



UNIONE EUROPEA
Fondo Sociale Europeo



Corso di Dottorato di ricerca
in Science and Technology of Bio and Nanomaterials
ciclo XXXVII

Tesi di Ricerca

New Green and Multifunctional Materials for the Protection of Cultural Heritage

Nuovi materiali green e multifunzionali per la protezione dei Beni Culturali
SSD: CHIM12

Coordinatore del Dottorato

Prof. Flavio Rizzolio

Supervisore

Prof.ssa Federica Menegazzo

Supervisore cotutela

Prof.ssa Elena Ghedini

Dottorando

Andrea Campostrini
Matricola 858327

La borsa di dottorato è stata cofinanziata con risorse del
Programma Operativo Nazionale Ricerca e Innovazione 2014-2020, risorse FSE REACT-EU
Azione IV.4 "Dottorati e contratti di ricerca su tematiche dell'innovazione"
e Azione IV.5 "Dottorati su tematiche Green"



UNIONE EUROPEA
Fondo Sociale Europeo



Università
Ca' Foscari
Venezia

PhD programme in
Science and Technology of Bio and Nanomaterials
cycle XXXVII

Research thesis

New Green and Multifunctional Materials for the Protection of Cultural Heritage

Nuovi materiali green e multifunzionali per la protezione dei Beni Culturali
SSD: CHIM12

PhD programme coordinator

Prof. Flavio Rizzolio

Supervisor

Prof. Federica Menegazzo

Co-supervisor

Prof. Elena Ghedini

PhD candidate

Andrea Campostrini
Student ID n. 858327

The PhD scholarship was co-funded with resources from the
National Operational Programme for Research and Innovation 2014-2020, FSE REACT-EU resources
Action IV.4 "Doctorates and research contracts on innovation topics"
and Action IV.5 "Doctorates on Green topics"

INDEX

Preface :	Science meets restorers: CATMAT LAB & UNISVE	1
Chapter I:	General introduction	4
	1. STONE MATERIALS AND THEIR DEGRADATION	5
	<i>Biodeterioration of stone materials</i>	
	<i>Strategies for improvement</i>	
	2. ANTIMICROBIAL PRODUCTS	10
	<i>Environmental and health issues of synthetic biocide treatments</i>	
	<i>Green antimicrobial alternatives</i>	
	3. NANOSTRUCTURED PROTECTIVE COATINGS	13
	<i>Polymeric coatings for stone protection</i>	
	<i>Inorganic nanostructured coatings</i>	
	<i>Photocatalysts as green materials for restoration</i>	
	Goals of the PhD thesis	19
	GOALS AND THESIS WORKFLOW	20
	GRAPHICAL ABSTRACT	21
Chapter II:	Enhancing Venetian traditional marmorino with TiO₂ and ZnO for antimicrobial protection – A case study	22
	Article published in <i>Next Materials</i> , Elsevier, 2025, 7, 100384 https://doi.org/10.1016/j.nxmte.2024.100384	

Chapter III:	Engineered silica NPs to hold and release the antimicrobial product Biotin T ®	48
	Article published in <i>Next Materials</i> , Elsevier, 2025, 7, 100373. https://doi.org/10.1016/j.nxmte.2024.100373	
Chapter IV:	Mesoporous silica and vegetal extracts combined as sustainable stone heritage protection against biodeterioration	77
	Article published in <i>Applied Microbiology and Biotechnology</i> , Springer Nature, 2025, 109, 99, 1 -15. https://doi.org/10.1007/s00253-025-13475-5	
Chapter V:	A nanostructured photocatalytic multifunctional coating for stone architectural materials	100
	Manuscript in preparation	
Chapter VI:	Conclusions	128
Bibliography		131
Acknowledgments		163
Glossary		164
Abstract		

PREFACE

SCIENCE MEETS RESTORERS: CATMAT LAB & UNISVE

Before getting into the details of my PhD work, I would like to tell you how this project was born thanks to the collaboration with the restoration company Uni.S.Ve.

Uni.S.Ve. Srl, *Unione Stuccatori Veneziani*, is a restoration company based in Venice, Italy, renowned for its commitment to preserving the city's rich architectural and artistic heritage. Founded in 2001 by master artisans and architects dedicated to safeguarding traditional Venetian techniques, the company specializes in the restoration and conservation of historic surfaces and in the creation of interior decorations inspired by the Venetian tradition.

Their expertise encompasses a wide range of artisanal practices, including plaster work, fresco restoration, marble plaster, gilding, and trompe l'œil. By combining historical methods with modern diagnostic tools, Uni.S.Ve. ensures the integrity and longevity of both architectural landmarks and private properties. The company's contributions to Venetian cultural heritage are amplified by its collaboration with academic institutions, fostering research, innovation, and the transmission of traditional craftsmanship.

Among its various collaborations, Uni.S.Ve. co-founded my PhD scholarship in partnership with the CATMAT research group, of the Department of Molecular Sciences and Nanosystems, at Ca' Foscari University of Venice as part of the doctoral scholarship of the Italian National Operational Program (PON) Research and Innovation 2014-2020, funded by REACT-EU ESF resources, action IV.4 "Doctorates and research contracts on innovation topics" and action IV.5 "Doctorates on Green topics". This PhD scholarship included, indeed, a six-month period working for the co-founder company, as well as six months of research abroad, which were carried out at the *Instituto Universitario de Restauración del Patrimonio* of the Universitat Politècnica de València, in Spain.

Thanks to the collaboration with Uni.S.Ve., I had the opportunity to observe firsthand the work of the restoration company, collaborating side-by-side with their expert personnel in the restoration sites, villas, museums, artisan laboratory, and administrative office (**Figure 1**).

Thanks to this collaboration, it was possible not only to interview the restorers about their work but also to experience firsthand the challenges faced during a restoration intervention and learn-by-doing about the problematics of this sector, especially trying to have a lower impact on the environment, that needs more and more precautions.

Indeed, the field of restoration faces the dual challenge of preserving cultural heritage while addressing contemporary environmental concerns. The integration of green and innovative technologies into restoration practices represents a critical step towards sustainable conservation. By reducing the environmental footprint of restoration activities, such technologies ensure the longevity of heritage assets, while aligning with global efforts to mitigate climate change.

A multidisciplinary approach is thus essential to achieve this balance. Collaboration between material scientists, conservation specialists, architects, restorers, chemists, and environmental engineers enables the development of cutting-edge solutions tailored to the specific needs of each project. From eco-friendly methods and bio-based materials to advanced diagnostic tools and energy-efficient techniques, such innovations provide a harmonious fusion of tradition and modernity. The adoption of green technologies in restoration extends beyond environmental benefits: it fosters a deeper understanding of historical materials, encourages the revitalization of traditional craftsmanship, and strengthens the connection between cultural heritage and contemporary society. The degradation phenomena threatening our cultural heritage in recent decades are indeed closely linked to environmental changes, which have profoundly transformed our habits and adversely impacted our health.

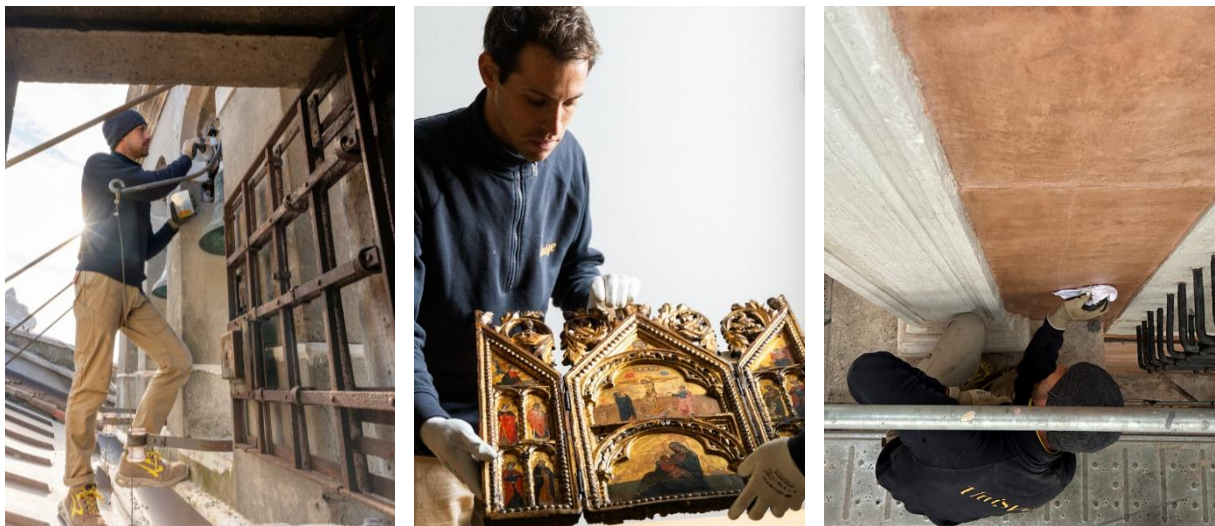


Figure 1: Photos by Joan Porcel Studio© of restoration interventions I participated in, carried out by Uni.S.Ve. Srl at the *Scuola Dalmata dei Santi Giorgio e Trifone* and *Museo Correr* in Venice, Italy

I.

GENERAL INTRODUCTION

1. STONE MATERIALS AND THEIR DEGRADATION

Stone materials are crucial elements of cultural heritage and architectural monuments, offering a tangible connection to history and human creativity. They have played a key role in the evolution of artistic expressions, thanks to their durability, versatility, and aesthetic, making them an ideal material to express various forms of art, from sculpture to architectural ornamentation [1]. Stone materials such as marble, granite, and limestone have historically been appreciated for their workability and capacity to capture intricate details, as shown in masterpieces from Antiquity till modern times (**Figure 1**) [2].



Figure 1: Photos of examples where stone materials were employed for their artistic purposes – (a) detail of composite marble pavement in St. Mark's Basilica (12th century), Venice, Italy – (b) detail of marble mosaic wall of Sheikh Zayed Grand Mosque (20th century), Abu Dhabi, United Arab Emirates – (c) detailed of intricately carved black basalt column of Ramappa Temple (13th century), Palampet, Telangana, India.

In modern architecture, the role of stone has evolved with the introduction of concrete and composite materials. While traditional stone continues to inspire architects for its timeless beauty and strength, contemporary designs often incorporate it in combination with cementitious materials [3–6]. This integration is particularly evident in facades, decorative claddings, and hybrid structures, where stone's natural appeal complements the versatility of reinforced concrete. Moreover, engineered stone products, such as agglomerates and thin stone veneers, have expanded the possibilities for lightweight applications, energy efficiency, and sustainable design in modern construction.

Despite their historical or modern applications, stone materials are highly susceptible to degradation phenomena influenced by a variety of factors [7]. Environmental conditions, such

as temperature variations, humidity, and air pollution, can lead to processes like freeze-thaw cycles, salt crystallization, and acid rain erosion, especially in the last decades as it has been well established by the scientific community [8–10].

Degradation phenomena were thus categorized into four categories based on the main origin of the phenomenon, which might be physical, chemical, biological, or anthropogenic; it is however important to emphasize that, in most cases, degradation is influenced by multiple factors (**Figure 2**).

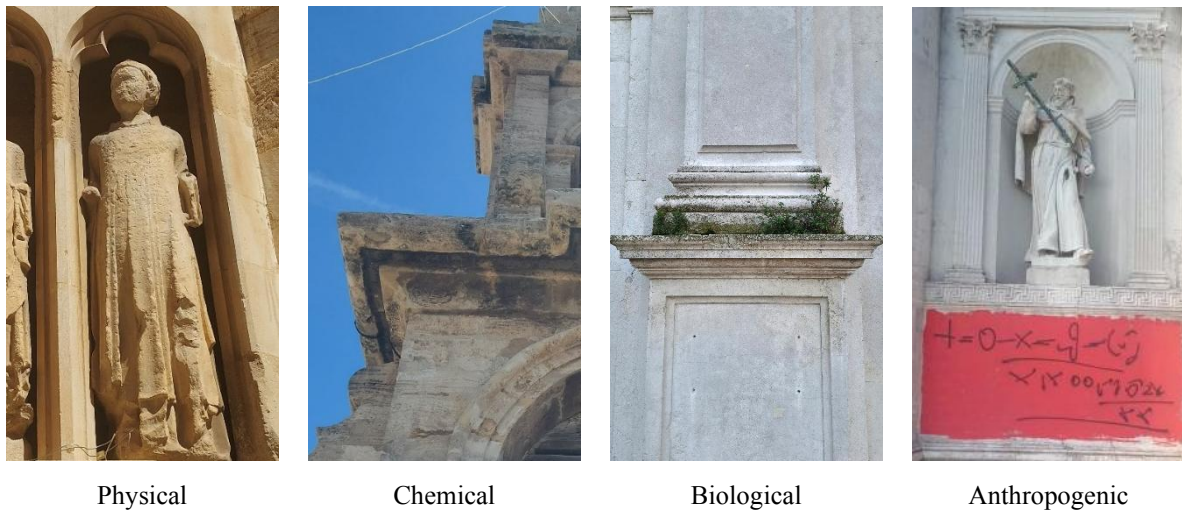


Figure 2: *Photos of degradation phenomena attributed to the four different categories based on the origins of the degradation (physical, chemical, biological, and anthropogenic).*

Physical degradation includes mechanical weathering, such as abrasion, cracking, and thermal expansion, often caused by environmental factors like temperature fluctuations and moisture. Chemical degradation involves reactions between the stone's mineral composition and external environmental agents, such as acid rain, salts, or pollutants, leading to processes like dissolution, efflorescence, or corrosion. Both forms of degradation threaten the integrity of cultural heritage, requiring careful preservation and conservation efforts [11,12]. Biological agents, including lichens, algae, and bacteria, contribute to biodeterioration by producing organic acids that interact with the stone's surface. Biodeterioration concerns the weakening of surfaces due to the activity of microorganisms, such as fungi or bacteria. These organisms can settle on the porous structure of stone, causing chemical and physical changes that weaken the material. Over time, this biological activity leads to disintegration, discoloration, and loss of structural integrity, posing significant challenges to the conservation of heritage sites [13–15]. Finally, the last category concerns the anthropogenic impacts on the material; graffiti and other

vandalic attacks on stone materials, as well as improper conservation practices, can aggravate the conservation state of stone structures leading to deterioration.

It is thus possible to state that stone materials, even if they might be more resistant than other artistic materials, can still suffer from different degradation phenomena and it is thus important to protect them. For example, marble and limestone, being composed primarily of calcium carbonate, are particularly vulnerable to acid rain, which dissolves the mineral matrix and results in surface pitting and loss of detail [16]. Granite, while more resistant, can suffer from mechanical stress and bio-colonization in humid environments [17]. Effective mitigation strategies include preventive conservation measures, such as controlling environmental exposure, employing protective coatings, and using compatible restoration materials to minimize further damage [18,19].

Biodeterioration of stone materials

Biodeterioration, in particular, is one of the most degradation processes, it is predominantly driven by microbial colonization and activity and it not only threatens the aesthetic and historical value of stone but also diminishes its structural stability, posing significant challenges to conservation efforts [20–22]. Effective preservation strategies must address both the biological agents and the conditions that promote their growth. Among the diverse microbial communities contributing to this phenomenon, fungi play a particularly destructive role, initiating complex biochemical and mechanical interactions with stone substrates [23].

Fungi thrive in diverse environmental conditions, making them effective colonizers of stone surfaces [24]. Airborne vegetative structures, including spores, conidia, and hyphal fragments, can readily settle on stone materials (**Figure 3**).

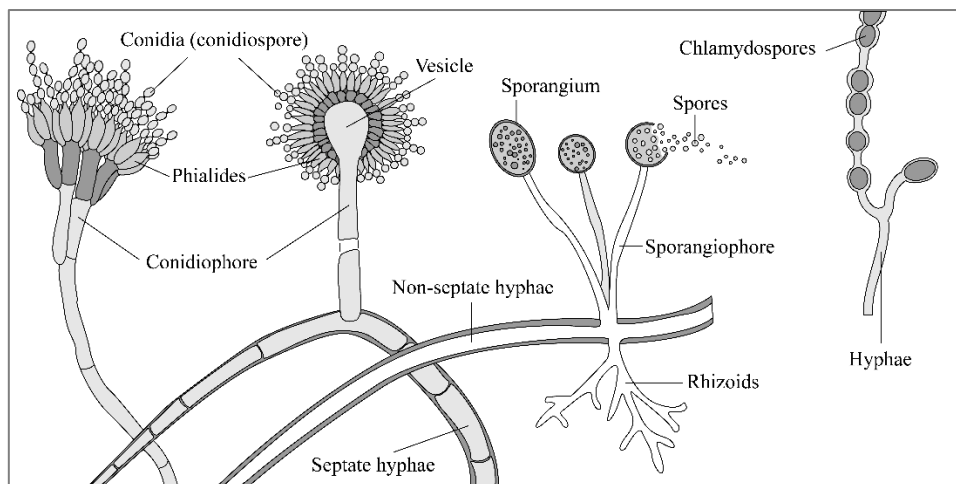


Figure 3: Main component and glossary of different fungal structure

Factors such as surface porosity, the presence of organic residues, and environmental conditions (*e.g.*, moisture, temperature, light, and air pollutants) can further enhance the bio-receptivity of stone surfaces [25–27]. Once established, fungal communities engage in processes that lead to both chemical and physical deterioration.

Fungal biodeterioration occurs through two main mechanisms: first, their capacity to prosper in environments with even low water content, allowing them to colonize diverse materials; and second the production and secretion of metabolic by-products, such as pigments, organic and inorganic acids, enzymes, and extracellular substances. These substances can alter the chemical and physical properties of materials, leading to processes of biocorrosion and penetration into artwork substrate [28–30]. Consequently, fungi are capable of colonizing, degrading, and transforming a wide range of materials used in monuments, artifacts, and items conserved in archives, museums, and historic buildings [31,32].

Environmental conditions play a crucial role in accelerating fungal colonization and biodeterioration. High humidity levels, temperature fluctuations, air pollution, and surface deposits such as dust and organic matter, all contribute to generate favorable conditions for fungal growth [33,34]. Furthermore, indoor environments, such as museums or storage facilities, are not exempt from biodeterioration risks: poor ventilation, localized moisture, and temperature instability can create microenvironments fertile for fungal colonization [35,36].

From a chemical point of view, some fungi can also secrete acidic metabolites, such as organic acids (oxalic, citric, and gluconic acids), that can react with the stone mineral composition. These reactions result in mineral dissolution, the formation of secondary by-products, and alterations in the stone's chemical composition. Enzymes produced by fungi further contribute to the degradation of the surface, easing deeper colonization. These biochemical interactions clearly weaken the stone's integrity and accelerate long-term deterioration [37–39]. Furthermore, fungi can interact with the substrate, inducing also physical damage: as fungal mycelia expand, they infiltrate microcracks and pores within the stone, exerting mechanical pressure. Over time, this pressure exacerbates pre-existing fractures, resulting in the loss of structural cohesion and surface detachment. The combined effect of biochemical weakening and mechanical stress can cause significant damage, even to historically significant and robust stone materials [40–42].

In addition to structural deterioration, fungal colonization significantly alters the visual appearance of stone surfaces. Many fungal species produce pigmented secondary metabolites,

including melanin, carotenoids, and anthraquinones, which stain the stone in a variety of colors. The discoloration ranges from dark black and brown hues associated with melanin to bright yellows, greens, and reds from other pigment-producing fungi [43–45].

The penetration of these pigments into porous or weathered substrates exacerbates the problem, creating stains that are difficult, if not impossible, to remove without compromising the stone's integrity. Moreover, the secretion of biofilms – a protective extracellular matrix produced by fungal communities – can trap additional pollutants and particles, further contributing to surface darkening and uneven coloration [46–48].

Improvement approaches

Effective ways to limit biodeterioration require a multidisciplinary approach that balances microbial control with the preservation of the historical and aesthetic integrity of the substrate [49]. Traditional methods, such as biocidal treatments, remain widely used but have significant limitations. Many chemical biocides are toxic to the environment, pose health risks to conservation professionals, and may leave residues that alter the stone's appearance or composition [50,51].

As an alternative, a growing interest in eco-friendly conservation methods has led to the study of natural substances, particularly vegetal extracts such as essential oils (EOs) [52,53]. These extracts contain bioactive compounds (e.g., terpenes, phenolic acids, and flavonoids) with potent antimicrobial properties. Their fungicidal activity can inhibit fungal growth, prevent recolonization, and mitigate aesthetic damage [54–56].

However, the direct application of essential oils presents challenges, including high volatility and potential interactions with the stone's surface. To address these limitations, advanced delivery systems have been developed, such as the one based on nanotechnology, which has emerged as a promising way to improve their efficacy by reducing the evaporation rate, enhancing stability, and minimizing the quantity employed [57–59]. Similarly, hydrogels and polymer-based nanocarriers have been employed to deliver vegetal extracts effectively while preserving the stone's aesthetic and structural properties. In addition to direct treatments, preventive measures are critical in combating biodeterioration; protective coatings embedded with antimicrobial agents offer another path for prevention, safeguarding stone surfaces without compromising their aesthetic or perceptible features [60–65].

Despite advancements, the conservation of stone materials remains a challenging endeavor. The variability in stone composition, environmental conditions, and microbial communities

necessitates site-specific strategies. The long-term effects of using natural products and nanotechnology in conservation treatments still require further investigations based on multidisciplinary approaches. Innovations such as bio-inspired materials, molecular analysis of microbial communities, and computational modeling of biodeterioration dynamics are leading the way for more effective and sustainable conservation practices [66–74]. Collaborative efforts between microbiologists, material scientists, and cultural heritage professionals are thus essential to preserving these invaluable artifacts.

2. ANTIMICROBIAL PRODUCTS

As previously mentioned, the study of microbial biodegradation poses a considerable challenge in the field of conservation science: the degradation of stone materials due to microbial activity represents a complex interplay of chemical, biological, and environmental factors. Addressing these phenomena often requires an interdisciplinary approach integrating microbiology, chemistry, physics, and conservation science knowledge [75,76].

The main kind of intervention usually carried out to deal with microbiological colonization over stone materials is related to cleaning actions with biocides and antimicrobial products [49]. However, most of these products are often toxic to the environment, especially when the washing water collection role is underestimated [77]. Another main issue is the operators' health risks they have to undergo while conducting the intervention.

Biodeterioration control methods can be divided into direct and indirect categories. Indirect methods focus on controlling microbial growth by acting on environmental factors that influence microbial activity and can be adjusted to inhibit growth. For example controlling the room temperature, humidity (thus moisture level), or air circulation can reduce the rate at which microorganisms proliferate. Direct methods, on the other hand, target the biodeteriogenic agents directly. These methods include mechanical means (such as using spatulas or brushes for removal), physical methods (like using 280-240 nm UV radiation), biological methods (such as enzymes that break down organic matter) and chemical methods (such as biocides). Restoration interventions often involve the use of biocides, which are composed of active ingredients, co-

formulates, and solvents. The choice of biocide should depend on the pathogenic organisms, the materials of the artifact, and its preservation state.

Environmental and health issues of synthetic biocide treatments

While biocides are an efficient way of fighting biodeterioration, they also pose significant risks to the environment. The chemicals that target the microorganisms can have harmful effects on the ecosystem, especially if not properly managed; thus, their use in restoration interventions can lead to involuntary contamination of the surrounding areas, including soil, water, and air [78–82].

For example, a lot of commonly used biocides, like benzalkonium chloride, are based on quaternary ammonium salts which are known to be harmful to aquatic life. Similarly, other biocides based on isothiazolinones, such as 2-octyl-4-isothiazolin-3-one (OIT) and 4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT), have strong toxicity to algae and other aquatic organisms [83,84]. Likewise, biocides containing carbamates, like IPBC (3-iodo-2-propynyl N-butyl carbamate), can also have harmful effects on non-target organisms, including insects, plants, and other microorganisms [85]. The main issue is that when all these biocides enter waterways through runoff or improper disposal, they can accumulate and persist in the environment, posing long-term risks [86,87].

Furthermore, the use of biocides poses health issues to restorers and other workers, who may be exposed through inhalation, skin contact, or ingestion, depending on the restoration methods used (e.g., spraying, brushing, or fumigation). The action of biocides, which block cell wall synthesis or damage DNA and RNA, can in fact also be toxic to humans.

It is known that several biocides commonly used in restoration interventions have associated health risks. Specifically, quaternary ammonium salts can cause irritation to the eyes, skin, and respiratory tract leading to chemical pneumonitis [88]. Isothiazolinones, such as 2-octyl-4-isothiazolin-3-one (OIT), are strong skin sensitizers and can cause allergic reactions: prolonged exposure to OIT has been linked to vascular and neurological diseases [89–91]. Biocides such as 3-iodo-2-propynyl N-butyl carbamate (IPBC) and 2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine (TCMSP) have been linked to allergic contact dermatitis and irritation of the skin and eyes [92–96]. The potential for biocide exposure to negatively impact human health therefore highlights the need for protective measures in restoration work or the use of less dangerous substances.

Green antimicrobial alternatives

The just-mentioned environmental and health risks, presented in the previous paragraphs, led to an increasing interest in using natural alternative substances: in recent years, the application of vegetal extracts, such as essential oils, has garnered attention as a promising strategy for combating microbial deterioration [97–100].

Essential oils (EOs) can be extracted from various plants and have been employed for their diverse therapeutic properties for a long time. In nature, they serve as the plant's natural defense mechanisms against microbial threats, therefore employing the intrinsic antimicrobial potential of essential oils has appeared as a convincing greener alternative path to synthetic chemical biocides, for safeguarding cultural artifacts from the harmful effects of fungal and bacterial colonizations [101]. One of the significant advantages of their use lies in their asserted fungicidal activity: their active constituents, such as terpenes and phenolic compounds, exhibit inhibitory effects on fungal growth and reproduction. EOs can indeed mitigate the risk of fungal colonization by disrupting cellular membranes and metabolic processes. In particular, their efficiency can be attributed to different destructive mechanisms of specific volatile molecules present within them; the most common active compounds are: α -pinene and β -pinene, thymol, carvacrol, cinnamaldehyde, linalool, eugenol, terpinene-4-ol, citronellal, geraniol, and camphor. These volatile antimicrobial molecules constitute a distinct and potent array of compounds, from terpenes to phenolic compounds and aldehydes, each class contributes uniquely to the overall antimicrobial efficacy of essential oils [102–104].

Terpenes represent a major class of antimicrobial volatile compounds present in essential oils, such as α -pinene and β -pinene (present in pine EO) that have demonstrated antimicrobial activity against a wide spectrum of pathogens, making them valuable contributors to the overall efficacy [105,106]. Phenolic compounds, including thymol (abundant in thyme EO) and carvacrol (predominant in oregano EO), interfere with microbial membranes and cellular processes, inhibiting bacterial and fungal growth [107–110]. Certain aldehydes, such as cinnamaldehyde (a key component in cinnamon bark EO), possess strong antibacterial and antifungal capabilities by disrupting microbial cell membranes [59,111]. The action of monoterpenoids, such as linalool (in lavender EO), involves interference mechanisms with the microbial membrane integrity [112,113]. Alcohols in essential oils, such as terpinen-4-ol (present in tea tree EO), are known for their efficacy against bacteria, fungi, and other microorganisms.

This dual action against fungi and bacteria positions essential oils as versatile agents for conserving cultural heritage materials vulnerable to microbial threats.

Despite their promising features, using essential oils in cultural heritage conservation is still challenging [114]. The concerns related to the potential alteration of artifact appearance, odor retention, and long-term efficacy require careful consideration in developing application protocols. Furthermore, the molecules with the highest antimicrobial action usually are the same and have high volatility and color; hence, the time efficacy of these products needs to be carefully considered. If for cleaning interventions, this is not a problem since essential oils can be trapped in cleaning poultices, when we are dealing with their preventive action, volatility is an issue. To avoid evaporation, options for the encapsulation of essential oil are proposed in the literature for protective coatings, for instance, embedding them in nanocarrier systems based on polymers (of different nature) or hydrogels. These systems are also studied for applications in the Cultural Heritage field.

3. NANOSTRUCTURED PROTECTIVE COATINGS

The preservation of stone materials, whether in historical monuments or modern architectural structures, is a critical challenge in cultural heritage conservation. Protective coatings are often employed to mitigate material degradation, forming a barrier from environmental and anthropogenic factors on stone surfaces [18,19,115]. To fulfill their goals effectively, ideal coatings should combine several attributes: durability, non-toxicity, hydrophobicity as well as water vapor permeability, chemical compatibility with the substrate, and minimal alteration of the stone's aesthetic properties, such as color and brightness. In recent years, most protective coatings were tailored to these requirements, however fulfilling all the desired criteria is hard and complex, therefore most products were developed and employed focusing on some of the characteristics, neglecting other less urgent but still important issues [116]. For this reason, continued research for innovation in this field is necessary. Among the most cutting-edge approaches, nanostructured coatings can be found, which take advantage of the unique properties of nanoscale-engineered materials to enhance the preservation of stone substrates.

Polymeric coatings for stone protection

Polymeric coatings represent a significant category of protective solutions, with several subtypes showing varying levels of effectiveness and limitations [117–119]. For example, epoxy-based coatings are known for their strong adhesion and ability to consolidate porous substrates and are generally employed to improve the structural integrity of stones. However, they are vulnerable to UV radiations, leading to discoloration and cracking, which coupled with their tendency to absorb water, limits their long-term effectiveness; additionally, their removal requires harsh chemical methods that risk damaging the underlying stone [120–123]. Acrylic-based coatings instead offer superior adhesion and transparency, enhancing stone strength while minimizing moisture penetration. Nonetheless, issues such as micropore formation, limited water resistance, and susceptibility to photo-oxidation pose risks to long-term performance. Their reliance on toxic solvents for removal further raises environmental and health concerns [124,125]. Polyurethane coatings are characterized by elasticity, impact resistance, and high gloss finishes and are usually employed in graffiti-resistant applications; however, their gloss deteriorates over time in outdoor conditions, necessitating frequent reapplications. Furthermore, the presence of toxic isocyanates in their traditional formulations restricts their use in environmentally sensitive areas [126–128]. Siloxane-based coatings exhibit excellent hydrophobicity, thermal stability, and resistance to chemical corrosion. Their non-permanent nature aligns with sustainable conservation practices, although hydrolysis over time can necessitate reapplication. The versatility of siloxanes, particularly in hybrid formulations, makes them a popular choice for stone material protection and consolidation [129–132].

Inorganic nanostructured coatings

Nanotechnology has revolutionized surface protection with the advent of inorganic nanostructured coatings, offering enhanced performance due to their nanoscale precision [133,134]. Hydrophobicity and self-cleaning properties were implemented: nanocoatings impart exceptional water-repellent properties, with some achieving super-hydrophobicity. This facilitates the rolling away of water droplets, which carry dust and particulates, thereby maintaining surface cleanliness. Furthermore, the robust barrier formed by nanocoatings resists staining from oils, chemicals, and graffiti, making them suitable for protecting monuments from vandalism [135,136].

Another interesting implementation is incorporating nanoparticles, such as titanium dioxide (TiO₂) or zinc oxide (ZnO), which enables coatings to harness photocatalytic properties and

antimicrobial properties. These materials can decompose harmful pollutants and exhibit antimicrobial effects, preventing biofilm formation and countering biodeterioration [137–139]. Other materials, such as silver (Ag) and copper (Cu) nanoparticles have demonstrated potent antimicrobial capabilities, further protecting stone substrates from microbial degradation [140–144]. Moreover, inorganic nanostructured ordered porous materials, such as SiO₂, have also attracted increasing scientific interest for their ability to interact with ions and molecules on their elevated surface area, depending on the distribution of pore sizes, shapes, and volumes. These materials may be produced through the self-assembly of surfactants, which mold the inorganic component and are characterized by an ordered mesostructure, very high surface area, and well-defined properties, making them ideal for encapsulating molecules of all kinds, from proteins to drugs to biocides. Mesoporous silicas such as MCM-41 and SBA-15 are highly efficient matrices in the design of controlled release systems due to characteristics like tunable pore size and the ordered structure [145].

The integration of nanoparticles into polymer matrices has also led to the development of hybrid nanocomposites. These systems combine the mechanical strength of polymers with the functional properties of nanoparticles, such as enhanced hydrophobicity and photocatalytic activity [146–148].

Photocatalysts as green materials for restoration

In recent decades, the urgent need to address environmental challenges has driven significant innovation in materials science and architecture. Photocatalysis, a process by which a material absorbs light and catalyzes chemical reactions, has opened new frontiers in material functionality. Titanium dioxide (TiO₂), a widely studied photocatalyst, exhibits exceptional properties, including its ability to degrade organic pollutants, eliminate harmful microorganisms, and facilitate self-cleaning [149,150]. When exposed to ultraviolet (UV) light, TiO₂ generates reactive oxygen species (ROS) that break down organic compounds and neutralize atmospheric pollutants, offering both aesthetic and health-related benefits (**Figure 4**) [151].

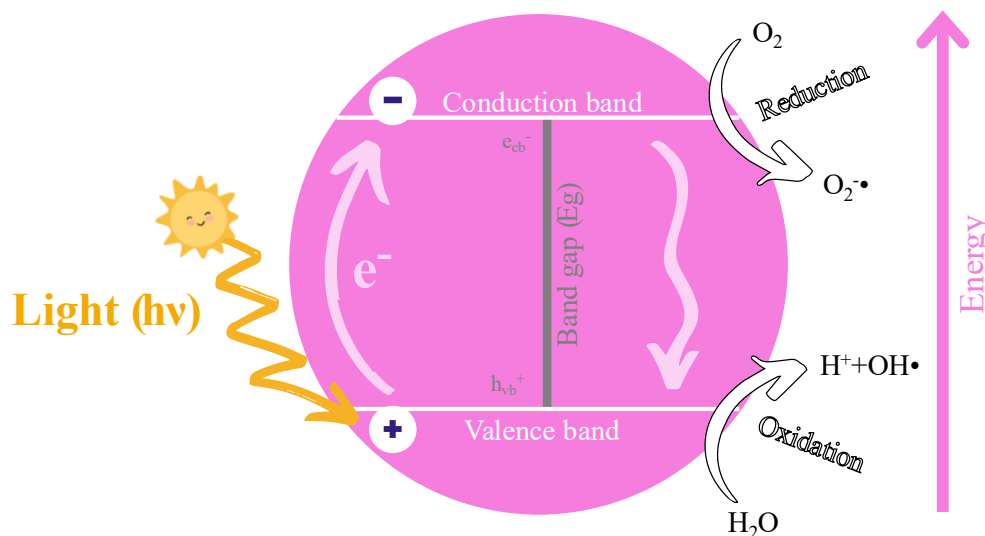


Figure 4: Scheme of the photocatalysis principles: (1) photon absorption, (2) jump to the conduction band, (3) electron holes formation, (4) reactions with e^- acceptors and e^- donors, and (5) free radicals' production

Photocatalytic materials, such as TiO_2 and ZnO , have been widely utilized in various paints and protective coatings applied to stone substrates [152]. Their ability to interact with organic deposits, pollutants, and other environmental residues has captured the attention of the scientific community as a means to address critical challenges, such as air pollution, self-cleaning surfaces, and energy efficiency [153–155].

In contemporary architecture, the development of these advanced materials aligns with global efforts to reduce carbon footprints, improve urban air quality, and create low-maintenance structures. The versatility of TiO_2 -based photocatalysts makes them suitable for a range of applications, from modern skyscrapers to urban infrastructure, such as in the Netherlands and Denmark, where TiO_2 -based asphalt was introduced to reduce NO_x emissions from vehicle exhaust and create cleaner urban environments [156–160].

Beyond its use in modern construction, TiO_2 also holds great promise for the preservation and restoration of historical buildings. Heritage structures, often exposed to pollutants, biological growth, and environmental wear, face unique challenges in maintaining their integrity and aesthetic value. Traditional cleaning and maintenance methods can be invasive, labor-intensive, and potentially harmful to delicate materials. TiO_2 -based coatings, on the other hand, offer a minimally invasive solution that respects the historical value of these buildings while enhancing their durability and appearance. Photocatalytic materials can be applied thus to stone, marble, and other surfaces to prevent the accumulation of deposits, reduce biological colonization, and

mitigate damage from air pollution. Moreover, these materials can be tailored to meet the specific conservation requirements of heritage sites, ensuring compatibility with original materials and aesthetic considerations [161–163].

For the just mentioned reasons, in recent years, the integration of TiO₂ into architectural and conservation practices has been introduced to address pressing challenges. For instance, in urban areas heavily impacted by air pollution, including nitrogen oxides (NO_x) and volatile organic compounds (VOCs), which contribute not only to respiratory problems but also to several degradation phenomena, TiO₂-based materials have been employed to neutralize these pollutants through photocatalysis [164–166]. A notable example is Palazzo Italia, built for the World Fair Expo 2015 in Milan, which was the first building in the world to use TiO₂-infused concrete to purify the surrounding air [167,168].

As discussed, TiO₂ has also been employed for surface maintenance and cleaning. Self-cleaning TiO₂-based coatings can break down organic matter and prevent dirt adhesion. Heritage structures facing degradation from pollution and biological colonization, which often require invasive and damaging restoration techniques, benefit from the application of TiO₂ coatings as a non-invasive protective layer [169–172]. To cite an example of heritage buildings, this technology has been studied on ornamental parts of the façades of the Markovits-Mathéser House in Oradea Municipality, Romania, where TiO₂ treatments have safeguarded the natural rock from microbiological growth [173].

Despite the several advantages of photocatalysts, certain drawbacks need to be addressed. One of the primary limitations is their dependency on ultraviolet (UV) light, which constitutes only a small fraction of natural sunlight, limiting the efficiency of outdoor applications. However, efforts are being made to develop visible-light-responsive photocatalysts, thanks to doping techniques for example. Moreover, the production of reactive oxygen species (ROS) during photocatalysis can pose challenges to material compatibility. In some cases, prolonged exposure to ROS may accelerate the degradation of the substrate materials. This risk is especially pertinent in the conservation of delicate heritage structures, where long-term stability is a critical concern [174,175]. Another significant consideration is the potential nanoparticle release into the environment, for this reason, their application and combination with other materials, able to hold them, need to be considered [176,177]. Lastly, the cost of nanostructured materials and their treatments may be prohibitive in some contexts, if compared with more traditional ones. At the same time TiO₂ and ZnO nanoparticles are one of the less expensive

materials in their context. While the long-term benefits of reduced maintenance and pollution mitigation may offset these costs, initial investment might remain a barrier.

Nevertheless, the remarkable photocatalytic and antimicrobial properties of TiO_2 , ZnO , essential oils, and vegetal extracts highlight their potential in addressing environmental challenges, from pollution mitigation to surface preservation, across both contemporary and historical architecture. As demonstrated in several applications, this technology successfully bridges innovation and tradition, enhancing sustainability and heritage conservation [178,179].

GOALS OF THE PHD THESIS

GOALS AND THESIS WORKFLOW

The principal aim of this PhD project was to develop a green multifunctional coating with antimicrobial properties for the protection of stone surfaces from biodeterioration phenomena.

The first part of the work focused on identifying green materials suitable for applications in the restoration of cultural heritage. In this context, photocatalytic nanoparticles, specifically TiO₂ and ZnO, were identified, characterized, and tested for their ability to inhibit fungal growth on a traditional Venetian plaster substrate (**Chapter II**).

Meanwhile, an in-depth study was conducted on other nanostructured materials to ultimately combine with photocatalysts. In particular, the research investigated the use of ordered mesoporous materials to act as nanocontainers for antimicrobial agents, with a focus on the synthesis, characterization, and functionalization of a SiO₂-based matrix capable of holding and gradually releasing a commercial biocide (**Chapter III**).

The next step involved studying and identifying a sustainable antimicrobial agent derived from vegetal sources or waste biomass, as a greener alternative to chemically synthesized biocides. In this regard, plant extracts and essential oils were combined with mesoporous materials and tested against microorganisms isolated from real-case cultural heritage stone samples (**Chapter IV**). Part of this work was conducted during a six-month research stay as a PhD visiting student at the *Instituto Universitario de Restauración del Patrimonio*, Universitat Politècnica de València (Spain).

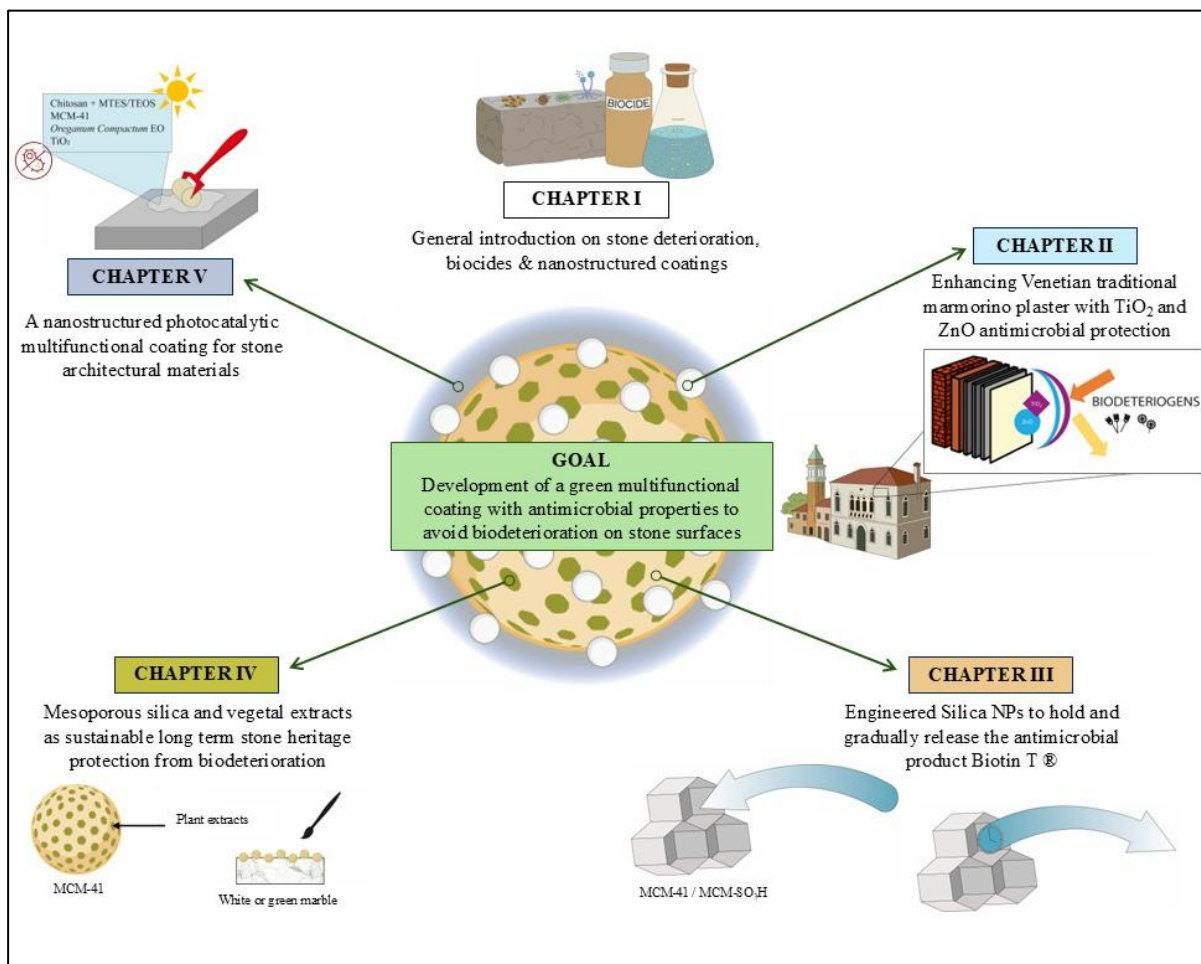
The final part of the work focused on optimizing the formulation of a coating capable of effectively delivering the active components, consisting of silica nanocontainers with the selected antimicrobial agent and photocatalysts. Final microbiological tests were also conducted to verify the compatibility of the formulation's ingredients (**Chapter V**).

The entire project was conducted with the support of Uni.S.Ve. Srl, who contributed to its development by drawing on their expertise, with the potential for the final formulation to be applied to a real case restoration intervention.

For each stage of the PhD work, a corresponding article, either published or submitted to international Peer reviewed scientific journals, is presented following the general introduction of the thesis.

GRAPHICAL ABSTRACT

NEW GREEN AND MULTIFUNCTIONAL MATERIALS FOR THE PROTECTION OF CULTURAL HERITAGE



II.

