradation were measured to evaluate the increase of fibers released in the water. At each time point, the water was filtered in mesocosm at 0.45 µm to test the ecotoxicological responses in three species belonging to different trophic levels. Both the seawater used and the negative controls of the mesocosms were analyzed for toxicity to the species tested. The endpoints tested were inhibition of algal growth (*Phaeodactylum tricornutum*), mortality (*Brachionus plicatilis*), and larval malformation during development (*Paracentrotus lividus*).

2.2. Mesocosms Treatments

After each measurement, the mesocosms were placed in the box-glove system, which was equipped with a system that could prevent nanoparticle contamination by double filtration of the inlet air. With this system, samples could be handled directly, and laboratory-related cross-contamination could be avoided (Figure 2). After removal of the wool tangle, the mesocosm water was filtered through a pre-weighed filter fiber disk (0.45 µm mesh filter; Whatman®, Buckinghamshire, UK) using a vacuum pump filtration device, which was oven-dried (105 ± 1 °C) to constant weight to determine TSS (mg/L), the results were normalised compared to results obtained on the negative control, and the effective contribution of wool fiber degradation over time was quantified.

In addition, the fibers inside the glove were observed using a stereomicroscope (80X, mod. SMZ 800 N; Nikon®, Melville, NY, USA) equipped with a camera connected to a computerized image analysis system (mod. DS -Fi2 high resolution color; Nikon®) and image analysis software (ACT-1; Nikon®). The microscope provided accurate micrometric measurements with known uncertainty calibrated with an accredited slide (LAT) and was used to measure wool fibers (dried at 40 ± 1 °C) whose infrared spectra were recorded as fingerprints with µFT- IR (microscope combined with infrared Fourier transform detector).

2.3. Physico–Chemical Analyses

TSS were determined according to the method APAT-CNR-IRSA 2090:2003, with the 0.45 µm filters collecting the contact water dried to constant weight in an oven (Memmert; mod. UN55) set at 105 ± 1 °C. Salinity (S, g/L), pH, and oxygen content (DO, mg/L) were measured using dedicated electrode probes, namely HI 763100 (Hanna Instruments), HI 10480 (Hanna Instruments), and HI98198 (Hanna Instruments).

The following methods were used to determine the measured values: AP-AT-CNR-IRSA 2070, man. 29/2003 (salinity), UNI EN ISO 10523:2012 (pH), and UNI EN ISO 5814:2013 (oxygen).

2.4. Chemical Microanalyses

The spectra of the studied microfibers were recorded using µFT- IR (mod. Nicolet i10 XM; Thermo Scientific®, Waltham, MA, USA) equipped with a liquid nitrogen cooled ATR, reflectance, and transmittance detector, allowing the determination of the spectral fingerprint of the studied materials. The spectra collected on 10 different fiber surfaces were used to determine the mean spectra of T0 on MW and MWc samples to create a reference spectra library. The same mean spectra were collected at each exposure time (T1–T7) and compared with the T0 spectra library to determine the percentage of identity between the compared spectra.

2.5. Ultrastructural Analyses

Fibers were analysed using a high-resolution scanning electron microscope (FESEM; Merlin II, Zeiss®, Oberkochen, Germany) equipped with a combined EDS/WDS microanalyzer for qualitative and quantitative high energy resolution analysis. FESEM is equipped with secondary and backscatter in lens electron detectors for image analysis up to sub-nanometric resolution. Moreover, a charge compensator allows microanalysis (EDS/WDS) of non-conducting and unprepared samples.

With this microanalytical platform, it is possible to characterize the microstructures, morphology, and elemental composition of samples. This technique proved to be of great importance to better focus ultrastructural changes and nanostructure of tested materials to determine the better recycling use and the general behaviour [15,16]. Observation of ultrastructure and morphology allowed changes to be highlighted during decomposition. Measurements with different exposure times were performed to evaluate the change in the mean value of the fiber width.

2.6. Ecotoxicological Assays

Filtered water samples were collected at each test time to evaluate ecotoxicological responses of three marine species from different trophic levels. Tests were conducted according to the methods indicated in Table 1. Unicellular algal species (*Phaeodactylum tricornutum*, growth inhibition after 72 h of exposure), rotifers (*Brachionus plicatilis*, mortality after 24 and 48 h of exposure), and echinoderms (*Paracentrotus lividus*, embryotoxicity after 72 h of exposure) were tested. Data collections were performed by stereomicroscopy (Nikon, mod. P-DSL-32) for *B. plicatilis* and *P. lividus*, while algal data were collected by spectrophotometry at the wavelength of 670 nm (Peak Instrument, mod. C-7100 S) using an internal calibration curve to relate measured absorbance values to cell densities. Negative and positive controls (exposure to a reference toxicant) were performed to verify the performance of the assay. Tests were considered acceptable only if the results of both positive and negative controls were consistent with responses reported in the method applied. At each exposure time, the negative controls of the mesocosms were tested as a sample and used to normalise the results of tested samples of the same exposure time.

Table 1. Ecotoxicological tests performed. In this table, species tested, methods, measured endpoint and effects, and measurement units are reported for both acute and chronic assays.

	Acute Toxicity (Type II)	**Acute Toxicity (Type II)**	**Chronic Toxicity (Type III)**
Species	*Brachionus plicatilis*	*Phaeodactylum tricornutum*	*Paracentrotus lividus*
Method	UNI EN ISO 19820:2016	UNI EN ISO 10253:2017	EPA/600/R-95-136/S15 and ISPRA 11/17
Endpoint	Mortality 24 and 48 h	Inhibition of growth 72 h	Embryotoxicity 72 h
Unit	%	%	%

2.7. Quality Assurance and Quality Control

The analyses were performed according to standardized official methods (UNI EN ISO, ICRAM, EPA, and APAT) in compliance with the UNI EN ISO 17025 guidelines. The instruments used for the measurements performed (electrodes and probes, μFT-IR, and spectrophotometer) were calibrated using standard reference materials or by LAT-accredited Centers.

