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: Dark alteration, FT-IR, LIF, Py-GC-MS, calcium oxalate film, biocleaning

Highlights

- The dark alteration was identified as a calcium oxalate film
- Cyanobacteria, bacilli, heterotrophic and cocci type bacteria were identified
- The film is probably due to both biological activity and residues of past treatments
- Results of the campaign highlighted the best chemical cleaning method
- The best protective agent was a non-filmy silane monomers aqueous emulsion

1. Introduction

Matera Cathedral was built in a Romanesque-Apulian style in the 12th century in the old part of Matera (Italy), called "*I Sassi"*, which is an agglomeration of dwellings made of the local rock (tufa) from the Gravina torrent valley. Matera lies at the western edge of a UNESCO World Heritage site.

The exterior walls of the cathedral are made from the local organogenic limestone. The lower part of the walls (up to 6-8 meters) have become darkened since at least the beginning of the 20th century (Figure 1). This is of major concern because of the serious aesthetic damage to one of the most important landmarks in Matera. No historical information could explain the alteration, for example whether it originated from past surface protection treatments or to unknown phenomena 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].

Due to the importance of the monument and the age of the dark alteration, an interdisciplinary team of experts in conservation science with complementary and multiple expertise and skills, including biologists, chemists, geologists, and physicists, was created in 2015 in order to plan an extensive analytical campaign, consisting of both *in situ* and *ex situ* analyses and cleaning tests (Table 1). A multi-technique approach prior to a conservation work is fundamental in order to consider and study the decay phenomena from various points of view. This strategy is also a good practice to reveal the artistic techniques, the provenance of materials, and the testing of restoration materials as evident in the recent literature [2–8]. In addition, the cleaning procedure represents a particularly delicate phase in terms of the monitoring, selection, and preventive evaluation of the effects produced by the intervention. Safety, both for the restorer and the environment as well as for the work of art, is of primary importance for deciding the best strategy.

During the first step of the campaign (Table 1), a non-invasive monitoring of the surfaces, based on the use of laser induced fluorescence (LIF), was planned in order to map the presence of biodecay phenomena and to verify a selection of cleaning methods, including a biocleaning treatment. The sampling of small fragments from the most representative altered areas was also performed and the samples were characterized by traditional and innovative techniques, in order to highlight signs of ongoing decay, which include inorganic and organic components and microorganisms. X-ray diffraction (XRD) and optical microscopy (OM) were used to highlight the mineralogical phases and to evaluate the stratigraphy of the mortar, Fourier transform infrared spectroscopy (FT-IR) was used to identify inorganic compounds, ion chromatography (IC) was used to quantify water-soluble salts, pyrolysis-gas-chromatography coupled with mass

spectrometry (Py-GC-MS) were used to characterize the organic fraction. The results guided the removal of the decay, depending on the presence of bio-colonisation or insoluble compounds.

Fig. 1. Facade of Matera Cathedral (Matera, Italy) with the dark alteration in the lower part of the structure (up to 6–8 m) and details of the niche with St. Eustache statue (above). The provenance of the samples (bottom).

The cleaning strategy was studied in Step 2 (Table 1), evaluating 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
Analyses	*In situ* analyses	Physical	LIF
	Ex situ analyses	Chemical	FT-IR, IC, OM observation, Py-GC-MS, XRD
		Biological	Microbial viable counts, ATP assay
Step 2	*Ex situ* test	Chemical cleaning	H_2O_2, Oxone®, TiO_2, UV
Cleaning		Biocleaning	Impact biogel
	In situ test	Ablation	Laser
		Chemical	AB57 Impact – poultice, H_2O_2
		Biocleaning	Impact biogel
Monitoring		Physical	Water absorption
		Optical	Colorimetric analysis
Protection		Chemical	FT-IR, Py-GC-MS
Step 3			
Cleaning	At *in situ* full scale	Chemical cleaning	Impact - poultice
Restoration		Ablation	Laser
		Chemical	Choice of the most effective protective agents

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 sampling points.

"Pietra di Matera" specimens

A block of 36 x 22 x 25 cm (about 40 kg) was sampled at 1-1.5 meters from the ground on the left external facade wall of the Cathedral and removed. It was cut horizontally by a dry mechanical saw into thinner specimen blocks (36 x 22 x 3.5 cm, each): the external blocks corresponding to the exposed surface were used for *ex situ* chemical and biocleaning treatments. The hole created by the removal was filled by another block of an aged piece of tufa rock of the same size [10].

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

2.1.2 *In situ* and *ex situ* analyses

Laser-induced fluorescence (LIF)

The LIF technique exploits a laser source, which is emitted at an appropriate wavelength and which induces a fluorescence emission from the surface of interest. The fluorescence emitted by the irradiated surface is analysed using a spectrometer in order to detect possible fluorescent compounds present on the surface: for example, photosynthetic pigments such as chlorophyll, which may indicate a biological photoautotrophic patina colonising the surface [11].

LIF spectra were acquired using a portable, optical fiber-coupled LIF spectrometer, which was developed in-house at IFAC-CNR. The prototype was specifically designed for an *in situ* operation and is battery-powered. The laser source emits at 405 nm, while the fluorescence spectrum is acquired in the spectral range of between 450 nm and 930 nm with a spectral sampling interval of 0.5 nm. The hand-held measuring head is optically coupled to the spectrometer and detects the fluorescence signal emitted from a 5mm diameter area.

