Conservation of calcareous stone monuments: screening different diammonium phosphate based formulations for countering phototrophic colonization

Stone degradation is a complex process induced by chemical, physical and/or biological factors. This process was accelerated in the last 50 years, by the worsening of the air quality, which caused acidic rains and an increasing of particulate deposition associated to contamination by soluble salts. Consolidation of degraded stone monuments is among the most important and at the same critical conservation goals. It is aimed at assuring higher physical stability, and therefore durability, to the monument. The inorganic consolidants have a good chemical-physical-mineralogical affinity with the stone material, and di-ammonium phosphate (DAP) is one of the last generation water soluble agent for the consolidation of the carbonate-calcareous stones. Nevertheless, because of its content in phosphor and nitrogen, in very special humid conditions/contexts DAP could favor biological growth. In order to counter this potential drawback different formulates based on DAP, used in conjunction with washing procedures with water or biocides, or by adding biocides directly to the DAP solution were tested in this experimental work. Two types of calcareous stones with different porosity were chosen for the experiments (Gioia marble and Gottardo stone). The best results were obtained when a mixture of diammonium phosphate and benzalkonium chloride (BAC) water solutions was applied.

Keywords: stone consolidation; diammonium phosphate; benzalkonium chloride; conservation; calcareous stone

Taxonomy: Interdisciplinary Application Areas, Archaeology, Surface Science, Conservation

Manuscript category: Information Technology in Cultural Heritage

Corresponding Author: oana adriana cuzman

Corresponding Author's Institution: ICVBC-CNR

Order of Authors: oana adriana cuzman, Beatriz Cano Barriuso, Guido Botticelli, Iacopo Osticioli, Piero Tiano, Mauro Matteini

Suggested reviewers: Monica Roldan, Petr Kotlik, oliviu boldura

Submission Files Included in this PDF

File Name [File Type]
Cover letter.docx [Cover Letter]
Manuscript.docx [Manuscript File]
Figures and captions.docx [Figure]
Tables and captions.docx [Table]
Supplementary Material.docx [e-Component]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.
Conservation of calcareous stone monuments: screening different diammonium phosphate based formulations for countering phototrophic colonization

Beatriz Cano Barriuso1, Guido Botticelli2, Oana Adriana Cuzman3, Iacopo Osticioli4, Piero Tiano3, Mauro Matteini3

1Department of Chemistry and Industrial Chemistry, University of Pisa, Via Giuseppe Moruzzi 13, 56124 Pisa (PI), bcanobb@gmail.com (student)
2International University of Art of Florence (UIA), Florence, Italy, g.botticelli@tin.it (associated)
3Institute for the Conservation and Valorization of Cultural Heritage, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy, mmatteini@icvbc.cnr.it (former director), tiano@icvbc.cnr.it (associated), cuzman@icvbc.cnr.it (researcher)
4Institute for the Applied Physics “Nello Carrara” – National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy, i.osticioli@ifac.cnr.it (researcher)

Abstract

Stone degradation is a complex process induced by chemical, physical and/or biological factors. This process was accelerated in the last 50 years, by the worsening of the air quality, which caused acidic rains and an increasing of particulate deposition associated to contamination by soluble salts. Consolidation of degraded stone monuments is among the most important and at the same critical conservation goals. It is aimed at assuring higher physical stability, and therefore durability, to the monument. The inorganic consolidants have a good chemical-physical-mineralogical affinity with the stone material, and di-ammonium phosphate (DAP) is one of the last generation water soluble agent for the consolidation of the carbonate-calcareous stones. Nevertheless, because of its content in phosphor and nitrogen, in very special humid conditions/contexts DAP could favor biological growth. In order to counter this potential drawback different formulate based on DAP, used in conjunction with washing procedures with water or biocides, or by adding biocides directly to the DAP solution were tested in this experimental work. Two types of calcareous stones with different porosity were chosen for the experiments (Gioia marble and Gottardo stone). The best results were obtained when a mixture of diammonium phosphate and benzalkonium chloride (BAC) water solutions was applied.

Keywords: stone consolidation, diammonium phosphate, benzalkonium chloride, conservation, calcareous stone
1. Research aims

The diammonium phosphate (DAP) was demonstrated to be a very appropriate consolidating agent for calcium carbonate based stones [1, 2, 3] due to the following main characteristics: (i) an effective consolidating action able to reach a sufficient depth within the stone; (ii) the predominant formation of hydroxyapatite as final stable and extremely insoluble consolidating product; (iii) the lack of toxicity; (iv) the changelessness of original stone color. Due to the presence of both nitrogen and phosphorus in the DAP molecule (\((\text{NH}_4)_2\text{HPO}_4\)), some drawback can happen in particularly humid contexts of application, as this may favor the development of biological growth. This would not be a problem if all the DAP applied would be involved, as expected (see below reaction 1), in the formation of calcium phosphate, which is insoluble and without any nutrient value. Nevertheless, some residual amount of non-reacted DAP must be taken into account. In the aim of preventing the above described drawback, different combinations of DAP mixed or rinsed afterwards with biocides (benzalkonium chloride (BAC) and Rocima103 (ROC) were tested. The leaching action of acidic water on treated with the above procedures was also tested. Green algae and cyanobacteria were used as phototrophic contaminating microorganisms in the tests.