Positive and negative controls were performed to ensure the data quality. Negative controls resulted in 0 ± 1.2% growth inhibition for *P. tricornutum*, 0% mortality for *B. plicatilis*, and 9.3 ± 1.2% mortality for *P. lividus*. Positive controls were performed with $K_2Cr_2O_7$ and resulted in an EC_{50} value of 24.0 mg/L (Cr^{2+}) with a range of 20.8–27.7 mg/L; and 347.2 mg/L ($K_2Cr_2O_7$) with a range of 331.5–363.6 mg/L respectively in *P. tricornutum*, and *B. plicatilis*. *P. lividus* assay was controlled with Cu^{2+}, and the EC_{50} value of the tested samples was 9.6 μg/L (5.1–17.9 μg/L).

Precision and accuracy were determined for all physico-chemical variables tested using standard reference materials, spiked natural matrix, and intercalibration exercises. Ultrastructural analysis (FESEM) was performed by the CERTEMA Centre of Excellence. BsRC (Accredia Lab. N. 1715L) performed microchemical and ecotoxicological analyses. The QA/QC technique included blank samples for chemical analysis, use of standard reference materials, and positive and negative controls.

2.8. Statistical Analyses

Results obtained on *P. lividus* were corrected according to the Abbott's formula as follows: Anomalies (%) = ((Anomalies in controls − Anomalies in tested sample)/Anomalies in controls) × 100. Collected data were analysed using Primer® statistical analyses routines to determine univariate relationships among tested variables. Ecotoxicological results

were analyzed by the software Trap (Toxicity Relationship Analysis Program), USEPA (TRAP1.30) to calculate EC_x values, when appropriate.

3. Results

The results of the mesocosm experiment are summarized in three different sections: physicochemical changes in MWool® fibers (changes in ultrastructure and variation in mean µFT-IR fingerprint of microfibers); effects of MWool® degradation on water (release of microparticles and changes in physicochemical parameters in water); and ecotoxicological effects on marine target species (three species from different trophic levels exposed to the contact water following standardized methods). In general, the fibers of MW showed a high density of colored wool mites and a more entropic fiber distribution compared with the fibers of MWc, which were more ordered and grayer in color.

3.1. Physical–Chemical Alteration of Wool Fibers

3.1.1. Microchemical Analyses

The analyses performed with µFT- IR showed a decrease in the average spectral match of the treated wool compared with the spectral characteristics of the unexposed wool (T0). This spectral decrease is shown in Figure 3. A significant average decrease of approximately −20.0% in spectral match was observed at T7 compared with T0 for the MW samples, while MWc decreased less (−12.6%).

3.1.2. Ultrastructural Analyses

Measurement of microfiber dimension (thickness, µm) performed on significant replicates of both tested wool types at T0 yielded mean values of 18.8 ± 2.1 µm (range 15.2–22.0 µm) for MW fibres and similar mean values of 18.1 ± 2.7 µm (range 15.0–21.9 µm) for worsted fibres (MWc). The differences between the two tested wool types were not significant according to both the *t*-Test ($p = 0.500$) and F-Test ($p = 0.441$). In contrast, measurements during the exposure period showed that fibre thickness increased significantly at T7 (24.0 ± 7.1 µm; range 15.5–31.0 µm), and both the *t*-Test ($p = 0.04$) and F-Test ($p < 0.01$) performed to compare T0 and T7 resulted in significance. In addition, ultrastructural analysis by FESEM showed a significant deterioration of the wool microstructure at T7, indicating that degradation processes were underway (Figure 4).

