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Sustainable Protective Strategies and Biocide Applications in the Restoration of Palazzo Centrale Dell'Università, Catania, Italy

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Abstract: The present work discusses the challenges and approaches involved in conserving cultural heritage (CH), specifically focusing on eco-friendly conservation methods and the management of biodeterioration. It highlights the need for innovative protocols that align with green conservation criteria, aiming to replace traditional, potentially harmful practices with sustainable alternatives. This study is based on the role of nanomaterials like halloysite in developing protective coatings for CH materials. Additionally, the issues of biological colonization on CH assets, the difficulties in controlling environmental factors affecting biodeterioration, and the use of direct methods in outdoor conservation were also evaluated. This work is specifically focused on a case study: the "Palazzo Centrale dell' Università" in Catania (Italy), where alternative, eco-friendly protectives and biocides have been tested on Hyblean limestones. After a preliminary study of the lithology and the forms of degradation which affect the whole monument, laboratory tests were carried out using the newly developed protective coatings on several types of Hyblean limestone in order to assess their efficacy and their impact on the stone. Furthermore, cleaning operations were also tested on-site by comparing an eco-friendly biocide with commercial counterparts in order to evaluate the effectiveness of the products and establish an efficient restoration protocol for future projects.

Keywords: cultural heritage; biodecay; nanoprotective biocides; limestones; aging test

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1. Introduction

The preservation of cultural heritage assets involves challenges in adopting eco-friendly conservation practices, particularly in developing innovative protocols that meet green conservation criteria. These protocols aim to replace conventional, potentially harmful methods with sustainable alternatives [1].

One aspect of this addresses the issue of biodeterioration of CH monuments and artifacts, which necessitates both an understanding of the causes and the development of methodologies to control unwanted biological growth [2].

To control biological colonization, direct methods (such as mechanical, physical, chemical, and biological) are often favored, especially in outdoor environments, due to difficulties in managing environmental factors like pH, humidity, temperature, and light [1,3–6]. In this scenario, all interventions have to be carefully planned in order to reduce all the factors that influence medium- and/or long-term conservation. While biodeteriogen treatment is a common restoration practice, creating efficient protocols remains a challenge for restorers. Regarding biocides, which are chemicals used to eliminate undesirable organisms, traditional products have been widely employed and still remain the most used practical solution [1,7], despite the potential risks to human health and the environment, as

EU reference databases indicate (https://echa.europa.eu/home, accessed on 7 July 2021). These products are effective against a broad spectrum of organisms and they can easily be removed after cleaning. However, recent research suggests that some of these products may also interfere with the interface of stone materials and, thus, there is growing interest in biologically derived biocides, like essential-oil-based solutions, as alternatives to traditional ones [8–10]. Among the traditional biocides, Preventol R180 (CTS), Rocima TM 103 (CTS), and Biotin R (CTS) are the chemical compounds most used by restorers and conservators in Italy [11]. Even though some ingredients of these products are now considered toxic, they are applied at low concentrations, which do not create significant risks for humans or the environment.

Furthermore, the removal of existing biomass is not sufficient to reduce the risks of unwanted recolonization. Indeed, after cleaning, the prevention procedure has to be planned in order to avoid new recolonization and/or to slow down the growth rate of biofilms [12–14]. This effect can be reached by the application of coating products.

In this respect, protective measures and nanotechnologies, specifically nanomaterials such as halloysite, have shown great potential in developing innovative methodologies for CH conservation. A detailed overview on nanostructured protective agents can be found in [15]. Nanostructured protective materials, when incorporated into coatings, exhibit good penetration, significantly contributing to the safeguarding of damaged stone substrates [16]. Besides a good water repellence feature, a protective coating should also exhibit good resistance to aging and, at the same time, induce negligible colorimetric variation on the treated surface.

In particular, protectives are substances with specific optical characteristics which are applied in the form of films on the surface of an artefact so as to protect it. The study and subsequent application of newly developed products are particularly relevant considering that, often, the treatments used in the past have caused damage to the treated surfaces [17]. Some commercial polysiloxane-based protectives pose a particular challenge in this regard, primarily due to their potential to exacerbate the crystallization of salts within the rock structure. This crystallization effect can lead to further damage to the stone materials when these protectives are applied [17].

Halloysite is a biocompatible naturally occurring aluminosilicate that can be extracted from different deposits in several regions. The nanotube size may vary from 50 to 100 nm for the external diameter and from 0.2 to 3 μm for the length [18–23]. Halloysite has received great interest for certain applications in several fields; for instance, there have been reports in the literature on both the thermal and mechanical properties of bioplastics when the nanotubes are homogeneously dispersed. Additionally, the inorganic nanotubes can be loaded with chemically and biologically active species for the design of nanostructured materials to be employed in cosmetic and health technology, food packaging, and for the protection of cultural heritage sites [24–29].

In the framework of the project Advanced Green Materials for Cultural Heritage (AGM for CuHe), which aimed at developing new technological and sustainable materials in the field of restoration, the cloister of the "*Palazzo Centrale dell'Università*" (Catania, Italy) has been selected as a case study for testing alternative and eco-friendly protectives and biocides. The present study was focused at understanding the effect of a protective product, based on halloysite nanoparticles, on limestones used as the building stones at the *Palazzo dell'Università Centrale* which are the most common lithotypes used in the baroque architecture in the "*Val di Noto*" area (Sicily, Italy). In order to assess the protection efficacy and the impact on the stone substrate before the on-site tests, preliminary laboratory analyses, including an accelerated aging test, were conducted on selected limestone from quarries. In parallel, since a significant portion of the case study shows considerable damage from biological degradation, tests with various biocides for cleaning operations were also conducted. The effectiveness of an essential-oil-based biocide was compared to other commercial ones with the aim of identifying an eco-friendly and effective solution that can be integrated into the operational protocol for future restoration endeavors.

Indeed, the approach to CH restoration interventions involves multiple steps, from the description of the building and the environment, to diagnosis (the assessment of building materials, their properties, and deterioration), and finally to therapeutic steps (choosing restoration treatments, and executing and controlling preservation measures). Establishing such a standardized operational protocol is crucial for future restoration projects.

Palazzo dell'Università Centrale: Historical Background

The *Palazzo Centrale dell'Università*, located in the old town of Catania, is part of a UNESCO World Heritage site. The palace dates back to 1696 and was reconstructed in the Baroque style by the architects *Francesco* and *Antonino Battaglia*, along with *Giovan Battista Vaccarini*, following the seismic event of 1693.

