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BIOTECHNOLOGY APPLIED TO HISTORIC STONEWORKS CONSERVATION: TESTING THE POTENTIAL HARMFULNESS OF TWO BIOLOGICAL BIOCIDES

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Abstract

Within restoration practices, the biodeterioration is a common and hard problem, in particular on historic stonework conservation, where, together with weathering actions, enlarge porosity and increase the decay. The chemical action with biocides is the more used method to remove biological patina on monumental stone but, during the time, that approach reveal hazardous environmental and health impacts. In recent time, innovative biotechnology methods have been developed but used only to a minor extent; that is due to the less information about the interaction of the new products with stone material. The aim of the research is to propose innovative and safe bio cleaning products for historic stonework conservation and define the level of security in the interaction with stone material. The two biological biocides, that has not been investigated previously, are Natria, a Bayer products based on pelargonic acid, and New FloorCleaner, based on Bacillus species. The specimens for our research are made up from an historical stone material (earlier twenty century handmade bricks) and we used the Normal UNI 11551-1:2014, the European protocol for the evaluation of a cleaning method in the Cultural Heritage. Our results show that biocleaning products are harmless: overall, the research demonstrates the opportunity to use these products in the conservation field, for the treatment of biological patina of historical brick, because do not highlight problems and damages and are environmentally sustainable.

Keywords: *Historic stonework; Preservation and Restoration; Pelargonic acid; Bacillus species; biological biocides.*

Introduction

All stone materials, both natural and artificial, undergo a natural aging associated with a progressive deterioration altering the original aesthetic and technological features. The biodeterioration, term coined by Hueck (1965), is "any undesirable change in the properties of a material caused by the vital activities of organisms". Organisms that attack the stone material can be of two different type: autotrophic organisms (some bacteria, algae, lichens, mosses and vascular plants), able to feed themselves using only simple inorganic substances and use stone as nutrient source; while heterotrophic organisms (some bacteria, fungi, animals), which feed on organic substances produced by other organisms, using the stone substrate only as a support

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for their growth. Stone material is part of natural biogeochemical cycles, when rock is deteriorated and reduced in various essential elements, such as nitrogen, carbon or sulphur, by chemical and physical actions: weathering exposure and water availability increase biodeterioration effects; for that reason mainly the outdoor artworks suffer the deterioration processes [1-4].

The biodeterioration is rarely caused by a single group of organisms and this depends on the complex interactions between different groups coexisting [5, 6]. The damage caused by microorganisms is closely related to the ability to adhere on the substrate; this is due to material characteristics and adhesion techniques of the microorganism. In the case of the stone material, hardness and mineralogical composition are important factors, in addition to the roughness and porosity of the surface, because irregularities are preferential points for adhesion [7, 8]. The more incisive bonding systems of microorganism are specialized structures as rhizines, rhizoids and roots that penetrate the surface (lichens, mosses and higher plants) or the biofilm formation that cement the body of the organism to the surfaces, such as algae. Biofilm is a polymeric substance, viscous and very dangerous that adheres closely on the substrate and is considerably important for the activation and development of alteration processes because inside them occurs a fluid retention and accumulation of aggressive metabolic compounds. The biofilm is able to maintain an environment very different from the surrounding, in terms of pH and chemical composition [7, 9]; biofilm may also collect particles and corrosive atmospheric pollutants able to increase the reaction rate of chemical corrosion [10]. Another characteristic of the microorganisms is their great adaptability to several environmental conditions and the capacity to thrive in every part of the biosphere, such in soil, on rock, in the oceans or in hot springs.

Therefore the biodeterioration is an hard problem in the stones conservation field, although the stone is considered as one of the most durable materials [1, 2, 8, 10-12]: a large percentage of the world's monuments is made by stone [13] and during the time were developed several treatments to contrast the damages of biological agents.

Within the last two centuries, thanks to industrial and technological discoveries, new materials and products come to light: compared to the old natural derivatives, restorers and conservators prefer synthetic products, with physical and mechanical properties more stable during the time. In particular the chemical industry had provided a large number of synthetic products used in agricultural field to contrast biological attack, such as herbicides and fungicides [14, 15]. These products have been adopted by restorers with appreciable results in biodeterioration control, but often operators ignore the potential harmfulness for health and environment, especially indoor, where the chemical residues are dispersed with difficulty [16-18].

Recently researchers have understood the toxicity of many synthetic products, widely used in the restoration of cultural heritage [19-21]. As a consequence, it appears clear that the use of new products derived from natural sources would be very welcome. In this context, the biology, biotechnology and green chemistry have a great and largely potential for the preservation and restoration of cultural heritage [22-24]. There are two approaches in this field: the development of polymers from renewable sources such as substitutes of petrochemical-based materials [25, 26]; and the application of competitive microorganisms to contrast the growth of the colony and damage action [10, 27, 28].

