2023 IMEKO TC-4 International Conference on
Metrology for Archaeology and Cultural Heritage
Rome, Italy, October 19-21, 2023

Long-lasting methods to prevent biodeterioration of stone monuments: new silica nanosystem coupled to natural biocide

Flavia Bartoli[1], Zohreh Hosseini[2], Alma Kumbaric[2], Giulia Caneva[2,3]

[1] Institute of Heritage Science (CNR-ISPC), National Research Council of Italy, Area della Ricerca di Roma 1, Montelibretti, Via Salaria Km 29,300, 00015 Rome, Italy, flavia.bartoli@cnr.it
[2] University of Roma Tre, Viale G. Marconi 446, 00149 Rome, Italy, seyedhzohreh.hosseini@uniroma3.it, alma.kumbaric@uniroma3.it
[3] National Biodiversity Future Center (NBFC), Università di Palermo, Piazza Marina 61, I - 90133 Palermo, Italy, giulia.caneva@uniroma3.it

Abstract **- Nanotechnology, specifically nanocompounds, shows promise due to their potential to reduce biocide quantities, operational times, and restoration costs. This study investigates the biodeterioration of stone materials and explores green, eco-sustainable alternatives to traditional biocides. In the context of the SUPERARE and GRAL projects, we assessed the long-term efficacy of two biocides, 2-mercaptobenzothiazole (MBT) and zosteric sodium salt (ZOS), applied via silica-nanosystems on various lithotypes. After three years, results reveal minimal recolonization by fungal and non-photosynthetic organisms, with MBT proving more effective than ZOS. Further analysis will determine if colonizing microorganisms cause physical or chemical damage. This research highlights the potential of green nanotechnology in stone conservation.**

I. INTRODUCTION

The phenomenon of biodeterioration of stone materials is particularly relevant when the environmental conditions, the supply of nutrients, and the edaphic conditions (bioreceptivity) favor biological growth [1-3]. Different treatment methods, such as UV, laser cleaning, microwaves, and heat shock treatments (HSTs) [4-7], as well as several biocidal substances have been used in combination with prevention strategies.

Considering the risk to human health and the environment, the use of traditional biocides to reduce biodeterioration phenomena is increasingly discouraged and, in the current national and international context, research for the conservation of cultural heritage is mainly focused to identify techniques that are as "green and eco-sustainable" as possible. The use of natural active compounds coupled to this nanotechnology seems a good way to pursue an eco-friendly, sustainable, and safe approach for the conservation of cultural heritage, reducing the amount of the bioactive compound and obtaining a satisfactory, long-lasting, antifouling action [8-10]. The literature reports the synthesis of different nanocontainers and loading techniques [10,11]. However, nanosystems based on mesoporous silica materials, applied in a multifunctional coating, as a controlled release biocide system over time, are reported only in a few cases [11-12]. In previous works, two different silica-based nanocontainers (Ns), namely a core-shell nanocapsule (NC) and a mesoporous nanoparticle (MNP), have been synthesized and characterized [13-14]. Previous preliminary in vitro cultures exposed to the nanopar- ticles showed a high antifouling activity associated with a slight biocidal activity against photosynthesizing microorganisms [12]. However, the efficiency of these Ns in the release control of the engaged biocides and the improvement of biocide efficiency, allowing a reduction in the quantities of biocide required, have not been sufficiently addressed.

In addition to the selection of non-hazardous biocides for humans and the environment, we are focusing on the development of nanotechnologies, since the application of a "nanocompound" would allow shorter operating times as well as greatly reduced quantities of biocides, with the obvious advantage for the environment and the health of operators. At the same time, these technologies would also make it possible to reduce the costs of restoration operations thanks to prolonged control over time.

The search for multifunctional products, therefore, represents an innovative approach capable of making significant improvements to restoration techniques. The present work, developed within the SUPERARE (SUPER - particelle per Rivestimenti Autopulenti e Antivegetativi a Rilascio lEnto) and then GRAL (GReen And Long-lasting stone conservation products) projects granted by the Lazio Region within the 2020 Research Groups Project, dealt with the problem of biological colonization of the Aurelian walls by testing in situ the efficacy of two biocides: (2-

mercaptobenzothiazole (MBT) and sodium zosterate (ZOS) applied through silica-nanosystems for the controlled release of these substances and in lower concentrations than with traditional methods. In particular, the main goal here was to verify the long-term effectiveness of the already-developed coating.

