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LCA as a Complementary Tool for the Evaluation of Biocolonization Management: The Case of Palazzo Rocca Costaguta

Letizia Berti [1,2,3], Francesco Arfelli [4], Federica Villa [2], Francesca Cappitelli [2], Davide Gulotta [5,*], Luca Ciacci [4], Elena Bernardi [4], Ivano Vassura [4], Fabrizio Passarini [4,*], Salvatore Napoli [6] and Sara Goidanich [3]

1 Department of Sciences of Antiquity, "La Sapienza" University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; letizia.berti@uniroma1.it
2 Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Luigi Mangiagalli 25, 20133 Milan, Italy; federica.villa@unimi.it (F.V.); francesca.cappitelli@unimi.it (F.C.)
3 Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Piazza Leonardo Da Vinci 32, 20133 Milan, Italy; sara.goidanich@polimi.it
4 Department of Industrial Chemistry "Toso Montanari", Università di Bologna, Via Piero Gobetti 85, 40129 Bologna, Italy; francesco.arfelli3@unibo.it (F.A.); luca.ciacci5@unibo.it (L.C.); elena.bernardi@unibo.it (E.B.); ivano.vassura@unibo.it (I.V.)
5 Getty Conservation Institute, Science Department, 1200 Getty Center Drive, Los Angeles, CA 90049, USA
6 CORES4N s.r.l, Via Raffaello Sanzio 6, Venegono Superiore, 21040 Varese, Italy; salvatore.napoli@cores4n.com
* Correspondence: dgulotta@getty.edu (D.G.); fabrizio.passarini@unibo.it (F.P.)

Abstract: The 17th Century *Casa a Ponente* of Palazzo Rocca Costaguta's wall provided an opportunity for an evaluation based on a Life Cycle Assessment (LCA) approach of conservation treatments aiming at removing biological colonization from built heritage surfaces. The investigated surfaces were historic plasters partially covered by a patchy green patina due to biofilm recolonization soon after a previous biocidal treatment. Areas of the biocolonized wall were treated by conservation professionals according to both conventional and "green" (i.e., exploiting natural active principles) biocidal products, including Preventol RI 50 (active substance benzalkonium chloride), Essenzio (active substance essential oregano oil), and hydrogen peroxide. Upon treatment, LCA analysis was conducted to evaluate the environmental impact of the different solutions, including a no-treatment option. LCA analysis was based on on-site investigations of the untreated wall surface with and without biofilm and following the biocidal treatment. The conservation treatment's impact on the mineral substrate was based on digital microscopy, colorimetry, and water contact angle measurements via an innovative portable method. The results highlighted the impacts of the different biocidal treatments, which, in some cases, have not completely removed the biofilm and, in some cases, have altered the surface properties of the plaster. This pointed out the opportunity to re-think conservation strategy, including LCA analysis as a complementary tool to assess the environmental impact of the different conservation treatments and procedures.

Keywords: biological recolonization; built heritage; Life Cycle Assessment; minimum intervention; environmental sustainability

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1. Introduction

Built heritage worldwide embeds critical and irreplaceable historical, aesthetic, and cultural values, contributing to creating and preserving local communities' identity and sense of belonging across generations. From an ecological point of view, it also provides dynamic ecosystems that support microbial life and preserve biodiversity [1]. Microorganisms grow on built heritage mineral surfaces exposed to the atmosphere, e.g., stone cladding, mortars, and plasters of historic buildings, as colorful sub-aerial biofilms (SABs) [2]. Thanks

to their extracellular products (Extracellular Polymeric Substance, EPS), SABs can adhere to mineral surfaces and better resist external stresses. The interactions between SABs and heritage surfaces have traditionally been considered primarily through the lens of the aesthetic alteration they cause and the possible physical–mechanical damage mechanisms associated with their development and, therefore, considered detrimental to the substrates [3,4], i.e., leading to biodeterioration. However, some recent empirical evidence has highlighted that SABs can also have a neutral or even protective role [1,5–13]. As a consequence, the actual impact and potential role of biofilms on the conservation of built heritage surfaces have become the object of an open debate, and the research on this novel topic is very active [14]. To this aim, we propose employing LCA analysis to support this ongoing discussion and possibly promote a shift in perspective toward more informed and sustainable approaches to biocolonization.

Life Cycle Assessment (LCA) is a tool employed to estimate the potential environmental impacts associated with products, processes, or activities and provides overcharging understanding and quantitative data for making informed decisions on the most environmentally friendly option(s) [15]. LCA is framed by the ISO standards 14040 [16] and 14044 [17] and has been increasingly applied to several research fields, although only a small number of studies deal with LCA analysis for the evaluation of conservation methods for cultural heritage [18–25]. Studies have assessed the ecotoxicity of products used in the treatment of cultural heritage assets, such as essential oils; however, these are limited to measuring LC50 values and do not apply the LCA methodology [26]. The limited research in this realm has so far focused on the evaluation of repair mortars and concretes [27], the use of recycled materials [28,29], consolidation treatments [30–32], or, more broadly, sustainable maintenance strategies for heritage buildings [33–36]; none has specifically investigated the theme of biocolonization removal.

The inherent complexity of heritage surfaces (e.g., material heterogeneity, weathering conditions, effects of past conservation treatments), as well as the specific and unique characteristics of conservation operations (e.g., methodologies adapted from neighboring fields, the application of conventional and innovative treatments, the impact of craftmanship and operators experience), are among the challenges hindering a systematic application of LCA in this sector, making data collection and the consequent estimation of environmental impacts complex. Developing reference databases for the LCA analysis specifically targeting conservation operations in the built heritage realm is a fundamental step in this direction and will support the researchers and practitioners' community in choosing appropriate treatment options, ultimately improving sustainability in the field (e.g., greener products and more eco-friendly treatment approaches).

To date, STICH (https://stich.culturalheritage.org, accessed on 20 July 2024, [37]) represents a reference resource, providing information on the main materials of interest to the cultural heritage community and enabling the calculation of their carbon footprint in relation to a unit of mass or volume used. However, the carbon footprint alone is not sufficient to obtain comprehensive environmental information encompassing the full spectrum of the impacts evaluated in LCA. Additionally, specific information regarding biocolonization removal is not available yet. In the context of LCA, when databases contain partial or incomplete information, the literature can be a reference source if studies conducted in similar contexts are available. However, to the best of our knowledge, no comparable studies have been identified that directly address the topics covered in this work, which, also because of this gap, represents an innovation in the field.

Different treatment approaches against biocolonization have been reported to present, including (i) chemical treatments with conventional biocides or nanoparticles; (ii) physical cleaning, such as mechanical removal, UV-C irradiation, gamma radiation, laser cleaning, heath shocking, and microwave or dry ice treatment; and (iii) the use of natural compounds [38]. Critical limitations with such approaches include no long-lasting effects generally reported, possible chromatic alterations, additional physical–mechanical stresses to the heritage materials, and the risk of undesired chemical interactions with specific com-

ponents of the treated substrates [39–41]. Therefore, a no-treatment strategy, i.e., preserving biofilms instead of removing them [11], should also be considered a viable option when no biodeteriorative effects are observed. This option could also better align, in some cases, with the golden criteria for improving sustainability in built heritage conservation [20,42–44], as well as with the ICOMOS's guidelines [45], recommending that "no actions should be undertaken without demonstrating that they are indispensable".