Fourier transform infrared spectroscopy (FT-IR)

The samples scraped off the surface were analyzed as KBr (Sigma-Aldrich FTIR Grade) pellets by an FTIR spectrophotometer BioRad Excalibur Series FTS 3000, DTGS detector, in transmission mode (4000 to 400cm^{-1}, 4cm^{-1} resolution, 16 scans). Samples C5 and C7 were analysed with 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 spectra were analysed with OPUS 7.2 software.

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 using Chromeleon software (version 6.7 SP1) which also provides data acquisition and data processing. 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).

Optical microscopy (OM)

Reflected light microscopy Polished cross-sections were obtained from fragments of collected samples. A Nikon Eclipse E600 Microscope was used, which was connected to a high-resolution digital camera and controlled by NIS Elements software for image analyses.

Transmitted light microscopy Petrographic observation of a 30 μm section was performed to obtain the main textural-compositional parameters of the C4 sample. An optical transmitted light polarized microscope, Zeiss AxioScope A.1 was used, with parallel and crossed nicols, and with 2.5X, 5X and 10X of magnification. The images acquired were processed with Axiovision software.

Pyrolysis gas chromatography mass spectrometry(Py-GC-MS)

A pyrolyser operating in constant temperature mode was used (multi-shot pyrolyzer® EGA/PY-3030D (Frontier Lab), which was 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. The mass spectrometer operated 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 an HP-5MS fused silica capillary column and the chromatographic parameters are reported in the literature [12].

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 were examined 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 as fungi and bacteria (PCA, Difco), and $BG_{11}M$ liquid medium for algae and cyanobacteria [13]. Another nutrient media, Nutrient Agar (NA, Difco) was used to isolate bacteria developed on PCA. The developed colonies were observed using the UV-light.

2. 2 Step 2

2.2.1 *Ex situ* and *in situ* cleaning tests

Ex situ chemical cleaning

In order to evaluate the cleaning of the dark area, advanced oxidation processes (AOPs) were considered [14,15]. These processes are designed to produce hydroxyl radicals which are highly efficient at destroying organic compound residues. AOPs therefore constitute a crucial technology for the degradation of non-easily removable organic

compounds. They 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) thus providing a powerful solution for the decrease or removal of residues [16–20].

For example, the entire surface of the "Pietra di Matera" specimen was split out into 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 a water solution (0.1 M concentration of active oxygen such as $KHSO_5$). A mixture of TiO_2 was obtained by sonicating 0.75 g anatase and 0.25 g rutile in 10 mL deionized water for 10 min. For the photochemical reactions, a photochemical multiray apparatus (Helios Italquartz; Milan, Italy) was used, which contained 10 UV lamps of 15 W power each of which emitted light at around 254 nm. All chemicals were purchased from Sigma Aldrich.

Microorganisms, media and ex situ biocleaning

On the basis of previous results, *P. stutzeri* strain A29 (DISTAM-DISTAAM Strain Collections, University of Milan - University of Molise, Italy), was used for the biocleaning [21,22]. In order to optimize the growth conditions for the cells to be applied to the altered stone surface, the biodegradation activity was assessed: the strain was incubated at 28°C for 24-36 hours, on mineral medium M9 and on the same medium supplemented by organic substances, such as linseed oil or egg (C.T.S. 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 3,000 ml total volume flask) and incubating it in a shaker (200 rpm) for 24 hours at 28°C. The cells were centrifuged at 7,000 rpm for 10 minutes at 4°C, washed twice with a 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 agar gel which needs a higher temperature before solidification [23]. 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 tufa rock surface using a sterile spatula. The entire piece was not covered in order to favour the aerobic conditions; the application times were optimised and ranged from 12 -24 hours, with a room temperature of 25ºC ± 2ºC.

Laser cleaning

The Nd:YAG Q-Switch laser group (C.T.S. srl, Altavilla Vicentina, Italy) had an integrated cooling system and microprocessor control panel for the laser beam frequency and power (wavelength, 1064 nm; energy, from 0 to 350 mJ; pulse duration, 10 ns; spot diameter 1-12 mm; power, 5 mW). The system was equipped with a 3 m optical fiber, easy to-use and particularly suitable for *in situ* works.

In situ chemical and biological treatments

In situ chemical cleaning tests on the outer wall of Matera cathedral were carried out adopting two different cleaning agents: H_2O_2 at 35% and AB57. The AB57 includes 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 apply to stone surfaces and frescoes (C.T.S. srl, Altavilla Vicentina, Italy).

The area selected for the *in situ* bioapplication was part of the external wall of the right-hand side of the cathedral and was about 2 m^2. Areas with the same size (25x40 cm) were identified 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, in the second control test of an untreated area, no carrier was used for comparison. For each area tested, about 0.3 kg of the delivery system was used, which had been stored in a sterile plastic box; the application and distribution were facilitated by sterile flat plastic spatulas, which made it possible to apply a thin layer of 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 follows:

i) Japanese paper, 9.0 g/m^2, natural, white, un-buffered (Klug Conservation, Walter GmbH & Co. KG., Immenstadt, Germany) was applied to protect the surface;

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 area. No other facilities were adopted (such as a plastic net or a plastic film to reduce water evaporation).