2. Introduction. Stone consolidation treatments and diamonium phosphate - state of the art

The water soluble inorganic consolidants have a good chemical and physical affinity with the stone material, interacting with the stone substratum by hydrolytic mechanisms, carbonation or other chemical interactions. The ammonium phosphate is one of the last generation inorganic consolidants. It is able to recover the lost cohesion due to weathering, getting the treated calcareous stones more durable [1-2]. The consolidating effect of DAP on limestone is based on the moderate formation of calcium phosphate, which is the effective consolidating agent, by involving a small percent of calcium carbonate in proximity of the surface of the stone material according to the following reaction:

\[
6(\text{NH}_4)_2\text{HPO}_4 (\text{s}) + 10\text{CaCO}_3 (\text{i}) \rightarrow \text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2 (\text{i}) + 6\text{NH}_3 (\text{g}) + 10\text{CO}_2 (\text{g}) + 8\text{H}_2\text{O} (\text{g})
\]  

(1)  

(s = soluble; i = insoluble; g = gas)
The presence of phosphorus and nitrogen can favor, in particularly humid environments, the development of microorganisms on stone material, with a consequence of organic matter accumulation and a faster succession of the biodiversity in time, inducing therefore an action of biodegradation on the stone material. The bioreceptivity is a key characteristic of a stone material to be colonized by biological growth [4].

In this paper, the efficiency of formulations of DAP in combination with two different biocides was tested on two different types of calcareous stones against a mix of phototrophic colonizers in laboratory conditions, using an accelerated growing chamber, in order to identify the most appropriate operative conditions for preventing by-colonization.

Some of the stone samples, after they have been treated with some of the combined formulations of DAP and biocide were exposed to sprays of acidic water to simulate an acid rain washing out effect, and therefore their resistance to bio-colonization was tested.

The series of procedures tested included: (i) stone-samples treated with DAP alone; (ii) samples treated with DAP followed by rinsing with water in order to remove the excess of not reacted DAP; (iii) samples treated with DAP followed by rinsing with one of the two biocides-solutions in order to remove the excess of not reacted DAP and, at the same time, to protect the stone against microbiological attack; (iv) samples treated with mixtures (solutions/suspensions) of DAP with one of the two biocides.

The behavior of each treatment was examined in relation with the type of stone as well (Gioia marble and Gottardo stone). The results obtained in interventions of consolidation of ancient monuments (façades, portals, sculptured elements) based on the combined use of DAP with biocides, are also reported.

### 3. Materials and methods

#### 3.1 - Stone materials selected for the tests

Two different types of calcareous stones were used: Gioia marble and Gottardo stone. Gioia marble has a polygonal granoblastic microstructure (350-400 µm mean dimension of the grains) and a low porosity. In order to simulate the condition of an aged marble it was decided to cause an artificial degradation of the samples by heating them at 300°C for 15 min [5]. The Gottardo stone is a variety of the Vicenza calcareous stone classified as biospartite, with the presence of microfossils, and higher porosity. The main physical characteristic of the two lithotypes are reported in Table 1 [6].
Slabs of initial dimensions 10x10x1 cm were cut of each type of stone. The consolidation treatment with DAP was carried out directly on these initial slabs. Successively, each slab was divided into 4 smaller samples (5x5x1 cm) for each condition anti-bio-colonization to be tested, according the program.

3.2 - Biocides used for the tests
Two different biocides (Rhom and Haas) were chosen as anti-colonization agents used in concentration of 2%: (i) Benzalkonium Chloride (BAC), - is a typical ammonium quaternary salt easily soluble in aqueous formulations (e.g. DAP solution) and one of the most commonly used for controlling the biological development in stone conservation [7,8]; (ii) Rocima 551 (ROC), is a water insoluble formulate (a combination of Methylisothiazolinone and Dithio-Methylbenzamide).

3.3 - Formulates and methods of application
Eight different combinations of formulates and treatment procedures were applied on the stone slabs (10x10 cm), while one slab for each type of stone remained untreated as a reference (Table 2). The consolidating DAP based formulates were applied by capillarity absorption, using cellulose poultices (50 g of cellulose pulp and 250 ml of the formulate). DAP was always used as 7% w/v water solution. The stone samples were placed upside down on the poultice for 24 h. Then, they were let to dry at room temperature for 48 h (Figure 1). After drying, two of them (2MA and 2GO) were rinsed with deionized water, other two (3MA and 3GO) with the biocide BAC and two (4MA and 4GO) with the biocide ROC, in the concentration shown in Table 2. For the rinsing procedures, the stone samples were placed with a 30° inclination and sprayed 4 times for 10 seconds, rotating each sample for 3 times after each spraying with 90° inclination. Four more samples were treated with DAP + biocide in mixture: 5MA and 5GO with DAP+BAC; 6MA and 6GO with DAP+ROC (Table 2).