However, in many cases, conservation treatments aiming at controlling and removing biocolonization and preventing further recolonization cannot be avoided, so more robust approaches to accurately assess the effectiveness of biocidal and preventive strategies are still needed [46,47]. Furthermore, a better understanding of the environmental impact of biocidal/cleaning treatments is critical to support practitioners in designing more informed and evidence-based conservation strategies and selecting the most appropriate treatment methods.

In this study, LCA has been applied to estimate the potential environmental impact of biocolonization removal from the plastered surfaces of *Casa a Ponente* of *Palazzo Rocca Costaguta* in Chiavari (Genova, Italy). A preliminary study assessed the presence of the SAB on the historic plasters and the related and measurable positive changes in surface wettability it induced, resulting in near-hydrophobic conditions of the colonized surface [48]. The area of interest was previously cleaned with biocidal treatments that did not prove to be long-lasting. Rapid recolonization followed the cleaning, and further bioremoval treatments were under discussion. In this context, the environmental impacts of additional biofilm removal operations had to be weighed against the limited durability shown by previous attempts, and alternative strategies needed to be evaluated, including a non-intervention option. Therefore, the main aim of this work was to evaluate the potentialities of LCA in supporting more informed treatment decisions for the case study and explore the challenges connected to its application for the assessment of three biocidal/cleaning treatments and a non-intervention option.

2. Materials and Methods

2.1. Casa a Ponente and Tested Areas

The *Casa a Ponente (West House)* dates back to the first half of the 17th century [49] with a building and conservation history that is poorly documented. The plastered wall under study has a north–east orientation and faces a botanical garden (Figure 1). It recently underwent a conservation intervention also aiming to remove a patchy green patina caused by SAB colonization. The biocidal treatment succeeded in removing the SABs but was not long-lasting. A few months after the treatment, the plastered wall experienced extensive biological recolonization. An area of such recolonization was selected for this study (Figure 1).

Figure 1. General view of *Palazzo Rocca* and the *Casa a Ponente* (white square), Chiavari (Italy). The yellow frame indicates the pilot area experiencing recolonization and is selected for the biocidal treatment applications and LCA evaluation.

2.2. Biocidal Treatments and Pilot Areas

Five different areas representative of various surface conditions were selected on the plastered wall. Three biocolonized areas of about 90 cm^2 with comparable biocolonization coverage have been identified and treated with commercial biocidal products, chosen based on the experience of the conservator responsible for the general conservation intervention (Table 1). The biocidal treatments were the following:

- Preventol RI 50 (IMAR ITALIA, Rome, Italy). A commercial biocide based on benzalkonium chloride. It has a broad-spectrum chemical biocidal effect, and it is widely used in biocleaning interventions [20,50];
- Essenzio (IBIX BIOCARE, Lugo, Ravenna, Italy). A mixture of essential oils (EOs), with oregano oil as the main component [51]. EOs are natural compounds that have gained recognition as potential biocides due to their strong antimicrobial activity, and their use in the field has increased as a supposed "greener" alternative to conventional chemical biocides [52,53];
- Hydrogen peroxide (Faichim s.r.l., San Giovanni Lupatoto, Verona, Italy). A well-established biocidal treatment with some known potential drawbacks, including surface discoloration (i.e., whitening) [38,50].

Chemicals such as benzalkonium chloride and hydrogen peroxide employed in this study have been extensively used in the past and are still widely employed by conservation practitioners despite some known disadvantages. In this study, they are used as reference conventional treatments to be compared against plant-derived biocides more recently introduced [54,55]. Among the formers, Essenzio has been proposed as a possible alternative to traditional chemicals by previous studies [56–58]. Treatment application procedures were defined according to manufacturer technical datasheets, when available, with adaptations based on the conservator's experience. All treatments were applied by brush for better control of the amount of product applied, according to common practice in laboratory and in situ treatment studies [59,60]. It is important to underline that the main purpose of the work was to evaluate the applicability and potentialities of the LCA tool in the context of biofilm management in cultural heritage conservation.

Table 1. Qualitative and quantitative information related to the treatment application.

Area	Chemical	Active Substance	Concentration	Volume [mL/cm^2]	Application Method
A	Preventol RI 50	Benzalkonium chloride	10% (in water)	0.30	By brush/3 repeated applications (2nd after 20 min, 3rd after few days)
B	Essenzio	Essential oregano oil	as provided by the supplier	0.30	By brush/3 repeated applications (2nd after 20 min, 3rd after few days)
C	Hydrogen peroxide	Hydrogen peroxide	130 volumes	0.10	By brush 20 min after application, the surface was cleaned with wet sponges (water) and brushed again (rigid bush)

Two additional areas were selected as a control: a biocolonized one where no treatment was applied (NT) and an uncolonized (NC) one (Figure 2).

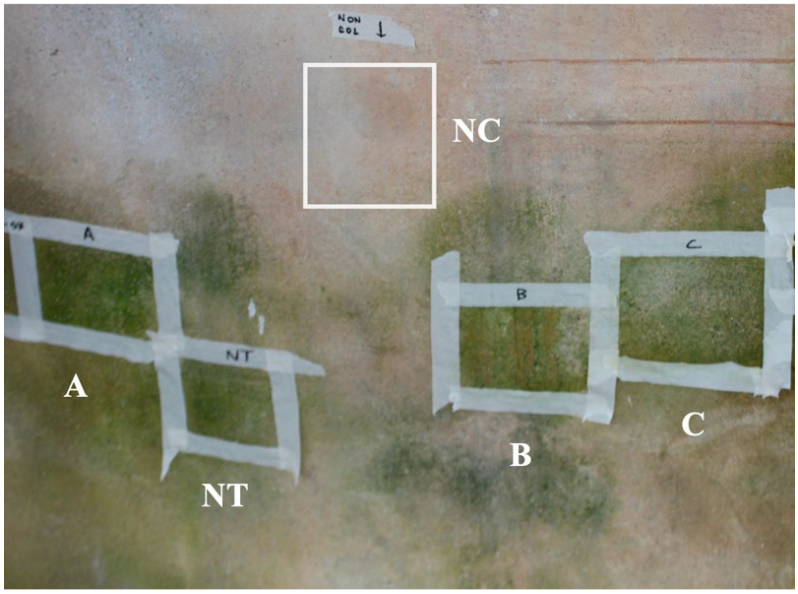

Figure 2. Image of the pilot areas of the Casa a Ponente's plastered wall for monitoring the different surface conditions: A = Preventol RI 50, B = Essenzio, C = hydrogen peroxide, NT = untreated biocolonized, NC = uncolonized. The average surface of each area is around 90 cm^2.

2.3. Characterisation Methods

The on-site observation and documentation of the plaster surface were performed using a DinoLite Premiere AM7013MT portable digital microscope (Torrance, CA, USA) equipped with a color CMOS sensor and a white light-emitting diode (LED) illumination. The percentage of colonized surface (% of biofilm coverage) was used to evaluate the treatment efficacy. Percentage values were calculated with the freeware software GIMP (v. 2.10.32) (GNU Image Manipulation Program, Berkeley, CA, USA) by processing 10 digital microscopy images per area.

