An interdisciplinary approach to a knowledge-based restoration: The dark alteration on Matera Cathedral (Italy)

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Abstract

An interdisciplinary analytical campaign was carried out on the exterior walls of the Santa Maria della Bruna and Sant'Eustachio Cathedral in Matera. Large areas of these walls have become darkened and the main objective was to evaluate the state of conservation of the stone material (a very porous, organogenic limestone called "Pietra di Matera"), and to suggest the best strategy for the current restoration. Several techniques were used in situ and ex situ-in laboratory analyses: X-ray diffraction, infrared spectroscopy, ion chromatography, pyrolysis/gas chromatography coupled with mass spectrometry, colour change measurements, laser-induced fluorescence together with biological techniques. Ex-situ and in situ cleaning tests were also carried out on the stone surface.
The results showed the presence of chlorophyll and bacteria on the surface, together with sulfation and calcium oxalate films as the main decay phenomena. In addition, the determination of saccharide and egg residues suggest both biological activity and past conservative treatments as the cause of oxalate films. Data obtained from the analyses proved to be very useful for the conservation work; a complex plan of restoration was adopted, including both traditional and innovative techniques (such as biocleaning, bacterial-gel and a laser system) together with a final evaluation of several protective methods.

Key words
Tuff dark alteration, FTIR, LIF, Py-GC-MS, calcium oxalate film, biocleaning

Highlights

- The dark alteration was identified as calcium oxalate film
- Cyanobacteria, bacilli, heterotrophic and cocci type bacteria were identified

- Films are probably due to both biological activity and residues of past treatments
- Results of the campaign suggested the best chemical cleaning method
- The most effective protective agent was identified

Introduction

Matera Cathedral was built in Romanesque-Apulian style in 12th century in the old part of Matera (Italy), called "*I Sassi"*, that is an agglomeration of almost completely abandoned dwellings made of the local rock (tuff) of the Gravina torrent valley. Matera lies at the western edge of an area called *Parco Archeologico Storico Naturale delle Chiese Rupestri del Materano*, which is listed in UNESCO World Heritage sites.

The exterior walls of the Cathedral, made of the local calcareous tuff, have shown a wide dark alteration in the lower part of the structure (up to 6-8 meters) since at least the beginning of 20th century (Figure 1). The phenomenon was worrying, for the serious aesthetic damage to one of the most important landmarks of Matera. No historical information could explain the alteration, for example whether it has originated from past surface protection treatments. The research on the sources of the dark alterations was no easy up today because it did not seem to relate only to a superficial film or to an unknown phenomenon affecting the stone inside.

Conservation work undertaken from 2014 to 2016 was a unique opportunity to examine the state of conservation of the surfaces, to provide new insights into the dark alteration and to propose a cleaning strategy. The characterization of the stone surfaces of monuments was very important in order to identify the decay phenomena, which could be both biological and chemical, and indirectly provides information on the conservation history of the building [1].

An interdisciplinary team of experts in Conservation Science-biologists, chemists, geologists, and physicists-thus was created in 2015 for planning an extensive analytical campaign, composed both by *on-site* and *ex situ* analyses and cleaning tests (Table 1). A multi-technique approach prior to a conservation work is fundamental to consider and study the decay phenomena from various points of view, being this strategy a good practice also for the knowledge of the artistic techniques, provenance of materials, testing of restoration materials as evident in the recent literature [2–8]. In this study, the importance of the monument and the age of the dark alteration has required a working group with complementary and multiple expertise and skill, to consider as many aspects as possible and to come up with a proposal with the effective cleaning method.

During the first step (Table 1), a non-invasive monitoring of the surfaces, based on the use of fluorescence (LIF) and colorimetric investigations, was planned in order to map the presence of biodecay phenomena and to verify a selection of cleaning methods, including a biocleaning treatment. The LIF technique is based on the induction of the fluorescence of the surface of interest by means of a laser source emitting at a suitable wavelength. The signal backscattered by the irradiated surface is collected and detected using a spectrometer. The experimental setup for the analyses of the Cathedral of Matera was tailored for recording the fluorescence signals both of the stone material and of photosynthetic pigments typical of photoautotrophic organisms. The results may enlighten the presence of chlorophyll, which is a clue of a biological patina colonizing the surface.

The sampling of small fragments from the most representative altered areas was performed as well and the samples were characterized by traditional and innovative techniques, seeking for the features of the on-going decay, that are inorganic and organic components and microorganisms. In particular, X-ray diffraction (XRD) and optical microscopy (OM) were used for pointing out the mineralogical phases and evaluating the stratigraphy of the mortar, Fourier transform infrared spectroscopy (FTIR) for the identification of inorganic compounds, ion chromatography (IC) for the quantification of water-soluble salts, pyrolysis-gas-chromatography coupled with mass spectrometry (Py-GC-MS) for characterizing the organic fraction. The results guided the practices of removal of the decay, depending on the presence of bio-colonisation or insoluble compounds.

The cleaning strategy was studied in Step 2 (Table 1), evaluatin bio- and chemical- methods both in ex situ tests on stone specimens and in in situ experiments, also considering ablation by laser. The in situ tests were monitored by evaluating the colour changes of the surface. In addition, various protective agents were tested and applied in situ, evaluating the results with non-invasive reflectance FT-IR and Py- GC–MS on micro-fragments.

The results from Steps 1 and 2 were used to define the best cleaning method and the best protective agent. This was carried out during Step 3, and was completed in 2017. The data concerning the choice and monitoring of the protective agents are discussed in detail elsewhere [9]. Only some aspects are mentioned in the conclusions of this paper, in order to emphasize the importance of a preliminary analytical campaign in protecting the stone surface.

Table 1
Description of the analytical campaign

Wall surface	**Phases and site**	**Methodology**	**Instruments/Methods/Materials**
Step 1	Sampling	Manual scalpel and cuts Physical	
Analyses	Analyses *on-site*	Optical	LIF, Colorimetric analysis, OM observation
	Analyses *ex situ*	Chemical	FTIR, XRD, IC, Py-GC-MS
		Biological	Microbial viable counts, ATP assay
Step 2	Test *ex situ*	Chemical cleaning	H_2O_2, UV, Oxone ®, TiO_2
Cleaning		Biocleaning	Impact biogel
	Test *on-site*	Physico-Mechanical	Micro-laser
		Chemical	H_2O_2, AB57 Impact - poultice
		Biocleaning	Impact biogel
Protection		Chemical	FTIR, Py-GC-MS
Monitoring		Physical	Water absorption
		Optical	Colorimetric analysis
Step 3		Chemical cleaning	Impact - poultice
Cleaning	At *on-site* full scale	Physico-Mechanical	Micro-laser
Restoration		Chemical	Final protective agents

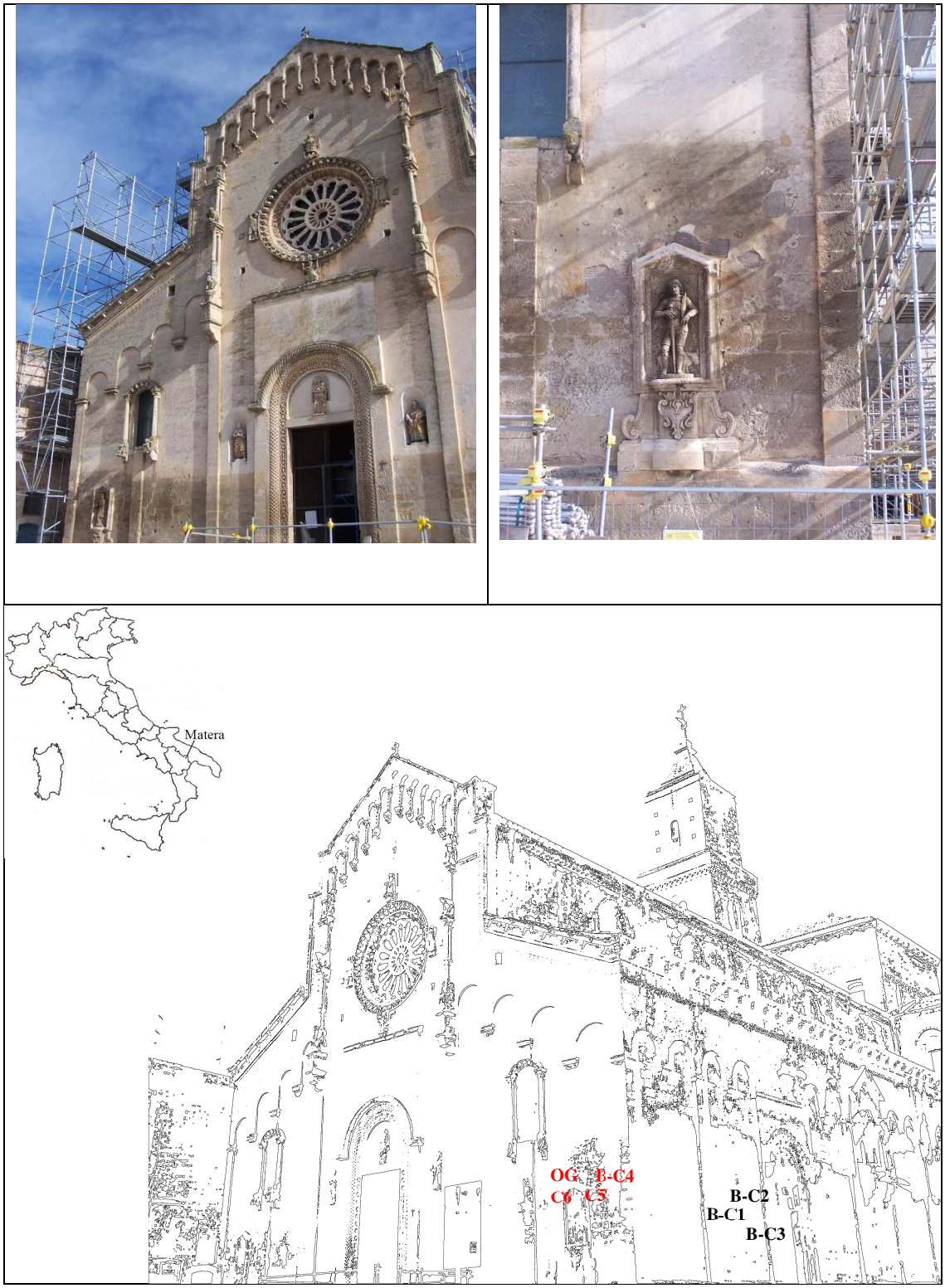

Figure 1 Exterior of the Matera Cathedral facade (Matera, Italy) with the evident dark alteration-in the lower part of the structure (up to 6-8 meters) (above). The provenance of the samples (bottom).

