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Eco-Friendly Detergent Based on Exhausted Edible Vegetable Oils: Impact on Marine and Freshwater Environments, a Case Study Focusing on SARS-CoV-2

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Abstract: On 30 January 2020, the World Health Organization declared a public health emergency of international concern due to the rapid spread among humans, on a global scale, of SARS-CoV-2, the virus responsible for COVID-19. Although international authorities have recommended the use of common detergents known to be effective against coronaviruses, one of the practices implemented to control the expansion of the virus has been the massive use of disinfectants on indoor and outdoor surfaces, a modality that has raised concern in the scientific community because of its impact on the aquatic environment. Considering possible future scenarios related to ongoing global change, in which further public health emergencies may become more frequent, and given the need to contribute to the identification of *eco-friendly* alternatives or strategies to mitigate the environmental and human health impacts of the massive use of disinfectants, the aim of this study was to quantify the effects of a liquid surface detergent based on exhausted edible oils of vegetable origin (*eco*-product). This was done by exposing organisms representing the main trophic levels of the marine and freshwater environment to the *eco*-detergent before and after a five-day biodegradation process, together with studies on biological oxygen demand and microbiology. The results indicated that the *eco*-product has potential antimicrobial activity and can be considered as a suitable alternative, although the use of a standardized agent for the production phase of the *eco*-product in liquid form is recommended to further reduce the impact on the aquatic environment. However, massive and indiscriminate use is a behavior to be discouraged, and limited and restricted use to appropriate areas and contexts is recommended.

Keywords: COVID-19; disinfectants; detergents; *eco-friendly* products; ecotoxicity; BOD_5; microbiology

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1. Introduction

In December 2019, outbreaks of pneumonia caused by SARS-CoV-2 (severe acute respiratory syndrome by coronavirus-2), the pathogen responsible for the disease named COVID-19 (coronavirus disease 2019), were recorded in Wuhan, a city in China's Hubei province [1–3]. Within a short time, the virus, which was distinguished by morbidity and mortality [4], spread among humans on a global scale. On 30 January 2020, the World Health Organization (WHO) declared a state of public health emergency of international concern, and on 11 March 2020, the COVID-19 epidemic was classified as a pandemic [5]. SARS-CoV-2 can be transmitted by direct contact with respiratory droplets, via aerosols, or infected surfaces; its survival may vary depending on the medium, surface, and environmental characteristics [6]. As an enveloped virus, it is more susceptible to standard disinfection methods such as alcohol solutions, chlorine products, and hydrogen peroxide [7].

The sense of panic and worry that characterized this period (often fuelled by misinformation, also spread by social media) has contributed to the extreme and inappropriate use

of disinfectants, raising concerns in the scientific community about the consequences for human health and the environment [8]. During the first three months of the public health emergency, *American's Poison Centres* recorded an increase in reports of exposure events to detergents and disinfectants [9]. In various parts of the world, intensive disinfection procedures were also carried out in urban public areas, spraying with commonly used disinfectants (chlorine-releasing agents, oxidizing agents, or quaternary ammonium cations), which are known to have toxic effects on terrestrial and aquatic organisms in both the short and long term [10].

China's Centre for Disease Control and Prevention (CCDC) has highlighted the risk of environmental pollution linked to repeated spraying of disinfectants on roads. Moreover, extensive chemical disinfection is almost useless in preventing the spread of COVID-19, given the low certainty of virus survival on surfaces such as pavement [11] The WHO has advised against outdoor spraying as a method of controlling the transmission of any pathogens, both because of the environmental and human health implications, and because the use of chemical disinfectants on uncleaned surfaces can lead to inactivation of the biocidal efficacy due to the presence of organic matter, dirt, or debris [7].

In both India [12] and China, chlorine solutions were the most widely used [13,14], with an estimated consumption of at least 2000 tons in the city of Wuhan alone [12]. Although chlorine has been recognized as a powerful disinfectant since the first half of the 19th century [15] due to its effectiveness in controlling waterborne diseases and its low cost [16], it is also recognized that prolonged exposure and intensive use in the environment can have significant consequences for human health and the ecosystem [17]. Chlorine solutions pose a threat to aquatic organisms by catalyzing the oxidation of proteins or the destruction of cell walls and, secondarily, by reacting with dissolved organic matter at the water surface to form a series of toxic by-products characterized by their ability to persist. Though disinfection practices have been implemented to control the virus, the massive use of disinfectants risks harming the world's aquatic ecosystems as their use to treat a wide range of outdoor and indoor surfaces results in their discharge into natural aquatic systems through direct or indirect wastewater [18].

Indeed, in order to mitigate the environmental impact of the massive use of biocides during the SARS-CoV-2 era, international authorities have recommended the use of common detergents, as these products are already known to be effective against coronaviruses. They are defined in Regulation (EC) 648/2004 as substances or mixtures with cleaning or sanitizing properties containing soaps and/or surfactants [11,19,20], which are amphiphilic substances with emulsifying properties that reduce surface tension at the interface between polar and non-polar matrices (e.g., water and oil). Applied to a surface, their hydrophobic domain interacts with the microbe's hydrophobic component, encapsulating it in micelles, while the hydrophilic heads of the surfactant bind to water, solubilizing it. These properties make detergents a viable alternative to known methods, as they can act on the outer lipid structure of the coronavirus, inactivating it [17]; however, when choosing a detergent, its chemical nature must be considered, as a product made from petroleum-derived raw materials can be a source of pollution, while the natural and biological origin of the ingredients (plant or microbiological) and biodegradability can further reduce harmful effects on the environment and human health [21].

COVID-19 is just one of the major epidemics of the 21st century. Global changes such as the climate crisis, demographic increase, urbanization, land use, and the intensification of trade in animals and plants may increase the risk of infectious diseases, and emergencies are likely to become more frequent [4].

In light of what has been discussed so far and given the need to contribute to the identification of *eco-friendly* alternatives or strategies to mitigate the environmental and human health impacts resulting from the massive use of disinfectants [6,12,18,22,23], the aim of this study was to quantify the effects of a liquid surface detergent based on exhausted edible oils of vegetable origin in toxicity tests, performed on representatives of the main trophic levels

of marine and freshwater environments before and after a five-day biodegradation process, in conjunction with Biological Oxygen Demand (BOD) and microbiological studies.

2. Materials and Methods

2.1. Tested Product

In order to help identify environmentally friendly alternatives, a liquid product under scientific investigation at the Bioscience Research Centre https://www.bsrc.it/ (accessed on 28 October 2024) was tested. The *eco*-product (which is given a generic name) was provided by the manufacturer (mentioned in the acknowledgments) with an information sheet and classified as a natural degreaser with detergent properties. The composition/information section on ingredients reports as follows:

"The product does not contain substances classified as hazardous to health or the environment according to the provisions of Regulation (EC) 1272/2008 (CLP) (as amended and adapted) in quantities that require declaration".

The *eco*-product was obtained from a saponification process of exhausted edible oils of vegetable origin collected from local communities in the Karst area of Trieste province (Friuli Venezia Giulia, Italy). The resulting solid soap was dissolved in tap water to obtain the liquid detergent product (*eco*).

For the purpose of this study, the pH of the *eco*-product was adjusted, if necessary, with 1 M HCl to ensure an optimal range for the survival of the organisms tested in the ecotoxicological assays and to exclude any effects due to this parameter.

2.2. Ecotoxicological Tests

Ecotoxicological tests (Table 1) were performed using two standardized batteries to test the toxicity of the *eco*-product in both marine and freshwater environments.

Table 1. The table reports the species tested, the methodology used, the endpoint measured, the unit in which it is expressed, and the toxicity (acute or chronic) for each reference environment (freshwater or marine).

System	**Marine and Freshwater**	**Marine**		**Freshwater**		
Species	*Aliivibrio fischeri*	*Phaeodactylum tricornutum*	*Paracentrotus lividus*	*Pseudokirchneriella subcapitata*	*Daphnia magna*	*Saccharomyces cerevisiae*
Method	UNI EN ISO 11348-1:2019 [24]	UNI EN ISO 10253:2017 [25]	Chapman et al. 1995; ISPRA 11/2017 [26,27]	UNI EN ISO 8692:2012 [28]	UNI EN ISO 6341:2013 [29]	non-standardized method
Endpoint	bioluminescence inhibition 5, 15 and 30 min	growth inhibition 72 h	abnormal larvae 72 h	growth inhibition 72 h	Immobility 24 and 48 h	growth rate and growth inibition 24 h
Unit	%	%	%	%	%	µ and I%
Toxicity	acute toxicity test	chronic toxicity test	acute toxicity test	chronic toxicity test	acute toxicity test	–

The battery representing the marine environment consists of *Aliivibrio fischeri* (method UNI EN ISO 11348-1:2019 [24]; acute toxicity test, endpoint: inhibition of bioluminescence), *Phaeodactylum tricornutum* (method UNI EN ISO 10253:2017 [25]; endpoint: growth inhibition), and *Paracentrotus lividus* (Chapman et al. 1995 [26] + ISPRA 11/2017 [27]; endpoint: larval development).

The freshwater battery is composed of *Aliivibrio fischeri* (method UNI EN ISO 11348-1:2019 [24]; acute toxicity test, endpoint: inhibition of bioluminescence), *Pseudokirchneriella subcapitata* (method UNI EN ISO 8692:2012 [28]; chronic toxicity test, endpoint: growth

inhibition), and *Daphnia magna* (method UNI EN ISO 6341:2013 [29]; acute toxicity test, endpoint: immobility). The choice of battery type is because they are standardized and regulated for the calculation of the ecotoxicological hazard index.