At different lengths of time from the bioapplication of viable bacterial cells (after 12 and 24 hours respectively), the biogel layers were removed by a 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 stone samples were collected for chemical and microbiological analyses. Three replicates (30 x 20 cm) were carried out for all biocleaning and control tests. During the bioapplication, the outdoor environmental temperature was recorded.

2.2.2 Monitoring of cleaning tests

Capillarity water absorption tests

To estimate the water uptake coefficient on the tuff surface of selected area, the contact sponge method, a non-destructive *in situ* methodology, was used [24]. The UNI Normal procedure requires using a sponge with a known density (Spontex type Calypso) soaked in water, weighed, and placed on the surface of the material for one minute with a given pressure, then weighed again [25].

Colorimetric analysis

Colour measurements (Miniscan with light D65; Hunter Lab, Bergamo, Italy), were taken to evaluate any chromatic changes in the paint of the biocleaned surface on several previously selected areas, over the short and medium term. The chromatic coordinates L* (lightness axis), a* (red/green axis), and b* (yellow–blue axis) of the wall surfaces were determined according to the CIE L*a*b* standard colour system [9, 21].

3. Results and Discussion

3.1 Step 1: *In situ* and *ex situ* analyses

3.1.1 LIF investigations

The LIF spectra were mainly acquired on selected ashlars of the southern wall and of the façade of the cathedral, focusing on those characterised by dark and clear areas. As an example, one of the examined ashlars is shown in Figure 2a.

For each ashlar, several LIF spectra were acquired in various spots of both the upper (clear) and lower (dark) part of the ashlar (Figure 2a). The spectra acquired on the dark part of the ashlar (blue-line spectra in Figure 2b) showed two main fluorescence contributions centred at about 510 nm and 610 nm. The spectra measured on the clear part of the ashlar (red-line spectra in Figure 2b) showed a similar spectral distribution to the one detected in the dark part (broad bands at about 510 nm and 610 nm), along with the typical fluorescence peak of chlorophyll at about 685 nm. In order to separate the fluorescence contribution of chlorophyll from the one due to the stone substrate, all the spectra were processed by interpolating the spectrum in the 640 nm - 800 nm spectral range (Figure 2c): the interpolated spectrum (blue line) was subtracted from the original one (red line) in order to determine the chlorophyll fluorescence spectrum (green line), which shows the typical main peak at about 685 nm and a shoulder at about 740 nm.

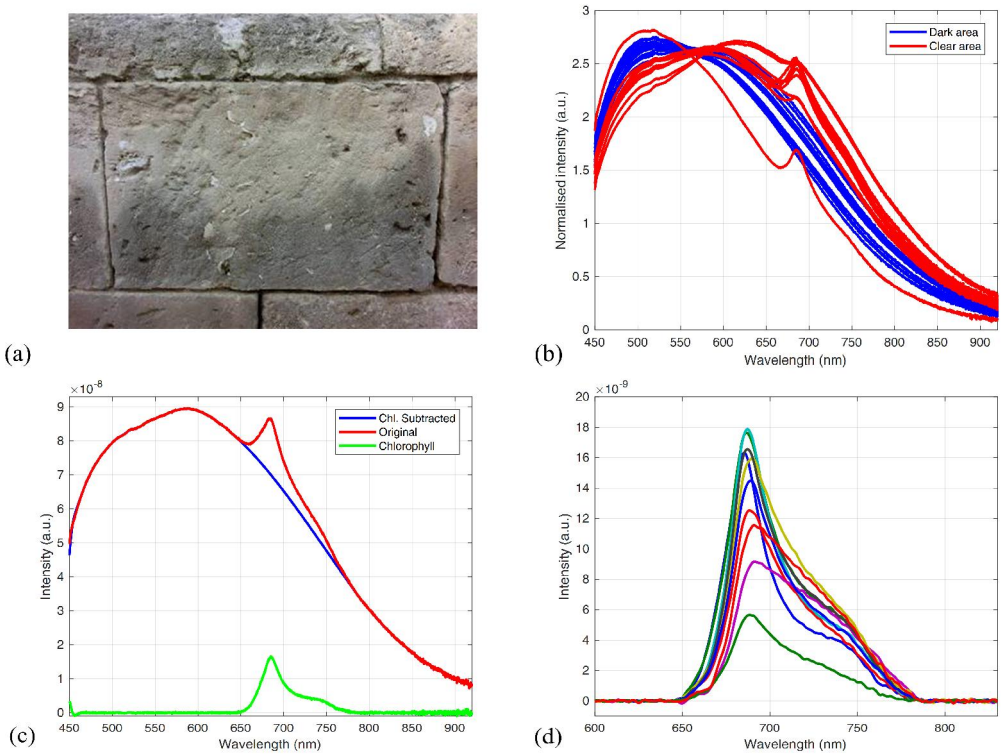

Fig. 2. LIF analysis of the ashlars of the southern wall: (a) Photographic image of one of the examined ashlars featuring the characteristic dark patina. (b) LIF spectra acquired in various spots of the ashlar, both in the upper and clear part of the ashlar (red lines) and in the lower and dark part 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.)