2. The experimental

2.1 Step 1

2.1.1 Sampling

Eight samples (C1-C7, OG) of "Pietra di Matera" and mortar (C4 sample) were taken from the stone surfaces of the southern wall and from the façade, both as powder and fragments scraped off the surface using a scalpel (Table 2). Selective sampling of C4 and C6 sample was carried out by a micro-lancet under an optical microscope, in order to analyse the three and two layers of the stratigraphy, respectively. The samples of powders for the biological analyses (B1-B4) were scraped off the surface with a sterile chisel (Table 2).
Figure 1 shows the points of sampling. The areas chosen for *on-site* analyses are shown too.

Table 2
Description of the samples

Sample label	Provenance	Description
C1	Southern wall	dark area, powder
C2		ochre area, powder
C3		ochre area, fragment
C4	Façade	ochre area, fragment of mortar
C5		dark area, powder
C6		ochre area, fragment
C7		ochre area, fragment
B1	Southern wall	powder (close to sample C1)
B2		powder (close to sample C2)
B3		powder (close to sample C3)
B4	Façade	powder (close to sample C4)
OG		ochre painted area, fragment

2.1.1.1 "Pietra di Matera" specimens
A noble tuff block (36 x 22 x 25 cm, 40 kg) was sampled at 1,5 meters from the ground on the left external facade wall of Cathedral and removed (Figure 2). It was cut horizontally by dry mechanical saw into thinner specimen blocks (36 x 22 x 3.5 cm, each; the external ones with the noble surface; the inners as artificial tests) and used for *ex situ* chemical and biocleaning treatments. The hole created by removal was filled by another block of a vile tuff of the same measurements [9].

Figure 2. *On-site* sampling of ancient tuff sandstone at left side of Matera Cathedral.

Laser-Induced Fluorescence (LIF)

The LIF technique used to acquire the measurements is based on the induction of the fluorescence of the surface of interest by means of a laser source emitting at a suitable wavelength. The signal backscattered by the irradiated surface is collected and detected using a spectrometer.
The instrumentation used to acquire the LIF spectra was an in-house developed fiber-optics-coupled portable LIF spectrometer powered by batteries. The laser source was chosen to optimise the induction of the fluorescence in the photosynthetic pigments, and particularly chlorophyll. The fluorescence signal was detected in the spectral range between 450 nm and 930 nm with a spectral sampling interval of approximately 0.5 nm. The optical and mechanical configuration of the handheld measuring head is configured to acquire the fluorescence signal on circular area of about 5 mm in diameter.

Optical Microscopy (OM)
Reflected light microscopy Polished cross-sections were obtained from fragments of some collected samples. A Nikon Eclipse E600 Microscope, connected to a high-resolution digital camera and controlled by NIS Elements Software for image analyses, was used.
Transmitted light microscopy Petrographic observation on a thin section of 30 μm thickness was performed to obtain the main textural-compositional parameters of the C4 plaster sample. An optical transmitted light polarized microscope, Zeiss AxioScope A.1, with parallel and cross nicols, with 2.5X, 5X and 10X of magnification was used. The acquired images were processed with Axiovision software.

Fourier transform infrared spectroscopy (FTIR)
The samples scraped off the paint layer were analyzed as KBr (Sigma-Aldrich FTIR Grade) pellets by a FTIR spectrophotometer BioRad Excalibur Series FTS 3000, DTGS detector, in the transmission mode (400 to 4000cm^{-1}, 4cm^{-1} resolution, 16 scans). The samples C5 and C7 were analysed by means of a portable Bruker Optics ALPHA FTIR Spectrometer equipped with SiC Globar source and DTGS detector, collecting 24 scans in ATR mode, with 4cm^{-1} resolution in the 4000-400 cm^{-1} range. The collected spectra were elaborated with an OPUS 7.2 Software.
Non-invasive FTIR measurements were carried out with an Alpha Bruker FTIR portable spectrophotometer in reflection mode for contactless measurements and a DTGS detector. Spectra were collected between 7000 and 400cm^{-1} with 4cm^{-1} resolution and 128 scans on a 4mm-diameter area. The average working distance from the surface was about 1 cm.

Ion Chromatography (IC)
Ion chromatography analyses were performed with a Dionex ICS-1000 instrument, equipped with a suppressed conductivity detector. For cation analyses, an IonPac® CS12 4x250 mm analytical column - which is specifically conceived for the analysis of alkali metals, alkaline earth metals and ammonium - and an IonPac® CG12 4x50 mm Guard Column. The eluent was methane sulfonic acid 20 mM and the suppressor was a cation self-generating suppressor 300x4mm (CSRS® 300). For anion analyses, an IonPac® AS 4A 4x250 mm analytical column and an IonPac® AG 4A 4x50 mm guard column were used. The eluent was an aqueous solution of Na_2CO_3 (1.8mM) and $NaHCO_3$ (1.7mM) and the suppressor was an anion self-generating suppressor 300x4mm (ASRS® 300). ICS-1000 operation is remote controlled by Chromeleon Software (version 6.7 SP1) that also provides data acquisition and data processing functions. For the chromatographic analyses, a given amount of water was added to a weighted amount of the sample. The solution was then stirred for 24 hours, decanted and filtered with hydrophilic PTFE filters (pore size 0.45 μm).

Pyrolisis Gas Chromatography Mass Spectrometry(Py-GC-MS)
A pyrolyser operating in constant temperature mode (multi-shot pyrolyzer® EGA/PY-3030D (Frontier Lab), connected to a split/splitless injector of a 6890N Network GC System gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), coupled with a 5973 Mass Selective

Detector (Agilent Technologies, Palo Alto, CA, USA) single quadrupole mass spectrometer, was used. The mass spectrometer was operating in the electron impact (EI) positive mode (70 eV).
The pyrolysis conditions employed for the analysis of the samples were: furnace temperature 500°C, interface temperature 300°C. In the pyrolysis cup, 2 µl of Hexamethyldisilazane (HMDS) was added to a few micrograms of each sample. The gas chromatograph was equipped with a HP-5MS fused silica capillary column and the chromatographic parameters are reported in literature [10,11].

X Ray Diffraction (XRD)
In order to identify the mineralogical composition, XRD analysis was performed. A PANalytical X'Pert PRO X-ray diffractometer with Cu anticathode (λ = 1.54 Å) was used, under the following conditions: current intensity of 30 mA, voltage 40 kV explored 2Θ range between 3 ° - 70 °, step size 0.02°, time to step 50 s and scan speed of 0.04 °/s. The instrument was equipped with X'Celerator multirevelatory and High Score data acquisition and interpretation software.

Biological analyses
The samples have been observed in transmitted and UV light with an optical microscope (Nikon Eclipse E600 with an UV-2A filter). A small aliquote of the collected powder (10 mg) was sown on three different nutrient media: potato dextrose agar for micromycetes (PDA, Difco), (plate count agar, for heterotrophs such fungi and bacteria (PCA, Difco), and $BG_{11}M$ liquid medium for algae and cyanobacteria [12]. Another nutrient media, nutrient agar (NA, Difco) was used for isolating bacteria developed on PCA. The isolated bacterial strains were gram-stained and observed in transmission mode, while unstained colonies were observed using the UV-light.

2.2 Step 2
Microorganism, media and ex situ biological test
On the basis of previous results, *P. stutzeri* strain A29 (DISTAM-DISTAAM Strains Collections, University of Milan - University of Molise, Italy), was used for the biocleaning processes [13,14]. In order to optimize growth conditions for the cells to be applied to the altered stone surface, an assessment was made of biodegradation activity: the strain was incubated at 28°C for 24-36 hours, on mineral medium M9 and on the same medium supplemented by organic substances, as linseed oil or egg (CTS srl, Altavilla Vicentina, Italy) at 0.5% v/v and 0.5% w/v, respectively.
Suspensions containing exponentially-growing bacteria, approximately 10^8 CFU ml^{-1} were obtained by inoculating 10 ml of an overnight broth-culture into 1,000 ml of fresh broth medium (in a flask of 3,000 ml total volume) and incubating it in a shaker (200 rpm) for 24 hours at 28°C. The cells were centrifuged at 7,000 g for 10 minutes at 4°C, washed twice with phosphate-buffered saline solution, and re-suspended in sterile distilled water, at pH 6.9; the final cell concentration was above 10^8 cells ml^{-1}, corresponding to an $O.D._{560\ nm}$ of about 1.6; pH was about 7.0 and the solution conductivity was about 947,1 µs cm^{-1}; the cells were stored at 4 °C before bio-applications.
In these biocleaning tests laponite was adopted as delivery system instead of the agar gel that needs higher temperature before solidification. The choice of this delivery system was made according to the requirements and limitations of each work of art [15]. In duplicate, laponite gel (control) and laponite gel including bacteria (laponite biogel, at 9.0 % w/v in the suspension bacterial cells) were employed. A 0.8 - 1.0 cm thick continuous layer of *P. stutzeri* A29 cells entrapped in the laponite delivery system was applied onto the *ex situ* noble tuff surface using a sterile spatula. The entire piece was uncovered with any layer plastic film in order to favour the aerobic conditions; the application times were optimised and ranged 12 -24 hours with a room temperature of 25ºC ± 2ºC.