The ecotoxicity test was also performed on *Saccharomyces cerevisiae*, the yeast representative of eukaryotic organisms, widely used in ecotoxicological studies and not subject to standardized procedures. The experiment was carried out both to obtain useful data on the response of the yeast to the tested substance and to obtain further information that could contribute to the implementation of a standardized method, considering the advantages (costs, storage methods, etc.) that characterize these organisms [30]. The experiment was performed according to the procedures published by the *Foundation Institute of Molecular Oncology ETS* [31] and the knowledge available in the scientific literature; the endpoint measured is growth rate and growth inhibition.

2.2.1. Test on Bacteria

Aliivibrio fischeri is a Gram-negative marine bacterium, rod-shaped, flagellated, non-pathogenic, and widely used in ecotoxicological studies of marine and freshwater ecosystems [32]. It is characterized by a natural bioluminescence that is directly proportional to the metabolic activity of the bacterial population. Bioluminescence decreases as a result of the decrease in enzyme activity due to toxicity, of which it provides a direct measure [33]. Freshly prepared bacteria (prepared from commercial batch number 19A4002A; Ecotox LDS, Milano, Italy) were used in the tests, and the percentage inhibition of bioluminescence (detected with a luminometer at 490 nm; Microtox, Ecotox LDS, Milano, Italy) was measured at the end of 5-, 15-, and 30-min exposure, at 15 ± 1 °C, up to a maximum of 90% of test substance concentration, in duplicate. The positive control was set up with 4.5 mg/L 3,5-dichlorophenol (3,5-DF; Sigma Aldrich, St. Louis, MO, USA, No. LRAC5200); the negative control for marine and freshwater environments consists of artificial seawater (ASW).

2.2.2. Test on Algae

The biological response of *Pseudokirchneriella subcapitata* and *Phaeodactylum tricornutum*, representatives of freshwater and marine primary producers, respectively, was measured as percent growth inhibition after 72 h of incubation, in triplicate. *P. subcapitata* (commercial batch number SC110918; Ecotox LDS, Milano, Italy)) was incubated at 23 ± 2 °C, while *P. tricornutum* (commercial batch number PT070219; Ecotox LDS, Milano, Italy) was incubated at 20 ± 2 °C, both under continuous illumination conditions (10,000 lux for sideway illumination). Percent growth inhibition was obtained from cell density, measured by spectrophotometer at 670 nm (Beckman Coulter DU730, Brea, CA, USA). To calibrate the spectrophotometric reading, a curve was generated in which cell density (obtained by counting algal cells in the standard Burker chamber) and absorbance are in direct proportion. For both organisms, the positive control was prepared with potassium dichromate ($K_2Cr_2O_7$; Cas NO.: 7778-50-9, Sigma Aldrich, St. Louis, MO, USA) and the negative control with an algal culture medium.

2.2.3. Test on Crustaceans

The biological response of primary consumers of freshwater environment was tested on *Daphnia magna* (Cladocera) using dormant eggs (ephippia) purchased from Ecotox LDS (Milano, Italy). After an incubation period of three days (maximum 90 h) at 21 ± 1 °C and 6000 lux, the neonates were pre-fed after hatching, transferred to test plates, and incubated at 20 ± 2 °C under a photoperiod of 16 h light and 8 h dark. The biological response was measured at 24 and 48 h and expressed as a percentage effect; neonates unable to swim within 15 s of slight agitation of the liquid, despite antennae movement, were considered immobile. Determination of immobile organisms was performed using a dissecting microscope (Optika, Ponteranica, Italy); the positive control was set up with $K_2Cr_2O_7$ and the negative control with AFW.

Regarding the freshwater environment, *D. magna* was tested in a further assay in which, following the same standardized procedures as described above, neonates were exposed not only to the product *as it is* (*eco*) but also to the *eco*-product prepared using standard AFW (*eco* AFW). The aim was to verify, the possible differences (in terms of biological response) resulting from the type of water used to prepare the liquid detergent; indeed, tap water may be subjected to various treatments (disinfection, sedimentation, etc.) that may affect its characteristics [34].

2.2.4. Test on Echinoderms

The biological response of primary consumers of the marine environment was investigated on the sea urchin *Paracentrotus lividus*, collected from the sea and raised in the laboratory. The test consists of quantifying the number of norm-formed pluteuses after exposure of zygotes (n = 100/mL) to the test substance for 72 h, at 18 ± 1 °C. The zygotes used in the assay were obtained by combining a sperm suspension with an oocyte suspension in a 10:1 ratio. After checking for fertilization, the solution containing zygotes and the test substance was incubated. After 72 h, the samples were fixed with 3 drops of buffered formaldehyde, and the number of normally developed larvae at the pluteus stage was determined under the microscope (objective 4× to 10×). Developmental anomalies reported in the reference manuals, such as undeveloped larvae, missing or different arm lengths, or body asymmetries, were considered. The positive control was set up with $Cu(NO_3)_2$ * $3H_2O$, and the negative control with filtered seawater (FSW).

2.2.5. Test on Yeasts (Non-Standardized Procedure)

The test was carried out on *Saccharomyces cerevisiae* in fresh format (UNIFERM—GmbH & CO. KG; Werne, Germania, https://uniferm.de/en/, accessed on 28 October 2024), sold for home use in 42 g cubes. To start the experiment with actively growing cells, a stock solution consisting of 0.5 g of yeast in 50 mL of culture medium (AFW + 20 g/L $C_6H_{12}O_6$) was prepared and incubated for 4 h in darkness, at 30 ± 0.5 °C.

The experiment was set up in 3 mL cuvettes (three replicates, including controls) consisting of 2930 µL of test substance diluted in culture medium (*eco*-product + AFW + $C_6H_{12}O_6$ 20 g/L) and 70 µL of yeast in culture medium. The solution level was marked at *T0* so that it could be topped up at the end of the incubation (*T24*). The following concentrations were tested: 100.0,10.0,1.0,0.1 mg/L. The pH and T °C were measured at *T0*.

The cuvettes were incubated at 30 ± 0.5 °C, in darkness, without caps [35].

The negative control was set up with AFW + $C_6H_{12}O_6$ (20 g/L) while the positive control was set up with a saturated salt solution (NaCl + AFW), as *S. cerevisiae* dies under extreme salt conditions [36]. The biological response to the tested substance was measured as growth rate (µ) and percentage of growth inhibition (I%), after 24 h, as follows:

$$\mu = \frac{\left[\ln\left(\frac{\text{cell number}}{\text{mL}}\right)_{T24} - \ln\left(\frac{\text{cell number}}{\text{mL}}\right)_{T0}\right]}{1}$$

$$I\% = \left(\frac{\mu_c - \mu_t}{\mu_c}\right) \times 100$$

where: μ = growth rate; μ_c = control growth rate; μ_t = treatment growth rate; and $I\%$ = inhibition rate (referring to ISO Standard 8692:2012 [28]). These values were obtained from the cell density (which was determined from the absorbance value at 600 nm measured with a spectrophotometer [37]), from which the blank value was subtracted. To calibrate the spectrophotometric reading, a curve was generated establishing the direct proportionality between cell density (obtained from Burker chamber counts) and absorbance based on five serial dilutions, with a decreasing dilution factor of 1:1. To determine the dilution factor, the cell density at the Burker chamber was checked to avoid high densities that would have made the counting process more difficult for the operator and affected

the final result. The volume of the yeast inoculum (70 µL) was determined considering the limits defined by the interpolation line.

2.3. Biological Oxygen Demand (BOD) and Ecotoxicity of Biodegradation Products

The ecotoxicological study of the *eco*-product was combined with an important indicator widely used for the study of organic water pollution, the Biological Oxygen Demand (BOD) [38]. The BOD (mg/L) corresponds to the amount of oxygen consumed by an inoculum of organisms in a sample at a temperature of 20 ± 0.5 °C (under dark conditions) [39]. It is used to estimate the degree of biodegradability of a substance [40,41], defined as the degradation or mineralization of organic matter by microbial and/or fungal activity [42]. However, *biodegradable* is not always synonymous with non-polluting, and a biodegradable substance may still cause damage to the ecosystem [43]. In fact, the ecotoxicological profile and environmental behavior of by-products resulting from biodegradation may differ from that of the parent product and may even be more toxic and persistent [44]. For this reason, the ecotoxicity tests were carried out both with the substance *as it is* (*eco*/eco AFW) and with the resulting liquid matrix after a five-day biodegradation process (*eco* BOD_5/*eco* AFW BOD_5), previously filtered to remove the bacterial component. Each organism was exposed to the *eco* and *eco* BOD_5 treatments in the same way as described in the Section 2.2, except for *S. cerevisiae*, which was exposed to the *eco* treatment exclusively. Only *D. magna* was exposed to the *eco*-product dissolved in AFW (*eco* AFW) and subjected to the biodegradation process (*eco* AFW BOD_5). A summary table of the organisms used, and their treatments can be found in the Supplementary Materials (Table S1).

The test volumes consisted of 360.0 mL of sample (at a concentration 10% higher than that to be tested to avoid the dilution effect at the time of bacterial inoculation) and 40.0 mL of bacterial inoculum obtained by filtering fresh and salt water, 1.0 g KOH.

The tests were performed in duplicate at detergent concentrations of 100.0 mg/L and 50.0 mg/L for both the freshwater (control+: glucose added to water; control−: natural filtered freshwater) and the marine system (control+: glucose added to water; control−: natural filtered seawater).