In this context, we want to investigate for the first time the action of two biological biocides, currently not employed in cultural heritage conservation, but with excellent characteristics of use:

- Natria: Bayer product based on pelargonic acid, used as a biocide for exterior surfaces such as garden path, walls or floors and is also used in organic farming;
- New FloorCleaner: product based on probiotics, is used by various cleaning companies for the sanitization of surfaces in hospitals. This product is distributed in Italy by

Atena-α (Ferrara, Italy) and is part of Probiotic Cleaning Hygiene System made by Chrisal_Cleaning products (Lommel, Belgium).

Natria is an herbicide with algaecide and fungicide properties, commercialized by Bayer Garden and the active ingredient is pelargonic acid, a nine-carbon fatty acid, isolated for the first time from the leaves of *Pelargonium roseum*, that causes an extremely rapid and nonselective burn-down of plants: it has been claimed to increase absorption of glyphosate while concurrently causing rapid desiccation of the tissues of treated plants. The action of Natria herbicide appears in a few hours after application, with leaf yellowing widespread and the desiccation of the affected parts in less than 24 hours. The herbicide exists in nature as essential oil and appears as colorless liquid, slightly soluble in water and with strong rancid smell [29]. The pelargonic acid not leaves residues and decomposes rapidly both on land and in the water environments: nowadays is legally employed in organic farming and in Italy it is currently used both in agriculture and for domestic use, such as cleaning of paths [30].

New FloorCleaner is a surface cleaner with probiotic products (PIP), consisting in spore of *Bacillus subtilis*, *B. megaterium* and *B. pumilus*, able to colonize the surfaces on which is applied and to control the proliferation of other bacterial species, based on the principle of biological competition (law of Gause, 1934). This principle lies in the fact that two different species (bacterial and/or fungal), insisting on the same ecological microcosm, cannot coexist in stable equilibrium if they refer to the same nutrient substrates: one of them, usually the less demanding nutritional factors, become dominant over the other and being able to cause extinction. From a microbiological point of view, for the surfaces treated with probiotic products, the existing biofilm is in fact replaced by a new type of biofilm, mainly formed by the novel microorganisms artificially placed with the cleaning products [31]. These procedures can then be connoted as "bio-stabilization techniques" of one species over another, therefore implying not a biocidal generalized action, if not as a final effect against specific microbial species [32, 33].

For understand the potential harmfulness of the two biological biocides, we used the protocol UNI Normal 11551-1:2014 “Methodology for the evaluation of a cleaning method” [34]. The test is done on handmade bricks, because this stone material is the more characteristic in the architecture of Emilia Romagna (Northern region of Italy): during the centuries people use row material easy to extract along the Po River and Apennines deposits. For this reason Ferrara, the most populated city near the River Po Delta, has become famous as “town of bricks” [35-37].

Experimental

For our research we evaluated the potential harmfulness of biocides on handmade bricks, produced in the beginning of the twentieth century, resulting from the demolition of a sugar manufacture chimney following the earthquake on 20 May 2012, at the Department of Engineering, Ferrara University (Italy) [38]. The stone material is a firebrick, with a very low open porosity, composed of sub-rounded pores with a size between 0.5 and 2 mm. The petrographic observation highlights the presence of small impurities (calcareous clasts and fragments of shells), probably due to the use of local clays [35], and the presence of micro-fragments of carbon (coal), added to the clay with the purpose to strengthen the bricks resistance on high temperatures [39]. Laboratory analyses were carried out at the Department of Physics and Earth Sciences of the University of Ferrara and surface analyses were made in the UNIFE TekneHub laboratories.

Stone Specimen Preparation

In according to UNI Normal 11551-1:2014 [34] procedure, we used 16 specimens, measuring 50×50×20 (± 3)mm, and another 4 measuring 50×100×20 (± 3)mm: the variability of measures is due to the manual cutting procedure, but not undermines the validity of the tests. Half of them will be used to test Natria and the other for New FloorCleaner. To obtain a smooth surface, the samples were treated with powder of silicon carbide (grain size P180 of the FEPA classification), subsequently washed with deionized water and dried in stove at 60°C for 24h; they were finally weighed and stored in a desiccator before treatment.

In order to define the stone surface variation, all samples were analyzed before and after treatment with biocides: specimens with smaller area were treated on all top surfaces and involved in every analysis; the other samples are half treated on top surface and investigated only with SEM.

The two biocides were solubilized in deionized water, according to the doses recommended by the manufacturer (Natria 200mL per liter, New FloorCleaner 10mL per liter). With a spray bottle, the solutions were then applied on the surfaces of the samples, in three applications every 48 hours; after all specimens are rinsed with deionized water. The aim of the treatment is to simulate a common cleaning action such as restoration procedure.

Water Absorption by Capillarity

The determination of water absorption by capillarity was carried out using the Normal UNI EN 15801: 2010 protocol [40]. The samples were laid in contact with water through a multiple layer of cotton wool; the measurements have been carried out at regular steps (1, 2, 3, 4, 5, 8, 13, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300min) and have been reported as Kg/cm^2 versus t ($s^{1/2}$). The amount of water absorbed by the specimen per unit area Q_i (Kg/m^2) at time t_i (s) is calculated as follows:

$$Q_i = \frac{m_i - m_0}{A} \quad (1)$$

Where m_0 and m_i are respectively mass of the sample dry and at time t_i, in Kg; A is the area of the specimen in contact with the water, in m^2.