While most of the spectra acquired in different spots of the clear part of the ashlar showed a typical chlorophyll fluorescence peak, none of the spectra measured on the dark part of the ashlar showed any fluorescence contribution that could be attributed to chlorophyll. All the spectra measured in the clear part of the ashlar were processed by

applying the procedure described in Figure 2c, and the retrieved chlorophyll fluorescence spectra are shown in Figure 2d. These results suggest the presence of an extended phototrophic biological patina in the upper part of the ashlar.

In order to further investigate the possible presence of a phototrophic patina also in the dark part of the ashlar, additional measurements were carried out after mechanically removing the very first layer of the dark patina and moistening the area with water. The aim was to expose and activate any phototrophic organisms underneath the dark patina. Also after this procedure, the LIF spectra showed no peak that could be attributed to chlorophyll.

The LIF technique was also used to study a plastered portion of the façade (Figure 3). The surveyed area included a plastered surface (Area A in Figure 3a), an area of stone ashlar from which the plaster was mechanically detached immediately before the measurements (Area B in Figure 3a), and an area with a large gap due to a previous plaster detachment (Area C in Figure 3a).

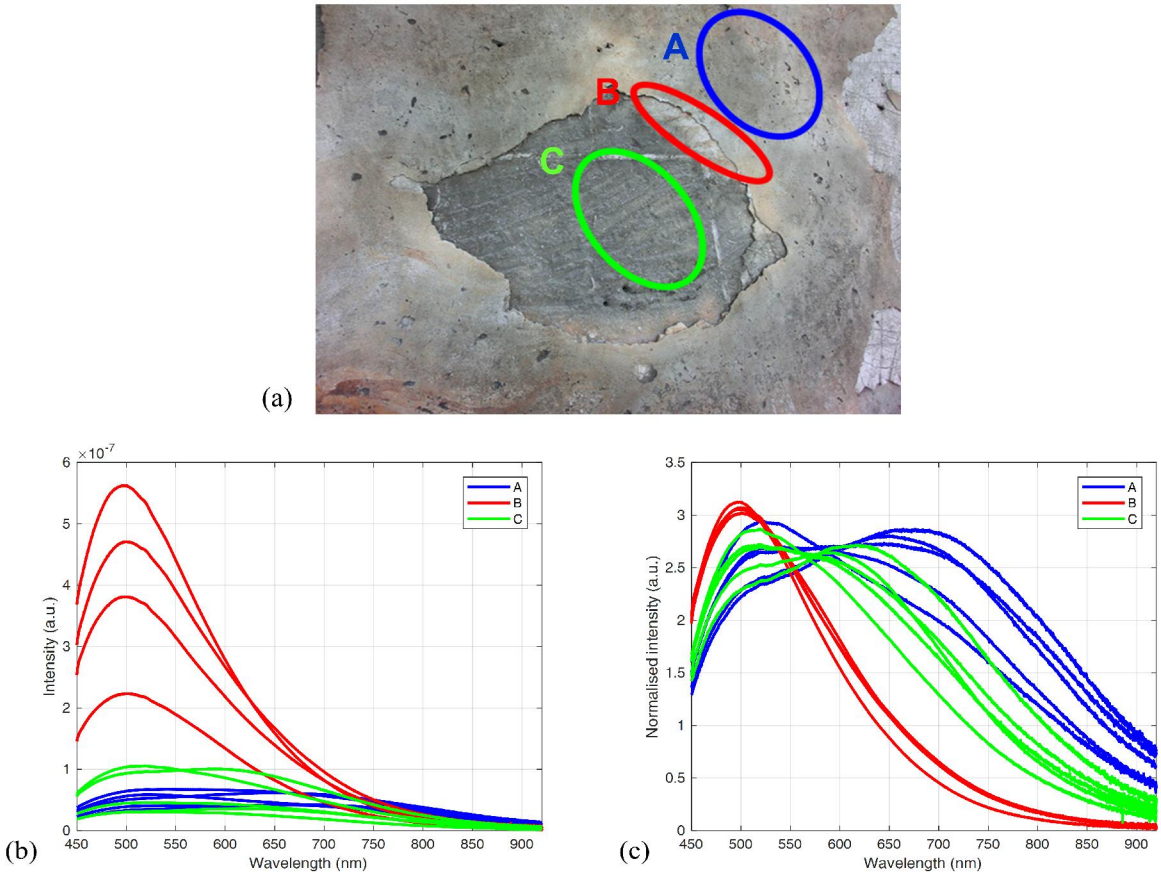

Fig. 3. LIF measurements on a plastered area of the cathedral's façade. (a) Photographic image of the examined area: three areas are highlighted in the photo: a plastered area (Area A), an area where the plaster was deliberately detached mechanically (Area B), and an area corresponding to a former gap 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 standarddeviation.

Figure 3b shows the LIF spectra acquired in the three different areas, while Figure 3c shows the same spectra normalised in intensity to the corresponding standard deviation in order to highlight their spectral distribution. Figure 3b shows that Area B (plaster removed before the measurement) has a high fluorescence efficiency, significantly greater than in the other two areas. Figure 3c shows that Area C has a very similar spectral distribution to that observed on the dark areas of other ashlars (Figure 3b, blue spectra), with two main fluorescence contributions at about 500-510 nm and 600-610 nm.