2.4. Microbiology (Total Bacterial Count, TBC)

The microbiological tests were carried out in order to verify the efficacy of the detergent on bacteria by counting the number of colonies (N./mL) after incubation in aerobic conditions (on culture medium) at 30 °C [45], 22 °C, and 37 °C [46] both before (−*eco*) and after (+*eco*) treatment with the *eco*-product, in two replicates.

2.5. Hazard Assessment

The hazard assessment was performed on standardized batteries (except *S. cerevisiae*) exposed to the *eco*-product (*eco*) and to the *eco*-product after five days of biodegradation (*eco* BOD_5), also incorporating the test carried out on *D. magna* with *eco*-product dissolved in AFW and *eco* AFW BOD_5.

2.5.1. Hazard Assessment in the Marine Environment

The Sediqualsoft 109.0® software (version 2.0) was used to estimate the ecotoxicological hazard in the marine environment. After guided data entry, the software returns information on the battery, providing the Hazard Quotient (HQ) and severity class of the ecotoxicological hazard. Hazard estimation was performed based on the worst-case scenario (100.0 mg/L of the *eco*-product).

2.5.2. Hazard Assessment in the Freshwater Environment

The procedure followed for calculating the hazard level refers to ISPRA 2013 [47]; the ecotoxicological hazard index is reported on a scale of 0–1 and is defined by the integrated index (TBI).

The results of each test have been indexed according to integration criteria that take into account the following variables and their relative weights:

- *E%:* effect percentage
- *FCS:* statistical correction factor
- *M*: ecological relevance of the examined matrix type
- *S*: severity of effect
- *O*: ecological representativeness of the organism used

A score (Ps_j) was calculated for each test and was determined on the basis of the weight and the score of the individual factors (*FCS*, *M*, *O*, *S*):

$$Ps_j = (FCS \times M \times O \times S)_j$$

The result of the individual assay (Ep_j) was therefore pondered:

$$Ep_j = (E\%)_j \times Ps_j$$

The total score of the assays making up the battery (*Eb*) is the sum of the individual scores (Ep_j) obtained for each organism:

$$Eb = \sum_{j=1}^{N} Ep_j$$

Eb values have been normalized to a relative scale between 0 and 1 by means of a normalization factor (*Fn*):

$$Fn = \frac{1}{100 \times \sum_{j=1}^{N} Ps_j}$$

The integrated toxicity battery index *(TBI)* is given by:

$$TBI = Eb \times Fn = \frac{\sum_{j=1}^{N} Ep_j}{100 \times \sum_{j=1}^{N} Ps_j}$$

TBI values are divided into four groups corresponding to four ecotoxicological hazard classes (Table 2). Hazard estimation was performed based on the worst-case scenario (100.0 mg/L of the *eco*-product).

Table 2. Ecotoxicological hazard scale defined based on the integrated index (TBI) compared to a scale of 0–1.

TBI	**Hazard**
$TBI \leq 0.1$	Absent/Negligible
$0.1 < TBI \leq 0.30$	Moderate
$0.3 < TBI \leq 0.5$	High
$TBI > 0.5$	Heavy

2.6. Quality Assurance

The experiments were carried out by the authors in an accredited laboratory UNI CEI EN ISO/IEC 17025:2018 (LAB n. 1715L). The test on *S. cerevisiae* was performed in the Ecotoxicology Laboratory of the Department of Life Sciences at the University of Trieste.

2.7. Statistical Analysis

2.7.1. Standardized Organisms

The biological response of the standardized organisms tested was analyzed using the *Toxicity Relationship Analysis Program software* (TRAP version 1.3a [48]), with the aim of estimating EC_{50} values (or EC_x) where possible. All data collected from the experiments

were recorded in a Microsoft Excel (Version 16.90) spreadsheet, analyzed, and reported in the form of graphs and tables, in terms of mean and standard deviation. The biological responses of organisms treated with the *eco*-product (*eco*), or *eco*-product dissolved in AFW (*eco* AFW), and the resulting matrix at the end of the biodegradation process (*eco* BOD_5/*eco* AFW BOD_5) were compared using Student's *t*-test to check for statistically significant differences between treatments (at the same concentration).

2.7.2. *S. cerevisiae*

The biological response of *S. cerevisiae* (non-standardized organism) was analyzed using the *IC50 calculator AAT Bioquest* [49] with the aim of estimating the EC_{50} where possible. The data obtained from the experiment were recorded on a Microsoft Excel (Version 16.90) spreadsheet for calculations and graphing.

A one-sample *t*-test was performed using R Studio software (R version 4.4.0 (24 April 2024)—'Puppy Cup') to check the difference between the growth rate (μ) of the samples (for each concentration) and that of the negative control (CNTR−), the latter being considered as the reference value. All results were expressed as mean and standard deviation.

3. Results

3.1. Ecotoxicological Assays (Standardized Batteries)

A summary table of the results obtained from standardized tests for each treatment (*eco*/*eco* BOD_5) is given in the Supplementary Materials (Table S1: species studied, controls, concentration [mg/L], mean biological response detected ± SD (%), EC_x [mg/L]).

3.1.1. Marine System

The effects of *eco* and *eco* BOD_5 in the marine environment were studied using a standardized multi-species battery consisting of representatives of the main trophic levels: *A. fischeri*, *P. tricornutum,* and *P. lividus*. The biological response (%) as a function of the concentration tested (mg/L) is plotted in Figure 1. In the case of *A. fischeri*, the response reported in the results refers to the maximum exposure time of 30 min; bioluminescence inhibition was observed at all concentrations tested, with statistically significant differences between the *eco* and *eco* BOD_5 treatments at 0.9 mg/L (*p*-value: 0.002/**) and 9.0 mg/L (*p*-value 0.001/***). In *P. tricornutum*, growth inhibition was observed at all concentrations in the *eco* treatment, while biostimulation was observed in the *eco* BOD_5 treatment at the lowest concentration tested, 6.25 mg/L, with a statistically significant difference to the *eco* treatment (*p*-value 0.0001/***). For *P. lividus*, the mean percentage of abnormal larvae is significantly different between *eco* and *eco* BOD_5 treatments (12.5 mg/L, *p*-value: 0.001/***; 25.0 mg/L, *p*-value: 0.040/*; 50.0 mg/L, *p*-value: 0.03/*), except at 100.0 mg/L, the highest concentration tested, where the biological response is comparable.

The EC_x values are reported in Figure 2/Marine system group; for none of the organisms in the battery, in both treatments (*eco* and *eco* BOD_5), an EC_{50} value within 100.0 mg/L was estimated (EC_{50} > 100.0 mg/L = substance not hazardous to aquatic organisms; Grabarczyk et al., 2020 [50] with reference to Directive EC93/67/EEC). The hazard assessment measured for both treatments (*eco* and *eco* BOD_5), at 100.0 mg/L, indicates a low level (HQ_{eco} = 1.48 and $HQ_{eco\ BOD5}$ = 1.38), where HQ is the Hazard Quotient assigned by the software to 1 of 5 hazard classes (absent, low, medium, high and very high).

The BOD_5 of the *eco*-product quantified after five days is given in Table 3/Marine system/*eco*. In the marine environment, at 100.0 mg/L, the *eco*-product assumes the highest BOD_5 values (42.30 mg/L) compared to the (much lower) values recorded for the freshwater system at the same concentration (BOD_5 *eco* = 9.55 mg/L; BOD5 *eco* AFW = 2.85 mg/L). At 50.0 mg/L, BOD_5 is 0.00 mg/L. All values are expressed in terms of mean and standard deviation.