Colorimetric measurements to evaluate the efficacy in SAB removal and the chromatic alteration induced by the treatments [61,62] were carried out using a Konica Minolta CM600D VIS-light reflectance spectrophotometer (Tokyo, Japan) in the 400–700 nm spectral range, equipped with an 8 mm aperture and a D65 standard illuminant at 8°. Both SCI and SCE conditions (specular component included and excluded) were recorded and 25 measurements per area were taken. The large number of repeated measurements per area were selected to account for the substrate heterogeneity, as recommended by the current standard UNI 15866:2010 [63]. The results were expressed in the CIE L*a*b* standard color space [63], which represents each color by means of the three parameters L*, a*, and b*. In this study, a* and b* are the most relevant chromatic parameters for evaluating the treatment's efficacy, and they are associated with changes in color saturation ranging from red to green and yellow to blue. The lightness L* was used to detect chromatic alteration of the plaster due to whitening or darkening effects. Pre- and post-treatment differences in these parameters were determined using the following equation:

$$\Delta L^* = L^*_{post} - L^*_{pre}$$

$$\Delta a^* = a^*_{post} - a^*_{pre}$$

$$\Delta b^* = b^*_{post} - b^*_{pre}$$

The global color change ΔE was also calculated as

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + (\Delta a^*)^2 + \left(\Delta b^*\right)^2}$$

From the colorimetric data, the overall biocidal results have been assessed by comparing data collected before (T0) and after treatment application (T6-month) in the same areas (A, B, and C). Measurements of the reference biocolonized (NT) and uncolonized (NC) surfaces, which did not undergo any treatments, were also conducted at the same time intervals of the treatment areas to account for possible contributions to the color variations due to the changed environmental conditions (e.g., relative humidity and temperature during the field campaign). Moreover, total color differences between each treated area (A, B, and C) and the uncolonized reference surface (NC) were also calculated at the 6-month monitoring interval to assess how the different treatment results compare to uncolonized conditions.

One micro-sample of each area was collected with a scalpel for the laboratory analysis. Samples were immediately stored in polyethylene containers and transferred to the lab. Polished cross-sections were prepared by embedding plaster fragments in the UV-cured resin Technovit® 2000 LC (Kulzer GmbH, Hanau, Germany). Micro-samples were studied using a Leica M250C stereomicroscope (Wetzlar, Germany), integrated with a Leica MC 150 HD digital camera for image capture. Polished cross-sections were observed by means of a Leica DM6M optical microscope in dark-field mode, equipped with a color digital camera Leica FLEXACAM C1.

The compositional features of the plaster were investigated by Fourier Transformed Infrared Spectroscopy (FTIR), using a Thermo Nicolet iN10 MX spectrometer with a diamond ATR accessory (Thermo Fisher Scientific Inc., Waltham, MA, USA). The collected plaster powder was previously homogenized in a mortar.

A Mobile Surface Analyzer (Krüss GmbH, Hamburg, Germany) was used for water contact angle (WCA) measurements to study the plaster wettability. Overall, 5 µL droplets of distilled water were dosed onto the target surface and a 60 s video was recorded with a 10 FPS frame rate for each measurement. The WCA was then determined on the first frame after the droplet stabilization, assuming negligible absorption at that moment [64]. WCA and full absorption times of the droplets (when possible) were calculated using the KRÜSS Advance software (v. 1.16). Each measurement was conducted in duplicates and repeated at three different points in the same area (6 measurements/area).

This characterization protocol of the wall surface was carried out before and six months after the treatment to evaluate (i) the overall effect of the biofilm growing on the plaster; (ii) the efficacy of the biocides action against the SABs; and (iii) any potential alteration and damage of the surface induced by the different treatments.

2.4. Sustainability of Biocolonization Removal

2.4.1. Methodology

According to the ISO standards 14040 and 14044 [16,17], LCA is a strategic technique used to identify and quantify the potential environmental impacts associated with a product or a system throughout its life cycle. By definition, the methodology is intended solely for estimating environmental impacts and, in the context of the present case study, does not allow for any evaluation of the qualitative and technical performance of the treatments applied nor of the direct effect on human health associated with emissions occurring during the use-phase. This study is based on the four LCA phases defined in the standards: (i) definition of the goal and scope, (ii) inventory analysis, (iii) impact assessment, and (iv) interpretation of the results [16,17].

2.4.2. Life Cycle Assessment

Goal and Scope Definition

Defining the goal and scope is a key element in LCA studies. The goal defines the intended application and the reasons for carrying out the study, the intended audience, and whether the results are to be used for internal purposes or disclosure to the public. The scope includes several elements related to the structure of the study and its main elements (i.e., functional unit, FU; system boundaries; allocation; and cut-off criteria).

In this study, LCA is applied to perform a quantitative evaluation of the environmental impact associated with biocolonization removal in the field of built heritage conservation. Two main goals drive this effort: (i) to evaluate the environmental impacts of the selected biocidal treatments for biocolonization removal and compare them and (ii) to support decision-makers about the most environmentally preferable strategy, including not removing biocolonization (no-treatment option).

The FU is a fundamental element in LCA defining the reference flow to which all inputs and outputs under assessment are referred throughout the system. The selected FU must ensure a reliable comparison between different products or processes. In the field of built heritage conservation and biocolonization removal, this issue is even more challenging due to the following variables to consider [20]: the chemical and physical characteristics of the substrate, the complexity of the biofilm community, the environmental parameters, and the conservation history of the surface.

Accordingly, the chosen FU is set here as the volume of chemicals (in applicable liquid form) employed to remove at least 90% of the biocolonized layer from 1 cm^2 of the surface (Table 1). In addition, the applied treatments must ensure that surfaces are not damaged beyond acceptable levels. Excessive damage would result in the treatment's exclusion from the comparison, as it would be considered unsuitable. Treatment durability was not considered due to the high influence of external factors (e.g., environmental conditions, weather events). Consequently, the obtained results can be adapted to different situations in which a greater or smaller number of treatment applications may be necessary due to different environmental contexts.

The study follows a "cradle-to-gate" approach, in which the system boundaries include raw material extraction and the production and supply of chemicals and materials used for the treatments. The "use" phase was excluded since the manual application of the treatments was considered to have a negligible impact, and the emissions in the atmosphere associated with the application and final waste treatment were not available as primary information.

A cut-off was applied to the application tools (i.e., brushes) since they are the same for all three applications and thus have an equivalent cradle-to-gate environmental impact. No allocation procedure was applied to the case study.

Life Cycle Inventory

The life cycle inventory (LCI) covers the identification and determination of the material and energy flows related to the analyzed system. It requires the collection of data (through direct measurements, calculations, or estimates) normalized to the FU. In this work, primary data were provided by the conservator, and they include the chemical composition and the amounts of each material used (Table 1). Complementary primary information was then gathered from technical datasheets of Preventol RI 50 and Essenzio and the final products have been modeled with the support of the Reaxys® (https://www.reaxys.com/, accessed on 3 September 2024) platform to simulate the reaction pathways. Regarding the essential oil, the product's technical datasheet indicates that the composition consists of 100% oregano oil. However, the literature reports that some quantities of thyme oil may also be present [51,56,65]. In the proposed study, it was decided to assume that the composition aligns with that reported in the technical data sheet (i.e., 100% oregano). This assumption does not impact the LCA results because the proxy used to determine the environmental impacts of oregano, drawn from Agribalyse database [66], does not show significant differences between the two alternatives [67] (i.e., pure oregano composition vs. blend of oregano as major component with minor thyme). More details about the described approach and data sources are reported in ESI 1. Records drawn from the Ecoinvent database [68] were used to model hydrogen peroxide. Additional background data regarding the raw materials supply, transportation, and production phase of the chemicals were complemented by Ecoinvent [68] and Agribalyse [66] databases, when not available elsewhere.