The spectra acquired in Area B are remarkably similar to the spectra that were acquired on a stone specimen obtained from an ashlar quarried out of an external wall of the cathedral (spectrum not shown in this paper). This suggests that the layer of plaster had in some way protected the stone from external agents, natural and/or artificial, which caused the darkening of large areas of the outer surface of the cathedral.

3.1.2 Chemical and mineralogical investigations

Optical microscopy

On the basis of observations in transmission mode, the bulk of C4 sample was constituted by an air-hardened calcitic lime binder with a homogeneous appearance and a micritic texture. The binder/aggregate ratio was 2:1. The grain size of the aggregate was bimodal (250-350 μm and 700-800 μm) and the shape of the grains was from sub-angular to sub rounded. Its composition was characterized by fragments of rock (i.e. "Pietra di Matera"), fossiliferous remains and micritic limestone. The porosity was due to the shrinkage cracks.

Samples C2 and C3, observed in reflection mode, were affected by a biological colonization producing a red fluorescence under UV light, due to chlorophyll. C6 sample was observed in stratigraphy which revealed a superficial layer (about 200 μm thickness) that had been altered, and a layer constituted by 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 mortar 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 3).

Table 3. Results of XRD analysis of the samples

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

Infrared spectroscopy

FT-IR spectra proved very effective in identifying the inorganic component of the samples. 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 cm^{-1} (out-of-plane bending vibration) and 712 cm^{-1} (in-plane

bending vibration) [27]. Alkaline nitrate and silicates were also present, as shown by the absorbance at 1384 and for the Si-O stretching band at 1033 cm^{-1}, respectively [28].

The spectra of samples C1, C5 and C7 presented the characteristic sharp peaks of gypsum ($CaSO_4 \cdot 2H_2O$), 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} [27]. Organic matter was found as traces in almost all the samples, with peaks of around 2982 and 2875 cm^{-1}, which could be ascribed to C-H signals, although FT-IR analysis cannot give a complete diagnosis without the support of more sensitive techniques.

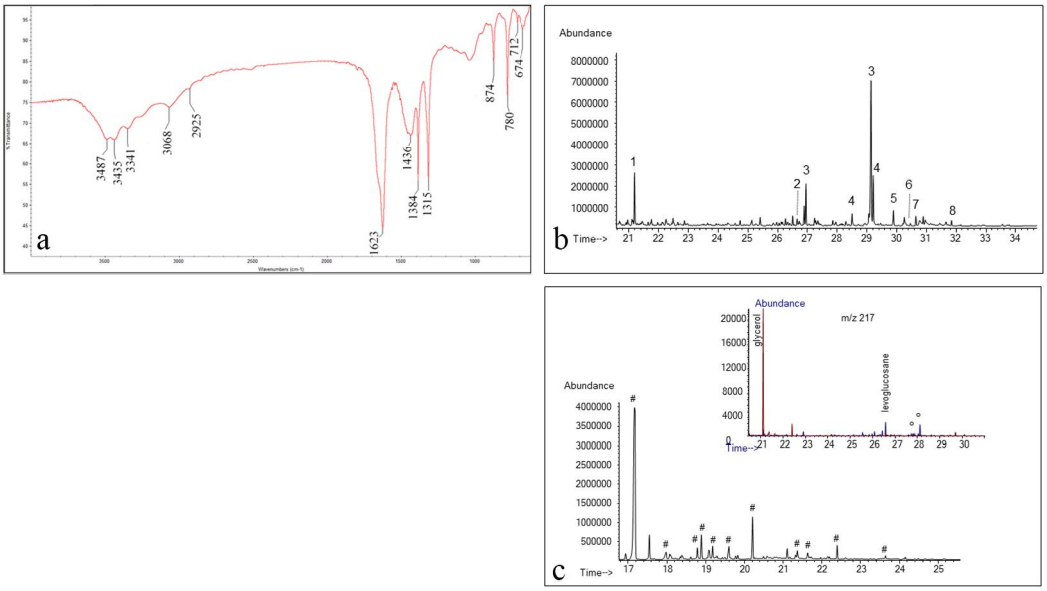

Fig. 4. (a) Transmission FT-IR spectrum of the external layer of sample C4, showing the presence of whewellite (3487, 3435, 3341, 3068, 1623, 1315, 780, and 674 cm^{-1}), calcite (1436, 874, and 712 cm^{-1}), alkalin nitrates (1384 cm^{-1}), traces of organic substances (2870–2980 cm^{-1}); (b) pyrogram of sample C3, with the most significant compounds identified: 1: glycerol, 2: levoglucosane, 3: arabinitol, 4: ribitol, 5: hexadecanenitrile, 6: mannitol, 7: palmitic acid, 8: octadecanenitrile, 9: inositol; (c) pyrogram of sample OG with the siloxane compounds marked (#) and in the upper part, the extract ion m/z 217, highlighting the markers of a saccharide source (glycerol, levoglucosane and other minors labelled with '°').

The three layers that formed sample C4 showed signals of calcite and traces of organic matter. The features determined in the spectra of the external layer suggested the mineralogical phase of calcium oxalate monohydrate, which is whewellite ($CaC_2O_4 \cdot H_2O$) (Figure 4a). This identification was due to the presence of very specific bands at 3487, 3435, 3341 and 3068 (O-H stretching bands), 1623 and 1315 (C=O stretching vibration), 780 and 674 cm^{-1} (O-C-O bending vibrations) [29]. The intermediate layer contained 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 was composed of gypsum, whewellite and organic compounds, whose peak pattern suggests a proteinaceous substance (2962, 2928, 2873, 1508, 1243 cm^{-1}).