Only stone samples 7MA - 7GO and 8MA - 8GO (DAP + biocide mixtures ) were exposed for 10 days to acidic water (for simulating acid rain) (Table 2) for testing the washing out resistance of the biocide present
in the formulates. For this operation, the system described in section 3.3 was used. The samples were daily moved clockwise one position for receiving a homogeneous exposure in between. They were gently sprinkled with acidic water (pH 5) prepared with H$_2$SO$_4$ 1M in water [9]. The acidic water was renewed after the first day in order to avoid its possible contamination due to the leaching of soluble substances, while for the remaining period the system was used in continuous circuit. As calcium carbonate of the stone samples reacted with the acid, the pH of the acidic water was daily controlled and adjusted to pH 5 when needed.

3.4 - Accelerated induced phototrophic colonization

The accelerated growing chamber (AGC) consists of a rectangular glass tank of 60 x 120 x 60 cm (Figure 2a) filled with the inoculum solution described below, for 5-6 cm height and sealed with a transparent plastic wrap. The stone samples were placed 5 cm above the water level, with a 30° inclination. A central tube is placed about 30-40 cm above the samples level. A diluted BG11 [10] nutrient media (1:3) containing the mix of phototrophic microorganisms is gently sprinkled 1h/day through the 8 sprinklers with 90° angle, in a closed loop system. A continuous aeration system is placed on the bottom of the AGC for shuffling and oxygenizing the microorganisms. The experiment ran for 2 months at 26-28°C, simulating 12h daylight cycles (50 μmol/s·m$^2$) at the stone level, alternated with 12 h nighttime. The inoculum was made of mixed phototrophic microorganisms sampled from natural developed blue-green patina on stone materials. The microbial concentration was about 28 g/L dry biomass and the biodiversity (Figure 2b-f) was composed of various species of algae (filamentous Tribonemales, unicellular green Chlorellales, diatoms Bacillariophyceae), and cyanobacteria (filamentous Nostocales, Oscillatoriales and coccoid Chroococcales).

3.5 - Stone characterization

The chemical composition of the stone sample surface was analyzed by FT-IR in reflection mode (ALPHA Bruker, measuring area Ø 6 mm), while the chemical characterization of the formulates, in transmittance mode, using KBr pellet and OPUS 7.2 software for the spectra acquisition.
Measures of Water Absorption Capacity are very important for evaluating the conservation state of a stone surface and the effect of the protective/consolidants treatments. Capillary rise test was performed according to UNI/NORMAL 10859, considering 10 minutes of absorption time on wetted filter paper discs (Filter-Lab 1300/80, Ø 90 mm) in a sealed box [11]. Water Absorption Capacity was calculated as the weight difference between the wet and the dry sample.

3.6 - Treatment efficiency evaluation of biological colonization

3.6.1 - Colorimetry

For assessing the color changes at the end of the biological colonization experiment, the central area (50 mm²) of each stone sample was measured for 3 times, using a tristimulus Minolta Chroma CR-200 Colorimeter and a metallic mask, rotating the sample after each measurement. The color difference (ΔE) between the values recorded at the beginning and the end of the experiment was calculated according to CIEL*a*b*, 1976 [12]. The samples were measured dry, after keeping them for 3h in the desiccator.

3.6.2 - Macro- and micro- observations

Macro images were acquired (Canon 7D camera, macro objective Canon EFS 60 mm) during the experiment for all the stone samples, in order to observe the visual changes in time due to the biological colonization. The morphologic analysis of the stone surfaces were carried out by means of an optical microscope Nikon Eclipse E600, a digital camera Nikon DXM1200F and plastic masks for each sample. For some samples, depending on the surface roughness, epifluorescence observation was carried out, using an Hg lamp and a UV-2A filter cube (excitation 330-380 nm, DM 400 nm, BA 420 nm).

3.6.3 - Chlorophyll Fluorescence Imaging

This technique permits to quantify the superficial phototrophic activity by imaging the chlorophyll fluorescence [13, 14] by using an HandyFluorCam FC 1000 H (Photon System Instruments, Brno, Czech Republic), with a false color scale. The Pulse Amplitude Modulated Fluorimetry measurements (PAM) were performed after 6, 8 and 9 weeks from the beginning of the experiment. The samples were kept in darkness.
for 1h before the measurement, to increase the yield of chlorophyll fluorescence. The investigated area of 16 cm² (corresponding to 69920 pixels) was always located in the center of the stone sample, and subdivided in 16 small sub-areas of 1cm², each considered for the quantification of the fluorescent pixels.

4. Results

4.1 - DAP formulates characterization

FT-IR analysis in reflectance mode confirmed the carbonatic nature of both limestones used in this research. FT-IR peaks at 1400, 720, 880, 1082, 1000 and 2500 cm⁻¹ are typical of calcium carbonate (SM1).

Residuals traces of DAP were observed in the spectra of both types of stone for all type of treatments, and its characteristic peaks (1080 cm⁻¹ and 553 cm⁻¹) were present even after the rinsing procedures. The lack of these peaks was noted only in the case of Gottardo stone treated with the formulate ‘DAP+ROC in mixture’ (groups 6 and 8). However, with FT-IR Spectroscopy it was not possible to see the characteristic peaks due to the formation of calcium phosphate, nor the ones of the biocides used, very probably because of their low relative concentration.