Ex situ chemical tests
In order to evaluate the cleaning of the altered brownish area, advanced oxidation processes (AOPs) were considered. They are designed to produce hydroxyl radicals that are highly efficient to destroy

organic compounds residues. Therefore, AOP constitute a crucial technology for the degradation of non-easily removable organic compounds. AOP combine, for example, ozone (O_3), ultraviolet irradiation (UV), hydrogen peroxide (H_2O_2) and/or (photo)catalysts (e.g., Fe ions, TiO_2, polyoxometalates) to offer a powerful solution for the decrease or the removal of residual [16–20].
For example, the entire surface of a noble tuff specimen was split out in vertical and horizontal regions. Several oxidation reagents were tested on the stone surface: H_2O_2, commercial Oxone®, TiO_2, UV light irradiation, H_2O_2+UV light and TiO_2+UV light. H_2O_2 was used at 35%; Oxone was dissolved in water solution (0.1 M concentration of active oxygen as $KHSO_5$). Mixture of TiO_2 was obtained sonicating 0.75 g anatase and 0.25 g rutile in 10 mL deionized water for 10 min.
For photochemical reactions, a photochemical multiray apparatus (Helios Italquartz; Milano, Italy) was used, which contained 10 UV lamps of 15 W power each that emitted light around 254 nm. All chemicals are purchased from Sigma Aldrich and used as such.

On-site chemical and biological treatments
On-site chemical cleaning tests on the outer wall of Matera Cathedral were carried out adopting two different cleaning agent; H_2O_2 at 35% and AB57 agent (Istituto Centrale del Restauro, ICR Rome) The AB57 include inorganic salts (ammonium bicarbonate, sodium bicarbonate, EDTA disodium salt, quaternary ammonium salt, carboxymethyl cellulose) in aqueous solutions with other reagents and inert materials for the preparation of cleaning mixtures and poultice to be applied to stone surfaces and frescoes (CTS srl, Altavilla Vicentina, Ialy).
The area selected for the *on-site* bioapplication was part of the external wall of the right side of the Cathedral and was about two square meters (Figure 3), distinguish area with same size (25x40 cm) as follows: i) Laponite delivery system +enriched with *P stutzeri* A29 cells (biogel); ii) Laponite carrier alone as a control using only the delivery system without bacterial cells (control 1, gel). Finally, the second control test of an untreated area, no carrier, was used as comparison. For each area tested about 0.3 kg of the delivery system, which had been stored in sterile plastic box, was used; the application and distribution was facilitated by sterile flat plastic spatulas, which made it possible to apply thin layer gel averaging 0.5 cm in depth.
Representative areas from around 25 cm^2 to around 1,000 cm^2 were selected and bio-application tests were performed as following:
i) Japanese paper, 9.0 g/m^2, natural, white, un-buffered (Klug Conservation, Walter GmbH & Co. KG., Immenstatd, Germany) was applied in respect to the artwork.
ii) Laponite and laponite-bacterial gels without or with suspension of *P. stutzeri* viable cells (biogel), respectively, were obtained by manually mixing components with a plastic spatula, for 5 min;
iii) The delivery systems (laponite-gel or laponite-bacteria gel, biogel) were stored at 4°C or immediately applied to the selected areas;
iv) Japanese paper, sterile water and the delivery system as negative controls (only laponite gel) were performed on each artwork. No other facilities were adopted (as plastic net or a plastic film to reduce evaporation of water).
At different lengths of time from the bioapplication of viable bacterial cells (after 12 and 24 hours respectively), the biogel layers were removed by soft brush from the surface of the stone wall and the areas were subjected to three gentle manual washes using a soft sponge soaked in distilled water. Samples from all the cleaned and control areas were removed after contact, and sample stone were collected for chemical and microbiological analysis. Three replicates (30 x 20 cm) were made for all biocleaning and control tests. During bioapplication, outdoor environmental temperature was recorded.

Physical cleaning (laser technology)
A laser group with integrated cooling system and microprocessor control panel for the laser beam frequency and power - a laser hand-piece with a 3 m optical fiber, easy to-use and particularly suitable for *on-site* works was adopted (mod LIGHT II Laser, CTS srl, Altavilla Vicentina, IT).

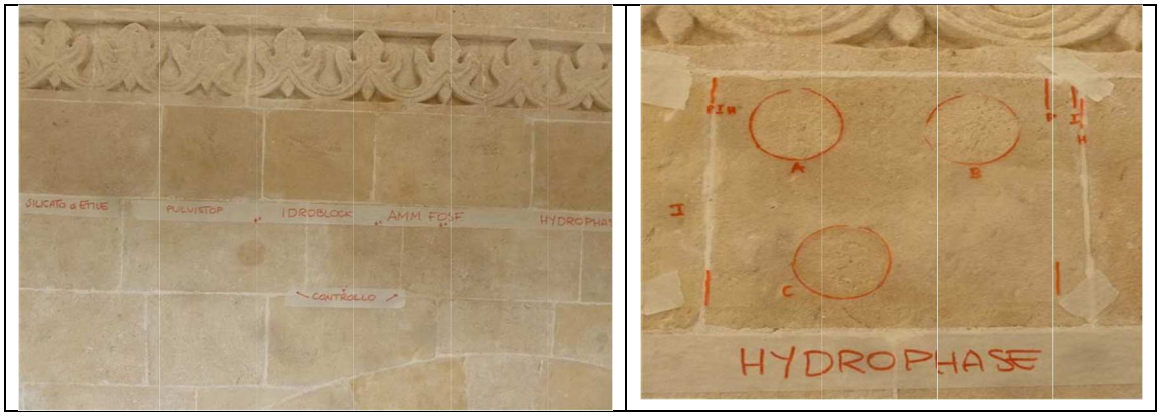

Figure 3. View of tuff stone area selected for the *on-site* treatment at Matera Cathedral (left). A particular of musk adopted on the wall to facilitate the re-position both of the colorimetric probe and the contact sponge (right).

2.2.2 Monitoring

Colour measurements

Colour measurements (Miniscan with light D65; Hunter Lab, Bergamo, Italy, and a portable Minolta, mod. Chromameter CR200), were adopted to evaluate any chromatic changes on the paint of the biocleaned surface on several previously selected areas, over the short and medium term. The chromatic coordinates L* (brightness), a* (difference between green and red), and b* (difference between yellow and blue) of the wall surfaces were determined according to the CIE L*a*b* standard colour system [9,21].

For each ancient tuff stone samples adopted both at *ex situ* lab trials and on the wall of the Cathedral as before described, several points of analysis (3-5) and (6-9), respectively, were selected; the instrument was set to automatically give the average value of the colorimetric coordinates (L*, a*, and b*) of the replicates (n. 3) measurements of each point, on each circle area selected. (Figure 3). The results of the chromatic change (Δ E*) was calculated by means of the following equation:

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

Finally, for each sample the (Δ E) values were mediated to obtain a single value and the standard deviation. The measurements were carried out on the same points, before and after the biological and chemical tests, by using an adequate mask.

Water uptake test (capillarity water adsorption)

To estimate a water uptake coefficient on the tuff surface of selected area, the contact sponge method, a non-destructive *on-site* methodology, was used [22]. The UNI Normal procedure requires to use a sponge with known density (Spontex1 type Calypso) soaked in water, weighed, placed on surface of the material for 1 minute, then weighed again [23]. Moreover, we adopted adequate paper disks (circular surface about 24 cm^2, diam. 6.0 cm) for the measurements, before and after the application of several treatment of cleaning and/or selected protective materials; for each treatment, mean of 3 replicated values were calculated (Figure 4).

3. Results and Discussion

3.1 Characterisation of the decay phenomena

3.1.1 LIF investigations

The LIF measurements were mainly performed on selected ashlars of southern wall and from the façade of the Cathedral, in particular those characterised by dark and clear areas (Figure 4).