Currently, the palace serves as the headquarters of the rector and central administration offices of the University of Catania, as well as the *Giambattista Caruso* Regional Library [30]. For its restoration, similar to many other buildings of that epoch, volcanic basalt rocks from Mount Etna and limestone from the Hyblean region were used, representing the distinctive bichrome characteristic of Etnean Baroque style.

The main façade was constructed in the 19th century by the architect *Mario Di Stefano*. All along the façade, the foundation is covered by massive basalt rocks, while the upper portion is composed of white limestone sourced from various formations in the Hyblean region—south Sicily—(i.e., the Palazzolo Formation, the Mt. Carruba Formation). The inner cloister, comprising two levels of arches and columns entirely constructed with limestone from the Mt. Carrubba Formation (*Lumachella* limestone), is an architectural masterpiece designed by Vaccarini. The paving of the cloister is one of the most precious treasures of the city of Catania. Created by *Francesco Fichera* in the early 20th century based on the designs by *Vaccarini*, this intricate artwork is composed of pebbles sourced from the Etna volcanoes and white slabs of Hyblean limestone (Ragusa Formation). These volcanic stones, each having a smooth, rounded shape, are precisely arranged like mosaic tiles, forming a beautiful figurative pattern showcasing floral and geometric motifs that are characteristic of Sicilian Baroque style. However, the paving represents the most damaged part of the building, due to biodeterioration. Even after the 2017 conservation–restoration work (https://www.archiviomultimedia.unict.it/palazzo-centrale-il-nuovo-chiostro.htm, accessed on 7 July 2021)), the pavement was severely affected by biodeterioration. This is due to both mechanical damage (substrate breakage and loss of cohesion) and aesthetic issues (formation of patinas and crusts) (Figure 1).

Figure 1. State of conservation of the paving in 2021.

In general, limestone rocks are more susceptible to weathering and degradation by atmospheric and biological agents compared to basaltic rocks. In this study, the effectiveness of nanoparticle-based protective treatment on the limestone that constitutes the palace was evaluated through laboratory tests on quarry samples. Subsequently small areas of the pavement were also subjected to tests of different biocide products in order to proceed with preliminary cleaning operation.

2. Experimental Procedures

2.1. Materials

2.1.1. Hyblean Limestones

A brief description of the selected limestone lithotypes from quarries is reported below.

Palazzolo Formation: This formation is composed of *Noto* and *Palazzolo Stone* which were extracted from the *Porcari* and *Famelio-Bagnato* quarries near *Noto* and *Palazzolo Acreide* (Syracuse, Sicily), respectively. They consist of two varieties: yellowish and white-cream colored [31–33]. Generally, their texture is mud-supported, containing allochemical components such as foraminifera, echinoderms, and peloids embedded within a micritic and microsparitic matrix. The yellowish variety exhibits a higher open porosity (41%) compared to the whitish one (35.74%), with respective average pore radii of 3.93 μm and 3.16 μm. Notably, the white-cream lithofacies exhibit greater physical–mechanical properties in comparison to the yellowish ones.

Ragusa Formation: *Ragusa Formation Stone* was taken from the *Canicarao* quarry near Comiso (Ragusa, Sicily). It is a compact-grained limestone rock with a yellowish color and an allochemical component making up 40–45% of the total composition. This stone is characterized by the presence of sparitic cement (15%), a porosity of 35%, and an open porosity of 25% [34].

Mt. Carrubba Formation: This formation is composed of *Lumachella* Stone, which was sampled near *Faro Capo Santa Croce* in the Augusta area (Syracuse, Sicily). This stone exhibits a grain-supported structure with a composition of 60% allochemical, 30% sparitic cement, and a porosity ranging from 30 to 40% [34]. Notably, it displays a relatively low capillarity-based absorption coefficient in comparison to the broader range of Hyblean calcarenites, alongside an open porosity of 20% [35].

2.1.2. Halloysite Nanoparticle Protective: Preparation and Application Methodology

The protective based on nanoparticles was developed by the Department of Physics and Chemistry of Palermo (University of Palermo). Halloysite is a clay mineral that belongs to the kaolin group; its structure and chemical composition is similar to that of kaolinite, but the unit layers of halloysite are separated by a monolayer of water molecules [36]. Minerals of this group were recently studied due to their potential as nano-containers for the encapsulation of various active ingredients [37]. The extensive characterization of the halloysite clay mineral employed in this work is provided in our paper [38]. The protective was prepared by the dispersion of wax microspheres in the order of a few microns, which have the functionality of protecting and making the treated surface more hydrophobic. A process called Pickering emulsion, a phenomenon of particular academic and industrial interest, was used to form the wax microspheres. The presence of a dense layer of particles at the droplet interface has an impact on the coalescent ability of the emulsion droplets; more simply, these are emulsions in which the dispersed phase is stabilized by solid nanoparticles. In this case, the solid nanoparticles are composed of halloysite, which, having a hollow tubular structure, can also be exploited to load active ingredients in order to achieve long-term antibacterial action.

The preparation process involves a series of steps:

- Water is heated (90 °C) and a certain amount of wax is added, which has a melting temperature of about 60 °C. The melting of the wax allows for the formation of dispersed oil droplets within the system under magnetic stirring.
- Halloysite is added, which stabilizes the dispersed droplets in the system.

- When the system appears sufficiently homogeneous, the heating is turned off and the system is allowed to cool to room temperature under magnetic stirring.
- The dispersed droplets solidify upon cooling and, due to the action of the halloysite, the spherical shape and size are preserved. Wax particles of tens of microns in size are obtained.
- Next, the film-forming polymer is added, which acts as a bonding agent. This film-forming polymer is composed of hydroxypropyl cellulose (HCP). The system becomes stable for months as no settling of particles is observed.

The resulting product can be brushed or sprayed on the surface.

The protective coating was applied by a brush on one side of the *Noto*, *Palazzolo*, and *Ragusa Formation Stone* block-shaped samples (Figure 2a). A total of 18 specimens were tested, with six representatives for each type of limestone. Post-application, the limestones were exposed to two distinct relative humidity conditions in order to observe their response to varying environmental settings. One treatment took place at a relative humidity of RH = 90 ± 5% and a temperature of 30 °C, while the other was conducted at a relative humidity of RH = 60 ± 5% and a temperature of 30 °C.