The Water Absorption Capacity of the Gottardo stone, a very porous stone, is about 5 times higher than the Gioia marble one. It was considerably reduced after DAP treatment, rinsed or not (groups 1, 2, 3, 4, 6, 8). The reduction was about 70 % in the case of Gioia marble and 80 % for the Gottardo stone, with respect to the reference untreated stone (Figure 3). The mixture DAP+BAC (groups 5 and 7) showed the highest water absorption decrease, of 80% in the case of Gioia marble and 85% for Gottardo stone in comparison with the reference (Figure 3).

[Figure 3]

The stone samples that have been exposed to acidic water (pH 5) were analysed by Optical Microscopy in reflection mode to observe possible morphological changes on the surface, and were also dry weighted before and after the exposure, to evaluate the possible loss of material as a result of the action of the acid on the carbonate. The Gioia marble stone group treated with DAP+BAC (7MA) and DAP+ROC (8MA) showed to be less affected by the action of acidic water with respect to the reference, while the Gottardo stone showed a higher attack by the acidic water, especially in the case of DAP+ROC treatment (8GO) (Figure 4c).
In fact, under the microscope a corrosion effect that significantly changed the roughness of the stone surfaces (Figure 4a-b) was observed.

[Figure 4]

4.2 - Biological colonization

A detailed discussion of the results obtained with PAM quantitative analysis, is reported in the sections 6 and 7. This paragraph was focused on the description of microbial diversity observed during the biological development, and on the colorimetric measurements of the bio-colonization, a method that brought additional information to those obtained with PAM.

The macroscopic observations after 2 months of experiment clearly revealed that biological colonization had a different development not only depending on the treatment applied on stone but also on the type of stone (Figure 5). As expected, the untreated Gottardo stone (0GO), due to its high porosity and a high specific surface area, showed a very heavy colonization with respect to the untreated Gioia marble, which was less colonized (0MA). In general, also after the various treatments, the colonization process appeared more evident in the case of Gottardo with respect to Gioia stone because of its higher bioreceptivity.

[Figure 5]

Figure 6 shows the percentage of the colonized surface by the phototrophs on each group of stone, considering the fluorescent pixels calculated with the FluorCam software. At the same experiment-time, the Gioia marble showed a surface colonization about 70% less than Gottardo’s.

One sample of the group 5GO (DAP + BAC in mixture), even if treated with the same procedure as the others, presented a darker stain, that corresponds to an island colonized by diatoms. Even at the moment of the treatment this area had behaved differently than the rest of the stone slabs, showing a lower absorption of the product then in the rest of the stone (SM2). The same heterogeneous colonization was observed on the DAP+BAC in mixture group (6GO), which was highlighted on intensely photrophic colonized areas, and areas without any trace of microorganisms (Figure 5b).
In the first step of the colonization process diatoms and green algae was observed independently of the kind of stone (Figure 7a-b). This was particularly evident in the non-treated samples. The biodiversity started to increase in time, and at the end of the experiment the filamentous species of cyanobacteria were dominant, especially on Gottardo stone, creating a phototrophic biofilm attached to the substrata (Figure 7f). In the net created by filamentous species such as Phormidium sp. (Figure 7f-h) and Leptolyngbya sp. (Figure 7e, i), the other species, unicellular, are still present (Figure 7g). However, on Gioia marble, together with filamentous cyanobacteria Leptolyngbya sp., also filamentous green algae were observed (Figure 2c). On this type of substrata, with lower porosity than the Gottardo stone, the unicellular species were still dominant at the end of experiment, such as diatoms, coccoid cyanobacteria and green algae (Figure 7c-d, i).

At the end of experiment, in parallel with the PAM measurements, colorimetric measurements were also performed with the aim of gathering some integrative evaluation on the biocolonization through the color variations of the various samples. The graph in Figure 8 reports only the data for the stone groups with ΔE values below 5, as considered potential ineffective against biological colonization; the ΔE values for which no visible change is seen is considered to be below 3. The treatments DAP+BAC in mixture (group 5MA) and DAP+ROC in mixture (group 6MA) resulted the most efficient (Figure 8). Most of the samples become yellower and greener. Only 6MA and 8GO become more white, while the rest, more dark.

5. In situ application

Besides the laboratory experiments illustrated in this paper, the DAP treatment in combination with a biocide has been applied on some monuments made of limestone. One significant example of these (and the first one case) concerns Palazzo Turati, a private 19th century building in Milan (Figure 9). The frames of the 32 windows in the courtyard of the palace are made of Gallina stone, a porous limestone which have been
consolidated with 7% DAP water solution. After drying, all the treated stone surface were rinsed with 10% BAC solution. A conservation treatment of the palace was carried out in 2012-2013 by a team of private restorers (Gasparoli s.r.l.) under the direction of architect Giovanni Pellegrinelli. Two of the authors (M. Matteini and G. Botticelli) were consultant of the direction. Before the restoration process the Gallina stone showed an advanced lack of cohesion due to a diffuse sulphatation induced by gypsum crystallization. The final result of the intervention was lead to the maintenance of its original color. Scientific examination confirmed the penetration of DAP within the stone micro-cracks (ICVBC/CNR, Milan). No event of biological growth has so far been observed. Two recently (2015/2016) very significant examples in which DAP was used (in mixture with BAC) are (i) the facing upper part of the famous Baroque Basilica of Santa Croce in Lecce; (ii) the marble Major Portal of the Cathedral of Monreale, near Palermo. In both cases excellent results were obtained although it is clearly early to make any evaluation on the effectiveness and durability of the bio-preventive treatment.