The test equipment is completed with a stopwatch with accuracy 1 sec and analytical balance with accuracy 0.01g; the laboratory temperature is stable on 22 ± 2°C.

Colorimetric analyses

The evaluation of color change on brick surface was carried out by Konica Minolta spectrophotometer CM-2300D with spherical geometry and horizontal alignment. It was carried out a total of six measurements per specimen, for everyone we measured a set of L*a*b* values (CIE 1976) [41-52].

The CIELAB color difference is expressed by the equation below

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

ΔL* - lightness difference.

Δa* - red/green difference.

Δb* - yellow/blue difference.

Colors are also described and located using the method of specifying their L*, C* and H* coordinates in according to the method described in the Normal UNI EN 15886:2010 protocol [42], namely CIE 1994 (abbreviated to CIE94), with symbol ΔE_{94}^*. The CIE94 formula is based on CIE lightness (ΔL*), Chroma (ΔC*), and hue (ΔH*) differences. It is important to determine the CIE94 because this incorporates factors that define the perception of color [43-52].

The samples were preconditioned before each series of measurements, in laboratory with constant temperature and humidity (22 ± 2°C, 45 ± 5% RH). It is preferred to use the mask with larger diameter (Ø 11mm), in order to have a measuring area wider and mitigate the effects due to lack of homogeneity on the surfaces.

Chemical analysis of the aqueous extract

An untreated specimen, A10_Natria and B10_NewFloorCleaner samples were immersed in 100mL of deionized water and placed in agitation on vibrating platform for 72 hours. The pH and EC (Electrical conductivity) were determined electrometrically by parametric probe, the pH meter Oranion 4-Star, produced by Thermo Scientific. Major cations and trace elements were detected by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a Thermo-Scientific X Series instrument [53]. Accuracy and precision, based on the repeated analyses of samples and standards, were better than 10% for all the considered parameters.

Surface analyses

Analyses were performed using the Stereo Microscope Optika SMZ 168TL with integrated camera for scanning images. For each sample were selected 5 points of analysis, photographed at 0.7X and 1.5X magnifications, with light in a perpendicular position to the surface (zenith) and in grazing light (30 ± 5°).

Four polished cross sections are extracted from 50×100×20mm samples: the profile is scanned by 7 points of analysis, at a constant distance, in order to see if the treatment has penetrated into the sample and caused damage. The analysis was carried out using SEM Zeiss EVO MA15 with Oxford Inca EDS analyser.

Results and Discussion

The treatment with the two biocides reveals a different activity: Natria stinks and leaves a white patina, whereas New FloorCleaner hasn't these problems. That is due in particular to Pelargonic acid and the timing of the treatment, higher than the one prescribed by the manufacturer. Despite during the analyses procedures, in particular through the process of the water absorption, smell and white patina of Natria disappears.

The results of water absorption analysis of the brick are in according with the performance of another raw material currently utilized by the Italian brick industry, with very low porosity and the significant percentage of micropores [54]. The water absorption results are illustrated in figure 1, Natria decreases the water absorption capacity of the brick, with waterproofing actions, because Pelargonic acid is an oil substance, but compared to a coating treatment [55] the percentage of reduction is very low, about 7% at 4 hour. New FloorCleaner reveals any changes.

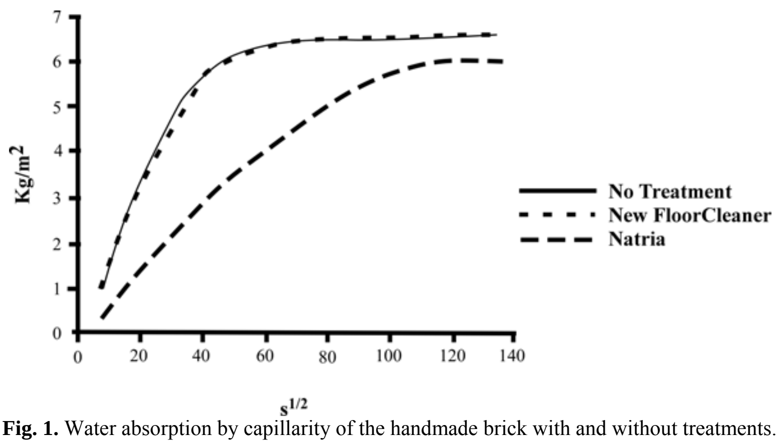

Fig. 1. Water absorption by capillarity of the handmade brick with and without treatments. Amount of absorbed water measured by weight at fixed time intervals: average values.