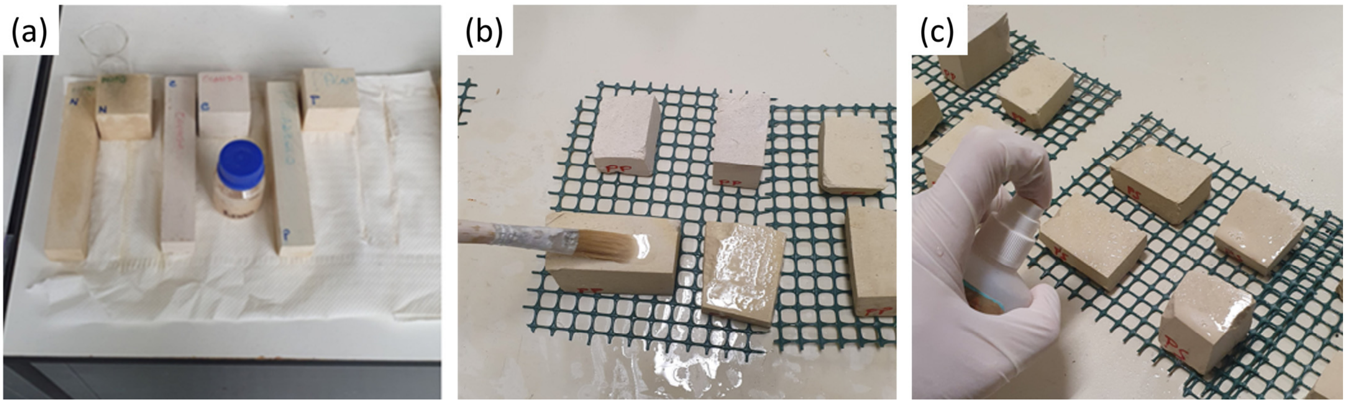

Figure 2. (**a**) Application of protective coating on *Noto*, *Palazzolo*, and Ragusa Formation stone by means of brush (**b**) and spray (**c**).

This same procedure was replicated within the laboratories of the Department of Biological, Geological and Environmental Sciences at the University of Catania for *Lumachella* stone: 24 specimens were treated either through brush application or the spraying method (as depicted in Figure 2b,c).

2.1.3. Biocides

In collaboration with a well-established restoration company (Piacenti S.P.A.), preliminary cleaning operations were investigated on the paving of the cloister.

Three distinct biocides on three selected small areas of the paving (Figure 3) were tested. Details of the applied product are as follows:

Biotin-R: A commercial product containing 3-iodo-2-propynylbutyl carbamate 10–25%, 2% 2-N-octil-2H-isotiazol-3-one 2.5–10%, 2-(2-butossietossi) ethanol 50–100%. This product boasts a wide spectrum of effectiveness against fungi, bacteria, and algae [11].

For the application, 15 mL of Biotin-R was diluted in 500 mL of white spirit.

Preventol RI80: Another commercial product, which contains alkyl-dimethyl-benzilam monium chloride (78–82%) and benzalkonium chloride (9–11%). Based on quaternary ammonium salts, this product employs a singular biocide [1,11]. For the application, 40 mL of Preventol was diluted in 600 mL of double-distilled water.

Biotersus—Opuntia 2MCL solution: Biotersus is an essential-oil-based product containing coridothymus capitatus, cinnamon, and carnation flower. The Opuntia 2MCL solution, derived from natural extracts of the common Sicilian prickly pear plant, was combined with the Biotersus solution to enhance its viscosity. This blended mixture provided by Preart srl (Catania, Italy) was used as supplied.

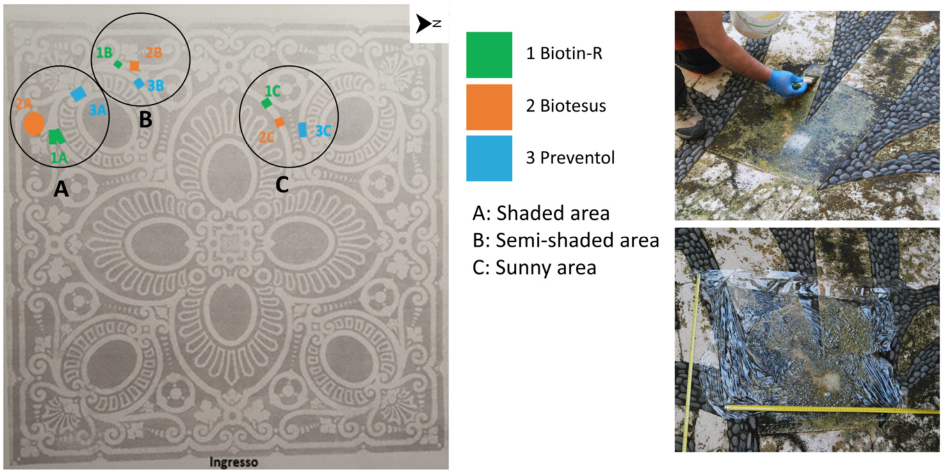

Figure 3. Draft depicting the cloister pavement layout and the specific zones marked for testing with diverse biocides.

A systematic mapping approach (Figure 3) was employed to monitor the application of biocides on the pavement. Each biocide was applied by brush to specific sections of the paving and these treated areas were carefully demarcated and recorded. This mapping process allows for accurate monitoring and assessment of the effects of each biocide on the biodeterioration and preservation of the paving. The labels A, B, and C correspond to different levels of sunlight exposure (A = shaded area; B = semi-shady area; C = sunny area). The numbers 1, 2, and 3 represent the specific biocide products (1 = Biotin-R; 2 = Biotersus; 3 = Preventol).

The three biocides were initially applied to selected areas at the end of April 2021 using a brush, followed by the treated sections being covered with plastic wrap for four days. This method served a dual purpose: preventing rapid evaporation of the biocides and protecting the area from potential rainfall.

Ten days after the biocides were applied, the treated areas were cleaned using a brush and sponge to evaluate the treatment effectiveness. Subsequently, one of the selected biocides was applied to the entire pavement in the middle of June 2021. A second application of the treatment was repeated at the end of November 2021, followed by another pavement cleaning at the beginning of December 2021. Cleaning was conducted using a water jet at a pressure of around seven bar and then mechanical removal of moss and lichens by means of a hard bristle brush.