Life Cycle Impact Assessment

Life cycle impact assessment (LCIA) is the phase where the LCI data are converted into potential environmental impacts through the application of scientific cause-effect models. LCIA consists of several stages, some of which are mandatory (i.e., classification and characterization) [17] and some others are optional. Classification assigns material/energy inputs and outputs to the relevant impact category (e.g., climate change), while characterization quantifies the contribution of each classified input and output to the respective impact categories and it aggregates the results in terms of the reference substance indicator (e.g., carbon dioxide equivalent (CO_2eq) for climate change). Several assessment methods are available and divided into two groups, based on the scope covered: "midpoint" methods that calculate the impacts due to the exhausted substances or damaging emissions and "endpoint" methods that are based on the probable damage that the resource consumption and emission release could determine on human health and the ecosystem [69]. Optional elements of normalization and weighting are excluded from this study.

The LCIA method selected in this analysis is ReCiPe 2016 [70], which includes 18 environmental midpoint impact categories in the evaluation, namely particulate matter formation potential (PMFP), global warming potential (GWP), stratospheric ozone depletion potential (ODP), ionizing radiation potential (IRP), tropospheric ozone formation potential (OFP), tropospheric ozone formation potential (ecosystem, OFP), terrestrial acidification potential (TAP), freshwater eutrophication potential (FEP), marine eutrophication potential (MEP), freshwater ecotoxicity potential (FETP), marine ecotoxicity potential (METP), human toxicity (cancer, HTPc), human toxicity (non-cancer, HTPnc), land occupation potential (LOP), mineral resources depletion potential (SOP), fossil resources scarcity potential (FFP), and water consumption potential (WCP). In accordance with the ReCiPe 2016 methodology, the single score is then calculated by summing the three endpoint categories, human health, ecosystem, and resources, once normalized to the unit of measurement (pts), in relation to the hierarchical perspective. The three categories were calculated through the grouping and weighting procedures outlined in the ReCiPe 2016 methodology using the Simapro software (v. 9.6) (PRé Sustainability, Utrecht, Netherlands). The choice of ReCiPe 2016 is primarily motivated by the broad acceptance of the method in the relevant literature and its wide coverage of complementary impact categories, which allows a thorough overview of the environmental effects, allowing the evaluation of an exhaustive spectrum of environmental impacts. In this work, the hierarchical perspective was adopted for normalization, which is considered the default cultural theory in LCA.

Uncertainty Analysis

Uncertainty evaluation was performed by means of Monte Carlo simulation. In total, 10,000 runs were carried out to determine how the intrinsic variability of the parameters and the quality of the data used in the modeling may affect the system outcomes both for midpoint and endpoint categories. The pedigree data quality matrix [71] was applied to the inventory data for geographical, temporal, and technological representativeness scores to assign uncertainty ranges, as reported in ESI 2.

3. Results and Discussion

3.1. Biofilm Characterisation and Assessment of the Plaster's Conditions Before and After Treatments

It has to be underlined that all the plaster's characterization results had to be analyzed taking into account the inherent substrate heterogeneity. Therefore, the analysis of the colorimetric data was performed considering the irregular distribution of the aggregate, which, in this case, is mainly composed of dark-colored grains surrounded by a much lighter binder phase [72]. Similarly, the substrate's roughness and chemical heterogeneity are non-ideal conditions for standard WCA measurements [73,74].

The plaster composition of the wall of *Casa a Ponente* is characterized by the presence of calcite, attributable to the carbonated lime binder, and quartz-silicate-based aggregates.

The results after cleaning confirmed that all treatments did not induce any change in the overall composition of the plaster.

The in situ microscopic observations of the plastered wall provided preliminary indications of the morphological features of the uncolonized surfaces with respect to the adjacent ones subjected to SAB colonization (Figure 3 NC, NT). In areas covered by the SAB, its distribution appears to be influenced by local mineralogical features. SAB is relatively uniform over the binder-rich areas, while it becomes very thin or absent over the aggregates (Figure 3 NT). Only epilithic growth with a thickness of around 40 mm was observed in the biocolonized area (Figure 3 NT, below), whereas areas identified as uncolonized after visual and in situ digital microscopy observation showed traces of endolithic growth (Figure 3 NC, below) in cross-section observation. Based on microscopy observation, both the epilithic and endolithic growths were not associated with the formation of cracks or fissures or showed any damage patterns consistent with loss of cohesion.

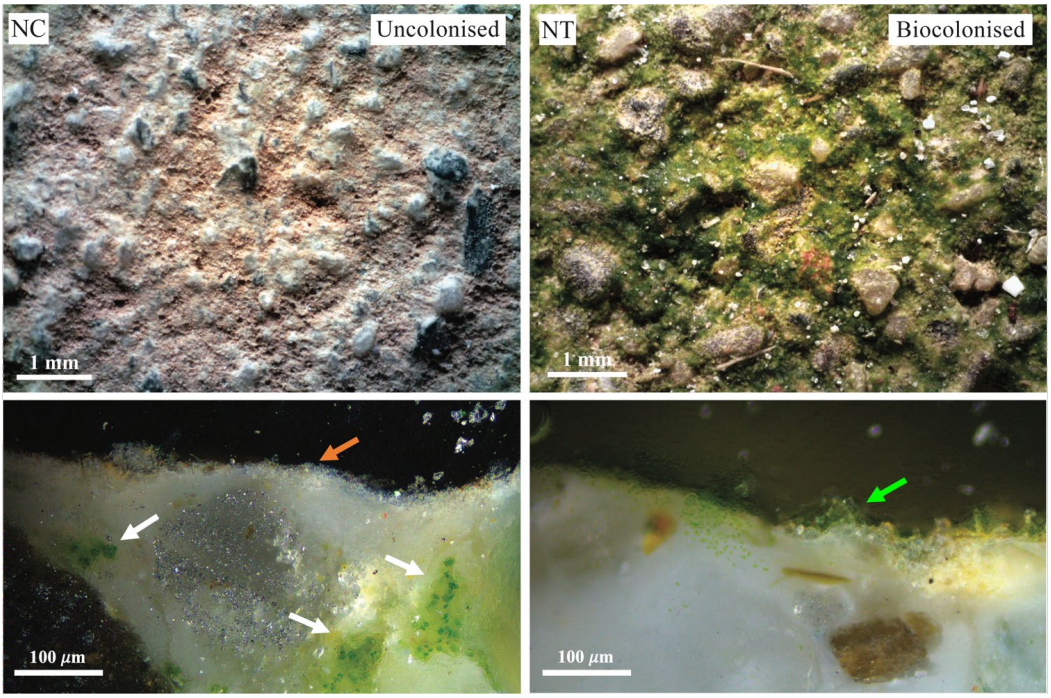

Figure 3. (**above**) In situ microscopy documentation of (NC) uncolonized and (NT) biocolonized area of the plastered wall of Casa a Ponente, and (**below**) optical microscopic observation of cross-sections, respectively, of the uncolonized and biocolonized areas: the white arrows indicate the endolithic SAB, the orange arrow indicates the plaster superficial binder layer (16.5 μm ± 3.4 thick), and the green arrow indicates the epilithic SAB.