Colorimetric data in the CIE L*a*b* system prove no significant color variation occurred throughout the test: in figure 2 we can see a color difference acceptable for the cultural heritage field, under the perceive color difference of the human eye ΔE* = 2/3 [41, 56]. The high standard deviations are due to the presences of calcite fragments, with a color different from the background. In some case after the treatment, when the calcite agglomerate is great, at first sight these zones appear more white: the L* values are invariant but a* values, are higher and therefore more red. The augmentation of red component can prove the sensation of whiter appearance of calcite conglomerate. The b* values no change in this component. The ΔE_{94}*, that describe the viewing reference conditions for perceived color difference, shows less values than ΔE* and confirms that the biocides activity is not invasive.

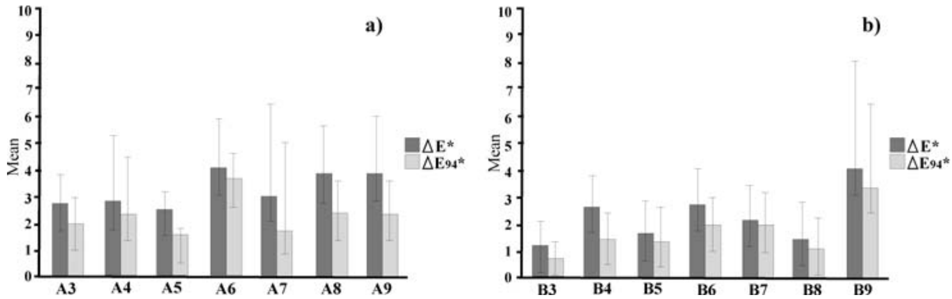

Fig. 2. Correlation between ΔE* and ΔE_{94}* color difference (a) in Natria treatment and (b) in New FloorCleaner treatment: average values.

The chemical analysis shows that the aqueous extract of the sample A10_Natria is comparable with the specimen without treatment: Natria have any chemical interaction with brick and few soluble components were detected in aqueous. Different speech instead for sample B10_NewFloorCleaner: compared to the aqueous extract of the untreated sample, there is a rise in conductivity (No_treatment 1.4mS/m, B10_NewFloorCleaner 5.45mS/m), sulphates have tripled (No_treatment 1,044ppm, B10_NewFloorCleaner 3,023ppm), while the cations calcium, sodium and magnesium are almost higher (No_treatment 810, 413 and 181ppm; B10_NewFloorCleaner 1451, 793 and 701ppm). The composition of the New FloorCleaner reveals the presence of cationic surfactants (< 5%) and anionic surfactants (< 5%) that cause this excess in ions correlated to salts (Fig. 3). However, there is no danger for environmental pollution, because the product is in accordance with European law [57] and its components are all biodegradable, as highlighted by the manufacturer's data sheet [58-60], but in future we have to do other investigations, because the regulation not take into account the effect of the product on the materials of historical and artistic interest, such as salts solubilization and efflorescence effects.

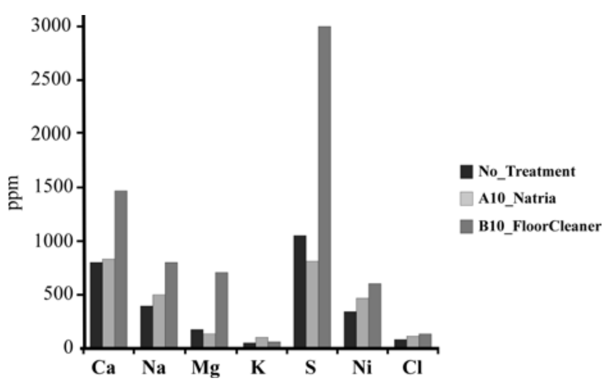

Fig. 3. Chemical analysis of the aqueous extract: the graph shows the main anionic and cationic species detected.

The surface analyses performed using the Stereo Microscope confirm the harmless of the Natria and New FloorCleaner treatment, because there are no damage and changes in the surface state. Only on specimens treat with Natria is possible to observe residues of the biocidal product thickened in the microporosity, although at the macroscopic level is not visible (Fig. 4).