6. Discussions

The macroscopic observations made after two months of experiment clearly revealed different results depending on the specific treatment employed as well as on stone type (Figure 5). The evaluation of the phototrophic colonization for all groups of stone samples with the macroscopic view and the PAM images acquired at 6, 8 and 9 weeks are presented in SM3..

The degree of colonization was related with the type of treatment and stone was performed on the base of PAM analysis (Figure 6), in order to acquire not only the final result, but also the progress, case by case, of the biological growth. Taking into account the total fluorescent pixels (SM4) measured with FluorCam software, the numeric values were normalized to 100, and a Biological Colonization index (BCi) was defined with the following formula:

\[ \text{BCi} = \frac{1}{\text{no. pixel}} \times 100000 \]  

(2)
It can be seen that the **BCi** is proportional with the colonization effectiveness: higher is BCi (0 to 100), greater is the biological colonization and, therefore, the lower is the effectiveness in preventing it.

Systematic comparisons between the various tested procedures were made, and expectations and preliminary comments on the results obtained were succinctly reported (Table 3).

[Table 3]

As regard the Gioa marble, the **BCi** values resulted generally low (Table 4). Despite repeated biological contamination over 8 weeks of experiment, this type of stone showed a general low bioreceptivity. Nevertheless, all the samples treated with anti-colonization processes showed lower biological colonization with respect to both the not treated stone samples and the sample treated with DAP. Rinsing with water the DAP residues has only slightly improved the anti-colonization properties, while rinsing with biocides showed much more effective results. However, the best procedure to counteract biological colonization was recorded with the use of biocides in mixture with DAP, especially with BAC.

[Table 4]

The apparently strange values of BCi recorded for the samples of Gioia marble treated with DAP, lower than those of the not-treated-reference samples, could be explained by considering the effect of consolidation caused by DAP. This induces a partial closure of pores and a minor roughness of marble, thus reducing water absorption/retention (Figure 3) and, consequently, microorganisms settlement.

As regard the Gottardo stone, the values reported in Table 4, show an evident high level of colonization indices than those of Gioia marble. This noteworthy difference is probably attributable to the decisive microstructural differences between the two types of stones and, in particular, to the heterogeneity and porosity (28,5%) of Gottardo stone, considerably greater than that of Gioia marble (1,5%) (Table 1). These characteristics induce in Gottardo stone a high capacity of water absorption and the presence of numerous loculi render the surface of this stone very bioreceptive. This explains the very high values recorded for
colonization indices. In retrospect, it can be observed that the forced conditions of accelerated contamination adopted in this research were probably excessive for this lithotype. On the other hand, such particularly high susceptibility to biocolonization - that now makes difficult to differentiate the anti-contamination effect due to the various bio-preventive systems - was not easily predictable. Nevertheless, by carefully examining the data (Table 4), important conclusions can still be drawn. The maximum of biological colonization, with no difference in between, was recorded for the non-treated samples and those treated with DAP. Washing with water does not change practically the situation, while washing with biocides shows some decrease of the colonization ability by microorganisms, which is better with ROC. Finally, even in the case of Gottardo stone, the use of biocides in mixture with DAP showed to be the most effective way to counteract bio-colonization, particularly remarkable in the case of DAP+BAC, but still significant for DAP+ROC.

Finally, the tests carried out using acidic water to simulate the possible leaching effect caused by acid rain on the biocides (Table 4) showed a good resistance to the leaching action in the case of Gioia marble, especially valid, again, for DAP in mixture with BAC, rather than with ROC.

As regard the Gottardo stone contradictory results were recorded. An increase in biological colonization, in agreement with the expectation, occurred with the formulation ‘DAP+BAC in mixture’ (in other words, this means that part of BAC was washed away), while the other mixture, DAP+ROC, even if partially leached by acidic rain, still showed good protection against bio-colonization.

A hypothetic explanation is that, although in the context of experimentation carried out ROC resulted, in general, a less satisfactory anti-biocolonization agent, compared with BAC, its non-solubility in water makes it more resistant to leaching of acid rain.

7. Conclusions

Different consolidation treatments for calcareous stones based on DAP, a substance with fertilizing properties which can induce bio-colonization in particularly humid contexts, have been studied in order to find the most appropriate DAP formulations able to counteract biological colonization induced by DAP residues not involved in the consolidation process. This result is possible when DPA is properly associated to biocide agents. The necessity to obtain rapid and indicative results to select the best formulation and/or
operative conditions for stone conservation, has led to design an artificial system for accelerating the phototrophic development of microorganisms which are considered pioneers of stone colonization. In this way, it was possible to distinguish between different possible formulations and operative conditions, in a relative short time. The experiment included also a procedure to evaluate the permanence of the biological growth protection in the aggressive condition typical of an urban centre, by exposure of the treated stone samples to an ‘artificial acid rain’ (pH5).