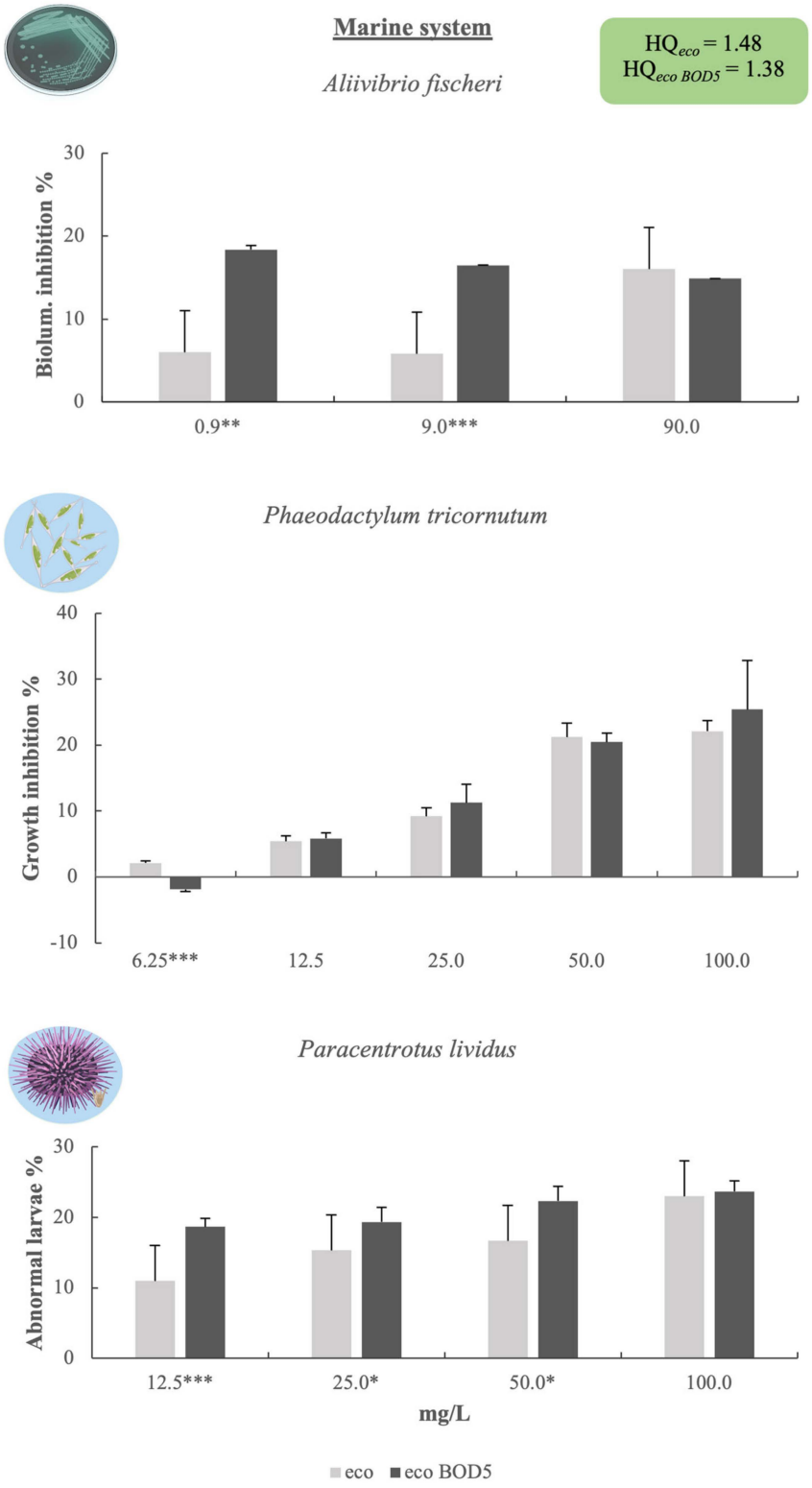

Figure 1. Biological response (%) measured as a function of tested concentrations (mg/L) of *eco* and *eco* BOD_5 for each battery organism representing the marine system. Significant differences detected between treatments (and the level of significance) are asterisked. *A. fischeri* is considered both a marine and freshwater indicator. The green box indicates the estimated Hazard Quotient (HQ_x) values for both treatments (*eco*/*eco* BOD_5), at 100.0 mg/L, corresponding to a low level of hazard for the marine system.

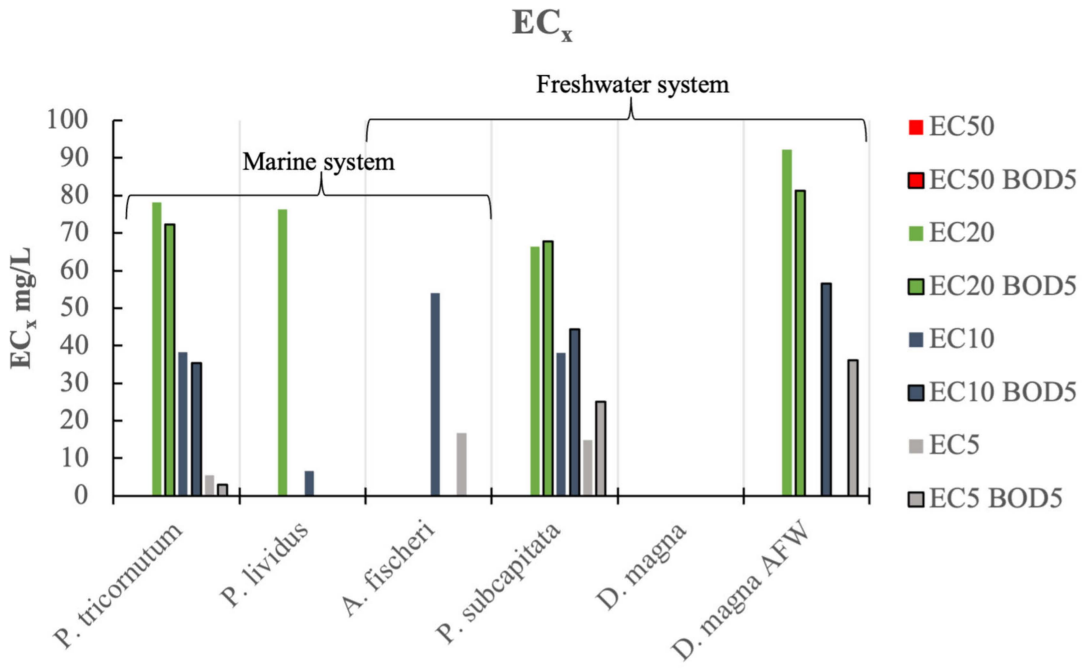

Figure 2. EC_x values (mg/L) calculated for each multi-species marine and freshwater battery exposed to the *eco*/*eco*-product BOD_5; *D. magna* was exposed to both the *eco*-product prepared with tap water (100% immobility and no detectable EC_x value) and the *eco*-product prepared with standard artificial freshwater (*D. magna* AFW).

Table 3. The table reports the biological oxygen demand measured after five days of incubation (BOD_5 mg/L) expressed as mean and standard deviation (±SD) at a concentration of 100.0 mg/L and 50.0 mg/L in both environments studied (marine and freshwater system), considering the *eco*-product prepared with tap water (*eco*) and standard artificial freshwater (*eco* AFW). In the marine environment, at 100.0 mg/L, the *eco*-product assumes the highest BOD_5 values (42.30 mg/L).

	Freshwater System				**Marine System**	
	eco		***eco* AFW**		***eco***	
Sample	**BOD_5 mg/L**	**± SD**	**BOD_5 mg/L**	**±SD**	**BOD_5 mg/L**	**±SD**
CNTR-	3.80	–	1.60	–	0.00	–
CNTR+	2.70	–	4.90	–	0.00	
[*eco*] 100.0 mg/L	9.55	0.35	2.85	0.35	42.30	1.70
[*eco*] 50.0 mg/L	6.80	1.13	1.60	0.00	0.00	0.00

3.1.2. Freshwater System

The effects of *eco* and *eco* BOD_5 in the freshwater environment were studied using a standardized multi-species battery consisting of representatives of the main trophic levels: *A. fischeri*, *P. subcapitata*, and *D. magna*. For *A. fischeri* and *D. magna*, the biological response reported in the results refers to the maximum exposure time of 30 min and 48 h. The response of the organisms as a function of the concentration tested is reported in Figure 3 The results obtained from the test on *A. fischeri* in the marine environment are also representative of the freshwater environment and confirm the same results reported in the previous section, with a statistically significant difference recorded at 0.9 mg/L and 9.0 mg/L. In the *P. subcapitata* test, growth inhibition was observed in both treatments (*eco*/*eco* BOD_5) without a statistically significant difference in the concentration range considered (6.25–100.0 mg/L), suggesting equivalence in terms of biological response. The most remarkable response was obtained from the *D. magna* test; in both treatments

(*eco*/*eco* BOD_5), 100% of the organisms were immobile in the concentration range tested (25.0–100.0 mg/L), resulting in an undetectable EC_{50} (Figure 2/*D. magna*).