ENHANCING VENETIAN TRADITIONAL MARMORINO WITH TiO_2 AND ZnO FOR ANTIMICROBIAL PROTECTION – A CASE STUDY

Post-print version of the Article published in *Next Materials*, Elsevier, 2025, 7, 100384

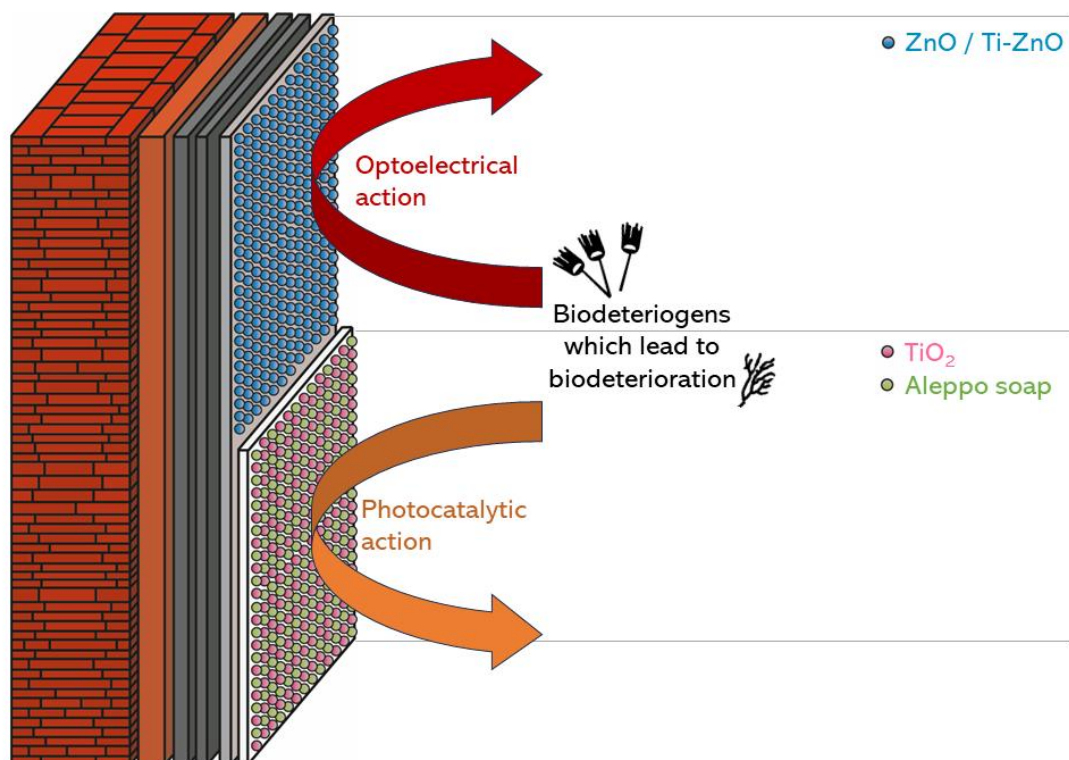
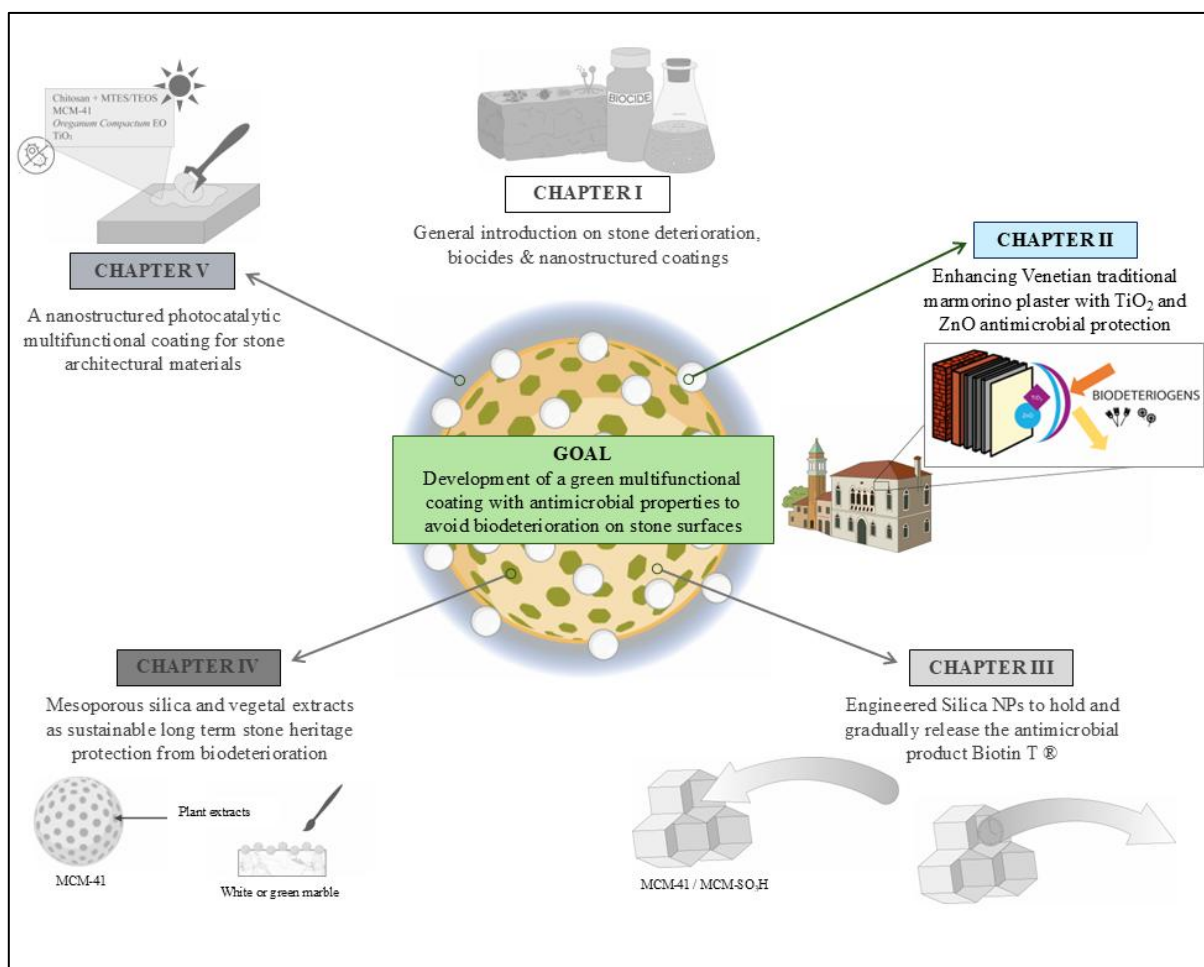
<https://doi.org/10.1016/j.nxmte.2024.100384>

This work, or part of it, has been presented to the following scientific conferences:

- International conference of the Italian Chemistry Society “Merk Young Chemists’ Symposium, MYCS 2022” (Rimini, November 21-23, 2022) – *Investigation of TiO₂ and ZnO-based photocatalysts to provide self-cleaning properties and inhibit microorganisms’ growth on traditional Venetian marmorino plasters*, A. Campostrini, D. Zanardo S. Manente, E. Ghedini, M. Signoretto, and F. Menegazzo
- International conference on analytical techniques in art and cultural heritage, TECHNART 2023 (Lisbon, May 07-12, 2023) – *High-Tech multifunctional marmorino plaster with antimicrobial and self-cleaning properties*, A. Campostrini, S. Manente, A. Di Michele, E. Ghedini, M. Signoretto, and F. Menegazzo
- 6th International Conference on Innovation in Art Research and Technology, InART2024 (Oslo, June 4-7, 2024) – *Preserving Venice's Heritage: An Innovative Safeguarding Approach Against Biodeterioration*, A. Campostrini, S. Manente, A. Di Michele, E. Ghedini, M. Signoretto, and F. Menegazzo

Part of this work was also published in **Intrecci, international journal of architectural conservation and restoration**, anno I (2022) n. 1, ISSN 2974-8577, pag. 35-49, *Marmorino e Fotocatalizzatori: un incontro tra tradizione e innovazione – Marmorino and photocatalysts: a meeting between tradition and innovation*, DOI: 10.57639/SIRA.INTR0104, L. Scappin, A. Campostrini, D. Zanardo, E. Ghedini, M. Signoretto, G. Berto, and F. Menegazzo.

Part of this work is going to be published also in **Ambiente e patrimonio a rischio: sfide tra conservazione e cambiamento**, Anteferma Ed., iNEST - Spoke 4 - Vol. 2.3, Chapter 5, *Innovazione e tradizione: i fotocatalizzatori per gli intonaci veneziani*, F. Menegazzo, A. Campostrini, S. Manente, E. Ghedini, A. Di Michele, and M. Signoretto.



Enhancing Venetian traditional marmorino with TiO₂ and ZnO for antimicrobial protection – A case study

Andrea Campostrini¹, Sabrina Manente¹, Elena Ghedini^{1*}, Alessandro Di Michele², and Federica Menegazzo¹

(1) Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30170 Venice, Italy

(2) Department of Physics and Geology, University of Perugia, Via A. Pascoli, 06123 Perugia, Italy

corresponding author: gelena@unive.it

Abstract

Venetian marmorino is a traditional plaster that has been used since the 15th century, in several palaces in Venice and ancient villas throughout the Veneto region. While this material has considerable durability, it remains susceptible to various surface degradation processes. An effective means to mitigate such events is through the application of photocatalytic coatings. These coatings employ just oxygen from the atmosphere and light as energy source to degrade both organic and inorganic environmental pollutants, confer self-cleaning properties, and reduce bacterial attacks or fungal growth.

In this work titania (TiO₂) and zinc oxide (ZnO) were incorporated into the marmorino, both as top coating and within the mortar itself. The biocidal efficacy of these additives was assessed through optical microscopic observations of fungal colonies grown on the marmorino mock-ups with differing compositions or finish layers. Two Fungi *Penicillium italicum* and *Cladosporium sphaerospermum* were selected as reference microorganisms attacking wall materials in Venice. Hence, a more detailed analysis utilizing electron microscopy (SEM) was conducted to confirm the biocidal properties.

The experimental results demonstrate that the development of a high-tech multifunctional marmorino capable not only of reducing environmental pollutants but of preventing surface degradation and inhibiting fungal growth. This inhibition leads to a reduction in biodegradation, resulting in a diminished ability to take root and lack of spore development.

Keywords: *Marmorino* plasters, photocatalysts, TiO₂, ZnO, biocidal properties, Venetian Palaces

1. Introduction

Venetian *marmorino* is a traditional plaster widely employed as wall coating in several ancient buildings in Venice and in the Veneto region [2,180–183]. *Marmorino* has been employed both as plaster for new constructions and as material for restoration interventions of ancient ones [184–186].

The stratigraphy of *marmorino* typically comprises multiple thin layers of sand-based plaster applied in a *fresco su fresco* technique without waiting for the lime putty ($\text{Ca}(\text{OH})_2$) to fully carbonate. Notably, the final mortar layer incorporates fine-grained marble powder (CaCO_3) alongside lime putty [187]. Traditionally, several natural substances such as soap, lime water, or natural oils were applied as finish layer, either before the carbonation process or afterward, to provide both protection and desired optical properties [188,189].

Despite its intrinsic protective features, *marmorino* surfaces exposed to modern pollutants face greater environmental challenges [12,190–198]. Volatile Organic Compound (VOC) pollutants, often deposited as thin layers not only contribute to chemical and physical degradation but also facilitate biodegradation processes [9,199]. Biodegradation includes all the undesirable changes in mater properties accelerated by microbial activity across various taxonomic groups [200,201].

The combination of environmental factors (*i.e.*, moisture, light, temperature, surface deposits, ventilation, capillary rise, and pollutants) eases the deterioration of the walls and the increase of the surface porosity [33,202,203]. This makes the surface more bio-receptive, encouraging the ecological flow growth of bacterial and fungal consortia, plant-like organisms, and even herbal to arbustive plants [13,23,31]. This heightened susceptibility to biodeterioration can foster the establishment of an ecosystem on stone surfaces. Biodeteriogens act on surfaces through different, but all dangerous, mechanisms [14,15,22,204].

While several approaches exist to remove such deposits and restore the surface, they often involve products applied after damage has occurred [205–207]. An appealing alternative is the use of materials capable of preventing deposit formation or chemically removing them as they begin to form [115]. Photocatalytic materials are well-suited for this purpose, as they can trigger beneficial chemical reactions employing atmospheric oxygen and sunlight energy. Photocatalysts can induce reactions that interact with common air pollutants, degrade surface degradation compounds, and even mitigate the growth of biological organisms that deposit, adhere, and proliferate on the wall surface [208].

Titanium dioxide (TiO₂) is the most employed photocatalytic material in various applications [209–211]. Consequently, it is also being investigated as component in photoactive wall coatings, either for its biocidal effect to inhibit biodeterioration or for its self-bleaching effect to prevent the formation of stains due to pollution or vandalism [212,213]. Zinc oxide (ZnO) is another semiconductor employed for its self-bleaching properties. Moreover, it is well-known for its potent biocidal properties in various applications [138,214]. As previously demonstrated by the authors [215], appropriate TiO₂ and ZnO photocatalysts do not cause color variation when applied to *marmorino* surfaces, thereby preserving the aesthetic integrity of the stone – an essential characteristic when dealing with Cultural Heritage materials [212]. Furthermore, they both demonstrated great self-cleaning surface characteristics, capable of degrading model stain compounds [215,216], such as methylene blue or methylene red, as well as real-case stain compounds like blue pen, various markers, or food residues [217]. Previous results also demonstrated that the combination of photocatalytic materials with Aleppo’s natural soap in the finish layer exhibited even higher efficacy [215]. This last aspect could also avoid the detachment of the photocatalysts from the surface, leading to a more stable material.

For these reasons, both TiO₂ and ZnO are employed and investigated for their antimicrobial activity to combat fungal proliferation even on stone surfaces [218–220]. Literature studies suggest that these materials can be applied primarily as a superficial coating on different types of stone surfaces [137,221–224] or even incorporated into the mortar in the case of concrete or plasters [225,226]. In the former case, the metal-based oxides primarily exploit photocatalytic phenomena, which cannot be fully used when incorporating the photocatalyst as a component of the mortar matrix. Nevertheless, studies report that employing metal oxides (*e.g.*, ZnO, CaO, MgO) within the material formulation, the particles still inhibit dangerous microorganisms’ growth [225]. Therefore, the antimicrobial phenomenon cannot exclusively be attributed to the photocatalytic actions of ZnO but rather to failures in the microorganisms’ proliferation and reproduction processes induced by the particle composition. The active oxygen species generated by the ZnO particles is considered the primary mechanism of antibacterial activity [227–231].

2. Research aim

In this work, our objective was to modify the formulation of Venetian *marmorino* plaster by incorporating TiO₂ and ZnO, aiming to confer inhibition properties against microorganisms’

growth. Titanium-based materials were employed for surface coating, while ZnO was integrated into the *marmorino* plaster formulation. Optical and electron microscopy (SEM) were employed to establish the inhibition action of ZnO and TiO₂ on fungal cultures inoculated on the surface of the *marmorino* mock-ups.

Previous works extensively characterized the photocatalytic plaster mock-ups and studied their activity towards self-cleaning features [215,217], while this study focuses on investigating the impact of added photocatalysts on the antimicrobial properties of these materials. To the best of the authors' knowledge, there are no specific studies regarding this aspect, applied to plaster-like stone materials.

3. Materials and methods

3.1 Materials

To synthesize the photocatalysts the reagents were used as-received: TiOSO₄ · xH₂O · yH₂SO₄, Zn(NO₃)₂ · 6H₂O, titanium (IV) isopropoxide, Na₂CO₃NH₃ aqueous solution, and isopropanol were purchased from Sigma Aldrich. The commercial photocatalysts employed were TiO₂ P25 from Evonik (Essen, Germany) and ZnO from CARLO ERBA Reagents S.r.l. (Milan, Italy). For what concerns the *marmorino* mock-ups preparation, the quicklime (CaO) and the marble powders (*impalpabile* and *spolverone* – CaCO₃) were provided by Uni.S.Ve. company (Venice, Italy).

For the microbiological growth, maintenance, and testing, the reagents were used as received: Sabouraud Dextrose Agar (SDA), Malt Extract Agar (MEA), and Nutrient Broth (NB) were purchased from Biolife (Milan, Italy), whereas the 5% saline solution (NaCl) was purchased from Sigma Aldrich.

Finally, for what concerns the SEM measurements samples preparation, Conducting Carbon Cement (Leit-C) was employed.

3.2 Methods

3.2.1 Photocatalysts preparation

The photocatalysts (TiO₂, ZnO, ZnO-TiO₂ composite) were prepared following a procedure previously reported by the authors [215,232,233] and labeled Ti-LAB, Zn-LAB, and ZnTi-LAB respectively. Briefly, a 1.2 M aqueous solution of TiOSO₄ precursor was added dropwise to 200 mL of H₂O, while maintaining the pH at a constant value of 5.5 using 9 M aqueous NH₃. Afterward, the suspension was aged at 60°C for 20 hours, washed on a Gooch filter, and air-

dried at 110°C for 18 hours. The resulting material was hence annealed at 400°C for 4 hours in an airflow of 30 mL/min and then labeled as Ti-LAB.

Similarly, laboratory-made ZnO was prepared using a 1.1 M aqueous solution of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 1.0 M Na_2CO_3 aqueous solution as the precipitating agent, while maintaining a pH of 9 during the precipitation process. This sample was labeled as Zn-LAB.

Finally, a zinc oxide-titanium dioxide composite material, containing a theoretical amount of 10% wt of TiO_2 , was prepared via wetness impregnation. This involved dissolving a proper amount of titanium precursor (Ti(IV) isopropoxide) in isopropanol. The impregnated sample was then air-dried at 110°C for 18 hours and subsequently annealed at 400°C for 4 hours in an airflow of 30 mL/min. In this way a core-shell like structure was obtained. This sample was labeled ZnTi-LAB.

The two benchmark products, used as references, were TiO_2 P25 from Evonik and ZnO from Carlo Erba, and were respectively labeled Ti-COM and Zn-COM.

In Table 1 are briefly reported the results previously obtained by the authors in the photocatalytic materials characterizations:

Table 1: photocatalytic materials characteristics

Sample	Crystallite size (nm)	Crystalline phase	BET surface area (m^2/g)
Ti-COM	20	anatase, rutile	52
Ti-LAB	9	anatase	120
Zn-COM	158	wurzite	5
Zn-LAB	15	wurzite	42
ZnTi-LAB	17	wurzite, anatase	33

3.2.2 Marmorino mock-ups preparation

The *marmorino* plaster mock-ups were prepared following the traditional Venetian recipe provided by the Uni.S.Ve. s.r.l. restoration company [217], applying different kinds of finish layers: no finish layer, traditional (Aleppo soap), Aleppo soap + TiO_2 .

Briefly, a laboratory-made Venetian *marmorino* was applied to a 20×20 cm *cotto* brick. Initially, an underlayer comprising coarse sand and lime putty in a 1:3 weight ratio was applied to a wet brick surface. Afterward, two layers of *marmorino* were applied. These layers consisted of *impalpabile*, a finely powdered marble (0.07 mm), *spolverone*, a coarser marble powder (1-3 mm), and lime putty in a 2:1:1 weight ratio. A third layer of *marmorino* was then applied, composed by combining 16 g of the aforementioned calcium-based mixture with either 4 g of photocatalyst or 4 g of *impalpabile*.

For what concerns the finish layers, the traditional Aleppo soap was compared with an innovative one characterized by Aleppo Soap enriched with TiO₂ NPs, namely Evonik P25 (Ti-COM). A comparison with a pristine, not treated sample was also carried out. The preparation of the traditional finish layer, 50 mg of Aleppo soap were diluted with 2.5 mL of H₂O, then placed in an ultrasonic bath for 30 minutes at $T = 50^{\circ}\text{C}$. They were subsequently collected and applied with a brush to the dedicated section. For the section reserved for the finish with Aleppo soap and Ti-COM, 50 mg of Aleppo soap were combined with 25 mg of Ti-COM, diluted in 2.5 mL of H₂O, and the same procedure as the previous application was followed. Finally, the mock-ups were left at RT for 20 days to dry and carbonate.

3.2.3 Microorganisms sampling campaign

To select the microorganisms to be used, a sampling campaign was conducted on real-case *marmorino* plasters found on the external walls of some palaces dating back approx. 1500-1700 in Venice (Figure 1S). The sampling process involved non-invasive methods using *Fungi-Tape*TM adhesive tape (Fisher Scientific) and sterile cotton swabs.

The *Fungi-Tape*TM samplings were examined under the optical microscope, while the cotton swabs were plated on Petri dishes with different cultivation mediums (SDA, MEA, NB, MEA+ NaCl 5%). The Petri dishes were then incubated at $21.5 \pm 2^{\circ}\text{C}$ for 7 days to allow initial mycelium development and monitored for up to 20 days for fungal colony growth until maturation (active and abundant sporulation).

At this stage, the identified Fungi, *Penicillium italicum* and *Cladosporium sphaerospermum*, were selected for the study based on their growth and morphology [234]. These Fungi were isolated and allowed to grow in pure axenic culture using MEA cultivation medium for 5 days in an incubator at $21.5 \pm 2^{\circ}\text{C}$, until optimal inoculation growth levels were reached.

3.2.4 Marmorino antimicrobial activity test

Based on literature [214,235], a system was developed to study the antimicrobial properties of the *marmorino* mock-ups. These mock-ups were tested by applying a fungi inoculum, cut using a microbiology cork borer, onto their surfaces, and observing the resulting fungal growth. The growth test spanned a month period, maintaining a humid environment and a controlled temperature of $23 \pm 2^{\circ}\text{C}$ to ease the Fungi spreading. The mock-ups were kept in a humid environment undergoing to natural nictemeral cycles, to better reproduce the influence of environmental factors.

During the growth test, fungal growth was evaluated through a photographic campaign of the plaster mock-ups. After the test concluded, more detailed microscope analyses were conducted. Initially, the mock-up surfaces were sampled using *Fungi-Tape*TM, which were then affixed onto glass slides for microscopy observation of the fungal growth state. The monitoring of fungal growth involved observations using a stereoscope Stemi SV 6 (ZEISS, Germany) and an optical microscope Axioplan (ZEISS, Germany) at different magnifications. Instantaneous images were acquired using an incorporated digital camera AxioCam ERc5s (ZEISS, Germany) and processed using the ZEN 2011 software (ZEISS, Germany).

Table 2 provides instructions for identifying the twelve samples. The first letter of the label (A, B, C, or D) denotes the *marmorino* plaster formulation used for making the tile, the number (1, 2, or 3) specifies the finish layer employed, and the last character (p or c) indicates the fungal genera inoculated onto the surface.

Table 2: Mock-ups and samples descriptions

Last <i>marmorino</i> plaster layer	Finish layer	Sample with <i>Penicillium italicum</i>	Sample with <i>Cladosporium sphaerospermum</i>
Marble powder + lime putty + <i>impalpabile</i> marble powder	No finish layer	A1p	A1c
	Traditional (Aleppo soap)	A2p	A2c
	Aleppo soap + TiO ₂	A3p	A3c
Marble powder + lime putty + Zn-COM	No finish layer	B1p	B1c
	Traditional (Aleppo soap)	B2p	B2c
	Aleppo soap + TiO ₂	B3p	B3c
Marble powder + lime putty + Zn-LAB	No finish layer	C1p	C1c
	Traditional (Aleppo soap)	C2p	C2c
	Aleppo soap + TiO ₂	C3p	C3c
Marble powder + lime putty + ZnTi-LAB	No finish layer	D1p	D1c
	Traditional (Aleppo soap)	D2p	D2c
	Aleppo soap + TiO ₂	D3p	D3c

For Scanning Electron Microscopy (SEM) analyses, a small sample was cut from the mock-up surface and affixed to the stub (sample holder) using conducting carbon cement. After drying, the sample was metalized with Chromium, and measurements were conducted. The SEM images were acquired using an FE-SEM (Field Emission Scanning Electron Microscopy) FEG LEO 1525 ZEISS, equipped with an in-lens detector for secondary electrons and operated at an accelerating voltage of 5 kV.

3.2.5 Photographs and Micrographs Interpretation

The interpretation of the naked eye observation was summarized considering the general development of the fungal colonization. Four levels were set, and each sample was labeled with “extensively distributed”, “distributed”, “poorly distributed”, or “not appreciable” depending on how the Fungi were spread over the surface. It is important to underline that when the sample was labeled with “not appreciable” it could be related to the high efficiency of the antimicrobial system as well as to the limit of this kind of investigation.

Likewise, for the microscopy observation of the glass slides the following classification was assigned to the samples: considering both the presence of the conidia and the hyphae it was possible to define once again the fungal growth. Hence a rank system of five steps was generated: a Fungi with “high fungal growth” is when no obstacle in the fungal growth was observed; a “good fungal growth” was defined when both conidia and hyphal structures and at least one of them inside the matrix of the substrate can be seen; an “only superficial fungal growth” was assigned to the samples where the colonization was visible over the whole sample, but there was not penetration into the substrate; then a “not propagated/inhibited fungal growth” was given to the samples when the colonization was present only around the inoculation point or when a block on the development of the Fungi was identified; and finally a “not rooted fungal growth” was assigned when the fungal growth was totally inhibited.

As for the SEM images, the fungal colonization was evaluated through the observation of the spores and the hyphae’s characteristics (*i.e.*, amount, size, freshness, and distribution): *massive*, when pervious spores and hyphae of normal diameter/thickness were present, resulting in the mycelium being in an excellent vegetative state; *good*, when both pervious and dehydrated spores and hyphae of sufficient size were present; *sufficient*, when mostly dehydrated spores and hyphae were present, although points of good rooting remained clearly visible in the mycelium; *regressed*, when only dehydrated, agglomerated, imploded spores and thin, dehydrated hyphae were visible.

4. Results

4.1 Fungi sampling campaign

From the sampling campaign conducted on the external walls of ancient palaces in the city of Venice presenting *marmorino* plaster in a discreet conservation state, it was possible to detect several Fungi species. *Penicillium italicum* (Fungi, Ascomycota, Eurotiales, Trichocomaceae) and *Cladosporium sphaerospermum* (Fungi Ascomycetes, Dothideomycetes, Cladosporiales) were then selected for this study, and their specific features are shown in Figures 2S and Figure 3S.

4.2 Photographic campaign and naked-eye observation

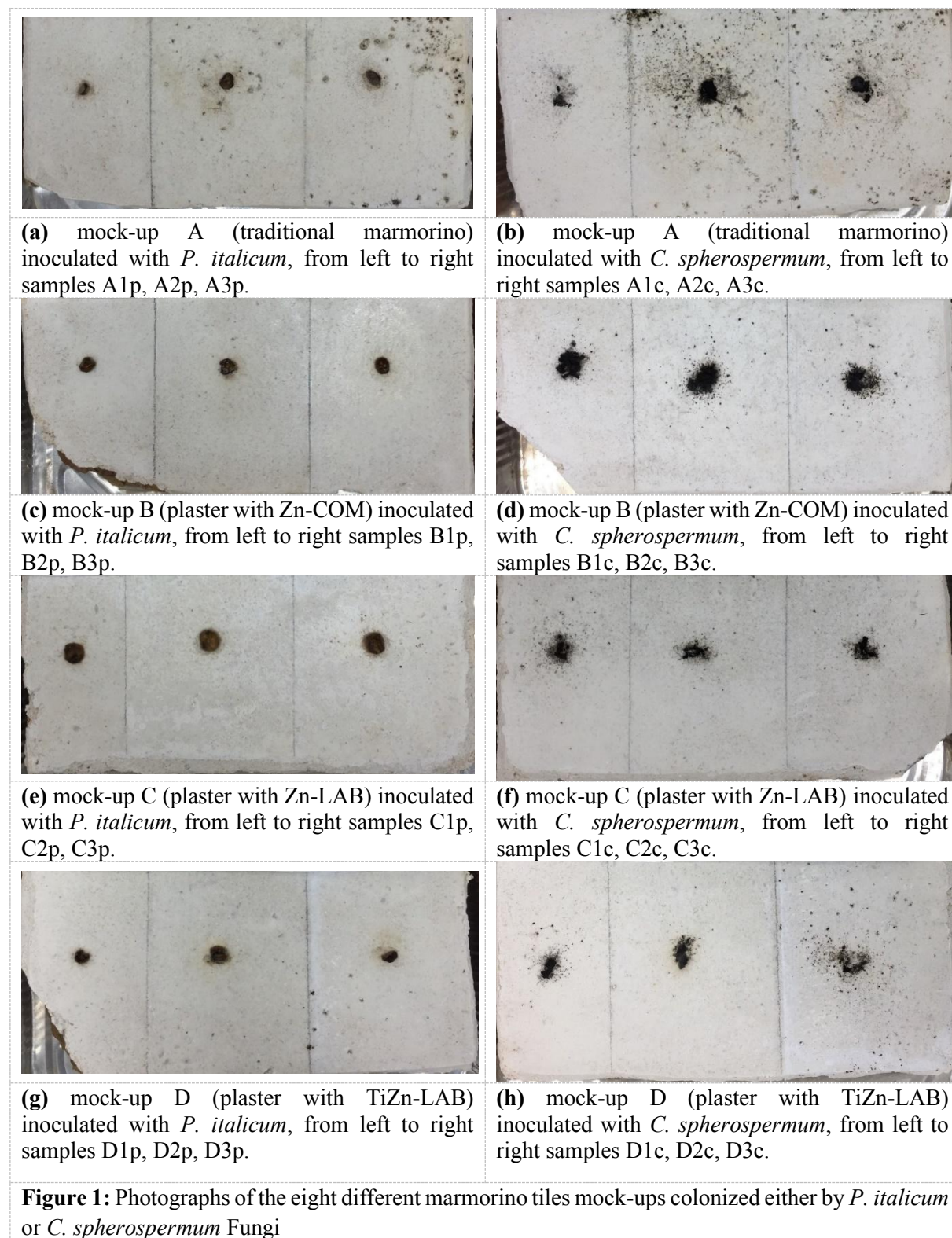
The results presented here pertain to pictures captured 30 days after the deposition of the Fungi inocula on the *marmorino* mock-ups, compared to those taken in the initial days following inoculation. The traditional *marmorino* tiles (mock-ups A) exhibited extensive microbiological colonization, noticeable even to the naked eye for both Fungi (Figure 1 – a, b). Samples A2p, A3p, A2c, and A3c displayed widespread growth across the samples, indicating unhindered growth and diffusion of the microorganisms on the traditional substrate. In contrast, samples A1p and A1c exhibited less visible distributed fungal growth, likely due to the different bio-receptivity of the substrate. The more porous nature of the substrate may have facilitated the proximity of Fungi growth just to the inoculum area.

Furthermore, it should be noted that *Penicillium* sp. is greatly affected by pH, requiring an acidic environment between 3.0 and 4.5 for optimal development, although it is capable of taking root even in seemingly more adverse conditions. Conversely, *Cladosporium*, while tolerating acidic environments up to pH 4.4, needs an environment around pH 6.0 for optimal growth [236].

Similar trends were observed for mock-ups B and C. Both Fungi exhibited distinct interactions with the substrate. While *P. italicum* did not exhibit any visible growth on the tiles in any of the different finish layer options, *C. sphaerospermum* showed less dispersed colonization of the plaster in the case of Zn-LAB compared to the Zn-COM (Figure 1 – c, d, e, f).

Finally, in the Ti-Zn-based tiles (mock-ups D), reduced growth of both *P. italicum* and *C. sphaerospermum* on the surfaces was observed. Additionally, the inoculum disk in the samples D3p and D3c (those with TiO₂ as finish layer) appeared to shrink or become not visible anymore (Figure 1 – g, h).

These results are preliminary insights, but further analysis through optic and electronic microscopy is necessary to gain a more complete understanding of the interaction between the microorganisms and the different *marmorino* tiles.



4.3 Microscope investigation of the glass slides

Figure 2 reports microscope investigations of glass slides from samples inoculated with the *Penicillium italicum* Fungus. In sample A1p, the presence of *P. italicum*'s conidia was evident in an ideal growth situation, although slightly smaller, often concatenated and intact; hyphae of the Fungus were also observed, but no conidiophore structures were present. Some contamination by external microorganisms, such as a cyanobacteria, likely originated from the environment where the tiles were stored, was noted.

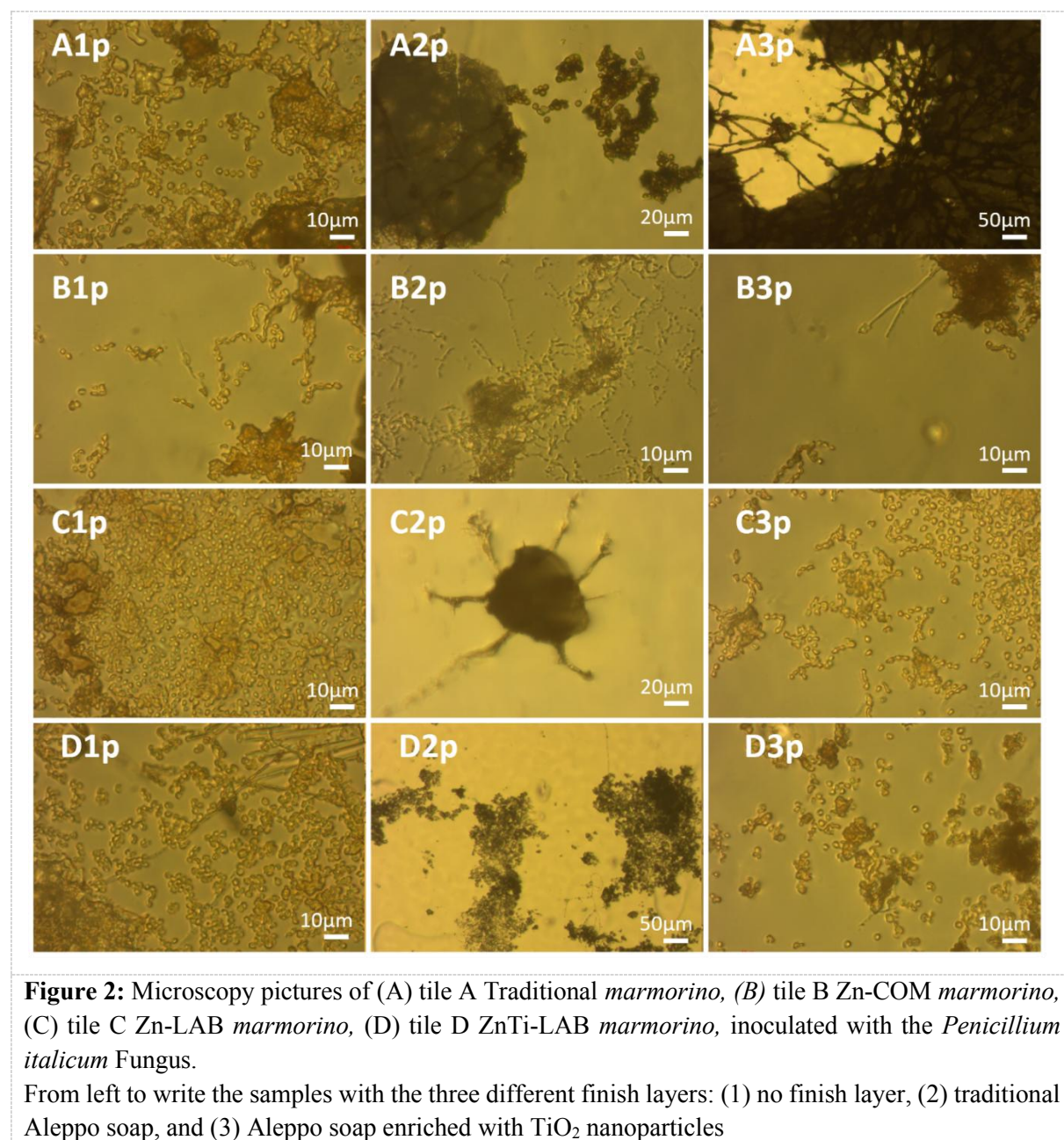
Sample A2p exhibited a Fungus in a good vegetative condition, with hyphae penetrating the *marmorino* matrix and intact chains of conidia visible. Additionally, biological consortium structures indicated the formation of a biofilm on the surface. Sample A3p showed a comparable situation to sample A2p, with slightly rounder and smaller conidia, and the presence of dry phialide structure with concatenated conidia nearby.

Regarding tile B inoculated with *P. italicum*, sample B1p revealed free conidia, sometimes still concatenated and dispersed in the plaster matrix, along with some hyphae exhibiting slight globular morphology, suggesting possible fungal growth deformation. Sample B2p also displayed a globular swelling structure and thin deformed hyphae penetrating the plaster matrix, with multiple inoculum points. In general, it was possible to notice very developed colonies in germination state with a young mycelium developed inside the matrix, with the uncommon presence of chlamydospores-like structures (*i.e.* thick-walled terminal or intercalary asexual resting propagule mostly for long-time survival) but already associated with *P. italicum* from some authors [237]; regarding the conidia, they were small and round, and concentrated near the inoculum area; it was also noticed an opaque surface, which might be linked to the presence of soap in the finish layer.

In sample B3p, filiform structures, likely underdeveloped hyphae, were observed. Performing an overview of the inoculum point and its vicinity, the conidia and the present hyphae were few, around 10-15% compared to the previous glass slides; the observed conidia were still concatenated, but only present around the inoculum area, meaning that there was no growth, *i.e.* it could be potentially inhibited.

Sample C1p showed few fresh and some dry dehydrated hyphae, numerous conidia still concatenated covering the substrate, and cell structures resembling a flat coenobium. Sample C2p exhibited a small structured mycelium with hyphae penetrating the plaster matrix. Sample C3p displayed smaller chained spores only around the inoculation point.

In sample D1p, only the footprint of the mycelium structure and some dendriform structures (probably hyphae) were visible at the inoculation point, with limited fungal growth outside this area. A comparable scenario was observed in sample D2p, with vegetative body and sporulation structures present, but absent from the center of the inoculation point; in other parts of the glass slide, it was instead possible to notice conidiophores and thin hyphae, also inside the plaster structure. Sample D3p exhibited a thin conspicuous hyphal system confined to the surface and some dried conidiophores.

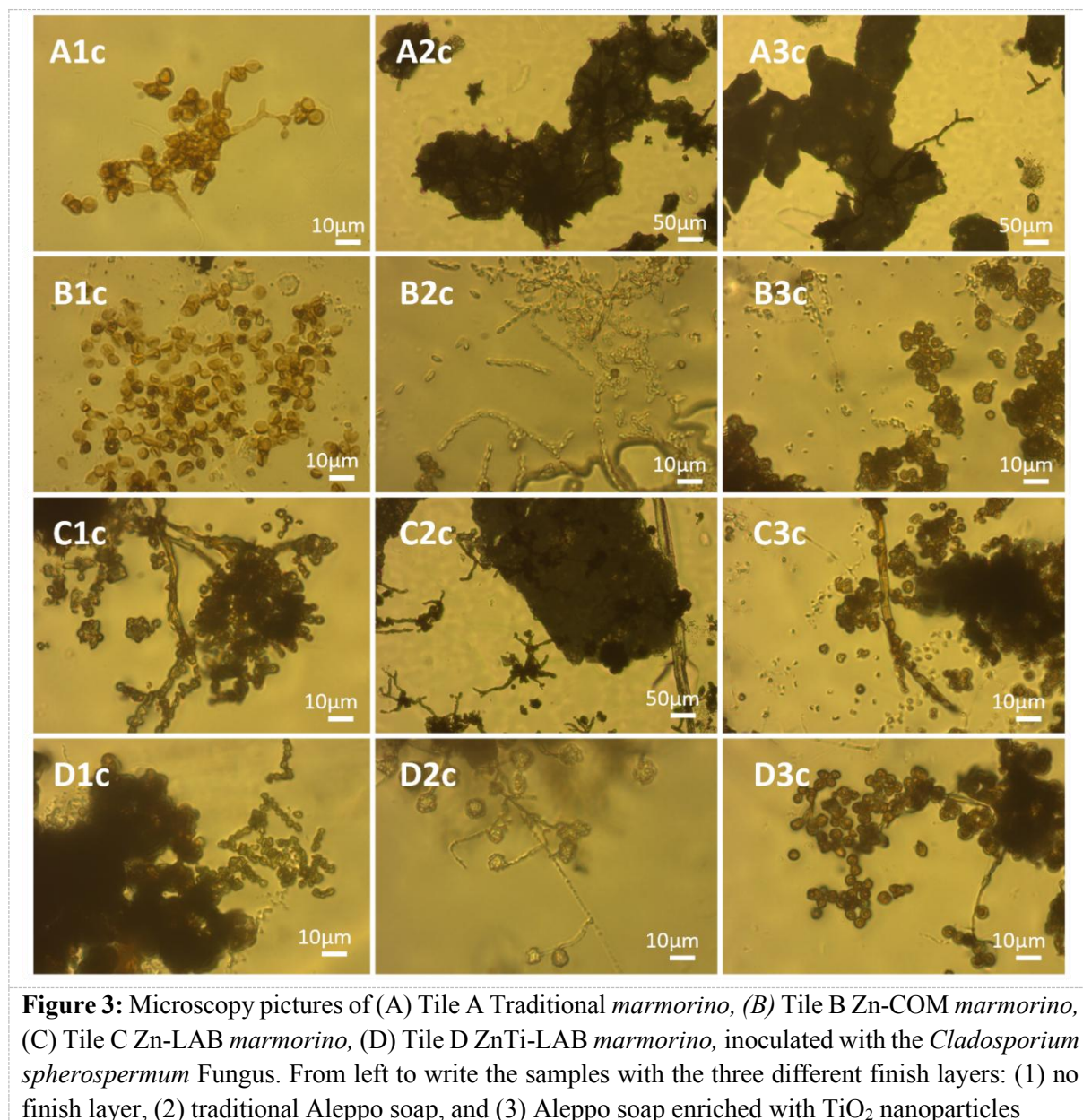


Regarding tile A inoculated with *Cladosporium sphaerospermum* (Figure 3), in sample A1c the Fungus exhibited a conspicuous vegetative thallus, spreading across the glass slide, accompanied by numerous hyphae and conidia. Sample A2c displayed pieces of compacted mycelium once again, but smaller scattered conidia were absent. The Fungus managed to penetrate the plaster substrate, resulting in a higher presence of *marmorino* flakes adhered to the *Fungi-Tape*TM. Sample A3c showcased an articulated-grained vegetative mycelium, with very few conidia present only in the inoculation point. The Fungus's growth appeared hindered, as the conidia seemed to have dried before sporulation.

In Sample B1c, a significant presence of small, mainly dehydrated conidia, and swollen broken hyphal cells was observed. Sample B2c exhibited fewer conidia than B1c, but more hyphae; the conidia were concentrated around the inoculation point, and hyphal systems were confined to this area, indicating limited propagation. A similar scenario was noted in sample B3c, with a compact but underdeveloped inoculum, as the Fungus did not propagate beyond the inoculation area. The conidia were dehydrated and unevenly arranged, and the mycelium was superficial and with thin hyphae.

In sample C1c, the Fungus presented subspherical scattered conidia, many of which were imploded, but no mycelium was visible. Conversely, in sample C2c, it appeared that the Fungus had initially taken root but then receded from the substrate, as evidenced by imploded and attached conidia, very thin hyphae, and a mycelium with short hyphal cells. Sample C3c showed no apparent root formation by the Fungus, since there is no mycelium present, with only a few thick hyphae and singularly scattered imploded small conidia, although fewer than in C2c.

Sample D1c displayed a densely compacted mycelium, but the Fungus was limited to the surface, with small and dehydrated conidia and hyphae exhibiting shorter cells. In sample D2c, the Fungus appeared mostly dehydrated, with interrupted growth phases of the mycelium, and sporulating elements with very small conidia were also visible. Finally, in sample D3c, a dehydrated Fungus with a developed mycelium was observed, primarily with hyphal forms over the matrix surface but not within, along with collapsed or imploded small conidia showing evident cavities. Some residual network structures, likely residual conidiophores, were also noted.



4.4 Scanning Electron Microscopy analyses

Scanning Electron Microscopy (SEM) proved to be a valuable technique for detailed examination of fungal growth across the various samples. Particular attention was focused towards assessing the presence and distribution of spores and hyphae on the surface, their morphological characteristics, and their condition.

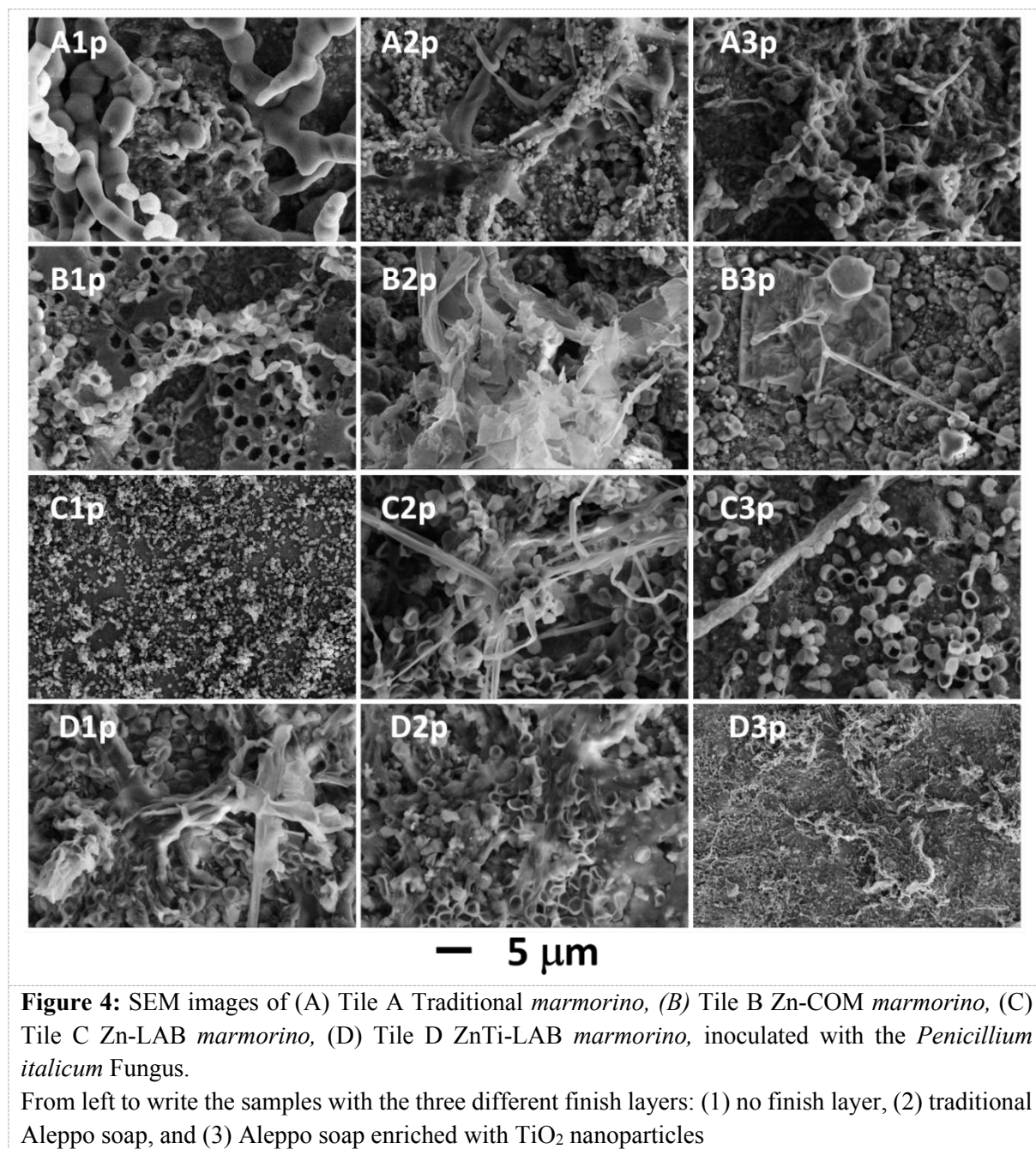
Regarding the *Penicillium italicum* Fungus (Figure 4), sample A1p exhibits thick globular phialides and a high concentration of spores distributed on the surface. In sample A2p, hyphae were observed scattered across the surface along with some dried spores. Additionally, irregular grains were noted over the *P. italicum* Fungus, potentially indicating substrate alteration caused

by the Fungus. Images from sample A3p depicted short and thick hyphae along with some characteristic concatenated spores, most of which appeared dried and exhibited shape deformations.