The results suggest that the dark alteration was mainly due to the presence of calcium oxalate films. Since the first famous occurrence on the Parthenon at the end of the 19th century [30], such films have been frequently observed in the Mediterranean basin on stone materials (mortars, mural paintings and sculptures) and various natural stones (mainly marble), and under several climatic conditions. The occurrence of calcium oxalate films has been well documented [2,4, 35–37]. These films, whose color ranges from ochre to black, are practically insoluble in water, and thus are stable and difficult to remove. The issue regarding the formation of these films has involved scientific communities [34,35] who have debated whether the origin is biological or chemical. A biological explanation would be that calcium oxalate comes from the biofilms on the stone surfaces, which produce oxalic acid that reacts with calcium carbonate from the

substrate or from deposits [36,37]. On the other hand, oxalic acid could originate from the oxidation of natural organic materials, such as oil, egg and milk, applied during previous conservation work on the surface [38,39]. Such conservation was carried out to artificial age new architectural elements, or to protect against natural decay, or for maintenance.

Ion chromatography

All samples from the south wall (samples C1, C2 and C3) were analyzed by Ion Chromatography (Table 4): samples C1 and C2 contained similar amounts of chloride (Cl^-), nitrate (NO_3^-) and sodium (Na^+). These ions, instead, were lower in sample C3. Sulfates (SO_4^{2-}) showed a significant amount only in sample C1. Finally, decreasing amounts of potassium (K^+) and calcium (Ca^{2+}) ions were found going from sample C1 to C2 to C3. These results suggest the presence of gypsum in sample C1, from the blackened area, and of soluble salts (probably sodium and potassium nitrate and chloride) in samples C1 and C2, from areas where the surface has clearly been contaminated by an external agent. Sample C3 was less affected.

Samples C5 and C6 from the façade were also analyzed by ion chromatography. Sample C5 contained a small amount of chloride, sodium and potassium, whereas nitrate, sulfate, calcium and magnesium were present in large amounts. The powder was probably contaminated by gypsum and soluble salts (presumably calcium and magnesium nitrate and/or organic nitrates). Sample C6 on the other hand, contained sodium, nitrate and sulfate in large amounts. Chloride, potassium and calcium were also present in significant amounts, highlighting that gypsum and soluble salts (particularly nitrates, presumably calcium and/or organic ones) were also present in this powder.

Table 4: IC sample analyses. All the reported amounts (mmol) refer to 100 mg of sample.

	Li^+	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	SO_4^{2-}
C1		$1.43 \cdot 10^{-2}$	$0.79 \cdot 10^{-2}$		$6.03 \cdot 10^{-2}$	$1.17 \cdot 10^{-2}$	$2.35 \cdot 10^{-2}$	$5.33 \cdot 10^{-2}$
C2		$1.23 \cdot 10^{-2}$	$0.31 \cdot 10^{-2}$		$5.93 \cdot 10^{-2}$	$1.15 \cdot 10^{-2}$	$2.13 \cdot 10^{-2}$	$0.09 \cdot 10^{-2}$
C3		$0.13 \cdot 10^{-2}$	$0.19 \cdot 10^{-2}$		$2.79 \cdot 10^{-2}$	$0.24 \cdot 10^{-2}$	$0.39 \cdot 10^{-2}$	$0.04 \cdot 10^{-2}$
C5		$0.31 \cdot 10^{-2}$	$0.09 \cdot 10^{-2}$	$1.76 \cdot 10^{-2}$	$3.79 \cdot 10^{-2}$	$0.55 \cdot 10^{-2}$	$1.92 \cdot 10^{-2}$	$7.24 \cdot 10^{-2}$
C6	$0.16 \cdot 10^{-2}$	$1.27 \cdot 10^{-2}$	$0.42 \cdot 10^{-2}$		$6.29 \cdot 10^{-2}$	$1.09 \cdot 10^{-2}$	$4.34 \cdot 10^{-2}$	$7.60 \cdot 10^{-2}$

Pyrolysis gas chromatography mass spectrometry (Py-GC-MS)

The pyrolysis performed on the sample with derivatization reaction *in situ* [12] led to 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 protein content. The hexadecanenitrile and octadecanenitrile markers of egg are shown in the pyrogram of sample C3, reported in Figure 4b as an example [12].

The results obtained from the samples collected from the ashlar were particularly interesting, which were investigated by LIF techniques in three different areas (C1, C2, and C3). The analyses highlighted the presence of saccharide material in the clear part of the ashlar (C2 and C3) but not in the darkest part (C1). Figure 4b for sample C3 also shows the markers of saccharide material (glycerol, levoglucosane, arabinitol, ribitol, mannitol, inositol). This material is most likely ascribable to the presence of plant cells; in fact, an *in situ* investigation by laser-induced fluorescence revealed chlorophyll. Natural resins, waxes or siccative oil were absent. Finally, the analysis of sample OG, which was assumed to be an ochre mortar that had been applied by the restorers, suggested that it was in fact a siloxane-based protective for

masonry. Figure 4c reports all the siloxane compounds identified, together with the markers of a trace of saccharide material.