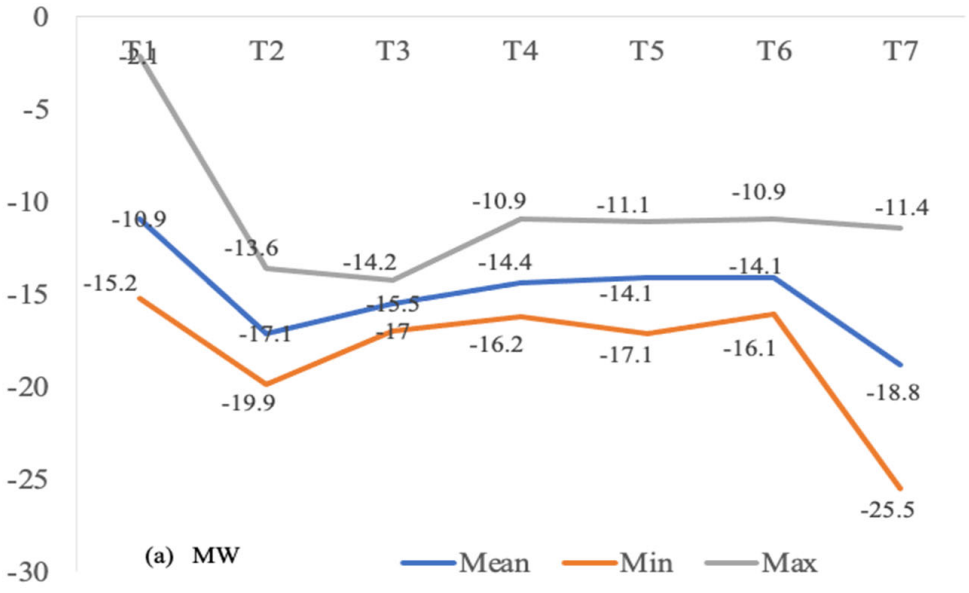

Figure 3. *Cont.*

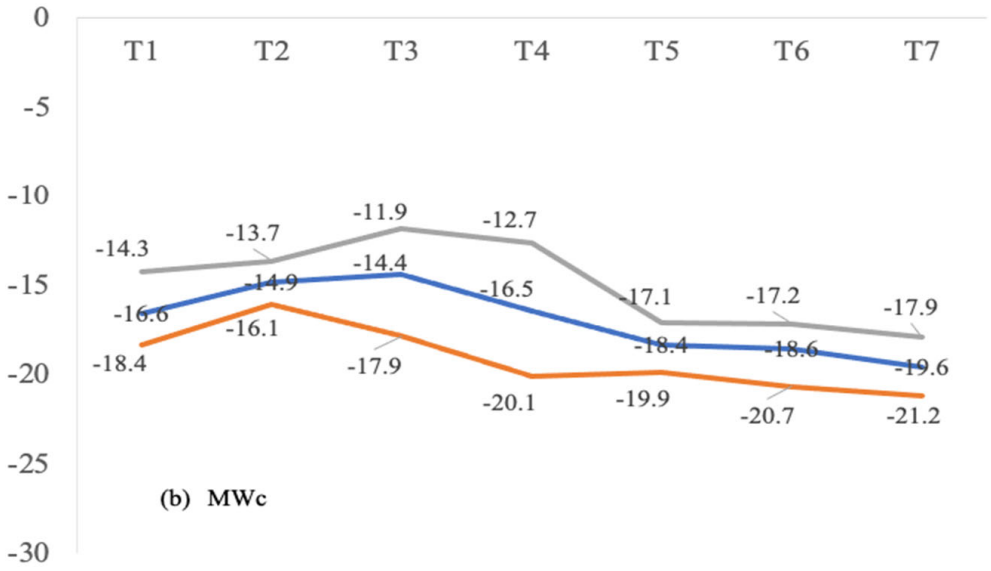

Figure 3. Reduction of spectral fingerprint matches of tested fibres during different experimental times compared with T0. Graphics represent the mean (blue line), maximum (gray line), and minimum (orange line) variation of recorded spectral match between fibres at T0 and fibres at different exposure times (T1–T7). Data are expressed as percentage of match. Negative values mean the occurrence of a decrease in spectral match between tested time and T0, indicating an increasing diversity among chemical fingerprints of fibres.