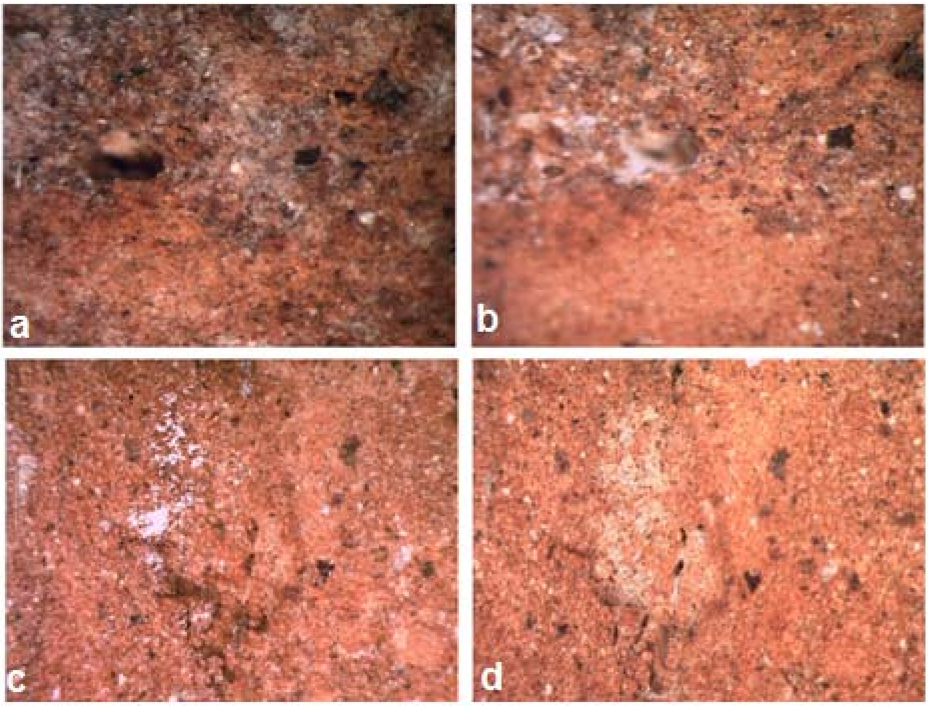

Fig. 4. Residues of pelargonic acid on treated specimens: sampler A7, point 5, magnification 0,7X (a) before and (b) after treatment; sampler A3, point 1, magnification 1,5X (c) before and (d) after treatment.
After treatment with Natria surfaces showed a white film.

The SEM analyses on polished cross sections not reveals variation along the profile of every specimen treated: this confirm that the biocides solution penetrated into the sample not caused damage and modification in samples microstructures.

Conclusions

In this paper, a new cleaning strategy based on biological biocides, Natria and New FloorCleaner has been proposed and optimized to removal biological patina on stone materials, such as handmade bricks produced in the early years of the 20th century. The analytical procedure is according to protocol Normal UNI 11551-1:2014 [34], and involves the analyses of water capillarity absorption rate, the amount of color changing by spectrophotometer, the chemical analyses of aqueous extract and the surface analyses with Stereo Microscope and SEM: all observations are carried out before and after the treatment with the biocides [61].

Generally the study suggested the potential use of these bio based products as novel biocides in the cultural heritage conservation, suitable for the treatment of biological patina of historical brick, because do not highlight problems and damages, thanks to the absence of interactions related to the treatment, such as decohesion, discoloration, or increase in porosity or fractures.

Another important feature of biocides Natria and New FloorCleaner is its safety for health and environment: this is very important because it responds to the challenges of the new approaches in terms of biotechnology and the necessity of the restorers to use sustainable products [23, 24]. The pollution from the use of traditional biocides method is typically more severe and persistent in indoor environment, but also in outdoor area is significant, especially in applications on stone materials placed in parks and gardens, where there is a risk of damage on precious and rare plants.

In addition to that, the treatment with these biological biocides is very easy and not requires applications with polyphasic approach, for example, combined with resin, polysaccharide, inhibitor substance or photodynamic treatment: simply, the diluted biocide is sprayed on the surface. An analyses of the cost of the biocleaning processes shows that the use of biological biocides Natria and New FloorCleaner is more convenient economically than the use of more common treatment or other biological approaches, such as enzymes or natural oil.

Despite the peculiarities of the two products, there are still limits to knowledge, in particular on the interaction between biocides and different contaminating species: it is well known that the Pelargonic acid in Natria is poorly bioselective [29], and the biodeteriogen control of New FloorCleaner is currently well known only in field of sanitizing environments, such as hospitals and waiting areas [31]. Furthermore, there are no studies that define the persistence in time of the treatment on the surface, in particular in outdoor zone more exposed on weathering agents, where can make the application less effective over the time.

The aim of this research is to study for the first time two biological biocides, currently no employing for conservation purposes; the future goal is to implement the research with the analysis of other stone materials and to improve knowledge on the interaction between the biocide and infesting species and test in situ the activity of biocides.

The results obtained in this research are anyway positive and the data confirm that Natria and New FloorCleaner can be used on all artificial stone compatible with the petrographic characteristics of the samples examined, while safeguarding the operators health, the environment state and, secondarily, the health of users of the cultural heritage.

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References

[1] T. Warscheid, T.W. Becker, M.A. Resende, *Biodeterioration of stone: a comparison between (Sub-)tropical and moderate climate zones,* **International Biodeterioration and Biodegradation**, **37**(1), 1996, p. 124.

[2] P.S. Griffin, N. Indictor, R.J. Koestler, *The biodeterioration of stone: a review of deterioration mechanisms, conservation case histories, and treatment,* **International Biodeterioration**, **28**(1-4), 1991, pp. 187-207.

[3] G. Ranalli, E. Zanardini, C. Sorlini, *Biodeterioration – Including Cultural Heritage*, **Encyclopedia of Microbiology** (Third Edition), edited by Moselio Schaechter, Academic Press, Oxford, 2009, pp. 191-205.