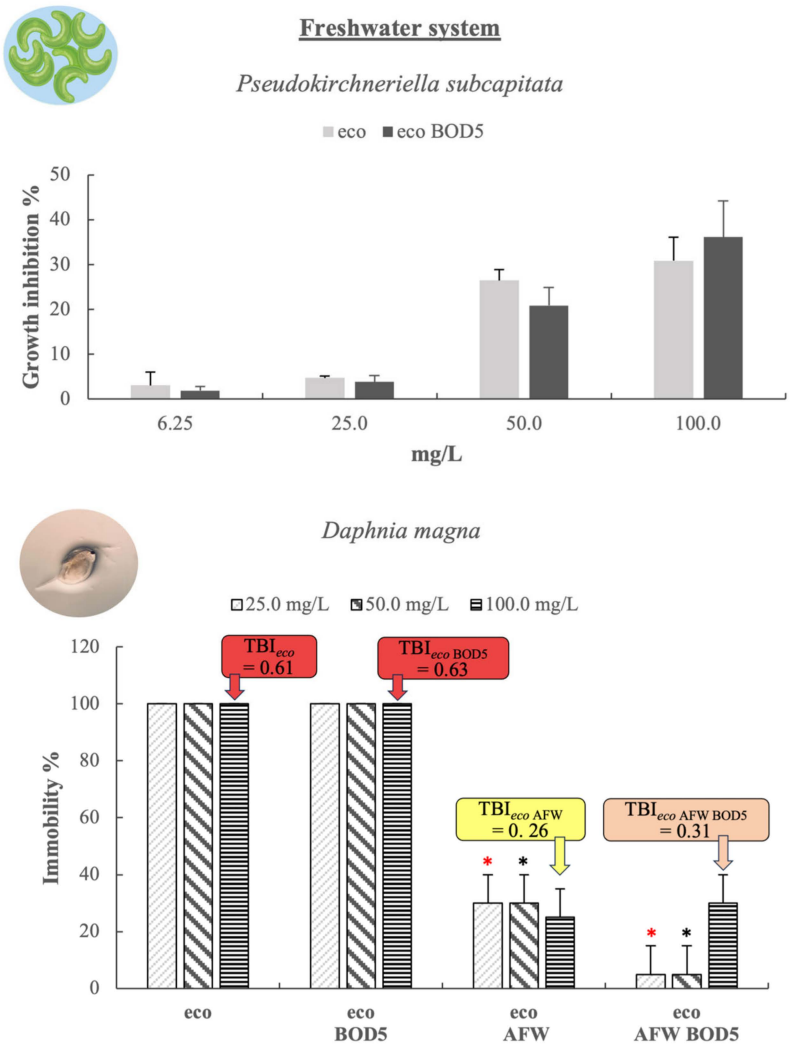

Figure 3. Biological response (%) as a function of *eco*/*eco* BOD_5 concentrations (mg/L) tested on representative freshwater organisms. Significant differences detected (and the level of significance) are asterisked. The response of *D. magna* is reported according to the treatments performed (tap water: *eco*/*eco* BOD_5; standard artificial freshwater: *eco* AFW/*eco* AFW BOD_5); of all the organisms in the batteries tested, only *D. magna* was exposed to the *eco*-product prepared with AFW. Estimated hazard index (TBI) is given for each treatment: heavy hazard is observed for the *eco* and *eco* BOD_5 treatments, moderate hazard for the *eco* AFW treatment, and high hazard for the *eco* AFW BOD_5 treatment. A conservative worst-case approach was used to estimate the index to the environment under consideration (100.0 mg/L of *eco*-product). The ecotoxicological hazard scale defined according to the TBI is reported in Table 2.

The EC_x values for the *Freshwater system* group are reported in Figure 2. For *A. fischeri* and *P. subcapitata*, no EC_{50} value was detected within 100.0 mg/L (EC_{50} > 100.0 mg/L = substance not hazardous to aquatic organisms). The level of ecotoxicological hazard estimated for the freshwater environment is heavy, both considering the *eco* treatment (TBI_{eco} = 0.61) and the *eco* BOD_5 treatment ($TBI_{eco\ BOD5}$ = 0.63); the estimate was based on a conservative worst-case approach (100.0 mg/L of *eco*-product). The BOD_5 of the *eco*-product quantified after five days is reported in Table 3/Freshwater system/*eco*. In fresh water, the *eco*-product at 100.0 mg/L has a BOD_5 of 9.55 mg/L; at 50.0 mg/L, the BOD_5 is 6.80 mg/L (BOD_5 is expressed as mean and standard deviation).

3.1.3. Additional Data: Test with *D. magna* and *Eco*-Product Dissolved in AFW (*Eco* AFW)

To investigate the possible causes of the biological response observed in *Daphnia magna* following treatment with *eco* and *eco* BOD_5 (100% immobility at all concentrations), the water used in the production phase of the liquid detergent was considered. An assay was set up in which neonates were exposed to the *eco*-product made with standard artificial freshwater (*eco* AFW/*eco* AFW BOD_5), instead of tap water. The results of the assay are reported in Figure 3; the highest value recorded (% immobility) was 30%. There is a statistically significant difference, in the biological response of *D. magna* to *eco* AFW and *eco* AFW BOD_5 at 25.0 mg/L (*p*-value: 0.04/*) and 50.0 mg/L (*p*-value: 0.04/*). The EC_x values are reported in Figure 2/*D. magna* AFW. For no treatment (*eco* AFW/*eco* AFW BOD_5), an EC_{50} value within 100.0 mg/L was estimated (EC_{50} > 100.0 mg/L = substance not hazardous to aquatic organisms). Based on this test, the hazard of the *eco*-product (made with AFW) in the freshwater environment was re-quantified; the results indicate a moderate level for the *eco* AFW treatment ($TBI_{eco\,AFW}$ = 0.26) and a high level for the *eco* AFW BOD_5 treatment ($TBI_{eco\,AFW\,BOD5}$ = 0.31). The BOD_5 of the *eco*-product dissolved in AFW quantified after five days is reported in Table 3/Freshwater system/*eco* AFW. At 100.0 mg/L, the BOD_5 is 2.85 mg/L; at 50.0 mg/L, the BOD_5 is 1.60 mg/(BOD_5 is expressed as mean and standard deviation).

3.1.4. *S. cerevisiae* (Non-Standardized Test)

Regarding the biological response detected in *S. cerevisiae* after 24 h (Table 4), the positive control (CNTR+ = AFW + NaCl) and the negative control (CNTR− = AFW + $C_6H_{12}O_6$) confirm the validity of the test, since in the first case the growth rate (μ) assumes a negative value due to the inability of *S. cerevisiae* to grow under extreme salinity conditions, whereas in the second case the colonies grow under the pH, T °C, and nutrient conditions imposed by the experiment. Although some growth (μ) of yeast colonies was detected in the concentration range considered ([*eco*] 100.0,10.0,1.0,0.1 mg/L), the inhibition rate (I%) assumed negative values, indicating inhibition of *S. cerevisiae* growth. However, the results of the one-sample *t*-test show a statistically significant difference between the growth rate of the control (CNTR–) and that of the sample only in the 100.0 mg/L *eco* treatment (*p*-value: 0.02/*), Supplementary Materials Figure S1; μ and I% are expressed as mean and standard deviation.

Table 4. Ecotoxicity test performed on *S. cerevisiae*; the table summarizes the treatments (CNTR−/CNTR+/[*eco*] mg/L), pH detected at T0 (pre-incubation), sample temperature at T0, growth rate (μ), and growth inhibition (I%) detected after 24 h, $\pm SD_x$, EC_{50} (not detected). The arrows to the left of the μ values indicate the colony growth trend (green = growth, red = no growth); the red arrows to the left of I% indicate the growth inhibition observed in all treatments (negative values/red arrow = growth inhibition; positive values/green arrow = biostimulation).

Treatment	pH T0	T °C T0	μ	$\pm SD_\mu$	I%	$\pm SD_{I\%}$	EC_{50}
CNTR−	7.8	20.5	↑ 0.80	0.02	-	-	–
CNTR+	6.5	20.5	↓ −0.21	0.03	↓ 126.53	3.56	
[*eco*] 100.0 mg/L	7.7	20.5	↑ 0.76	0.01	↓ 5.01	1.65	
[*eco*] 10.0 mg/L	7.6	20.5	↑ 0.75	0.05	↓ 6.07	6.25	
[*eco*] 1.0 mg/L	7.6	20.5	↑ 0.77	0.04	↓ 3.24	5.33	
[*eco*] 0.1 mg/L	7.6	20.5	↑ 0.78	0.03	↓ 2.70	3.43	

3.2. Microbiology

The results of the tests performed to estimate the Total Bacterial Count (TBC), or the total number of bacterial colonies expressed in N./mL (where N. is the number of colonies counted), in a growth medium before (−*eco*) and after treatment with the *eco*-product (+*eco*), considering three exposure temperatures (22 °C, 30 °C, 37 °C), are reported in Table 5 and Supplementary Materials Figure S2 in terms of mean and standard deviation. After the treatment +*eco*, the percentage variation (Δ%) in total bacterial count (TBC) amounts to

−96% at 22 °C, −96% at 30 °C, and −95% at 37 °C. As can be inferred from the results, the addition of the *eco*-product to the medium has a considerable effect on the number of bacterial colonies, suggesting a certain biocidal activity against the treated species.

Table 5. Total Bacterial Count (TBC) expressed as number of colonies counted (N./mL) at 22–30–37 °C before and after treatment with the *eco*-product (−eco/+eco) in the culture medium, reported as mean, ±SD, and % difference (Δ%).

TBC	22 °C		30 °C		37 °C	
	Mean	± SD	Mean	± SD	Mean	± SD
−eco	121.0	18.4	165.5	9.2	151.0	7.1
+eco	4.5	0.7	7.0	2.8	7.5	2.1
Δ%	−96%		−96%		−95%	

4. Discussion

SARS-CoV-2 had a severe impact on the health of thousands of people, the economy, social life [51], and the environment [52]. Strategies to prevent the spread of the virus have been defined by international authorities who, in addition to social distancing and travel restrictions, have recommended the use of personal protective equipment and common disinfectants (chlorine solutions, alcohol solutions, hydrogen peroxide, quaternary ammonium compounds, etc.) [7]. Disinfectants, in particular, have been used extensively in both outdoor and indoor environments, and although they are known to be effective against a wide range of organisms, they pose a threat to aquatic ecosystems that are reached directly or indirectly by wastewater [18].

Although the efficacy of these substances is undeniable, SARS-CoV-2 belongs to the category of enveloped viruses and is therefore susceptible to common detergents, whose soaps and/or surfactants interact with the lipid surface of the virus and inactivate it. For this reason, detergents are a class of products recommended by international authorities, both for their efficacy and because the biocidal action of a disinfectant applied to a surface that has not been previously cleaned may be compromised by the presence of organic matter or dirt [11,19,20].

Considering that the ongoing global changes may influence the increasing frequency of epidemic phenomena [4], it is necessary to adopt strategies that can mitigate the impacts on the aquatic environment resulting from the intensive use of substances known for their effects [6,12,18,22,23].

The aim of this study was to contribute to this cause by investigating the effects on the marine and freshwater environment resulting from the use of a surface detergent of natural origin, produced from exhausted edible vegetable oils, without substances of petrochemical origin, in conjunction with studies on biodegradability and microbiology.