Importantly, the results showed that not all treatments were able to completely remove the green SAB that was still partly preserved particularly in the binder-rich areas surrounding the aggregates, as indicated by the arrows in Figure 4A,B. In these areas, the biocolonization was already more intense before the biocidal treatments due to favorable conditions, such as mineralogical composition, a more porous microstructure with respect to the quartz-rich aggregates, and geometric features. The binder-rich areas showed a slightly receded front compared to aggregates, which are more exposed and, therefore, provide some protection to the former from rain runoff. Moreover, because of such geometrical features, the mechanical action to remove SAB in the binder-rich area following the biocide application can be less effective. This issue has been observed in previous studies, in which treatments were found to be not always effective in completely removing the SAB from microcracks, porosities, and along the grain boundaries [40,41,75]. This residual superficial biomass may have a role in subsequent recolonization depending also on the location and the climatic condition of the different sites (i.e., a garden environment easily promotes new

biocolonization) [75,76]. It is important to underline that the evaluation of the efficacy of the treatments was not the main aim of this study and that further tests would be necessary for a comprehensive comparison of the efficacy of the different treatments. As previously specified, all treatments were applied by brush for better control of the amount of product applied. The reported results, therefore, are specific to the procedure and amount of product used for the biological removal of the biofilm of *Casa a Ponente* and are not generalized conclusions on treatments' efficacy and possible alterations of the substrates. In most cases, the plaster morphology appeared altered after treatment (Figure 4A–C) if compared to the uncolonized reference (Figure 4 NC), inducing further recession of the binder-rich areas. As a result of this, the aggregates appeared more exposed and visible than in the uncolonized surface, which was confirmed by the colorimetric analysis reported below. The mechanical cleaning step with a rigid brush and a sponge following the biocide application caused the loss of some plaster (Figure 4A–C). This is in agreement with results reported in the literature, where an increase in roughness induced by biocidal treatments has often been observed because of mechanical stresses and the resulting erosion induced by brushing [39–41,76].

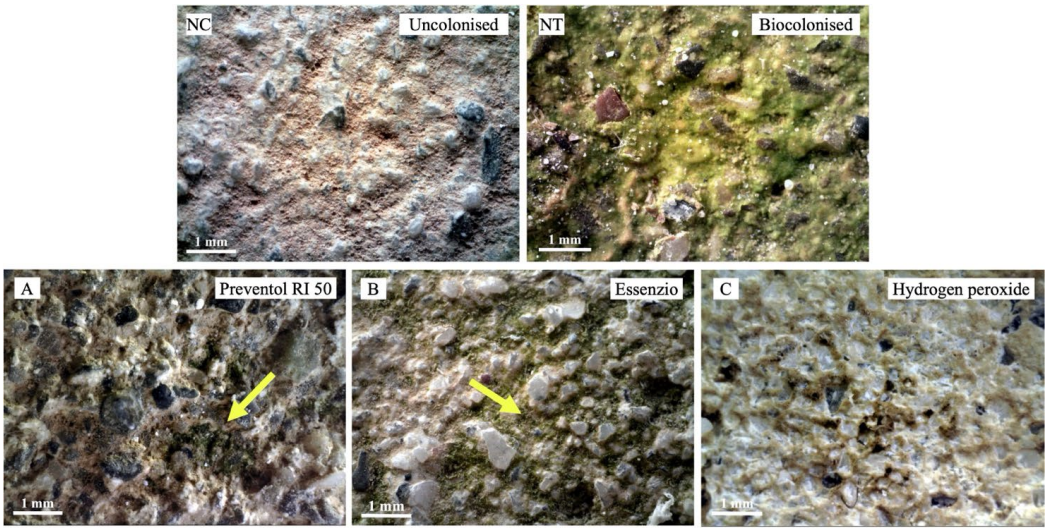

Figure 4. On-site microscopy documentation of reference uncolonized plaster (NC), biocolonized (NT), and treated (**A**–**C**). Arrows indicate the areas where the SAB was still preserved after the treatments.

The efficacy of the biocides was evaluated by image analysis of the in situ digital microscopy documentation. The initial biofilm coverage expressed as a percentage of the total surface was 85.8% ± 2.2 for the biocolonized reference area (NT). The extent of biocolonization was reduced by more than 90% by Preventol RI 50 and hydrogen peroxide, with hydrogen peroxide < Preventol RI 50 and by around 60% by Essenzio (Figure 5). Considering the application conditions, Preventol RI 50 proved to be the most effective biocide in terms of biofilm removal. It is important to emphasize that the aim of this characterization was to allow comparability of the alternatives, by guaranteeing acceptable biofilm removal and minor damage to the surface. Further investigations would be required for a more comprehensive comparison of the tested products in terms of both efficacy and potential damage to the treated substrates.

Biocides based on quaternary ammonium salts have been reported to be the most effective in a number of works [62,77] while the so-called "green" biocides such as essential oils showed, in some cases, lower efficacy in biofilms removal especially in the long-term [77,78]. It is worth noting that, according to the conservator's experience, a fourth application of Essenzio two weeks after the first one could improve biocidal efficacy. This additional step was not performed for the case study. Based on this assumption, it is reasonable to assume that a higher final bioremoval result could have been reached with a

fourth application. In light of this, it was considered that all treatments met the minimum requirements for application, and it was worth including Essenzio in the LCA evaluation.

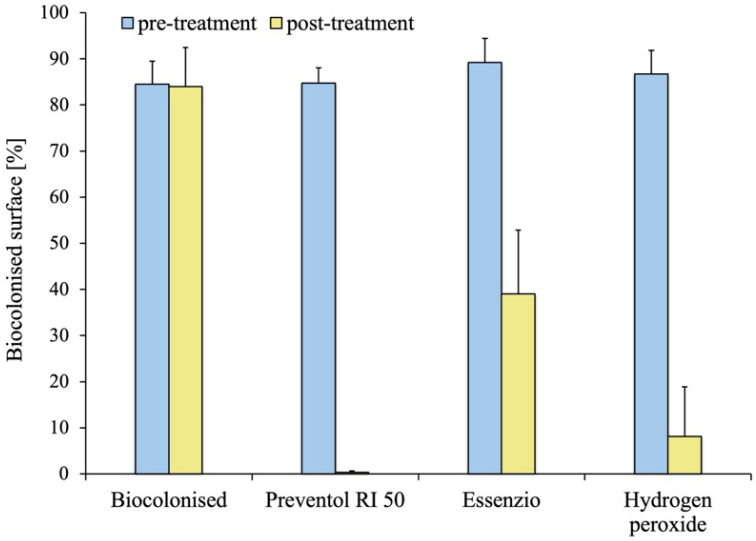

Figure 5. Percentage of biocolonized surface with respect to the total surface of each pilot area before (T0) the biocidal treatment and 6 months after (T-6month).