[4] K.Sterflinger, G. Piñar, *Microbial deterioration of cultural heritage and works of art — tilting at windmills?,* **Appllied Microbiology and Biotechnology**, **97**(22), 2013, pp. 9637–9646.

[5] F. Bartoli, A. Casanova Municchia, Y. Futagami, H. Kashiwadani, K.H. Moon, G. Caneva, *Biological colonization patterns on the ruins of Angkor temples (Cambodia) in the biodeterioration vs bioprotection debate*, **International Biodeterioration and Biodegradation**, **96**, 2014, pp. 157-165.
[6] M.T. Mendes, S. Pereira, T. Ferreira, J. Mirão, A. Candeias, , *In situ preservation and restoration of architectural tiles, materials and procedures: Results of an international survey,* **International Journal of Conservation Science, 6**(1), 2015, pp. 51-62.
[7] A.Z. Miller, P. Sanmartín, L. Pereira-Pardo, A. Dionísio, C. Saiz-Jimenez, M.F. Macedo, B. Prieto, *Bioreceptivity of building stones: A review*, **Science of the Total Environment**, **426**, 2012, pp. 1–12.
[8] M. Korkanç, A. Savran, *Impact of the surface roughness of stones used in historical buildings on biodeterioration*, **Construction and Building Materials**, **80**, 2015, pp. 279-294.
[9] M.E. Young, H.L. Alakomi, I. Fortune, A.A. Gorbushina, W.E. Krumbein, I. Maxwell, C. McCullagh, P. Robertson, M. Saarela, J.Valero, M. Vendrell, *Development of a biocidal treatment regime to inhibit biological growths on cultural heritage: BIODAM*, **Environmental Geology**, **56**(3-4), 2008, pp. 631–641.
[10] E. Zanardini, P. Abbruscato, N. Ghedini, M. Realini, C. Sorlini, *Influence of atmospheric pollutants on the biodeterioration of stone*, **International Biodeterioration and Biodegradation**, **45**(1-2), 2000, pp. 35-42.
[11] T. Warscheid, J. Braams, *Biodeterioration of stone: a review*, **International Biodeterioration and Biodegradation**, **46**(4), 2000, pp. 343-368.
[12] J. Monge-Nájera, B. Morera-Brenes, *Biodeterioration and biodegradation of Roman monuments: A comparison of the current status of 18th century paintings by the Canalettos,* **International Journal of Conservation Science**, **5**(1), 2014, pp. 3-8.
[13] S. Scheerer, O. Ortega-Morales, C. Gaylarde, *Microbial deterioration of stone monuments-an updated overview*, **Advances in Applied Microbiology**, **66**, 2009, pp. 97-139.
[14] B. Achilladelis, A. Schwarzkopf, M. Cines, *A study of innovation in the pesticide industry: Analysis of the innovation record of an industrial sector,* **Research Policy**, **16**(2-4), 1987, pp. 175-212.
[15] N. Colombani, M. Mastrocicco, D. Di Giuseppe, B. Faccini, M. Coltorti, *Batch and column experiments on nutrient leaching in soils amended with Italian natural zeolitites*, **Catena**, **127**, 2015, pp. 64-71.
[16] S. Nava, F. Becherini, A. Bernardi, A. Bonazza, M. Chiari, I. García-Orellana, F. Lucarelli, N. Ludwig, A. Migliori, C. Sabbioni, R. Udisti, G. Valli, R. Vecchi, *An integrated approach to assess air pollution threats to cultural heritage in a semi-confined environment: The case study of Michelozzo's Courtyard in Florence (Italy),* **Science of the Total Environment**, **408**(6), 2010, pp. 1403-1413.
[17] G. Liqun, G. Yanqun, *Study on Building Materials and Indoor Pollution*, **Procedia Engineering**, **21**, 2011, pp. 789-794.
[18] A. Unger, *Decontamination and "deconsolidation" of historical wood preservatives and wood consolidants in cultural heritage*, **Journal of Cultural Heritage**, **13**(3), 2012, pp. 196–202.
[19] M. Gherardi, A. Gordiani, A. Proietto, *Chemical exposure measurements in art restoration*, **Journal of Chemical Health and Safety**, **14**(6), 2007, pp. 4-7.
[20] M. Falkiewicz-Dulik, K. Janda, G. Wypych, **Handbook of Material Biodegradation, Biodeterioration, and Biostabilization,** Toronto, 2010, pp. 309 – 316.
[21] M. Silva, T. Rosado, D. Teixeira, A. Candeias, A.T. Caldeira, *Production of green biocides for cultural heritage - novel biotechnological solutions,* **International Journal of Conservation Science, 6**(SI), 2015, pp. 519-530.
[22] J.L. Ramírez, M.A. Santana, I. Galindo-Castro, A. Gonzalez, *The role of biotechnology in art preservation*, **Trends in Biotechnology**, **23**(12), 2005, pp. 584-588.