The results of the ecotoxicological tests of the two multispecies batteries exposed to *eco* and *eco* BOD_5 do not show a uniform and statistically significant difference between these two treatments. Looking at Figure 1 (Marine system), compared to the *eco* treatment (*eco*), the biological response (%) to the biodegradation treatment (*eco* BOD5) is significantly higher for both *A. fischeri* (0.9–9.0 mg/L) and *P. lividus* (12.5 mg/L; 25.0 mg/L; 50.0 mg/L) to be comparable to the highest concentration tested (90.0 mg/L in *A. fischeri* and 100.0 mg/L in *P. lividus*). It is plausible to deduce that bacteria and primary consumers are more sensitive to the biodegradation product, whereas in *P. tricornutum* (as a representative of primary producers) no significant difference between treatments is observed, except at 6.25 mg/L, with a biostimulatory effect detected in the *eco* BOD_5 treatment.

According to ISPRA 2013 [47], biostimulation (higher sample response than negative control) may indicate first stress in response to low contaminant concentrations. However, although differences in the biological response can be observed that vary between organisms and treatments, the impact of the *eco*-product and *eco*-BOD_5 (after five days of biodegradation) in the marine environment is summarized by the Hazard Quotient (HQ)

estimated for both treatments (at 100.0 mg/L of *eco*-product), which indicates a low level of hazard. This result is consistent with the measured EC_x values (Figure 2). Considering that no EC_{50} values were estimated within 100.0 mg/L, the tested substance is not hazardous to aquatic organisms either before or at the end of a five-day biodegradation process.

Regarding the tests carried out on freshwater organisms, the results recorded with *P. subcapitata*, as a primary producer, indicate the inhibition exerted by both treatments (*eco*/*eco* BOD_5), but no significant differences in terms of biological response were found in relation to the biodegradation process. The results observed in *D. magna*, an organism known for its sensitivity [53–55], suggest a radical effect induced by exposure to *eco* and *eco* BOD_5 treatments (100% immobility at all tested concentrations). The cause was investigated in the water used in the production phase of the liquid *eco*-product, considering the treatments carried out for water treatment, which could be the cause of the observed effect [34].

Indeed, tap water seems to be a crucial factor for the biological response of *D. magna*, since the exposure of the organisms to the liquid *eco*-product prepared with standardized artificial freshwater (*eco* AFW/*eco* AFW BOD_5) demonstrated such a biological response that it can be inferred that the water quality reduces the toxicity of the product (EC_{50} > 100.0 mg/L = the tested substance is not considered hazardous to aquatic organisms), indicating the need to use a standardized medium in the production phase of the *eco*-product in liquid form. Furthermore, a statistically significant difference is observed in the percentage of response between *eco* AFW and *eco* AFW BOD_5 (% immobility detected in *eco* AFW > % immobility detected in *eco* AFW BOD_5), suggesting that the use of a standardized medium in the production phase has an effect not only on the biological response but also on biodegradability process.

The impact of the *eco*-product on the freshwater environment can be summarized by the integrated hazard index (TBI), which varies depending on the treatment to which *D. magna* was exposed. The index indicates a heavy level of hazard when *D. magna* is exposed to *eco* and *eco* BOD_5 treatments, a moderate level when *D. magna* is exposed to *eco* AFW treatment, and a high level when *D. magna* is exposed to *eco* AFW BOD_5 treatment. These results also demonstrate the importance of using a standardized medium for the production phase of the *eco*-product in liquid form, and the test on *D. magna* particularly highlighted this aspect.

It should be noted that the estimated hazard level for the freshwater environment is based on a conservative approach considering the worst-case scenario, i.e., a worst-case environmental *eco*-product concentration of 100.0 mg/L; according to Smith et al. 2020 [17], surfactants represent only a fraction of the total detergent composition and the limits for emission to surface waters should be kept at $\leq$ 2 mg/L (D. Lgs. 152/06 [56]). These considerations suggest that the scenario on which the hazard assessment was based (100.0 mg/L), considering *D. magna* was exposed to *eco* AFW and *eco* AFW BOD_5, may be unrealistic, although it can be considered as a reference when discussing the massive use of a product. Under normal conditions (concentrations less than 100.0 mg/L), the *eco*-product (*eco* AFW BOD_5) stimulates a lower percentage of immobility after five days of biodegradation, and considering that the TBI is an index that integrates the weights of the species that make up the battery, it is plausible to expect a further reduction in hazard (Figure 3).

In fact, if we carry out the calculation of the integrated TBI index (freshwater environment), considering the multispecies battery with *D. magna* exposed to the *eco* AFW and *eco* AFW BOD_5 treatment, considering an environmental concentration of 25.0 mg/L of *eco* AFW, the risk is absent ($TBI_{eco\,AFW}$ = 0.06), while in the *eco* AFW BOD_5 treatment the risk is moderate ($TBI_{eco\,AFW\,BOD5}$ = 0.13), confirming the expected decrease. The same cannot be said when considering the risk associated with the multispecies battery with *D. magna* exposed to the *eco* and *eco* BOD_5 treatments, in which the risk recalculated at 25.0 mg/L is high (TBI_{eco}= 0.48) and heavy ($TBI_{eco\,BOD5}$ = 0.50) because it is conditioned by the weight of the biological response recorded in *D. magna* at 25.0 mg/L (100% of immobile organisms, see Figure 3, *eco*/*eco* BOD_5 treatment).

The *eco*-product appears to be a potential *eco-friendly* candidate for conventional disinfection methods applied to the coronavirus group, also demonstrating potential antimicrobial activity, as measured by total bacteria count tests, which indicated an average load reduction of −96% at 22 °C, −96% at 30 °C, and −95% at 37 °C (Table 5). However, it must be highlighted that a product can only be released to the market with the biocide label after approval by the European Community in accordance with *Regulation (eu) no. 528/2012 of the European Parliament and of the Council of 22 May 2012* [57], considering the European context.

Products of secondary origin are part of the circular economy, a model of production and consumption inspired by a regenerative and evolutionary system. In light of this concept, exhausted edible vegetable oil is a raw material of secondary origin, and its reuse helps to reduce the environmental impact associated with these substances, which are hazardous waste if not properly disposed of. In addition, their application in the production of detergents can mitigate the impacts associated with the use of oils imported from other countries, with the added benefit of valorizing a local *zero km* waste product. The choice to use detergent with surfactants of natural origin means reducing both the environmental effects associated with the extraction and refining processes of surfactants of a petrochemical nature and the effect of their persistence [58].

With reference to BOD_5 (Table 3), an important indicator of water quality that corresponds to the amount of oxygen required by microorganisms to decompose the organic matter present in a water sample over a period of five days at a standard temperature of 20 °C, it can be stated that the *eco*-product (*eco*/*eco* AFW), at 100.0 mg/L, in the freshwater environment is within the limits established by the *Testo Unico Ambientale, D.L. 3 April 2006 n. 152* (BOD_5 limit $\leq$ 40. 0 mg/L with reference to the emission limits for discharges into surface waters, taking into account the context of the Italian territory) [56]. The BOD_5 value detected on the *eco*-product (100.0 mg/L) in the marine system shows an exceedance of this limit equal to 2.30 mg/L (in terms of average values) and, although this environment plays an important role in the dilution and dispersion of pollutants [59], this information must be considered in the light of possible massive use in relation to potential pollution of organic nature. However, at 50.0 mg/L of *eco*-product, the BOD_5 value is considerably reduced to 0.0 mg/L. It should also be taken into account that the BOD_5 values recorded in this study cannot be considered as unambiguously predictive and must be considered in relation to the experimental conditions (type of inoculum, T °C, etc.).

Finally, *S. cerevisiae*, an organism already widely used in ecotoxicological assays and potentially useful for the detection of toxic substances in the aquatic environment [60,61], was tested in a range of *eco*-product concentrations (0.1–100.0 mg/L) with the aim of collecting data on the biological response with a view to possible standardization of a method potentially advantageous due to the numerous characteristics associated with the use of yeasts (low cost, easy storage methods, high level of knowledge, possibility of reducing the use of animals in testing, etc.). Although negative values of inhibition rate (I%) indicate inhibition of *S. cerevisiae* growth (μ) associated with treatment with the *eco*-product at all concentrations (Table 4), the one-sample *t*-test shows a statistically significant difference in terms of growth between control (CNTR–) and sample at 100.0 mg/L (*p*-value: 0.02/*), suggesting statistical significance in terms of inhibition only at 100.0 mg/L, which in turn suggests a lack of toxicity of the *eco*-product in consideration of an undetected EC_{50}. It can be concluded that *S. cerevisiae* shows a response compatible with that of the organisms tested in the standardized multi-species batteries, for which no EC_{50} values were detected with the exception of *D. magna* treated with *eco*/*eco* BOD_5, for which specific considerations were made and the EC_{50} was not detected due to the measured biological response (100% immobility between 25.0 and 100.0 mg/L).

5. Conclusions

In conclusion, based on the results of the investigation conducted on the *eco*-product, analyzed from the perspective of a potential *eco-friendly* alternative to the disinfection

procedures implemented during the SARS-CoV-2 era (an enveloped virus and therefore susceptible even to common detergents), it can be considered as a suitable alternative. However, this study highlights the need to use a standardized agent for the production phase of the *eco*-product in liquid form to mitigate the impact on the aquatic environment, especially considering the biological response detected by the tests conducted on the multispecies battery representative of freshwater systems. A further element to be considered is the massive and indiscriminate use; a product with *eco-friendly* potential does not justify its misuse, and limited and restricted applications in appropriate areas and contexts are recommended.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments11110242/s1, Figure S1: Saccharomyces cerevisiae (growth rate and growth inhibition); Figure S2: Total bacteria count; Table S1: Summary table (standardized methods).