Sample B1p showcased a surface highly colonized by *P. italicum*, featuring chains of spores and thin, elongated hyphae. Notably, a compact layer with perforation was observed, indicating the presence of *P. italicum* colonies both underneath and atop it. Conversely, in sample B2p, a scarce distribution of spores was observed, except near the inoculation point. Additionally, a transparent-whiteish element resembling an agglomeration of really dried and broken hyphae that create a cloth-like effect, likely due to Aleppo soap finish layer, was noted. Similarly, sample B3p exhibited a modest presence of spores and hyphae, primarily around the inoculation site.

In sample C1p, a significant number of smaller spores and dried hyphae were observed, although with a non-homogenous distribution on the surface, suggesting uneven growth. Sample C2p displayed small, occasionally agglomerated spores, and dried, elongated hyphae. Conversely, in sample C3p, both the number of hyphae and spores were lower, with spores appearing small and sufficiently dry to fracture.

Sample D1p presented a high density of spores homogeneously distributed on the surface, while hyphae were scarce, dried, and fragmented, forming a flake-like structure. In sample D2p, small spores embedded in a mucilaginous-like structure were visible, possibly associated with Aleppo soap agglomerations or a densely compact hyphal network. Finally, sample D3p finally exhibited a small quantity of dry, agglomerated spores along with long, thin, and dried hyphae.



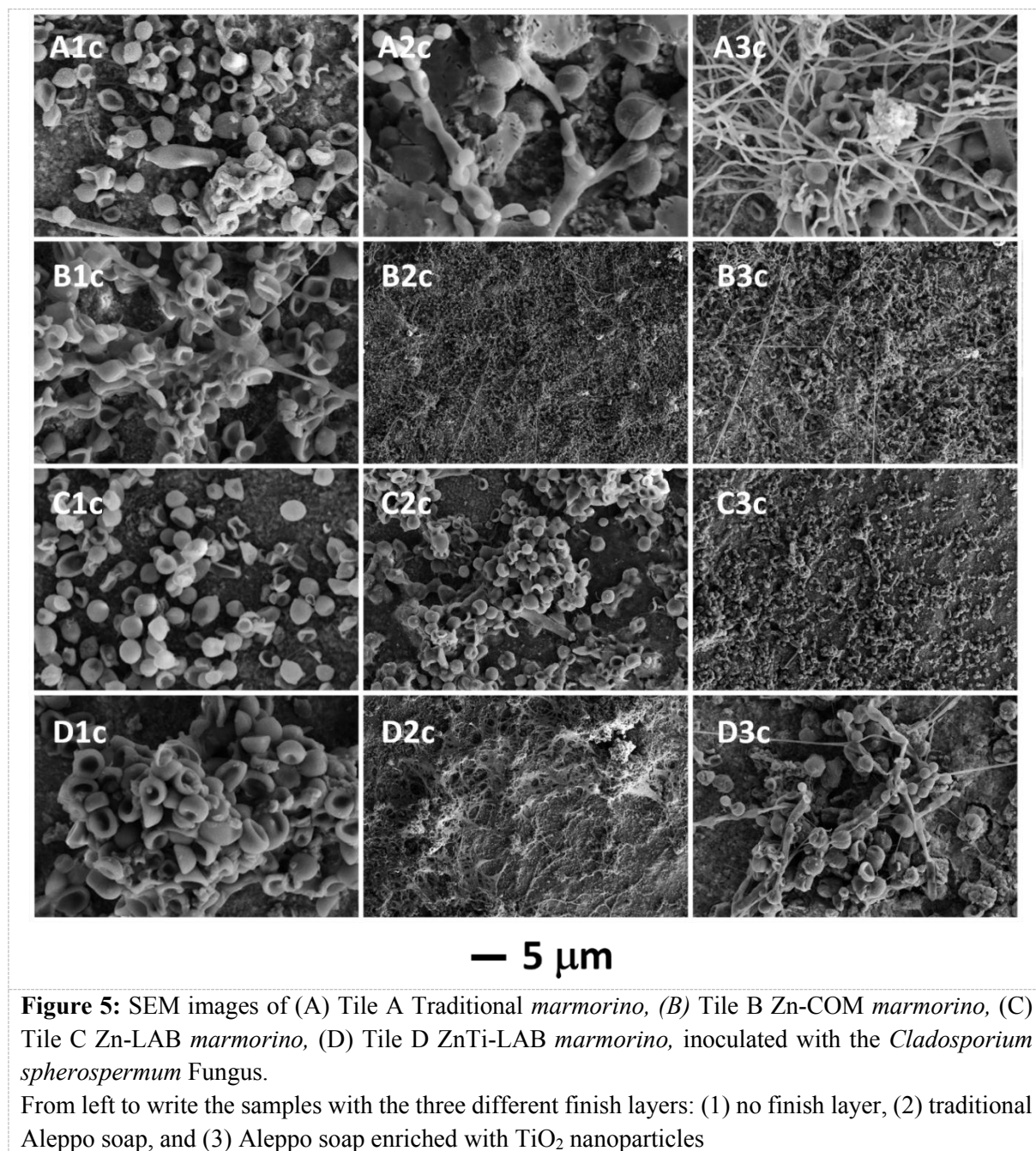
Regarding the *marmorino* mock-up tiles colonized by *Cladosporium sphaerospermum* (Figure 5), a situation similar to that described for the *Penicillium italicum* colonization was observed. On sample A1c, fungal spores of various dimensions and hyphae were visible, distributed over the surface. In sample A2c, ramoconidia were once again observed, along with hyphae. Notably, spherical structures suggestive of spores in germination were present. In sample A3c, there was a generally significant decrease in the number of spores, which appeared deformed, with only

few very thin hyphae visible. It is also interesting to note the presence of crystals probably directly produced by the Fungus [238].

In sample B1c, a high concentration of spores was evident, with surficial hyphae indicating a developed mycelium that subsequently dried up. Sample B2c exhibited conglomerates of dry spores, small spores, and really elongated hyphal systems. In sample B3c, dry spores conglomerates were again present, although smaller in size, with a general decrease in pore size. Long and dry hyphal structures were also visible.

Sample C1c displayed equally dispersed small spores but no evidence of hyphal network. Similarly, in sample C2c, small spores were present without long hyphae, but hyphal cells were observed. Likewise, in sample C3c, the absence of hyphae and the presence of small drier spores were notable.

In sample D1c, conglomerates of imploded, dry, medium-sized spores were unevenly distributed on the surface, with no visible hyphae. In sample D2c, spores of varying dimensions were observed, inserted in a mucilaginous-like structure, as seen in the *P. italicum* inoculation in sample D2p. Finally, in sample D3c, conglomerates of smaller and larger spores were present.



5. Discussion

To better deal with the high amount of results, mainly based on image interpretation, the parameters taken into account were summarized, as described in paragraph 3.2.5, and were used to design Table 3. In this way, it was possible to show the results and efficiently underline the effect of the synergetic combination of photocatalysts in contrasting the biodeterioration on the *marmorino* plaster mock-ups.

Table 3: Summary of the obtained results

Marmorino plaster mixture	Finish layer	Sample	Naked-eye observation (colonization level)	Microscope glass-slide (fungal growth)	SEM images
Traditional	No finish layer	A1p	Not appreciable	High	massive
		A1c	Not appreciable	High	massive
	Traditional (Aleppo soap)	A2p	Extensively distributed	Good	good
		A2c	Extensively distributed	Good	massive
	Aleppo soap + TiO ₂	A3p	Extensively distributed	Good	sufficient
		A3c	Extensively distributed	Inhibited/not propagated	good
Enriched with Zn-COM	No finish layer	B1p	Not appreciable	Good	good
		B1c	Distributed	High	good
	Traditional (Aleppo soap)	B2p	Not appreciable	Good	good
		B2c	Distributed	Inhibited/not propagated	sufficient
	Aleppo soap + TiO ₂	B3p	Not appreciable	Inhibited/not propagated	regressed
		B3c	Distributed	Inhibited/not propagated	regressed
Enriched with Zn-LAB	No finish layer	C1p	Not appreciable	Only superficial	sufficient
		C1c	Poorly distributed	Only superficial	good/sufficient
	Traditional (Aleppo soap)	C2p	Not appreciable	Good	sufficient
		C2c	Poorly distributed	Inhibited/not propagated	good/sufficient
	Aleppo soap + TiO ₂	C3p	Not appreciable	Inhibited/not propagated	regressed
		C3c	Poorly distributed	Not rooted	regressed
Enriched with ZnTi-LAB	No finish layer	D1p	Poorly distributed	Inhibited/not propagated	sufficient
		D1c	Poorly distributed	Only superficial	sufficient
	Traditional (Aleppo soap)	D2p	Poorly distributed	Inhibited/not propagated	regressed
		D2c	Poorly distributed	Inhibited/not propagated	regressed
	Aleppo soap + TiO ₂	D3p	Poorly distributed	Only superficial	sufficient
		D3c	Poorly distributed	Inhibited/not propagated	sufficient/regressed

Investigating the effect of the different photocatalysts, it was observed that the incorporation of ZnO in the plaster mixture, as well as the enrichment with TiO₂ in the finish layer, generally endowed the plaster with antimicrobial features or hindered fungal proliferation in some extent. Specifically, in the case of Zn-base materials in the plaster mixture, better results were obtained with laboratory-made photocatalysts. This correlation can be attributed to the photoactivity of zinc oxides; all the three samples present optoelectronic properties which were correlated with their activity, resulting in an increasing level of activity from Zn-COM, to Zn-LAB, and finally to ZnTi-LAB, as it has been previously demonstrated by the authors [215,217]. In Zn-COM and Zn-LAB *marmorino*, Zn–OH surface groups delay photoactivity by trapping photoexcited charge carriers on the hydroxyl group, thereby diminishing activity by insulation and acting as an electrical barrier for charge transfer. On the other hand, in ZnTi-LAB, the passivation of Zn–OH surface groups and the increased number of heterojunctions enhance the activity by reducing the excited states quench, thereby implementing charge separation [239,240].

Furthermore, it was previously observed that the Zn-COM sample exhibited a Ca(OH)₂-rich surface, which could act as insulating layer, reducing free radical production and thereby limiting a potential way to arm the biological proliferation. The ZnTi-LAB nanomaterial, comprising a zinc oxide core and a titanium dioxide shell, previously demonstrated the most effective self-bleaching activity due to enhanced heterojunction formation among the photoactive phases [215]. For the same reasons, a higher arming interaction with the biological materials could explain the obtained superior results.

In absence of finish layer, the susceptibility to biological attacks is heightened. Applying a finish layer reduces surface bio-receptivity by reducing available pores. However, it is important to note that while surface hydrophobicity increases with the application of soap, applying organic material on an already carbonated plaster surface could serve as metabolic source for microorganisms. This could explain the microbial proliferation in samples treated with Aleppo soap finish layer.

On the other hand, adding TiO₂ NPs to Aleppo soap resulted in improved antimicrobial effects, inhibiting or even preventing microbiological colonization in most cases. This confirms the antimicrobial features of TiO₂, which are mainly attributed to the generation of free radicals that compromise the structural and functional integrity of microorganism cells, ultimately leading to pathogenic cell death or significant reduction in growth and proliferation.

Moreover, the chemical and electrical features of TiO₂ contribute to its antimicrobial action. Its good electric conductivity can damage cell membranes and inhibit metabolic pathways, thereby reducing the ability of microorganisms to obtain energy and nutrients for reproduction, including spore germination and the formation of new fungal colonies [241,242]. This aspect is particularly emphasized in the samples containing metal oxides in both in the plaster mixture and finish layer, creating a hostile environment for microbiological proliferation through a synergetic effect.

6. Conclusions

This study investigated the antimicrobial properties of TiO₂ and ZnO against two Fungi, *Penicillium italicum* and *Cladosporium sphaerospermum*, directly sampled from real-case scenarios. The photocatalysts were incorporated into the formulation of Venetian traditional *marmorino*, with different finish layers, allowing for a comparison between a traditional layer of Aleppo soap and innovative TiO₂-based one.

For both Fungi, the presence of zinc oxides in the plaster mixture helped create an unfavorable environment for fungal proliferation. The best results were obtained with the lab-made ZnTi-LAB. Regarding the finish layer, the addition of TiO₂ to Aleppo soap increased the antimicrobial effect, strengthening the surface's ability to contrast fungal growth. Given the intrinsic features of the two metals oxides, their antimicrobial activity was correlated with their photocatalytic and optoelectrical properties.

In conclusion, this study demonstrated the feasibility of conferring antimicrobial properties to Venetian *marmorino* plaster through the use of photocatalysts, effectively merging historical traditional materials with scientific innovation.

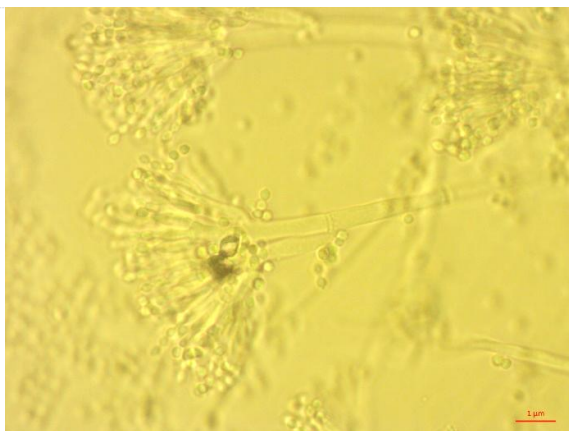
Acknowledgments

The authors would like to thank Mr. Giorgio Berto (Uni.S.Ve. s.r.l., Venice, Italy) and Arch. Luca Scappin (I.U.A.V. University of Venice, Venice, Italy) for their collaboration and technical assistance with the *marmorino* plaster tiles preparation and historical background. Furthermore, we would like to acknowledge Ms. Elisa Lanaro for her practical contributions to this work. Andrea Campostrini finally thanks the “PON Ricerca e Innovazione 2014–2020” and “Fondo per la promozione e lo sviluppo delle politiche del Programma Nazionale per la Ricerca (PNR) 2021–2027” for financing his Ph.D. scholarship.

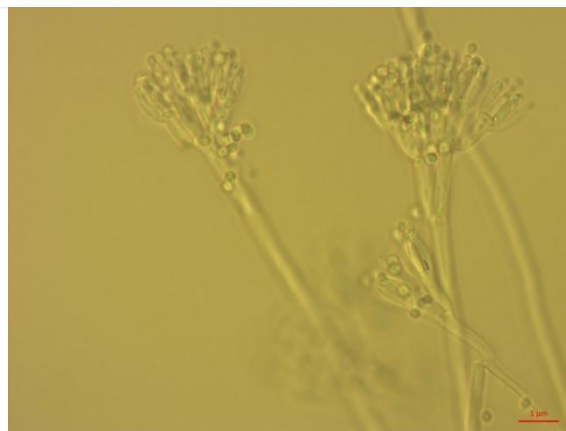
Appendix A – Supporting information



Figure 1S: Venice map with indicated sampling campaign point on the ancient palaces in the areas of *Campo San Tomà*, *Campo San Stin*, *Campo Sant'Aponal*, and *Calle dei Botteri*. On the right microorganisms sampling from the historical marmorino plasters (approx. 1500-1700).

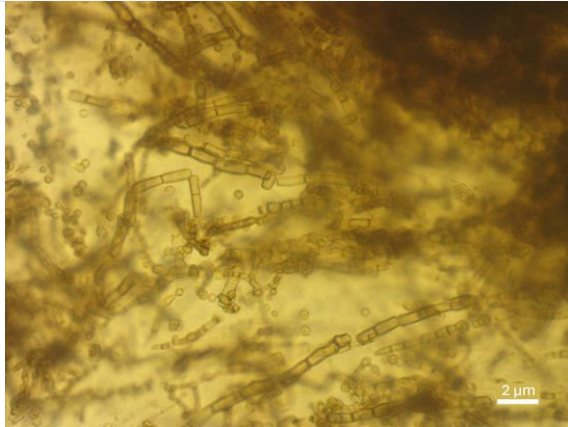


A.

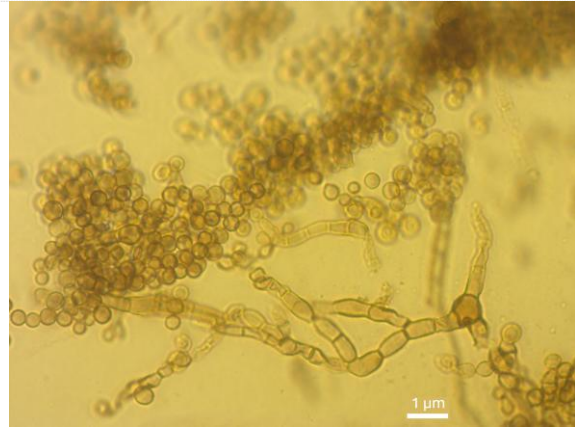


B.

Figures 2SA and 2SB: Hyaline, terverticillate conidiophores of *Penicillium italicum* (Ascomicota, Eurotiales, Trichocomaceae); evident branches; slender phialides with short but distinct necks; subglobose, smooth-walled conidia.



A.



B.

Figures 3SA and 3SB: *Cladosporium sphaerospermum*: conidiophores with simple (*on the left*) or branching (*on the right*) chains of ramoconidia on a peculiar tree-like conformation, both with typical spherical spores. (conidia).

III.

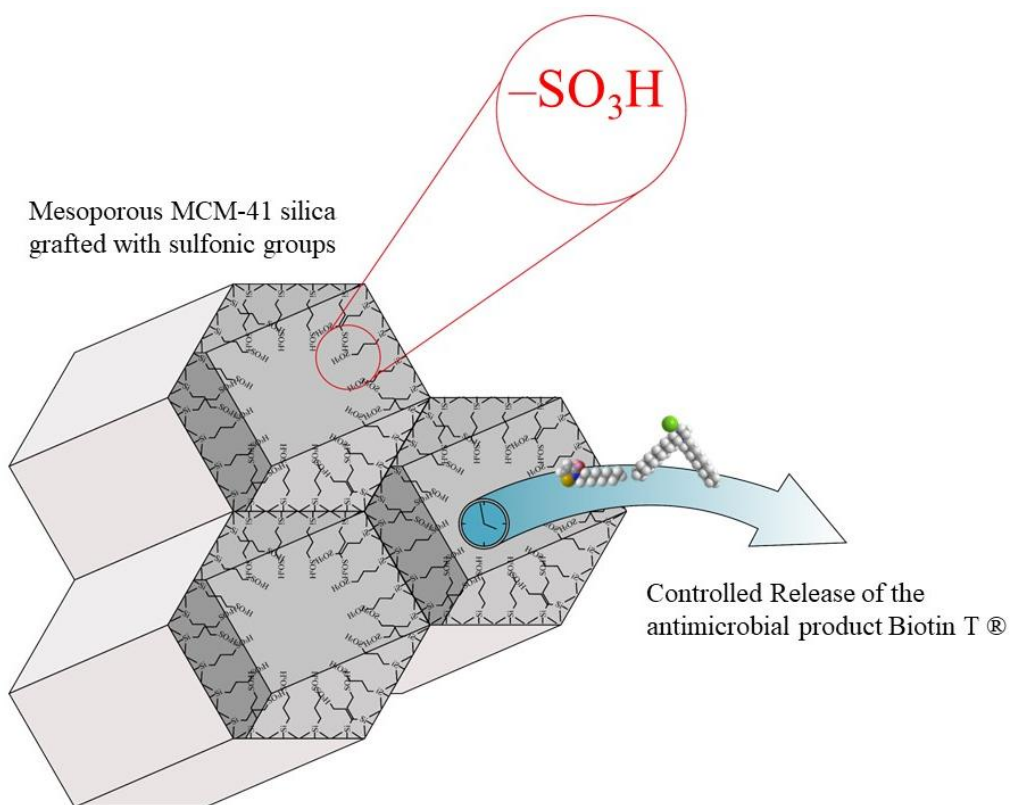
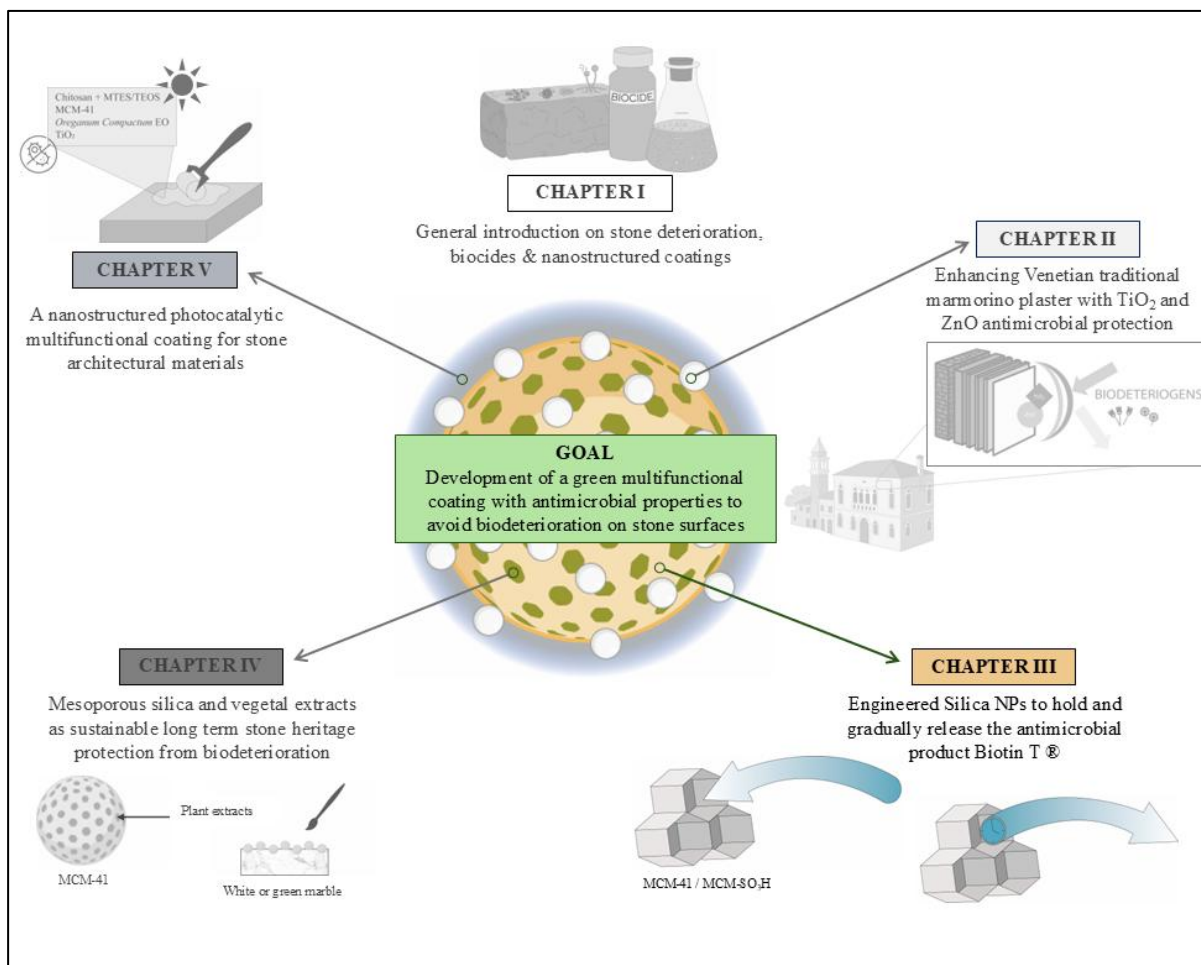
ENGINEERED SILICA NPS TO HOLD AND RELEASE THE ANTIMICROBIAL PRODUCT BIOTIN T ®

Post-print version of the article published in *Next Materials*, Elsevier, **2025**, 7, 100373.

<https://doi.org/10.1016/j.nxmte.2024.100373>

This work, or part of it, has been presented at the following scientific conferences:

- 5th international conference on Nanotechnology based Innovative applications for the Environment, NINE2023 (Venice, June 25-28, 2023) – *Silica nanocontainers antimicrobial coating for sustainable Built Heritage protection*, A. Campostrini, E. Ghedini, M. Signoretto, and F. Menegazzo
- National congress of the Italian National Interuniversity Consortium of Materials Science and Technology “XIV Convegno INSTM” (Cagliari, June 9-12, 2024) – *Functionalized Silica NPs for the controlled release of antimicrobials to protect Cultural Heritage from biodeterioration*, F. Menegazzo, A. Campostrini, T. Botrè, S. Manente, G. Cruciani, A. Giordana, G. Cerrato, M. Signoretto, and E. Ghedini



Engineered silica NPs to hold and release the antimicrobial product Biotin T ®

Andrea Campostrini ¹, Elena Ghedini ^{1,*}, Teresa Botrè ¹, Sabrina Manente ¹, Alessia Giordana ², Giuseppina Cerrato ², Giuseppe Cruciani ³, Alex W. Robertson ⁴, Michela Signoretto ¹, and Federica Menegazzo ¹

¹ *Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy;*

² *Chemistry Department, University of Turin and NIS Interdept. Centre, Via P. Giuria 7, 10125 Turin, Italy;*

³ *Physics and Earth Sciences Department, University of Ferrara, Via Giuseppe Saragat, 1, 44122 Ferrara, Italy;*

⁴ *Department of Physics, University of Warwick, Coventry CV4 7AL, United Kingdom (UK);*

*Correspondence: gelena@unive.it; Tel.: (+39) 041 234 8552

Abstract: While the preservation of culturally significant materials is increasingly recognized as important within the scientific community, it remains closely tied to traditional practices and the empiric knowledge of small handicraft companies. These procedures are usually highly effective, but, especially when dealing with biological degradation phenomena, they are often not updated to the latest scientific innovations and hence do not always consider the impact of their use on the environment. MCM-41 silica-based nanoparticles were employed as nanocontainers to encapsulate and later release the antimicrobial agent Biotin T ®. Specifically, the silica nanoparticles were modified with sulphonic groups to functionalize the silica structure and its interaction with the antimicrobial compound, thereby aiming to regulate its release. Microbiological tests were conducted to determine Biotin T ® antimicrobial activity at low concentrations. The nanomaterials were characterized by N₂ physisorption, XRD, TPO, TG/TDA, Raman IR/ATR spectroscopy, SEM, EDS, and HR-TEM, whereas Biotin T ® release was studied through UV spectroscopy. The functionalized silica nanoparticle-based matrix can encapsulate and gradually release the commercial biocidal. Two of the matrices, MCM-41 and MCM-SO₃H, exhibited different properties after functionalization, with both maintaining the original structure but leading to a higher interaction with the antimicrobial product.

Keywords: silica nanoparticles; sulphonic group; Biotin T; antimicrobial; Cultural Heritage

1. Introduction

Stone cultural heritage materials are often subject to degradation phenomena that are related to the environment they are exposed to. It is hence crucial to protect them and to improve the products employed to reduce the degradation effects [147,191,243].

Biodegradation is considered one of the most dangerous factors that could alter the material conservation state; it has a high aesthetical negative impact and can be one of the first steps of a series of more complex phenomena that could lead to the complete loss of the artwork [13,22,244].

Various methods to remove environmental deposits and restore the original surface are currently employed; however, they primarily imply the application of products only once the material has already been highly colonized and/or damaged [245,246]. Antimicrobial commercial products are hence usually applied for cleaning interventions, without considering their potential preventive power [247]. Numerous products are employed in the cleaning and preventing interventions in the field of conservation and restoration; one of the most commonly used for the treatment of stone surfaces has the commercial name of “Biotin T”, which is normally employed for cleaning interventions at low concentrations, around 1-3% [248,249]. Its composition is based on two components: a quaternary ammonium salt (didecyldimethylammonium chloride – DDAC) and the molecule of 2-octyl-2H-isothiazol-3-one (OIT), reported in Figure 1; their action is respectively related to fungicide and antimicrobial actions [84,250,251].

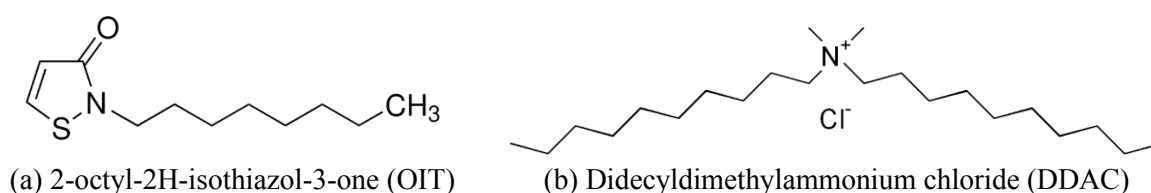


Figure 1. Biotin T ® main active molecules: 2-octyl-2H-isothiazol-3-one (a), and Didecyldimethylammonium chloride (b).

Subsequent to a cleaning intervention, a protective coating should be applied to minimize further attacks on the stone surface, provided that it must not alter the surface properties or chromatic characteristics of the substrate [18,19,148].

Nanostructured protective coatings are typically employed for this aim, exploiting the appealing features of nanomaterials [115,116,134,252,253]. Nanoparticles (NPs), usually metal oxides, have been studied since the 80s for their applications in conservation science. This growing

interest is related to the high chemical and aesthetical compatibility that these materials have. Moreover, they can present lower toxicity towards the environment and the operator [133]. The presence of NPs on the surface could have both a chemical and a physical effect; the NPs' elevated surface area ratio can confer the material with high reactivity, which could lead to favorable interaction with the environment, such as self-cleaning features. From a physical point of view, instead, the NPs' presence could enhance the surface hydrophobicity, creating a micro-roughness that could lead water to form droplets over the surface instead of being absorbed by the material. Other appealing characteristics are related to the possibility of modifying their properties by changing the porosity, size, and shape or grafting them with functional groups. Nanoparticles that are highly functionalizable and present the just reported characteristics are hence widely employed in several fields, such as in the development of hi-tech conveying systems of biologically active molecules.

Embedding active substances in nanosized materials to control their release is a well-known technique, in what is generally referred to as Drug Delivery System (DDS). Indeed, the term DDS is usually used to describe the conveying of bioactive molecules, such as drugs, in a controlled and targeted way. It is an application that is highly employed in the medical and pharmaceutical fields, but which can be employed in many other sectors. These kinds of systems can grant to the formulation a modulated release over time, regulating its kinetic to maximize the efficiency and reducing the number of treatments, and hence the costs [254–256]. Matrices are fundamental in the formulation of a DDS because they are responsible for controlling the release of the active molecule, which might be modulated both by chemical and/or physical interactions. Matrices' parameters can also be modified to further vary the release control by changing the structure and the material morphology, surface functional groups, material degradation, material hydrophobicity or hydrophilicity, or even the capability to interact with the active substance without modifying its activity.

In this field, particularly noteworthy are porous silica nanoparticles (SiO_2 NPs), which have been widely employed to encapsulate biomolecules and enzymes in different fields of application [257,258]; these matrices exhibit high biocompatibility and biodegradability, resistance to microbial attacks, and are characterized by high mechanical strength, elevated thermal stability, and negligible swelling in organic solvents compared to organic polymers. These features contribute to making this type of material an excellent carrier in many systems, including biological ones, as they allow reaching the target without degradation and protect the

active agent until it is released to perform its function [145,259,260]. For these materials, a high surface area, a large pore volume, and an appropriate size of the pores are essential requirements to encapsulate a desired amount of the active substance. Through the appropriate modification of these parameters, it is possible to modulate the loading and release of the active ingredient [261–264].

Mesoporous silicas, such as MCM-41 and SBA-15, are highly efficient matrices in the design of a controlled release system, owing to features such as pore size and their ordered structure [265–269].

The surface area of mesoporous silicas is composed of the sum of the external and porous surface areas. Its high value (approximately 700-1000 m²/g) results in a significant reactivity of the material, allowing for the encapsulation of a large quantity of molecules within it [270]. Silica contains silanol groups (Si-OH), and increasing the temperature leads to their disappearance [271]. Therefore, subjecting silica to high temperatures enhances its hydrophobic character. In this scenario, the adsorption process is characterized by hydrophobic van der Waals dispersive forces. Another intriguing aspect concerning the surface of silicas is that upon contact with aqueous solutions of varying ionic strength and pH, silanols can undergo deprotonation, resulting in a negative surface charge (SiO⁻) that facilitates the adsorption of ions and polar molecules from the solution [272].

The ability to modify the surface of silica is a key feature that makes this material highly versatile. It is possible to tailor material characteristics according to specific needs by introducing a wide variety of functional groups. These functional groups may include amines, thiols, carboxyl, or sulfonic groups. Functionalization is a common procedure when aiming to modulate the properties of controlled release in mesoporous silicas for DDS. The goal is to enhance the interaction between the functional groups of the matrix and the active agent incorporated within it. The two most commonly employed procedures for functionalization are co-condensation and grafting. These techniques, depending on the application field, enable the modification of the matrix to enhance its interaction with the molecule to be transported.

Hence, the incorporation of the biocide within the pores of the grafted silica matrix would allow for its controlled release over time, thereby reducing the frequency of cleaning interventions on the surface of the target stone. Furthermore, by maximizing the interaction with the biocide and modulating its release, the proliferation of microorganisms' colonies over the stone surface would be avoided or at least reduced. Additionally, the use of silica nanocapsules in a protective

coating would also contribute to modulating its final properties, enhancing, for example, its hydrophobicity.

Therefore, the goal of this study is to functionalize silica nanoparticles with sulphonic functional groups, aiming to modulate the release by modifying the silica matrix structure and its interaction with the antimicrobial commercial product Biotin T®. In particular, attention was focused on MCM-41 silica NPs.

2. Materials and Methods

2.1. MCM-41 Synthesis

Mesoporous MCM-41 was synthesized using TEOS (Tetraethyl orthosilicate; CAS 78-10-4) as the silica source and hexadecyltrimethylammonium bromide (CTAB; CAS 57-09-0) as the template in NaOH (CAS 1310-73-2) basic solution. The mixture was continuously stirred at 300 rpm for 3 h at 25 °C and crystallized in a Teflon autoclave at 75 °C for 70 h, keeping pH = 11. The mixture was then filtered, washed, dried at 25 °C for 20 h, and finally calcined in air (50 mL/min) at 510 °C (0.5 °C/min rate) for 6 hours.

All the materials employed for this step were purchased from Sigma-Aldrich® (Merck Life Science S.r.l., Milan, Italy).

2.2. MCM-41 grafting with -SO₃H groups

A post-grafting method was used to functionalize the above MCM-41 material, following the procedure reported by previous work from the authors [273]. (3-Mercaptopropyl)trimethoxysilane (MPTMS; CAS 4420-74-0) was used as grafting agent and a NaCl (CAS 7647-14-5) solution 0.2 M was used as reaction solvent: 1 g of MCM-41 was dissolved in 30 mL of saline solution. MPTMS was added to the mixture exhibiting a 2:1 M ratio of SiO₂ to 1 MPTMS and the mixture was stirred at 1000 rpm for 24 h at 90 °C. The solid was then washed in water (three portions of 20 mL) and dried for 18 h at 70 °C. The material was labeled MCM-SH. Then, mild oxidation with 30 wt.% H₂O₂ (CAS 7722-84-1) using a 2:0.11 M ratio of SiO₂ to H₂O₂ was performed stirring at 500 rpm for 24 h at 30 °C. The sulfonated solid was filtered, washed with methanol (CAS 67-56-1), and dried at RT for 18 h. The final material was labeled MCM-SO₃H.

All the materials employed for this step were purchased from Sigma-Aldrich® (Merck Life Science S.r.l., Milan, Italy).

2.3. Impregnation with Biotin T ®

The nanomaterials MCM-41 and MCM-SO₃H were impregnated by Incipient Wetness Impregnation (IWI) method with an aqueous solution of Biotin T ® 30%. In order to ensure optimal impregnation of the biocide, a series of intermediate drying steps at 30 °C were carried out. The silica matrices were both impregnated with Biotin T ® till the maximum of their absorption capability, leading to a concentration of Biotin T ® equal to 1.3 mL/g for MCM-41 and 0.5 mL/g for MCM-SO₃H. The two samples were respectively labeled MCM-41-BT and MCM-SO₃H-BT.

The antimicrobial product Biotin T ® was purchased from CTS S.r.l., Altavilla Vicentina, Italy.

2.4. Microbiological Sampling campaign

To select the microorganisms to be used, a sampling campaign has been conducted on real-case built heritage stone materials present in the city of Venice, Italy; precisely, from the Istria stone well in *Campo dei Frari (Sestiere San Polo)* and from the external wall of *San Giacomo dall'Orto Church (Sestiere Santa Croce)* – IX century.

The samplings were carried out by non-invasive methods performed with adhesive tape *Fungi-Tape*TM (Fisher Scientific) and sterile cotton swabs. The samplings were directly plated on Petri dishes with Malt Extract Agar (MEA, Biolife). The Petri dishes were incubated at 21.5 ± 2 °C for 7 days for initial mycelium development and then monitored for fungal colony utilization until maturation (active and abundant sporulation) of the fungal colonies. At this stage, it was identified the Fungus *Cladosporium* sp. which was selected for this study. It was hence isolated and let grow in pure axenic culture with MEA cultivation medium for 5 days in incubator at 21.5 ± 2 °C, reaching an optimal inoculation growth level with active sporulating conidiophores.

2.5. Biotin T ® microbiological test

To test the antimicrobial properties of Biotin T ® and identify its Minimal Inhibitory Concentration (MIC), a preliminary challenge test based on the principles of the Kirby-Bauer diffusion test method was conducted [274]. The test's main intent was to assess that even a small amount of Biotin T ®, as the quantity released by the silica NPs, was sufficient to inhibit fungal growth. It is, in fact, known from literature that the encapsulation in nanomaterials does not limit the antimicrobial features of the active molecules [275].

To conduct the test, first some *Cladosporium* sp. inocula were placed in a 25 mL PBS buffer solution and vortexed for 3 minutes at 2000 rpm. Then, spore counting in the solution was performed using a Bürker counting chamber, repeating the procedure with three counting replicates ($n = 3$) to obtain an average of 5000 spores/mL [276]. Afterward, 500 μ L of the spore-containing solution was taken and inoculated onto aerated Petri dishes ($\varnothing = 90$ mm) containing Malt Extract Agar (MEA – Sigma-Aldrich® Merck Life Science S.r.l., Milan, Italy) culture medium, spreading on the surface with an L-shaped loop (Hockey stick). Three sterile paper discs ($\varnothing = 15$ mm) made of cellulose pulp were then placed on each Petri dish, onto which 20 μ L of Biotin T ® was absorbed at the volume concentrations of 0.1%, 0.3%, 1%, 3%, 10%, 30%. Petri dishes without the deposition of the biocide were also prepared and kept as references (*control*). All tests were performed in triplicate.

Depending on the growth of the Fungi, the MIC of the biocide can be determined, and thus, the amount of silica NPs required for an effective release of Biotin T ®.

To analyze the inhibition area, it was measured the average between the shorter and the largest radius of the circle where there was no fungal growth for each paper disc; then the inhibition area was calculated. The measurement was done on pictures taken at the end of the test (after 7 days) and processed through Adobe Illustrator CC software.

2.6. Characterization of the silica NPs

Specific surface areas and pore size distributions were evaluated by N₂ adsorption/desorption isotherms at -196 °C using a Tristar II Plus Micromeritics instrument. The samples underwent a pre-treatment at 70 °C prior to the analysis. The surface area was calculated using the B.E.T. method, whereas pore size distribution was determined by the B.J.H. method, applied to the N₂ desorption branch of the isotherm. For all the surface area data the error reported is lower than 2%.

X-ray powder diffraction (XRD) analyses were carried out by a Bruker D8 Advance Da Vinci automatic diffractometer using a Cu sealed X-ray tube and equipped with a LynxEye XE strip detector with a Ni filter on the diffracted beam.

Total acid sites were determined by the following titration method: 50 mg of powder was dissolved in a 10 mL 0.02 M NaOH solution, stirring for 1 h. The contents were then filtered, diluted with 10 mL of H₂O, and then titrated with 10 mL of a 0.02 M HCl solution, using phenolphthalein as a pH indicator. The amount of acid used to titrate the solution allowed the

determination of the number of acidic groups present in MCM-41 and MCM-SO₃H that reacted with NaOH.

Temperature Programmed Oxidation (TPO) measurements were carried out with an Anton Paar High Vacuum Adsorption Analyzer, autosorb iQ series C-MP. The samples (100 mg) were heated with a temperature rate of 10 °C/min from 20 to 1000 °C in a 5% O₂/He flow (40 mL/min). The effluent gases were analyzed by a built-in TCD detector. All temperature programmed analyses were repeated twice to validate the results.

Thermal analyses (TG/DTA) were performed on a NETZSCH STA 409 PC/PG instrument in air flux (20 mL/min) using a temperature rate set at 5 °C/min in the 25-800 °C temperature range.

Raman spectra were recorded on pure samples using an FT-Raman Instrument (Bruker Vertex 70 spectrometer, equipped with the RAM II accessory and a Ge detector) by exciting with a 1064 nm Nd:YAG laser, recording 3000 scans with a resolution of 4 cm⁻¹.

Attenuated Total Reflectance (ATR) infrared spectroscopy was employed to identify the presence of IR-active vibrations attributable to functional groups that could confirm the occurrence of matrix grafting. The instrument used for the analyses is a Bruker Vertex 70 spectrometer equipped with a Harrick MVP2 ATR cell (64 scans, resolution 4 cm⁻¹), and a DTGS detector. All data sets were processed with the software Origin 2021 by OriginLab, Northampton, MA, USA.

Scanning Electron Microscopy (SEM), coupled with Energy Dispersive X-ray Spectroscopy (EDS), and High-Resolution Transmission Electron Microscopy (HRTEM) analyses were employed to study the morphological modification of the silica matrices, to study the nanoparticles' dimensions, and to confirm the effective addition of the –SO₃H functional groups and the impregnation with the antimicrobial product. The instruments used for these analyses were a Zeis Gemini SEM with Oxford Instruments SDD EDS detector and a JEOL 2100 HRTEM with Gatan OneView detector. SEM images were acquired at an accelerating voltage of 5 kV with the secondary electron detector, and EDS maps were taken at an accelerating voltage of 10 kV. SEM samples were prepared by spreading a thin layer of sample powder onto sticky conducting carbon tape. HRTEM imaging was performed at an accelerating voltage of 200 kV. HRTEM samples were prepared by sonicating the sample in ethanol solvent for 5 minutes, and then subsequently drop-casting onto a holey carbon copper grid (Agar

Scientific) and left to dry overnight. Data was collected using the Electron Microscopy Research Technology Platform at the University of Warwick.

2.7. Study of the Release System

To evaluate the ability to release the active components of Biotin T ®, from both unmodified and grafted silica NPs, a customized release test was optimized. In this test, various aqueous solutions of MCM-41 and MCM-SO₃H of equal concentration were shaken over a time interval ranging from 30 minutes to 30 days. Subsequently, the solutions were filtered (0.2 µm filter) and analyzed using UV-visible spectrophotometry to identify and quantify the concentration of biocide molecules in the sample solutions.

The test was carried out to simulate the slower release that would take place in much more time when inserted into an antimicrobial protective coating formulation.

The powder samples of MCM-41 and MCM-SO₃ were dissolved in 3 mL of distilled water in a quantity to achieve a potential maximum concentration of Biotin T ® at 0.5% in the solution. UV-visible spectroscopy analyses were conducted using an Agilent Technologies Cary300 UV-vis spectrophotometer. The analyses were run through the Cary WinUV Scan software 4.20. The absorbance peak around 250-300 nm of the OIT molecule was used as a reference for the presence of Biotin T ®. The study has been conducted in triplicate with an error lower than 5%. All data sets were processed with the software Origin 2021 by OriginLab, Northampton, MA, USA.

3. Results and Discussion

The selection of MCM-41 as silica nanocontainer was based on the size of Biotin T ® molecules (~1 nm) and the diameters of the nanopores; which are approximately 3 nm for MCM-41 and broader for other kinds of ordered mesoporous silica [277,278]. Therefore, the study was centered on nanomaterials with smaller pores, to effectively hold and interact with the commercial biocide. The surface of silica can be modified ad hoc to tune the interaction with the host molecules didecyldimethylammonium chloride (DDAC) and 2-octyl-2H-isothiazol-3-one (OIT).

3.1. Biotin T ® activity microbiological test

The microbiological test was conducted to obtain preliminary information regarding the biocidal efficacy of various concentrations of Biotin T ® solutions, with the aim of

understanding the amount of impregnated silica NPs needed to ensure effective antimicrobial action revisiting the principles of the Kirby-Bauer Diffusion-Test Method (*i.e.* the antimicrobial diffuses from the disk into the agar and the fungal growth is inhibited at a distance from the disk that is proportional to its specific susceptibility).

From the photos taken after 7 days from inoculation, as illustrated in Figure 2, it is evident that the inhibition area – *i.e.*, the area where the growth of *Cladosporium* sp. Fungi is not observed – increases with the concentrations of Biotin T ® solutions. The picture of the negative control is also provided for comparison (Figure 2 a). These solutions demonstrate low effectiveness at concentrations $\geq 0.1\%$ (Figure 2 b); when the concentration exceeds 1% (Figure 2 d), there is no observable fungal growth (complete inhibition), *i.e.* no spore germination and/or mycelium growth and proliferation all around (radially) the Biotin T ® impregnated paper discs.