2.2. Analytical Instruments

2.2.1. Colorimetry

In order to assess the effect of the protective on the stone surface, colorimetric analyses were carried out by means of a portable colorimeter (Konica Minolta CM-600d/CM2500d). All measurements were made in D65 illuminant and 10-degree observer conditions in order to quantify chromatic alterations in stones; color parameters were calculated with the CIELAB 1976 formula [39]. The CIELAB system comprises three chromatic coordinates, L*, a*, and b*, where L* is the lightness/darkness coordinate, a* is the red/green coordinate (+a* indicating red and −a* green), and b* is the yellow/blue coordinate (+b* indicating yellow and −b* blue). Colorimetric measurements were also performed to evaluate the color differences (ΔE*) of the stone surfaces due to treatment, according to $\Delta E^* = \mathrm{sqrt}\,[(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]$ [40].

2.2.2. Accelerated Aging Test by Salt Mist Chamber

The test for the determination of resistance to aging by salt mist was carried out by using a climatic chamber, Angelantoni DCTC 600 PN, according to the procedure described in the UNI EN 12370 (2020) standard [41]. Cubic specimens were dried in an oven for 24 h at a temperature of 70 ± 5 °C, then they were left to cool for 1 h at room temperature in a desiccator and finally weighed to assess the dry mass. The saline solution was prepared by dissolving 10 wt% of highly pure sodium chloride in 90 wt% of deionized water. A total of 60 cycles were carried out; however, every 15 cycles, the specimens were taken from the chamber for visual inspection. Each cycle consisted of 4 h of exposure to salt mist followed by 8 h of drying, with a chamber temperature set at 35 °C.

At the end of the test, the samples were removed from the chamber and immersed in deionized water in order to remove any deposited salts. Salt removal is considered complete when the electrical conductivity of the solution in contact with the test samples does not exceed twice the value of the original deionized water. Subsequently, the specimens were placed in an oven to dry at a temperature of 70 °C to constant mass, and then they were left to cool to room temperature for 2 h and weighed.

2.2.3. Contact Angle Measurements

In order to measure the wettability of the surfaces after the nanoprotective application, the water contact angle measurements were carried out. Measurements were conducted through the contact angle apparatus (OCA 20, Data Physics Instruments, Filderstadt, Germany) equipped with a video measuring system with a high-resolution CCD camera. The contact angle (ϑ) of water was measured by the sessile drop method at 25.0 ± 0.1 °C.

2.2.4. UV Fluorescence Images with Nile Red Probe

In order to assess possible wax residues of the protective, it is possible to carry out a UV light analysis of surfaces wetted with ‘Nile red’. ‘Nile red’ is a fluorescent substance that, when irradiated by UV radiation, emits fluorescence in the presence of hydrophobic domains (in this case, in the presence of wax). The method of analysis involves the application of a few drops of Nile red on each sample using a simple pipette. The drops of Nile red were placed on the treated and untreated portions of limestone, with the aim of verifying the presence of wax residues or the total absence of wax. In order to assess the difference between the different samples, they were placed inside a box irradiated by UV radiation.

2.2.5. Peeling Test

The Scotch Tape Test (STT), introduced in the field of cultural heritage conservation to verify the degree of cohesion of historical materials, consists of adhering a portion of adhesive tape to the surface to be studied, its subsequent removal, and the assessment of the amount of material removed by peeling [42]. In the present study, the STT was used to test the adhesion of the nanostructured protective tape to the stone substrates. An adhesive tape of approximately 2 × 2 cm was used for the peeling test. Three measurements were taken in order to obtain a statistically more complete response on different areas of the sample surface. The adhesive tape was placed on the surface of the samples and then a weight was placed above them for approximately 90 s in order to ensure the adhesion of the tape. The adhesive tape was finally peeled away from the surface, attempting to keep it in a normal position at an angle of approximately 90°. This procedure was performed on the surface of samples treated with the protective and after the test in a climatic chamber. Tests were compared with the original rock surface. All the portions of adhesive tape removed from the surfaces of the samples were then stuck onto a dark card in order to observe macroscopically the differences between the various samples.

3. Results

3.1. Preliminary Laboratory Tests for the Development of Halloysite-Based Protective

3.1.1. Assessment of Hydrophobic Features

The hydrophobic features of the nano halloysite-based coating were evaluated through contact angle measurements on the selected limestone. The results are reported in Figure 4 and Table 1. By comparing the droplet profiles (Figure 4) of the uncoated limestone with those of the coated limestone, it becomes evident that all the specimens display heightened hydrophobicity subsequent to the application of the protective coating.

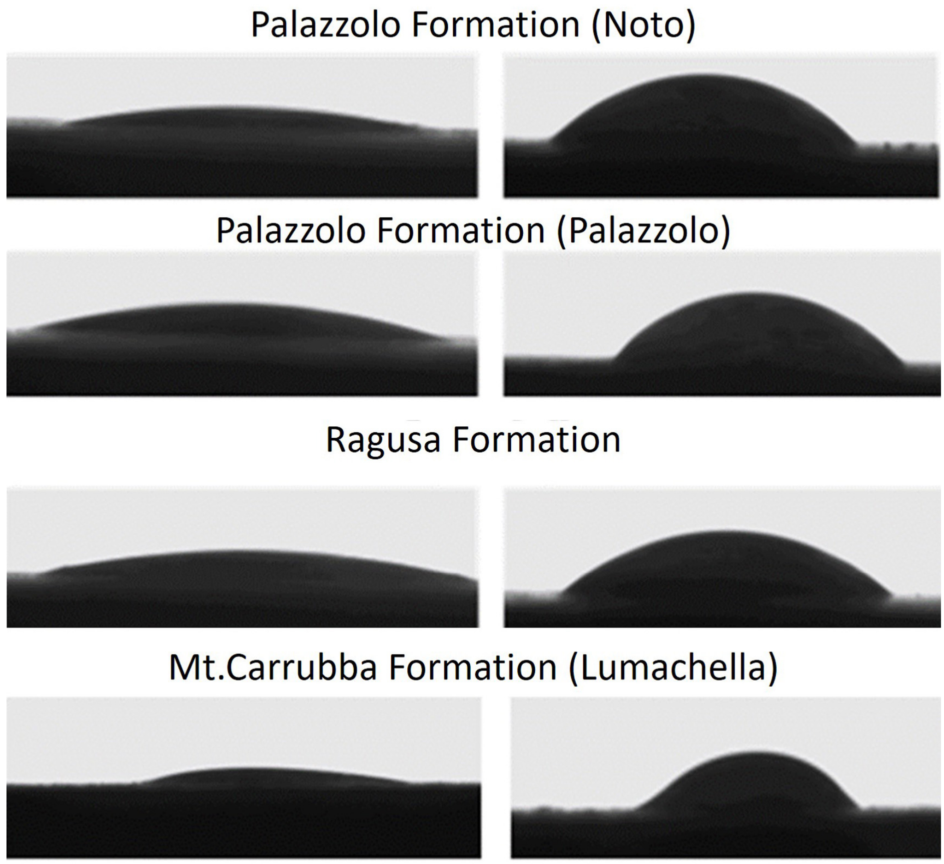

Figure 4. Droplet profile before and after protective coating; measurements were carried out at the laboratories of the University of Palermo.