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References

1. Helmy, Y.A.; Fawzy, M.; Elaswad, A.; Sobieh, A.; Kenney, S.P.; Shehata, A.A. The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. *J. Clin. Med.* **2020**, *9*, 1225. [CrossRef] [PubMed]
2. Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H.; Wang, W.; Song, H.; Huang, B.; Zhu, N.; et al. Genomic Characterisation and Epidemiology of 2019 Novel Coronavirus: Implications for Virus Origins and Receptor Binding. *Lancet* **2020**, *395*, 565–574. [CrossRef] [PubMed]
3. Worobey, M.; Levy, J.I.; Malpica Serrano, L.; Crits-Christoph, A.; Pekar, J.E.; Goldstein, S.A.; Rasmussen, A.L.; Kraemer, M.U.G.; Newman, C.; Koopmans, M.P.G.; et al. The Huanan Seafood Wholesale Market in Wuhan Was the Early Epicenter of the COVID-19 Pandemic. *Science* **2022**, *377*, 951–959. [CrossRef] [PubMed]
4. Baker, R.E.; Mahmud, A.S.; Miller, I.F.; Rajeev, M.; Rasambainarivo, F.; Rice, B.L.; Takahashi, S.; Tatem, A.J.; Wagner, C.E.; Wang, L.-F.; et al. Infectious Disease in an Era of Global Change. *Nat. Rev. Microbiol.* **2022**, *20*, 193–205. [CrossRef] [PubMed]
5. World Health Organization. *Report of the Review Committee Regarding Standing Recommendations for COVID-19*; World Health Organization: Geneva, Switzerland, 2023.
6. Hiep, N.T.; Nguyen, M.-K.; Nhut, H.T.; Hung, N.T.Q.; Manh, N.C.; Lin, C.; Chang, S.W.; Um, M.J.; Nguyen, D.D. A Review on Sterilization Methods of Environmental Decontamination to Prevent the Coronavirus SARS-CoV-2 (COVID-19 Virus): A New Challenge Towards Eco-Friendly Solutions. *Sci. Total Environ.* **2023**, *904*, 166021. [CrossRef]
7. World Health Organization. *Cleaning and Disinfection of Environmental Surfaces in the Context of COVID-19*; World Health Organization: Geneva, Switzerland, 2020.
8. Rai, N.K.; Ashok, A.; Akondi, B.R. Consequences of Chemical Impact of Disinfectants: Safe Preventive Measures Against COVID-19. *Crit. Rev. Toxicol.* **2020**, *50*, 513–520. [CrossRef]
9. Chang, A. Cleaning and Disinfectant Chemical Exposures and Temporal Associations with COVID-19—National Poison Data System, United States, January 1, 2020–March 31, 2020. *MMWR Morb. Mortal. Wkly. Rep.* **2020**, *69*, 496–498. [CrossRef]

10. Nabi, G.; Wang, Y.; Hao, Y.; Khan, S.; Wu, Y.; Li, D. Massive Use of Disinfectants against COVID-19 Poses Potential Risks to Urban Wildlife. *Environ. Res.* **2020**, *188*, 109916. [CrossRef]
11. Draisci, R.; Crebelli, R.; Attias, L.; Baldassarri, L.; Marcello, I.; Cresti, R.; Abenavoli, C.; Lucentini, L.; La Rosa, G.; Veschetti, E.; et al. *Raccomandazioni per La Disinfezione Di Ambienti Esterni e Superfici Stradali per La Prevenzione Della Trasmissione Dell'Infezione Da SARS-CoV-2. Gruppo Di Lavoro ISS Biocidi COVID-19 Gruppo Di Lavoro ISS Ambiente e Rifiuti COVID-19*; Istituto Superiore di Sanità (ISS): Roma, Italy, 2020.
12. Bhat, S.A.; Sher, F.; Kumar, R.; Karahmet, E.; Haq, S.A.U.; Zafar, A.; Lima, E.C. Environmental and Health Impacts of Spraying COVID-19 Disinfectants with Associated Challenges. *Environ. Sci. Pollut. Res.* **2022**, *29*, 85648–85657. [CrossRef]
13. Li, Z.; Song, G.; Bi, Y.; Gao, W.; He, A.; Lu, Y.; Wang, Y.; Jiang, G. Occurrence and Distribution of Disinfection Byproducts in Domestic Wastewater Effluent, Tap Water, and Surface Water During the SARS-CoV-2 Pandemic in China. *Environ. Sci. Technol.* **2021**, *55*, 4103–4114. [CrossRef]
14. Zhang, Z.; Zhou, Y.; Han, L.; Guo, X.; Wu, Z.; Fang, J.; Hou, B.; Cai, Y.; Jiang, J.; Yang, Z. Impacts of COVID-19 Pandemic on the Aquatic Environment Associated with Disinfection Byproducts and Pharmaceuticals. *Sci. Total Environ.* **2022**, *811*, 151409. [CrossRef] [PubMed]
15. Block, S.S. *Disinfection, Sterilization, and Preservation*; Lippincott Williams & Wilkins: Philadelphia, PL, USA, 2001; ISBN 0683307401.
16. Nozaic, D.J. Chlorine: Is It Really so Bad and What Are the Alternatives? *Water Sa* **2004**, *30*, 18–24. [CrossRef]
17. Smith, M.L.; Gandolfi, S.; Coshall, P.M.; Rahman, P.K.S.M. Biosurfactants: A COVID-19 Perspective. *Front. Microbiol.* **2020**, *11*, 1341. [CrossRef] [PubMed]
18. Dhama, K.; Patel, S.K.; Kumar, R.; Masand, R.; Rana, J.; Yatoo, M.I.; Tiwari, R.; Sharun, K.; Mohapatra, R.K.; Natesan, S.; et al. The Role of Disinfectants and Sanitizers During COVID-19 Pandemic: Advantages and Deleterious Effects on Humans and the Environment. *Environ. Sci. Pollut. Res.* **2021**, *28*, 34211–34228. [CrossRef] [PubMed]
19. Draisci, R.; Abenavoli, C.; Attias, L.; Baldassarri, L.; Marina Costamagna, F.; Cresti, R.; Famele, M.; Fava, L.; Ferranti, C.; Giordano, F.; et al. *Raccomandazioni Ad Interim Sui Disinfettanti Nell'attuale Emergenza COVID-19: Presidi Medico Chirurgici e Biocidi Gruppo Di Lavoro ISS Biocidi COVID-19 Con La Collaborazione Di Raffaella Perrone Ministero Della Salute*; Istituto Superiore di Sanità (ISS): Roma, Italy, 2020.
20. Draisci, R.; Attias, L.; Baldassarri, L.; Catone, T.; Cresti, R.; Fidente, R.M.; Marcello, I.; Buonanno, G.; Bertinato, L.; Bochicchio, F.; et al. *Raccomandazioni Ad Interim Sulla Sanificazione Di Strutture Non Sanitarie Nell'attuale Emergenza COVID-19: Ambienti/Superfici. Aggiornamento Del Rapporto ISS COVID-19 n. 25/2020. Versione Del 20 Maggio 2021*; Istituto Superiore di Sanità (ISS): Roma, Italy, 2021.
21. Farias, C.B.B.; Almeida, F.C.G.; Silva, I.A.; Souza, T.C.; Meira, H.M.; Soares da Silva, R.d.C.F.; Luna, J.M.; Santos, V.A.; Converti, A.; Banat, I.M.; et al. Production of Green Surfactants: Market Prospects. *Electron. J. Biotechnol.* **2021**, *51*, 28–39. [CrossRef]
22. Dewey, H.M.; Jones, J.M.; Keating, M.R.; Budhathoki-Uprety, J. Increased Use of Disinfectants During the COVID-19 Pandemic and Its Potential Impacts on Health and Safety. *ACS Chem. Health Saf.* **2022**, *29*, 27–38. [CrossRef]
23. Duane, B.; Pilling, J.; Saget, S.; Ashley, P.; Pinhas, A.R.; Lyne, A. Hand Hygiene with Hand Sanitizer versus Handwashing: What Are the Planetary Health Consequences? *Environ. Sci. Pollut. Res.* **2022**, *29*, 48736–48747. [CrossRef]
24. *UNI EN ISO 11348-1:2019*; Water Quality—Determination of the Inhibitory Effect of Water Samples on the Light Emission of Luminescent Bacteria (Luminescent Bacteria Test)—Part 1: Method Using Freshly Prepared Bacteria. Ente Nazionale Italiano di Unificazione: Milano, Italy, 2019.
25. *UNI EN ISO 10253:2017*; Water Quality—Marine Algal Growth Inhibition Test with Phaeodactylum Tricornutum and Skeletonema Costatum. Ente Nazionale Italiano di Unificazione: Milano, Italy, 2017.
26. Chapman, G.A.; Denton, D.L.; Lazorchak, J.M. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms*; US EPA, Office of Research and Development, National Exposure Research: Washington, DC, USA, 1995.
27. ISPRA. Saggi Di Fecondazione e Saggi Di Sviluppo Embrionale Con Il Riccio Di Mare PARACENTROTUS LIVIDUS (LAMARCK)(ECHINODERMATA: ECHINOIDEA) Quaderni Di Ecotossicologia 11/2017. 2017. Available online: https://www.certifico.com/guide-ispra/242-consulting/ambiente/documenti-ambiente/documenti-ambiente-ispra/5484-ispra-quaderno-di-ecotossiologia-11-17 (accessed on 13 September 2024).
28. *UNI EN ISO 8692:2012*; Water Quality—Freshwater Algal Growth Inhibition Test with Unicellular Green Algae. Ente Nazionale Italiano di Unificazione: Milano, Italy, 2012.
29. *UNI EN ISO 6341:2013*; Water Quality—Determination of the Inhibition of the Mobility of Daphnia Magna Straus (Cladocera, Crustacea)—Acute Toxicity Test. Ente Nazionale Italiano di Unificazione: Milano, Italy, 2013.
30. Braconi, D.; Bernardini, G.; Santucci, A. Saccharomyces Cerevisiae as a Model in Ecotoxicological Studies: A Post-Genomics Perspective. *J. Proteom.* **2016**, *137*, 19–34. [CrossRef]
31. IFOM—Istituto Fondazione di Oncologia Molecolare Ets Protocolli: Il Lievito. Available online: https://www-new.ifom.eu/it/ifom-outreach/risorse-digitali/protocolli/organismi-modello/lievito.php (accessed on 6 June 2024).
32. Abbas, M.; Adil, M.; Ehtisham-ul-Haque, S.; Munir, B.; Yameen, M.; Ghaffar, A.; Shar, G.A.; Asif Tahir, M.; Iqbal, M. Vibrio Fischeri Bioluminescence Inhibition Assay for Ecotoxicity Assessment: A Review. *Sci. Total Environ.* **2018**, *626*, 1295–1309. [CrossRef]