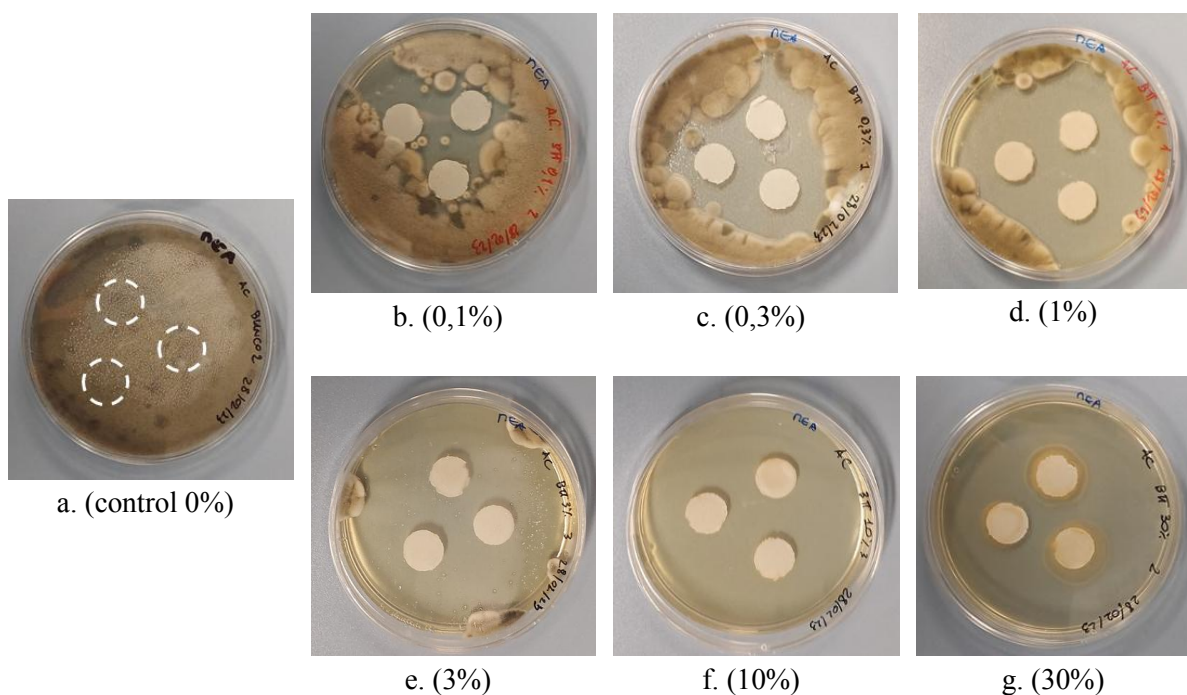


Figure 2. Photos of the microbiological test with the different concentrations of Biotin T ®: control 0% (a), 0.1% (b), 0.3% (c), 1% (d), 3% (e), 10% (f), 30% (g) – the pictures were taken after 7 days from inoculation with *Cladosporium* sp. Fungi.

Table 1 reports data on the inhibition areas of the samples; the result obtained for each sample corresponds to the average of the results from the three cellulose disks impregnated with Biotin T ® within the three Petri dishes. For the concentrations at 10% and 30%, it is reported the whole area of the Petri dish, since growth was not identified.

Table 1. Inhibition area of Biotin T ® in different concentrations over *Cladosporium* sp. Fungi.

Sample	Inhibition Area (cm ²)	Standard Deviation (SD)
Negative control	0	± 0
0.1 %	30	± 5
0.3 %	77	± 6
1 %	185	± 6
3 %	304	± 6
10 %	(max)	± 0
30 %	(max)	± 0

Regarding the negative control, the inhibition area corresponds to 0, as the Fungi completely cover the Petri dishes, including the cellulose disks. With the 0.1% sample, the average inhibition area is lower than that of the cellulose disk impregnated with Biotin T ® (~18 cm²); indeed, the Fungi were able to colonize part of the cellulose disks. From the 0.3% (Figure 2 c) concentration onward, a notably higher inhibition area is observed, increasing further with 1%, concentration at which complete radial inhibition is observed (Figure 2 d) with subsequent confluent growth. This result is confirmed with the 3% sample (Figure 2 e) and reaching total inhibition on the overall Petri area with the 10% and 30% samples (Figure 2 f and 2 g).

These results indicate that the biocide is effective even at low concentrations. Therefore, in the case of gradual release, the minimum concentration necessary to exert antimicrobial action can be easily achieved.

3.2. Characterizations of the silica matrices

The central part of the study was focused on the characterization of both MCM-41 and the functionalized silica matrix MCM-SO₃H, also focusing on the intermediate step MCM-SH. Their different structure and features are indeed closely related to their delivery performances. Furthermore, some analyses were carried out on the impregnated samples with Biotin T ® (MCM-41-BT and MCM-SO₃H-BT).

3.2.1. N₂ physisorption

The analysis was employed to evaluate the surface characteristics of the materials. As shown in Figure 3 a, both analyzed samples exhibit a Type IV isotherm, indicative of MCM-41 typical hysteresis. It is noticeable that, at the same relative pressure (p/p°), the amount of N₂ absorbed in the MCM-41 pores is higher than that absorbed by the MCM-SO₃H ones. This suggests a

successful grafting procedure: the loss in volume and surface area could be due to the covalently bonded functional groups that partially occupy the material's pores, as confirmed by the BJH curve, shown in Figure 3 b. MCM-41 presents a BET surface area of 920 m²/g and a BJH cumulative pore volume of 0.750 cm³/g. After the grafting procedure, these values significantly decrease, resulting in a BET surface area of 413 m²/g and a BJH cumulative pore volume of 0.205 cm³/g for MCM-SO₃H.

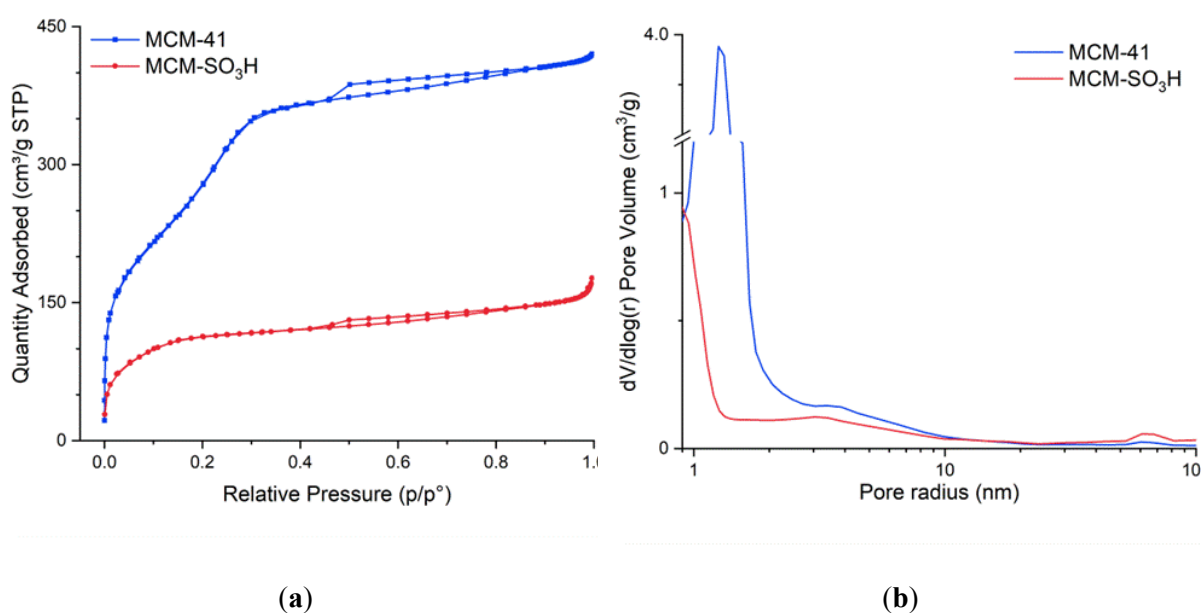


Figure 3. N₂ physisorption graphs of MCM-41 (in blue) and MCM-SO₃H (in red): (a) Hysteresis graphs; (b) Chart depicting the pore volume as a function of pore size distribution.

Concerning the pore size of the two materials, the analysis reveals that the average pore diameter for both samples is approximately 3 nm. However, it can be observed that in the case of MCM-SO₃H the hysteresis begins to form at slightly lower pressures compared to the MCM-41. This suggests a more heterogeneous distribution of pore sizes in the material (Figure 3 b). It is therefore possible that the grafting treatment might have partially altered the material's structure. To further investigate the influence of the grafting procedure on the structure of the silica matrices, X-ray diffraction measurements were also performed.

3.2.2. X-ray diffraction (XRD)

In Figure 4 a significant loss of the long-range order can be observed due to the action of the grafting treatment on the porous structure of MCM-41. This is shown by a drastic intensity

reduction of the main peak and the disappearance of secondary peaks in the MCM-SO₃H profile.

Furthermore, in comparison to the initial d-spacing of 36.8 Å, there is an initial contraction in the MCM-SH structure, reducing the d-spacing to 35.0 Å, followed by a second contraction in MCM-SO₃H, which has a d-spacing of 33.5 Å.

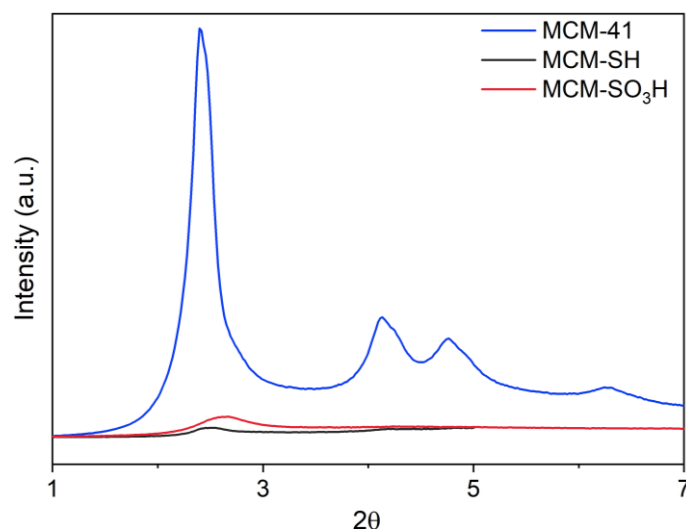


Figure 4. X-ray diffraction graph of three samples: MCM-41 (in blue), MCM-SH (in black), and MCM-SO₃H (in red).

3.2.3. Analysis of the acid sites by back-titrations

The effectiveness of the grafting procedure was verified through a synergy of approaches. The first involved a preliminary analysis to roughly assess the sulfonic group grafted on the sample: a back-titration test was conducted, as it represents a quick and straightforward investigation method: 50 mg of MCM-41 were titrated with 1.35 mL of HCl, corresponding to $0.27 \cdot 10^{-4}$ mol of acid. Using the relationship (1):

$$\text{moles of NaOH} - \text{acid groups moles} = \text{moles of HCl}, \quad (1)$$

it was possible to determine the number of moles of acidic groups in the material that reacted with NaOH, namely $1.73 \cdot 10^{-4}$ mol.

As for the back-titration of MCM-SO₃H, 1.05 mL of HCl was required, equivalent to $0.21 \cdot 10^{-4}$ mol. Using the same relationship (1), the moles of acidic groups that reacted were $1.79 \cdot 10^{-4}$ mol.

Comparing the two results, it is noticeable that in the case of the basic solution containing MCM-41 a lower number of acidic groups reacted with NaOH. This could be attributed to the lower acidity of the –OH groups present on the surface of MCM-41, compared to the –SO₃H groups added after grafting.

3.2.4. Temperature Programmed Oxidation (TPO)

The Temperature-Programmed Oxidation (TPO) technique was employed to determine, based on the increasing temperature and consumption of the oxidizing mixture, the presence of oxidizable species in the analyzed samples. In the graph reported in Figure 5, it can be observed that the three matrices exhibit very different behaviors as the temperature increases. In detail, the MCM-41 curve does not show significant peaks, unlike MCM-SH and MCM-SO₃H; this aspect can be attributed to the decomposition of chemical species.

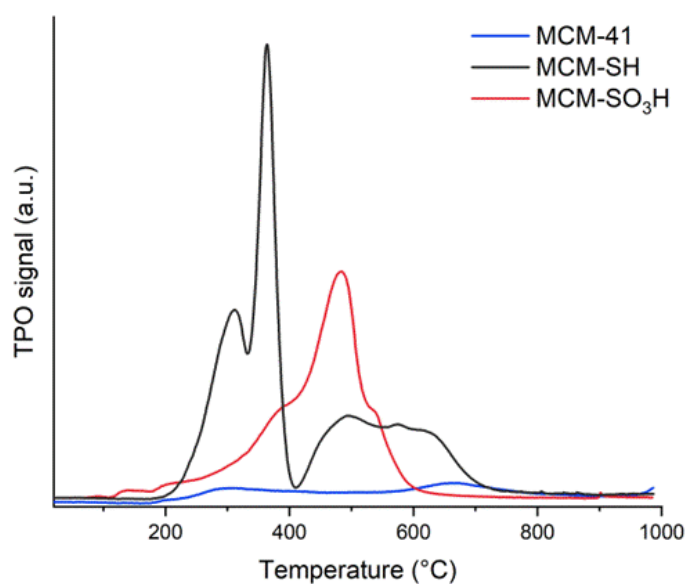


Figure 5. TPO graph of MCM-41 samples (in blue), MCM-SH (in black), and MCM-SO₃H (in red).

Regarding the other two samples, hypotheses can be made by referring to what is present in the literature [279]. In the curve of MCM-SH, a very intense peak is noticeable just before 400 °C;

it can be attributed to the oxidation of the –SH groups linked to the silica surface. The broadband after 400 °C is probably due to the overlapping of different processes involving different species present in the sample. Two shoulders are recognizable in the band: the first, at around 500 °C, could be due to the decomposition of the sulfonic groups formed after the oxidation of thiols, while the second at 550 – 600 °C may be due to the decomposition of the alkyl chain directly bonded to the silica [279].

These hypotheses can be supported by the analysis of MCM-SO₃H, whose curve shows an intense peak at around 500 °C, which can be attributed to the decomposition of propyl-sulfonic groups linked to the material. The curve also exhibits two shoulders that develop at temperatures comparable to the peaks in the MCM-SH profile. The first, around 400 °C, is probably due to the oxidation of –SH groups that were not completely oxidized during the H₂O₂ procedure and have lower intensity compared to those present in the MCM-SH profile. The second shoulder, at around 550 °C, is associated with the decomposition of alkyl chains bonded to the material.

To support and complement the information obtained from the TPO analysis, Thermogravimetric (TG) and Differential Thermal Analysis (DTA) were performed and are discussed in the following section.

3.2.5 Thermal analyses (TG/DTA)

Thermogravimetric analysis (TG) and Differential Thermal Analysis (DTA) (in Figure 6) allowed the evaluation of the mass losses (%) of the three different samples as the temperature increased. These losses were associated with exothermic or endothermic processes leading to the degradation of chemical species.

In the TG analysis, in all three samples, a first weight loss up to 200 °C is noticeable: it could be associated with the evaporation of interstitial H₂O present in the pores of the materials. In the case of the MCM-41, this is the only percentage loss recorded. These weight losses are reflected in the DTA, where endothermic processes are evident up to 150 °C, likely they were needed to provide the necessary energy for water to evaporate from the material's pores. In the case of the MCM-SO₃H, the weight loss is higher than the others, probably because it contained a greater amount of H₂O; this is justified by the DTA analysis in which the associated endothermic process peak is more pronounced than those of the other two samples.

In the MCM-SH TG analysis, a second weight loss between 200 °C and 400 °C was attributed to the decomposition of –SH groups, followed by a third weight loss, up to 700 °C, which was

attributed to the decomposition of the alkyl chains linked to them. In the DTA analysis, it is possible to observe the development of exothermic processes in the temperature range of 100 - 700°C, ascribed to the decomposition of these chemical species.

Regarding MCM-SO₃H, the second significant weight loss in the DTA, approximately between 200 °C and 600 °C, is associated with exothermic processes that could have involved the decomposition of various species, such as the –SH groups that were not oxidized during grafting, –SO₃H groups, and the alkyl chains bonded to the silica.

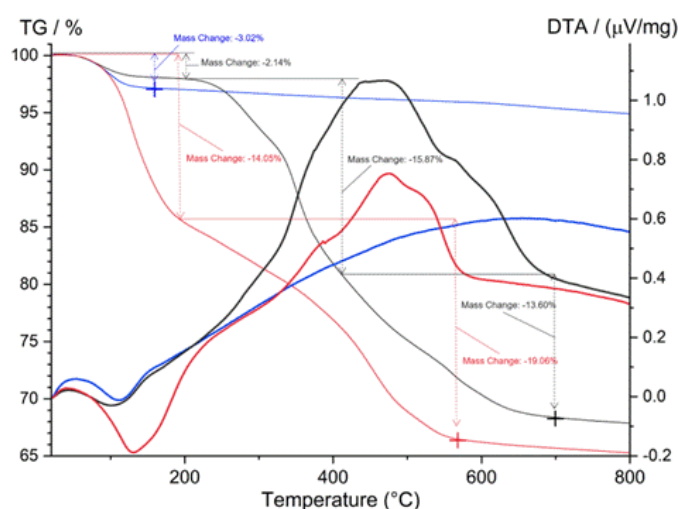


Figure 6. Graph depicting TG and DTA analyses of three different samples: MCM-41 (in blue), MCM-SH (in black), and MCM-SO₃H (in red).

3.2.6 Raman Spectroscopy

Through Raman and ATR spectroscopic analyses the surface properties of the silica matrices, before and after grafting, as well as the functional groups contained in them, were studied.

Regarding Raman spectroscopy, in the graph shown in Figure 7, it can be observed that, while MCM-41 exhibits almost no detectable vibrations, the spectrum of MCM-SH shows a very intense signal at 2570 cm⁻¹ attributable to the presence of thiol groups (–SH). This signal disappears following oxidation in MCM-SO₃H, revealing the signal of sulfonic groups (–SO₃H) at around 1050 cm⁻¹.

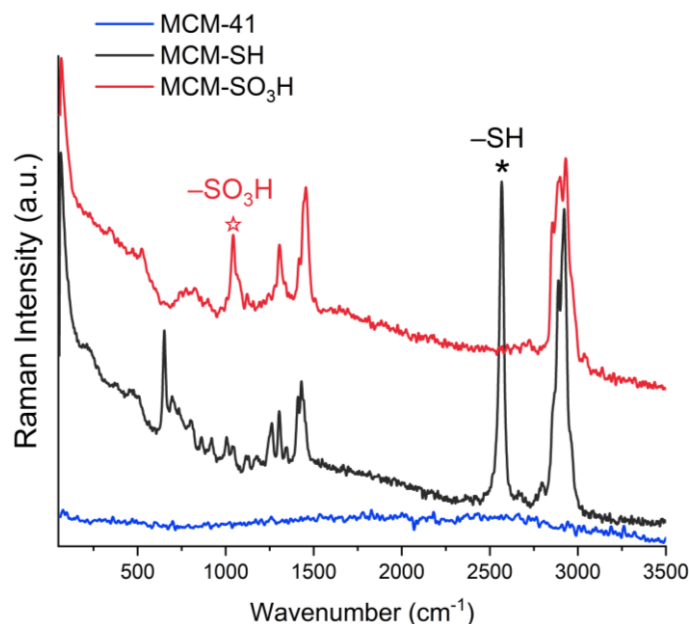


Figure 7. Raman spectra of three samples: MCM-41 (in blue), MCM-SH (in black), and MCM-SO₃H (in red).

3.2.7. Attenuated Total Reflectance (ATR) infra-red spectroscopy

ATR spectrum of MCM-41 presents the characteristic band of silica, in particular an intense and broad band centered at 1060 cm^{-1} related to the asymmetric stretching O–Si–O units, and other weaker signals at ~ 800 and 410 cm^{-1} attributable to symmetric stretching and bending modes of O–Si–O.

In the ATR spectrum of MCM-SH (in Figure 8 a) there is a signal at around 2900 cm^{-1} attributable to the C–H stretching of the aliphatic chains bonded to the material's surface. This signal becomes weaker and undefined after oxidation, also due to the presence of a larger amount of water, if compared to the other two samples. After grafting, in MCM-SH and MCM-SO₃H, two additional signals at 1240 cm^{-1} and 690 cm^{-1} are singled out, respectively attributable to the C–S vibrations of the functional groups.

In the spectrum of commercial Biotin T ® (50% in water solution) only the signals of aliphatic chains, present in both DDAC and OIT molecules, are recognizable. The intense stretching mode of methyl and methylen groups below 3000 cm^{-1} and corresponding bending modes around 1460 cm^{-1} are evident in samples containing Biotin T ®, MCM-41-BT, and MCM-SO₃H-BT, confirming the effectiveness of the impregnation method (Figure 8 b).

Differential spectra were obtained (in Figure 8 c), trying to do some consideration on the nature of interaction between surface and impregnated molecules. For MCM-41-BT we observed that

impregnation consume the signal of isolated surface silanol, so we can suppose that for plain MCM-41 the impregnation mechanism involved, almost in part, the formation of H bond between isolated Si-OH and carbonyl of OIT. MCM-SO₃H does not present free silanols, that were involved in grafting with MPTMS, and impregnation seems to consume part of H-bonding. For sample containing Biotin T[®] differential spectra evidence also modification of silica modes, probably related to a slight modification of material network. No consideration can be done on sulfonate groups, which signals are superimposed to intense silica mode.

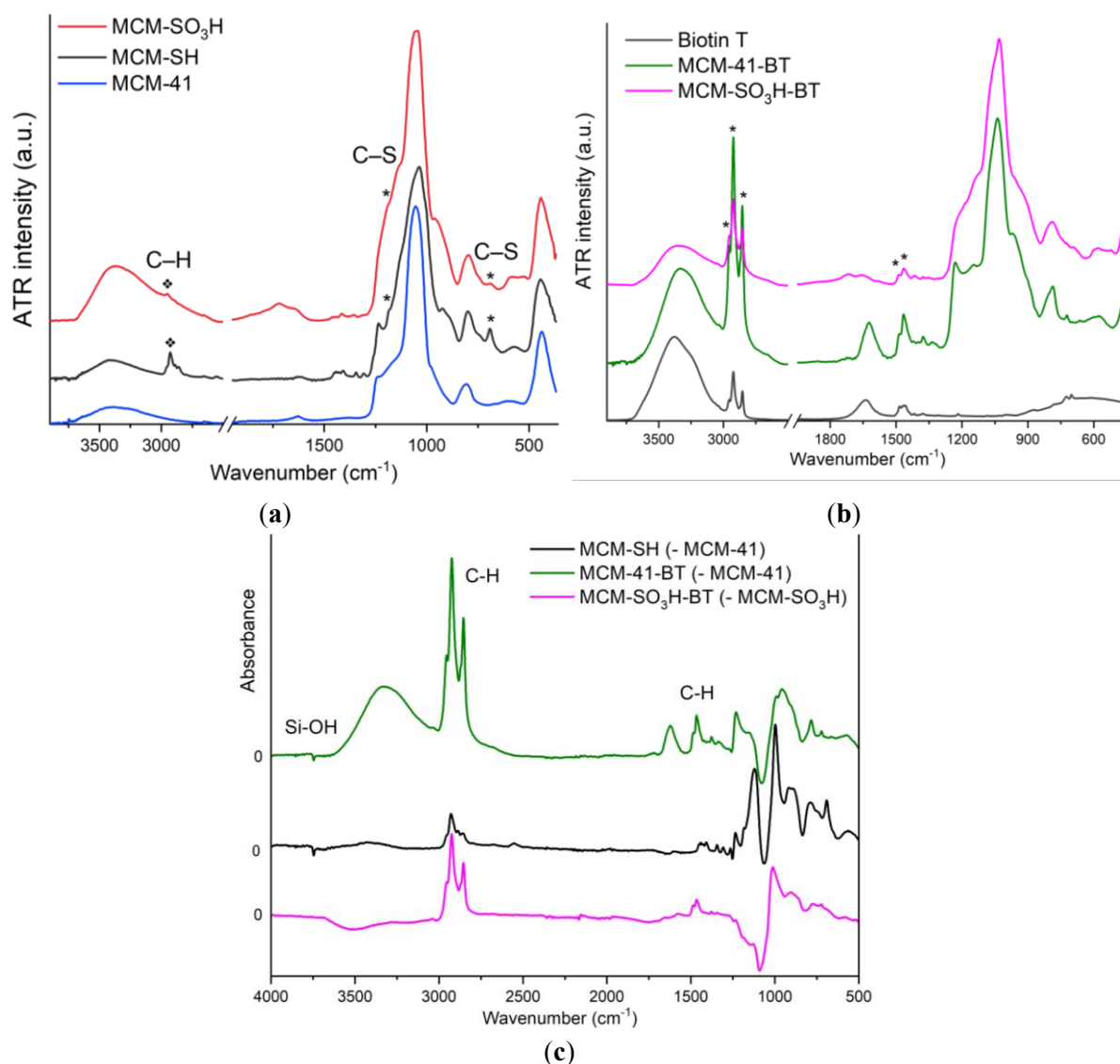


Figure 8. (a) ATR spectra of three samples: MCM-41 (in blue), MCM-SH (in black), MCM-SO₃H (in red); (b) ATR spectra of the impregnated samples MCM-41-BT (in green) and MCM-SO₃H-BT (in pink) compared with the ATR spectra of Biotin T[®] (in black) with highlighted the Biotin T[®] related signal; (c) Differential spectra of the samples MCM-SH (in black) and of the impregnated sample MCM-41-BT (in green) and MCM-SO₃H-BT (in pink).

3.2.8. Scanning Electron Microscopy (SEM) and High-Resolution Transmission Electron Microscopy (HRTEM)

The electron microscopy techniques (*i.e.*, SEM and TEM) were employed to study the morphology and topography of the silica matrices. Figure 9 reports both SEM and TEM images referred to the MCM-41 mesoporous silica matrix. The SEM micrograph (section a) shows that the nanoparticles possess a spherical shape with an average size of around 500 nm. From the HRTEM micrograph (section b) it can be seen that this material has the characteristic short-range-ordered mesoporous structure of hexagonal symmetry with alternating channels and siliceous network. The pore width, as determined from HRTEM micrographs is approximately 3 nm.

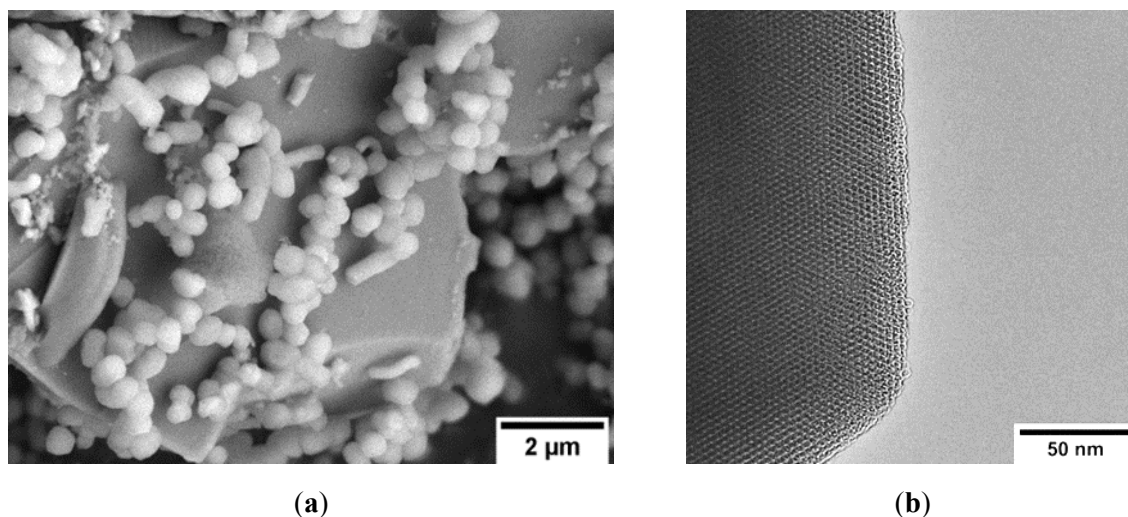
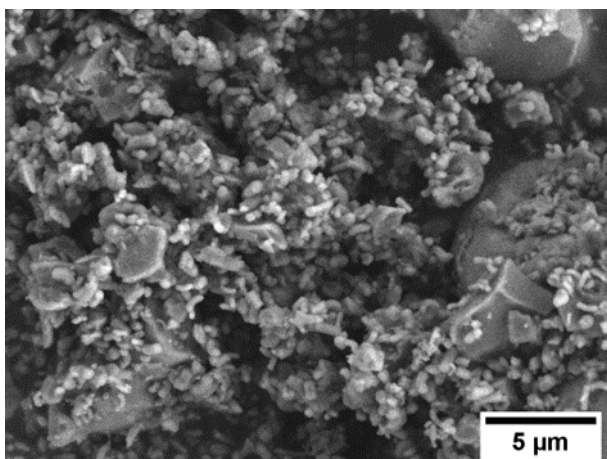
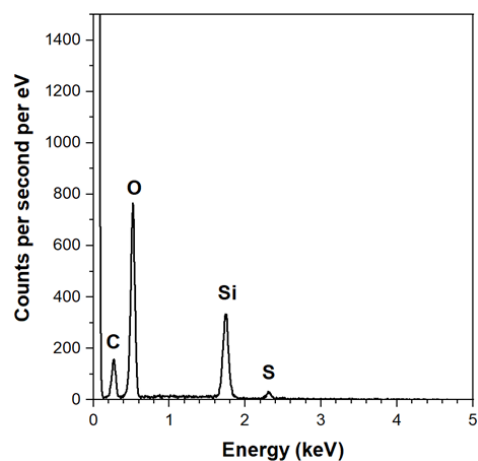


Figure 9. (a) SEM micrograph of the sample MCM-41; (b) HRTEM micrographs of the sample MCM-41: the view of the material along the hexagonal axis.

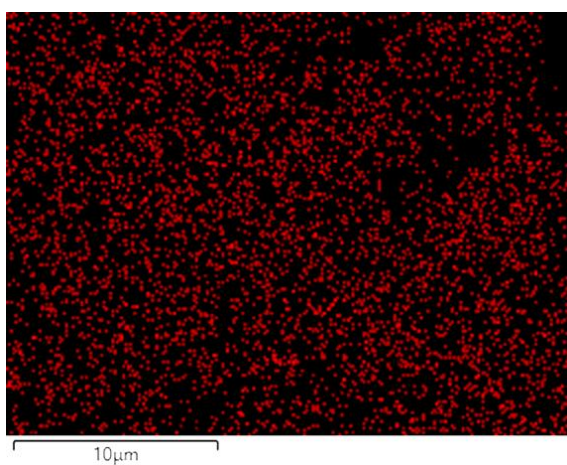
For MCM-SO₃H, the SEM micrographs were coupled with an Energy-Dispersive X-ray Spectroscopy (EDS) analysis. Figure 10 reports the SEM micrograph of the sample (a) and the Map Sum Spectrum (b) confirming the presence of sulfur (S), besides oxygen (O) and silicon (Si); from the elemental mapping it can be noticed that S (e) is as homogeneously distributed as Si (c) and O (d); this suggests a correct dispersion of the grafting treatment along the material. Moreover, from the HRTEM micrograph (Figure 10 – f) both the hexagonal structure, also present in MCM-41, and the view perpendicular to the hexagonal axis can be identified. This characterization confirms that, even though the material underwent modification, the fundamental original structure has been preserved.



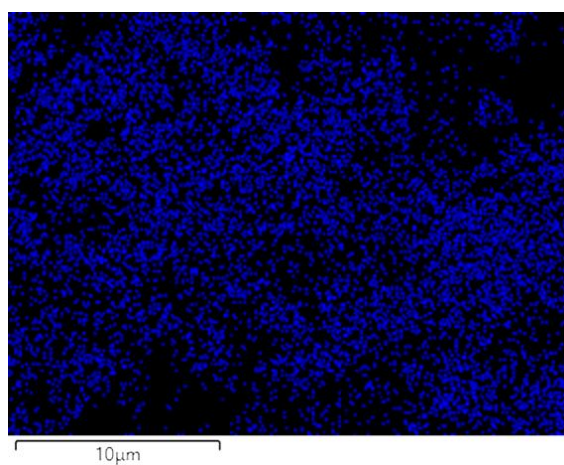
(a)



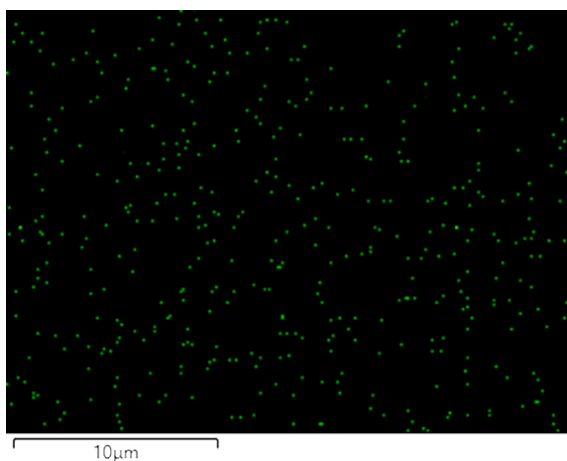
(b)



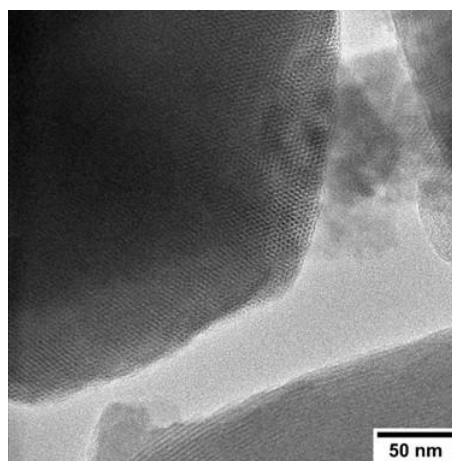
(c)



(d)



(e)

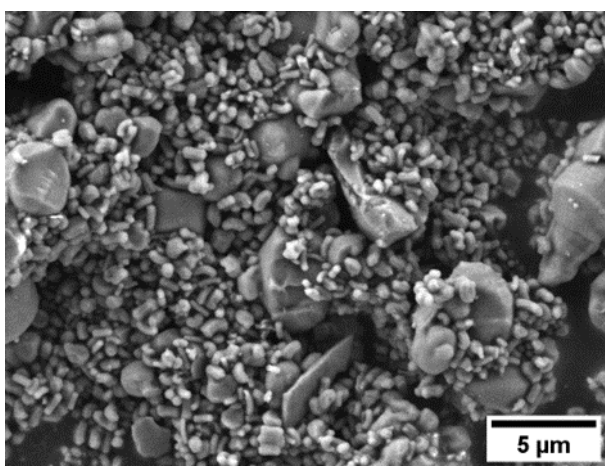


(f)

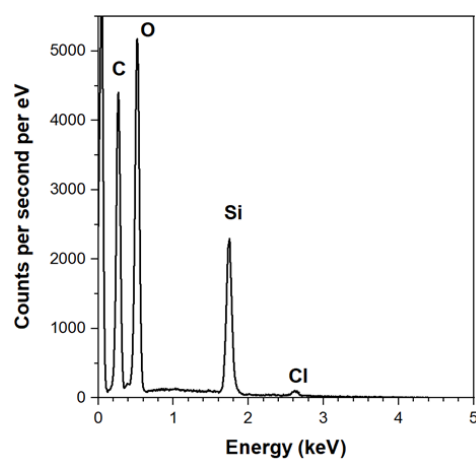
Figure 10. (a) SEM micrograph of the sample MCM-SO₃H; (b) total EDS map sum spectrum; (c) EDS elemental mapping of Si; (d) EDS elemental mapping of O; (e) EDS elemental mapping of S; (f) TEM micrograph of the sample MCM-SO₃H, showing the view both along (top) and perpendicular (bottom) to the hexagonal axis.

The SEM micrographs of sample MCM-41-BT were coupled with an EDS analysis as well. Figure 10 reports the SEM micrograph of the sample (a) and the total map sum spectrum (b) confirming the presence of chlorine (Cl), besides O and Si. From the elemental mapping, it can be noticed that chlorine is well distributed as Si (c) and O (d); this suggests a homogeneity in the impregnation treatment.

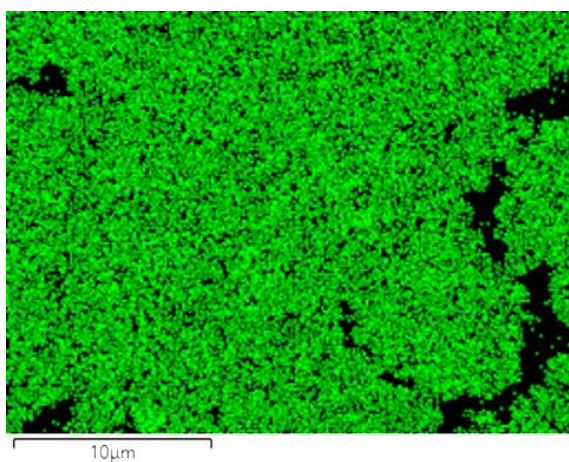
From the HRTEM micrograph (Figure 11 – f) the characteristic MCM-41 structure can be identified, from the view perpendicular to the hexagonal axis. This characterization confirms that the fundamental original structure has been once again preserved.



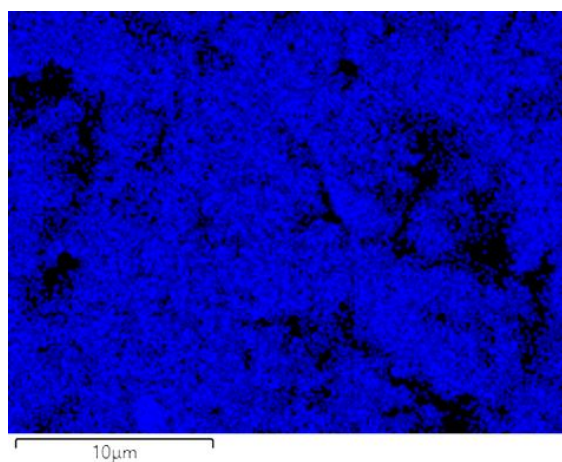
(a)



(b)



(c)



(d)

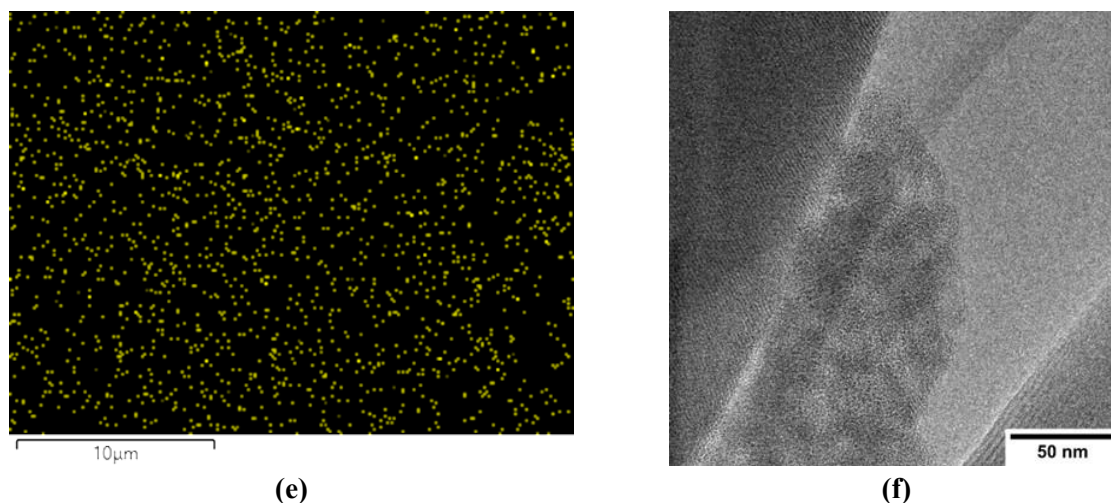
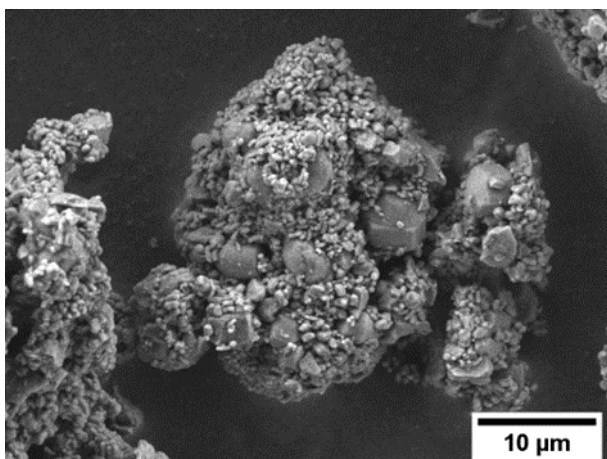


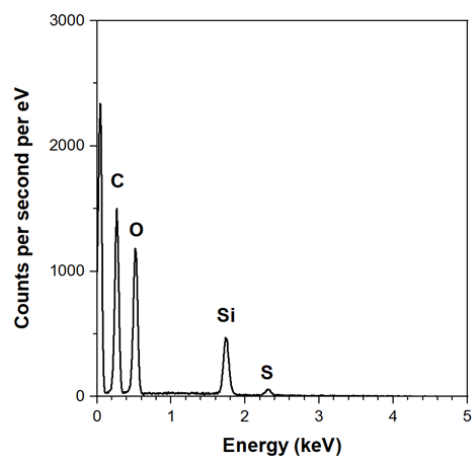
Figure 11. (a) SEM micrograph of the sample MCM-41-BT; (b) EDS map sum spectrum; (c) EDS elemental mapping of Si; (d) EDS elemental mapping of O; (e) EDS elemental mapping of Cl; (f) TEM micrograph of the sample MCM-41-BT.

Figure 12 shows the SEM (a), along with an EDS analysis (b-e), and the TEM (f) micrographs of sample MCM-SO₃H-BT. From the elemental mapping, it is possible to see, besides Si (c) and O (d), the homogeneous distribution of S (e). In this case, the presence of Cl was not detected, probably due to its low amount in the sample: considering that Biotin T ® concentration in the matrix is 0.5 mL/g and that the DDAC molecule, containing Cl, is generally present at 40-60% in the product, the amount of DDAC in the analyzed sample would be around 0.2-0.3%. Furthermore, the low elemental amount of Cl in the molecule must be considered. Therefore, it could be stated that the low amount of Cl is the main responsible for its absence in the EDS map.

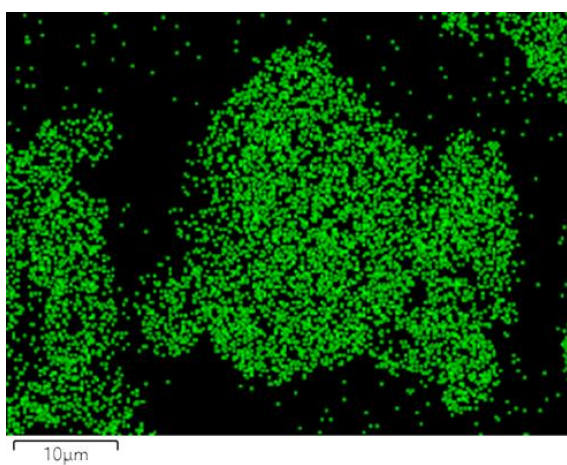
From the HRTEM micrograph (Figure 12 – f) the characteristic MCM-41 particle shape can be identified, suggesting that the fundamental original structure has been once again preserved. It is worth mentioning that in both impregnated samples, MCM-41-BT and MCM-SO₃H-BT, the organic compounds of Biotin T ® cause interference, making the sample beam sensitive, and thus resulting in less defined HRTEM images. It was however possible to identify the material characteristic planes, which appear as pale parallel lines in the micrographs.



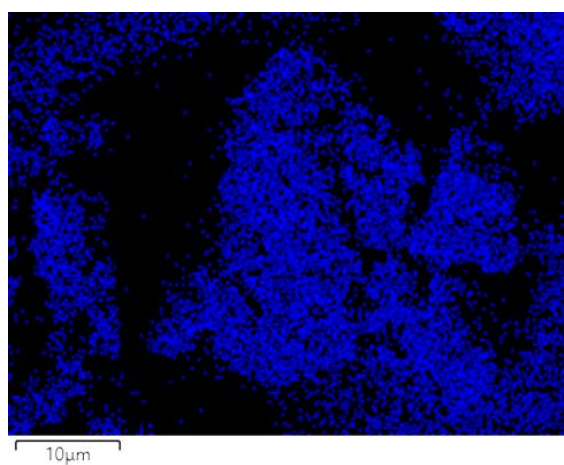
(a)



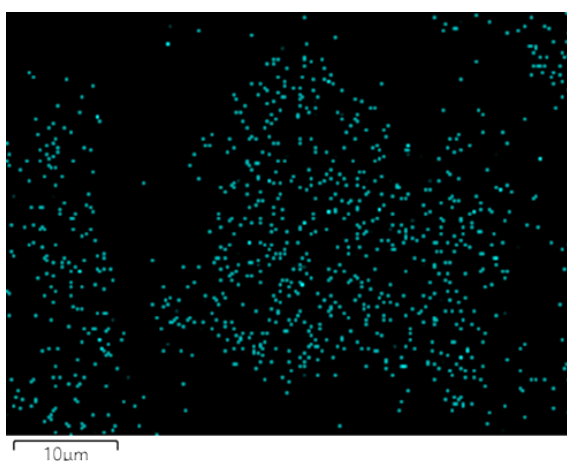
(b)



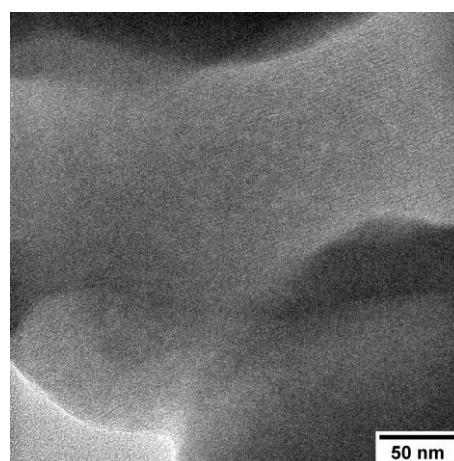
(c)



(d)



(e)



(f)

Figure 12. (a) SEM image of the sample MCM-SO₃H-BT; (b) EDS map sum spectrum; (c) EDS elemental mapping of Si; (d) EDS elemental mapping of O; (e) EDS elemental mapping of S; (f) TEM image of the sample MCM-SO₃H-BT.