3.1.3 Biological investigations

The powders of four samples, collected from the south wall (B1, B2, B3) and the façade (B4), were observed in epifluorescence with an optical microscope. They showed a red fluorescence due to the chlorophyll of photoautotrophs, and a yellow, orange or blue fluorescence ascribable to the presence of other unidentified organic products (Figure 5). Sample B4 had a lower microbial contamination than the other samples characterised by a greater biodiversity, especially compared to sample B3 (Table 5). The fungal strain *Alternaria* sp. was present in almost all the samples, except B4.

Table 5. The microbial count/presence observed on the three nutrient media; CFU (colony forming units): +/- = scarce, + = present, ++ = frequent, +++ = abundant.

Nutrient media	Microbial count (CFU/100 mg)			
	Sample B1	Sample B2	Sample B3	Sample B4
PDA (fungi)	1 F	2 F	3 F	1 F
PCA (fungi and bacteria)	2 F	1 F	5 F	0 F
	41 B	37 B	52 B	1 B
$BG_{11}M$ (phototrophs)	++	+++	+++	+/-

In the nutrient media $BG_{11}M$ used for the development of phototrophic microorganisms, the coccoid cyanobacteria of the family of *Xenococcaceae,* with spherical mucilaginous colonies were dominant in all the samples, except for sample B4 where the presence of phototrophs was insignificant. Light and deep pink pigmented bacteria (cocci and rod-shaped) were observed, above all in samples B1, B2 and B3 in the heterotrophic nutrient media (PCA). Some of these bacterial colonies showed a pink (B1 sample) or yellow (B2 sample) autofluorescence.

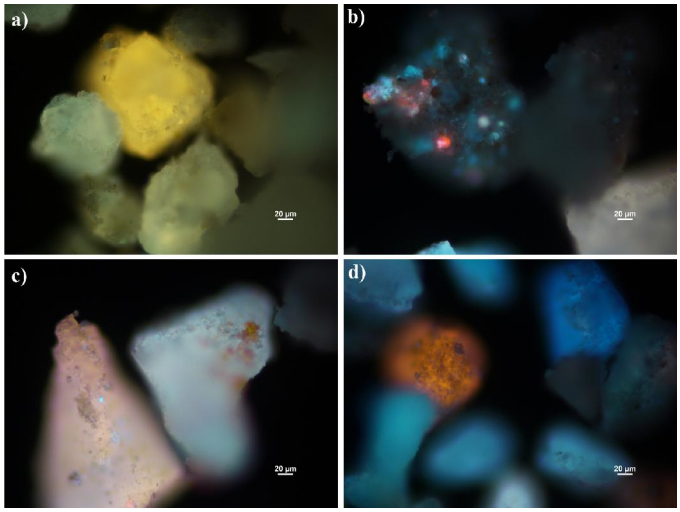

Fig. 5. Autofluorescence examples of the powder samples B1 (above, left), B2 (above, right), B3 (bottom, left) and B4 (bottom, right).

3.2 Step 2

3.2.1 *Ex situ* and *in situ* cleaning tests

Ex situ chemical and biological cleaning

Preliminary laboratory cleaning treatments were applied and tested on a block of "Pietra di Matera" extracted from the cathedral, with UV light at 254 nm for 72 h, or without exposure (at 24 h), as previously described. Table 6 summarizes the ΔE* values, after treatment by chemical and biological agents.

Studies using advanced oxidation processes (AOPs) for the removal, reduction and/or mineralization of organic pollutants have shown good results in soils, water and wastewater treatments. AOPs can work as an alternative or complementary method in traditional treatment cleaning in several contexts. In addition highly reactive free radicals, especially hydroxyl radicals (OH) generated via chemical (O_3/H_2O_2, O_3/OH^-), photochemical (UV/O_3, O_3/H_2O_2) reactions, act as the main oxidant, producing simple, relatively harmless inorganic molecules [14]. Under optimum operation conditions, (i.e, adequate contact time), it is possible to mineralize the residual contaminant to CO_2 and H_2O, which means that AOPs can be defined as "environmentally friendly" [15].

Table 6. Colour changes (ΔE*) measured on an *ex situ* block of "Pietra di Matera", 24-72 hours after treatment 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 showed that the most effective chemical treatments were H_2O_2 + UV, Oxone and TiO_2 + UV, respectively. However, the area treated with oxone was ruined in some points, therefore this oxidant was discarded. Only UV radiation had no effect compared to the control (blank). In addition, the area treated with TiO_2 appeared to be "coated" with white pigment, which remained the same also after a washing with deionized water. Conversely, when treated with H_2O_2, all the dark alteration was bleached, and the cleaning efficiency was much greater when H_2O_2 was combined with UV exposure (72 h). H_2O_2 is a green and environmentally friendly reagent because the only by-products are water and molecular oxygen. Furthermore, H_2O_2 is not an acidic compound and can also be utilized on limestone.

After 48 h from the biological treatment, the water had spontaneously evaporated, and the gel was manually removed with a soft brush. The results from colorimetric measurements showed the efficacy of the biocleaning agents when laponite biogel enriched with *P. stutzeri* viable cells was applied for 48 h.