Table 1. Values of contact angle before and after coating treatment (University of Palermo).

Sample	Contact Angle Uncoated	Contact Angle Coated
Palazzolo Formation (*Noto*)	8.6°	48.2°
Ragusa Formation	15.4°	43.5°
Palazzolo Formation (*Palazzolo*)	16.0°	50.0°
Mt Carrubba Formation (*Lumachella*)	19.0°	54.0°

Before treatment, the average contact angle values indicate a hydrophilic characteristic for all the limestone samples (contact angle values being <90°), whereas a significant increase in hydrophobic behavior is evident for all specimens following the application of the coating treatment. This effect can potentially be attributed to the presence of paraffin wax within the formulation.

After exposing the Noto, Palazzolo, and Ragusa Formation stones to two distinct relative humidity conditions (RH = 60 ± 5% and RH = 90 ± 5% at 30 °C) for a duration of 60 days, it is macroscopically evident that variations in environmental conditions do not lead to observable modifications in the visual appearance of the specimens. The results of these visual observations are presented in Figure 5.

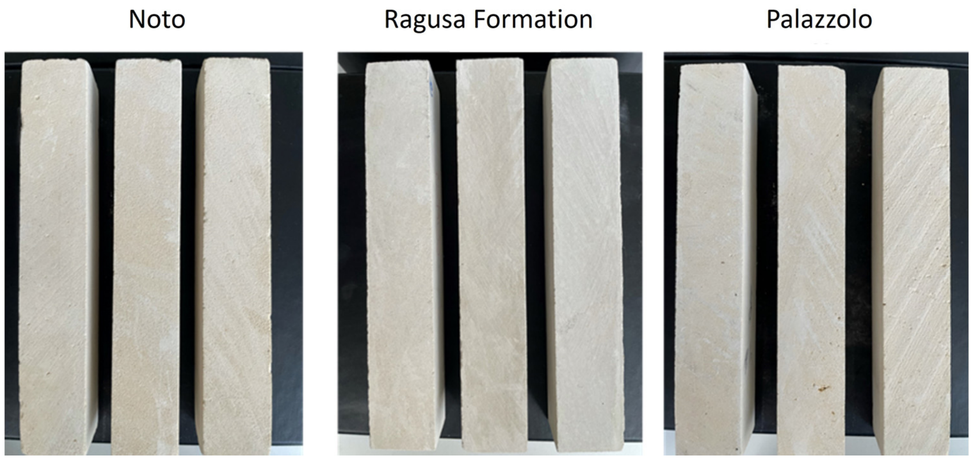

Figure 5. Macroscopic photographs of the samples following exposure to different environmental conditions. In each photograph, the first sample is untreated (RH = 60 ± 5%), and the other two are treated and exposed to RH = 60 ± 5% and RH = 90 ± 5%, respectively. The temperature is always 30 °C.

Macroscopically, it has been observed that humidity exposure has no discernible impact on the appearance of the samples. Additionally, the contact angle measurements conducted on the aged samples demonstrate that wettability remains unaltered.

3.1.2. Colorimetric Analyses

The shifts in color become readily apparent with a ΔE* < 3. In the majority of the available literature, the so-called JND (just noticeable difference) is recognized as the threshold at which a difference in color becomes perceptible. This threshold is typically considered to be around 2.3 [40]. However, depending on the materials which are observed, different JND values can be found, e.g., [43,44].

Here, the difference in color between the surfaces of the *Noto*, *Palazzolo*, and *Ragusa Formation* observed after the exposure (RH = 60 ± 5% and RH = 90 ± 5% at 30 °C) and their original color being below 4 indicates that the colorimetric changes are slightly perceptible by human eye, in accordance with the findings of [45].

Colorimetric analyses were also conducted on *Lumachella*, differentiating between samples treated with the protective coating applied via brush (labeled as PNP) and those treated with the spray method (labeled as PNS), see Table 2.

Table 2. Average values of CIELAB colorimetric coordinates and color differences (ΔE*) calculated for both uncoated *Lumachella* samples and those coated using the brush and spray methods.

Lumachella Stone	L* (Mean)	L* (σ)	a* (Mean)	a* (σ)	b* (Mean)	b* (σ)	ΔE*
Uncoated	77.5	4.2	1.8	0.2	15.1	2.2	
Coated by brush (PNP)	81.1	0.7	1.9	0.7	12.6	2.2	4.38
Coated by spray (PNS)	79.9	2.0	1.9	0.6	12.3	1.9	3.68

These data were compared with measurements from uncoated samples. To ensure accuracy, four measurements were taken for each sample with three samples for each set.

In particular, an increase in the brightness value (L*) was observed on the samples treated both by brush and spray (L* = 81.1, L* = 79.9, respectively) in comparison to the uncoated samples (L* = 77.5). Furthermore, a slight average decrease in the b* parameter was also observed in the coated samples (b*= 12.6 and 12.3) compared to the uncoated ones

($b^* = 15.14$). No significant changes were observed regarding the a* parameter between the coated ($a^* = 1.9$) and uncoated samples ($a^* = 1.8$). On the other hand, it is observed that the b* parameter (where $+b^*$ indicates yellow and $-b^*$ indicates blue) tends to decrease, shifting towards a less yellowish color. As denoted by the ΔE^* values, the differences in the colors between the uncoated and coated samples with both application methods are around 4.

Based on the thresholds stipulated by Mokrzycki and Tatol [46], differences in color are considered perceptible but still acceptable. Nevertheless, the obtained color changes are an improvement on those with other hydrophobic coatings reported in the literature (e.g., Manoudis et al., 2009 [47]).