In summary, from SEM, EDS, and TEM analyses it was possible to highlight that the functionalized silica matrix MCM-SO₃H does not differ in structure from the original MCM-41. Additionally, the –SO₃H groups, identified through the S element, were successfully homogeneously distributed over the material. For what concerns the impregnated matrices MCM-41-BT and MCM-SO₃H-BT, it was possible to notice the presence of Cl, representing the antimicrobial product Biotin T ®, that is didecyldimethylammonium chloride (DDAC) molecules.

3.3. Study of the Release Systems

After studying the properties of the silica nanocontainers, their performance was assessed through in vitro release tests to verify their capacity to deliver the biocide product.

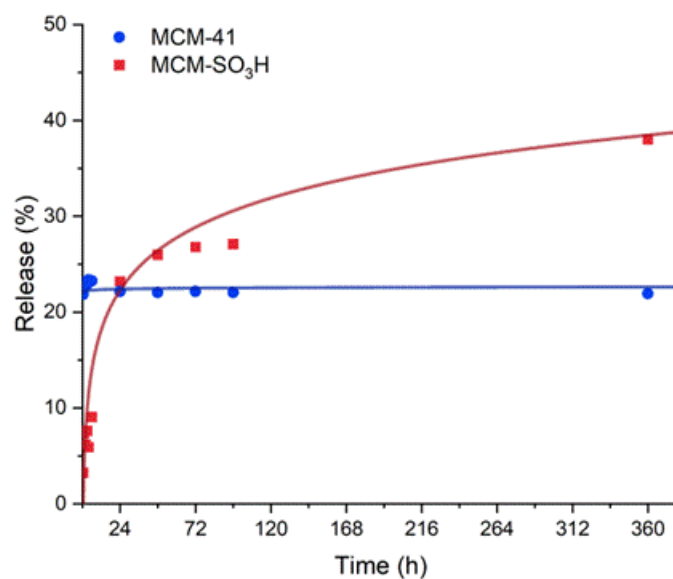
From UV-visible analysis, it was possible to determine, through the absorbance values of the molecule 2-octyl-2H-isothiazol-3-one (OIT), the concentration of Biotin T ® present in the various solutions at specific intervals over time, allowing for the evaluation of the effectiveness of the MCM-41 and MCM-SO₃H matrices.

From the graph in Figure 8 a, it can be observed that with MCM-41 the biocide is rapidly released into the solution with a percentage of ~22% within the first hour, maintaining a constant concentration for 15 days (360 hours).

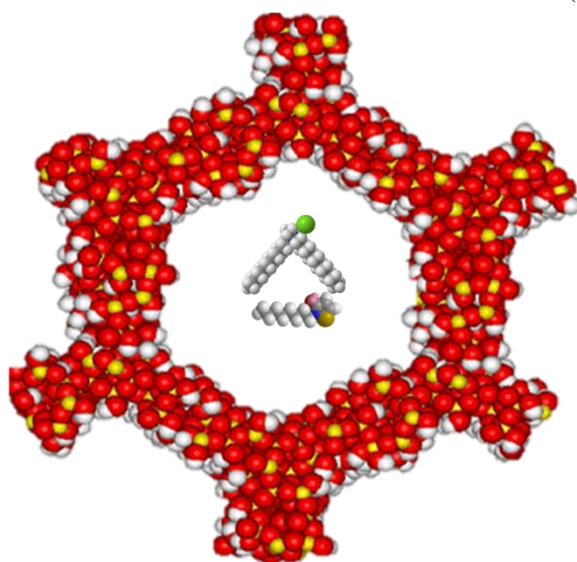
On the other hand, with MCM-SO₃H, the concentration of Biotin T ® in the solution progressively increases over time, reaching ~38% after 15 days (360 hours). For this sample it was also carried out a 30-day sample: in this case, the concentration further increased to 46%. Thus, OIT is released in greater quantity and more gradually from MCM-SO₃H compared to MCM-41, where, after a certain threshold the OIT concentration does not increase further but remains constant.

Since a controlled release system is influenced by various factors, including pores size and shape and the chemical-physical interactions that can occur between the matrix and the active component, it is possible to hypothesize that MCM-SO₃H offers a less ordered structure that favors the release of a greater quantity of biocide compared to MCM-41 (Figure 13 b, c). The porosity of the modified sample, which appears more heterogeneous with a certain percentage of smaller pores compared to those of the unmodified sample, probably explains the slower release kinetics in the initial phase. The less ordered porous structure, while controlling release, facilitates the diffusion of a greater amount of the active compound. Additionally, the release is influenced by the different nature of the nanocapsules: the weak interaction with the sulfonate

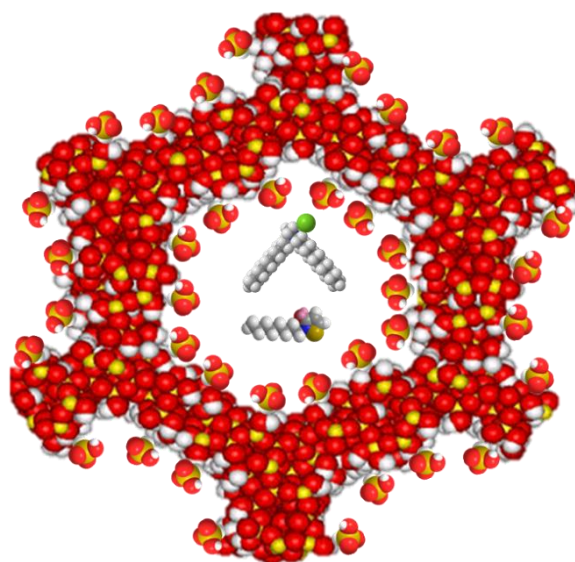
surface groups also promotes gradual release. They could interact through a hydrogen bond that may form between the hydrogen atom attached to the sulfonic groups and the numerous nonbonding electron pairs present on the ring of the OIT molecule. Another boost to the release is the increased affinity with the release medium, which in this case is aqueous. The surface of MCM-SO₃H is certainly more compatible with the medium and therefore likely promotes the release of a greater quantity of the active ingredient.



(a)



(b)



(c)

Figure 13. (a) The concentration of Biotin T® in aqueous solution over time; (b) 3D representation of the MCM-41 structure containing the two molecules composing Biotin T®; (c) 3D representation of the MCM-SO₃H structure containing the two molecules composing Biotin T®.

Regarding the determination of the DDAC molecule, although it was not possible to quantify its concentration in the solution, the UV-visible absorbance spectra show a progressive increase in the absorption band intensity over time in the MCM-SO₃H sample, which could justify a gradual release of the molecule from the matrix pores.

4. Conclusions

Within this work, a multifunctional silica NP-based matrix was successfully synthesized and functionalized that, due to its characteristics, allows the encapsulation and a gradual release of the commercial biocidal product Biotin T[®], commonly employed in the stone restoration field. The material is aimed to be suspended into a polymeric-based formulation as the principal component of an antimicrobial protective coating for stone surfaces. This work aims indeed to limit or prevent the growth of microorganism colonies on stone surfaces, preserving them from biodeterioration phenomena and minimizing the need for cleaning interventions. Additionally, the proposed approach is not only environmentally greener but also more sustainable for the health of the operators and economically.

CRedit authorship contribution statement

Andrea Campostrini: Formal analysis, Conceptualization, Data curation, Writing – original draft, Preparation, Visualization. Elena Ghedini: Conceptualization, Validation, Methodology, Writing – review & editing. Teresa Botrè: Formal analysis, Visualization. Sabrina Manente: Formal analysis. Alessia Giordana: Formal analysis, Investigation. Giuseppina Cerrato: Writing – review & editing. Giuseppe Cruciani: Formal analysis. Alex W. Robertson: Formal analysis, Writing – review & editing. Michela Signoretto: Resources, Supervision, Writing – review & editing. Federica Menegazzo: Resources, Conceptualization, Supervision, Writing – review & editing.

Acknowledgments: The authors would like to acknowledge Mr. Nicola Calderaro and Ms. Tania Fantinel for their practical contributions to this work. Andrea Campostrini finally would like to thank the “PON Ricerca e Innovazione 2014–2020”, “Fondo per la promozione e lo sviluppo delle politiche del Programma Nazionale per la Ricerca (PNR) 2021–2027”, and the restoration company Uni.S.Ve. S.r.l. of Venice (Italy) for financing his Ph.D. scholarship.

IV.

MESOPOROUS SILICA AND VEGETAL EXTRACTS COMBINED AS SUSTAINABLE STONE HERITAGE PROTECTION AGAINST BIODETERIORATION

Post-print version of the article published in *Applied Microbiology and Biotechnology*,

Springer Nature, 2025, 109, 99, 1 -15.

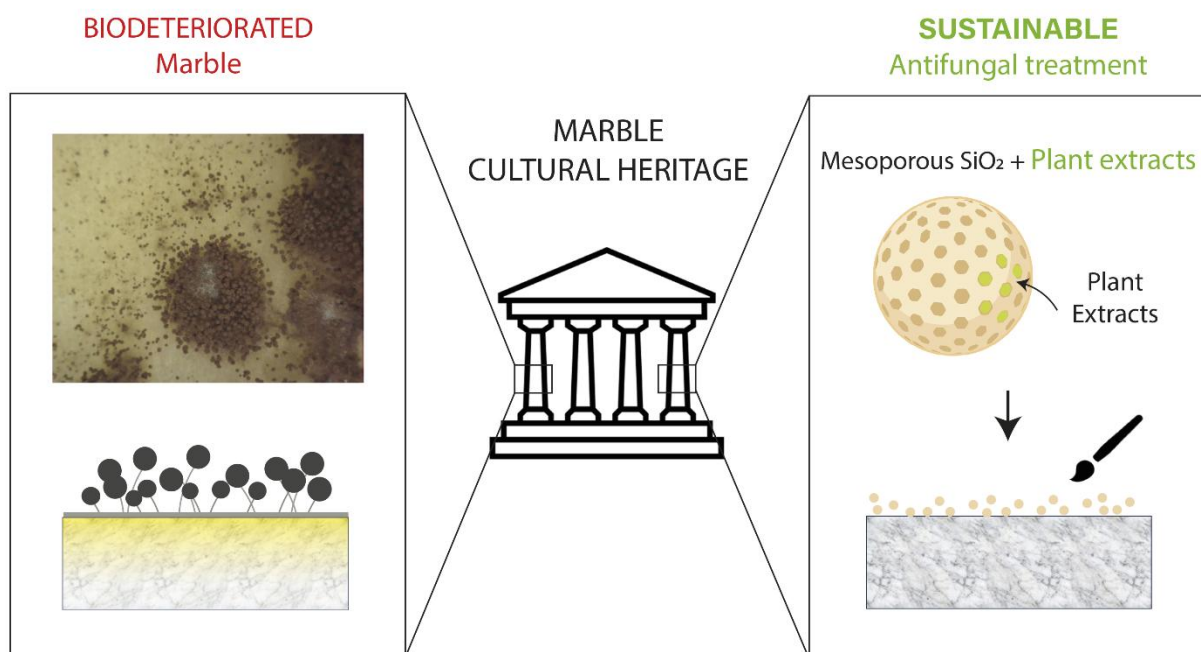
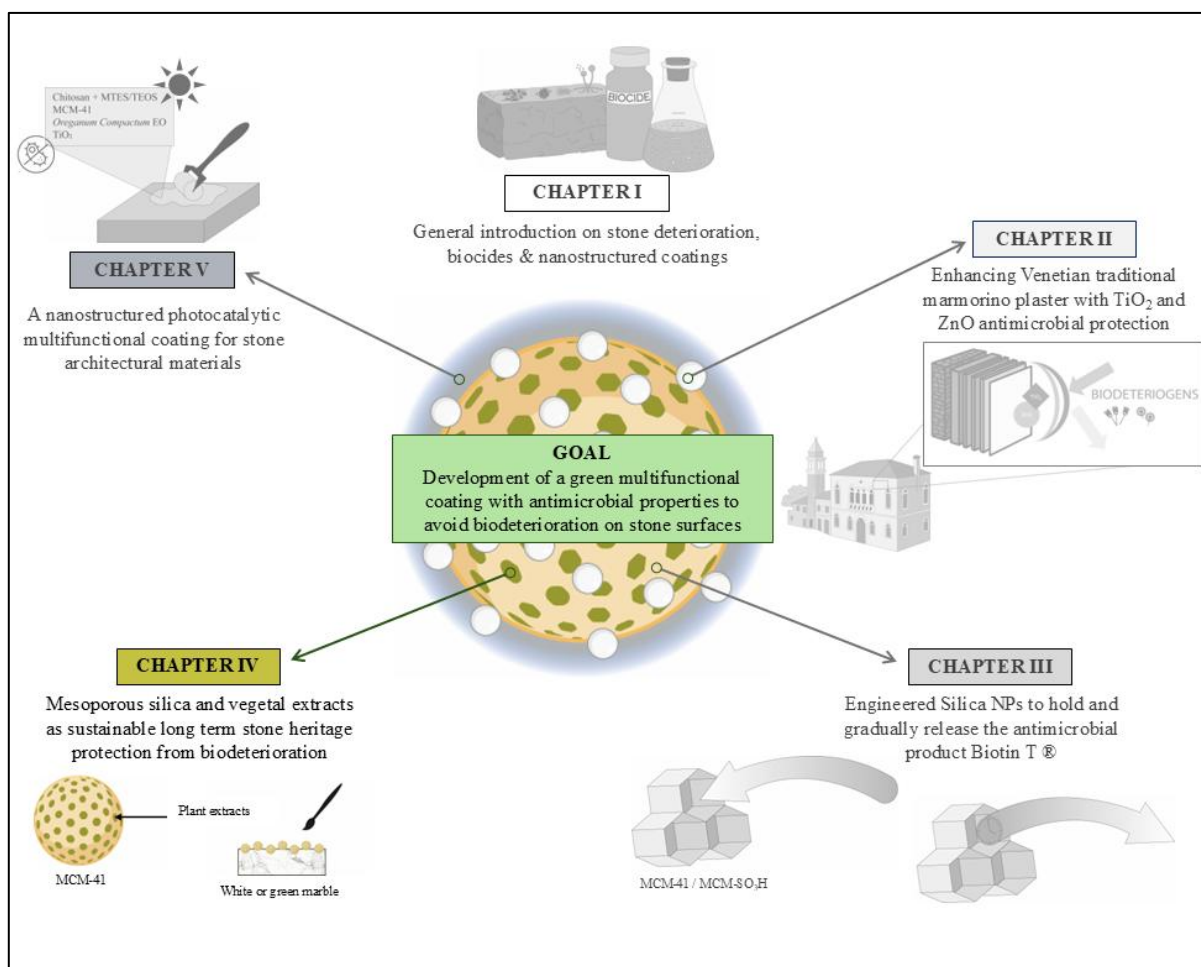
<https://doi.org/10.1007/s00253-025-13475-5>

This work, or part of it, has been presented at the following scientific conferences:

- Italian national conference “X Workshop Gruppo Interdivisionale Green Chemistry – Chimica Sostenibile: 1^a Giornata Congiunta con la Divisione di Chimica Industriale” (Florence, October 6, 2023) – *Essential oils and vegetal extracts as a greener alternative to antimicrobial products in the restoration of cultural heritage stone materials*, A. Campostrini, P. Bosch-Roig, E. Ghedini, M. Signoretto, and F. Menegazzo
- International PhD Academy at Venice International University (VIU): “Preserving and Safeguarding the Beauty of Cultural Heritage: Fundamentals, Methods and Applications of state-of-the-Art Diagnostic Tools Using Optical, X-Ray and Particle Probes” (Venice, November 06-10, 2023) – *Safeguarding our Heritage stone materials: fighting biodeterioration with sustainable plant-based solutions*, A. Campostrini, A. Sala-Luis, P. Bosch-Roig, E. Ghedini, M. Signoretto, and F. Menegazzo
- 6th International Conference on Innovation in Art Research and Technology – InART2024” (Oslo, June 4-7, 2024) – *Exploring Eco-Friendly Antimicrobial Solutions for Cultural Heritage Stone Conservation*, A. Campostrini, A. Sala-Luis, P. Bosch-Roig, E. Ghedini, M. Signoretto, and F. Menegazzo
- National conference of the Italian Chemistry Society “Chemistry: Elements of Future – SCI2024 – XXVIII National Congress” (Milan, August 26-30, 2024) – *Sustainable biodeterioration prevention for Built Heritage stone surfaces: increasing essential oils antimicrobial features through encapsulation with SiO₂-based NPs*, A. Campostrini, A. Sala-Luis, P. Bosch-Roig, E. Ghedini, M. Signoretto, and F. Menegazzo
- International conference “Ciencia y Arte: Ciencias y tecnologías aplicadas a la conservación del patrimonio” (Madrid, October 29-31, 2024) – *Hidrogeles y enzimas para la eliminación de pigmentos fúngicos en mármol blanco de Carrara*, J.A. Castañeda, P. Bosch-Roig, A. Sala-Luis, A. Campostrini, and G. Piñar
- Research seminar “Patrimonio a rischio: sfide tra conservazione e cambiamento” (Venice, March 31, 2025) – *Estratti naturali e nanomateriali per proteggere le superfici lapidee dal biodeterioramento*, A. Campostrini

Part of this work was also published in **ARCHÉ**, n. 16-17-18 (2023), SEPARATA 25 AÑOS DEL IRP, ISSN 1887-3960 / 2445-1150, p. 106-109, *Desarrollo de un recubrimiento sostenible elaborado por nanopartículas de sílice y biocidas naturales para la protección a largo plazo de superficies pétreas del patrimonio cultural frente al biodeterioro*, P. Bosch Roig, A. Campostrini, and A. Sala Luis.

Part of this work is going to be published also in **Ambiente e patrimonio a rischio: sfide tra conservazione e cambiamento**, Anteferma Ed., iNEST - Spoke 4 - Vol. 2.3, Chapter 5, *Estratti naturali e nanomateriali per proteggere le superfici lapidee dal biodeterioramento*, A. Campostrini, P. Bosch-Roig, A. Sala-Luis, E. Ghedini, S. Manente, M. Signoretto, and F. Menegazzo.



Mesoporous silica and vegetal extracts combined as sustainable stone heritage protection against biodeterioration

Andrea Campostrini ^{a,b}, Agustí Sala-Luis ^a, Pilar Bosch-Roig ^{*,a}, Elena Ghedini ^b, Michela Signoretto ^b, and Federica Menegazzo ^b

^a Instituto Universitario de Restauración del Patrimonio, Universitat Politècnica de València, Camí de Vera s/n, 46022 Valencia, Spain

^b CatMat Lab, Department of Molecular Sciences and Nanosystems, Venice Ca' Foscari University and INSTM RUVE, Via Torino 155, 30172 Venice, Italy

*corresponding author: mabosroi@upvnet.upv.es

Authors' ORCID ID

Andrea Campostrini: 0000-0002-9684-8510 – andrea.campostrini@unive.it

Agustí Sala-Luis: 0000-0002-1310-7240 – agsalui@bbaa.upv.es

Pilar Bosch-Roig: 0000-0003-2019-399X – mabosroi@upvnet.upv.es

Elena Ghedini: 0000-0001-5789-8163 – gelen@unive.it

Michela Signoretto: 0000-0002-0051-2968 – miky@unive.it

Federica Menegazzo: 0000-0001-8023-6379 – federica.menegazzo@unive.it

Abstract: Since biodeterioration is considered one of the main issues related to the conservation of cultural heritage stone materials, an investigation was conducted into preventive sustainable antimicrobial alternatives to protect the stone surfaces. The study focuses on using MCM-41 mesoporous silica particles and vegetal extracts: the mesoporous materials act as nanocontainers encapsulating the extracts, which instead serve as green antimicrobial compounds to inhibit microbiological proliferation. In this way, the antimicrobial features of the extracts are sustained for a more extended period, reducing the evaporation rate and diminishing the quantity required; the amount necessary to achieve the minimum inhibitory concentration was reduced due to the decrease in evaporation. Moreover, since the MCM-41 can host a higher quantity of product than is necessary to exert the antimicrobial effect, the duration of activity is further prolonged, releasing the extracts over time.

Specifically, the mesoporous particles were impregnated with the vegetal extract of limonene and the essential oils of thyme and oregano. *In vitro* microbiological tests were conducted on two fungi (*i.e.*, *Aspergillus tubingensis* and *Penicillium chrysogenum*), taken as model microorganisms from real-case scenarios.

A combination of mesoporous silica and vegetal extracts was employed to develop a protective coating for stone surfaces, and tests were conducted on marble mock-ups.

The promising synergic results show that this system could be of interest for preventing microbiological growth over stone surfaces, avoiding a visible aesthetic impact, being non-toxic for the environment or the operator, and preventing the extract from evaporating but holding it for a controlled release.

Key Points:

- Green antimicrobial system using porous silica as nanocontainer for plant extracts
- Encapsulated vegetal extracts to inhibit microbial growth on stone surfaces
- Stable and efficient coating against fungal species *in vitro* and on marble mock-ups

Keywords: Essential oils, Biodeterioration, Stone protection, Sustainable materials, Long-term effect, mesoporous MCM-41

Introduction

The degradation of stone materials represents a significant concern in preserving cultural heritage and architectural structures [1,7,12,191]. Among the numerous factors contributing to this deterioration, microorganisms have emerged as a critical agent in the biodegradation process, both for outdoor and indoor scenarios [20,76]. Since biodeterioration is a critical issue in safeguarding Cultural Heritage materials, different techniques have been employed to limit or reduce it [81,116,217,280].

Microorganisms, such as fungi, play a fundamental role in the biodeterioration of stone materials; their ability to colonize and thrive on the surface of stones is helped by organic matter, moisture, and favorable environmental conditions [33,281]. As microorganisms proliferate, the morphology of stone surfaces undergoes profound alterations. Surface pitting, chromatic alterations, and loss of structural cohesion are common manifestations of microbial biodegradation [204,282]. These alterations compromise the aesthetic and structural integrity of the stone materials, requiring extensive conservation measures. The study of the degradation of stone materials due to microbial activity represents a complex interplay of physico-chemical, biological, and environmental factors [22,244,283]. Addressing these challenges requires an interdisciplinary approach integrating microbiology, chemistry, environmental science, and conservation & restoration science.

The intervention usually undertaken to deal with microbiological colonization over stone materials is related to cleaning treatments with biocides [219,284,285]. However, most of these products are toxic not only for the target organisms but also for the environment and the operator [84,246]. This led to an increasing interest in using natural substances: in recent years, the application of vegetal extracts, such as essential oils, has garnered attention as a promising strategy for combating microbial deterioration [56,62–64,74,286].

Vegetal extracts can derive from various plant sources and have long been employed for their diverse therapeutic properties. One of the notable advantages lies in their asserted fungicidal activity: their active molecules, such as terpenes and phenolic compounds, exhibit inhibitory effects on fungal growth and reproduction. They could, therefore, mitigate the risk of fungal colonization on various surfaces, including those of historical significance [65,68,71,101]. This action against fungi places vegetal extracts as versatile agents for conserving cultural heritage materials vulnerable to microbial threats [66,73,100,287].

Vegetal extracts such as essential oils (EOs) are also applied in the cultural heritage field, especially for cleaning treatments or prevention. The volatile antimicrobial molecules present in essential oils constitute a distinct and potent array of compounds.

Despite their features, using EOs in cultural heritage conservation is challenging. The concerns related to the potential alteration of artifact appearance, odor retention, operators' exposures, and long-term stability require careful consideration in developing application protocols. Furthermore, the molecules with the highest antimicrobial action usually are the same and have high volatility; hence, the time efficacy of these products needs to be carefully considered. If volatility is not a problem for cleaning interventions since they can be trapped in cleaning poultices, it becomes an issue when dealing with preventive actions. To avoid evaporation, options for encapsulating EOs are proposed in the literature, such as embedding them in nanocarrier systems based on polymers (of different nature) or hydrogels. These systems have also been studied for applications in cultural heritage [249,288,289]. Another advantage of encapsulating EOs is the reduction of exposure for operators. Although their toxicity is considered low to moderate, some components of EOs can be toxic in high amounts [286,290,291]. Encapsulation can help reduce the possibility of direct contact with these substances for operators, thereby increasing worker safety [292,293].

Another promising way is to embed vegetal extracts in mesoporous matrices with a structure able to host substances, such as MCM-41 mesoporous silica. These materials are widely used in various applications, including coatings for cultural heritage materials [213,294–296].

Therefore, in this research, three vegetal extracts have been embedded in mesoporous SiO₂ to study their antifungal preventive power. The investigated system aims to exploit the antimicrobial power of the extracts' active molecules, avoiding evaporation and color-related issues, thanks to mesoporous silica's morphological ability to store and later release the antimicrobial vegetal extracts. Specifically, MCM-41 particles were employed to hold and release the vegetal extract of limonene and the essential oils of *Origanum compactum* and *Thymus vulgaris*.

The decision on which silica nanocontainer to focus on was based on the diameters of the nanopores, which are approximately 3 nm for MCM-41 and, for instance, 6 nm for SBA-15 [263,277,278]. Meanwhile, concerning the vegetal extracts, the choice fell on employing extracts, already tested by different authors in literature and employed in the conservation field, derived from plants highly diffused and endemic to the Mediterranean region in an optic of

environmental sustainability and availability from Spain and Italy, the two countries partners in this work [297–299].

Materials and methods

MCM-41 Synthesis

Mesoporous MCM-41 was synthesized using TEOS (Tetraethyl orthosilicate; CAS 78-10-4) as a silica source and hexadecyltrimethylammonium bromide (CTAB; CAS 57-09-0) as a template in NaOH (CAS 1310-73-2) basic solution. To reach a material with a pore diameter of ~4 nm the mixture was continuously stirred at 300 rpm for 3 h at 25 °C and crystallized in a Teflon autoclave at 75 °C for 70 h, keeping pH = 11 [300]. The mixture was then filtered, washed, dried at 25 °C for 20 h, and finally calcined in air (50 mL/min) at 510 °C (0.5 °C/min rate) for 6 hours [301].

All the materials employed for this step were purchased from Sigma-Aldrich® (Merck Life Science S.r.l., Milan, Italy).

Impregnation with vegetal extracts

MCM-41 particles were impregnated, till the maximum of their absorption capability, by the Incipient wetness impregnation (IWI) method with the vegetal extract of limonene and the essential oils of *Origanum compactum* and *Thymus vulgaris* CT linalool, leading to a concentration of ~1.6 mL g⁻¹, once dried at RT. Briefly, a known amount of extract was poured on the silica powder and amalgamated with a glass spatula. In order to ensure optimal impregnation of the extract, a series of intermediate drying steps at RT were carried out.

The limonene extract was donated by Commercial Xúquer (S.A.R.L.), Valencia, Spain. The essential oils *Origanum compactum* and *Thymus vulgaris* CT linalool were purchased from Pranarôm International (S.A.), Ghislenghien, Belgium (BE). Limonene is a pure component present in different *Citrus spp.* EOs [302], while oregano EO main component is carvacrol [303,304] and the main component of thyme EO is linalool, as indicated in the commercial product information [305,306].

Microbiological Sampling campaign

Sampling campaigns have been conducted on real-case-built heritage stone materials in Valencia, Spain to select the microorganisms to be used,. Precisely, as shown in Figure 1, from marble parts of four statues from the Sculpture Museum of the *Universitat Politècnica de*

València, Vera Campus (MUCAES- UPV): Statue Homenaje a la Dra. Juana Portaceli, Statue Arquitectura del Silencio, Statue Homenaje a Manolo, Statue Torso H. (1989-2007).

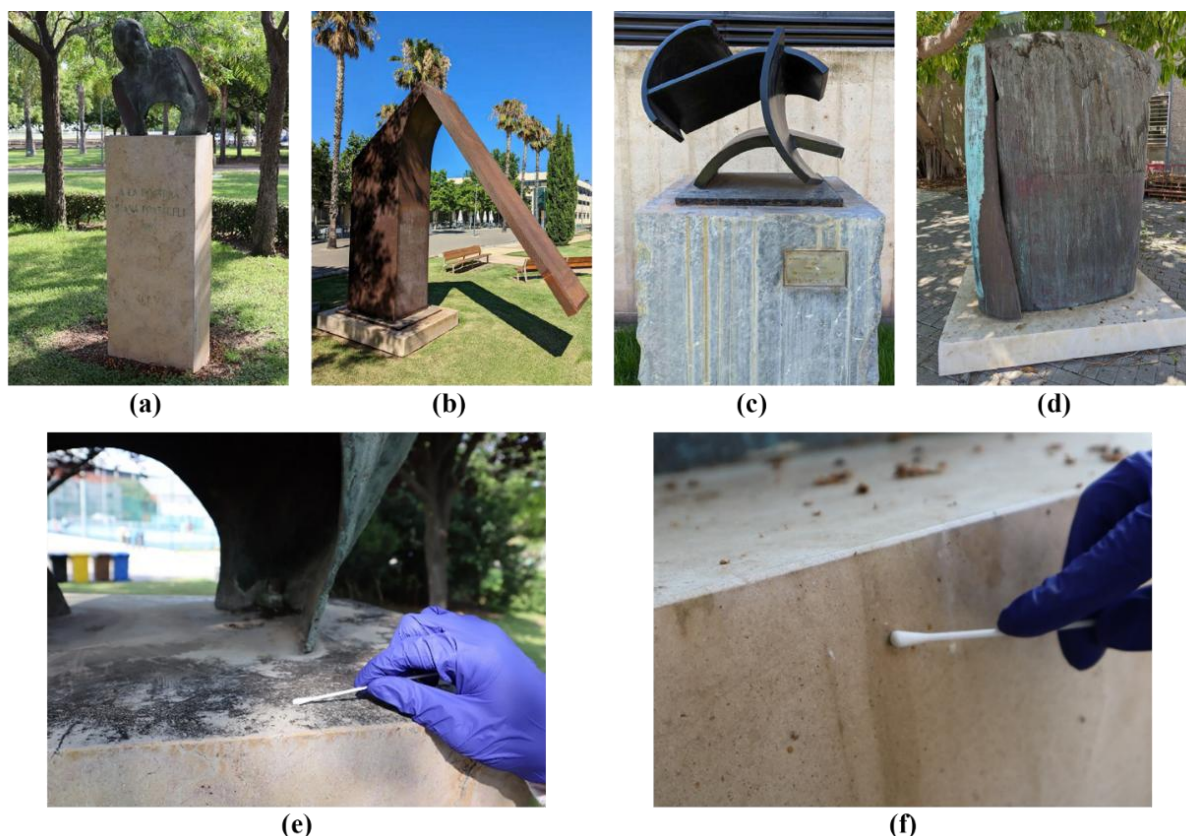


Fig. 1: Statues from MUCAES- UPV: *Statue Homenaje a la Dra. Juana Portaceli* (a), *Statue Arquitectura del Silencio* (b), *Statue Homenaje a Manolo* (c), *Statue Torso H.* (d), and pictures of the sampling campaign (e, f)

The samplings were executed by non-invasive methods, rubbing humid sterile cotton swabs on the stone surfaces [307]. The samplings were plated on Petri dishes with Sabouraud Dextrose Agar with Chloramphenicol (SDA, purchased from Sharlab, Barcelona, Spain). The Petri dishes were incubated at 28 °C for 3 days for initial mycelium development and then monitored until the fungal colonies' maturation (active and abundant sporulation).

Fungal isolation and molecular identification

Based on the morphological characteristics of the fungal colonies obtained from the stones, the two most frequent fungi were selected and isolated for this study. The selected fungi were let grow in pure culture with an SDA cultivation medium in an incubator (Binder INC, Bohemia,

EUA) at 28 °C for 3 days, reaching an optimal inoculation growth level with active sporulating conidiophores.

Genomic DNA was purified from the pure cultures and stored on the Whatman FTA™ Indicating Card for fungal isolates by STAB Vida Lda. (Portugal). Fragments of the fungal rDNA gene containing the ITS and D1/D2 regions were PCR amplified using an ITS5/LR6 set of primers. FNA Sanger of the amplified products were aligned to generate consensus sequence and checked against the GenBank by BLAST of consensus sequence against NCBI database, applying a similarity score of $\geq 99.0\%$. Furthermore, the β -Tubulin genomic region has been sequence and analyzed as the gene of choice for species-level differentiation within *Aspergillus* and *Penicillium* genera, especially when the ITS (Internal Transcribed Spacer) region sequence alone is insufficient as a single marker to distinguish closely related species. First amplification and subsequent partial sequencing of the β -Tubulin gene (with bidirectional reads) were performed using the primers Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTG TAGTGACCCTTGGC-3') [308]. Then the sequences were compared using BLAST (Basic Local Alignment Search Tool) analysis [309] against the NCBI public reference database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The percentages of similarity, the extent of overlapping fragments, and the names of microorganisms with the highest sequence identity were finally collected. Finally, the isolated strains used in this study were deposited in the CECT publicly accessible culture collection belonging to the WDCM.

Minimal Inhibition Concentration Determination by Poisoned Food Technique

The Poisoned Food Technique method was used to determine the Minimal Inhibition Concentration (MIC). The method is based on mixing, within the cultivation medium, fungal growth inhibitors or potentially toxic substances [281]. The medium was prepared by dissolving SDA powder in water (65 g/L) in the autoclave at 120 °C for 20 minutes in a flask and then poured into different falcon tubes. At this stage, different amounts of vegetal extract, *free* or encapsulated in the MCM-41 SiO₂, were added to the falcon tubes to reach the desired extract concentrations (from 0.05% to 5% (v/v) – corresponding to ~0.45 mg/mL to ~45 mg/mL). The *poisoned* cultivation media were poured into Petri dishes ($\varnothing = 55$ mm).

After medium solidification, a small circular inoculum ($\varnothing = 6$ mm) was cut from fresh, pure SDA fungal colonies and placed in the Petri disk's center, making the colonized side of the circular inoculum touch the medium. They were hence incubated at 28 °C for 7 days, monitoring mycelium development: the presence of growth indicates that the tested product

concentration does not inhibit the microorganism's growth. Control tests were carried out with just SDA and with SDA with MCM-41 mesoporous nanoparticles (without vegetal extracts). All tests were performed in triplicate. Pictures were taken after 3 and 7 days to evaluate the antimicrobial efficacy with a Canon EOS M50 camera. To establish the growth inhibition, the diameter of colonies was measured using ImageJ, then the percentage of inhibition was calculated, as reported in Literature [310,311], using the formula (1):

$$Inhibition (\%) = \frac{\varnothing_{Control} - \varnothing_{Test}}{\varnothing_{Control}} \times 100 \quad (1)$$

Evaporation tests

To evaluate the volatility of the active molecules composing the three vegetal extracts an evaporation test was carried out for each extract separately [312]: 100 μ L of extract were placed in a Petri glass dish, keeping track of the weight loss over time with an analytical balance.

The test was repeated in the same conditions, with the best-performing encapsulated extract, to evaluate the decrease in volatility after impregnation. Therefore, 1 g of MCM-41 mesoporous SiO₂ was impregnated with 1600 μ L of extract and then weighted over time. The weight measure was carried out before starting the evaporation test (T_0) and then repeated at different periods (T_n), where n corresponded to every 3 minutes for the first hour, then every hour till 6 hours, and later at 1-2-3-7-14-28 days. All data sets were processed with the software Origin 2021 by OriginLab, Northampton, MA, USA. The percentage of evaporation was determined using the formula (2):

$$evaporation (\%) = \frac{weight\ EOs\ T_n}{weight\ T_0} \times 100 \quad (2)$$

Data analysis

Statistical analyses were conducted to summarize the data, including the calculation of means and standard deviations. All measurements and tests were performed in triplicate to ensure reliability and reproducibility. Outliers were identified and excluded to maintain data integrity. Microsoft Excel was used for data processing and computation to ensure accuracy and consistency in the results.

Coating on marble mock-ups

To evaluate the applicative aim of the presented combination of vegetal extract and SiO₂ MCM-41, the impregnated particles were dispersed in a 1:1 mixture of glycerol and 96% ethanol. This solution was then applied at a rate of 2 L/m² on the upper face of sterilized white Carrara and green marble mock-ups.

The quantity of impregnated SiO₂ varied to develop two coating formulations, resulting in coverage levels of 0.7 g/m² or 7 g/m² on 5×5 cm stone surfaces, corresponding to 0.1% (0.09 mg/mL) or 1% (0.9 mg/mL) of plant extract. These formulations were therefore named COT-01 (0.1% vegetal extract and SiO₂ MCM-41) and COT-1 (1% vegetal extract and SiO₂ MCM-41). While the ratio of plant extract to SiO₂ was maintained constant, the amount of impregnated SiO₂ in the coating formulations was varied (17 mg/mL or 170 mg/mL).

Microbiological test over coated marble mock-ups

To evaluate the antimicrobial activity of the treatment with the impregnated MCM-41 SiO₂, the coated mock-ups were placed in some sterile cylindric recipients where SDA growth medium was poured till reaching the mock-up surface level. Once the medium was solidified, it was inoculated with fungal spores and covered the cylinder with a cotton lid to let oxygen pass through. The cylinders were incubated at 28 °C for 3 days for initial mycelium development and then monitored at Room Temperature (RT) for 60 days with a Canon EOS M50 camera.

Results

Microbiological Sampling campaign and fungi identification

The sampling campaign led to the selection of two fungi as model microorganisms, distinguished by their appearance: a black fungus (Figure 2a) with white hyphae and black spores, and a green fungus (Figure 2b) with white hyphae and green spores. Initially, morphological identification using optical microscopy classified them as *Aspergillus sp.* and *Penicillium sp.*, respectively. Subsequently, molecular sequencing confirmed their species as *Aspergillus tubingensis* CECT 21302, showing 99.82% similarity (554/555 bp) with sequence HQ632707 (reference strain IHEM 13662) and 99.80% similarity (507/508 bp) with sequence EF661086 (type strain NRRL 4875), and *Penicillium chrysogenum* CECT 21303 exhibiting 100% similarity (477/477 bp) with sequence MZ078823 (strain 426E).

The selected and isolated microorganisms were molecular sequencing and therefore identified as *Aspergillus tubingensis* (CECT 21302) and *Penicillium chrysogenum* (CECT 21303).

It is worth mentioning that both *Penicillium* sp. and *Aspergillus* sp. are two of the most common fungal genera found when dealing with the biodeterioration of stone surfaces; therefore, they have been selected for this study.



Fig. 2: Pictures of the pure colonies of the two model microorganisms taken as reference for this study: *Aspergillus tubingensis* (CECT 21302) (a) and *Penicillium chrysogenum* (CECT 21303) (b)

Minimal Inhibition Concentration Determination by Poisoned Food Technique

Minimal Inhibition Concentration (MIC) can be defined as the minimum amount of antimicrobial required to get a total inhibition. The Poisoned Food Technique determined MIC; first, *free* vegetal extracts were studied and, afterward, compared with their use encapsulated in the MCM-41 mesoporous silica matrix.

Figure 3 presents the pictures of the test results carried out on the *free* vegetal extracts, whereas Table 1 reports the relative average percentage of fungal inhibition, measured by the given formula.

Concerning the results obtained with the *free* oregano essential oil (OEO) with both fungi, it was noticed that with a concentration of 0.1% or higher, there was no fungal development over the Petri dish. A test with a lower concentration of 0.05% was also conducted; in this case, mycelium development was instead noticed. The *free* oregano EO MIC was therefore set at 0.1%.

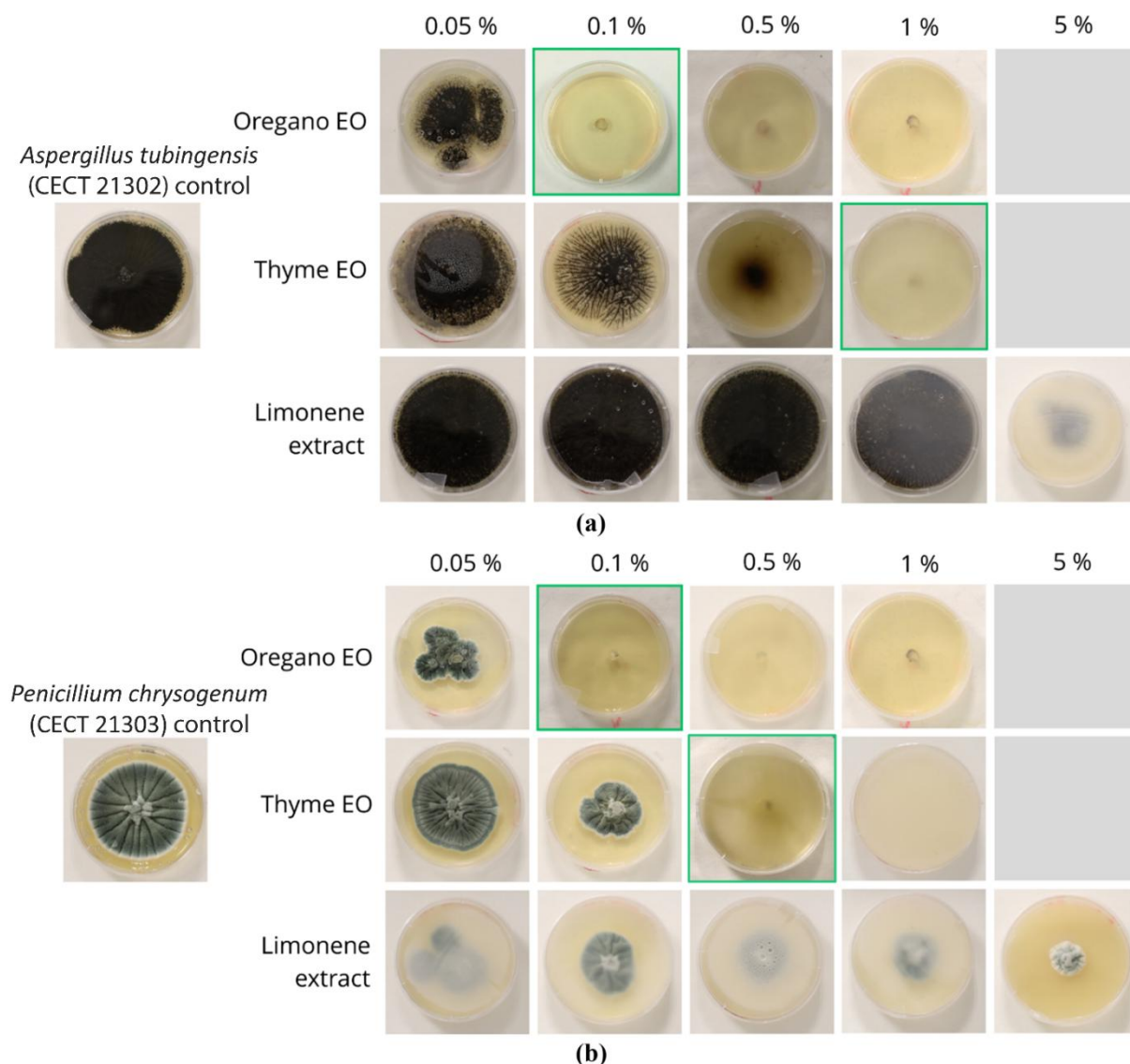


Fig. 3: Pictures of the two reference fungi *A. tubingensis* (a) and *P. chrysogenum* (b) grown on an SDA medium with *free* vegetal extracts in different concentrations, following the poisoned-food technique. Highlighted in green is the relative Minimum Inhibitory Concentration (MIC)

Table 1 – Percentage fungal inhibition of *A. tubingensis* and *P. chrysogenum* following the poisoned-food technique with *free* vegetal extracts

		Concentration of EO				
		0.05%	0.1%	0.5%	1%	5%
<i>A. tubingensis</i>	Oregano EO	29%	100%	100%	100%	nd
	Thyme EO	0%	11%	79%	100%	nd
	Limonene extract	0%	0%	0%	0%	84%
<i>P. chrysogenum</i>	Oregano EO	68%	100%	100%	100%	nd
	Thyme EO	33%	68%	100%	100%	nd
	Limonene extract	57%	68%	77%	79%	88%

The samples' average SD was < 1.5%
nd no data

For what concerns *free* thyme EO (TEO), for the *A. tubingensis*, complete inhibition was reached at a higher concentration, equal to 1%. It can be observed that at a concentration of 0.5%, partial inhibition of the mycelium development is present. For *P. chrysogenum*, complete inhibition was set at 0.5%, and at 0.1%, partial inhibition was instead observed.

Partial inhibition might prevent a high spread of microorganism over the surface but will not lead to an acceptable solution over time. The *free* thyme EO MIC was thus set at 0.5% for *P. chrysogenum* and 1% for *A. tubingensis*.

Finally, concerning the *free* limonene extract results, it was noticed that none of the concentrations previously studied successfully inhibited fungal growth. Therefore, the extract concentration was further increased to 5%. In this case, a delay in mycelium development was observed only for *A. tubingensis*. Nevertheless, it was insufficient to inhibit fungal growth totally. The concentration was not further increased since it would lead to a remarkably high amount of vegetal extract. Furthermore, it was possible to notice that the volatile components released by the limonene extract were degrading the Petri dishes' plastic lids, making them permanently opaque.

Figure 4 presents the test results on the vegetal extracts encapsulated in the MCM-41 mesoporous silica particles, whereas Table 2 reports the relative average percentage fungal inhibition.