II. MATERIAL AND METHODS

At first, to verify the long-term effectiveness of nanostructured coatings, we selected four lithotypes: Carrara marble, travertine, mortar, and brick. The relative specimens (size 5x5x1 cm) were subjected to aging consisting of two cycles of heating at 600 °C and a bath in distilled water for 24 hours, with the aim of increasing their porosity. Then, we selected two different biocides, namely 2-mercaptobenzothiazole (MBT) and zosteric sodium salt (ZOS), previously tested [8].

The MBT is a well-known biocide used in the conservation of cultural heritage as an antifungal and, in general, anticryptogamical compound [7,15]. Zosteric sodium salt (ZS) is a natural product antifoulant (NPA), which is less studied, but a promising product in this context. Zosteric acid and its sodium salt are produced and released by *Zostera marina* L., which is a wide-ranging marine flowering plant in the Northern Hemisphere, and its antifouling capability is related to the sulfate group present in the chemical structure [8,16].

According to Ruggiero [11], we synthesized two different silica nanocontainers, namely core-shell nanocapsules (NC) and mesoporous nanoparticles (MNP) (Figure 1). Briefly, to synthesize the silica nanocapsules (NC), the following compounds were adopted: water, cetyltrimethylammonium bromide (CTAB, Aldrich, Italy) as a cationic surfactant, ammonia solution (NH_3aq 30%, Aldrich) as a basic catalyst, tetraethoxysilane (TEOS, Aldrich) as a silica precursor, and diethyl ether (Et_2O, Aldrich) as a cosolvent. All the chemicals were analytical grade and were used without further purification [8].

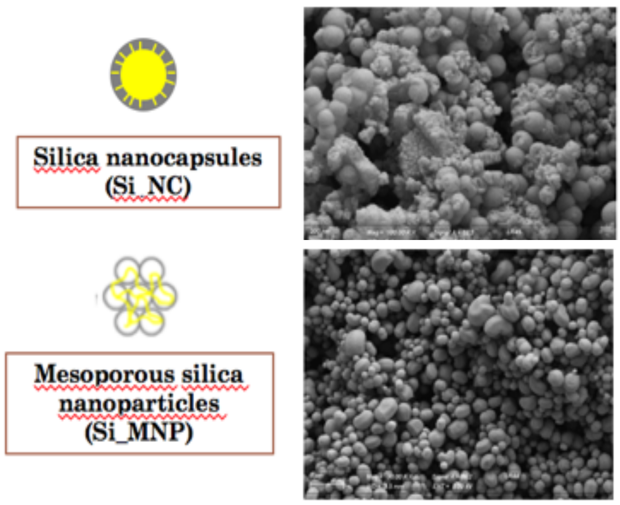

Fig. 1. Morphology of silica nanosystems at SEM

These nanoparticles were dispersed in a coating, prepared following [17] and [14] by using TEOS, ethanol (Carlo Erba Reagents S.r.l., Cornaredo, Italy), poly (dimethylsiloxane) hydroxyl- terminated (PDMS-OH, Sigma-Aldrich, St. Louis, MO, USA), and n-octylamine (Sigma-Aldrich, St. Louis, MO, USA) as a non-ionic surfactant, without any further purification [18].

To characterize the biocidal efficacy of coatings, we collected biological patinas in the area of the Aurelian Walls near Porta San Sebastiano, and samples, which were cultivated using a BG-11 liquid media (Sigma–Aldrich) at 25°C in a BG-11 liquid media (Sigma–Aldrich) at 25°C in conditions of natural sunlight, as previously reported [8]. Then, we proceeded with the identification of the patina constituents on a morphological basis with an optical microscope and molecular techniques.

Fig. 2. North exposure of Aurelian Walls near Porta San Sebastiano, and in situ test placement.

To perform the in-situ evaluation, for each lithotype we prepared tiles with the two biocides [and the two nanosystems (NC and MNP)], and one control without coating application. At this stage, we Afterward, dispersed the loaded nanodevices in a multifunctional TEOS-based coating also containing TiO_2 nanoparticles as photocatalytic agents [11]. Considering the previous results of Bartoli [19], which assessed that a significant recolonization starts from the third year after the applications, an acceleration of the natural colonization was performed. For such aim, we inoculated the mixed culture of microorganisms previously collected and after one week from the inoculum, we exposed the tiles near Porta San Sebastiano, on a North exposure of the Aurelian Walls (Figure 2).