The colorimetric data provided complementary information on the residual presence of biofilms on the treated surface and on the overall cleaning results. Table 2 reports the detected colorimetric data comparing (a) each area before and after treatment (monitoring intervals T-6month compared to T0) to investigate the effectiveness of biocolonization removal, including the reference biocolonized (NT) and uncolonized (NC) areas to detect any additional contributions to color change due to changed environmental conditions (Supplementary Materials, Figure S1) and (b) the treated areas (A, B, and C) compared to the reference uncolonized one (NC) were all evaluated 6 months after the treatment application to understand the biocides' ability to restore surface color conditions closer to the uncolonized plaster upon biofilm removal. In this study, Δa* was the most relevant parameter associated with the epilithic colonization removal effectiveness. In Table 2a, a shift toward a* positive value was observed. It highlighted the effectiveness of Preventol RI 50 and hydrogen peroxide and the weakest effect of the Essenzio. Positive values of Δa* indeed indicated a reduction in green tone (Table 2a) and a similar trend was observed on the treated granite cloister of the Monastery of San Martino Pinario (Santiago de Compostela, Spain), previously colonized by a phototrophic biofilm [9,62]. An increase in L* values can be only partly associated with the efficacy of biofilm removal. The treatments have indeed uncovered the aggregates, removing the biofilm and a part of the whitish binder layer. Thus, a greater or lesser brightening cannot be directly associated with biofilm removal. Furthermore, Δb* remained constant for the biocolonized area and the areas treated with Preventol RI 50 and Essenzio but decreased for the areas treated with hydrogen peroxide. For this area, the increase in L* values and the decrease in b* values indicated an overall whitening of the surfaces and confirmed the chromatic alteration of the plaster caused by the treatment: ΔL* and Δb* were, respectively, the highest and the lowest despite the incomplete removal of the SAB. Different climatic conditions during the two on-site investigations and the ones of the days before could have affected the number of microbial pigments [79], also explaining the small colorimetric variation in the biocolonized area. This effect was limited for uncolonized areas due to the little impact of the relative humidity on the color of mineral surfaces. Since a* and b* values for the area treated with Preventol RI 50 were very close to the ones of the uncolonized (NC) area (Δa* and Δb* around 1), Preventol RI 50 proved to be able to bring the color coordinates closest to the reference uncolonized area, although the surface remains slightly darker

compared to the uncolonized one, as indicated by the ΔL* value (Table 2b). With Essenzio treatment as well, the surface resulted in being darker than the uncolonized one. In this case, a much more marked variation in the a* component due to persisting biocoloniziation after treatment was observed. Variation in the L* coordinate is very limited for hydrogen peroxide, which, on the other, hand has the greatest Δb*, corresponding to a reduction in the yellow component.

Table 2. Results of the colorimetric analysis. ΔL*, Δa*, Δb*, and ΔE* were calculated for (**a**) in the same area before (t0) and after treatment (T6-month), for (**b**) between each treatment area and the reference uncolonized one (NC) at 6-month monitoring interval. Bold numbers indicate the highest value for each colorimetric variation.

(a)	**Post-Treatment (T6-Month)—Pre-Treatment (T0)**			
	ΔL*	**Δa***	**Δb***	**ΔE**
Biocolonized	6.04	3.29	−6.51	9.47
Preventol RI 50	12.81	**8.15**	−6.30	16.44
Essenzio	10.43	3.58	−5.90	12.50
Hydrogen peroxide	**19.38**	7.28	**−10.92**	**23.41**
Uncolonized	0.31	0.00	-0.91	0.96
(b)	**Post-Treatment (T6-Month)—Uncolonized (NC, T6-Month)**			
	ΔL*	**Δa***	**Δb***	**ΔE**
Preventol RI 50	**−8.21**	−0.40	−1.16	8.30
Essenzio	−7.22	**−4.28**	−0.69	**8.43**
Hydrogen peroxide	−1.30	−2.52	**−2.71**	3.92

Previous investigations have shown that the presence of SAB on the plastered wall of the *Casa a Ponente* significantly increased the contact angle and reduced the water absorption rate (Figure 6) [48]. The biocolonized surfaces presented an initial WCA of 123 ± 17° and a drop absorption time > 60 s. While the literature is extensive on the application of WCA for assessing protective treatments of built heritage surfaces [80], field applications of the method are still limited [48,81]. In this study, portable contact angle measurements were used for the first time to assess the effect of biocidal treatments on plaster wettability and water absorption behavior. Overall, all biocides changed the surface wettability by reducing the initial contact angle values compared to biocolonization conditions (Table 3). The most relevant change occurred upon treatment with hydrogen peroxide, leading to WCA values comparable with the uncolonized area, despite the residual presence of SAB as indicated by digital microscopy observations. This peculiar behavior can be explained by considering the almost complete removal of the outermost plaster layer due to brushing, which exposed a fresh surface more similar to the uncolonized one. In the area treated with Essenzio, despite the residual biofilm presence indicated by the colorimetric and surface coverage data, a significant reduction in the WCA was also observed, although the mean value was still >90°. Similarly, a WCA mean value around 100° was measured in the area treated with Preventol RI 50, which was the most effective biocide in removing the biofilm. In this case, the presence of residual EPS on the surface, which might go undetected after digital microscopy observations and color measurements, can also affect WCA when biocolonization seems mostly removed. Therefore, WCA measurement can indeed represent a key complementary method to assess biocolonization removal by targeting features that could go undetected with other on-site investigations.

The treatments had an even greater impact on liquid water absorption. Drop absorption times of all treatments were reduced by at least half compared to the reference biocolonized area, for which absorption times were always >60 s (Table 3 and Figure 6); this means that the drop was not completely absorbed within the 60 s defined as the recording

time. Water absorption is a key indicator in all surface treatment results, and it is still challenging to measure on-site. So far, contact sponge tests have been used to investigate alterations in water absorption associated with biocidal treatments [77,78]. However, such a method requires larger contact surfaces and water volumes than the portable micro-drops-based WCA one [82]. Consequently, it can have a greater impact on the tested surface, particularly in the presence of water-susceptible biofilms, and requires larger testing areas allowing for an adequate number of replicated measurements. Therefore, in situ WCA measurements via micro-drops represent a promising and less invasive option for studying water-related properties of real architectural surfaces.

Table 3. The average values and standard deviations of water contact angle (WCA) and drop absorption time of the treated areas compared to the biocolonized and the uncolonized ones. Drop absorption time > 60 s means that the drop was not completely absorbed within the 60 s defined as the recording time.