33. Anselmi, S.; Cavallo, A.; Del Rio, L.; Renzi, M. Impact of Global Change on Environmental Hazards of Different Clays: A Case Study on Aliivibrio Fischeri. *J. Hazard. Mater.* **2023**, *457*, 131806. [CrossRef]
34. Dinelli, E.; Lima, A.; Albanese, S.; Birke, M.; Cicchella, D.; Giaccio, L.; Valera, P.; De Vivo, B. Comparative Study Between Bottled Mineral and Tap Water in Italy. *J. Geochem. Explor.* **2012**, *112*, 368–389. [CrossRef]
35. Mazzoleni, S.; Landi, C.; Cartenì, F.; de Alteriis, E.; Giannino, F.; Paciello, L.; Parascandola, P. A Novel Process-Based Model of Microbial Growth: Self-Inhibition in Saccharomyces Cerevisiae Aerobic Fed-Batch Cultures. *Microb. Cell Fact.* **2015**, *14*, 109. [CrossRef] [PubMed]
36. Blomberg, A. Metabolic Surprises in Saccharomyces Cerevisiae During Adaptation to Saline Conditions: Questions, Some Answers and a Model. *FEMS Microbiol. Lett.* **2000**, *182*, 1–8. [CrossRef]
37. Tecniche Ottiche Nel Monitoraggio Real-Time Della Crescita Di Colture Batteriche. Available online: https://we.riseup.net/assets/528713/tecniche-ottiche.pdf (accessed on 6 June 2024).
38. Vigiak, O.; Grizzetti, B.; Udias-Moinelo, A.; Zanni, M.; Dorati, C.; Bouraoui, F.; Pistocchi, A. Predicting Biochemical Oxygen Demand in European Freshwater Bodies. *Sci. Total Environ.* **2019**, *666*, 1089–1105. [CrossRef]
39. Hach, C.C.; Klein, R.L., Jr.; Gibbs, C.R. Biochemical Oxygen Demand. *Tech. Monogr.* **1997**, *7*, 1–22.
40. Hufschmid, A.; Becker-Van Slooten, K.; Strawczynski, A.; Vioget, P.; Parra, S.; Pulgarin, C. BOD5 Measurements of Water Presenting Inhibitory Cu^{2+}.: Implications in Using of BOD to Evaluate Biodegradability of Industrial Wastewaters. *Chemosphere* **2003**, *50*, 171–176. [CrossRef]
41. Rudaru, D.G.; Lucaciu, I.E.; Fulgheci, A.M. Correlation Between BOD5 and COD—Biodegradability Indicator of Wastewater. *Rom. J. Ecol. Environ. Chem.* **2022**, *4*, 80–86. [CrossRef]
42. Briassoulis, D.; Babou, M.; Mistriotis, A.; Hiskakis, M. *KBBPPS Knowledge Based Bio-Based Products' Pre-Standardization Work Package 6 Biodegradability Deliverable N° 6.1: Report on Current Relevant Biodegradation and Ecotoxicity Standards Public*; KBBPPS: Bruxelles, Belgio, 2015.
43. Kogawa, A.C.; Cernic, B.G.; do Couto, L.G.D.; Salgado, H.R.N. Synthetic Detergents: 100 Years of History. *Saudi Pharm. J.* **2017**, *25*, 934–938. [CrossRef]
44. la Farré, M.; Pérez, S.; Kantiani, L.; Barceló, D. Fate and Toxicity of Emerging Pollutants, Their Metabolites and Transformation Products in the Aquatic Environment. *TrAC-Trends Anal. Chem.* **2008**, *27*, 991–1007. [CrossRef]
45. Istituto Superiore di Sanità. *Metodi Di Analisi per Il Controllo Microbiologico Degli Alimenti. Rapporti ISTISAN 96/35*; Istituto Superiore di Sanità: Roma, Italy, 1996.
46. Istituto Superiore di Sanità. *Metodi Analitici Di Riferimento per Le Acque Destinate al Consumo Umano Sensi Del DL.vo 31/2001. Metodi Microbiologici. Rapporti ISTISAN 07/5*; Istituto Superiore di Sanità: Roma, Italy, 2007.
47. ISPRA. Batterie di saggi ecotossicologici per sedimenti e acque interne. In *I Manuali di Ecotossicologia 88/2013*; ISPRA: Roma, Italy, 2013.
48. Environmental Protection Agency (EPA) TRAP (Toxicity Relationship Analysis Program). Available online: https://archive.epa.gov/med/med_archive_03/web/html/trap.html (accessed on 6 June 2024).
49. AAT Bioquest, Inc. IC50 Calculator. Available online: https://www.aatbio.com/tools/ic50-calculator (accessed on 6 June 2024).
50. Grabarczyk, Ł.; Mulkiewicz, E.; Stolte, S.; Puckowski, A.; Pazda, M.; Stepnowski, P.; Białk-Bielińska, A. Ecotoxicity Screening Evaluation of Selected Pharmaceuticals and Their Transformation Products towards Various Organisms. *Environ. Sci. Pollut. Res.* **2020**, *27*, 26103–26114. [CrossRef]
51. Haleem, A.; Javaid, M.; Vaishya, R. Effects of COVID-19 Pandemic in Daily Life. *Curr. Med. Res. Pract.* **2020**, *10*, 78–79. [CrossRef] [PubMed]
52. Zambrano-Monserrate, M.A.; Ruano, M.A.; Sanchez-Alcalde, L. Indirect Effects of COVID-19 on the Environment. *Sci. Total Environ.* **2020**, *728*, 138813. [CrossRef] [PubMed]
53. Martins, J.; Oliva Teles, L.; Vasconcelos, V. Assays with Daphnia Magna and Danio Rerio as Alert Systems in Aquatic Toxicology. *Environ. Int.* **2007**, *33*, 414–425. [CrossRef] [PubMed]
54. Bownik, A. Daphnia Swimming Behaviour as a Biomarker in Toxicity Assessment: A Review. *Sci. Total Environ.* **2017**, *601–602*, 194–205. [CrossRef] [PubMed]
55. Tkaczyk, A.; Bownik, A.; Dudka, J.; Kowal, K.; Ślaska, B. Daphnia Magna Model in the Toxicity Assessment of Pharmaceuticals: A Review. *Sci. Total Environ.* **2021**, *763*, 143038. [CrossRef]
56. Ministero dell'Ambiente e della Tutela del Territorio e del Mare. *Decreto Legislativo 3 Aprile 2006, n. 152*; Norme in materia ambientale; Gazzetta Ufficiale della Repubblica Italiana: Roma, Italy, 2006.
57. European Union Regulation (EU). No 528/2012 of the European Parliament and of the Council of 22 May 2012 Concerning the Making Available on the Market and Use of Biocidal Products Text with EEA Relevance. Available online: https://eur-lex.europa.eu/eli/reg/2012/528/oj (accessed on 6 June 2024).
58. Lucchetti, M.G.; Paolotti, L.; Rocchi, L.; Boggia, A. The Role of Environmental Evaluation within Circular Economy: An Application of Life Cycle Assessment (LCA) Method in the Detergents Sector. *Environ. Clim. Technol.* **2019**, *23*, 238–257. [CrossRef]
59. Danovaro, R. *Biologia Marina. Biodiversità E Funzionamento Degli Ecosistemi Marini*, 2nd ed.; SpA, D.A.S., Ed.; UTET Università: Torino, Italy, 2019; ISBN 108860085314/13978-8860085313.

60. Rumlova, L.; Dolezalova, J. A New Biological Test Utilising the Yeast Saccharomyces Cerevisiae for the Rapid Detection of Toxic Substances in Water. *Environ. Toxicol. Pharmacol.* **2012**, *33*, 459–464. [CrossRef]
61. Dolezalova, J.; Rumlova, L. A New Biological Test of Water Toxicity-Yeast Saccharomyces Cerevisiae Conductometric Test. *Environ. Toxicol. Pharmacol.* **2014**, *38*, 977–981. [CrossRef]