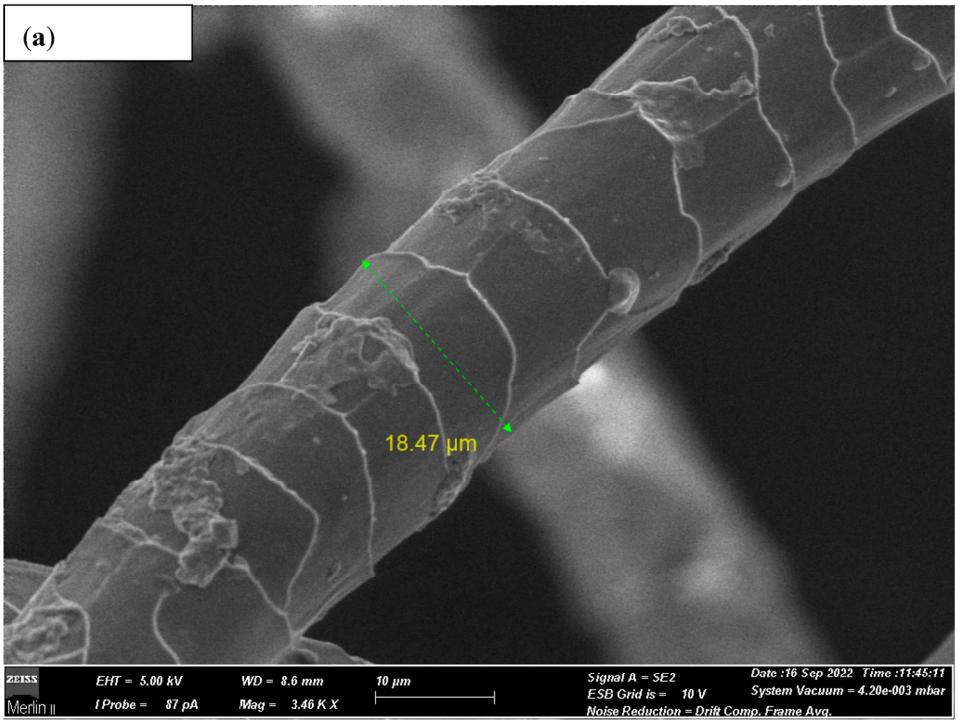

Figure 4. *Cont.*

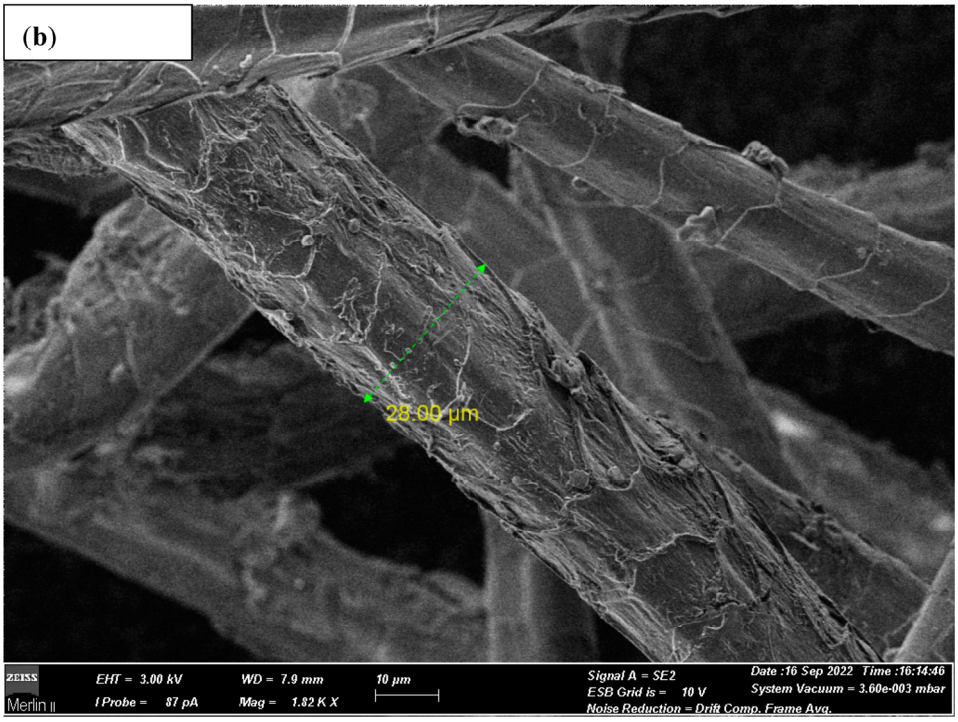

Figure 4. FESEM microphotographs selected for this purpose. (**a**) (T1) and (**b**) (T7) of tested MWool® fibers with evidence of measured thickness.

3.2. Effects of Wool Degradation on Water

Release of Microfibers in Water

The total amount of suspended solids (TSS) measured in water after each exposure time (T1–T7) is shown in Table 2 as statistical values (mean, standard deviation, and range min-max of variation). Significant values associated with the *t*-Test and F-Test are also shown in bold. A significant difference between replicates was found only occasionally in MW (T3; *t*-Test) and MWc (T7; F-Test) samples.

Comparing the exposure times with the values recorded at T1, significant differences were found starting at T3 for the MW samples and T5 for the MWc samples. To verify the statistically significant differences both between the times of the individual samples and between the two types of wool, the *t*-Test and F-Test were performed. No statistically significant differences were found between the two wool samples, and, consequently, very similar behaviour of the two tested wool types was observed. The MWc sample showed statistically significant changes at time points T1, T3, T4, T5, T6, and T7 for the *t*-Test and at time points T2, T4, T5, T6, and T7 for the F-Test. MW showed statistically significant changes at time points T2, T3, T4, T5, T6, and T7 for the *t*-Test and at time points T3, T4, T5, T6, and T7 for the F-Test. Concerning features of released microfibers, microscope analyses of TSS filters showed that microfibers released in water have variable length within 35–3932 µm with a modal value included within 300–500 µm.

Table 2. Microfibres release in water during the exposure. (a) Total suspended solids released in water (mg/L) in both tested MW and MWc wools. Data are expressed as mean, standard deviation (SD), and minimum and maximum recorded values ($n = 3$). Negative control values (control mesocosms) ranged within 2.0 (T1) and 9.6 (T7) mg/L; results are not normalised compared with negative controls. Results were tested according to Student's *t*-Test and F-Test to evaluate significant of differences between the two different tested wools (MW, and MWc, (b)) and between T0 and other exposure times (c). Significant values are highlighted in bold ($p < 0.05$; $p < 0.01$).

(a)		**T0**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Natural Water		0.3							
MWc	Mean	1.4	5.2	4.3	36.6	58.3	56.5	34.7	53.5
	SD	0.1	3.0	0.7	6.8	27.4	13.5	9.3	0.5
	Min	1.4	1.9	3.6	30.5	36.9	41.1	24.4	52.9
	Max	1.5	7.9	5.0	43.9	89.1	66.4	42.4	53.7
MW	Mean	2.0	4.1	9.0	8.9	25.8	42.9	42.8	85.8
	SD	0.9	2.0	4.1	6.4	18.2	10.1	22.2	35.0
	Min	1.3	2.5	4.3	3.6	12.8	32.5	20.6	45.5
	Max	3.1	6.4	11.3	15.9	46.6	52.7	65.1	108.7
(b)		**T0**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
MW vs. MWc	*t*-Test	0.26	0.66	0.11	0.05	0.13	0.53	0.46	0.24
	F-Test	0.62	0.65	0.32	0.10	0.34	0.71	0.55	0.67
(c)			**T0–T1**	**T0–T2**	**T0–T3**	**T0–T4**	**T0–T5**	**T0–T6**	**T0–T7**
t-Test	MWc		0.05	0.06	**0.01**	**0.01**	**0.01**	**0.01**	**0.03**
	MW		0.13	**0.03**	**0.04**	**0.02**	**0.01**	**0.01**	**0.01**
F-Test	MWc		0.05	**0.01**	0.06	**0.04**	**0.01**	**<0.01**	**<0.01**
	MW		0.25	0.05	**0.02**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

3.3. Effects of Pigment Release in Water

Pigment Release in Water

The sample showed that red and pink fibres resulted particularly depigmented at the end of exposure releasing pigments in contact water. This release needed to be studied from an ecotoxicological point of view to evaluate possible effects on the biotic component that may be associated with the early stages of decomposition. In sample MW (Figure 5), a loss of pigmentation was already observed at time T1, and the contact water became bright red.

Table 3 shows the mean values (SD) of pH, salinity, and oxygen content for MW and MWc samples; data are grouped by exposure time (T1–T7). In Table, the values of the experimental negative controls (mean and SD, calculated for all exposure times T1–T7) and the values recorded in filtered natural seawater (MW) are indicated. Results of statistical tests (*t*-Test, and F-Test) are also reported.