[23] P. Fernandes, Applied *microbiology and biotechnology in the conservation of stone cultural heritage materials*, **Applied Microbiology and Biotechnology**, **73**(2), 2006, pp. 291–296.
[24] P. Bosch-Roig, G. Ranalli, *The safety of biocleaning technologies for cultural heritage*, **Frontiers in Microbiology**, **5**, 2014, pp. 1-3.
[25] R. Giorgi, M. Baglioni, D. Berti, P. Baglioni, *New Methodologies for the Conservation of Cultural Heritage: Micellar Solutions, Microemulsions, and Hydroxide Nanoparticles*, **Accounts of Chemical Research**, **43**(6), 2010, pp. 695-704.
[26] M. Stupar, M.L. Grbić, A. Džamić, N. Unković, M. Ristić, A. Jelikić, J. Vukojević, *Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects*, **South African Journal of Botany**, **93**, 2014, pp. 118–124.
[27] G. Ranalli, G. Alfano, C. Belli, G. Lustrato, M.P. Colombini, I. Bonaduce, E. Zanardini, P. Abbruscato, F. Cappitelli, C. Sorlini, *Biotechnology applied to cultural heritage: biorestoration of frescoes using viable bacterial cells and enzymes*, **Journal of Applied Microbiology**, **98**(1), 2006, pp. 73–83.
[28] F. Valentini, A. Diamanti, G. Palleschi, *New bio-cleaning strategies on porous building materials affected by biodeterioration event,* **Applied Surface Science**, **256**(22), 2010, pp. 6550–6563.
[29] A.P. Wendy, W. Jingrui, K.H. Kriton, *Translocation, and metabolism of Glufosinate in Five Weed Species as Influenced by Ammonium Sulfate and Pelargonic Acid*, **Weed Science**, **47**(6), 1999, pp. 636-643.
[30] * * *, **Che cos'è Natria**, http://www.bayergarden.it/Natria [accessed on 16 June 2015].
[31] S. Mazzacane, G. Finzi, L. Aparo, P.G. Balboni, A. Vandini, L. Lanzoni, M.T. Camerada, M. Coccagna, *The Sanitation of Hospital Stays: New Strategies For The Reduction of HAIs*, **Health Management**, **14**(3), 2014, 1-8.
[32] P. Wattiau, M. E. Renard, P. Ledent, V. Debois, G. Blackman, S.N. Agathos, *A PCR test to identify Bacillus subtilis and closely related species and its application to the monitoring of wastewater biotreatment*, **Appllied Microbiology and Biotechnology**, **56**(5-6), 2001, pp. 816–819.
[33] E. Korenblum, G.V. Sebastián, M.M. Paiva, C.M.L.M. Coutinho, F.C.M. Magalhães, B.M. Peyton, L. Seldin, *Action of antimicrobial substances produced by different oil reservoir Bacillus strains against biofilm formation*, **Appllied Microbiology and Biotechnology**, **79**(1), 2008, pp. 97–103.
[34] * * *, **Cultural Heritage – Stone Materials – Methodology for the Evaluation of a Cleaning Method;** Part 1: Analytical protocol aimed examination of the potential harmfulness, UNI 11551-1:2014
[35] E. Marrocchino, D. Rapti-Caputo, C. Vaccaro, *Chemical–mineralogical characterisation as useful tool in the assessment of the decay of the Mesola Castle (Ferrara, Italy),* **Construction and Building Materials**, **24**(12), 2010, pp. 2672-2683.
[36] S. Bruni, M.R. Bovolenta, V. Finoli, M. Leis, C. Vaccaro, L. Volpe, *Study of conservative conditions of "Great graffito" made by Rosario Murabito in Villa Ottolenghi (Acqui Terme, Alessandria - Italy),* **Proceedings of the National Meeting Science for Contemporary Art**, 2011, pp. 5-13.
[37] M. Leis, E. Marrocchino, C. Vaccaro, L. Volpe, *Ricerche storiche e conservative del patrimonio rurale della provincia di Ferrara: il caso studio di villa La Mensa (Sabbioncello San Vittore, Copparo, Ferrara),* **Annali dell'Università di Ferrara - Museologia Scientifica e Naturalistica**, **8**(1), 2012, pp. 91-98.
[38] F. Minghini, G. Milani, A. Tralli, *Seismic risk assessment of a 50 m high masonry chimney using advanced analysis techniques*, **Engineering Structures**, **69**, 2014, pp. 255-270.
[39] P. Asokan, M. Saxena, S.R. Asolekar, *Coal combustion residues-environmental implications and recycling potentials*, **Resources, Conservation and Recycling**, **43**(3), 2005, pp. 239-262.