Then, we monitored the progress of the colonization, starting from February 2019 to June 2023. To highlight chromatic changes in the surfaces, and thus explain the timing of recolonization, we collected images using a field microscope in visible and UV light, and we carried out colorimetric measurements. In parallel, to our *in-situ* analysis, the analysis of fisico-chemical properties of the treated materials are also investigated, and will be discussed in another paper.

III. RESULTS AND DISCUSSION

The in-progress research adds new data on the efficacy over time of synthesized coatings based on TiO2 and two different silica nanodevices, encapsulating a commercial biocide (2-mercaptobenzothiaole, MBT) and a natural antifoulant product (zosteric sodium salt, ZS) [18,14]. In the biofilms that develop on the surfaces of the materials, a dominance of photosynthetic microorganisms has been detected, including green algae and cyanobacteria, and in particular, *Chlorococcum* sp. and *Leptolyngbya* sp., associated with a modest presence of meristematic fungi. Molecular characterization is in progress. After more than 3 years from the exposure of the treated specimens (started in the SUPERARE project) only a slow and minimal recolonization by fungal organisms and other non-photosynthetic heterotrophic organi sms can be observed, which suggests a functionality of the coating loaded with the tested biocides towards photosynthetic organisms. Currently, a different degree of colonization is also observed between the different stone materials and the

different systems/biocides. To date, the mortar and brick substrates responded better to the application of the coating and, among the two biocides, MBT results are more efficient than the zosterate sodium (Figure 3).

These results confirm earlier research indicating that the ZS in the pure substance lacked significant biological activity against algal cells at the tested concentrations [8]. However, uploading it in nanosystem have a certain biological efficiency by inhibiting microorganism adhesion and interfering with their interaction with surfaces rather than directly killing them [20,21]. Indeed, an improvement in the quantity of zosterate in the nanosystem can give better results for the second eco-friendly biocide.

Moreover, the different efficiency observed between the NC and MNP systems highlights that the second demonstrates higher efficiency. This distinction appears to