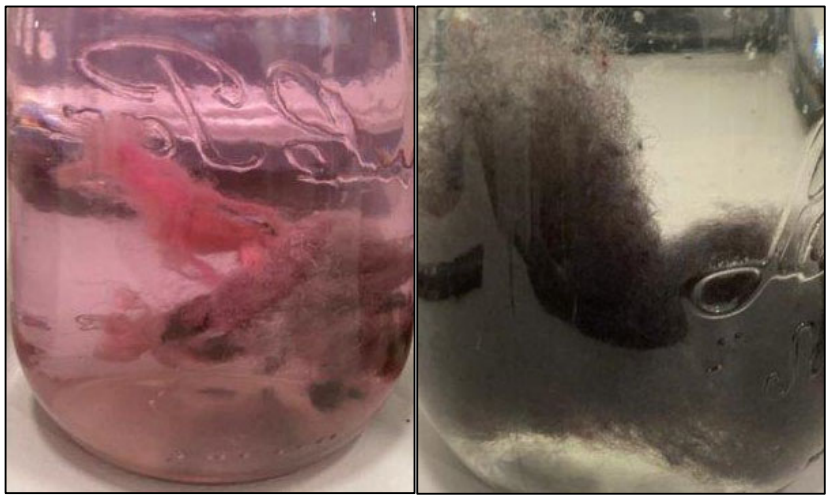

Figure 5. Pigment release in exposure water. In this figure, MWool® (MW, **left** side) is compared with MWool® carded (MWc, **right** side) after the same exposure time (T1). The release of pigment in contact water was recorded (MW).

3.4. Ecotoxicity of Exposure Water

Ecotoxicological tests allow for the evaluation of the associated effect on the marine environment coming from chemicals released during biodegradation process of the wool under tested conditions. The results of the ecotoxicological analyses performed on filtered exposure water (pore size 0.45 µm), exposing a battery of three marine species from different trophic levels are presented in Table 4.

Table 3. Physical–chemical parameters measured in tested contact water at different exposure times (T0–T7). Marine water used to perform tests showed the following values: 7.94 (pH) of pH, 30.9 (g/L) of salinity, and 7.2 mg/L (O_2) of oxygen. Mean and standard deviation (SD) are reported for both tested wools, and *t*-Test and F-Test comparing pairs of exposure times are also reported. Significant values ($p < 0.05$) are reported in bold.

	MWc						**MW**					
	pH		**S (g/L)**		**O_2 (mg/L)**		**pH**		**S (g/L)**		**O_2 (mg/L)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
T0	7.83	0.06	28.1	1.2	6.3	0.1	7.83	0.08	28.1	1.7	6.3	0.6
T1	7.75	0.09	28.4	1.8	6.6	0.4	7.70	0.08	28.7	1.3	6.4	0.3
T2	7.68	0.10	29.0	1.0	6.4	0.5	7.79	0.17	28.9	1.2	6.5	0.4
T3	7.81	0.17	29.1	0.8	5.8	0.1	7.77	0.14	29.4	0.8	6.7	0.5
T4	7.67	0.07	29.2	1.0	6.5	0.3	7.84	0.21	28.9	0.5	6.5	0.5
T5	7.78	0.21	28.9	0.3	6.1	0.3	7.78	0.12	28.4	0.7	6.4	0.1
T6	7.77	0.02	28.5	0.3	6.4	0.4	7.92	0.10	28.9	0.3	6.7	0.2
T7	7.83	0.06	28.6	0.5	6.5	0.9	7.83	0.13	28.5	0.3	6.2	0.1
Max	7.83	0.21	29.2	1.8	6.6	0.9	7.92	0.21	29.4	1.7	6.7	0.6
Min	7.67	0.02	28.1	0.3	5.8	0.1	7.70	0.08	28.1	0.3	6.2	0.1

Table 3. *Cont.*

	***t*-TEST**			***t*-TEST**		
	pH	**S (g/L)**	**O_2 (mg/L)**	**pH**	**S (g/L)**	**O_2 (mg/L)**
T0–T1	0.3	0.9	0.3	0.4	0.8	0.8
T0–T2	0.1	0.4	0.7	0.8	0.7	1.0
T0–T3	0.9	0.3	<0.01	0.9	0.4	0.6
T0–T4	0.03	0.3	0.3	0.6	0.7	1.0
T0–T5	0.7	0.4	0.2	0.8	1.0	0.8
T0–T6	0.2	0.6	0.8	0.1	0.7	0.5
T0–T7	1.0	0.6	0.8	0.4	0.9	0.4
	F-TEST			**F-TEST**		
	pH	**S (g/L)**	**O_2 (mg/L)**	**pH**	**S (g/L)**	**O_2 (mg/L)**
T0–T1	<0.01	0.6	0.2	1.0	0.8	0.5
T0–T2	0.52	0.8	0.1	0.3	0.7	0.7
T0–T3	0.23	0.6	0.5	0.5	0.4	0.8
T0–T4	0.91	0.8	0.2	0.2	0.2	0.9
T0–T5	0.15	0.1	0.3	0.6	0.3	0.1
T0–T6	0.25	0.1	0.1	0.7	<0.01	0.3
T0–T7	1.00	0.3	0.0	0.5	<0.01	0.1

Table 4. Results of ecotoxicological tests performed in MWool® of both MW and MWc types. Results are expressed as mean percentage (%) and standard deviation (SD) for all tested species (n = 3 experimental replicates). Furthermore, data are reported as inhibition of growth (I; SD) in *Phaeodactylum tricornutum*, mortality (Mean and SD after 24 and 48 h of exposure) in *Brachionus plicatilis*, and larval anomalies (Mean, SD, and Mean° corrected according to Abbott's formula for anomalies recorded in negative controls) in *Paracentrotus lividus*. Values recorded at each exposure time (T0-T7) are expressed as mean and min-max range of variation in the experimental replicates of mesocosms tested.