[40] * * *, **Conservation of Cultural Property - Test Methods - Determination of Water Absorption by Capillarity,** UNI EN 15801:2010.
[41] J. Schanda, **Colorimetry**, Wiley-Interscience John Wiley & Sons Inc., 2007, p. 56.
[42] * * *, **Conservation of Cultural Property - Test Methods - Colour Measurement of Surfaces Specifies a test Method to Measure the Surface Colour Changes**, UNI EN 15886:2010
[43] F.M. Helmi, Y.K. Hefni, *Using nanocomposites in the consolidation and protection of sandstone*, **International Journal of Conservation Science**, **7**(1), 2016, pp. 29-40.
[44] G.V. Atodiresei, I.G. Sandu, E.A. Tulbure, V. Vasilache, R. Butnaru, *Chromatic characterization in CieLab system for natural dyed materials, prior activation in atmospheric plasma type DBD*, **Revista de Chimie**, **64**(2), 2013, p. 165-169.
[45] S.S. Darwish, Evaluation of the effectiveness of some consolidants used for the treatment of the XIXth century Egyptian cemetery wall painting, **International Journal of Conservation Science, 4**(4), 2013, pp. 413-422.
[46] D. Grossi, E.A. Del Lama, J. Garcia-Talegon, A.C. Inigo, S. Vicente-Tavera, *Evaluation of Colorimetric Changes in the Itaquera Granite of the Ramos de Azevedo Monument, Sao Paulo, Brazil*, **International Journal of Conservation Science, 6**(3), 2015, pp. 313-322.
[47] V. Pelin, I. Sandu, S. Gurlui, M. Branzila, V. Vasilache, E. Bors, I.G. Sandu, *Preliminary Investigation of Various Old Geomaterials Treated with Hydrophobic Pellicle*, **Color Research and Application**, 2016, DOI: 10.1002/col.22043.
[48] A.M. Saviuc-Paval, I. Sandu, I.M. Popa, I.C.A. Sandu, V. Vasilache, I.G. Sandu, *Obtaining and Characterization of Ceramic Pigments for Polychrome Artistic Elements II. Microscopic and colorimetric analysis,* **Revista de Chimie**, **63**(2), 2012, pp. 170-178.
[49] A.M. Saviuc-Paval, A.V. Sandu, I.M. Popa, I.C.A. Sandu, A.P. Bertea, I. Sandu, *Colorimetric and microscopic study of the thermal behavior of new ceramic pigments,* **Microscopy Research and Technique, 76**(6), 2013, pp. 564-571.
[50] F. Valentini, A. Diamanti, G. Palleschi, *New bio-cleaning strategies on porous building materials affected by biodeterioration event*, **Applied Surface Science, 256**(22), 2010, pp. 6550-6563.
[51] C. Pereira, T. Busani, L.C. Branco, I. Joosten, I.C.A. Sandu, *Nondestructive characterization and enzyme cleaning of painted surfaces: Assessment from the macro to nano level*, **Microscopy and Microanalysis**, **19**(6), 2013, pp. 1632-1644.
[52] S. Hrdlickova Kuckova, M. Crhova Krizkova, C.L.C. Pereira, R. Hynek, O. Lavrova, T. Busani, L.C. Branco, I.C.A. Sandu, *Assessment of green cleaning effectiveness on polychrome surfaces by MALDI-TOF mass spectrometry and microscopic imaging,* **Microscopy Research and Technique**, **77**(8), 2014, pp. 574-585.
[53] * * *, **SCP Science**, http//www.scpscience.com [accessed on 16 June 2015].
[54] M. Raimondo, M. Dondi, D. Gardini, G. Guarini, M. Mazzanti, *Predicting the initial rate of water absorption in clay bricks*, **Construction and Building Materials**, **23**(7), 2009, pp. 2623–2630.
[55] G. Alessandrini, M. Aglietto, V. Castelvetro, F. Ciardelli, R. Peruzzi, L. Toniolo, *Comparative Evaluation of Fluorinated and Unfluorinated Acrylic Copolymers as Water-Repellent Coating Materials for Stone*, **Journal of Applied Polymer Science**, **76**(6), 2000, pp. 962–977.
[56] S.S. Guan, M.R. Luo, *A colour-difference formula for assessing large colour differences*, **Color Research and Application**, **24**(5), 1999, pp. 344–355.
[57] * * *, **The European Parliament and of the Council of 31 March 2004 on Detergents, Regulation** (EC) No 648/2004.
[58] M.J. Scott, M.N. Jones, *The biodegradation of surfactants in the environment*, **Biochimica et Biophysica Acta (BBA) - Biomembranes**, **1508**(1-2), 2000, pp. 235-251.

[59] T. Cserháti, E. Forgács, G. Oros, *Biological activity and environmental impact of anionic surfactants*, **Environment International**, **28**(5), 2002, pp. 337-348.
[60] G. Ying, Fate*, behavior and effects of surfactants and their degradation products in the environment*, **Environment International**, **32**(3), 2006, pp. 417-431.
[61] I.C.A. Sandu, E. Murta, R. Veiga, V.S.F. Muralha, M. Pereira, S. Kuckova, T. Busani, *An innovative, interdisciplinary, and multi-technique study of gilding and painting techniques in the decoration of the main altarpiece of Miranda do Douro Cathedral (XVII-XVIIIth centuries, Portugal),* **Microscopy Research and Technique, 76**(7), 2013, pp. 733-743.

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