	WCA [°]			Drop Absorption Time [s]		
Biocolonized	123	±	17	>60		
Preventol RI 50	109	±	15	24	±	8
Essenzio	96	±	9	18	±	4
Hydrogen peroxide	66	±	7	9	±	2
Uncolonized	46	±	17	9	±	4

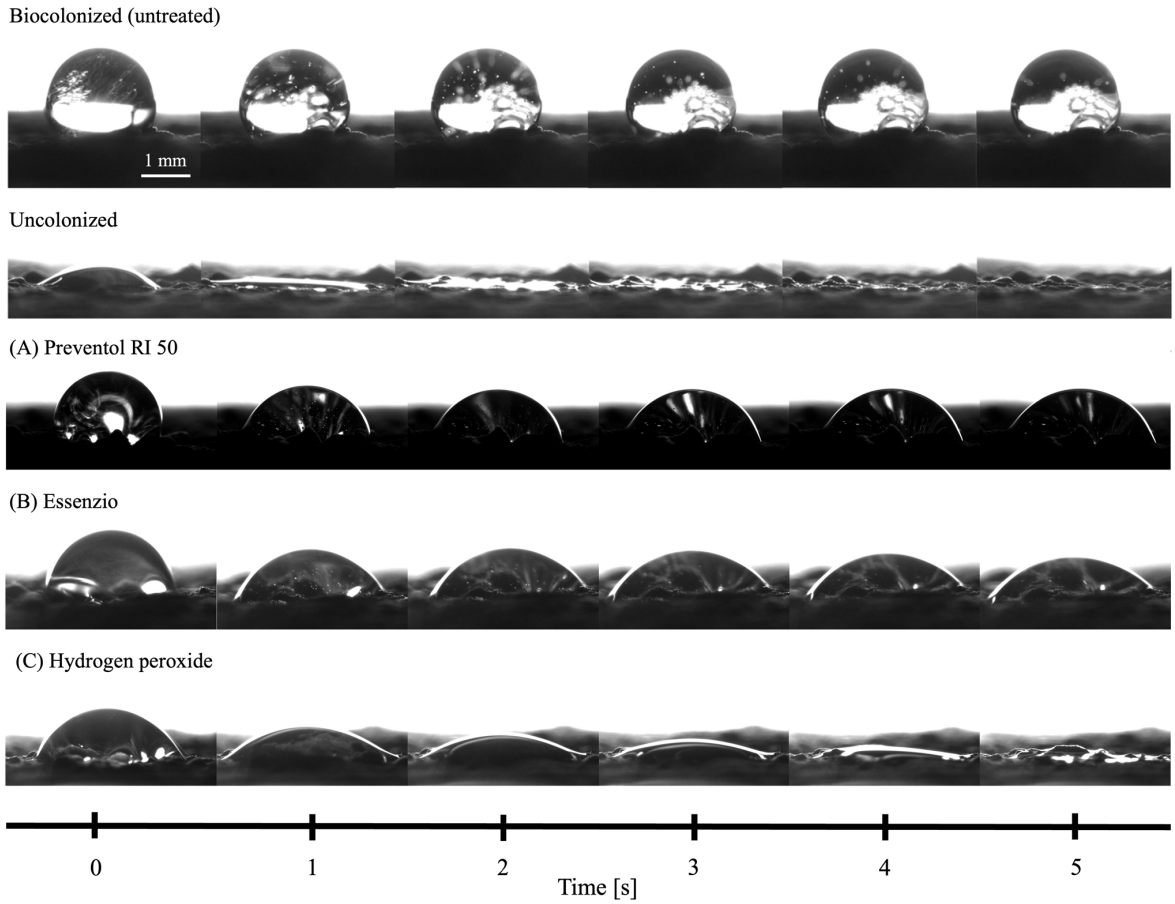

Figure 6. WCA video frames corresponding to the first 5 s after drop dosing onto the biocolonized and the uncolonized area and the one treated with (A) Preventol RI 50; (B) Essenzio; and (C) hydrogen peroxide.

3.2. LCA Results

Historical buildings constitute a large part of the Global Cultural Heritage, and their preservation has an impact, not only from a cultural, social, and economic point of view but also from an environmental one due to the required periodical conservation works [20]. These aspects have been also mainstreamed by the United Nations in the Sustainable Development Goal "Sustainable Cities and Communities" within the "Agenda 2030" plan of action [83]. Cities play a crucial role in achieving this goal because (i) more than 60% of humanity presently lives in cities, and this number is expected to grow [83] and (ii) up to 80% of the European buildings that will be inhabited by 2050 have already been constructed [84] and require maintenance and/or renovation.

In Figure 7 (and Supplementary Materials, Table S6 of the ESI), the three treatment alternatives are compared at the midpoint (a) and endpoint (b) levels. All treatments ensured an acceptable outcome in terms of biofilm removal and negligible or quite limited damage; therefore, all three were included in the LCA evaluation. The results are normalized to 1 cm^2, and the LCA -results are presented on a relative basis: for each impact category, the bar of the most impacting alternative was set at 100%, with the other bars being scaled to it proportionally. The impact values are reported at the top of each bar. Overall, the potential environmental impacts associated with the volume of the chemicals employed to achieve an acceptable cleaning efficiency when treating 1 cm^2 of biocolonized surface provide a relative preference for hydrogen peroxide, as exemplified by GWP, for 15 out of 18 midpoint categories. However, as reported in Section 3.1, treatment with hydrogen peroxide was associated with surface alteration of the plaster, making it arguable whether this treatment can be appropriate. Benzalkonium chloride was demonstrated to be less preferable than oregano oil for its potential contribution to categories related to toxicity (i.e., FETP, METP, and HTPnc) and FFP. In contrast, oregano oil showed the highest impact for the remaining 13 categories. It is emphasized that categories evaluating the toxicity (i.e., HTPc, HTPnc, FETP, METP, TETP) do not describe the toxicity effects associated with the application of the biocide, but the comprehensive emissions occurring in all the phases of the life cycle of the analyzed alternatives. Toxicity effects on the operator during the treatment application should be investigated through the application of risk assessment, which lies outside the scope of the present study [85,86]. Furthermore, as stated in the Goal and Scope Definition, the application phase (i.e., use) is excluded from the system boundaries due to a lack of information.

The endpoint results mostly confirmed the midpoint outcomes, identifying the oregano oil as the main impacting treatment alternative for all the categories examined, i.e., human health, ecosystem quality, and resource scarcity. These findings remark that the employment of nature-derived products is not always a synonym for lower environmental impact [87] than synthetic products. According to the Agribalyse database [66], the environmental hotspots of oregano oil mainly occur during the cultivation phase of the oregano plant, with the extraction phase representing only 3% of the single score and 6% of the GWP impacts. The main contributing phases of benzalkonium chloride and hydrogen peroxide are the synthesis of the chemicals. The LCA results should be intended as a first indication of environmental preferability since absolute ranking is seldom universally definable. LCI models and LCIA results should be revised wherever there is an optimization of the described removal procedures that involve a significant change in the volumes used or a different synthesis route applied to the investigated chemicals (e.g., oregano oil derived from biowaste rather than from direct plant cultivation). Uncertainty analysis confirms the trends observed for the midpoint results, except for the IRP and WCP categories. However, since such uncertainty values are observed only for these two categories, it could be inferred that they do not depend on the inventory data quality. These two categories are also involved in the computation of the human health endpoint, making it challenging to assess the environmental preference for such an endpoint unambiguously. Uncertainty analysis results are reported in ESI 3.