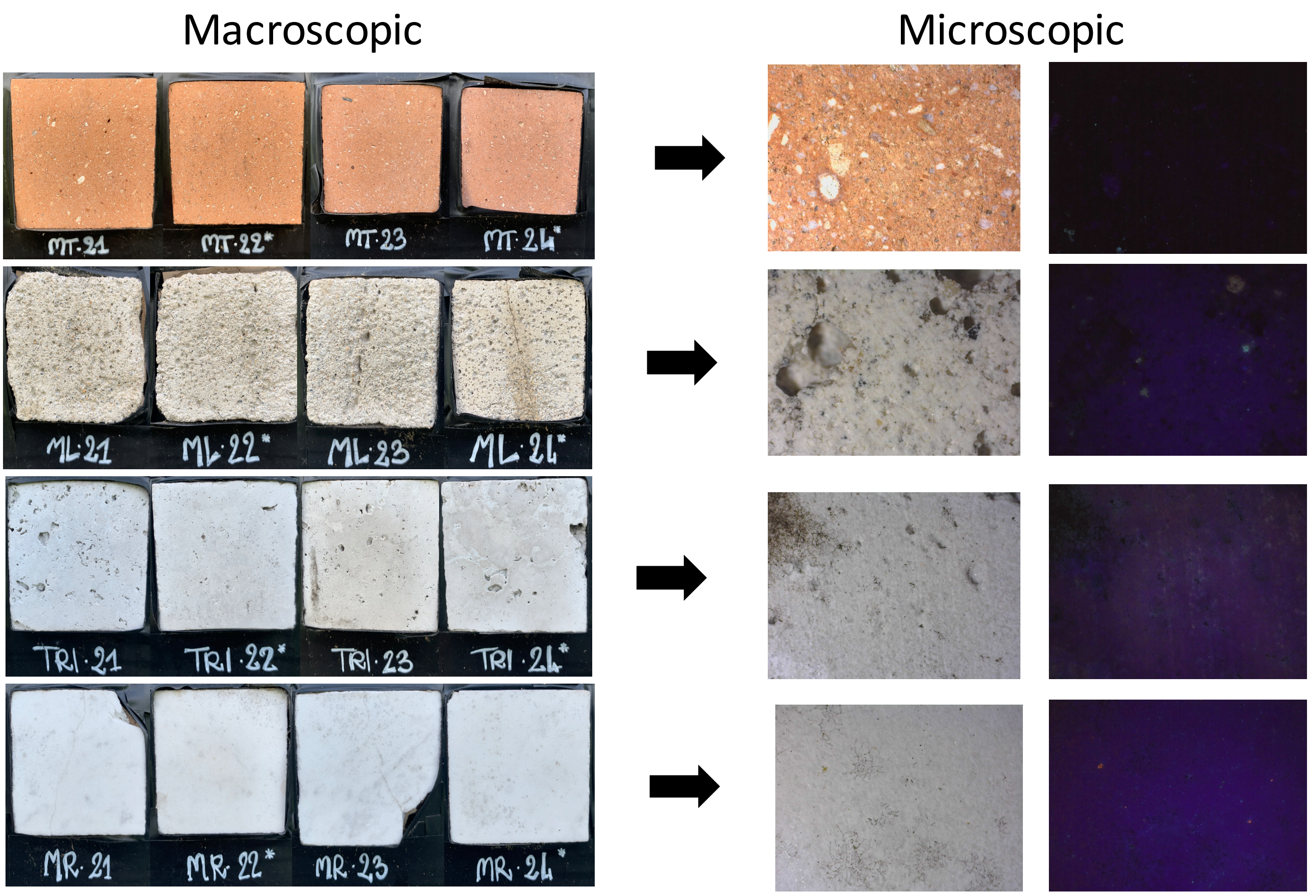

Fig. 3. Macroscopic and stereo-microscopic images (at 65X) of the tiles treated with coatings uploaded with MBT in May 2023.

be linked to the nanocontainers' structural characteristics [8, 11]. Notably, the self-assembly method employed for these two nanosystems differs; the mesoporous system features hexagonally packed cylindrical mesopores, while the nanocapsule boasts a core-shell structure. These differing structures seem to elucidate why, within the short term, MNP exhibited greater efficiency compared to NC, as the biocide compounds appeared to be less trapped and were released more quickly [8,11].

Analysis of the possible biodeteriogenic action of colonizing microorganisms is underway to determine whether, in addition to causing a visible blackening of the surface, they are also causing physical or chemical damage to the stone materials.

IV. CONCLUSION

We believe that research in this area is useful and very promising, even if the data obtained are still preliminary. To advance our understanding, it is crucial to conduct more detailed investigations into the following aspects: the ability of the coatings to withstand heat, how effectively biocides are loaded into and released from the nanocapsules, and the long-term stability of these materials.

REFERENCES

[1] G.Caneva, M.p.Nugari, O.Salvadori, "Plant biology for cultural heritage: biodeterioration and conservation", Getty Publications, 2008.

[2] N.Cutler, H.Viles, "Eukaryotic microorganisms and stone biodeterioration." Geomicrobiology Journal, vol. 27, No. 6-7, 2010, pp. 630-646.

[3] A.Z.Miller, P.Sanmartín, L.Pereira-Pardo, A.Dionísio, C.Sáiz-Jiménez, M.F.Macedo, B.Prieto, "Bioreceptivity of building stones: A review." Science of the total environment, vol. 426, 2012, pp. 1-12.

[4] M.Tretiach, S.Bertuzzi, F.Candotto Carniel. "Heat shock treatments: a new safe approach against lichen growth on outdoor stone surfaces." Environmental science & technology, vol. 46, No. 12, 2012, pp. 6851-6859.

[5] M.Mascalchi, I.Osticioli, C.Riminesi, O.A.Cuzman, B. Salvadori, S. Siano, "Preliminary investigation of combined laser and microwave treatment for stone biodeterioration." Studies in Conservation, vol. 60, 2015, pp. S19-S27.

[6] G.Caneva, M.Tescari, "Stone biodeterioration: Treatments and preventive conservation." In Proc. of the 2017 International Symposium of Stone Conservation, Conservation Technologies for Stone Cultural Heritages: Status and Future Prospects, September 2017, Seoul, Korea vol. 21.

[7] M.R.Fidanza, G.Caneva, "Natural biocides for the conservation of stone cultural heritage: A review." Journal of Cultural Heritage, vol. 38, 2019, pp. 271-286.