MW		***P. tricornutum***		***B. plicatilis***				***P. lividus***		
		I	**SD**	**Mean (24 h)**	**SD (24 h)**	**Mean (48 h)**	**SD (48 h)**	**Mean**	**SD**	**Mean°**
T0	**Mean**	−9.4	1.1	0.0	0.0	0.0	0.0	7.1	1.3	-
	Min	−10.2	1.1	0.0	0.0	0.0	0.0	5.7	0.6	0.0
	Max	−8.1	2.2	0.0	0.0	0.0	0.0	8.0	1.5	0.0
T1	**Mean**	4.2	1.1	3.3	5.8	3.3	5.8	16.1	2.3	7.5
	Min	3.2	0.8	0.0	0.0	0.0	0.0	14.3	1.5	5.5
	Max	5.3	3.4	10.0	11.0	10.0	11.0	18.7	5.0	10.3
T2	**Mean**	3.7	2.1	3.3	5.8	3.3	5.8	16.6	1.3	8.0
	Min	1.4	0.7	0.0	0.0	0.0	0.0	15.7	0.0	7.0
	Max	5.3	1.6	10.0	11.0	10.0	11.0	18.0	1.7	9.6
T3	**Mean**	2.7	1.2	6.7	5.8	6.7	5.8	17.3	2.6	8.8
	Min	1.4	1.9	0.0	0.0	0.0	0.0	14.3	0.6	5.5
	Max	3.6	3.3	10.0	11.0	10.0	11.0	19.0	4.5	10.7
T4	**Mean**	3.6	0.4	13.3	5.8	13.3	5.8	18.9	1.9	10.5
	Min	3.2	2.0	10.0	0.0	10.0	0.0	16.7	2.6	8.1
	Max	4.0	3.4	20.0	11.0	20.0	11.0	20.0	4.6	11.8

Table 4. *Cont.*

MW		*P. tricornutum*		*B. plicatilis*				*P. lividus*		
		I	SD	Mean (24 h)	SD (24 h)	Mean (48 h)	SD (48 h)	Mean	SD	Mean°
T5	Mean	3.2	0.8	16.7	5.8	16.7	5.8	19.0	1.8	10.7
	Min	2.3	1.9	10.0	0.0	10.0	0.0	17.0	1.5	8.5
	Max	3.7	4.0	20.0	11.0	20.0	11.0	20.3	2.6	12.1
T6	Mean	3.9	0.6	13.3	5.8	13.3	5.8	17.2	1.6	8.7
	Min	3.5	1.5	10.0	0.0	10.0	0.0	15.3	1.0	6.6
	Max	4.5	3.5	20.0	11.0	20.0	11.0	18.3	2.1	9.9
T7	Mean	3.5	1.6	30.0	0.0	30.0	0.0	19.7	1.5	11.4
	Min	2.3	1.6	30.0	11.0	30.0	11.0	18.0	0.0	9.6
	Max	5.3	3.2	30.0	11.0	30.0	11.0	21.0	3.6	12.9
MWc		*P. tricornutum*		*B. plicatilis*				*P. lividus*		
		I	SD	Mean (24 h)	SD (24 h)	Mean (48 h)	SD (48 h)	Mean	SD	Mean°
T0	Mean	−9.3	1.3	0.0	0.0	0.0	0.0	7.3	0.6	-
	Min	−10.2	0.9	0.0	0.0	0.0	0.0	7.0	1.0	0.0
	Max	−7.8	2.2	0.0	0.0	0.0	0.0	8.0	2.0	0.0
T1	Mean	3.0	1.5	0.0	0.0	0.0	0.0	20.6	0.8	12.4
	Min	1.4	1.3	0.0	0.0	0.0	0.0	19.7	2.0	11.4
	Max	4.5	3.5	0.0	0.0	0.0	0.0	21.0	3.6	12.9
T2	Mean	2.9	0.3	3.3	5.8	3.3	5.8	19.0	2.7	10.7
	Min	2.6	0.7	0.0	0.0	0.0	0.0	16.3	2.5	7.7
	Max	3.1	1.3	10.0	11.0	10.0	11.0	21.7	6.0	13.6
T3	Mean	3.6	1.2	3.3	5.8	3.3	5.8	19.3	3.9	11.0
	Min	2.2	1.3	0.0	0.0	0.0	0.0	16.0	1.5	7.4
	Max	4.4	2.8	10.0	11.0	10.0	11.0	23.7	3.6	15.8
T4	Mean	3.5	0.9	13.3	5.8	13.3	5.8	17.8	3.0	9.3
	Min	2.7	0.8	10.0	0.0	10.0	0.0	14.7	0.6	5.9
	Max	4.4	1.9	20.0	11.0	20.0	11.0	20.7	2.5	12.5
T5	Mean	3.4	1.4	16.7	5.8	16.7	5.8	18.1	0.5	9.7
	Min	2.2	0.7	10.0	0.0	10.0	0.0	17.7	2.9	9.2
	Max	4.9	2.1	20.0	11.0	20.0	11.0	18.7	3.6	10.3
T6	Mean	3.0	0.9	13.3	5.8	13.3	5.8	17.8	2.7	9.3
	Min	1.9	0.8	10.0	0.0	10.0	0.0	14.7	1.2	5.9
	Max	3.5	3.4	20.0	11.0	20.0	11.0	19.7	4.0	11.4
T7	Mean	3.6	1.9	23.3	5.8	23.3	5.8	17.0	0.6	8.5
	Min	2.3	1.4	20.0	0.0	20.0	0.0	16.3	1.5	7.7
	Max	5.8	3.2	30.0	11.0	30.0	11.0	17.3	3.2	8.8

The ecotoxicological assays were tested in triplicate per each mesocosm and reported as the mean (SD) for MWool® type tested at each exposure time. The values of the negative control of the assay (seawater) and the negative control of the experiment (exposure water) were also given as a reference for each exposure time (T0–T7). The dyed contact water (MW) allowed for the evaluation the ecotoxicity of the pigments released by the degradation processes.

- MW. The lowest percent change was observed in the species *P. tricornutum* at time point T3 (I% 2.7 ± 1.2), while the highest change occurred in *B. plicatilis* at time point T7 (30% ± 0 after 24 and 48 h). In *P. lividus*, the greatest change was observed at time T1 (Δ% from T0 16.1%), while the least change occurred at time T7 (Δ% from T6 of +2.5%).