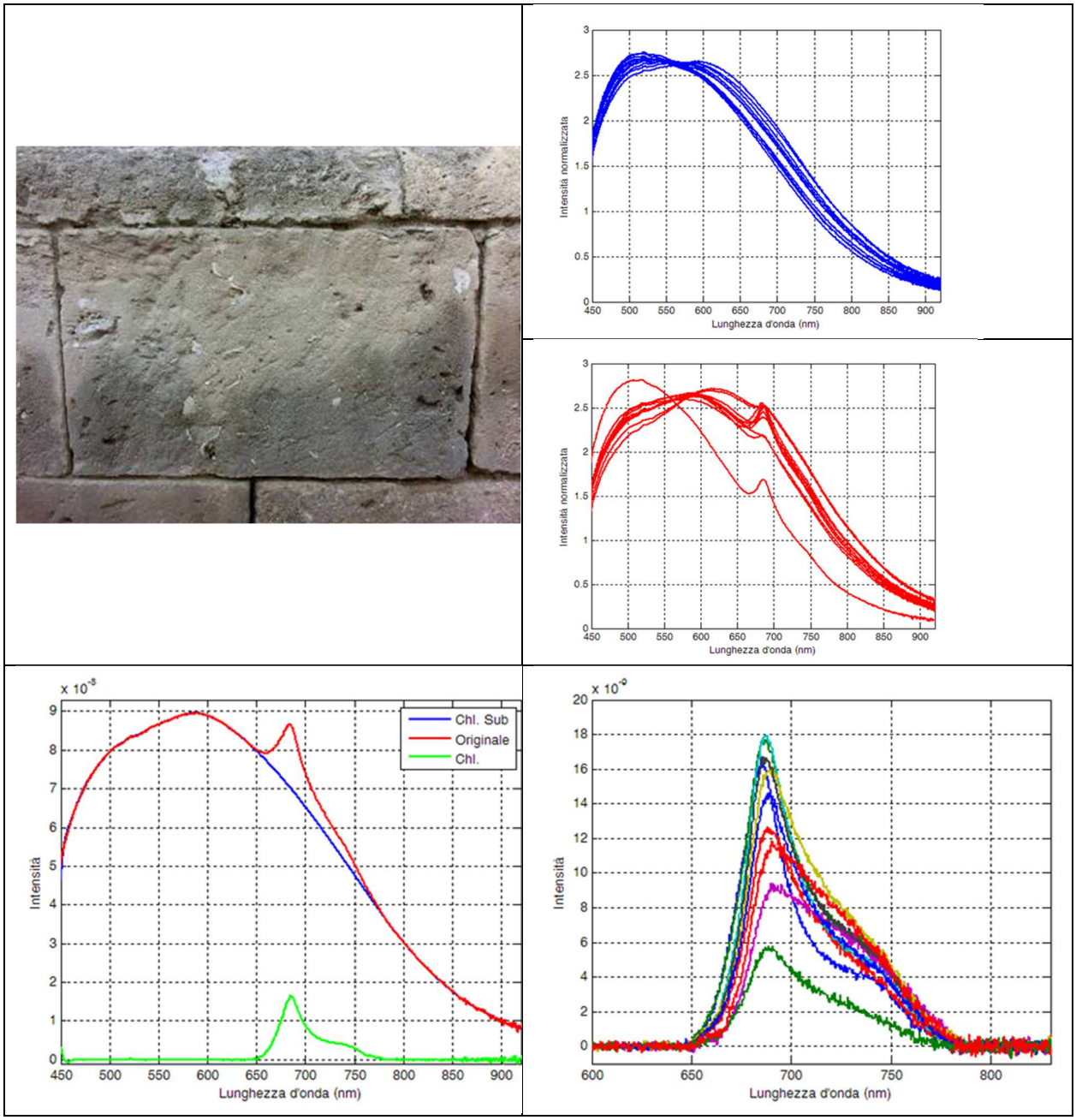

Figure 4. LIF analysis of the ashlars of the southern wall: (a) Photographic image of one of the examined ashlars featuring the characteristic dark patina. (b) LIF spectra acquired in various spots of the ashlar, both in the upper and clear part of the ashlar (red lines) and in the lower and dark part of the ashlar (blue lines). (c) Procedure applied to the acquired LIF spectra (red line) in order to separate the fluorescence contribution of chlorophyll (green line) from that of the stone substrate (blue line).
(d) Fluorescence of chlorophyll retrieved from the LIF spectra acquired in various spots of the dark area. All the LIF spectra were normalised in intensity to their standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Typically, the measurements have been carried out on both the upper (clearer) and lower (darker) part of the ashlars (Figure 4). The spectra were characterized by the presence of two main contributions of fluorescence, in a variable ratio, describable as two broad bands centred at about 510 nm and 610 nm. In these spectra the presence of the typical fluorescence peaks of photosynthetic pigments such

as chlorophyll could not be detected. The spectra measured on the upper, clearer, part of the ashlar was characterized by a spectral distribution of the substrate similar to the one detected in the darkest part (broad bands centred at about 510 nm and 610 nm). In these spectra, however, it is present the typical fluorescence spectrum of chlorophyll that is characterized by the typical peak signal centred at about 685 nm and by a spectral shoulder of lesser intensity at about 740 nm. In order to better highlight the typical chlorophyll fluorescence signal, the measured spectra have been further elaborated. On the basis of measurements made in the surface areas not subject to the presence of biological patinas, it is possible to observe that in the spectral range of interest, between about 640 nm and 800 nm, there are no particular structures in the fluorescence signal. The spectra were processed, by means of interpolation and reconstruction procedures, so as to estimate the spectral distribution of fluorescence due to chlorophyll. In Figure 4a is shown an example of application of this processing technique. In red it is shown as actually measured fluorescence spectrum that is characterized by the presence of the peak of chlorophyll fluorescence. In blue it is shown the result of the interpolation procedure allowing to estimate the spectrum without the chlorophyll contribution. In green it is shown the fluorescence signal related to chlorophyll clearly highlighting the main contributions at 685 nm and 740 nm. Figure 4b shows the chlorophyll fluorescence spectra obtained by applying this procedure to the spectra acquired on the lower part of the ashlar.

These fluorescence signals suggest the presence of a phototrophic biological patina. We have to notice that it was possible to detect the presence of phototrophic patinas exclusively on the upper and clearer part of the ashlar. In order to verify the possible presence of phototrophic organisms in detectable concentration also in the lower and darker part of the ashlar, other fluorescence measurements were carried out after the execution of some mechanical procedures capable to put in evidence the eventual presence of a biological patina that could be inactive and/or not located on the very surface of the ashlar. The applied procedures consisted, in sequence, in the mechanical removal of the very first superficial layer of the dark patina and in moistening with water. Also after this procedure, it was not possible to detect the presence of chlorophyll by means of its fluorescence signal. In some case further test measurements were carried out on the darkest part of the ashlar to verify the lack of the chlorophyll fluorescence signal, determining the presence of the fluorescence signal of such a pigment only in correspondence of alveolus with a diameter of between 4 and 6 mm. Furthermore, the spectral distribution of fluorescence associated to the stone substrate inside the alveolus is distinctly different from that of the surrounding area.

Some investigations were carried out also on the St. Eustace statue, which was positioned on the façade. The measurements were carried out on the right shoulder of the statue and on a yellowed area of the aedicule where the statue was housed, just close to samples C6 and C7. The spectra acquired on the shoulder show a main fluorescence contribution with a maximum at about 500 nm and a secondary lower intensity contribution in the 700-750 nm spectral region. The spectra acquired on the aedicule area show a very different spectral distribution, very broad and unstructured, with a maximum between 590 nm and 650 nm.

A plastered portion of the façade was analysed too (Figure 5). The surveyed area includes the plastered surface (circled in blue), a portion of stone ashlar from which the plaster was mechanically detached immediately before the measurements (circled in red), and an area in the major plaster gap caused in the past for a plaster detachment (circled in green).

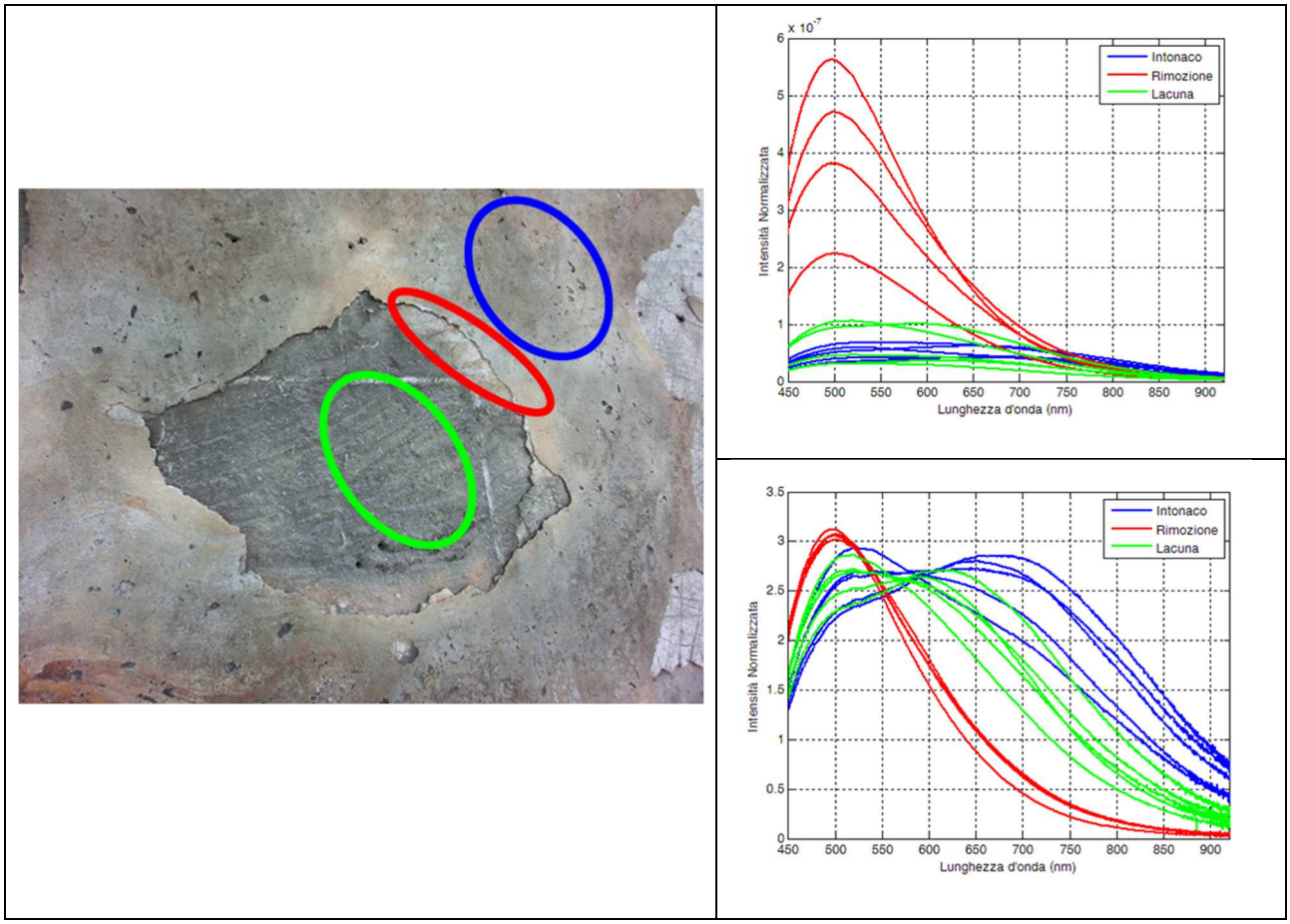

Figure 5. LIF measurements on a plastered area of the cathedral's façade. (a) Photographic image of the examined area: three areas are highlighted in the photo: a plastered area (Area A), an area where the plaster was deliberately detached mechanically (Area B), and an area corresponding to a former gap in the plaster (Area C).
(b) LIF spectra measured in the three different areas. (c) LIF spectra measured in the three different areas and normalised in intensity to the relative standard deviation.

Figure 6 shows the fluorescence spectra acquired on the three different areas. By comparing the intensities and spectral distributions of these spectra, it is apparent that the surface exposed because of the removal of the plaster is characterized by a fluorescence efficiency significantly greater than both the plastered one and that measured in the area with a lack of plaster caused in the past, as shown by the spectral distribution. The spectra of the 'old' gap showed a spectral distribution substantially similar to that observed on the darker areas of other ashlars being characterized by two main fluorescence contributions at about 500-510 nm and 600-610 nm. The spectra acquired in the area in which the plaster was manually removed are instead remarkably similar to those acquired on the stone specimen not treated. This points out that the layer of plaster had in some way protected the stone from external agents, natural and / or artificial, that caused browning of extended surfaces of the outer surface of the Cathedral.