3.1.3. Accelerated Aging in a Climatic Salt Spray Chamber

As *Lumachella* exhibited the highest contact angle values among all the limestone types after the application of the protective coating, it was selected for the accelerated aging test in a salt spray chamber following the UNI EN 12370:2001 standards [48]. The protective coating was applied both by brush and by spray for comparison. After completing 30 cycles, which represents half of the entire test duration, no fractures or apparent volume reductions were observed (see Figure 6a).

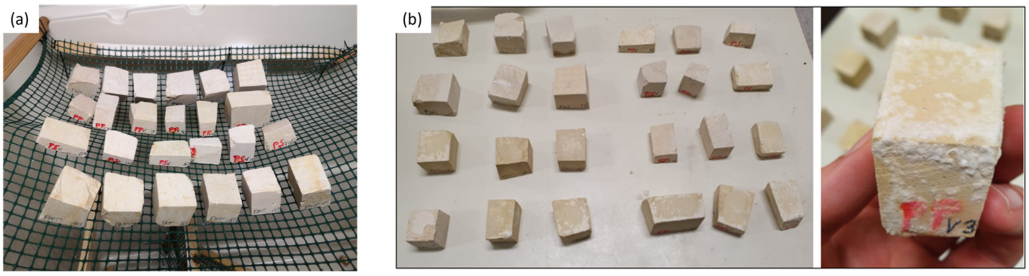

Figure 6. (**a**) Samples of *Lumachella* stone after 30 cycles in a climatic salt spray chamber; (**b**) salt formation observed on *Lumachella* stone after exposure to the climatic salt spray chamber (60 cycles).

At the end of the 60-cycle test, the samples were removed from the chamber and subjected to visual examination. The surfaces of the limestones exhibited significant salt deposits; however, no cracks or mechanical alterations were detected (see Figure 6b).

After the washing procedure to remove excess salts, contact angle measurements were conducted on both brush- and spray-coated *Lumachella*. The sample treated with the protective and subjected to accelerated aging showed a contact angle of 31°, which is lower than that measured before the aging treatment (Table 2). The lower value observed in the aged samples may be attributed to the potential washout of the protective coating during aging cycles, followed by the process of salt removal and drying.

The results of the 'Nile red' tests under UV light are reported in Figure 7. The images reveal the presence of residual wax on the limestone subjected to both the brush (a and b) and spray applications of the protective coating. Any wax residues, and, therefore, the presence of the protective coating, were assessed through UV light analysis of surfaces wetted with 'Nile red'. The results of the analysis revealed that the quantity of residual wax on the treated samples after accelerated aging is insignificant, as the fluorescence effect was not evidenced (Figure 7a,b).

Figure 7c displays, on one side, a portion of an untreated sample (left) and, on the other side, a portion of a treated sample (right) to verify the actual presence of dissolved wax within the protective coating. The quantity of residual wax on the samples that have not undergone accelerated aging testing shows a clearly visible fluorescence effect.

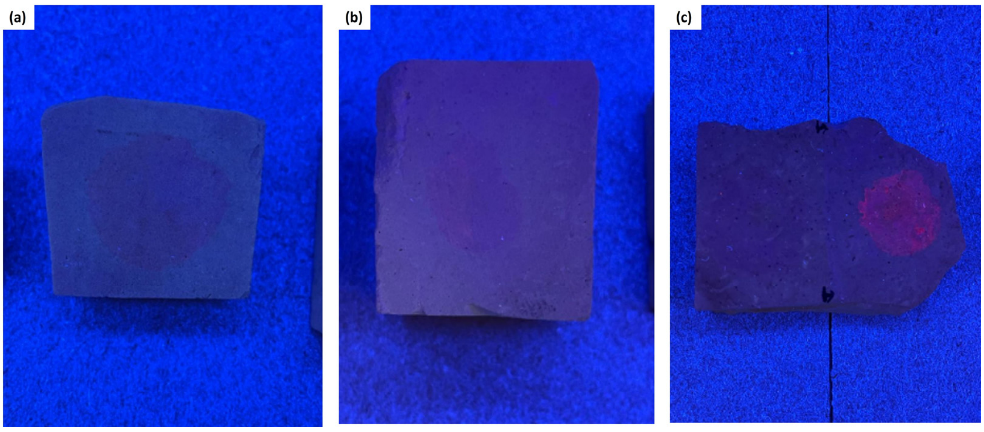

Figure 7. Nile red test under UV light on *Lumachella* samples: (**a**,**b**) after accelerating aging test; (**c**) *Lumachella* treated solely with the protective coating.

Figure 7a–c demonstrate a distinction between the samples treated solely with the protective coating and those treated and exposed to accelerated aging. This difference is likely attributed to the effect of salt spray during the weathering process, causing the degradation of the coating. Additionally, the subsequent sample preparation, which involves salt removal through washing and drying procedures, may contribute to this effect, as evidenced by a slightly visible fluorescent response.

3.2. Scotch Tape Test (STT) Results

The Scotch Tape Test (STT), employed to assess the efficacy of treatments on deteriorated stone surfaces [42], has proven to be a useful tool in evaluating the level of adhesion and the residues of the coating applied to *Lumachella* Stone.

Based on visual observation, regardless of the application method, it is evident that the surfaces of the coated samples before aging (Figure 8a) appear cleaner compared to the aged ones (PBA and PSA, protective applied by brush and spray, respectively, Figure 8b,c). This variance could be due to alterations in the stone surfaces resulting from treatment in the climatic chamber. The results also suggest that the samples treated with the protective coating via brush application (PBA, Figure 8b) show a higher residual component on the tape after accelerated aging, in contrast to the samples to which the protective coating was sprayed (PSA, Figure 8c). This observation underscores that the brush application method allows for a quantitatively more generous application of the product to the surfaces, which inevitably results in the deeper penetration of the product on the surface of the treated rock. This is particularly pronounced in highly absorbent materials like limestone.