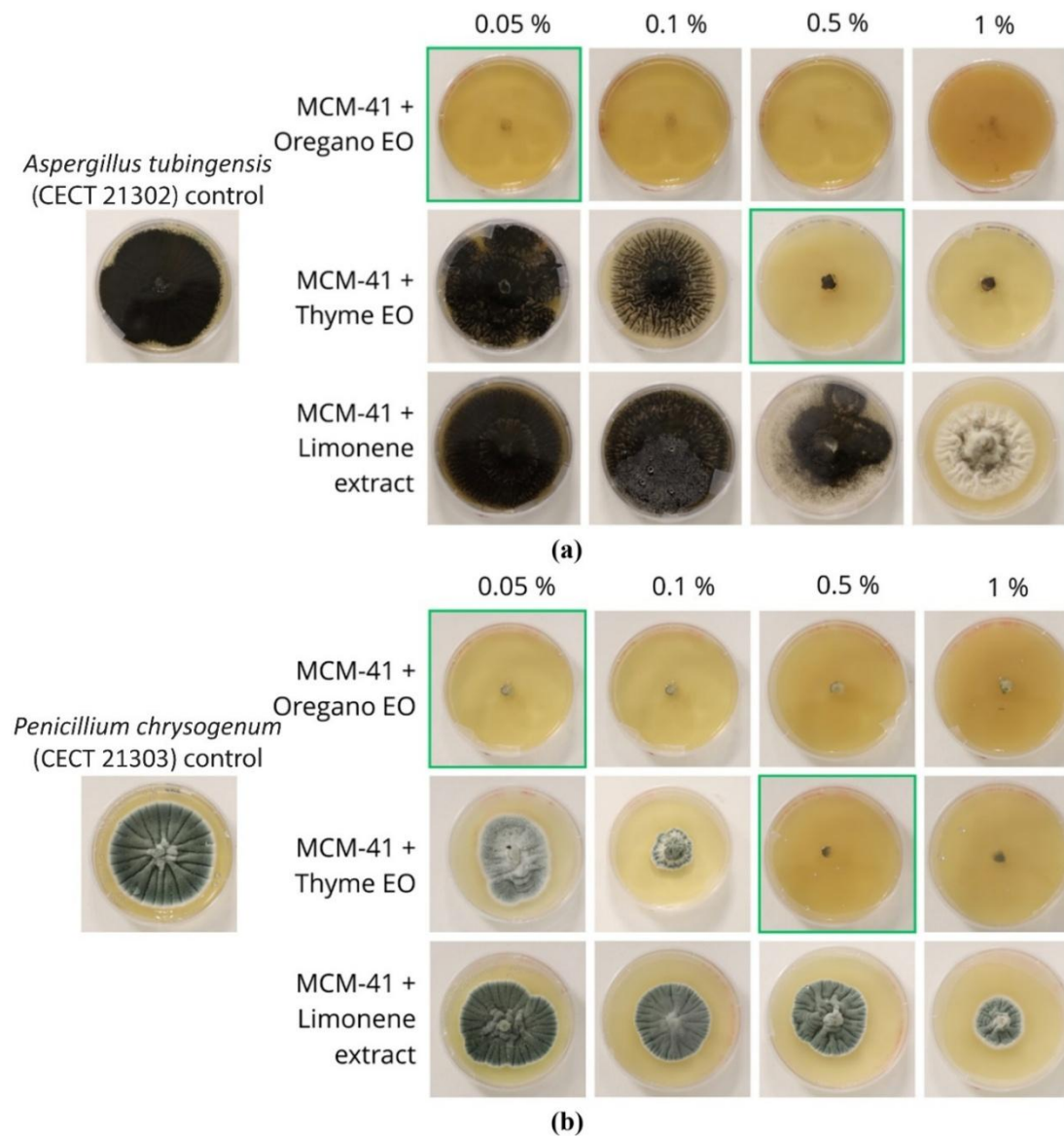


Fig. 4: Pictures of the two reference fungi *A. tubingensis* (a) and *P. chrysogenum* (b) grown on an SDA medium with vegetal extracts encapsulated in the MCM-41 SiO₂ in different concentrations, following the poisoned-food technique. Highlighted in green is the relative Minimum Inhibitory Concentration (MIC).

Table 2 – Percentage fungal inhibition of *A. tubingensis* and *P. chrysogenum* following the poisoned-food technique with encapsulated vegetal extracts in MCM-41 SiO₂

		Concentration of vegetal extract			
		0.05%	0.1%	0.5%	1%
<i>A. tubingensis</i>	MCM-41 + Oregano EO	100%	100%	100%	100%
	MCM-41 + Thyme EO	15%	29%	100%	100%
	MCM-41 + Limonene extract	0%	0%	13%	38%
<i>P. chrysogenum</i>	MCM-41 + Oregano EO	100%	100%	100%	100%
	MCM-41 + Thyme EO	58%	90%	100%	100%
	MCM-41 + Limonene extract	46%	63%	64%	83%

The samples' average SD was < 1.5%

Regarding the encapsulated oregano EO, at the concentration of 0.1%, previously identified as MIC for the non-encapsulated system, no fungal development was observed for either *A. tubingensis* or *P. chrysogenum*. The concentration was therefore halved to 0.05%, which again did not let the fungi develop in the poisoned medium. Thus, the MIC for the encapsulated oregano EO was set at 0.05% for both fungi.

Equivalent results were obtained for the encapsulated thyme EO, for which fungal growth was not noticed at concentrations of 1%. Considering the halved concentration obtained with the encapsulated Oregano EO, an encapsulated thyme EO concentration of 0.5% was evaluated. This concentration was enough to totally inhibit mycelium development in the Petri for both fungi. Tests at the lower concentrations of 0.1% and 0.05% were also conducted; in this case, after 7 days, fungal growth was observed. Therefore, the encapsulated thyme EO MIC was set at 0.5% for both fungi.

Regarding the tests conducted with the particles holding the limonene extract, the fungal growth was visible in all the evaluated concentrations; therefore, it was not possible to determine the MIC neither for the encapsulated limonene extract. However, comparing the results obtained from the *free* extract, it is possible to notice that for *A. tubingensis* (CECT 21302), at the concentration of 1%, the mycelium development and the spore formation are delayed in the encapsulated sample. Concerning instead the *P. chrysogenum* (CECT 21303), a reduction in the overall fungal development is visible proportionally to the increase of the extract concentration; another interesting detail is that the Petri dishes of the encapsulated sample are not opaque, suggesting a higher retention of the volatile molecules by the MCM-41 mesoporous particles.

The control tests with the two fungi growth in SDA alone or with SDA and MCM-41 mesoporous SiO₂ without vegetal extract showed a complete Petri dish growth after 3-7 days incubation at 28°C.

In summary, as described in Table 3, the MIC for oregano EO is 0.1% (9 mg/mL) when *free* and 0.05% (4.5 mg/mL) when encapsulated; the MIC for thyme EO is 0.5% (42.5 mg/mL) both *free* and encapsulated; and finally, the MIC for Limonene was not possible to be identified with the parameters employed in this study, nor when *free* nor encapsulated.

Table 3 – Minimal Inhibition Concentration (MIC) of the three vegetal extracts for the studied microorganisms

	<i>A. tubingensis</i> (CECT 21302)	<i>P. chrysogenum</i> (CECT 21303)
<i>Origanum compactum</i> EO	0.1 %	0.1%
<i>Origanum compactum</i> EO + MCM-41	0.05%	0.05%
<i>Thymus vulgaris CT linalool</i> EO	1 %	0.5%
<i>Thymus vulgaris CT linalool</i> EO + MCM-41	0.5%	0.5%
Limonene extract	> 5%	> 5%
Limonene extract + MCM-41	> 1%	> 1%

Overall, considering the *in vitro* results and the obtained MIC, the best performing vegetal extract resulted be *Origanum compactum* EO, which encapsulated in the mesoporous MCM-41 SiO₂ showed very promising results and was therefore selected for the test on marble mock-up surfaces.

Evaporation tests

Figure 5a shows the evaporation curves of the *free* vegetal extracts over 28 days. It can be noticed that limonene extract and thyme EO have a similar evaporation trend; within the first hours, they both almost reach complete evaporation, whereas *free* oregano EO has a slower trend, going beyond 95% after around 14 days and reaching 100% after 28 days. In Figure 5b, the curves of the *free* oregano EO (Free OEO) are compared with the encapsulated in the mesoporous MCM-41 SiO₂ (MCM-OEO); it can be noticed that the oregano EO evaporation is reduced by around 30% after 28 days, the mesopores of MCM-41 can indeed trap the volatile molecules of the EO and prolongate the evaporation time.

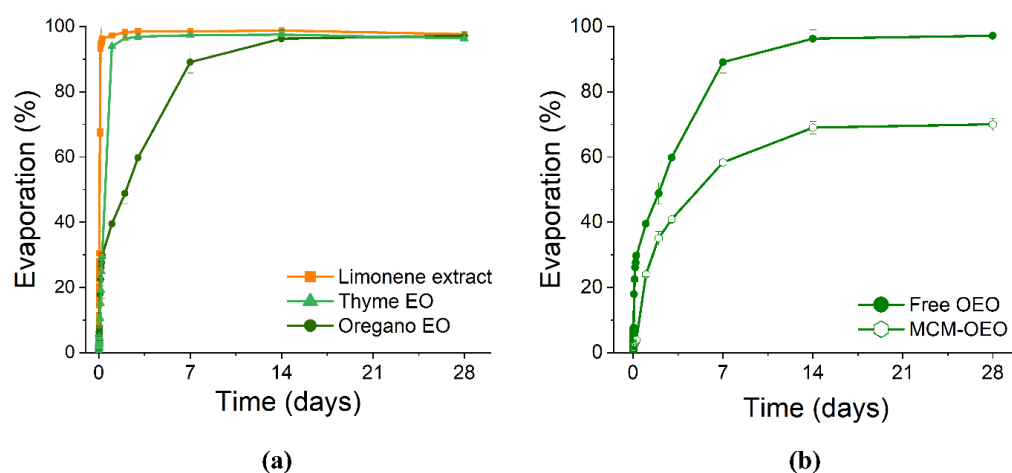


Fig. 5: Graph reporting the evaporation curves of the three *free* vegetal extracts (a) and comparison between the evaporation curve of *free* and MCM-41 SiO₂ encapsulated oregano essential oil (b)

Microbiological test on coated marble mock-ups

The fungal growth on the uncoated marble mock-ups was abundant for both fungi, presenting mycelium in the vegetative state expanding over the surface. Figure 6 illustrates this phenomenon, particularly visible in the white marble mock-ups.

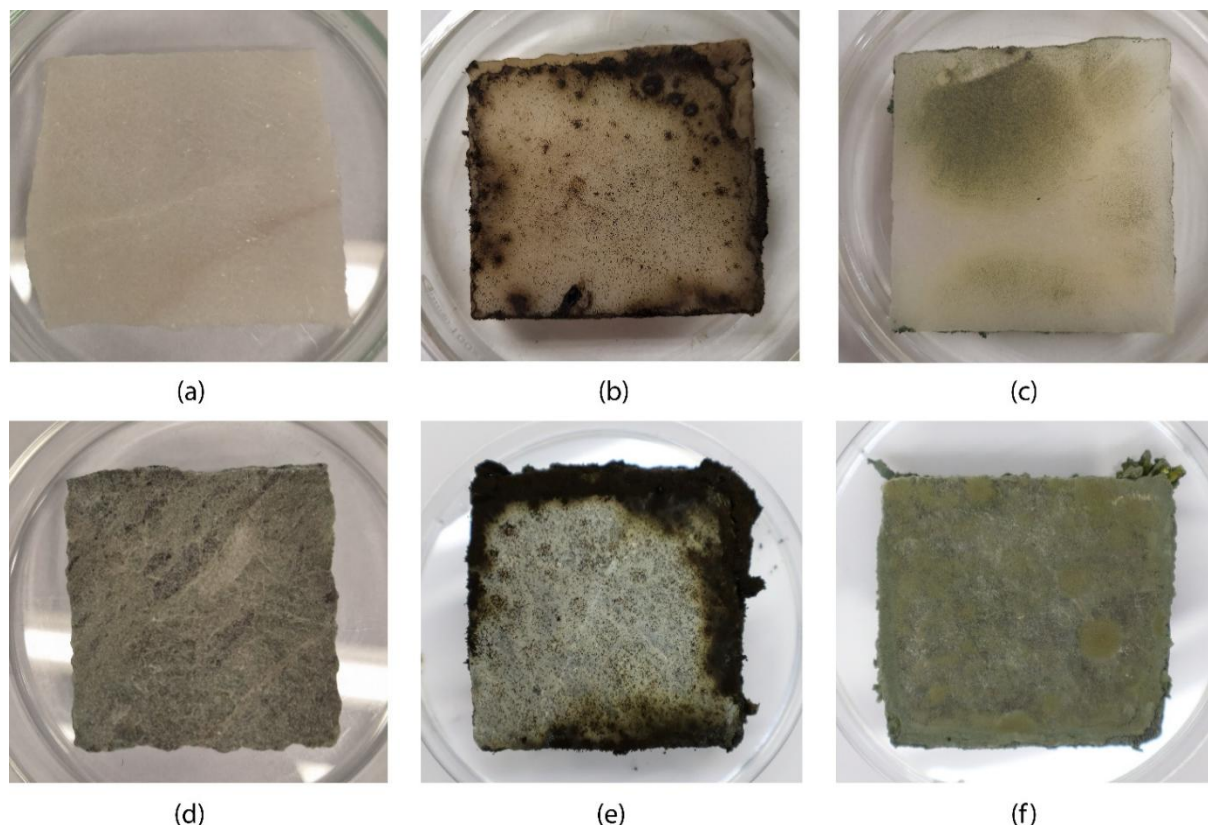


Fig. 6: Pictures of the white marble mock-ups (a, b, c) and the green marble mock-ups (c, d, f) before (a, d) and after being colonized by the fungi *A. tubingensis* (b, e) and *P. chrysogenum* (c, f). Pictures were taken after one month from inoculation.

For clarity, the results obtained from the mock-ups treated with the two coatings (*i.e.*, COT-01 and COT-1), containing MCM-41 mesoporous SiO₂ and *Origanum compactum* EO, will be reported starting from those of COT-01 (with 0.1% EO) and following with those of COT-1 (with 1% EO).

The test conducted with the *A. tubingensis* showed that, after two months, the surfaces treated with COT-01 were partially colonized by the fungi (Figure 7b), particularly visible on the white marble, where dark little dots on the surface, likely spores in a germination state, were observed. Comparable results were observed with *P. chrysogenum*, where the COT-01 coating was once again insufficient to inhibit fungal growth (Figure 7c), particularly visible on the green marble mock-up where the white hyphae had no trouble colonizing the surface. It is also interesting to

notice that the *free* oregano EO, according to the evaporation curves, has fully evaporated in one month. However, when encapsulated, it persists for a longer period, at least twice as long, according to these tests conducted on the samples over two months.

On the other hand, the COT-1 coating, which contained more EO-impregnated MCM-41, was more effective in preventing fungal colonization. Specifically, regarding *A. tubingensis*, it can be observed that the fungi are confined on the agar on the side of the mock-up (Figure 7e). As for *P. chrysogenum*, there is an overall reduction in colonization on the surface (Figure 7f); however, a greenish patina can be seen on the sides of the mock-ups, especially on the white marble one.

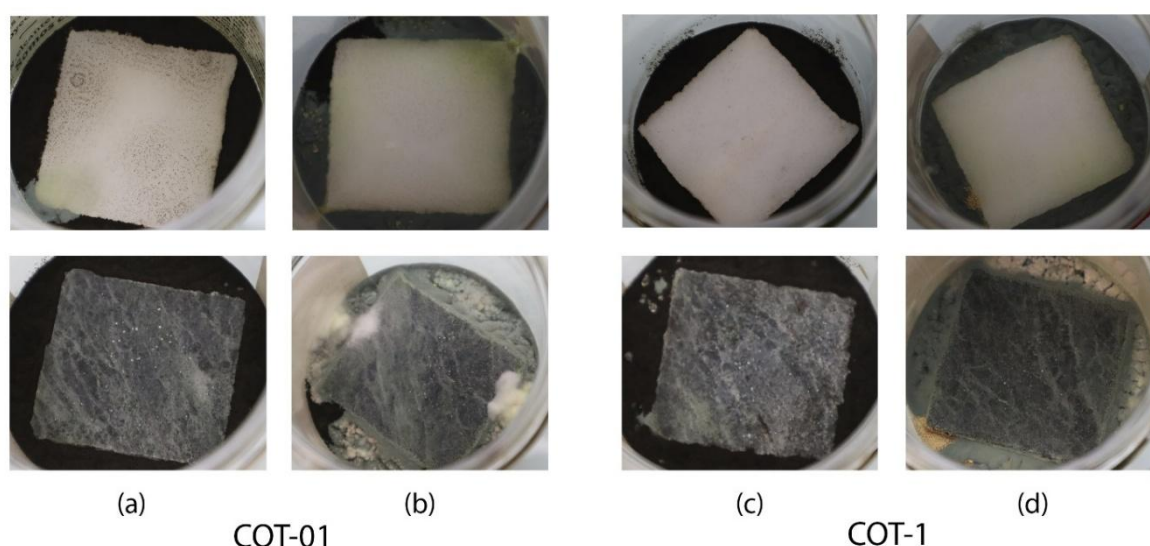


Fig. 7: The white Carrara marble (upper) and green marble (lower) mock-ups treated with the coating COT-01 and further colonization by *A. tubingensis* (a) and *P. chrysogenum* (b); and the white Carrara marble and green marble mock-ups treated with COT-1 and further colonization by *A. tubingensis* (c), and *P. chrysogenum* (d); pictures were taken after 60 days.

Discussion

The antimicrobial inhibitory potential of three plant extracts (limonene, *Origanum compactum*, and *Thymus vulgaris*) was evaluated when *free* or encapsulated in mesoporous MCM-41 silica (SiO_2), determining the plant extracts evaporation rate and Minimal Inhibition Concentration (MIC) against two fungal strains (*Aspergillus tubingensis* and *Penicillium chrysogenum*) isolated from real-case stone heritage materials.

The results indicated that encapsulation of the extracts led to a reduction in the MIC or the general growth of the fungi; it is, in fact, known from the literature that encapsulating such

vegetal extract can increase its antimicrobial activity [57,58,72,313]. Specifically, embedding the oregano essential oil into the MCM-41 mesoporous SiO₂, a halving in the MIC of the studied fungi was noticed. Overall, oregano essential oil exhibited the most favorable performance at the lowest concentration, thanks to its established antimicrobial ability (Plati and Paraskevopoulou, 2022; Milagres de Almeida et al., 2023). Subsequently, an evaporation test on the MCM-41 impregnated with oregano EO was conducted, and the result was a reduction in the evaporation time. The outcomes suggest a correlation between volatility and antifungal activity: it is known that volatile molecules contribute to the antimicrobial efficacy of the extracts [68,314]. Therefore, encapsulation within the silica matrix diminishes evaporation and enhances the antimicrobial activity, suggesting prolonged efficacy over time, as already mentioned in literature in other fields of application [103,107,315]. Indeed, the *free* oregano EO showed the slowest evaporation rate, further diminishing when encapsulated. This phenomenon can be easily linked to the MCM-41 silica ability to physically hold the active compounds into its ordered porous structure and later gradually release them [300].

Consequently, oregano EO encapsulated in mesoporous MCM-41 silica (SiO₂) was subsequently tested on white and green marble mock-ups as the principal antifungal preventive component of coatings treatments at two concentrations (0.1% and 1%).

In the *in vitro* test, the growth medium was treated with the extracts, effectively inhibiting fungal growth. In contrast, for the test on marble mock-ups, only Sabouraud Dextrose Agar (SDA) with Chloramphenicol was applied around the mock-ups, providing a fertile environment for fungal proliferation. Therefore, the quantity of EO-encapsulated MCM-41 in the coatings was adjusted to achieve an oregano EO concentration matching the MIC of *free* oregano (0.1%) and a higher concentration (1%).

Therefore, two coating bases were developed, COT-01 and COT-1, depending on the concentration of MCM-41 impregnated with oregano EO corresponding to 0.1% and 1% of *free* oregano EO, starting from a coating base made of a mixture of glycerol and ethanol, as previously studied by the authors (Lamuraglia et al., 2023).

The coating COT-01 was insufficient to inhibit fungal growth, whereas the coating COT-1 showed better efficacy in limiting microbiological proliferation. This disparity can be attributed to the distribution and concentration of MCM-41 particles within the coatings. In COT-01, the SiO₂ particles may need to be adequately spaced or uniformly dispersed across the surface. Conversely, the COT-1 coating contained more impregnated MCM-41, resulting in elevated

antimicrobial activity. Overall, the quantity of SiO₂ employed in COT-1 proved sufficient to limit fungal proliferation on the marble surfaces.

The difference between *in vitro* (MIC 0.05%) and “*in vivo*” results (MIC 1%) can be explained by underlining the difference in the two systems: in the *in vitro* test, the impregnated SiO₂ was directly dispersed in the cultivation medium where a 6 mm diameter fungal inoculum was physically placed, whereas in the “*in vivo* test” the impregnated SiO₂ used in the coatings needed to inhibit the growth of a higher amount of fungal colonies, since the cultivation medium around the stone mock-up was not “poisoned” and therefore the fungi had no problem in proliferating there.

This study highlights the potential of mesoporous MCM-41 silica encapsulating vegetal extracts as a sustainable and effective solution for inhibiting microbiological growth on cultural heritage stone surfaces. Impregnating these mesoporous particles with limonene, thyme, and oregano extracts reduced the Minimal Inhibition Concentration (MIC) required to prevent fungal proliferation, indicating enhanced antimicrobial efficacy. Moreover, the encapsulation process mitigated evaporation rates, prolonging the duration of antimicrobial activity while minimizing the quantity of extract needed [61,316]. The results with oregano essential oil (EO) were particularly promising, demonstrating the most favorable performance regarding antimicrobial activity and evaporation rate reduction.

Further tests conducted on marble mock-ups, similarly shown in literature by Ramadan S. [317], confirmed the efficacy of MCM-41 mesoporous SiO₂-encapsulated oregano EO as a component of the base of the protective coatings. The coating containing 1% oregano EO (COT-1) exhibited superior antimicrobial activity compared to 0.1% (COT-01). The disparity in effectiveness between coatings with different concentrations can be attributed to variations in distribution, the quantity of impregnated particles, and the related quantity of plant extract. Coatings with a higher concentration of impregnated MCM-41 demonstrated better antimicrobial performance, underling the importance of uniform dispersion across the surface. The findings suggest that the combination of MCM-41 mesoporous SiO₂ and vegetal extracts holds significant promise for inhibiting microbiological growth on stone surfaces, offering a sustainable alternative to traditional antimicrobial treatments. Even though mesoporous materials technology is highly studied in other fields, such as the pharmaceutical one [318–320], its employment in combination with natural extracts and its application in conservation science is still a novelty. In fact, in the literature, a lot of recent works related to the use of

essential oils for their antimicrobial features can be found. Nevertheless, the field of application, the aim, or the release system employed (if present) is different. A.P. Santo [62], in their paper, use essential oils to contrast biodeterioration on marble, showing how EOs can be employed as sustainable biocides with similar efficiency in comparison with traditional products. However, the EOs are employed for cleaning interventions: the antimicrobial action is not aimed to last for a long period. Other authors have also studied similar applications, such as M. Spada [65] or R. Candela [71]. The encapsulation of EOs is presented by G. Granata [287], M. Kapustová [321], or by F. Plati [60] employing polymer-based nanocapsules to host essential oils, for application mainly in the alimentary field. Meanwhile, I. Romano [69] presents the use of polymer-encapsulated EOs in the field of stone cultural heritage. However, the latter nanostructured materials are totally different from the one presented in this study since they are not aimed for a long-term release apart from their different chemical and physical characteristics. The same can be said for the study presented by R. Ranaldi [74] which employed EOs in hydrogel suspensions for the restoration of stone cultural heritage.

This approach addresses the critical issue of biodeterioration in cultural heritage conservation, which, as previously described, is one of the main issues to deal with in a restoration intervention [75] and presents opportunities for broader applications in architectural preservation and restoration efforts. Further research into optimizing the coating formulation and application techniques will be considered for future steps of this work.

Acknowledgments

The authors thank the Catedra EMR-CPA (Estudios y Métodos de Restauración restoration company) for contributing to the project. And thank the Sculpture Museum of the Universitat Politècnica de València Vera Campus (MUCAES- UPV) for permitting us to sample the statues. Furthermore, the authors thank Ms. Eva Gomis from Commercial Xúquer (S.A.R.L.) for the limonene extract. Andrea Campostrini would like to thank the “PON R&I 2014–2020”, “Fondo per la promozione e lo sviluppo delle politiche del Programma Nazionale per la Ricerca (PNR) 2021–2027”, and the restoration company Uni.S.Ve. S.r.l. of Venice (Italy) for financing his Ph.D. scholarship. Agustí Sala-Luis would like to thank the Generalitat Valenciana for funding his Ph.D. scholarship (CIACIF/2022/179).

Authors contributions

AC and PBR conceived and designed the research. AC and AS conducted the formal analysis, investigation, and visualization. PBR validated the data. AC wrote the original draft of the manuscript. AC, AS, PBR, EG, and FM reviewed and edited the manuscript. PBR, MS, and FM contributed resources. PBR, EG, and FM provided supervision. PBR and FM administered the project. All authors read and approved the manuscript.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper. Other eventual supplementary information is available from the authors on reasonable request.

Compliance with Ethical Standards

Conflict of interest:

Andrea Campostrini declares that he has no conflict of interest.

Agustí Sala-Luis declares that he has no conflict of interest.

Pilar Bosch-Roig declares that she has no conflict of interest.

Elena Ghedini declares that she has no conflict of interest.

Michela Signoretto declares that she has no conflict of interest.

Federica Menegazzo declares that she has no conflict of interest.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

V.

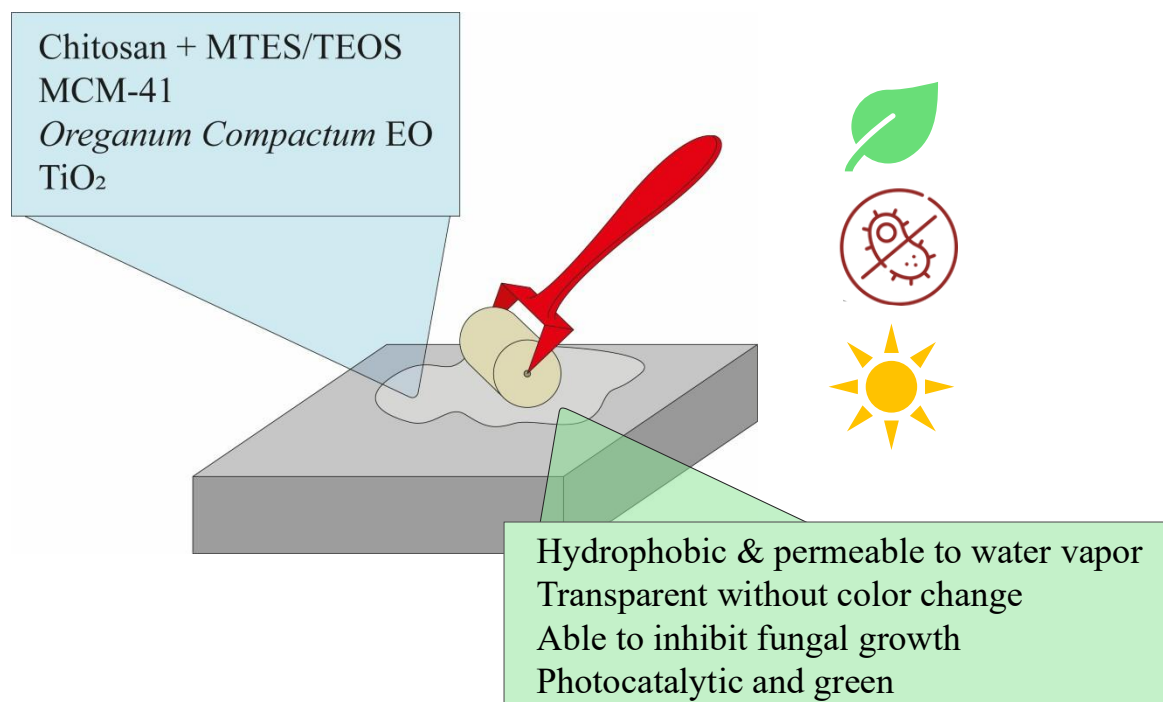
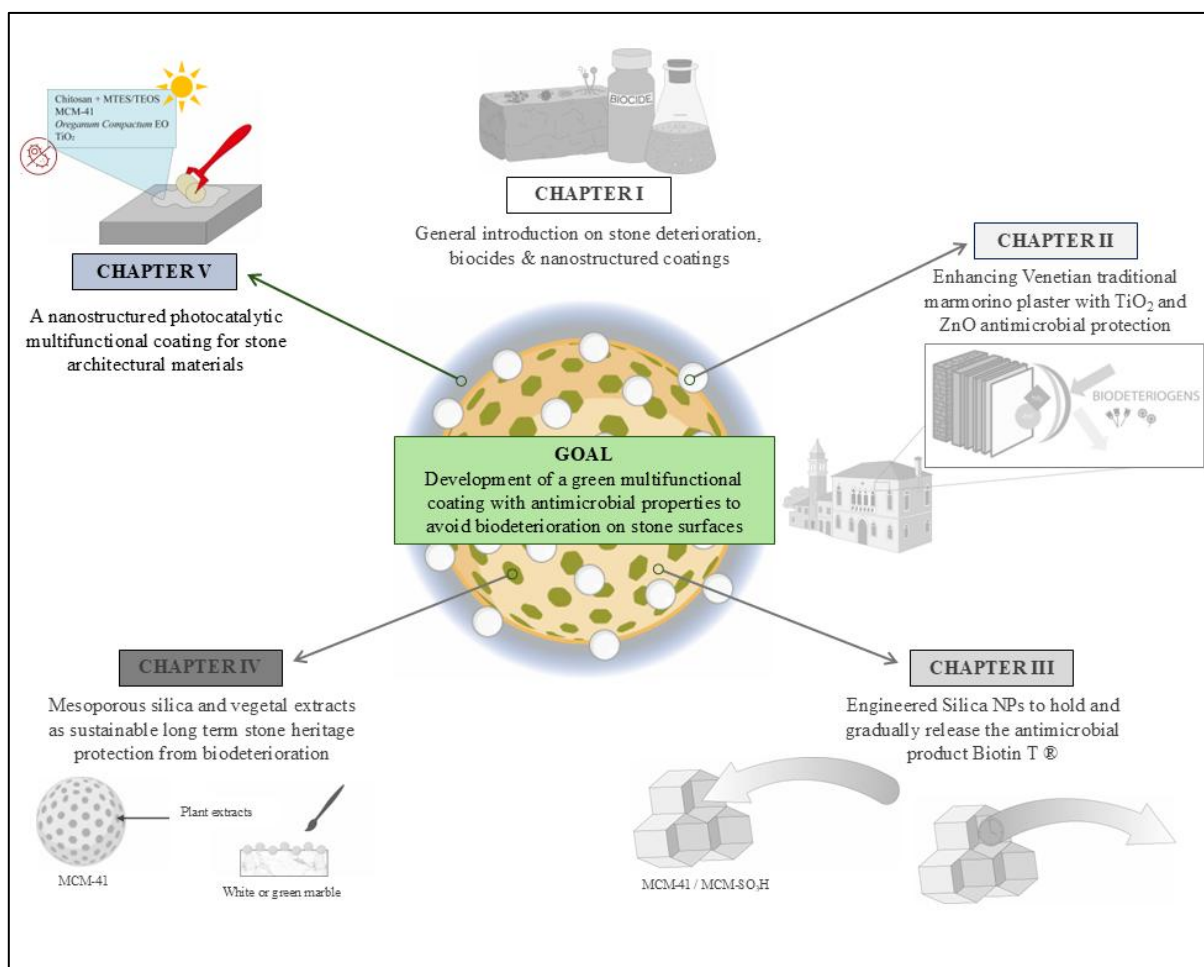
**A NANOSTRUCTURED PHOTOCATALYTIC
MULTIFUNCTIONAL COATING FOR STONE
ARCHITECTURAL MATERIALS**

Manuscript in preparation

This work, or part of it, has been presented at the following scientific conferences:

- International Congress Chemistry for Cultural Heritage 7th ChemCH Congress (Bratislava, July 2-5, 2024) – *TiO₂ enriched SiO₂ Nanocontainers for the protection of stone surfaces*, A. Campostrini, T. Botrè, E. Ghedini, M. Signoretto, and F. Menegazzo
- National conference of the Italian Chemistry Society “Chemistry: Elements of Future – SCI2024 – XXVIII National Congress” (Milan, August 26-30, 2024) – *TiO₂-loaded MCM-41 for surface stone protection*, T. Botrè, A. Campostrini, E. Ghedini, M. Signoretto, and F. Menegazzo
- International conference on analytical techniques in art and cultural heritage, TECHNART 2025 (Perugia, May 06-09, 2025) – *Advanced hybrid coatings with TiO₂ and SiO₂ for the preservation of architectural heritage*, A. Campostrini, T. Botrè, E. Ghedini, A.W. Robertson, M. Signoretto, and F. Menegazzo
- International conference of Architecture, Engineering and Design for Monuments Safeguarding – AID Monuments 2025 (Perugia, May 21-24, 2025) – *Hybrid coating embedding TiO₂ and SiO₂ nanostructured materials for the protection of Built Heritage*, T. Botrè, A. Campostrini, E. Ghedini, M. Signoretto, and F. Menegazzo
- 13th International Conference on Environmental Catalysis – ICEC 2025 (Isola delle Femmine, June 2-5, 2025) – *Photocatalysts and essential oils: could they ever be friends?*, A. Campostrini, T. Botrè, S. Manente, A. Sala-Luis, P. Bosch-Roig, E. Ghedini, M. Signoretto, and F. Menegazzo

Part of this work will also be submitted to **Lecture Notes in Civil Engineering**, Springer, T. Botrè, A. Campostrini, E. Ghedini, M. Signoretto, and F. Menegazzo.



A nanostructured photocatalytic multifunctional coating for stone architectural materials

Andrea Campostrini¹, Teresa Botrè¹, Elena Ghedini^{1,2}, Sabrina Manente¹, Alex W. Robertson³, Pilar Bosh-Roig⁴, Michela Signoretto¹, and Federica Menegazzo^{1,*}

¹ Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy;

² Department of Chemistry, University of Bari Aldo Moro, Via Edoardo Orabona 4, 70121 Bari, Italy;

³ Department of Physics, University of Warwick, Coventry CV4 7AL, United Kingdom;

⁴ Instituto Universitario de Restauración del Patrimonio, Universitat Politècnica de València, Camí de Vera s/n, 46022 Valencia, Spain;

* Correspondence: federica.menegazzo@unive.it; Tel.: (+39) 0412348552

Abstract

Stone materials have historically been employed as construction resources due to their exceptional strength and durability. However, their exposure to outdoor environments results in gradual decay caused by chemical, physical, and biological agents. Among these, biodeterioration poses a significant threat to the conservation of architectural artifacts. Preventive actions, such as the application of protective coatings, are crucial to mitigate these degradation phenomena.

This study aims to formulate a hybrid protective coating by combining titanium dioxide (TiO₂) nanoparticles with mesoporous silica (SiO₂) MCM-41 nanocontainers impregnated with *Origanum compactum* essential oil (EO), offering the synergy of antimicrobial and photocatalytic properties.

Different TiO₂/SiO₂ materials were synthesized and characterized by N₂ physisorption, SEM-EDS, and HRTEM to evaluate their structural properties and the TiO₂ dispersion. Photocatalytic activity was evaluated in liquid to assess the best-performing TiO₂/SiO₂ composite material. The latter was employed to hold the *Origanum compactum* EO and it was, finally, introduced in a hybrid hydrogel composed of chitosan and a mixture of silicon alkoxides (MTES and TEOS) to formulate the protective coating. The coating's features were thus evaluated by assessing hydrophobicity, water permeability, colorimetry, and antimicrobial efficacy of the product, once applied to stone mock-ups. The proposed hybrid coating represents a multifunctional, eco-friendly solution for protecting stone architectural materials. Combining antimicrobial and self-cleaning functionalities, it provides a sustainable approach to preserving cultural heritage, while minimizing environmental impact and operator risks.

Keywords: Silica nanocontainers, Titanium dioxide (TiO₂), protective coating, antimicrobial, hybrid hydrogel

1. Introduction

Stone materials, such as granite, limestone, sandstone, and marble, are widely used in the construction and architectural sector due to their durability and aesthetic properties. However, these materials are prone to degradation mainly caused by several weathering mechanisms: mechanical, chemical, and biological agents can lead to structural and aesthetic deterioration [7,12,42]. The protection of stone surfaces is therefore critical, especially for those exposed outdoors [322]. In this context, protective coatings play a crucial role in avoiding direct contact with the substrate and enriching the surface with innovative useful features [123]. Ideally, protective coatings should be durable, transparent, non-toxic for the operator, sustainable, and hydrophobic while permeable to water vapor [18,116,117,122]. Water indeed is one of the principal factors that lead to several deterioration phenomena; among these, surely biodeterioration plays a crucial role [13,20]. For this reason, another interesting aspect that a protective coating might have is the ability to inhibit microbiological growth [37,246].

Recent advancements in nanotechnology have led to the development of innovative coatings, based on nanostructured materials, that enhance the self-cleaning and protective properties of treated surfaces [82,115,134,253]. An example is titanium dioxide (TiO_2) which is a well-known photocatalyst with excellent photocatalytic and self-cleaning properties, making it suitable for stone protection [150,154,177,216,218]. Strictly linked to its photocatalytic activity, it has also been investigated for its antimicrobial properties in several fields of application [153,163,212,323]. Nevertheless, due to its white coloration, its application in the cultural heritage field is still challenging.

Another interesting sustainable way to combat biodeterioration is the use of natural substances, such as essential oils (EOs) [56,61,80,288]. Their wide and high concentration of active molecules makes them good candidates to act as antimicrobial agents against the microorganisms that easily proliferate on stone surfaces [46,75]. Nevertheless, the high volatility of their active molecules and the color stain they can leave on the surface limited their use to cleaning interventions maintaining a short time of contact with the substrate and possibly not exploiting their full potential. In this sense, different ways for their embedment into nanostructured matrices or polymers have been studied for applications in the industrial pharmaceutical field, whereas their use in restoration is still a developing novelty [58,67,275,295].

In this regard, nanocapsules of ordered mesoporous silica (SiO_2) are a perfect candidate. Mesoporous materials are in fact known for their ability to interact with ions and molecules on both their external and internal surfaces, making them able to encapsulate and later release an active [145,267,268]. For this feature, they are highly investigated in several fields of application, especially as drug delivery systems (DDS). These materials are produced through the self-assembly of surfactants, which mold the inorganic component and are characterized by an ordered mesostructure, high surface area, and well-defined properties, making them ideal for encapsulating molecules of all kinds, from proteins to drugs to biocides [289]. Mesoporous silicas, such as MCM-41, are highly efficient matrices in the design of controlled release systems due to characteristics like pore size and ordered structure [145,277].

This study focuses on the development of a novel hybrid protective coating based on mesoporous silica MCM-41 nanocontainers, enriched with TiO_2 and loaded with *Origanum compactum* essential oil. These materials would be then embedded in an organic-inorganic hybrid matrix composed of chitosan and a mixture of silicon alkoxides (methyltriethoxysilane and tetraethylorthosilicate). The proposed formulation maximizes the beneficial properties of each individual component while minimizing potential side effects that may occur when used separately. In fact, the formulation would be able to exploit the photocatalytic activity of titania, while also harnessing the encapsulation of a natural antimicrobial compound thanks to the mesoporous silica, avoiding undesired color, evaporation, or health-related issues.

2. Materials and Methods

The protective coating was developed through a multi-step process, beginning with the synthesis of mesoporous silica (MCM-41), the subsequent impregnation with titanium dioxide (TiO_2) nanoparticles and essential oil of oregano, and finally the formulation of the hybrid hydrogel matrix.

2.1 Synthesis of MCM-41

The synthesis of silica mesoporous particles MCM-41 was performed using a procedure previously reported by the authors [300,324,325]. Briefly, MCM-41 was synthesized using tetraethyl orthosilicate (TEOS) as a silica source in an alkaline medium with hexadecyltrimethylammonium bromide (CTAB) as a template in NaOH basic solution. The molar ratio of the reagents is 1 SiO_2 : 0.12 CTAB : 0.53 NaOH : 590 H_2O . The mixture was

stirred, aged, and then calcined at 510 °C with a rate of 0.5 °C/min in flowing air (50 mL min⁻¹) for 6 hours to remove the template and achieve the mesoporous structure.

All the materials employed for this step were purchased from Sigma-Aldrich® (Merck Life Science S.r.l., Milan, Italy).

2.2 Impregnation with titanium dioxide

The incipient wetness impregnation (IWI) method was employed to load MCM-41 with 20 wt.% TiO₂ [326,327] The IWI was performed using three commercially available TiO₂ samples: Evonik P25, usually regarded as benchmark photocatalyst [328], Kronos VLP, and Mirkat 211. The main features of these three materials are reported in **Table 1** [329].

The TiO₂-enriched MCM-41 samples were respectively labelled as MP (MCM-41 with Evonik P25 TiO₂), MM (MCM-41 with Mirkat 211 TiO₂), and MK (MCM-41 with Kronos VLP TiO₂), and were then characterized to assess their structural integrity and surface properties.

Table 1 – Textural properties of commercial titania samples

<i>Sample</i>	<i>Specific surface area (m² g⁻¹)</i>	<i>Pore size range (nm)</i>	<i>Pore volume (mL g⁻¹)</i>	<i>Crystallite size (nm)</i>	<i>Band gap (eV)</i>	<i>Crystal phase</i>
Evonik P25	52	4-80	0,13	40	3.15	Rutile and anatase
MIRKAT 211	217	4-10	0,27	11	3.25	Anatase
KRONOS VLP	269	4-10	0,30	15	3.21	Anatase

2.3 Impregnation with oregano essential oil and TiO₂

The MCM-41 mesoporous silica was first impregnated by the Incipient Wetness Impregnation (IWI) method with the *Origanum compactum* essential oil (EO), leading to a concentration of 1.6 mL g⁻¹, once dried at T < 30 °C. The *O. compactum* essential oil was purchased from Pranarôm International (S.A.), Ghislenghien, Belgium (BE), and chosen for its antimicrobial and biocide properties [330–332]. The material was thus labeled as MO.

The material was then further impregnated with 20 wt.% of Evonik P25 TiO₂, following the same procedure previously described. The sample prepared was thus labeled as MOT (MCM-41 with oregano EO and Evonik P25 TiO₂). Evonik P25 was selected for this formulation since it is the material which presented the highest photocatalytic activity also when combined with MCM-41 SiO₂.

2.4 Formulation of protective coating

A hybrid organic-inorganic hydrogel was selected as the best-suited matrix for supporting the MOT material. For the organic component, the attention was focused on the use of the biopolymer chitosan, whereas for the inorganic part, a mixture of 70:30 of methyltriethoxysilane (MTES) and tetraethylorthosilicate (TEOS) was employed as SiO_2 source in the sol-gel process (**Figure 1**).

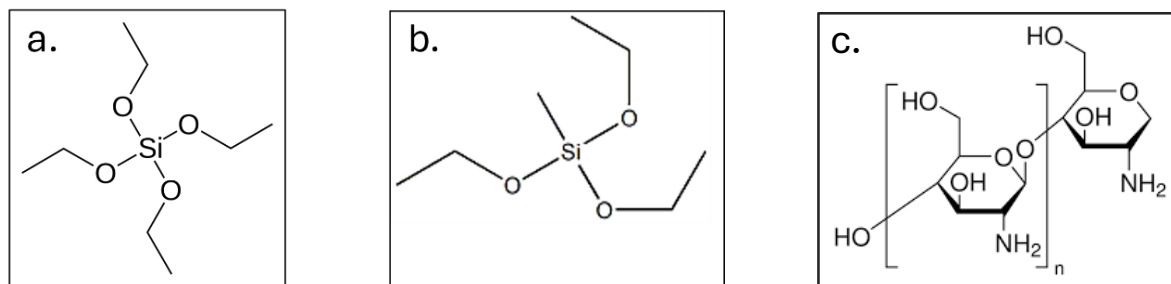


Figure 1: Chemical structures of TEOS (a), MTES (b), and chitosan monomer (c)

For this preparation, 0.7 mL of MTES and 0.3 mL of TEOS were mixed in 4 mL of CH_3COOH 0.1 M solution with two drops of 0.2 M HCl, acting as the gelation catalyst. Then it was subjected to ultrasonic treatment for 40 minutes to initiate hydrolysis and condensation reactions. 40 mg of MOT was then added, and the suspension was ultrasonicated for 1 hour. In parallel, 30 mg of chitosan powder was dissolved in 3.5 mL of CH_3COOH 0.1 M solution, where 1 mL of HCl 0.4 M was added to act as a gelation catalyst. The chitosan solution was stirred at 800-900 rpm for 1 hour until complete dissolution.

Finally, the MTES-TEOS mixture containing MOT was added to the chitosan solution. The resulting formulation was stirred at 300 rpm for 30 minutes to ensure interaction between the silica network and the chitosan. This process resulted in the formation of the hybrid hydrogel protective coating.

The final formulation was then labeled as C-MOT.

As a reference for C-MOT, hydrogels were prepared using only MO, MP, and without any mesoporous SiO_2 . These three formulations were therefore labeled C-MO, C-MP, and C, respectively.

For clarity, the different coating formulation components are summarized in **Table 2**.

Table 2 – Composition of the different coating formulations, where the full dot (●) represents the presence of the component, whereas the empty one (○) represents its absence.

Coating name	Hydrogel chitosan/MTES-TEOS	MCM-41	<i>Origanum compactum</i> EO	TiO ₂
C	●	○	○	○
C-MO	●	●	●	○
C-MP	●	●	○	●
C-MOT	●	●	●	●

2.5 Characterization of the SiO₂-TiO₂ samples

2.5.1 N₂ physisorption technique

Nitrogen physisorption technique was employed to study specific surface areas and pore size distributions of the materials by evaluating N₂ adsorption/desorption isotherms at -196 °C using a Tristar II Plus Micromeritics instrument. The samples underwent a pre-treatment at 200 °C before the analysis. The surface area was calculated using the B.E.T. method, whereas pore size distribution was determined by the B.J.H. method, applied to the N₂ desorption branch of the isotherm. For all the surface area data the error reported is lower than 2%.