3.1.2 Biological investigations

The powder samples, observed with an optical microscope in epifluorescence showed red fluorescence due to the chlorophyll of photoautotrophs, while the yellow, orange or blue fluorescence could be attributed to the presence of other not identified organic products (Figure 6).

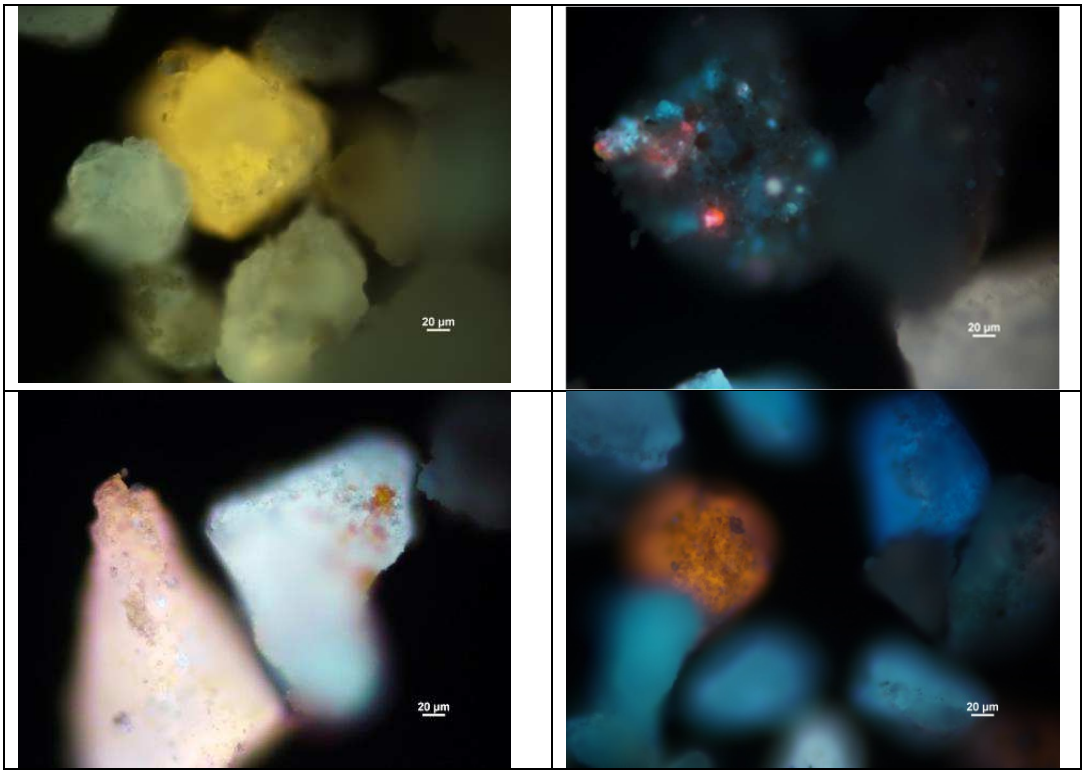

Figure 6. Autofluorescence examples of the powder samples B1 (above, left), B2 (above, right), B3 (bottom, left) and B4 (bottom, right).

The sample B4 had a lower microbial contamination with respect to the other samples with a greater biodiversity, especially for the sample B3 (Table 3). The fungal strain *Alternaria* sp. was present almost all the samples, except B4.

Table 3

The microbial count/presence observed on the three nutrient media; CFU (colony forming units): +/- = scarce, + = present, ++ = frequent, +++ = abundant.

Media/ samples	Microbial count (CFU/100 mg)			
	B1	B2	B3	B4
PDA (fungal)	1 fungal	2 fungal	3 fungal	1 fungal
PCA (fungal and bacterial)	2 fungal 41 bacterial	1 fungal 37 bacterial	5 fungal 52 bacterial	0 fungal 1 bacterial
BG_{11M} (phototrophs)	++	+++	+++	+/-

In the nutrient media used for the development of phototrophic microorganisms, the coccoid cyanobacteria of the family of *Xenococcaceae,* with spherical mucilaginous colonies was dominant in all the samples, except for B4 where the presence of phototrophs was insignificant. Other than cyanobacteria were detected, in the heterotrophic nutrient media, light and deep pink pigmented bacteria (cocci and rod-shaped), mostly in samples B1, B1 and B3. Some of these bacterial colonies have an autofluorescence: pink in sample B1 and yellow in sample B2.

3.1.3 Chemical and mineralogical investigations

Optical Microscopy

On the basis of observations in transmission mode, the plaster is constituted by an air hardening calcic lime binder with a homogeneous aspect and a micritic texture. The binder/aggregate ratio is 2:1. The grain size of the aggregate is bimodal (250-350 mm and 700-800 mm) and the shape of the grains is from sub-angular to sub rounded. Its composition is characterized by fragments of rock (i.e. Matera stone), fossiliferous remains and micrite limestone. The porosity is due to the shrinkage cracks.
Regarding to C2 and C3 samples, observed in reflection mode, were interested by a biological colonization giving a red fluorescence under UV light, due to chlorophyll. C6 sample was observed in stratigraphy: it is possible to distinguish an alteration superficial layer (about 200 μm thickness) and a layer constituted by the sound carbonatic stone. Biological colonization was not identified.

X-Ray Diffraction

XRD analyses revealed the presence of whewellite ($CaC_2O_4 \cdot H_2O$, calcium oxalate monohydrate) in the samples coming from the facade, with the exception of the plaster C4. Gypsum was detected in C1 (from southern wall) and in C6 and C7 (from façade). Calcite and quartz are present in all analyzed samples (Table 4).

Table 4
Results of FTIR and XRD analysis of the samples

Sample label		Composition
C1		calcite, gypsum, alkaline nitrate, silicates, traces of quartz and organic compounds
C2		calcite, quartz, alkaline nitrate, silicates, traces of clay minerals and organic compounds
C3		calcite, quartz, alkaline nitrate, silicates, organic compounds
C4	plaster	calcite, traces of quartz
	external layer	calcite, whewellite, traces of organic compounds
	inner layer	calcite, gypsum, traces of organic compounds
C5		calcite, gypsum, silicates, traces of whewellite and quartz
C6	bulk	calcite, gypsum, whewellite, traces of quartz
	external layer	gypsum, whewellite, traces of organic compounds
C7		calcite, gypsum, whewellite, silicates
OG		calcite, quartz, whewellite, traces of goethite

Ionic Chromatography

All samples coming from Southern Wall (samples C1, C2 and C3) were analyzed by means of ionic chromatography: samples C1 and C2 contain analogous amounts of chloride (Cl^-), nitrates (NO_3^-) and sodium (Na^+). The amounts of such ions, instead, are lower in sample C3. Sulfates (SO_4^{2-}) are relevant only in sample C1. Finally, decreasing amounts of potassium (K^+) and calcium (Ca^{2+}) ions are revealed passing from sample C1 to C2 and to C3. These results suggest the presence of gypsum in sample C1, coming from blackened area, and of soluble salts (probably sodium and potassium nitrate and chloride) in samples C1 and C2, coming from areas where the surface is evidently contaminated by external agent, more than C3.
Sample C5 contains low amount of chloride, sodium and potassium, whereas nitrates, sulfates, calcium and magnesium are present in high amount. Stone powder is so contaminated by gypsum and soluble salts (presumably calcium and magnesium nitrate and/or organic nitrates).
Sample C6 on the contrary contains sodium, nitrates and sulfates in big amounts. Chloride, potassium and calcium are present in quite significant amount, too. Gypsum and soluble salts (particularly nitrates, presumably calcium and/or organic ones) are present in this powder too.

Infrared Spectroscopy

FTIR spectra proved very effective in identifying the inorganic component of samples (Table 4).

In all the samples the presence of calcium carbonate, in the mineralogical form of calcite, was suggested by an asymmetric C=O stretching band around 1420 cm^{-1} and by the absorbance at 873 (out-of-plane bending vibration) and 712 cm^{-1} (in-plane bending vibration) [24]. Alkaline nitrate and silicates were present as well, as shown by the absorbance at 1384 and for the Si-O stretching band at 1033 cm^{-1} respectively [25].

The spectra of samples C1, C5 and C7 present the characteristic sharp peaks of gypsum ($CaSO_4$ $2H_2O$), of hydroxyl stretching bands (3545 and 3408 cm^{-1}) and bending vibrations (1689 and 1622 cm^{-1}), for the S-O asymmetric stretching modes at 1145 and 1116 cm^{-1}, and bending mode at 669 cm^{-1} [24]. Organic matter was found as traces almost in all the samples, with peaks around 2982 and 2875 cm^{-1}, which could be ascribed to CH signals, although FTIR analysis cannot be diagnostic without the support of more sensitive techniques.

The three layers which form sample C4 show the signals of calcite and of traces of organic matter mentioned before. The features determined in the spectra of the external layer suggest the presence of calcium oxalate monohydrate mineralogical phase, that is whewellite ($CaC_2O_4 \cdot H_2O$) (Figure 7). The identification occurred due to the presence of very specific bands at 3487, 3435, 3341 and 3068 (OH stretching bands), 1623 and 1315 (CO stretching vibration), 780 and 674 cm^{-1} (OCO bending vibrations), which are shown in Figure 13 [26]. The inner layer contains gypsum, as shown by the typical peaks, which have already been discussed. The same absorbance signals of whewellite were recognized in the spectra of samples C5 and C7.

The external layer of sample C6 is composed by gypsum, whewellite and organic compounds, whose pattern of peaks might suggest a proteinaceous substance (2962, 2928, 2873, 1508, 1243 cm^{-1}).