The various treatments showed a different behavior as regard the capability to counteract biological colonization, depending also on the type of calcareous stone used in the tests: Gioia marble and Gottardo stone (Table 4).

The experimental tests conducted in this research allowed to acquire important results about the use of the consolidation treatment for carbonatic stones based on di-ammonium phosphate (DAP). For preventing an undesired biological growth that can happen when the treatment is applied in particularly humid contexts various anti-biocolonization treatments were taken into consideration and a selection was made. The phenomenon of bio-colonization is induced by possible residues of unreacted DAP that remain in the stone porosity. The tests showed that simple washing with water is insufficient to remove these residues of DAP.

The use of biocides appeared to be crucial. As an overall result, benzalkonium chloride emerges as the most appropriate preventive agent anti-biocolonization. Beside its efficiency, its solubility in water makes it far more suitable for the practical use in restoration, being able to be mixed without problems with the aqueous solution of DAP.

The experimentation put in evidence that between the use of biocides as washing agents or in mixture with DAP, the latter mode appears to be the most effective. However, the other mode is not excluded. The restoration practice will be able to say the last word on this matter.

In real operative cases, the biological colonization conditions will rarely be such aggressive as those adopted in this work, and therefore the results will be expected to be more optimistic on whatever stone type, included those highly porous. Further experimental laboratory has to be performed for establishing the most appropriate concentrations of the active agents and to test the behavior of other biocides, possibly even more effective.
Acknowledgements

The authors thank E. Pinzani (ISE-CNR) and C. Capriolo (ICVBC-CNR) for their contribution in the construction of the ‘accelerated chamber’ and S. Siano (IFAC-CNR) for the given possibility of using the PAM fluorimeter.

References

[1] M. Matteini, S. Rescic, F. Fratini, G. Botticelli. Ammonium phosphates as consolidating agents for carbonatic stone materials used in architecture and cultural heritage: preliminary research, Int J Architect Herit, 5 (2011) 717-736. doi: 10.1080/15583058.2010.495445

[2] M. Matteini, C. Colombo, G. Botticelli, M. Casati, C. Conti, R. Negrotti, E. Possenti, M. Realini. Ammonium phosphates to consolidate carbonatic stone materials: an inorganic-mineral treatment greatly promising. In: Online Proceedings of the Conference BUILT HERITAGE 2013 Monitoring Conservation and Management, Milan, Italy 18-20 November 2013, M. Boriani et al. (eds), Politecnico di Milano, Centro per la Conservazione e Valorizzazione dei Beni Culturali, Milano, 2013, pp. 1278-1286

[3] G. Graziani, E. Sassoni, E. Franzoni. Consolidation of porous carbonate stones by an innovative phosphate treatment: mechanical strengthening and physical-microstructural compatibility in comparison with TEOS-based treatments, Herit Sci, 3 (2015) 1-6. doi: 10.1186/s40494-014-0031-0

[4] O. Guillitte. Bioreceptivity: a new concept for building ecology studies, Sci Tot Environ 167 (1995) 215-220

[5] A. Bertagnini, M. Franzini, C. Gratziu, M. Spampinato. Il marmo cotto in natura e nei monumenti, RENDICONTI Società Italiana di Mineralogia e Petrologia, 39 (1984) 39-46

[6] online database http://www.italithos.uniroma3.it/

[7] P. Tiano. Biodegradation of Cultural Heritage: Decay Mechanisms and control Methods. In: Conservation of stone and other materials. Thiel MJ (ed), RILEM/UNESCO Paris, E & FN Spon Press, London, 1993, pp 573-580

[8] K. Sterflinger, G. Piñar. Microbial deterioration of cultural heritage and works of art-tilting at windmills?, Mini Review, Appl Micorbial Biotechnol 97 (2013) 9637-46. doi: 10.1007/s00253-013-5283
[9] H. B. Fan, Y. H. Wang. Effects of simulated acid rain on germination, foliar damage, chlorophyll contents and seeding growth of five hardwood species growing in China, Forest Ecol Managem 126 (2000) 321-329. doi: PII: S0378-1127(99)00103-6

[10] R. Rippka, J. Deruelles, J. B. Waterbury, M. Herdman, R. Stanier. Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J Gen Microbiol 111 (1979) 1–61, doi: 10.1099/00221287-111-1-1.

[11] AA.VV, Materiali lapidei naturali ed artificiali. Determinazione dell’assorbimento di acqua per capillarità, Doc. UNI/NORMAL10859 (2000)

[12] BS EN 15886:2010, Conservation of cultural property. Test methods. Color measurement of surfaces (2010)

[13] A. Eggert, N. Häubner, S. Klaus, U. Karsten, R. Schumann. Quantification of algal biofilms colonising building materials: chlorophyll a measured by PAM-fluorometry as a biomass parameter, Biofouling, 22 (2006) 79-90. doi: 10.1080/08927010600579090

[14] M. Mascalchi, I. Osticioli, C. Riminesi, O. A. Cuzman, B. Salvadori, S. Siano, Preliminary investigation of combined laser and microwave treatment for stone biodeterioration, Stud Conserv, 60 supplement 1 (2015) S19-S27. doi: 10.1179/0039363015Z.00000000203
Figure 1. The treatment procedure used on Gioia marble and Gottardo stone for the application of the formulates (a) and for the rinsing operations (b).