- MWc. In *P. lividus*, the greatest change was observed at time T1 (Δ% from T0 of 180.3%), and the least at time T7 (Δ% 131.8). In *P. tricornutum*, the greatest change was observed at time point T7 (Δ% 175.9), and the least at time point T2 (Δ% 166.9). In *B. plicatilis*, the greatest change was observed at time point T7 (Δ% 318.2), and the least at time point T1 (Δ% 0.0).

4. Discussion

4.1. General Features

Wool is an organic molecule of biological origin; its chemical composition is variable but made up of approximately 97% proteins, 2% structural lipids (40% sterols, such as cholesterol and demosterol, 30% polar lipids, such as cholesterol sulphate, and ceramides, glycosphingolipids, and 25% fatty acids, such as stearic, palmitic, oleic, and 18-methylicosanoic acid), and 1% from other substances (mineral salts, nucleic acids, and carbohydrates). The elemental composition of wool is approximately 50% carbon, 22–25% oxygen, 16–17% nitrogen, 7% hydrogen, and 3–4% sulphur [18,19]. Natural proteins are divided into fibrous proteins and globular proteins, among which the former (α- and β-keratin, collagen, and elastin) are insoluble in water and form the basis of the structure of various types of animal tissues, while the latter, soluble in water, are found in the structure of enzymes and other functional proteins, such as albumins. Wool proteins are all α-keratins, which are classified according to their amino acid composition [18,19]. The wool structure is complex showing a stratified cuticle (epicuticle, exocuticle, and endocuticle) and a cortex (orthocortex, paracortex, macrofibrils, and microfibrils) [18]. Protein structure is made by high-sulphur proteins, high-glycine–tyrosine protein, and several families of low-sulphur proteins; microfibrils, are made largely by low-sulphur proteins [18].

4.2. Degradation of Wool in Marine Environment

Seawater is generally basic, with a pH of 8.05–8.16, although progressive acidification of ocean pH is expected to reach 7.76 in response to global change with the increase of CO_2 emissions [20]. Oxygen conditions in seawater vary widely, according to water depth (e.g., high oxygen levels in the intertidal zone and hypoxic levels in the deep ocean) and geographical location (e.g., >5 mL/L in Northern Atlantic Ocean and <1 mL/L in deep water) [21]. Concerning decomposition, wool is considered a heat-resistant material; the high content of proteins consisting of S-S ligands is difficult for bacteria to degrade [18–22]. Wool can be dissolved in water; therefore, a chemical reaction with moist heat results in complete dissolution of the wool fibre by hydrolysis, and it is more resistant at a pH between 5 and 7. Structural changes begin with the breakdown of cysteine bridges and lead to progressive structural loss; these changes occur more readily in a basic environment [22]. In well-controlled aquatic biodegradation experiments, cotton and rayon microfibers resulted to degrade under aerobic conditions, whereas polyester microfibers are considered persist in the environment for long periods of time [8]. Cellulosic textile materials resulted in being less resistant than non-cellulosic ones (such wool) to biological degradation by microorganisms due to its molecular structure and surface [10] also found in soils.

Our results on wool supported the biodegradation of this type of textile in marine water environments, even if phenomena occurred slowly and required longer time laps. As described above, degradation of wool in the marine environment is a progressive and slow process with sequential and incremental phases. In contact waters, a decrease in pH is observed after exposure to wool, which could be related to the release of molecules with acidic action into the solution. This phenomenon, which in an open marine environment would certainly be buffered by the carbonate–bicarbonate equilibrium [23] and, therefore, would not be detectable, is not compensated in the closed experimental system and likely allows us to identify early stages of structural degradation of the natural proteins that make up the wool fibres.

The results of this study show that the pigments from the samples of MW are released upon contact with water starting at T1, resulting in light pink/red colored water. In the

marine ecosystem, fibres from MWool® fabrics are released starting from T3 (MW) and T5 (MWc), with no significant differences between MW and MWc. This effect could be due to a higher content of particles from air (i.e., dust, pollen, etc.) that could be accumulated in MW wool, which is significantly lower in carded MWc types. The fibres released from the degradation process of wool became significant starting from T5. The analyses performed with µFT-IR showed a decrease in the mean spectral match between T0 and T7 that resulted within 12.6% (MW) and 20.0% (MWc). Exposure to seawater resulted in increased formation of micropores in the wool fibre, as evidenced by ultrastructural analyses, fibre breakage, flake loss, and the occurrence of swelling [24]. This occurrence is independent of the spatial arrangement of MWool® (MW and MWc are similar) but is related to the duration of seawater exposure. The degradation processes significantly lowered the pH of the water at T1 and T2, resulting in acidification of the MW and MWc types. In contrast, acidification was lower from T3 to T7, when the measured pH values were close to 8.00 and similar to those of seawater, indicating that the early acidification process lost intensity during the exposure time.

The release of fibres was related to the exposure time and showed that the microfibers released (length between 35–3932 µm; modal value 300–500 µm) were consistent with the values reported in the literature for the mechanical stress of fibres during washing of textiles, that resulted in a mean value of 360–660 µm [4]. This dimensional range represents the largest amount of microfiber released from the exposed wool weight upon contact with water, highlighting the need for more in-depth studies focused on the behaviour of this specific fibre dimension in aquatic ecosystems, as they are representative. The same research report that type of fibres constituting the yarns and their twist, influenced the release of microfibres during washing, and this occurrence could explain some differences reported between MW and MWc concerning fibres released during the exposure time. A recent study has shown that fiber cohesion and the presence of shorter and irregular fibers in the textile mass, characterized by lower strength, lead to higher release [6], which could be enhanced by decomposition processes and breaks in the ultrastructure of wool during the exposure period, leading to an exponential release of microparticles in combination with wool treatments in the case of MW, as shown in Figure 6.