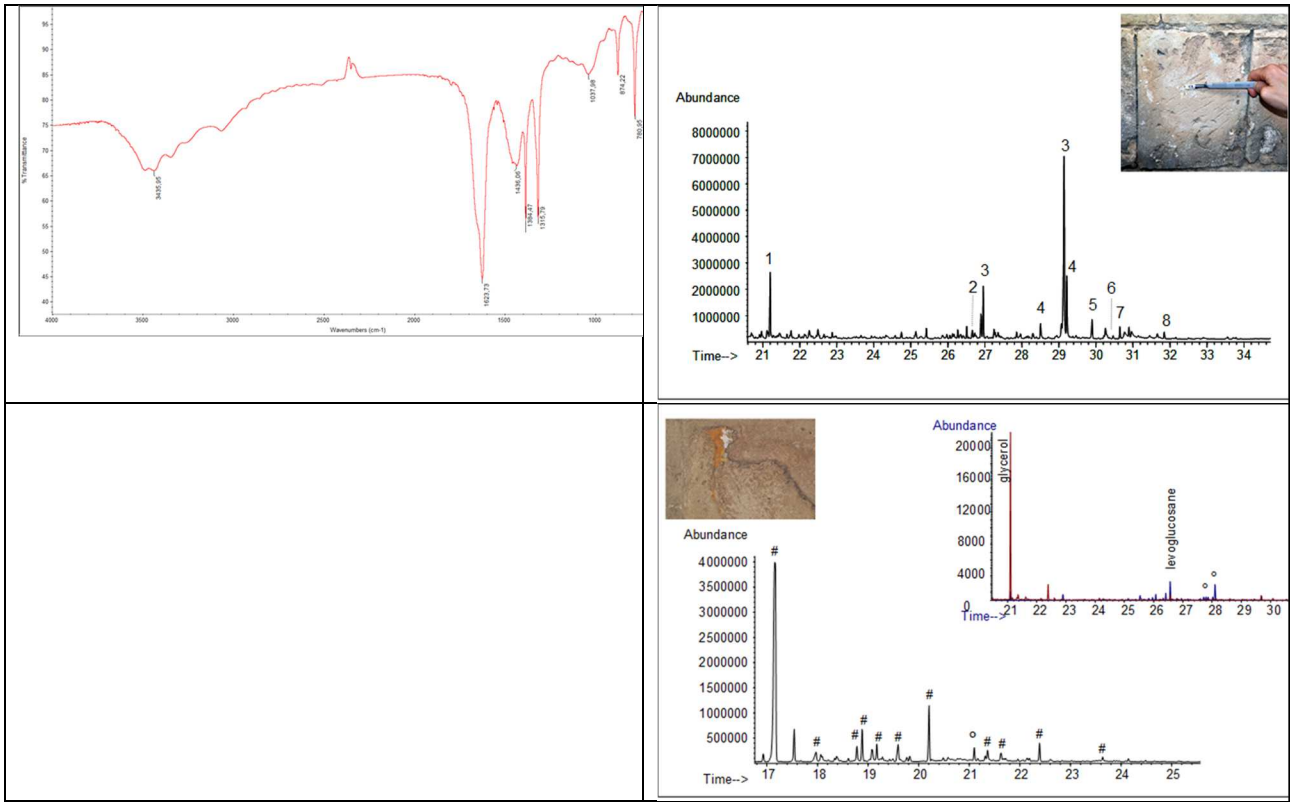

Figure 7. Transmission FTIR spectrum of the external layer of sample C4 (left), showing the presence of whewellite (3487, 3435, 3341, 3068, 1623, 1315, 780, and 674 cm^{-1}), calcite (1436, 874, and 712 cm^{-1}), alkalin nitrates (1384 cm^{-1}), traces of organic substances (2870-2980 cm^{-1}). Pyrogram of sample C3 (right, above), with the most significant compounds identified: 1: glycerol, 2: levoglucosane, 3: arabinitol, 4: ribitol, 5: hesadecanenitrile, 6: mannitol, 7: palmitic acid, 8: octadecanenitrile, 9: inositol. Pyrogram of sample OG (right, bottom), with marked the siloxane compounds (#) together with the markers of a saccharide source (glycerol, levoglucosane and two unknown markers).

Pyrolisis Gas Chromatography Mass Spectrometry (Py-GC-MS)

The pyrolisis performed on the sample with derivatisation reaction *in situ* [11] allowed the characterisation of the origin of the organic materials in the thick crust and patina of the façade.
All the samples analyzed presented a variable content of protein. The markers hexadecanenitrile and octadecanenitrile of egg are shown in the pyrogram of sample C3, reported in Figure 7 as an example. Particularly interesting are the results obtained from the samples collected from the ashlar which has been investigated by means of LIF techniques in three different areas (samples C1, C2, and C3). The analyses highlighted the presence of saccharide material in the clear part of the ashlar (samples C2 and C3) but not in the darkest one (sample C1). In Figure 7 also the markers of saccharide material are evidenced (glycerol, levoglucosane, arabinitol, ribitol, mannitol, inositol). Most likely, this material is to be ascribable to the presence of plant cells, as evidenced by the finding of chlorophyll by the LIF *on-site* investigation. Considering that the saccharide material is absent in the black part (sample C1), and that sulfates are more abundant in the black area, we can hypothesize that they inhibit the growth of chlorophylls. The formation of calcium oxalate is possibly originated by the presence of the proteinaceous material [27–29] which has been probably applied in a *beverone* with conservation purpose. In fact, being the egg most abundant in the blacker area, we can explain the presence of darker and thick crust on the bottom of the cathedral walls, with an easier accessibility for such conservation treatment during the time. Natural resins, waxes or siccative oil are absent. Finally, the analysis of sample OG (Figure 7), supposed to be an ochre mortar applied by the restorers, suggested to be a protective siloxane based for masonry. In Figure 7 are reported all the siloxane compounds identified, together with the markers of a trace of saccharide material.

3.2 Cleaning and protection tests

3.2.1 Assessment on e*x situ* laboratory tests

The preliminary laboratory treatments have been applied and tested on ancient noble surface tuff stone of the Cathedral, with UV light at 254 nm for 72 hours, or without exposition (at 24 hours), as previously described. The ΔE* values measured on ancient tuff stone, after treatments by chemical and biological agents, are summarized in Table 5.

Table 5
Colour changes (ΔE*) measured on *ex situ* tuff stone Matera Cathedral, at 24-72 hours after treatments with chemical or bio-cleaning agents. Average ± SD for 3 points obtained in each area.

Processes	Treatment	Δ E*
	Blank	0.1 ± 0.1
	TiO_2	2.2 ± 0.3
Chemical	TiO_2, + UV	3.7 ± 0.4
	UV	0.1 ± 0.2
	H_2O_2	3.4 ± 0.2
	H_2O_2 + UV	4.9 ± 0.4
	Oxone	4.2 ± 0.5
Biological	Laponite gel	2.2 ± 0.4
	Laponite biogel	3.9 ± 0.3

The colorimetric measurements on the tuff stone showed that the most effective chemical treatments were H_2O_2 + UV, Oxone and TiO_2 + UV, respectively. However, the tuff area processed with Oxone resulted, at some points, ruined, so we discarded this oxidant.
We noted that only UV radiation had no effect as compared to the control (blank). Moreover, area treated with TiO_2 appeared "coated" with white pigment; it was washed by deionized water, but the band remained white. On the contrary, when tuff area was processed with H_2O_2, the brownish

alteration was bleached in all surface, raising the cleaning effect when H_2O_2 was combined with UV exposition (72 h). H_2O_2 is a green and environmental friendly reagent because the only by-products are water and molecular oxygen; furthermore, H_2O_2 is not an acidic compound and can be utilized also on the limestone.

The results of the biological treatment were determined at 48 hours, after the soft removal of laponite gel residues by manual brush, when the stone surface was dried by spontaneous evaporation of water initially contained in the laponite gel. The results by colorimetric measurements showed the effective of biocleaning agents when laponite biogel enriched of *P. stutzeri* viable cells was applied for 48 h.

3.2.2 *On-site* tests on Matera Cathedral wall

The results on the application of chemical (AB57 mixtures-poultice and H_2O_2) and biological (laponite biogel) treatments onto *on-site* selected areas (Table 6) indicate a good compatibility of all treatments, where the colorimetric measurements performed on the area tested showed ΔE* values over the threshold of 3 units; so, the chromatic changes were more than 3 so visible by the naked eye and as consequence the treated areas at the end of the treatments (i.e, after AB57 poultice or gel removal) appear affected by less dark alteration (Figure 8). Moreover, when biogel (laponite + bacteria) was adopted the chromatic change ΔE* average values were higher 4.75 $\pm$ 1.40 compared to the 4.1 $\pm$ 1.28 of laponite alone, but with no statistical evidence. However, in both cases, wide range of SD values suggesting that the irregular and not homogeneous surface of tuff stone with large pits and caves, empty spaces, due to loss of materials, exfoliation and decay of surface material, greatly affected the final results.

In the case of chemical cleaning methods, the chromatic change values between the use of H_2O_2 or AB57 poultice were higher (ΔE* of 4.95 and 4.65, respectively) and statistically comparable each other.

Table 6

Colour changes (ΔE*) and Water uptake (g $\pm$ SD results at *on-site* sandstone artwork Matera Cathedral, with chemical or bio-cleaning agents, measured at 48 h, after application.