Figure 2. The accelerated growing chamber (a) and the biodiversity present in the 1:3 diluted BG11 nutrient media observed under optical microscope: unicellular (b) and filamentous green algae (c), coccoid (d) and filamentous cyanobacteria (d, e) such as *Leptolyngbya* sp. and *Nostoc* sp., and diatoms (f).
Figure 3. Water Absorption Capacity of the treated and untreated stone samples.

Figure 4. Morphology of the stone surface of Gioia marble (a) and Gottardo stone (b) before (left) and after (right) exposure to acidic water at pH5, and (graph on the right) loss of weight recorded for each sample subjected to the same procedure.
Figure 5. The slabs of Gioia marble (a) and Gottardo stone (b) after 2 months of experiment.

Figure 6. The percentage of the colonized surface by the phototrophs on each group of Gioia marble (a) and Gottardo stone (b), calculated as an average of the four specimens (x, y,z,w) of each group. The percentage was estimated taking into account the values of the fluorescent pixels revealed by PAM measurements in SM4.
Figure 7. Biological colonization in the first weeks (a, b) and after 2 month of accelerated biological growth (c-f), and the main species found on the samples as main colonizers: filamentous cyanobacteria *Phormidium* sp. (g, h) and *Leptolyngbya* sp. (i), the green algae *Chlorella* sp. (g) and the diatom *Navicula* sp. (i).

Figure 8. Color variation at the end of experiment.
Figure 9. The cloister of the 19th century Palazzo Turati (in Milan) and (right) detail of the big windows on which DAP rinsed with BAC was used (2012)
**Table 1.** Physical characteristics of Gioia marble and Gottardo stone, where $\gamma = \text{absolute density}$, $\gamma_s = \text{apparent density}$, $P = \text{total open porosity}$, and $\text{CIV} = \text{the volumetric imbibition coefficient}$

| Lithotype          | $\gamma$ (g/cm³) | $\gamma_s$ (g/cm³) | $P$ (%) | CIV (%) |
|--------------------|-------------------|--------------------|---------|---------|
| Gioia marble       | 2.71 ± 0.10       | 2.67 ± 0.10        | 1.5 ± 0.2 | 0.54± 0.15 |
| Gottardo stone     | 2.70 ± 0.20       | 1.923              | 28.51   | 23.6± 0.24 |

**Table 2.** The whole set of treatments applied to each stone group.

| sample code | treatment applied (g/100cm³ solution) as cellulose poultices | rinsing procedures | exposed to acidic water |
|-------------|---------------------------------------------------------------|-------------------|-------------------------|
|             |                                                               | deionized water   | biocide 2% (g/100g solution) |                  |
|             |                                                               |                   |                          |                  |
| 0MA         | not treated                                                  |                   |                          |                  |
| 1MA         | DAP (7%)                                                     |                   |                          |                  |
| 2MA         | DAP (7%)                                                     |                   |                          |                  |
| 3MA         | DAP (7%)                                                     |                   |                          |                  |
| 4MA         | DAP (7%)                                                     |                   |                          |                  |
| 5MA         | DAP (7%) + BAC (2%)                                          |                   |                          |                  |
| 6MA         | DAP (7%) + ROC (2%)                                          |                   |                          |                  |
| 7MA         | DAP (7%) + BAC (2%)                                          |                   |                          |                  |
| 8MA         | DAP (7%) + ROC (2%)                                          |                   |                          |                  |

**Table 3.** Comparison between the efficacy expected/observed for all types of treatments tested, where n.t. = not treated (stone reference), BCi = Biological Colonization index; w = weeks.