2.5.2 Electron microscopy techniques

Scanning Electron Microscopy (SEM), coupled with Energy Dispersive X-ray Spectroscopy (EDS), and High-Resolution Transmission Electron Microscopy (HR-TEM) analyses were employed to study the morphological modification of the silica matrices, to study the nanostructured pores' dimensions, and to confirm the effective addition of the TiO₂. The analyses were conducted using a Zeiss Gemini SEM equipped with an Oxford Instruments SDD EDS detector and a JEOL 2100 HRTEM with a Gatan OneView detector. SEM images were gained at an accelerating voltage of 5 kV with the secondary electron detector, while EDS maps were captured at an accelerating voltage of 10 kV. SEM sample preparation involved spreading a thin layer of powdered sample onto adhesive conductive carbon tape. HRTEM imaging was instead carried out at an accelerating voltage of 200 kV. For HRTEM samples, the material was sonicated in ethanol for 5 minutes, then drop-cast onto a holey carbon copper grid (Agar Scientific) and left to air dry overnight. Data was collected using the Electron Microscopy Research Technology Platform at the University of Warwick.

2.6 Photocatalytic Activity Tests

The photocatalytic efficiency was evaluated by measuring the degradation under UV light of methylene blue (MB), chosen as model standard compound sensitive to photocatalytic degradation [215,333–335]. UV-visible spectroscopy was finally employed to monitor the degradation process [336,337].

The test was conducted as follows: [217,338] 125 mL of standard aqueous solution of MB 1×10^{-5} M was placed under magnetic stirring at 350 rpm. In the absence of illumination, 20 mg of the sample was poured into the MB solution and left stirring for 15 minutes until a homogeneous solution was obtained. The solution was then irradiated with a 125 W mercury UV lamp with an irradiance of 40 W m^{-2} (spectral range 315–400 nm, HG100; Jelosil Srl) and 5 mL of the solution was withdrawn at regular intervals (0, 5, 10, 20, 40, 60, 90, 120 minutes). All samples were diluted with 5 mL of Milli-Q water and finally analyzed to monitor the degradation of MB over time with a Cary5000 UV-Vis spectrophotometer (Agilent Technologies). All data sets were processed with the software OriginPro 2021 by OriginLab, Northampton, MA, USA.

2.7 Application of the protective coating formulations

The coatings were applied in sterile conditions on previously sterilized white Carrara marble and green Serpentine marble mock-ups and on laboratory-prepared concrete mock-ups. For each mock-up, the top surface was treated with 0.021 mL/cm^2 of product, on average. The application was carried out using a roller to ensure consistent and even application of the coatings, while the other surfaces remained untreated. The concrete mock-ups were composed of sand, lime, and cement in a ratio of 4:2:1. These mock-ups were prepared using silicone molds, obtaining $5 \times 5 \times 2.5 \text{ cm}$ samples. The marble mock-ups, also of surface 5×5 , were sterilized by a 60-minute cycle at 120°C in an autoclave (HICLAVE HV, 110 L, HMC Europe) to then carry out microbiological tests.

2.8 Evaluation of the protective coating formulations properties

Once the coatings were applied to the mock-ups, various tests were carried out to evaluate the properties of the formulations.

2.8.1 Hydrophobicity and water vapor permeability tests

The hydrophobic properties of the treated surfaces were investigated by measuring the contact angle, following the UNI EN 15802 standard [339]. Briefly, $10 \mu\text{L}$ of deionized H_2O was deposited on the surfaces and after 10 seconds registered with a Celestron Deluxe Digital

Handheld Microscope. The related contact angle was subsequently calculated, using ImageJ software.

The water vapor permeability test was carried out following the procedure described in the UNI EN 15803 standard [340]. In summary, the mock-ups were placed in a container, with 50 mL of a 93% KNO₃ solution, and sealed with microcrystalline wax, a refined paraffin. The test was performed both on treated and untreated mock-ups to compare the water vapor permeability. The weight of each sample was registered daily, as well as the Relative Humidity (RH%) and the T.

2.8.2 Colorimetric analysis

Color measurements were conducted with a Konica Minolta CM-26dG spectrophotometer both before and after the application of the protective coating. Results were elaborated by the software Spectra Magic NX. The total color variation, expressed as ΔE , was calculated by the following equation:

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

where ΔL^* , Δa^* and Δb^* are the differences between the values obtained on the same sample before and after the application of the coating.

For the colorimetric evaluation, the Specular Component Included (SCI) data were considered.

2.8.3 Antimicrobial properties

For this test *Penicillium chrysogenum* and *Aspergillus niger*, both fungi Ascomycota Eurotiales, were chosen as model pathogen microorganisms. In particular, the two fungi come from a real-case scenario in Ca' Cappello, also known as Palazzo Cappello Layard, a historical palace part of the Ca' Foscari University of Venice. The fungi were sampled non-invasively by sterile swabs and let grow in pure culture in Petri dishes ($\varnothing = 100$ mm) with an SDA cultivation medium in an incubator (VELP, Refrigerated Thermostats FOC 225E) at 18 °C for 7 days, reaching an optimal inoculation growth level with active sporulating conidiophores.

The test was based on a system, consolidated by the authors, used for monitoring fungal growth. Specifically, to evaluate the antimicrobial activity of the various formulation treatments, each coated mock-up was placed in a sterile cylindrical container filled with SDA growth medium up to the surface level of the mock-up. Once the medium solidified, it was inoculated with 1 mL of fungal spore solution (approx. 500 ± 25 spores mL⁻¹), and the container was covered

with a cotton and aluminum foil lid and secured with an elastic band. **Figure 2** reports the main components of the setup. All the parts were sterilized in an autoclave (HICLAVE HV, 110 L, HMC Europe) for 60 minutes at a temperature of 120 °C before the test started.

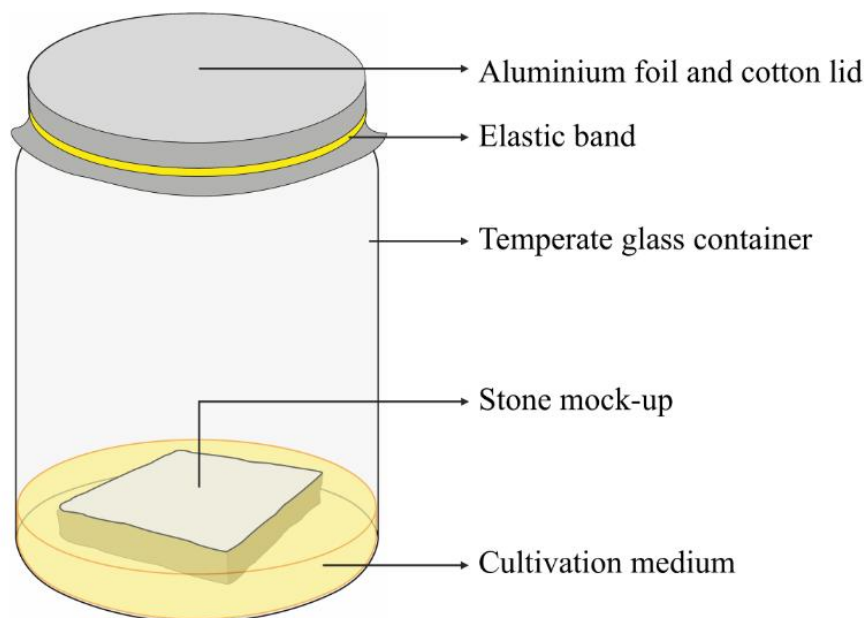


Figure 2: Schematic representation of the system set-up for the microbiological test

The containers were monitored at room temperature (RT) for up to 60 days, with pictures taken at regular intervals (2, 7, 14, 35, and 60 days) to evaluate fungal growth. In the end, a sampling by Fungi-Tape™ was carried out on the center of the mock-ups to microscopically evaluate the fungal growth.

For this test white Carrara marble and green Serpentine marble mock-ups were employed, repeating the test in triplicate ($n=3$). C-MOT coating was tested, keeping as reference the mock-ups treated with C-MO, C-MP, C, and some uncoated ones.

3. Results

3.1 Characterization of the $\text{SiO}_2/\text{TiO}_2$ materials

The samples' isotherms and pore size distributions are shown in **Figures 3a and 3b** respectively; whereas **Table 3** reports the surface area, average pore diameter, and pore volume of all the samples. The nitrogen physisorption analysis of the MCM-41 sample confirms its successful synthesis, exhibiting an average pore diameter of 2.7 nm, a high surface area of

1249 m² g⁻¹, and a well-defined mesoporous structure, as evidenced by its characteristic Type IV isotherm and hysteresis loop. For the titania-impregnated samples (MP, MM, MK), the isotherms maintain a Type IV shape but with reduced adsorbed gas volume. The widening and partial change in the shape of the loop can be associated with a change in the shape and size of the mesopores, making them less available, likely due to the partial blockage of the pores by the titania nanoparticles [341].

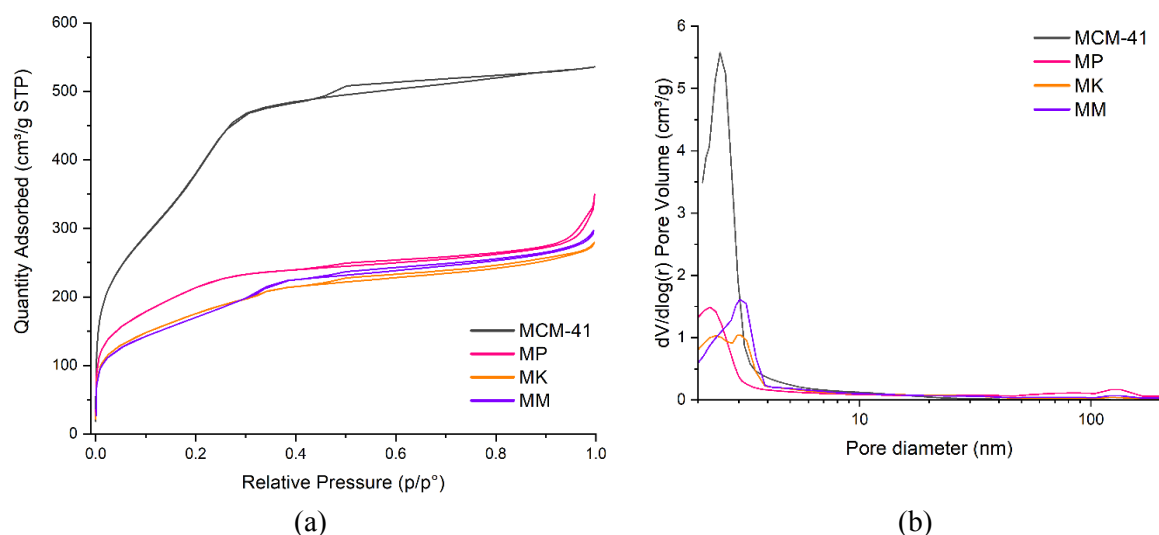


Figure 3: N₂ adsorption-desorption isotherm (a) and pore radius distribution (b) of MCM-41 and TiO₂-enriched MCM-41 samples

Table 6: specific area, average pore size, and pore volume of MCM-41, MP, MM, and MK

	Surface area (m ² g ⁻¹)	Average pore diameter (nm)	Pore volume (cm ³ g ⁻¹)
MCM-41	1249	2.7	0.85
MP	763	3.2	0.45
MM	617	3.3	0.45
MK	626	3.1	0.39

Overall, in all samples, the addition of titania allowed the maintenance of proper surface area and porosity values without significantly compromising the silica texture. This is essential for photocatalytic applications as it ensures adequate adsorption surface area. To further confirm these results, electron microscopy analyses were conducted.

Figure 4a displays a micrograph of the MCM-41 sample, highlighting an overall well-defined morphology, and uniform shapes, indicative of a highly ordered morphology of the SiO₂ particles. **Figure 4b** provides instead a higher magnification view, focusing on the

arrangement of the mesoporous channels within the MCM-41 sample, showing a periodic arrangement of pores. The regular, hexagonal pattern of the pores confirms the successful formation of the mesoporous silica framework. This hexagonal packing is a defining characteristic of MCM-41, indicating the material's high degree of structural regularity.

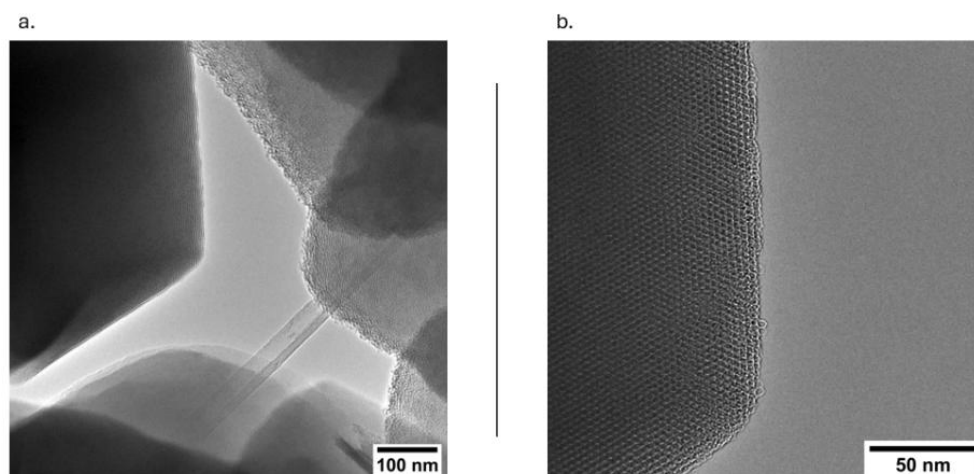


Figure 4: HRTEM micrographs of MCM-41 sample

The HRTEM micrographs, presented in **Figure 5**, of the samples MP, MM, and MK reveal the excellent structural integrity preservation of the MCM-41 structure post-impregnation with the TiO_2 nanoparticles. Of particular interest are the distinct and parallel lattice fringes, which suggest the presence of crystalline TiO_2 on the silica surface, partially covering it. These fringes are indicative of well-dispersed TiO_2 nanoparticles, likely in the anatase or rutile phase [342]. Therefore, the HRTEM analysis confirms that the MCM-41 structure remains unaffected by the impregnation with the three TiO_2 and their ordered nature is preserved across all samples.

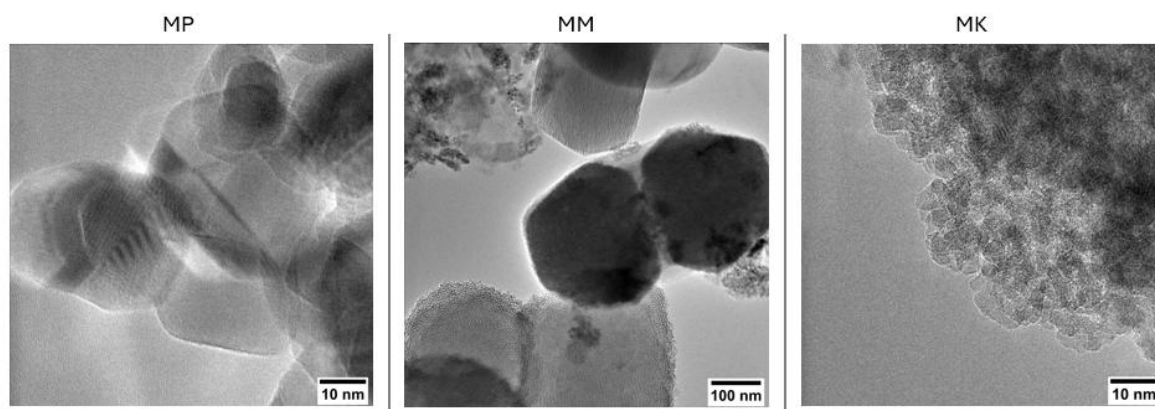


Figure 5: HRTEM micrographs of MP, MM, and MK

The SEM micrograph of MCM-41, presented in the top left corner of **Figure 6**, shows particles with spherical shapes, consistent with the expected morphology of MCM-41. The surface texture appears mostly smooth, with minor irregularities. The size (ranging from 100 to 500 nm) and shape of the material are critical factors influencing the properties of the final protective coating, such as transparency, hydrophobicity, homogeneity, and viscosity. Specifically, the spherical shape form could significantly affect the roughness of the final coating, which subsequently reduces wettability and increases impermeability [343,344].

The same characteristics can be found in the samples impregnated with TiO_2 , where the uniform distribution of titanium can be observed, overlapping the EDS map to the SEM micrographs (**Figure 6**). The consistent Ti signal across the sample suggests that TiO_2 is homogeneously distributed on the external surface of the silica material; if TiO_2 NPs were confined to the internal pores, its signal would likely appear less uniform and more localized to specific regions. The EDS maps also reveal an overlapping presence of Si and O across all the samples. Silicon, associated with the MCM-41 framework, and oxygen, present in both silica and titania structures, demonstrate the thorough integration of titania within the silica matrix.

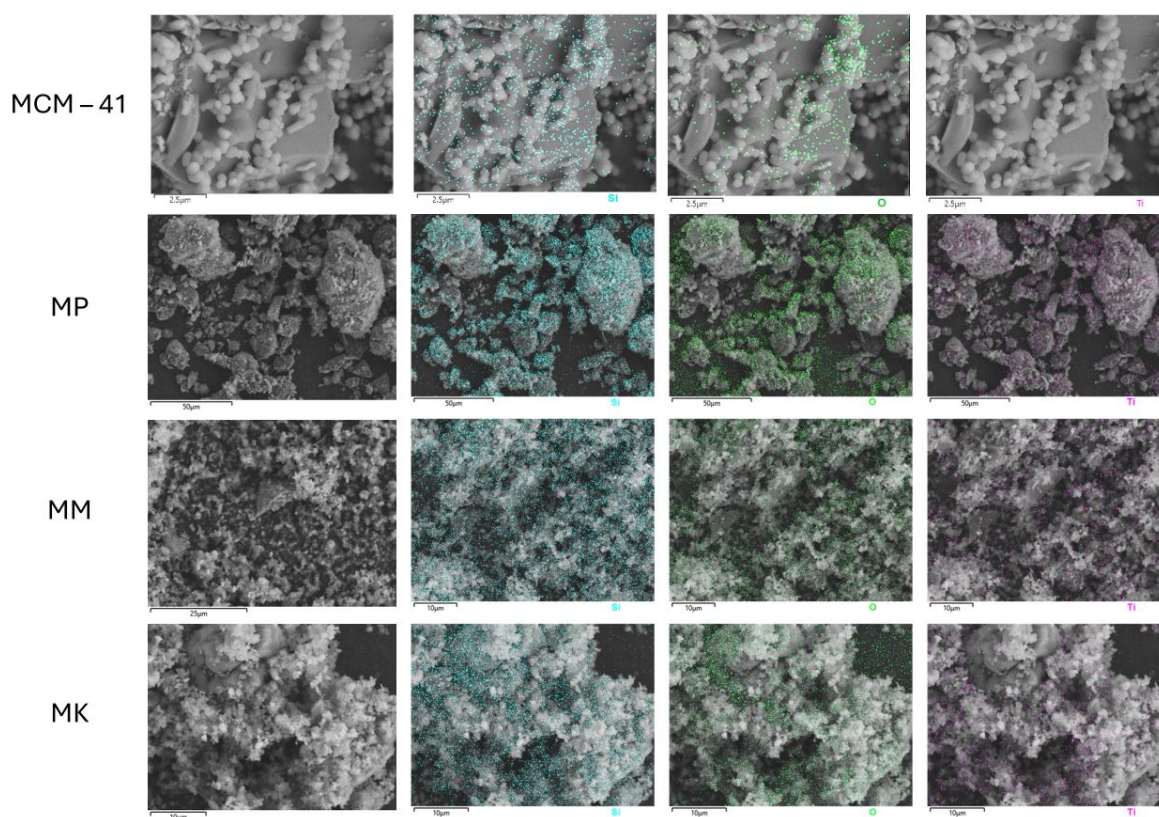


Figure 6: SEM micrographs of MCM-41, MP, MM, and MK, overlapped to the EDS maps identifying Silicon (blue marked), Oxygen (green marked), and Titanium (purple marked)

3.2 Photocatalytic activity test

To evaluate the photoactivity of TiO₂/SiO₂ composite materials, the samples were tested for their ability to degrade Methylene Blue (MB) under UV light. **Figure 7** illustrates the MB degradation profiles for pure MCM-41, MP, MM, and MK, with degradation efficiency plotted as MB's relative concentration (C/C_0) over time.

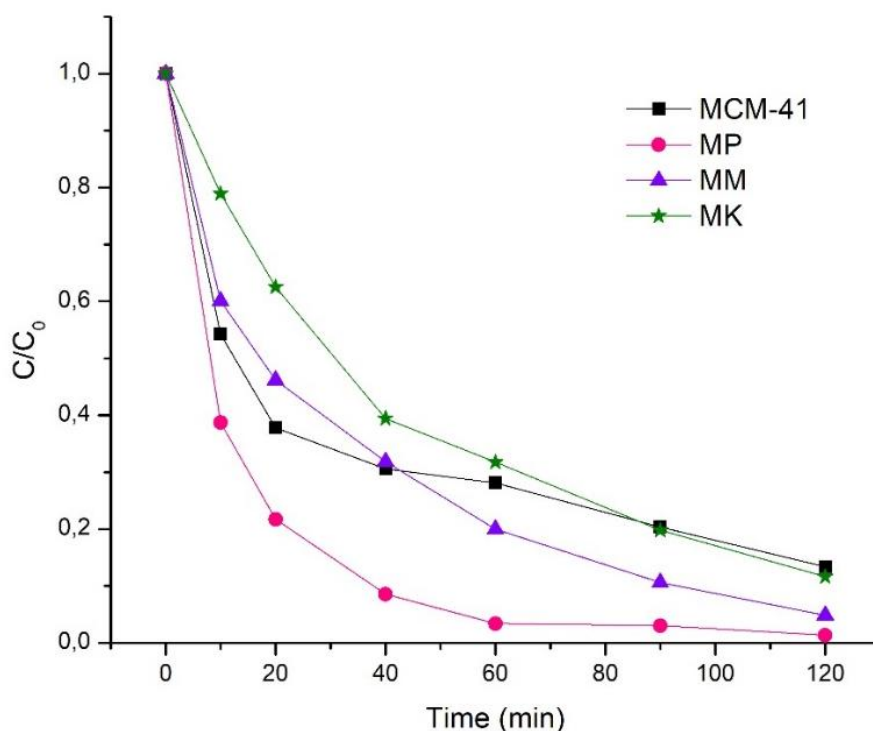


Figure 7: Graph of the degradation of Methylene Blue under UV light using MCM-41, MP, MM, and MK

The MP sample, containing P25 TiO₂, showed the highest photocatalytic activity, as highlighted by the steepest decline in MB concentration. Within 60 minutes, MP achieved nearly complete methylene blue degradation, outperforming MM, MK, and clearly pure MCM-41.

The process is governed by two simultaneous mechanisms: adsorption and degradation. Therefore, not only the photocatalytic properties but also the surface area of the materials used play a crucial role. For instance, MCM-41, despite lacking photocatalytic properties, still showed a decrease in the concentration of methylene blue through an adsorption mechanism by its mesoporous structure. This adsorption removed methylene blue molecules from the solution, leading to an apparent decrease in concentration. In fact, the dye molecules present in the solution can be absorbed by the mesoporous structure, as well as photocatalytically degraded

by the titania. These two phenomena act in combination, leading to a general discoloration of the solution.

Nevertheless, observing the residual solid SiO_2 , it is possible to partially discriminate the results. The TiO_2 NPs provide active sites for photocatalysis upon exposure to light, resulting in the degradation of methylene blue molecules not only in the solution but also once absorbed. The degradation of the absorbed methylene blue into the TiO_2 -enriched silica particles was indeed evident only for sample MP, whereas for MM and MK the particles deposited on the bottom of the tube remained blue, as can be appreciated in **Figure 8**.

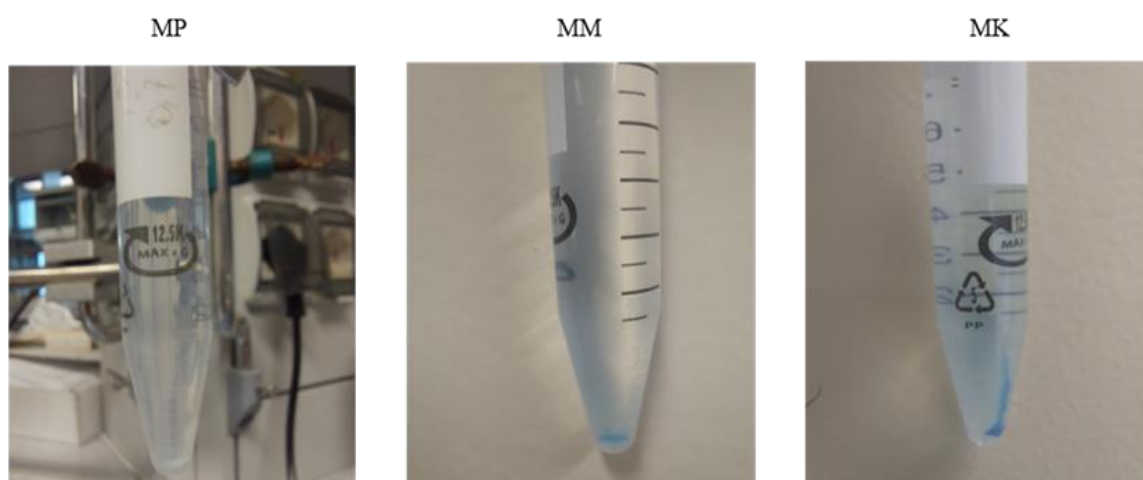


Figure 8: tubes with respectively MP, MM, and MK samples after 120 minutes of exposure to UV light

The superior performance of MP could be attributed to Evonik P25 TiO_2 's mix of anatase (80%) and rutile (20%) crystalline phases, known to enhance photocatalytic activity. The anatase phase has a band gap of 3.2 eV, while the rutile phase has 3.0 eV, enabling efficient light utilization and reduced electron-hole recombination.

To ensure photocatalytic activity was retained after interaction with *Origanum compactum* essential oil (EO), the same photodegradation test was performed also on the EO-impregnated sample, enriched with Evonik P25 (MOT). **Figure 9** thus shows the methylene blue degradation curves for MOT and MP over 300 minutes. MOT maintained photocatalytic activity but required 5 hours to completely degrade the methylene blue, compared to MP's faster rate. The initial methylene blue concentration decrease in MOT suggests significant adsorption on the material's surface and pores, though the presence of the EO slightly hindered methylene blue diffusion to titania active sites, slowing degradation.

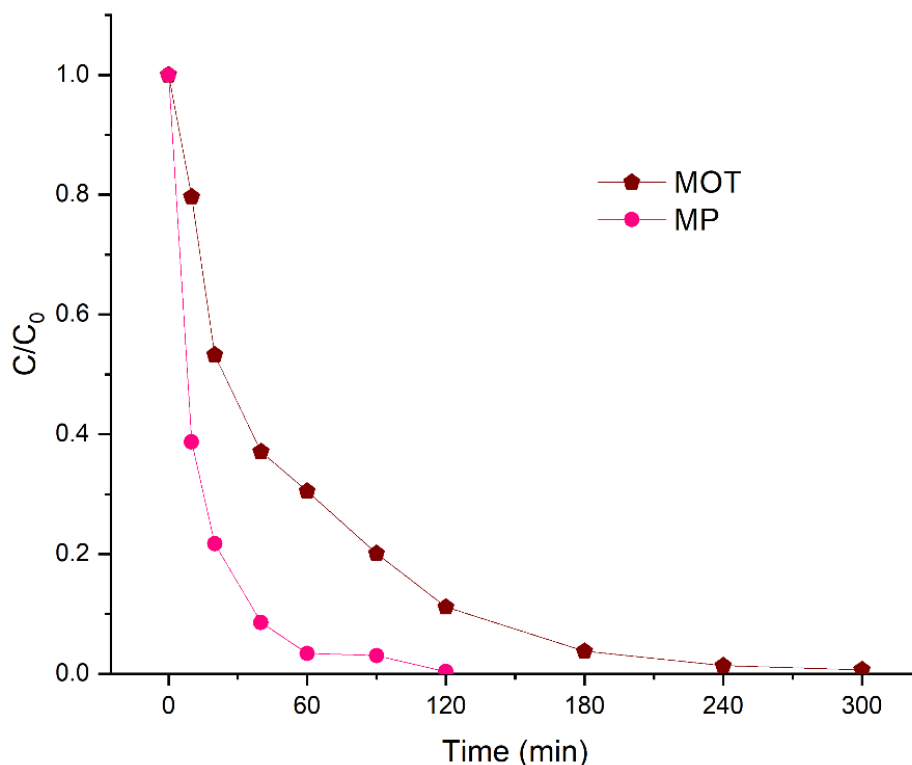


Figure 9: degradation of Methylene Blue under UV light using MOT

Concluding, it is possible to state that despite this slower rate, the essential oil did not block the photocatalytic activity of the TiO_2 , as the titania nanoparticles, located on the MOT material's surface, continued to promote methylene blue degradation under UV light. Thus, the preserved photocatalytic efficiency of MOT made the material suitable for application in the protective coating formulation.

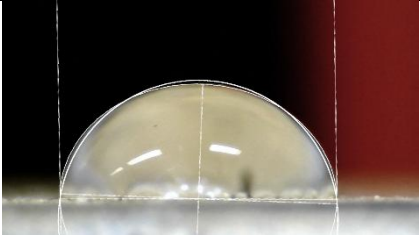
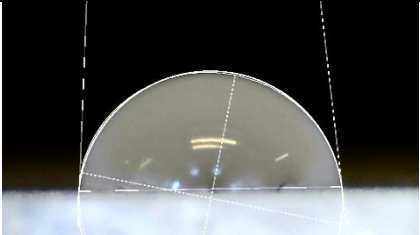
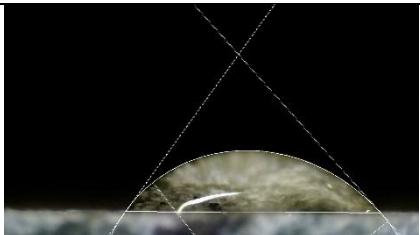
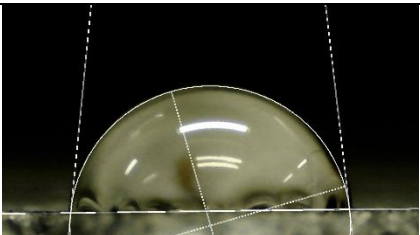
3.3 Evaluation of the protective coating formulation properties

The MCM-41 nanocapsules with oregano EO and Evonik P25 TiO_2 were embedded in the chitosan and MTES/TESO matrix protective coating formulation, named C-MOT. After applying it to the surface of the mock-ups, various tests were conducted to assess the coating's properties.

Hydrophobicity and water vapor permeability measures

To assess the hydrophobic properties of the formulation, the contact angle was measured following the standard methodology outlined in UNI EN 15802. **Table 7** shows the pictures taken of the water droplets once fallen on the untreated (on the left) and treated surfaces (on the right) for each substrate; the contact angle measurements are reported under each picture.

Table 7: Pictures and contact angle values of the sample surfaces before (on the left) and after (on the right) the application of the C-MOT coating treatment

Material	Before treatment	After treatment
Concrete		
	0.0°	90° ± 1
White Carrara Marble		
	70° ± 1	88° ± 1
Green Serpentine Marble		
	58° ± 1	86° ± 1

As it is possible to observe, before the treatments the substrates act differently depending on their porosity, which in the concrete is known to be way higher than in the two marbles. For this reason, the concrete mock-up surface before the treatment completely absorbs the water with a contact angle of 0°. Anyhow, in the case of concrete, the increase of contact angle is relevant, passing from 0° to 90°. Also, in the case of the marble mock-ups, it is possible to notice a rise in contact angle, increasing from 70° to 87° for the white Carrara and from 58° to 86° for the green serpentine marble. These results thus highlight a substantial improvement in the general hydrophobicity following the coating treatment. This increment in hydrophobicity could be correlated to the presence of the mesoporous SiO₂, which could make a microrugosity on the surface, decreasing the area of contact between the liquid and the substrate: a similar effect to that observed on superhydrophobic surfaces (such as the Cassie-Baxter model) can occur, where air trapped between the surface roughness prevents water from making contact with the surface [345]. At the same time, the presence of the hydrogel formulation could also

work in this sense: the MTES present in the formulation has nonpolar side chains (methyl groups) that tend to increase the hydrophobicity of the surface.

Anyway, these promising hydrophobicity results need to be considered along with the water vapor permeability ones, since these two parameters are strictly connected.

Figure 10 represents a histogram with the water vapor permeability (δ_p) data of the three materials before and after the application of the C-MOT coating treatment. It is possible to notice a slight decrease in permeability after the application, but in all cases lower than 20%; keeping in mind that the UNI EN 15803 standard does not establish a fixed percentage threshold for the acceptable variation in water vapor permeability after the application of a protective treatment. However, in the field of conservation and according to best restoration practices, a reduction in water vapor permeability of less than 30-40% compared to the untreated material is generally considered acceptable [116,346].

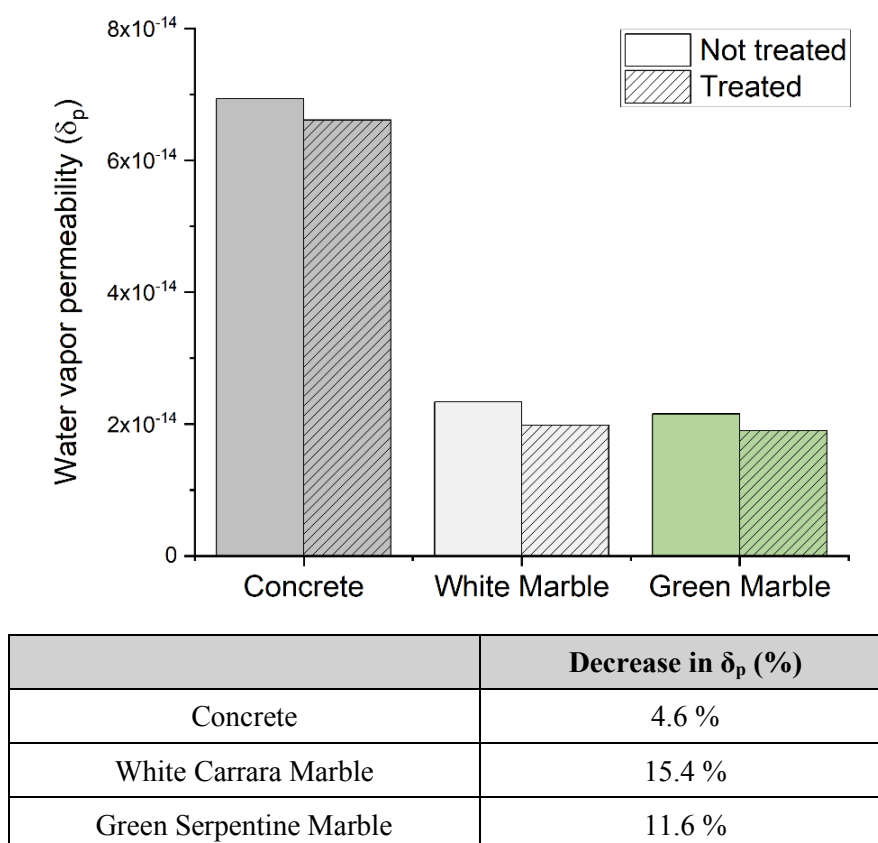


Figure 10: histogram of the water vapor permeability values (δ_p) with relative decrease % of each material underneath

Another interesting aspect that can be noticed, is the high difference in water vapor permeability between the concrete and the marble samples. This difference can be surely attributed to the diverse composition of the materials and can be correlated to other characteristics, such as the porosity, which for concrete is usually around 10-20%, whereas for marble is between 0.3% and 1.5% [347–349].

Colorimetric Analyses

To examine potential chromatic alterations, colorimetric analyses were performed. Color measurements were taken at three distinct points on the mock-ups' surface both before and after the coating was applied. **Table 8** presents the colorimetric data in the CIELAB color space, along with the ΔE values comparing the surface appearance before and after the application of the coating.

Table 8: colorimetric coordinates in the CIELAB color space and ΔE

Material		L*	a*	b*	ΔE	Mean ΔE
Concrete	Point A (before)	75.50	1.10	9.40	2	3
	Point A (after)	73.17	1.35	8.72		
	Point B (before)	75.47	1.00	8.91	3	
	Point B (after)	72.56	1.26	9.20		
	Point C (before)	74.89	1.19	9.22	3	
	Point C (after)	74.40	1.47	6.95		
White Carrara Marble	Point A (before)	68,61	-1,25	-3,65	3	3
	Point A (after)	65,27	-1,29	-3,16		
	Point B (before)	69,96	-0,76	-1,31	2	
	Point B (after)	67,68	-0,75	-0,7		
	Point C (before)	68,5	-1,05	-2,37	3	
	Point C (after)	65,25	-1,03	-2,59		
Green Serpentine Marble	Point A (before)	42,41	-1,29	0,32	2	3
	Point A (after)	40,56	-1,44	0,66		
	Point B (before)	42,77	-1,43	4,03	4	
	Point B (after)	38,62	-1,87	3,91		
	Point C (before)	46,01	-1,9	3,65	3	
	Point C (after)	42,84	-2,82	4,41		

Examining the ΔE , which represents the color variation and is expressed in integers, it is possible to observe that, considering the mean value for each material, the color variation is below the detection threshold of the naked human eye ($\Delta E > 3$); therefore, the application of the protective coating does not significantly change the appearance of the substrates.

Considering the overall experimental evidence and the characterization performed on the C-MOT coating formulation and its constituent materials, it is possible to state that a protective coating with promising features was developed for application on various stone substrates. The TiO₂/SiO₂ materials exhibited good homogeneity and a strong ability to degrade model stain compounds, even when combined with oregano essential oil. Furthermore, once incorporated into the hydrogel formulation, it demonstrated excellent performance, enhancing hydrophobicity without significantly altering the water vapor permeability or aesthetics of the surface. To finally validate the formulation features, an in-depth study of the coating components' antimicrobial activity was necessary.

3.5 Evaluation of the antimicrobial activity

The monitoring of fungal growth of both molds *Penicillium chrysogenum* and *Aspergillus niger* on pristine white Carrara and green Serpentine marble mock-ups revealed rapid growth in the cultivation medium during the first few days, followed by a stationary growth phase once the marble surfaces became partially covered.

Figure 11 presents pictures of one mock-up of each type, inoculated with *P. chrysogenum* or *A. niger*, over the 60-day period.

Regarding *P. chrysogenum* colonization on white marble, widespread coverage is observed by day 14, with complete colonization confirmed by day 60. This well-established colonization is further evidenced by the release of pigments onto the stone surface, which takes on a yellowish color, a characteristic effect of *P. chrysogenum* [350,351]. Although no visible color change is appreciable in the green Serpentine marble mock-up, a similar growth pattern emerges, with fungal development already noticeable on the surface by day 14.

For *A. niger* on white marble, complete surface coverage is observed within the first 7 days, with dark spots (*i.e.* conidiophores) becoming evident and intensifying over time. By day 14, the white surface begins to take on a yellowish hue, which gradually darkens to a brownish coloration by day 60. *A. niger* is known to produce brown and yellowish pigments, such as melanin, which may be then absorbed by the marble substrate [352,353]. In contrast, no color change is appreciable in the green serpentine marble mock-up, although a speckled appearance emerges from day 14 and becomes more intense by day 60, suggesting a similar growth pattern.

For comparison, **Figure 12** presents the pictures of the mock-ups treated with the final C-MOT formulation, which consists of a chitosan-MTES/TEOS hydrogel and MCM-41 SiO₂ impregnated with *Origanum compactum* essential oil and enriched with Evonik P25 TiO₂ NPs.

As before, the cultivation medium surrounding both marble mock-ups was inoculated with *P. chrysogenum* or *A. niger*. These images reveal a stark contrast to the untreated samples. While *P. chrysogenum* growth is still noticeable in some areas, *A. niger* development is significantly inhibited.

Analyzing the pictures of the mock-ups inoculated with *P. chrysogenum*, it is evident that the C-MOT formulation effectively inhibits fungal colonization already from day 14. This period could be associated with favorable atmospheric precipitation conditions, which may facilitate the washout and subsequent removal of colonizing spores from the stone surface. However, in later images, particularly those of the white Carrara marble mock-ups, a slight yellowing of the surface is observed, suggesting that the formulation might not completely prevent fungal growth or that pigments produced by the fungi in the cultivation medium may have penetrated the substrate from the sides.

In contrast, the mock-ups inoculated with *A. niger* exhibit a near-total inhibition of fungal growth when treated with the C-MOT formulation. By the end of the 60-day monitoring period, the samples remain almost free of fungal coverage, even though the surfaces of the substrates treated with this coating exhibit a light pale-yellow hue.

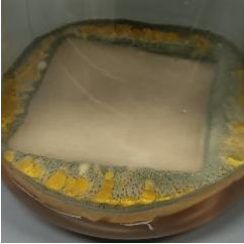
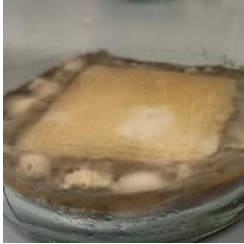
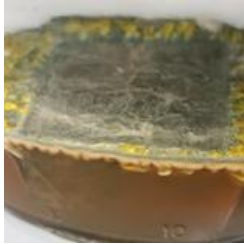
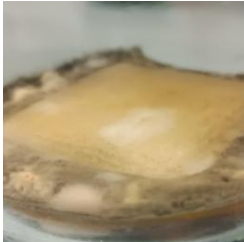
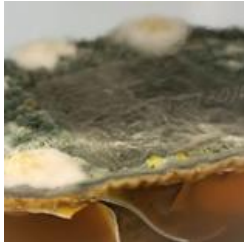
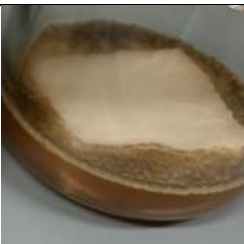
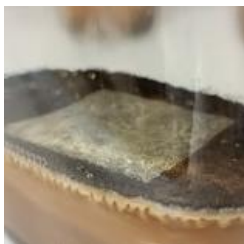
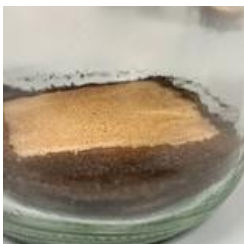
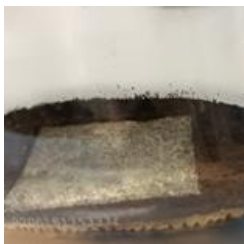
	PRISTINE WHITE MARBLE		PRISTINE GREEN MARBLE	
<i>Penicillium chrysogenum</i>				
	Day 2	Day 28	Day 2	Day 28
				
	Day 7	Day 35	Day 7	Day 35
				
	Day 14	Day 60	Day 14	Day 60
<i>Aspergillus niger</i>				
	Day 2	Day 28	Day 2	Day 28
				
	Day 7	Day 35	Day 7	Day 35
				
	Day 14	Day 60	Day 14	Day 60

Figure 11: Photos taken at 2, 7, 14, 28, 35, and 60 days after the inoculation on pristine mock-ups

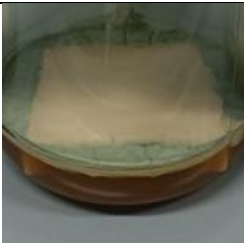
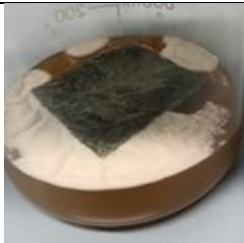
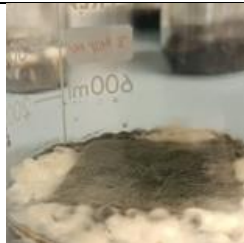
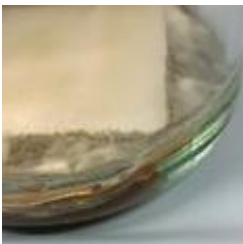
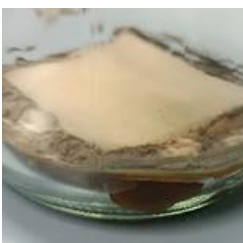
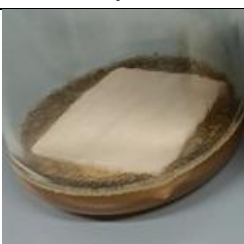
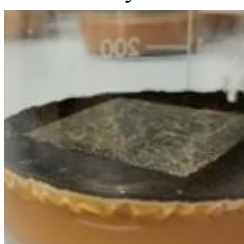
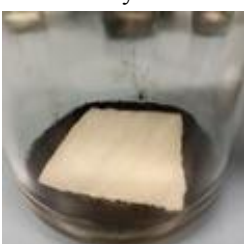
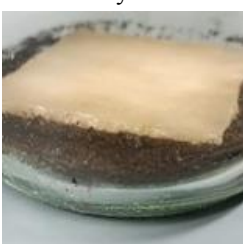
	WHITE MARBLE TREATED WITH C-MOT		GREEN MARBLE TREATED WITH C-MOT	
<i>Penicillium chrysogenum</i>				
	Day 2	Day 28	Day 2	Day 28
				
	Day 7	Day 35	Day 7	Day 35
				
	Day 14	Day 60	Day 14	Day 60
<i>Aspergillus niger</i>				
	Day 2	Day 28	Day 2	Day 28
				
	Day 7	Day 35	Day 7	Day 35
				
	Day 14	Day 60	Day 14	Day 60

Figure 12: Photos taken at 2, 7, 14, 28, 35, and 60 days after the inoculation on mock-ups treated with C-MOT

To fully understand the role of the formulation's different components, the positive results obtained with C-MOT were compared with those obtained with C, C-MP, and C-MO formulations. This approach allows us to differentiate the influence of the chitosan-MTES/TEOS hydrogel from that of *Origanum compactum* essential oil and Evonik P25 TiO₂ nanoparticles. Since the clearest results were previously obtained with the white Carrara marble mock-ups, this material was selected as the reference for comparison.

From **Figure 13**, it can be observed that the mock-ups treated with the C formulation exhibit a low level of colonization after 14 days. However, some dark spots begin to appear, and by day 60, the surface darkens with a yellowish tone. When silica and TiO₂ are added (C-MP formulation), a reduction in colonization is observed at day 14. However, this is not sufficient to prevent the surface yellowing, as seen in the pictures taken on day 60, particularly in the case of *A. niger*.

Examining the C-MO formulation, it was observed that even after 60 days, the mock-up surfaces appear free of fungal colonization. However, a comparison with the C-MOT formulation pictures reveals that the latter maintains a cleaner surface with lighter tones.