[8] F.Bartoli, M.Zuena, A.Sodo, G.Caneva, "The efficiency of biocidal silica nanosystems for the conservation of stone monuments: Comparative in vitro tests against epilithic green algae." Applied Sciences, vol. 11, No. 15, 2021, pp. 6804.

[9] M.Silva, T.Rosado, D.Teixeira, A.Candeias, A.T.Caldeira, "Production of green biocides for cultural heritage. Novel biotechnological solutions." Int. J. Conserv. Sci. vol. 6, 2015, pp. 519–530.

[10] L.McCusker, F.Liebau, G.Engelhardt, "Nomenclature of structural and compositional characteristics of ordered microporous and mesoporous materials with inorganic hosts (IUPAC Recommendations 2001)." Pure Appl. Chem. Vol. 73, 2009, pp. 381e394.

[11] L.Ruggiero, E.Di Bartolomeo, T.Gasperi, I.Luisetto, A.Talone, F.Zurlo, D.Peddis, M.A.Ricci, A.Sodo, "Silica nanosystems for active antifouling protection: nanocapsules and mesoporous nanoparticles in controlled release applications." Journal of Alloys and Compounds, vol. 798, 2019, pp. 144-148.

[12] A.Kuznetsova, P.M.Domingues, T.Silva, A.Almeida, M.L.Zheludkevich, J.Tedim, M.G.S.Ferreira, A.Cunha, "Antimicrobial activity of 2-mercaptobenzothiazole released from environmentally friendly nanostructured layered double hydroxides." J. Appl. Microbiol. Vol. 122, 2017, pp.1207–1218.

[13] L.Ruggiero, F.Bartoli, M.R.Fidanza, F.Zurlo, E.Marconi, T.Gasperi, S.Tuti, L.Crociani, E.Di Bartolomeo, G.Caneva, et al. "Encapsulation of environmentally-friendly biocides in silica nanosystems for multifunctional coatings." Appl. Surf. Sci. vol. 514. 2020, pp.145908.

[14] L.Ruggiero, M.R.Fidanza, M.Iorio, L.Tortora, G.Caneva, M.R.Ricci, A.Sodo. "Synthesis and characterization of TEOS coating added with innovative antifouling silica nanocontainers and TiO2 nanoparticles." Frontiers in Materials, vol. 7, 2020, pp. 185.

[15] G.Caneva, M.P.Nugari, D.Pinna, O.Salvadori, "Il Controllo del Degrado Biologico: I Biocidi nel Restauro dei Materiali Lapidei." Nardini Editore: Fiesole, Italy, 1996.

[16] B.M.Z. Newby, T.Cutright, C.A.Barrios, Q.Xu, "Zosteric acid—An effective antifoulant for reducing fresh water bacterial attachment on coatings." JCT research, vol. 3, 2006, pp. 69-76.

[17] F.Xu, D.Li, Q.Zhang, H.Zhang, J.Xu, "Effects of addition of colloidal silica particles on TEOS-based stone protection using n-octylamine as a catalyst". Prog. Org. Coatvol 75, . 2012, pp. 429–434

[18] M.Zuena, L.Ruggiero, G.Caneva, F.Bartoli, G.Della Ventura, M.A.Ricci, A.Sodo, "Assessment of Stone Protective Coatings with a Novel Eco-Friendly

Encapsulated Biocide", Coatings, vol. 11, No. 9, 2021, pp. 1109.

[19] F.Bartoli, A.Casanova Municchia, M.Leotta, S.Luciano, G.Caneva, "Biological recolonization dynamics: Kentridge's artwork disappearing along the Tiber embankments (Rome, Italy)." International Biodeterioration & Biodegradation, vol. 160, 2021, pp. 105214.

[20] T. Geiger, P.Delavy, R.Hany, J.Schleuniger, M.Zinn, "Encapsulated zosteric acid embedded in poly [3-hydroxyalkanoate] coatings—Protection against biofouling." Polym. Bull. Vol. 52, 2004, pp. 65–72

[21] C.Cattò, S.Dell'Orto, F.Villa, S.Villa, A.Gelain, A.Vitali, V.Marzano, S.Baroni, F.Forlani, F.CAppitelli, "Unravelling the Structural and Molecular Basis Responsible for the Anti-Biofilm Activity of Zosteric Acid." PLoS ONE, Vol.10, 2015, e0131519.