| No. | treatments | comparison in between // samples | surface colonization index | preliminary comments | expectation |
|-----|------------|----------------------------------|---------------------------|-----------------------|-------------|
| 1   | treated with DAP // not treated (reference stone samples) | MA Gioia marble GO Gottardo stone | 6w | 8w | results out of expectation: the BC is low at 6w and medium-low at 8w, but it is higher for the reference samples (n.t.) than in those treated with DAP | BC should be significant in both cases, although higher on the samples treated with DAP, due to some possible DAP residues that favor the BC |
|     | 1MA (DAP) // 0MA (n.t.) | 4 | 20 | | |
|     | 1GO (DAP) // 0GO (n.t.) | 90 | 99 | results partially in agreement with expectation: high BC on all samples, with no significant differences between the reference samples (n.t.) and those treated with DAP | BC should be higher on the samples treated with BAC than in those treated with DAP and then rinsed with water |
|     | 2MA (DAP→H₂O) // 1MA (DAP) | 2 | 14 | results in agreement with the expectations: the BC is generally low, but smaller in the case of rinsed samples both at 6w and 8w | |
|     | 2GO (DAP→H₂O) // 1GO (DAP) | 96 | 100 | results partially in agreement with the expectations: the BC is very high, with no significant difference between the two treatments | |
|   | treated with DAP, then rinsed with biocides // treated with DAP, then rinsed with water | 3MA (DAP→BAC) | 4MA (DAP→ROC) // 2MA (DAP→H₂O) | 3GO (DAP→BAC) | 4GO (DAP→ROC) // 2GO (DAP→H₂O) | results in agreement with the expectations: the BC is almost inexistent at 6w after rinsing with both biocides, and had a low development in the following 2w | the use of a biocide to rinse DAP residues for preventing BC should be more effective than rinsing only with water |
|---|---|---|---|---|---|---|---|
| 3 | treated with DAP, then rinsed with BAC // treated with DAP, then rinsed with ROC | 3MA (DAP→BAC) // 4MA (DAP→ROC) | 0 (mean) 6 (mean) | 2 (mean) 14 (mean) | 45 (mean) 86 (mean) | results partially in agreement with the expectations: the BC is lower for the samples rinsed with the biocides than with those rinsed water. Nevertheless, at 8w the BC is high also for the samples rinsed with biocides | results in agreement with the expectations: both biocides showed an excellent efficacy when used for rinsing the DAP residues |
| 4 | treated with DAP mixed with biocides // treated with DAP rinsed with biocides | 5MA (DAP+BAC) // 3MA (DAP→BAC) // 6MA (DAP+ROC) // 4MA (DAP→ROC) | 0 1 (mean) 2 (mean) | 0 3 (mean) 6 (mean) | 75 95 (mean) 16 77 (mean) | results scarcely in agreement with the expectations: rinsing with BAC resulted anyhow ineffective with this kind of stone, and ROC was able to reduce BC only at 6w | results in agreement with the expectations: the BC is almost inexistent for both mixture formulates, at 6w and 8w |
| 5 | treated with DAP mixed with biocides, then exposed to acidic rain // treated with DAP, mixed with biocides | 7MA (DAP+BAC→acidic water) // 5MA (DAP+BAC) // 8MA (DAP+ROC→acidic water) // 6MA (DAP+ROC) | 1 10 (mean) 2 (mean) | 0 1 (mean) 2 (mean) | 9 32 (mean) 1 (mean) | results in agreement with the expectations: the BC is higher in samples exposed to acidic water for both biocides, although BAC is more resistant to leaching caused by acid rain. | biocides in mixture with DAP could be more efficient than biocides used for rinsing |
| 6 | treated with DAP, mixed with biocides, then exposed to acidic rain // treated with DAP, mixed with biocides | 7GO (DAP+BAC→acidic water) // 5GO (DAP+BAC) // 8GO (DAP+ROC→acidic water) // 6GO (DAP+ROC) | 5 34 (mean) 1 (mean) | 7 11 (mean) 2 (mean) | 3 11 (mean) 1 (mean) | results out of expectations: the BC is lower for the samples exposed to the acidic water treatment | theoretically, the exposure to acidic water could wash out the biocide and consequently increase the BC |

Results out of expectations: the BC is lower for the samples exposed to the acidic water treatment.
Table 4. Biological colonization behavior of two types of calcareous stones treated with DAP in various formulations and/or operative conditions.

| No. | Treatments                                              | Gioia marble |     | Gottardo stone |     |
|-----|---------------------------------------------------------|--------------|-----|----------------|-----|
|     |                                                         | 6 weeks      | 8 weeks | 6 weeks | 8 weeks |
| 1   | not treated (n.t.)                                      | 8            | 23   | 93          | 100   |
| 2   | treated with DAP                                        | 4            | 20   | 90          | 99    |
| 3   | DAP, then rinsed with water                             | 2            | 14   | 96          | 100   |
| 4   | DAP, then rinsed with BAC                               | 0            | 9    | 75          | 95    |
| 5   | DAP, then rinsed with ROC                               | 0            | 3    | 16          | 77    |
| 6   | DAP mixed with BAC                                      | 0            | 1    | 7           | 11    |
| 7   | DAP mixed with ROC                                      | 1            | 4    | 37          | 60    |
| 8   | DAP mixed with BAC, then exposed to acidic water        | 1            | 10   | 5           | 34    |
| 9   | DAP mixed with ROC, then exposed to acidic water        | 9            | 32   | 3           | 11    |
SM1. The transmittance (DAP, BAC or ROC) and reflectance spectra (all the rest) of Gioia marble and Gottardo stones treated as reported in Table 2.

| Treatment Description | Gioia marble | Gottardo stone |
|-----------------------|--------------|----------------|
| DAP treatment (group 1) | ![Graph](image1) | ![Graph](image2) |
| DAP treatment rinsed with water, BAC or ROC (group 2, 3, 4) | ![Graph](image3) | ![Graph](image4) |
| Mixed DAP and biocides (BAC or ROC) treatment (group 5, 6, 7, 8) | ![Graph](image5) | ![Graph](image6) |
SM2. The 5GO Gottardo stone sample before cutting, immediately after the poultice removal of DAP+BAC (a) and the same sample at the end of the experiment (b).
SM3. The samples for each group of treatment tested in this research with the images acquired by a digital camera and by PAM-imaging fluorimeter
GOTTARDO STONE
SM4. The fluorescent pixels quantified by PAM-imaging.