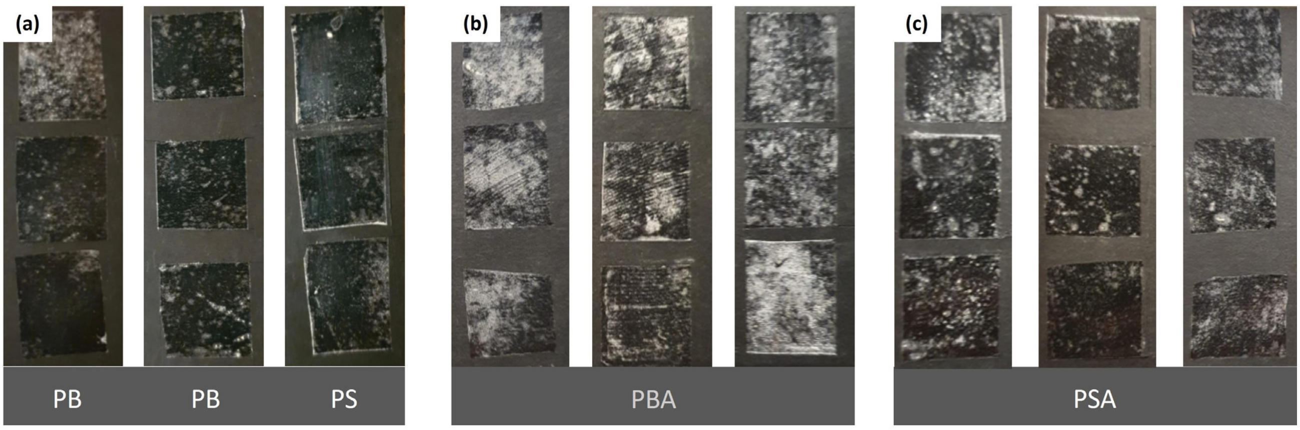

Figure 8. Scotch Tape Test on *Lumachella* stone coated samples via brush and spray application method, respectively, before and after aging treatments. PB = protective applied by brush;

PS = protective applied by spray; PBA = protective applied by brush, aged; PSA = protective applied by spray, aged. On the other hand, the residual material observed in Figure 8 after the Scotch Tape Test could potentially be attributed to either the salts formed during aging or residues from the coating itself. It is possible that the application process could influence the substrate in a way that promotes salt formation, or that improper drying or curing of the coating could leave residues. Factors such as the application method, drying conditions, and substrate preparation could provide valuable insights into salt formation and help optimize the coating process to minimize this effect.

3.3. Biocide Testing and Application on the Pavement

In order to subsequently test the protective directly on-site, specific cleaning actions were required for the paving of the cloister. In this section will be described the operational phase and the results from the tests of the different biocides.

Firstly, in situ observations were conducted in order to identify the materials employed and the degradation forms associated. The results are depicted in Figure 9a,b. As indicated by the map (Figure 9a), the pavement exclusively comprises volcanic (grey) and limestone (white) as the primary materials. Volcanic cobbles exhibited limited decay, generally showing minor missing sections or alterations in color attributed to normal outdoor exposure (Figure 9a). On the other hand, the limestones were subjected to significant biodeterioration, primarily driven by the adherence of microorganisms like algae, mosses, and lichens to the stone surface. In certain areas, this leads to a weakening of mechanical strength, resulting in stone cohesion loss and breakage, as well as aesthetic damage through the formation of patinas and crusts (Figure 9a,b).

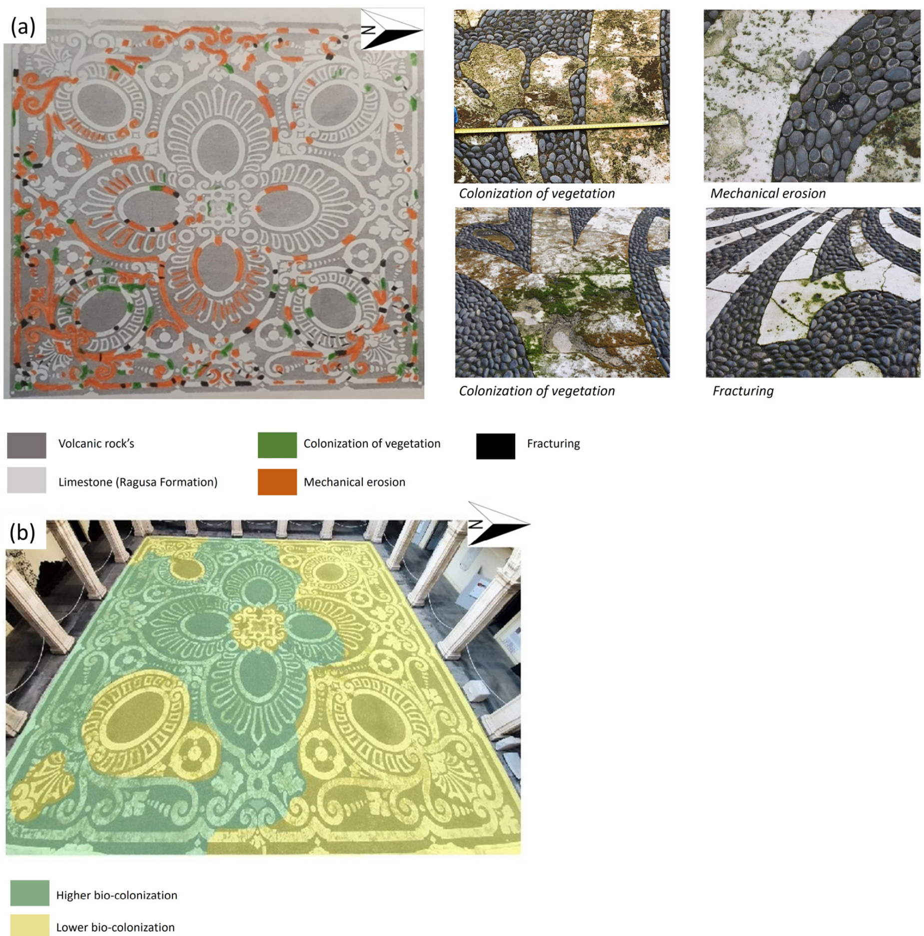

Figure 9. Schemes of the state of conservation of the pavement: (**a**) map of material type and degradation forms with some visual examples; (**b**) map of biodeterioration intensity using ArcMap.

An idea of the extent of the biodeterioration is given by the map in Figure 9b, created using ArcMap software (Version 10.7.1. 2019). The map clearly demonstrates that the bio-colonization is not uniformly distributed due to the exposure of different stone materials to the effects of sunlight.

In Situ Biocide Test Application and Cleaning Operations

The specific areas selected for the biocide application tests were delineated in the Experimental Procedures section (Section 2 and reported in Figure 2). For simplicity, images related only to the shaded area in relation to the various biocide treatments are presented below (Figure 10a–i). Four days after the application of the biocides, the plastic films were removed. As depicted in Figure 10b,e,h, a noticeable color change became evident for all the biocide products. The once bright green color of the algae changed from its original brightness to shades of brown and reddish, an alteration supported by colorimetric analyses (see following section).