Area	Treatments	ΔE*	Water uptake
1-3	Control/Blank	0.70 $\pm$ 0.10	0.6 $\pm$ 0.1
4-5, 8-10	AB57 mixtures and poultice	4.65 $\pm$ 0.50	3.4 $\pm$ 0.3
6	Laponite gel	4.1 $\pm$ 1.28	2.5 $\pm$ 1.4
7	Laponite biogel	4.75 $\pm$ 1.40	2.9 $\pm$ 1.9
11	H_2O_2	4.95 $\pm$ 0.70	3.0 $\pm$ 0.6

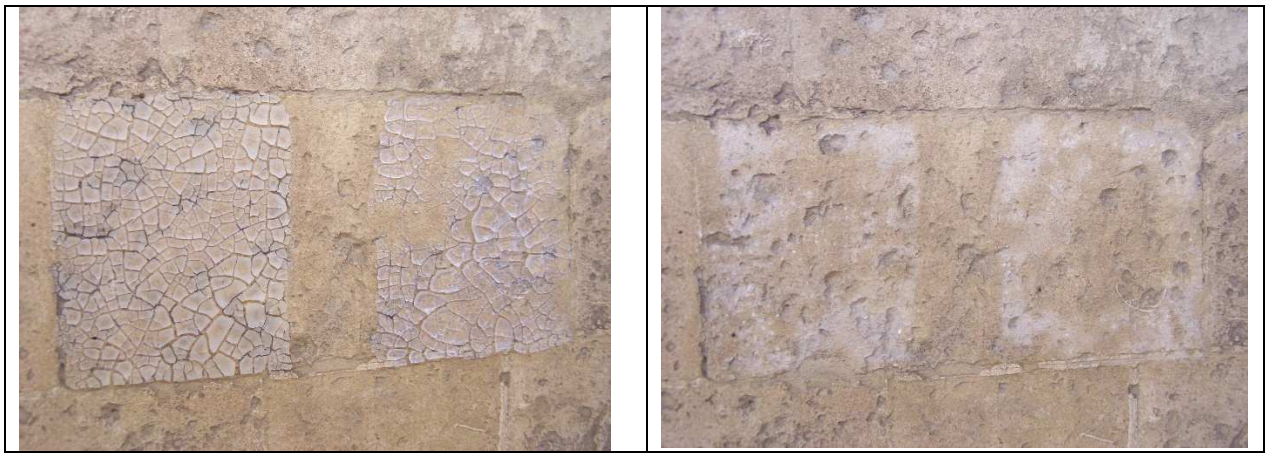

Figure 8 *On-site* biocleaning test performed on the lateral tuff wall of Matera Cathedral; laponite biogel bacteria, at 48 hours of the bioapplication, before (left) and after (right) the soft biogel removal.

Moreover, Table 6 shows the values of water uptake (mean+SD in g) by lithoid surface of the left gate near the column, before and after the several tests performed.
The calculated data in the treated areas are meaningful different from those made in reference ones (control/blank). In the case of chemical cleaning (AB57) the highest value was obtained with a little range of SD (3.4 + 0.3), demonstrating the high performance of cleaning over all the surface treated. In other cases, the application of laponite gel and laponite biogel bacteria appear to influence at intermediate level the stone substrate in term of water uptake, but with higher DS values, probably due to the irregular of the surface and the presence of *lacunes*. Finally, the values of water uptake after the H_2O_2 treatment appear not meaningful different from those previously cited.

3.2.3 Physical cleaning by laser technology

Within restoration activities, which are carried out on works of art of historic and artistic interest, cleaning represents a particularly delicate phase. It is a well-known that the possibility of control, selectivity, preventive evaluation of the effects produced by the intervention, harmlessness both for the restorer and the environment as well as for the work of art, are primary conditions and necessary, for adopting cleaning systems with suitable characteristics for combining the different requirements. The use of laser technology, (a type of physical treatment) has given positive results in this field, adopted especially on limited area on tuff stone surface of Matera Cathedral altered by hard layers of grey-brown crusts that still present even after previously cleaning treatments above cited (Figure 9). As consequence, in fact, the laser cleaning proved to be: i) selective, the shotted beam is absorbed by dirt without affecting the substrata; ii) graduated, the beam power can be selected according to the operator's needs: iii) high quality, when laser was used even on some degraded artworks without the need to pre-consolidate.

Figure 9 Micro laser-device and technology adopted on limited area on tuff stone surface of Matera Cathedral

3.2.4 Protective tests

Some stone ashlars were treated with ammonium oxalate and ammonium phosphate and treated with the protective agents Pulvistop, Idroblock and Hydrophase Acqua. The surfaces were monitored by non-invasive FTIR spectroscopy (Figure 10) and Py-GC-MS.

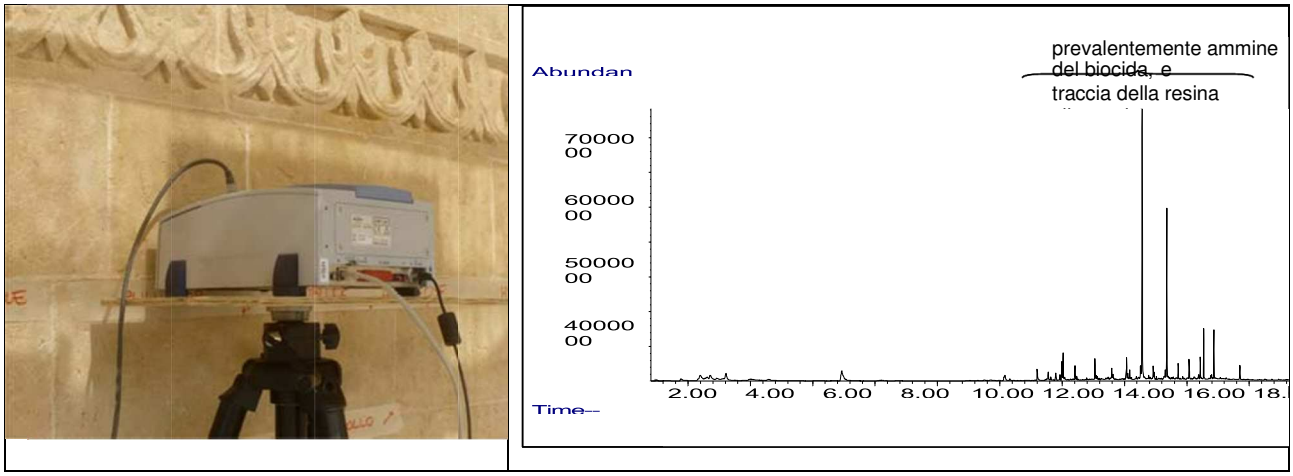

Figure 10 Non-invasive FTIR analysis of a ashlar (left). Pyrogram (right).

FTIR results showed the presence of calcite due to the stone substrate. Only in case of the area treated with ammonium oxalate, other weak bands were present. The absorbance at 1318 and 784cm^{-1} were ascribed to calcium oxalate, being the CO stretching and OCO bending vibrations respectively [26]. When protective agents were used the SiC signal at 1267cm^{-1} was observed, and in case of Pulvistop the absorbance at 1737cm^{-1} due to acrylic resin was evident.

Coming to the Py-GC-MS analysis of micro-fragments of the surface after two months since the application of the protective agents, signals of decay products were absent. The residues of agents Idroblock and Hydrophase Acqua on the surface were much less than the biocide (Figure 10). Test on new formulation of Hydrophase Acqua showed the presence of siloxane, suggesting the use of the type tested on-site.

3.2.5 *Water uptake*

The results obtained to evaluate the water uptake coefficient on the tuff surface of selected area, by the contact sponge method, showed the best results was obtained with *Hydrophase acqua* [water uptake 1.58 mg/(min*cm^2)], if compared to the other protective agents tested (article in submission) and to the control area [water uptake 90.05 mg/(min*cm^2)].

The methodology adopted implies manual skill and experience to produce a coherent set of data; the variables involved are in fact not only the imposed pressure but also the compression mechanics; the control on the applied pressure allowed reproducible measurements too.

4. Conclusions

This study provides a systematic investigation of the Cathedral of Matera by biological and chemical techniques, also non-invasive, which succeeded in identifying the dark alteration of the external walls as calcium oxalate film.

Since the first famous occurrence on the Parthenon at the end of 19th century [30], the phenomenon of calcium oxalate films has been frequently observed in the Mediterranean basin on stone materials (mortars, mural paintings and sculptures) and natural stones of various natures, mainly marble and in several climatic conditions. These films, whose color ranges from ochre to black, are very insoluble in water, thus stable and hardly to remove. The issue about the formation processes of these films has involved the scientific communities at the end of the last century [31,32] debating on the biological or chemical origin. According to the former, calcium oxalate comes from the biofilms on the stone

surfaces, producing oxalic acid that reacts with calcium carbonate from the substrate or from deposits [33,34]. On the contrary, oxalic acid could originate from the oxidation of natural organic materials, such as oil, egg and milk, applied in the past on the surface [27,28], being past conservation treatment well documented in the literature for various purposes, such as artificial ageing of new architectural elements, protection against the natural decay, maintenance works.

The occurrence of calcium oxalate films has been documented until recent years [2,4,35–37]. In the specific case of the Cathedral of Matera, the two mechanisms could actually occur simultaneously on the stone surface, for the presence of biological activity (cyanobacteria, pigmented heterotrophic bacteria, bacilli and cocci type bacteria) and of residues of organic compounds, which seem to indicate that past conservation treatments were applied. In the specific case of sample C4, the sequence of layers might suggest a past conservation work, when the surface degraded to gypsum was covered by a mixture of calcite and organic compounds, that transformed into calcium oxalate. Where the so-called *beverone*-a mixture of natural organic compounds applied on the stone surface in form of thin film-was not applied, the sulfates, more abundant respect to the calcium oxalate, possibly prevented the growth of microorganisms. LIF measurements in fact showed an 'antagonist' behaviour between the presence of phototrophic biodeteriogens and the presence of the dark alteration of the stones: the chlorophyll is present almost exclusively in areas without dark alteration. Therefore the mechanism of formation of calcium oxalate could be exclusively chemical.

The analytical campaign was effective to manage and guide the cleaning tests carried out on stone specimens treated with different cleaning methods. The application of AB57, followed by washing with deionized water, was the most effective method. The test with laser Nd: YAG (El.En) was effective too, but it was carried out only on the carved elements because the cleaning time proved to be too long.

Coming to the protective agents, the results suggested the use of Hydrophase acqua, that shows the best performance as does not cross-link and does not film, its colour and composition are constant and gives a good water repellency to the surface. Considering the high porosity of the stone, the surface should be treated again in 3-4 years.

Our multi-technique approach was useful for the whole conservation work, in particular the removal of the dark alteration. Figure 11 shows the final appearance of the Cathedral, which will be one of symbols of Matera, that was proclaimed Matera the European Capital of Culture for 2019.

Figure 11 The Cathedral of Matera before (left) and after (right) the conservation work here reported.

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REFERENCES

[1] AA.VV., Science and Art: A Future for Stone, in: J. Hughes, T. Howind (Eds.), Proc. 13th Int. Congr. Deterior. Conserv. Stone, University of the West of Scotland, Paisley, 2016: p. 684.