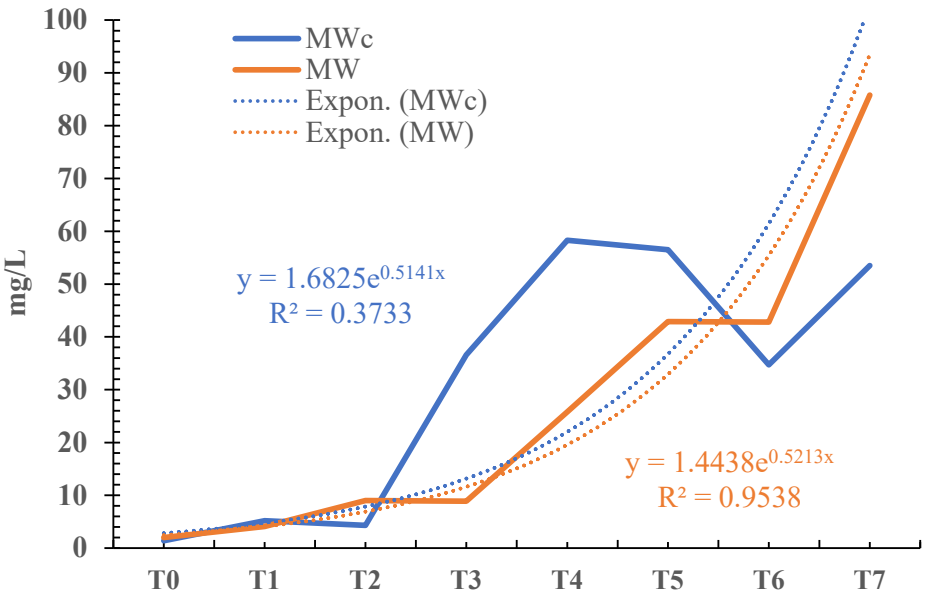

Figure 6. Mathematical model of release of microparticles from tested materials during the exposure time. TSS data are expressed as mg/L of microfibres released. Correlations among the model and recorded data are reported with equations as R^2 values. Orange line represents MW wool, while blue line represents MWc wool. Equations are reported respectively in blue (MWc) and orange (MW) colours.

The recent literature showed that textile design could affect fibre releases from textiles into the environment to both air and water [9], supporting the recorded differences in our study among MW (exponential) and MWc (early exponential T0–T3, polynomial T4–T7) behaviour concerning fibre releases during the exposure time.

Recent research performed on MWool® carbon footprint showed that 1 kg of this type of recycled fabric has a carbon footprint of 0.1–0.9 kg CO_2 eq., while producing virgin fibers determines a release of 10–10^3 kg CO_2 eq. [11], reducing impacts affecting climate change. Furthermore, our results showed that biodegradation, even if it is a very slow process, started in marine ecosystem within the experimental time lap considered in this study.

4.3. Ecotoxicological Effects

Ecotoxicological tests represent an important approach to obtain integrated responses to the effects of complex and unknown mixtures of chemicals released in environmental matrices on representative species of the marine trophic web [16]. The tested species showed that biodegradation of the tested wool textiles by marine microorganisms generates by-products and release of pigments in seawater, which are not capable of having significant effects on marine trophic webs.

The observed ecotoxicological effects are below the EC_{50} value at each exposure time tested, both at MW and MWc. Interestingly, even pigmented water associated with MW tissue did not show measurable EC_{50} effects at any time of contact. Early developmental stages of *P. lividus* are sensitive and representative impacts on marine ecosystems [25–27].

Ecotoxicological results showed that the degradation process of tested MWool® samples under the experimental exposure condition does not significantly affect tested species, owing to different marine trophic webs. This result means that also under experimental conditions that are conservative for the environment due to the high concentration of wool per liter of water and exposed without water dilution, chemicals released from tested wools are ineffective for tested species.

4.4. Pro and Cons and Future Developments

The mesocosm approach provides solid and statistically significant data under controlled laboratory conditions. This is both a strength and a weakness of this type of study. Under controlled conditions, we were able to demonstrate the occurrence of biodegradation processes that show that degradation of natural wool microfibers can occur in marine ecosystems. However, standardisation of mesocosm studies require defining some variables and fixing them to exclude interferences. This led to a loss of realism since natural ecosystems are much more complex and variable than mesocosms. For example, under controlled laboratory conditions, the light cycle and light exposure are standardised and fixed—UV radiation can affect the degradation of wool fibers—and in natural environments, both latitude and water depth can vary significantly with respect to the effects of UV exposure. Although the much more realistic conditions could be represented by an in situ test, we are persuaded that this approach is unethical with respect to the specific objective of this study because it could result in the dispersal of a potentially hazardous substance (tested samples) at high concentrations into open ecosystems, leading to possible unpredictable effects; moreover, the mesocosm approach represents a better cost–benefit ratio to achieve the specific objectives of this research. The strength of this study is that the ecotoxicity of chemicals released during the degradation process could be monitored and tested on a selection of standard species that can be considered representative of the different trophic levels of marine ecosystems. However, trophic webs are much more complex than the three levels tested, and further studies will be improved to evaluate much more variable and variability to better represent the effects in an open marine ecosystem. Nevertheless, the results of this study are encouraging and support a degradation process of the tested materials in marine ecosystems.

5. Conclusions

The decomposition of MWool® (MW) and MWool® carded (MWc) products in aquatic environments is a slow process due to both the properties of the wool's structural protein and the presence of specific physicochemical properties of the marine environment, which result in slow yet occurring decomposition rates. The decomposition process of the tested wool begins with the loss of colour and swelling of the microfibers. The results showed that the tested samples did not exhibit relevant ecotoxicological effects during the exposure period on the species (*Phaeodactylum tricornutum*, *Brachionus plicatilis*, and *Paracentrotus lividus*) tested in this study.

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