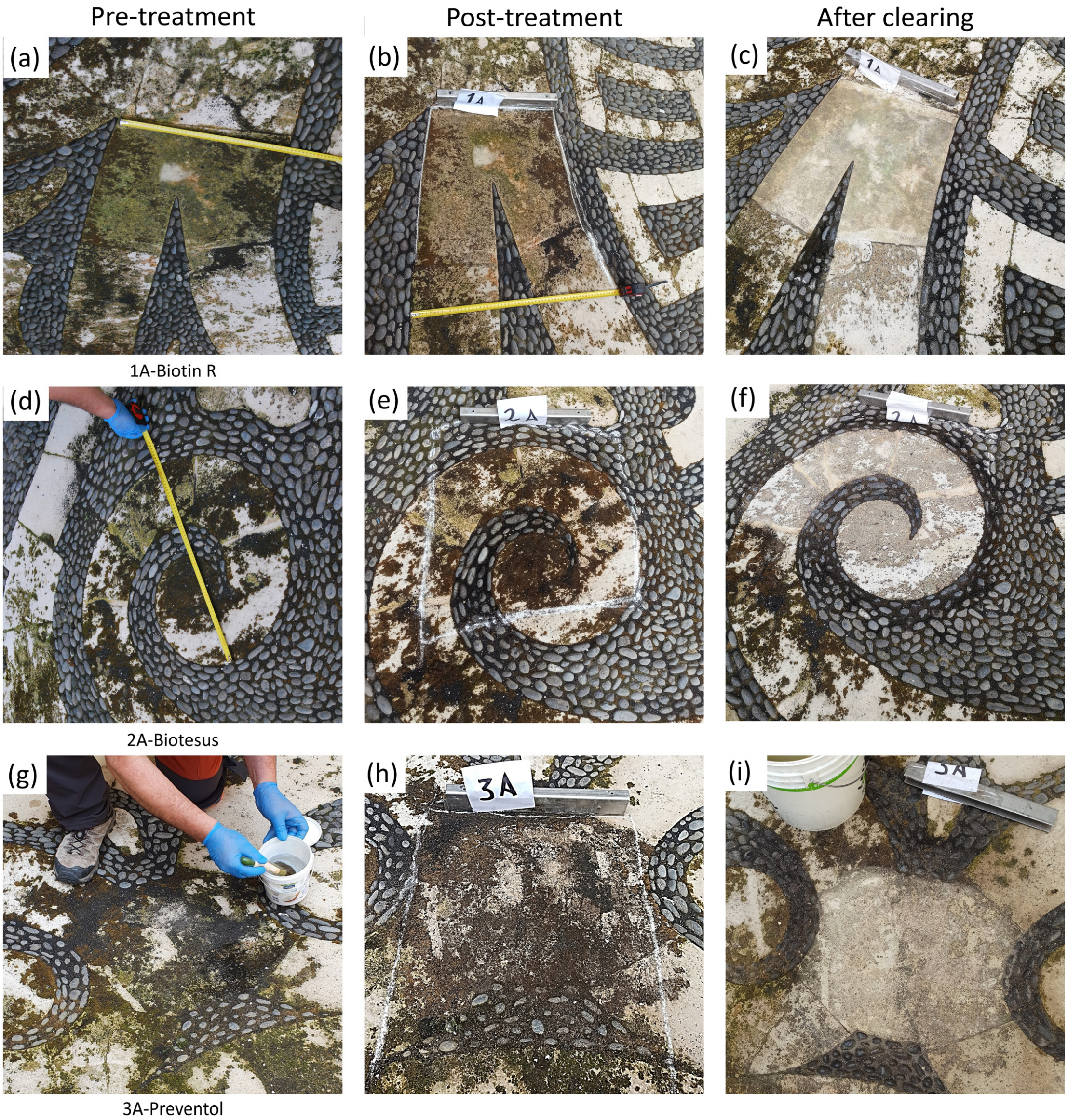

Figure 10. Visual observations of shaded areas before and after biocidal treatment application. (**a**,**d**,**g**): application of the three biocides; (**b**,**e**,**h**): stone surface four days after treatment; (**c**,**f**,**i**) stone surface after cleaning.

After ten days, the residual patina was removed using brushes and sponges. The visual results of the surfaces after these procedures are shown in Figure 10c,f,i. Nevertheless, subsequent to the cleaning procedures, some parts of the limestone substrate displayed signs of staining and occasional erosion.

Considering the results and the eco-friendly approach of the intervention, Biotersus biocide was chosen for application on the whole pavement (Figure 11). The application of the biocide was followed by a subsequent cleaning operation phase. The biocide application and cleaning operations were carried out twice over a span of seven months, encompassing the summer season (June–September) and immediately after the initial autumn rains (September–October). The effectiveness of the biocide is still under monitoring to continue the study of its efficacy over time.

Figure 11. Application of Biotersus on the whole paving and cleaning operations.

4. Conclusions

In this study, a halloysite-based protective was applied on limestone materials. Laboratory tests were firstly conducted to assess the product's effectiveness on the limestone with a view for use in future on-site testing.

In general, the results obtained at the laboratory scale of the samples treated with the halloysite-based protective have shown that they may not be particularly durable when subjected to repeated washing and temperatures exceeding 60 °C. This is independent of the method used for applying the protective coating to the substrate. Therefore, it may be preferable to use this product on surfaces not excessively exposed to sunlight and rain but perhaps in semi-open environments. However, the fact that the protective is not durable under these conditions does not necessarily have to be considered a negative result. In the field of CH, the reversibility of applied products is one of their most appreciated aspects. With increasing attention paid to environmental sustainability, the theme of product reversibility has extended to CH conservation, promoting the development of eco-friendly and easily applicable and removable technologies that are progressing in the field of restoration, which is the focus of this study. Based on these results, the application of the protective treatment will proceed on small sections of the columns of the cloister, which are entirely composed of *Lumachella* Stone and on the paving of the cloister after the cleaning operation procedure. In this context, the results of the tests focused on the application of biocides, particularly addressing the cloister's pavement, which, as observed, suffers from severe biodeterioration, have enabled the identification of the most effective eco-friendly biocide based on essential oils. This can be easily applied with complete safety for both operators and the environment. This result undoubtedly marks the first step towards the development of an operational protocol for future cleaning and restoration procedures.

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