[2] C. Genestar, C. Pons, J.C. Cerro, V. Cerdà, Different decay patterns observed in a nineteenth-century building (Palma, Spain), Environ. Sci. Pollut. Res. 21 (2014) 8663–8672.

[3] T. Rosado, A. Reis, J. Mirão, A. Candeias, P. Vandenabeele, A.T. Caldeira, Pink! Why not? On the unusual colour of Évora Cathedral, Int. Biodeterior. Biodegradation. 94 (2014) 121–127. doi:10.1016/j.ibiod.2014.07.010.

[4] D. Pinna, M. Galeotti, A. Rizzo, Brownish alterations on the marble statues in the church of Orsanmichele in Florence: what is their origin?, Herit. Sci. 3 (2015) 7. doi:10.1186/s40494-015-0038-1.

[5] M. Lettieri, M. Masieri, Surface characterization and effectiveness evaluation of anti-graffiti coatings on highly porous stone materials, Appl. Surf. Sci. 288 (2014) 466–477.

736 doi:10.1016/j.apsusc.2013.10.056.
737 [6] V. Crupi, G. Galli, M.F. La Russa, F. Longo, G. Maisano, D. Majolino, M. Malagodi, A.
738 Pezzino, M. Ricca, B. Rossi, S.A. Ruffolo, V. Venuti, Multi-technique investigation of
739 Roman decorated plasters from Villa dei Quintili (Rome, Italy), Appl. Surf. Sci. 349 (2015)
740 924–930. doi:10.1016/j.apsusc.2015.05.074.
741 [7] R.-M. Ion, D. Turcanu-Caruţiu, R.-C. Fierăscu, I. Fierăscu, I.-R. Bunghez, M.-L. Ion, S.
742 Teodorescu, G. Vasilievici, V. Rădiţoiu, Caoxite-hydroxyapatite composition as
743 consolidating material for the chalk stone from Basarabi–Murfatlar churches ensemble, Appl.
744 Surf. Sci. 358 (2015) 612–618. doi:10.1016/j.apsusc.2015.08.196.
745 [8] M. Ricca, C.M. Belfiore, S.A. Ruffolo, D. Barca, M.A. De Buergo, G.M. Crisci, M.F. La
746 Russa, Multi-analytical approach applied to the provenance study of marbles used as
747 covering slabs in the archaeological submerged site of Baia (Naples, Italy): The case of the
748 “Villa con ingresso a protiro,” Appl. Surf. Sci. 357 (2015) 1369–1379.
749 doi:10.1016/j.apsusc.2015.10.002.
750 [9] G. Alfano, G. Lustrato, C. Belli, E. Zanardini, F. Cappitelli, E. Mello, C. Sorlini, G. Ranalli,
751 The bioremoval of nitrate and sulfate alterations on artistic stonework: The case-study of
752 Matera Cathedral after six years from the treatment, Int. Biodeterior. Biodegradation. 65
753 (2011) 1004–1011. doi:10.1016/j.ibiod.2011.07.010.
754 [10] A. Lluveras, I. Bonaduce, A. Andreotti, M.P. Colombini, GC/MS analytical procedure for the
755 characterization of glycerolipids, natural waxes, terpenoid resins, proteinaceous and
756 polysaccharide materials in the same paint microsample avoiding interferences from
757 inorganic media, Anal. Chem. 82 (2010) 376–386. doi:10.1021/ac902141m.
758 [11] S. Orsini, F. Parlanti, I. Bonaduce, Analytical pyrolysis of proteins in samples from artistic
759 and archaeological objects, J. Anal. Appl. Pyrolysis. 124 (2017) 643–657.
760 [12] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments,
761 strain histories and properties of pure cultures of cyanobacteria, Microbiology. 111 (1979) 1–
762 61.
763 [13] G. Ranalli, G. Alfano, C. Belli, G. Lustrato, M.P. Colombini, I. Bonaduce, E. Zanardini, P.
764 Abbruscato, F. Cappitelli, C. Sorlini, Biotechnology applied to cultural heritage:
765 biorestoration of frescoes using viable bacterial cells and enzymes, J. Appl. Microbiol. 98
766 (2005) 73–83. doi:10.1111/j.1365-2672.2004.02429.x.
767 [14] G. Lustrato, G. Alfano, A. Andreotti, M.P. Colombini, G. Ranalli, Fast biocleaning of
768 mediaeval frescoes using viable bacterial cells, Int. Biodeterior. Biodegradation. 69 (2012)
769 51–61. doi:10.1016/j.ibiod.2011.12.010.
770 [15] P. Bosch-Roig, G. Lustrato, E. Zanardini, G. Ranalli, Biocleaning of Cultural Heritage stone
771 surfaces and frescoes: which delivery system can be the most appropriate?, Ann. Microbiol.
772 65 (2015) 1227–1241. doi:10.1007/s13213-014-0938-4.
773 [16] M. Bressan, L. Liberatore, N. d’Alessandro, L. Tonucci, C. Belli, G. Ranalli, Improved
774 combined chemical and biological treatments of olive oil mill wastewaters, J. Agric. Food
775 Chem. 52 (2004) 1228–1233.
776 [17] L. Liberatore, M. Bressan, C. Belli, G. Lustrato, G. Ranalli, Chemical and biological
777 combined treatments for the removal of pesticides from wastewaters, Water, Air, Soil Pollut.
778 223 (2012) 4751–4759. doi:10.1007/s11270-012-1230-5.
779 [18] L. Garrel, M. Bonetti, L. Tonucci, N. d’Alessandro, M. Bressan, Photosensitized degradation
780 of cyclohexanol by Fe (III) species in alkaline aqueous media, J. Photochem. Photobiol. A
781 Chem. 179 (2006) 193–199.
782 [19] P. D’Ambrosio, L. Tonucci, N. d’Alessandro, A. Morvillo, S. Sortino, M. Bressan, Water-
783 soluble transition-metal-phthalocyanines as singlet oxygen photosensitizers in ene reactions,
784 Eur. J. Inorg. Chem. (2011) 503–509.
785 [20] L. Tonucci, F. Coccia, M. Bressan, N. d’Alessandro, Mild photocatalysed and catalysed
786 green oxidation of lignin: a useful pathway to low-molecular-weight derivatives, Waste

Biomass Valor. 3 (2012) 165–174.
[21] UNI-EN 15886:2000. Conservation of cultural property - test methods - colour measurement of surfaces, (1994).
[22] UNI 11432. Beni culturali Materiali lapidei naturali ed artificiali - Misura della capacita di assorbimento di acqua mediante spugna di contatto, (2011) 6.
[23] P. Tiano, C. Pardini, Valutazione in situ dei trattamenti protettivi per il materiale lapideo: proposta di una nuova semplice metodologia, Arkos Sci. E Restauro Dell'architettura. 5 (2004) 30–36.
[24] M.R. Derrick, D. Stulik, J.M. Landry, Infrared Spectroscopy in Conservation Science, The Getty Conservation Institute, Los Angeles, 1999.
[25] M.J. Wilson, Clay mineralogy: spectroscopic and chemical determinative methods, Chapman & Hall, London, 1994. https://books.google.it/books?id=-EVKpdzHQq8C.
[26] I. Petrov, B. Šoptrajanov, Infrared spectrum of whewellite, Spectrochim. Acta Part A Mol. Spectrosc. 31 (1975) 309–316.
[27] F. Cariati, L. Rampazzi, L. Toniolo, A. Pozzi, Calcium oxalate films on stone surfaces: experimental assessment of the chemical formation, Stud. Conserv. 45 (2000) 180–188. doi:10.2307/1506764.
[28] L. Rampazzi, A. Andreotti, I. Bonaduce, M.P. Colombini, C. Colombo, L. Toniolo, Analytical investigation of calcium oxalate films on marble monuments, Talanta. 63 (2004) 967–977.
[29] R. Bugini, C. Corti, L. Folli, L. Rampazzi, Unveiling the Use of Creta in Roman Plasters: Analysis of Clay Wall Paintings From Brixia (Italy), Archaeometry. 59 (2017) 84–95. doi:10.1111/arcm.12254.
[30] J. Liebig, Ueber den Thierschit, Ann. Der Chemie Und Pharm. 86 (1853) 113–115. doi:10.1002/jlac.18530860110.
[31] M. Realini, L. Toniolo, eds., The Oxalate Films in the Conservation of Works of Art, in: Editeam, 1996. https://books.google.it/books?id=tmTqAAAACAAJ.
[32] A. VV., International Symposium on the Oxalate Films: Origin and Significance in the Conservation of Works of Art, in: 1989. https://books.google.it/books?id=G4B_AQAACAAJ.
[33] M. Vendrell-Saz, W.E. Krumbein, C. Urzi, M. Garcia-Vallès, Are patinas of Mediterranean monuments really related to the rock substrate?, in: 8th Int. Congr. Deterior. Conserv. Stone, Berlin, 30 Sept.-4 Oct. 1996, 1996: pp. 609–624.
[34] M. Garcia-Vallès, M. Vendrell-Saz, J. Molera, F. Blazquez, Interaction of rock and atmosphere: patinas on Mediterranean monuments, Environ. Geol. 36 (1998) 137–149.
[35] T. Rosado, M. Gil, J. Mirão, A. Candeias, A.T. Caldeira, Oxalate biofilm formation in mural paintings due to microorganisms – A comprehensive study, Int. Biodeterior. Biodegradation. 85 (2013) 1–7.
[36] I. Arrizabalaga, O. Gómez-Laserna, J. Aramendia, G. Arana, J.M. Madariaga, Applicability of a Diffuse Reflectance Infrared Fourier Transform handheld spectrometer to perform in situ analyses on Cultural Heritage materials, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 129 (2014) 259–267.
[37] A. Bonazza, C. Natali, N. Ghedini, C. Vaccaro, C. Sabbioni, Oxalate Patinas on Stone Monuments in the Venetian Lagoon: Characterization and Origin, Int. J. Archit. Herit. 9 (2015) 542–552. doi:10.1080/15583058.2013.837546.