Laser cleaning

Laser technology was used on a limited area of the carved surface of the cathedral that had been altered by hard layers of dark films that were still present even after the previous cleaning treatments cited above (Figure 6). The laser cleaning proved to be: i) selective, the laser radiation was absorbed by the superficial dirt without affecting the substrata; ii) adjustable, the beam power, frequency and laser spot diameter could be selected according to the operator's needs; iii) highly effective without the need to pre-consolidate the stone.

Fig. 6. Laser cleaning of a limited area on the tufa stone surface of the Matera Cathedral.

In situ chemical and biological treatments

The *in situ* chemical (AB57 mixtures-poultice and H_2O_2) and biological (laponite biogel) treatments showed similar results in terms of ΔE* (Table 7). The chromatic changes were over the value of 3-which is the threshold to be perceptible to the naked eye- and the dark alteration appeared less visible (Figure 7). In addition, when laponite biogel (laponite + bacteria) was used, the average chromatic change ΔE* values were higher (4.75 $\pm$ 1.40) compared to the use of the laponite alone (4.1 $\pm$ 1.28), but with no statistical significance. However, in both cases, the wide range of SD values suggested that the irregular and not homogeneous surface of the stone with large pits, empty spaces, due to loss of materials, exfoliation and decay of the surface material, greatly affected the final results. In the case of the chemical cleaning methods, ΔE* values measured after the treatments with H_2O_2 or AB57 poultice were higher respect to the laponite but with no statistical significance.

Table 7. Colour changes (ΔE*) and capillary water absorptions g $\pm$ SD results in *in situ* sandstone artwork of Matera Cathedral, with chemical or bio-cleaning agents, measured at 48 h, after application.

Area	Treatments	ΔE*	Water capillary absorption
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

Fig. 7. In situ biocleaning test performed on the lateral wall of Matera cathedral; laponite biogel bacteria, after 48 h of bioapplication, before (left) and after (right) the soft biogel removal.

Table 7 shows the water absorption test values by contact sponge (mean values +SD in g), before and after the various tests on the stone surface of the left gate close to the column.

The data from the treated areas are significantly different from the reference data (control/blank). In the case of the chemical cleaning, the highest value was obtained with AB57, demonstrating the high cleaning performance over all the treated surface.

In case of the application of laponite gel and laponite biogel bacteria, the water uptake values showed higher SD values probably due to the irregularity of the surface and the presence of *lacunes*. Finally, the water capillary absorption values after the H_2O_2 treatment did not appear significantly different from those previously cited.

3.2.2 Monitoring of the cleaning tests

Water absorption

The capillary water absorption coefficient on the tufa surface of selected areas was evaluated by the contact sponge method. The levels of ammonium oxalate and ammonium phosphate, which are commonly used for this kind of stone, were determined along with the more recent siloxane emulsion with the medium/high molecular weight synthetic protective agents, Pulvistop, Idroblock and Hydrophase Acqua. The best results were obtained with *Hydrophase acqua* [water uptake 1.58 mg/(min*cm^2)], compared to the other protective agents tested [9] and to the control area [water uptake 90.05 mg/(min*cm^2)]. The tests and the monitoring of the surfaces by non-invasive FT-IR spectroscopy and Py-GC-MS confirmed the efficacy of *Hydrophase acqua*, which showed the best performance in terms of its capacity to penetrate the stone, resistance to UV irradiation, and absence of decay [9].

Fig. 8. Matera Cathedral before (left) and after (right) the conservation work reported here.

4. Conclusions

A systematic investigation of the external walls of Matera cathedral by biological, chemical and also non-invasive techniques, identified the dark alteration as a calcium oxalate film.

In terms of the debate regarding the chemical and biological origin of calcium oxalate, our study revealed that the two mechanisms may actually have occurred simultaneously on the stone surface. In fact, the results revealed the presence of biological activity (cyanobacteria, pigmented heterotrophic bacteria, bacilli and cocci type bacteria) as well as residues of proteinaceous material [38–40], which seem to indicate that past conservation treatments had been applied. As egg was most abundant in the blacker area, the presence of a darker and thick crust on the bottom of the walls, may be due the easier accessibility for such conservation work over time. In sample C4, the sequence of layers suggests past conservation work, when the surface of the mortar degraded to gypsum was covered by a mixture of calcite and organic compounds, which was then transformed into calcium oxalate. Where the mixture (known as *beverone*) of natural organic compounds applied on the stone surface in form of a thin film was not applied, the sulfates, which were more abundant compared to the calcium oxalate, may have prevented the growth of microorganisms. LIF measurements in fact showed a competitive behaviour between the presence of phototrophic biodeteriogens and the dark alteration of the stones: chlorophyll was present almost exclusively in areas without a dark alteration. The formation of calcium oxalate may therefore be exclusively chemical.

The analytical campaign was effective in managing and guiding the cleaning tests carried out on the stone specimens treated with different cleaning methods. Using AB57, followed by washing with deionized water, was the most effective method. The test with an Nd:YAG laser was also effective, however it was carried out only on the carved elements because the cleaning time proved to be too long due the large size of the cathedral. In addition, *Hydrophase acqua* was found to be the best protective agent.

Our multi-technique approach was thus useful for the whole conservation work, and in particular the removal of the dark alteration. Figure 8 shows the final appearance of the cathedral, which is one of the symbols of Matera and which was proclaimed the European Capital of Culture for 2019.

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