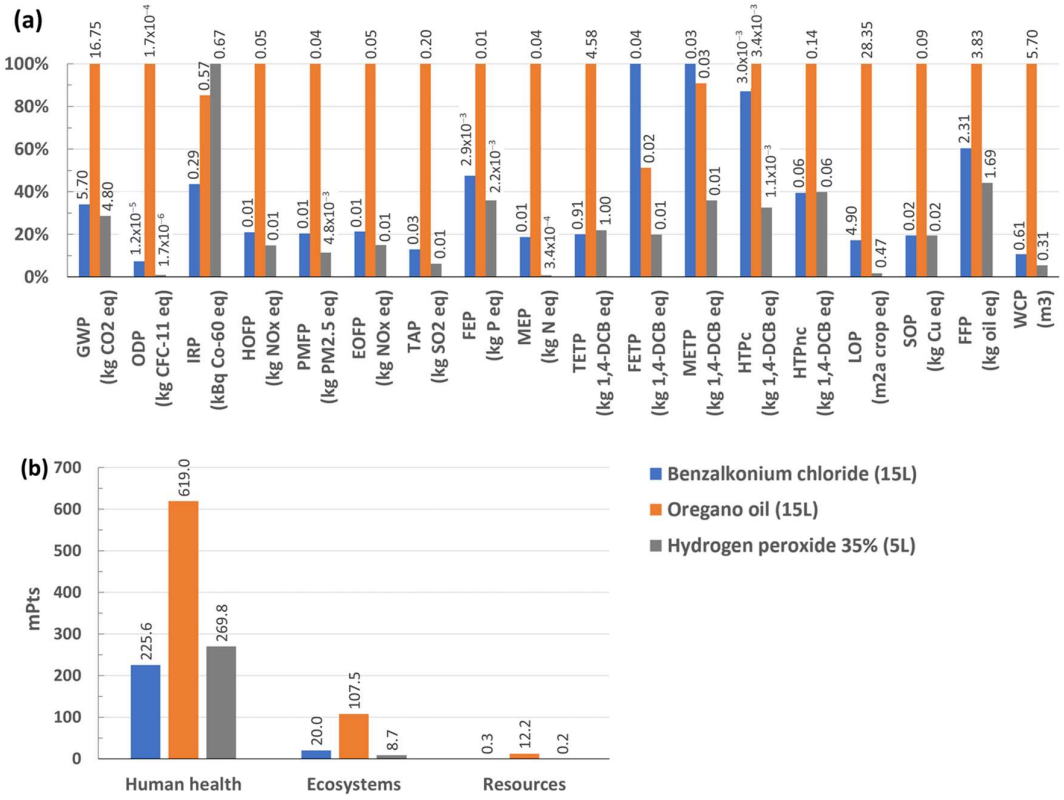

Figure 7. Life Cycle Impact Assessment at the midpoint level (**a**) and endpoint level (**b**) of the three alternatives according to the ReCiPe 2016 method.

As known, LCA results are specifically related to the selected functional unit. This aspect is particularly critical in the field of cultural heritage, as it requires establishing parameters or thresholds that enable treatments to be evaluated on a comparable level (e.g., the minimum percentage of biofilm removal, acceptable color change on the surface, damage limits, and so on). It follows that treatments failing to meet minimum requirements must be excluded from the LCA evaluation, as they are considered effectively unsuitable and should not be accounted for in the assessment. Treatments being compared must always ensure that biofilm removal does not cause material damage, thereby upholding the primary objective of cultural heritage preservation. In this context, the proposed study also aims to provide guidelines to facilitate future applications of LCA in the conservation of cultural heritage.

In addition to the three treatment alternatives previously described, a no-treatment option should also be considered. The overall characterization results showed that the SAB on the recolonized plastered wall of *Casa a Ponente* of Palazzo Rocca in Chiavari is mainly epilithic and its growth does not appear to be associated with cracks or fissures formation, nor linked to loss of cohesion. Moreover, the WCA results showed that the SAB induces near-hydrophobic characteristics on the surface. A no-treatment option, and thus no usage of products for bioremoval, would result in a zero-environmental impact, making it an attractive choice both from a conservation and sustainability perspective. This would fit into the criteria of safety, health, and minimum intervention that drive the conservation of built heritage [20,42]. On the other hand, when dealing with objects of cultural interest, the aesthetical impact of SABs generally plays a critical role in treatment decision-making. So far, the presence of a bioprotective visible patina has only been deemed acceptable in very specific contexts (e.g., archaeological sites, monumental cemeteries, and vernacular architecture).

For this case study, preserving the historical plasters is the main priority, and LCA can be considered a complementary tool supporting informed decision-making on the most

effective and environmentally sustainable treatment approach. As common in conservation practice, a suitable balance must be identified between a satisfactory outcome in terms of treatment quality and cost-effectiveness and minimized environmental impact associated with the type of intervention. For instance, LCA can support practitioners in weighing options with comparable high-quality results based on the lowest environmental impact. Additionally, LCA's ability to assess the entire life cycle of the treatment materials highlights the advantages and potential shortcomings of using alternative options obtained by non-conventional sources (i.e., biomass or waste) that could be further explored in conservation.

4. Conclusions

The main aim of this work was to evaluate the potentialities of LCA in supporting more informed treatment decisions for the case study and explore the challenges connected to its application for the assessment of three biocidal/cleaning treatments. The treatments being compared must show adequate efficacy and always ensure that biofilm removal does not cause material damage. It follows that treatments failing to meet the minimum requirements must be excluded from the LCA evaluation, as they are considered effectively unsuitable and should not be accounted for in the assessment.

All tested treatments were considered adequate to be included in the LCA assessment. Preventol RI 50 (benzalkonium chloride) was found to be the most effective treatment in biofilm removal. Hydrogen peroxide also had a removal efficacy above 90%, but it caused some physical alterations to the plaster surface. Essenzio (essential oil of oregano) has the lowest efficacy, and a higher number of applications is needed to obtain good results in terms of biocolonization removal. In this case, it is important to underline that the evaluation of the efficacy of the treatments was mainly aimed at verifying that they could guarantee acceptable biofilm removal and minor damage to the surface. Further investigations would be required for a more comprehensive comparison of the tested products in terms of both efficacy and potential damage to the treated substrates.

The LCA analysis allowed us to compare the environmental impact of the biocidal treatments. The environmental impacts of treatments are proportional to the volume applied, which is dependent on the removal efficiency of the chemicals employed. Concerning the volumes applied in the case study, the findings show a generally better environmental performance for hydrogen peroxide and benzalkonium chloride (including the GWP category and the single score) over the essential oil. However, the environmental impact associated with the use of Essenzio may be reduced by intervening in the cultivation phase of oregano and the potential additional components of the blend (thyme).

The option of preserving the biofilm, i.e., no-treatment option, should also be taken into account due to the resulting zero-environmental impact, and it is supported by negligible/low microbially-induced deteriorating effects and an environmental context (i.e., NE exposure of the plastered wall and proximity to the botanical garden) providing particularly favorable conditions for recolonization. However, further investigations and long-term monitoring of the SAB's evolution will be needed to better understand its impact on the historical plasters.

The complexity of built heritage sites, the lack of dedicated resources for the application of LCA to historic preservation treatments, and the heterogeneity of materials and surface conditions require a case-by-case approach that also depends on the specific environmental and cultural contexts. LCA proved to be effective in better understanding the environmental impact of different conservation treatments in the case study, all initially deemed suitable by conservation professionals, and has the potential for broader application in similar contexts, supporting decision-making on the most effective and sustainable biocidal strategies. LCA is becoming an important supporting tool to assess the sustainability of conservation treatment materials and could be complemented by integrating information from the application of risk assessment methodologies, providing additional data on the direct effects on the operator's health and safety.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/heritage7120318/s1, Figure S1: ΔE values before (t0) and after treatment (T6-month) of the different pilot areas; ESI 1: Preventol RI 50, Essenzio and hydrogen peroxide inventories, composed by Tables S1–S4; ESI 2: Pedigree matrix, composed by Table S5; ESI 3: Environmental impacts, composed by Table S6a–c. References [88–92] are cited in the Supplementary Materials.

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