Considering these findings, it is likely that the primary active component in the C-MOT formulation responsible for inhibiting fungal growth is *Origanum compactum* essential oil. The active antimicrobial molecules in this extract once encapsulated in mesoporous MCM-41 silica, are released over an extended period, exerting a prolonged inhibitory effect on fungal proliferation, both regarding the development of the vegetative mycelium and the initiation of a sporulation phase. The addition of TiO₂ nanoparticles (NPs) also enhances the overall antimicrobial activity, as titania possesses biocidal properties, as demonstrated in the C-MP coating. However, in the C-MP formulation, the antimicrobial activity of titania may not be sufficiently strong or fully exploited. This limitation may be due to the absence of direct UV radiation, which is often necessary to activate titania's full photocatalytic potential. Testing under UV radiation was not feasible in this study, as it would have independently influenced fungal proliferation.

Nonetheless, in the C-MOT coating, the combined inhibitory effects of encapsulated *Origanum compactum* essential oil and TiO₂ nanoparticles result in a synergistic antimicrobial action, enhancing overall effectiveness. Therefore, the combination of both active components in C-MOT presents a promising strategy for developing durable antimicrobial coatings, particularly

for cultural heritage conservation and other applications requiring persistent biodeteriogens prevention.

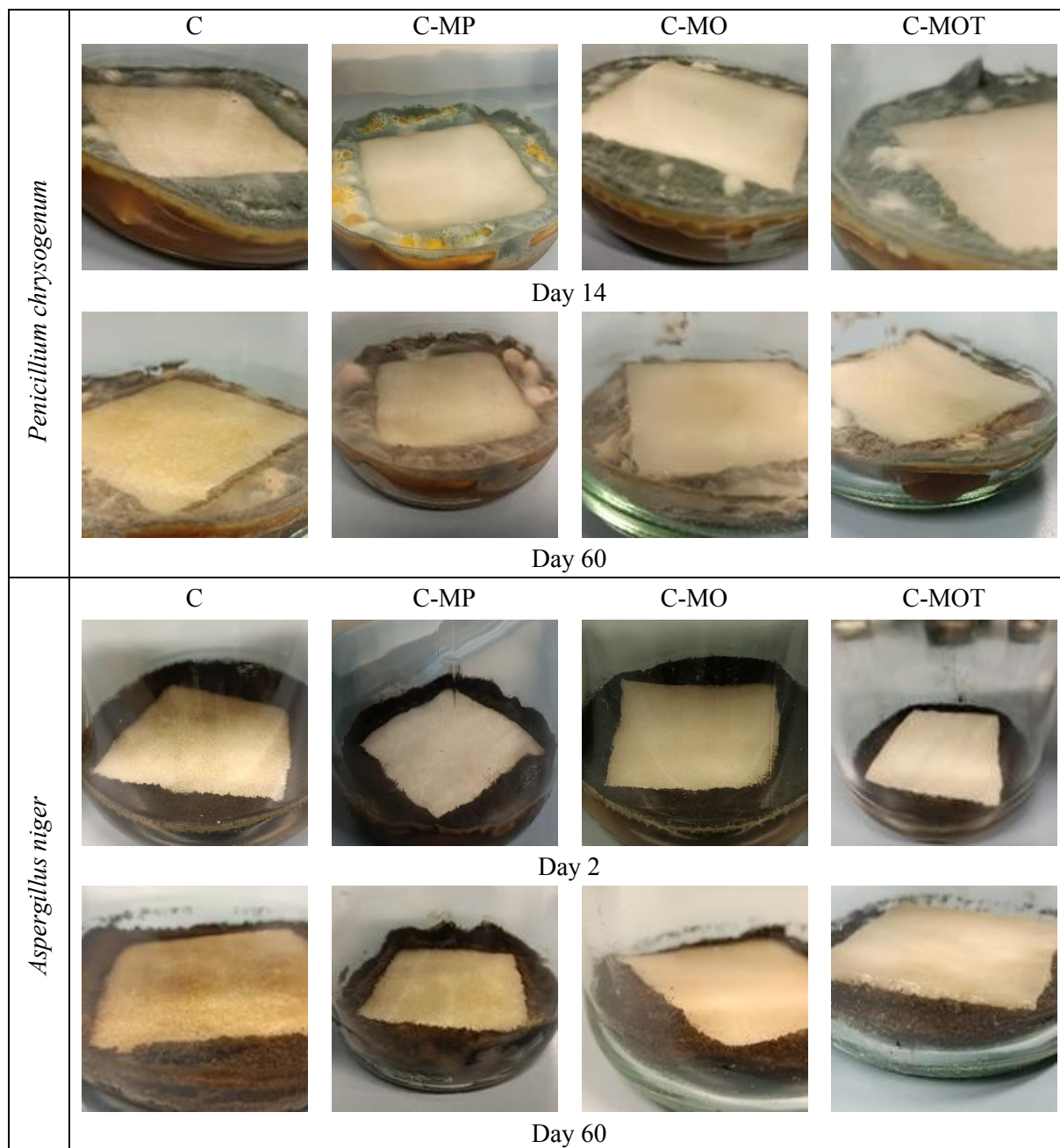


Figure 13: Photographs taken after 14 and 60 days from inoculation of *Penicillium chrysogenum* and *Aspergillus niger* fungi with white Carrara marble mock-ups previously treated with the different coating formulations

5. Conclusions

Within this work it was developed a nanostructured photocatalytic multifunctional coating based on a chitosan-MTES/TEOS hydrogel, embedding mesoporous MCM-41 loaded with TiO_2 and oregano essential oil. The innovative formulation presents a promising solution for the preservation of stone materials since the combination of essential oils and TiO_2 within the MCM-41 matrix provides a multifunctional and synergic antimicrobial effect. The mesoporous SiO_2 MCM-41 is indeed able to hold and gradually release the active, while the inhibition effect over microbial growth of the oregano EO is increased by the photocatalytic activity of Evonik P25, known for its efficient degradation of pollutants.

The hybrid hydrogel matrix, combining silica and chitosan, proved to be an effective carrier for the nanoparticles and the essential oil, ensuring good filming and viscosity properties and good adhesion to the stone surface. The coating not only protects the surface from pollution and microbial degradation phenomena but also provides hydrophobicity to the surface while maintaining water vapor permeability and color stability.

In conclusion, these findings highlight the potential of this multifunctional coating as a promising advancement in stone material preservation, offering enhanced protection against microbial degradation, pollutants, and moisture while maintaining the material's aesthetic and structural integrity.

Acknowledgments

The authors would like to thank Ms. Valentina A. Rizzolio for her practical contributions to part of this work and acknowledge the financial support of the “PON Research and Innovation 2014–2020”, the “Fund for the Promotion and Development of PNR Policies 2021–2027”, and the restoration company *Uni.S.Ve. Srl* of Venice (Italy) for financing Andrea Campostrini’s PhD scholarship.

VI.

CONCLUSIONS

CONCLUSIONS

In this PhD research thesis stone preservation has been investigated, aiming at developing a protective green multifunctional coating for stone surfaces to limit biodeterioration phenomena. In this final section, the key findings and contributions of this doctoral thesis are presented, emphasizing their relevance to the field of conservation science.

The research for sustainable green materials for application in the cultural heritage field found an interesting match in the use of photocatalysts. In this sense, the application of photocatalytic nanoparticles on stone materials demonstrated their sustainable potential, exploiting sunlight energy, in enriching the surface with self-cleaning properties and in inhibiting fungal growth. Through this study it was possible to integrate traditional methods with new technologies, developing an innovative application to implement prevention strategies to limit biodeterioration phenomena, linking the photocatalytic and optoelectrical properties of TiO₂ and ZnO nanoparticles with their antimicrobial activity. In this sense, the use of TiO₂ NPs exhibited a highly promising trajectory towards establishing a material to be added into the final formulation presented in this thesis. Nevertheless, while the use of these nanoparticles showed promising results, it is important to consider that their efficacy is also closely tied to activation through environmental conditions such as light, which may limit their effectiveness in darker and less exposed areas. In the pursuit of developing a green sustainable material to work together with the TiO₂ NPs, through a chemical-induced interaction with the biodeteriogens, was needed. In this sense the use of MCM-41 silica nanocontainer was a significant achievement, to avoid the direct use of synthetic biocide, which could harm the environment and the operators' health. In particular, the combination of vegetal extracts with ordered mesoporous SiO₂ was a greener viable alternative to traditional synthetic biocides, which could also increase antimicrobial activity and extended the agents' effectiveness. The fact that these outcomes were confirmed both *in vitro* and applied on marble mock-ups, showed how these materials might be a great innovative tool also for real-case applications.

The final formulation of the hybrid coating system – integrating the biocidal activity of the vegetal extract, the structural properties of ordered mesoporous silica MCM-41, and the photocatalytic activity of TiO₂ NPs into a cohesive functional product – constitutes a valuable advancement, effectively addressing a key knowledge gap in this field. The coating formulation demonstrated compatibility with various stone materials and showed significant antimicrobial activity, suggesting an amplified constructive synergy. The need to be in line with the

restoration gold standards for protective coating was satisfied, since the formulation led to an increase of the surface hydrophobicity without altering the surface's aesthetical appearance or its water vapor permeability. The continuous dialogue with restorers, acting as end-users, to compare and validate the formulation was crucial in determining its practical applicability. This significant achievement positions the formulations as not only potential game-changers in the field of stone surfaces protection but also as a testament to the feasibility and effectiveness of eco-conscious multidisciplinary strategies in advancing the field of heritage science. This innovative accomplishment not only drives progress in the field but also highlights the significant influence of this study on the future of fresco conservation.

Concluding, the synergy of various materials and techniques, some already employed in restoration, has made it possible to enhance their effectiveness while overcoming certain inherent limitations. This achievement has been reached through an interdisciplinary approach that integrates green chemistry, microbiology, nanotechnology, and the craftsmanship of master restorers. In the future, the same approach would need to be applied to surpass the limitations of the current formulation, refine it, assess its scalability, and applicability in restoration projects of real-world artworks.

BIBLIOGRAPHY

- [1] S. Morena, F. Bordese, E. Caliano, S. Freda, E. De Feo, S. Barba, Architectural Survey Techniques for Degradation Diagnostics. An Application for the Cultural Heritage, The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences XLVI-M-1–2021 (2021) 449–454. <https://doi.org/10.5194/isprs-archives-XLVI-M-1-2021-449-2021>.
- [2] N. Huse, W. Wolters, The art of Renaissance Venice: architecture, sculpture, and painting, 1460-1590, Choice Reviews Online 29 (1991) 29-0081-29–0081. <https://doi.org/10.5860/CHOICE.29-0081>.
- [3] Þ. Pétursdóttir, Concrete matters: Ruins of modernity and the things called heritage, Journal of Social Archaeology 13 (2013) 31–53. <https://doi.org/10.1177/1469605312456342>.
- [4] B. Ciarkowski, Post-war modernist architecture in Poland as part of the european heritage of twentieth- -century concrete-based architecture, Czasopismo Techniczne (2019) 5–18. <https://doi.org/10.4467/2353737XCT.19.077.10856>.
- [5] L. Bertolini, M. Carsana, M. Gastaldi, F. Lollini, E. Redaelli, Corrosion assessment and restoration strategies of reinforced concrete buildings of the cultural heritage, Materials and Corrosion 62 (2011) 146–154. <https://doi.org/10.1002/maco.201005773>.
- [6] H.A. Doğan, Perception of the Modern Movement in Architecture as Cultural Heritage, Art History & Criticism 16 (2020) 65–78. <https://doi.org/10.2478/mik-2020-0004>.
- [7] K. Elert, C. Rodriguez-Navarro, Degradation and conservation of clay-containing stone: A review, Constr Build Mater 330 (2022) 127226. <https://doi.org/10.1016/j.conbuildmat.2022.127226>.
- [8] M.L. Brusseau, A.D. Matthias, A.C. Comrie, S.A. Musil, Atmospheric Pollution, Environmental and Pollution Science (2019) 293–309. <https://doi.org/10.1016/B978-0-12-814719-1.00017-3>.
- [9] C. Rodriguez-Navarro, E. Sebastian, Role of particulate matter from vehicle exhaust on porous building stones (limestone) sulfation, Science of The Total Environment 187 (1996) 79–91. [https://doi.org/10.1016/0048-9697\(96\)05124-8](https://doi.org/10.1016/0048-9697(96)05124-8).
- [10] F. Becherini, J. Cassar, M. Galea, A. Bernardi, Evaluation of the shelters over the prehistoric Megalithic Temples of Malta: environmental considerations, Environ Earth Sci 75 (2016) 1079. <https://doi.org/10.1007/s12665-016-5875-z>.
- [11] Y. Zhang, D. Cui, X. Bao, S. Liu, H. Guo, B. Li, Exfoliation of stone cultural relics under the microclimate in the Yongling Mausoleum of the Former Shu Dynasty, Herit Sci 10 (2022) 155. <https://doi.org/10.1186/s40494-022-00788-1>.
- [12] B.J. Smith, M. Gomez-Heras, S. McCabe, Understanding the decay of stone-built cultural heritage, Progress in Physical Geography: Earth and Environment 32 (2008) 439–461. <https://doi.org/10.1177/0309133308098119>.
- [13] T. Warscheid, J. Braams, Biodeterioration of stone: a review, Int Biodeterior Biodegradation 46 (2000) 343–368. [https://doi.org/10.1016/S0964-8305\(00\)00109-8](https://doi.org/10.1016/S0964-8305(00)00109-8).

- [14] M.L. Coutinho, A.Z. Miller, M.F. Macedo, Biological colonization and biodeterioration of architectural ceramic materials: An overview, *J Cult Herit* 16 (2015) 759–777. <https://doi.org/10.1016/j.culher.2015.01.006>.
- [15] A. Negi, I.P. Sarethy, Microbial Biodeterioration of Cultural Heritage: Events, Colonization, and Analyses, *Microb Ecol* 78 (2019) 1014–1029. <https://doi.org/10.1007/s00248-019-01366-y>.
- [16] S. Sivalingam, G. Vishal, B. Anush, Environmental and health effects of acid rain, in: *Health and Environmental Effects of Ambient Air Pollution*, Elsevier, 2024: pp. 91–107. <https://doi.org/10.1016/B978-0-443-16088-2.00007-7>.
- [17] A.C. Hernandez, J. Sanjurjo-Sánchez, C. Alves, C.A.M. Figueiredo, Study of the Geochemical Decay and Environmental Causes of Granite Stone Surfaces in the Built Heritage of Barbanza Peninsula (Galicia, NW Spain), *Coatings* 14 (2024) 169. <https://doi.org/10.3390/coatings14020169>.
- [18] A. Artesani, F. Di Turo, M. Zucchelli, A. Traviglia, Recent Advances in Protective Coatings for Cultural Heritage—An Overview, *Coatings* 10 (2020) 217. <https://doi.org/10.3390/coatings10030217>.
- [19] V. Gomes, A. Dionísio, J.S. Pozo-Antonio, Conservation strategies against graffiti vandalism on Cultural Heritage stones: Protective coatings and cleaning methods, *Prog Org Coat* 113 (2017) 90–109. <https://doi.org/10.1016/J.PORGCOAT.2017.08.010>.
- [20] 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 (1991) 187–207. [https://doi.org/10.1016/0265-3036\(91\)90042-P](https://doi.org/10.1016/0265-3036(91)90042-P).
- [21] T.C. Dakal, P.K. Arora, Evaluation of potential of molecular and physical techniques in studying biodeterioration, *Rev Environ Sci Biotechnol* 11 (2012) 71–104. <https://doi.org/10.1007/s11157-012-9264-0>.
- [22] L. Schröer, N. Boon, T. De Kock, V. Cnudde, The capabilities of bacteria and archaea to alter natural building stones – A review, *Int Biodeterior Biodegradation* 165 (2021) 105329. <https://doi.org/10.1016/J.IBIOD.2021.105329>.
- [23] F. De Leo, A. Marchetta, C. Urzì, Black Fungi on Stone-Built Heritage: Current Knowledge and Future Outlook, *Applied Sciences* 12 (2022) 3969. <https://doi.org/10.3390/app12083969>.
- [24] J.C. Zak, H.G. Wildman, Fungi in stressful environments, in: G.M. Mueller, G.F. Bills, M.S. Foster (Eds.), *Biodiversity of Fungi: Inventory and Monitoring Methods*, Elsevier, 2004: pp. 303–315. <https://doi.org/10.1016/B978-012509551-8/50017-9>.
- [25] O.A. Shilova, D.Y. Vlasov, T.V. Khamova, M.S. Zelenskaya, O.V. Frank-Kamenetskaya, Microbiologically induced deterioration and protection of outdoor stone monuments, in: *Biodegradation and Biodeterioration At the Nanoscale*, Elsevier, 2022: pp. 339–367. <https://doi.org/10.1016/B978-0-12-823970-4.00015-4>.
- [26] J. Trovão, A. Portugal, The impact of stone position and location on the microbiome of a marble statue, *The Microbe* 2 (2024) 100040. <https://doi.org/10.1016/j.microb.2024.100040>.
- [27] P. Tyagi, R.K. Verma, N. Jain, Fungal degradation of cultural heritage monuments and management options, *Curr Sci* 121 (2021) 1553. <https://doi.org/10.18520/cs/v121/i12/1553-1560>.

- [28] G. Gómez-Alarcón, M.L. Muñoz, M. Flores, Excretion of organic acids by fungal strains isolated from decayed sandstone, *Int Biodeterior Biodegradation* 34 (1994) 169–180. [https://doi.org/10.1016/0964-8305\(94\)90006-X](https://doi.org/10.1016/0964-8305(94)90006-X).
- [29] K. Sterflinger, Fungi as Geologic Agents, *Geomicrobiol J* 17 (2000) 97–124. <https://doi.org/10.1080/01490450050023791>.
- [30] S. Görs, R. Schumann, N. Häubner, U. Karsten, Fungal and algal biomass in biofilms on artificial surfaces quantified by ergosterol and chlorophyll a as biomarkers, *Int Biodeterior Biodegradation* 60 (2007) 50–59. <https://doi.org/10.1016/j.ibiod.2006.10.003>.
- [31] K. Sterflinger, Fungi: Their role in deterioration of cultural heritage, *Fungal Biol Rev* 24 (2010) 47–55. <https://doi.org/10.1016/J.FBR.2010.03.003>.
- [32] M.W. Lee, M.-L.E. Florian, Fungal Facts: Solving Fungal Problems in Heritage Collections, *Journal of the American Institute for Conservation* 43 (2004) 114. <https://doi.org/10.2307/3179856>.
- [33] E. Stanaszek-Tomal, Environmental Factors Causing the Development of Microorganisms on the Surfaces of National Cultural Monuments Made of Mineral Building Materials—Review, *Coatings* 10 (2020) 1203. <https://doi.org/10.3390/coatings10121203>.
- [34] L. Zucconi, F. Canini, D. Isola, G. Caneva, Fungi Affecting Wall Paintings of Historical Value: A Worldwide Meta-Analysis of Their Detected Diversity, *Applied Sciences* 12 (2022) 2988. <https://doi.org/10.3390/app12062988>.
- [35] X. Liu, R.J. Koestler, T. Warscheid, Y. Katayama, J.-D. Gu, Microbial deterioration and sustainable conservation of stone monuments and buildings, *Nat Sustain* 3 (2020) 991–1004. <https://doi.org/10.1038/s41893-020-00602-5>.
- [36] W. Pathomsiriwong, S. Aroonsrimorakot, W. Taratima, P. Maneerattanarungroj, P. Reanprayoon, Exploring airborne fungal contaminations and air quality pollution in nine ancient stone temples, Surin, Thailand, *Environmental Science and Pollution Research* 31 (2024) 33733–33751. <https://doi.org/10.1007/s11356-024-33310-0>.
- [37] N.S. Geweely, New frontiers review of some recent conservation techniques of organic and inorganic archaeological artefacts against microbial deterioration, *Front Microbiol* 14 (2023). <https://doi.org/10.3389/fmicb.2023.1146582>.
- [38] Y. Wang, H. Zhang, X. Liu, X. Liu, W. Song, Fungal communities in the biofilms colonizing the basalt sculptures of the Leizhou Stone Dogs and assessment of a conservation measure, *Herit Sci* 9 (2021) 36. <https://doi.org/10.1186/s40494-021-00508-1>.
- [39] N.C. Silva, A.R. Madureira, M. Pintado, P.R. Moreira, Biocontamination and diversity of epilithic bacteria and fungi colonising outdoor stone and mortar sculptures, *Appl Microbiol Biotechnol* 106 (2022) 3811–3828. <https://doi.org/10.1007/s00253-022-11957-4>.
- [40] J. Shakir, S. Ahmedi, S. Pandey, N. Manzoor, Fungal Infestation and Antifungal Treatment of Organic Heritage Objects, in: *Advances in Antifungal Drug Development*, Springer Nature Singapore, Singapore, 2024: pp. 675–702. https://doi.org/10.1007/978-981-97-5165-5_26.
- [41] Ž. Savković, M. Stupar, N. Unković, A. Knežević, J. Vukojević, M. Ljaljević Grbić, Fungal Deterioration of Cultural Heritage Objects, in: *Biodegradation Technology of Organic and Inorganic Pollutants*, IntechOpen, 2022. <https://doi.org/10.5772/intechopen.98620>.

- [42] M. Tokmak, M. Dal, Classification of Physical, Chemical and Biological Deteriorations Observed in Ankara Stone Monuments, *International Journal of Pure and Applied Sciences* 6 (2020) 8–16. <https://doi.org/10.29132/ijpas.718466>.
- [43] J. Pavlović, Z. Farkas, L. Kraková, D. Pangallo, Color Stains on Paper: Fungal Pigments, Synthetic Dyes and Their Hypothetical Removal by Enzymatic Approaches, *Applied Sciences* 12 (2022) 9991. <https://doi.org/10.3390/app12199991>.
- [44] M. Afroz Toma, Md.H. Rahman, Md.S. Rahman, M. Arif, K.H.M.N.H. Nazir, L. Dufossé, Fungal Pigments: Carotenoids, Riboflavin, and Polyketides with Diverse Applications, *Journal of Fungi* 9 (2023) 454. <https://doi.org/10.3390/jof9040454>.
- [45] H. Meruvu, J.C. dos Santos, Colors of life: a review on fungal pigments, *Crit Rev Biotechnol* 41 (2021) 1153–1177. <https://doi.org/10.1080/07388551.2021.1901647>.
- [46] J. Trovão, A. Portugal, Current Knowledge on the Fungal Degradation Abilities Profiled through Biodeteriorative Plate Essays, *Applied Sciences* 11 (2021) 4196. <https://doi.org/10.3390/app11094196>.
- [47] E. Loukou, N.F. Jensen, L. Rohde, B. Andersen, Damp Buildings: Associated Fungi and How to Find Them, *Journal of Fungi* 10 (2024) 108. <https://doi.org/10.3390/jof10020108>.
- [48] K. V. Sazanova, M.S. Zelenskaya, A.D. Vlasov, S.Yu. Bobir, K.L. Yakkonen, D.Yu. Vlasov, Microorganisms in Superficial Deposits on the Stone Monuments in Saint Petersburg, *Microorganisms* 10 (2022) 316. <https://doi.org/10.3390/microorganisms10020316>.
- [49] F. Cappitelli, C. Cattò, F. Villa, The Control of Cultural Heritage Microbial Deterioration, *Microorganisms* 8 (2020) 1542. <https://doi.org/10.3390/microorganisms8101542>.
- [50] R.C. Fierascu, M. Doni, I. Fierascu, Selected Aspects Regarding the Restoration/Conservation of Traditional Wood and Masonry Building Materials: A Short Overview of the Last Decade Findings, *Applied Sciences* 10 (2020) 1164. <https://doi.org/10.3390/app10031164>.
- [51] M. Baglioni, G. Poggi, D. Chelazzi, P. Baglioni, Advanced Materials in Cultural Heritage Conservation, *Molecules* 26 (2021) 3967. <https://doi.org/10.3390/molecules26133967>.
- [52] F. Menicucci, E. Palagano, M. Michelozzi, A. Ienco, Essential Oils for the Conservation of Paper Items, *Molecules* 28 (2023) 5003. <https://doi.org/10.3390/molecules28135003>.
- [53] F. Palla, M. Bruno, F. Mercurio, A. Tantillo, V. Rotolo, Essential Oils as Natural Biocides in Conservation of Cultural Heritage, *Molecules* 25 (2020) 730. <https://doi.org/10.3390/molecules25030730>.
- [54] E. Stashenko, J. René Martínez, Essential Oils and the Circular Bioeconomy, in: 2024. <https://doi.org/10.5772/intechopen.112958>.
- [55] D'agostino, Tesse, Frippiat, Machouart, Debourgogne, Essential Oils and Their Natural Active Compounds Presenting Antifungal Properties, *Molecules* 24 (2019) 3713. <https://doi.org/10.3390/molecules24203713>.
- [56] J. Cai, R. Yan, J. Shi, J. Chen, M. Long, W. Wu, K. Kuca, Antifungal and mycotoxin detoxification ability of essential oils: A review, *Phytotherapy Research* 36 (2022) 62–72. <https://doi.org/10.1002/ptr.7281>.

- [57] A. El Asbahani, K. Miladi, W. Badri, M. Sala, E.H.A. Addi, H. Casabianca, A. El Mousadik, D. Hartmann, A. Jilale, F.N.R. Renaud, A. Elaissari, Essential oils: From extraction to encapsulation, *Int J Pharm* 483 (2015) 220–243. <https://doi.org/10.1016/j.ijpharm.2014.12.069>.
- [58] S.K. Sundar, J.K. Parikh, Advances and trends in encapsulation of essential oils, *Int J Pharm* 635 (2023) 122668. <https://doi.org/10.1016/j.ijpharm.2023.122668>.
- [59] M.M. Badr, M.E.I. Badawy, N.E.M. Taktak, Preparation, characterization, and antimicrobial activity of cinnamon essential oil and cinnamaldehyde nanoemulsions, *Journal of Essential Oil Research* 34 (2022) 544–558. <https://doi.org/10.1080/10412905.2022.2107100>.
- [60] F. Plati, A. Paraskevopoulou, Micro- and Nano-encapsulation as Tools for Essential Oils Advantages' Exploitation in Food Applications: the Case of Oregano Essential Oil, *Food Bioproc Tech* 15 (2022) 949–977. <https://doi.org/10.1007/s11947-021-02746-4>.
- [61] A. Mutlu-Ingok, D. Devecioglu, D.N. Dikmetas, F. Karbancioglu-Guler, E. Capanoglu, Antibacterial, Antifungal, Antimycotoxigenic, and Antioxidant Activities of Essential Oils: An Updated Review, *Molecules* 25 (2020) 4711. <https://doi.org/10.3390/molecules25204711>.
- [62] A.P. Santo, B. Agostini, O.A. Cuzman, M. Michelozzi, T. Salvatici, B. Perito, Essential oils to contrast biodeterioration of the external marble of Florence Cathedral, *Science of the Total Environment* 877 (2023). <https://doi.org/10.1016/j.scitotenv.2023.162913>.
- [63] S. Macedo-Arantes, A. Piçarra, A.T. Caldeira, A.E. Candeias, M.R. Martins, Essential oils of Portuguese flavouring plants: potential as green biocides in cultural heritage, *The European Physical Journal Plus* 136 (2021) 1106. <https://doi.org/10.1140/epjp/s13360-021-02018-2>.
- [64] R. Russo, F. Palla, Plant Essential Oils as Biocides in Sustainable Strategies for the Conservation of Cultural Heritage, *Sustainability* 15 (2023) 8522. <https://doi.org/10.3390/su15118522>.
- [65] M. Spada, O.A. Cuzman, I. Tosini, M. Galeotti, F. Sorella, Essential oils mixtures as an eco-friendly biocidal solution for a marble statue restoration, *Int Biodeterior Biodegradation* 163 (2021). <https://doi.org/10.1016/j.ibiod.2021.105280>.
- [66] S. Antunes Filho, M.S. dos Santos, O.A.L. dos Santos, B.P. Backx, M.-L. Soran, O. Oprea, I. Lung, A. Stegarescu, M. Bououdina, Biosynthesis of Nanoparticles Using Plant Extracts and Essential Oils, *Molecules* 28 (2023) 3060. <https://doi.org/10.3390/molecules28073060>.
- [67] G.G. Lenetha, A Brief Overview On The Essential Oils' Encapsulation With Biodegradable Polymers, 2022.
- [68] C.M. Sanchis, P. Bosch-Roig, B.C. Moliner, A.Z. Miller, Antifungal properties of oregano and clove volatile essential oils tested on biodeteriorated archaeological mummified skin, *J Cult Herit* 61 (2023) 40–47. <https://doi.org/10.1016/j.culher.2023.02.006>.
- [69] I. Romano, G. Granata, A. Poli, I. Finore, E. Napoli, C. Geraci, Inhibition of bacterial growth on marble stone of 18th century by treatment of nanoencapsulated essential oils, *Int Biodeterior Biodegradation* 148 (2020). <https://doi.org/10.1016/j.ibiod.2020.104909>.
- [70] M. Veneranda, L. Blanco-Zubiaguirre, G. Roselli, G. Di Girolami, K. Castro, J.M. Madariaga, Evaluating the exploitability of several essential oils constituents as a novel biological treatment against cultural heritage biocolonization, *Microchemical Journal* 138 (2018) 1–6. <https://doi.org/10.1016/j.microc.2017.12.019>.

- [71] R.G. Candela, F. Maggi, G. Lazzara, S. Rosselli, M. Bruno, The essential oil of *Thymbra capitata* and its application as a biocide on stone and derived surfaces, *Plants* 8 (2019). <https://doi.org/10.3390/plants8090300>.
- [72] I. Lobato-Guarnido, G. Luzón, F. Ríos, M. Fernández-Serrano, Synthesis and Characterization of Environmentally Friendly Chitosan–Arabic Gum Nanoparticles for Encapsulation of Oregano Essential Oil in Pickering Emulsion, *Nanomaterials* 13 (2023) 2651. <https://doi.org/10.3390/nano13192651>.
- [73] M. EL-Hefny, W. Abo Elgat, A. Al-Huqail, H. Ali, Essential and Recovery Oils from *Matricaria chamomilla* Flowers as Environmentally Friendly Fungicides Against Four Fungi Isolated from Cultural Heritage Objects, *Processes* 7 (2019) 809. <https://doi.org/10.3390/pr7110809>.
- [74] R. Ranaldi, L. Rugnini, F. Gabriele, N. Spreti, C. Casieri, G. Di Marco, A. Gismondi, L. Bruno, Plant essential oils suspended into hydrogel: Development of an easy-to-use protocol for the restoration of stone cultural heritage, *Int Biodeterior Biodegradation* 172 (2022). <https://doi.org/10.1016/j.ibiod.2022.105436>.
- [75] G. Ranalli, E. Zanardini, C. Sorlini, Biodeterioration – Including Cultural Heritage, in: Reference Module in Life Sciences, Elsevier, 2018. <https://doi.org/10.1016/B978-0-12-809633-8.13016-X>.
- [76] E. Di Carlo, G. Barresi, F. Palla, Biodeterioration, in: *Biotechnology and Conservation of Cultural Heritage*, Springer International Publishing, Cham, 2022: pp. 1–30. https://doi.org/10.1007/978-3-030-97585-2_1.
- [77] L. Kumar, N. Kapoor, A. Tiwari, Biotechnology to Restoration and Conservation, in: *Microbial Biotechnology Approaches to Monuments of Cultural Heritage*, Springer Singapore, Singapore, 2020: pp. 151–175. https://doi.org/10.1007/978-981-15-3401-0_9.
- [78] M.L. Coutinho, A.Z. Miller, P.M. Martin-Sanchez, J. Mirão, A. Gomez-Bolea, B. Machado-Moreira, L. Cerqueira-Alves, V. Jurado, C. Saiz-Jimenez, A. Lima, A.J.L. Phillips, F. Pina, M.F. Macedo, A multiproxy approach to evaluate biocidal treatments on biodeteriorated majolica glazed tiles, *Environ Microbiol* 18 (2016) 4794–4816. <https://doi.org/10.1111/1462-2920.13380>.
- [79] S.E. Favero-Longo, R. Benesperi, S. Bertuzzi, E. Bianchi, G. Buffa, P. Giordani, S. Loppi, P. Malaspina, E. Matteucci, L. Paoli, S. Ravera, A. Roccardi, A. Segimiro, A. Vannini, Species- and site-specific efficacy of commercial biocides and application solvents against lichens, *Int Biodeterior Biodegradation* 123 (2017) 127–137. <https://doi.org/10.1016/j.ibiod.2017.06.009>.
- [80] M.A. Kakakhel, F. Wu, J.-D. Gu, H. Feng, K. Shah, W. Wang, Controlling biodeterioration of cultural heritage objects with biocides: A review, *Int Biodeterior Biodegradation* 143 (2019) 104721. <https://doi.org/10.1016/j.ibiod.2019.104721>.
- [81] S. Lo Schiavo, F. De Leo, C. Urzì, Present and Future Perspectives for Biocides and Antifouling Products for Stone-Built Cultural Heritage: Ionic Liquids as a Challenging Alternative, *Applied Sciences* 10 (2020) 6568. <https://doi.org/10.3390/app10186568>.
- [82] I. Franco-Castillo, L. Hierro, J.M. de la Fuente, A. Seral-Ascaso, S.G. Mitchell, Perspectives for antimicrobial nanomaterials in cultural heritage conservation, *Chem* 7 (2021) 629–669. <https://doi.org/10.1016/j.chempr.2021.01.006>.

- [83] J. Arning, R. Dringen, M. Schmidt, A. Thiessen, S. Stolte, M. Matzke, U. Bottin-Weber, B. Caesar-Geertz, B. Jastorff, J. Ranke, Structure–activity relationships for the impact of selected isothiazol-3-one biocides on glutathione metabolism and glutathione reductase of the human liver cell line Hep G2, *Toxicology* 246 (2008) 203–212. <https://doi.org/10.1016/j.tox.2008.01.011>.
- [84] V. Silva, C. Silva, P. Soares, E.M. Garrido, F. Borges, J. Garrido, Isothiazolinone Biocides: Chemistry, Biological, and Toxicity Profiles, *Molecules* 25 (2020) 991. <https://doi.org/10.3390/molecules25040991>.
- [85] S. Badreshia, J.G. Marks, Iodopropynyl butylcarbamate, *American Journal of Contact Dermatitis* 13 (2002) 77–79. <https://doi.org/10.1053/ajcd.2002.30728>.
- [86] B.G. de Campos, J. Figueiredo, F. Perina, D.M. de S. Abessa, S. Loureiro, R. Martins, Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened?, *Crit Rev Environ Sci Technol* 52 (2022) 3179–3210. <https://doi.org/10.1080/10643389.2021.1910003>.
- [87] F.A. Guardiola, A. Cuesta, J. Meseguer, M.A. Esteban, Risks of Using Antifouling Biocides in Aquaculture, *Int J Mol Sci* 13 (2012) 1541–1560. <https://doi.org/10.3390/ijms13021541>.
- [88] V.M. Varnai, J. Macan, A. Ljubicic Calusic, L. Prester, B. Kanceljak Macan, Upper respiratory impairment in restorers of cultural heritage, *Occup Med (Chic Ill)* 61 (2011) 45–52. <https://doi.org/10.1093/occmed/kqq170>.
- [89] J.F. Schwensen, J.D. Johansen, Isothiazolinones, in: *Kanerva's Occupational Dermatology*, Springer International Publishing, Cham, 2020: pp. 507–520. https://doi.org/10.1007/978-3-319-68617-2_216.
- [90] R. Alvarez-Sánchez, D. Basketter, C. Pease, J.-P. Lepoittevin, Studies of Chemical Selectivity of Hapten, Reactivity, and Skin Sensitization Potency. 3. Synthesis and Studies on the Reactivity toward Model Nucleophiles of the ¹³C-Labeled Skin Sensitizers, 5-Chloro-2-methylisothiazol-3-one (MCI) and 2-Methylisothiazol-3-one (MI), *Chem Res Toxicol* 16 (2003) 627–636. <https://doi.org/10.1021/tx0256634>.
- [91] R. Alvarez-Sánchez, D. Basketter, C. Pease, J.-P. Lepoittevin, Covalent binding of the ¹³C-labeled skin sensitizers 5-chloro-2-methylisothiazol-3-one (MCI) and 2-methylisothiazol-3-one (MI) to a model peptide and glutathione, *Bioorg Med Chem Lett* 14 (2004) 365–368. <https://doi.org/10.1016/j.bmcl.2003.11.002>.
- [92] D. Sasseville, A. Balbul, P. Kwong, K. Yu, Contact sensitization to pyridine derivatives, *Contact Dermatitis* 35 (1996) 100–125. <https://doi.org/10.1111/j.1600-0536.1996.tb02299.x>.
- [93] T. Inoue, A. Yagami, A. Sano, M. Nakagawa, M. Abe, A. Mori, K. Sasaki, K. Matsunaga, Contact dermatitis because of antimicrobial coating desk mat, *Contact Dermatitis* 58 (2008) 123–124. <https://doi.org/10.1111/j.1600-0536.2007.01202.x>.
- [94] A. Gushi, T. Kanekura, Y. Katahira, H. Miyoshi, T. Kanzaki, Contact dermatitis from the antimicrobial coating of a desk mat, *Contact Dermatitis* 48 (2003) 347–348. <https://doi.org/10.1034/j.1600-0536.2003.00150.x>.
- [95] C.J. le Coz, P. Caussade, A. Bottlaender, Occupational contact dermatitis from methyl-terpyridine in a chemistry laboratory technician, *Contact Dermatitis* 38 (1998) 214–215. <https://doi.org/10.1111/j.1600-0536.1998.tb05712.x>.

- [96] W.-K. Huh, Y. Masuji, J. Tada, J. Arata, M. Kaniwa, Allergic contact dermatitis from a pyridine derivative in polyvinyl chloride leather, *American Journal of Contact Dermatitis* 12 (2001) 35–37. <https://doi.org/10.1053/ajcd.2000.8184>.
- [97] A. Sil, K. Pramanik, P. Samantaray, Md.F. Mondal, V. Yadav, Essential oils: A boon towards eco-friendly management of phytopathogenic fungi, *J Entomol Zool Stud* 8 (2020) 1884–1891.
- [98] J. Sharifi-Rad, A. Sureda, G. Tenore, M. Daglia, M. Sharifi-Rad, M. Valussi, R. Tundis, M. Sharifi-Rad, M. Loizzo, A. Ademiluyi, R. Sharifi-Rad, S. Ayatollahi, M. Iriti, Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems, *Molecules* 22 (2017) 70. <https://doi.org/10.3390/molecules22010070>.
- [99] C. Barbieri, P. Borsotto, Essential Oils: Market and Legislation, in: *Potential of Essential Oils*, InTech, 2018. <https://doi.org/10.5772/intechopen.77725>.
- [100] M. Stupar, M.Lj. 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) 118–124. <https://doi.org/10.1016/j.sajb.2014.03.016>.
- [101] S.E. Favero-Longo, M. Laurenzi Tabasso, F. Brigadeci, M.-C. Capua, A. Morelli, P. Pastorello, M. Sohrabi, A. Askari Chaverdi, P. Callieri, A first assessment of the biocidal efficacy of plant essential oils against lichens on stone cultural heritage, and the importance of evaluating suitable application protocols, *J Cult Herit* 55 (2022) 68–77. <https://doi.org/10.1016/j.culher.2022.02.006>.
- [102] K.H.C. Baser, G. Buchbauer, *Handbook of Essential Oils: Science, Technology, and Applications*, 2nd ed., CRC Press, Boca Raton, USA, 2015. <https://doi.org/10.1201/b19393>.
- [103] Y. El Abdali, A.M. Mahraz, G. Beniaich, I. Mssillou, M. Chebaibi, Y.A. Bin Jordan, A. Lahkimi, H.-A. Nafidi, M.A.M. Aboul-Soud, M. Bourhia, A. Bouia, Essential oils of *Origanum compactum* Benth: Chemical characterization, in vitro, in silico, antioxidant, and antibacterial activities, *Open Chem* 21 (2023). <https://doi.org/10.1515/chem-2022-0282>.
- [104] V. Parasuraman, A.M. Sharmin, M.A. Vijaya Anand, A.S. Sivakumar, D. Surendhiran, G. Sharesh, S. Kim, Fabrication and bacterial inhibitory activity of essential oil linalool loaded biocapsules against *Escherichia coli*, *J Drug Deliv Sci Technol* 74 (2022) 103495. <https://doi.org/10.1016/j.jddst.2022.103495>.
- [105] M. Allenspach, C. Valder, D. Flamm, C. Steuer, Authenticity control of pine sylvestris essential oil by chiral gas chromatographic analysis of α -pinene, *Sci Rep* 11 (2021) 16923. <https://doi.org/10.1038/s41598-021-96356-x>.
- [106] T.Q. Machado, J.R.S. Felisberto, E.F. Guimarães, G.A. de Queiroz, A.C.C. da Fonseca, Y.J. Ramos, A.M. Marques, D. de L. Moreira, B.K. Robbs, Apoptotic effect of β -pinene on oral squamous cell carcinoma as one of the major compounds from essential oil of medicinal plant *Piper rivinoides* Kunth, *Nat Prod Res* 36 (2022) 1636–1640. <https://doi.org/10.1080/14786419.2021.1895148>.
- [107] I. Rodriguez-Garcia, B.A. Silva-Espinoza, L.A. Ortega-Ramirez, J.M. Leyva, M.W. Siddiqui, M.R. Cruz-Valenzuela, G.A. Gonzalez-Aguilar, J.F. Ayala-Zavala, Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products, *Crit Rev Food Sci Nutr* 56 (2016) 1717–1727. <https://doi.org/10.1080/10408398.2013.800832>.

- [108] A.G. de Souza, N.M.A. dos Santos, R.F. da Silva Torin, D. dos Santos Rosa, Synergic antimicrobial properties of Carvacrol essential oil and montmorillonite in biodegradable starch films, *Int J Biol Macromol* 164 (2020) 1737–1747. <https://doi.org/10.1016/j.ijbiomac.2020.07.226>.
- [109] H. Hou, X. Zhang, T. Zhao, L. Zhou, Effects of *Origanum vulgare* essential oil and its two main components, carvacrol and thymol, on the plant pathogen *Botrytis cinerea*, *PeerJ* 8 (2020) e9626. <https://doi.org/10.7717/peerj.9626>.
- [110] J. Milagres de Almeida, B.L. Crippa, V.V. Martins Alencar de Souza, V.P. Perez Alonso, E. da Motta Santos Júnior, C. Siqueira Franco Picone, A.S. Prata, N.C. Cirone Silva, Antimicrobial action of Oregano, Thyme, Clove, Cinnamon and Black pepper essential oils free and encapsulated against foodborne pathogens, *Food Control* 144 (2023) 109356. <https://doi.org/10.1016/j.foodcont.2022.109356>.
- [111] J. Fabbri, M.A. Maggiore, P.E. Pensel, G.M. Denegri, M.C. Elissondo, In vitro efficacy study of Cinnamomum zeylanicum essential oil and cinnamaldehyde against the larval stage of Echinococcus granulosus, *Exp Parasitol* 214 (2020) 107904. <https://doi.org/10.1016/j.exppara.2020.107904>.
- [112] V.K. Rai, P. Sinha, K.S. Yadav, A. Shukla, A. Saxena, D.U. Bawankule, S. Tandon, F. Khan, C.S. Chanotiya, N.P. Yadav, Anti-psoriatic effect of Lavandula angustifolia essential oil and its major components linalool and linalyl acetate, *J Ethnopharmacol* 261 (2020) 113127. <https://doi.org/10.1016/j.jep.2020.113127>.
- [113] H.-T. Chang, C.-Y. Lin, L.-S. Hsu, S.-T. Chang, Thermal Degradation of Linalool-Chemotype Cinnamomum osmophloeum Leaf Essential Oil and Its Stabilization by Microencapsulation with β -Cyclodextrin, *Molecules* 26 (2021) 409. <https://doi.org/10.3390/molecules26020409>.
- [114] B. Paolino, M.C. Sorrentino, S. Pacifico, Greener solutions for biodeterioration of organic-media cultural heritage: where are we?, *Herit Sci* 12 (2024) 334. <https://doi.org/10.1186/s40494-024-01442-8>.
- [115] S.A. Ruffolo, M.F. La Russa, Nanostructured Coatings for Stone Protection: An Overview, *Front Mater* 6 (2019). <https://doi.org/10.3389/fmats.2019.00147>.
- [116] R. Lamuraglia, A. Campostrini, E. Ghedini, A. De Lorenzi Pezzolo, A. Di Michele, G. Franceschin, F. Menegazzo, M. Signoretto, A. Traviglia, A New Green Coating for the Protection of Frescoes: From the Synthesis to the Performances Evaluation, *Coatings* 13 (2023) 277. <https://doi.org/10.3390/coatings13020277>.
- [117] Y. Ocak, A. Sofuoglu, F. Tihminlioglu, H. Böke, Protection of marble surfaces by using biodegradable polymers as coating agent, *Prog Org Coat* 66 (2009) 213–220. <https://doi.org/10.1016/j.porgcoat.2009.07.007>.
- [118] M. Sadat-Shojai, A. Ershad-Langroudi, Polymeric coatings for protection of historic monuments: Opportunities and challenges, *J Appl Polym Sci* 112 (2009) 2535–2551. <https://doi.org/10.1002/app.29801>.
- [119] T. Fistos, I. Fierascu, M. Doni, I.E. Chican, R.C. Fierascu, A Short Overview of Recent Developments in the Application of Polymeric Materials for the Conservation of Stone Cultural Heritage Elements, *Materials* 15 (2022) 6294. <https://doi.org/10.3390/ma15186294>.

- [120] Z. Chen, X. Liu, H. Chen, J. Li, X. Wang, J. Zhu, Application of epoxy resin in cultural relics protection, *Chinese Chemical Letters* 35 (2024) 109194. <https://doi.org/10.1016/j.cclet.2023.109194>.
- [121] F. Cappitelli, F. Villa, P. Sanmartín, Interactions of microorganisms and synthetic polymers in cultural heritage conservation, *Int Biodeterior Biodegradation* 163 (2021) 105282. <https://doi.org/10.1016/j.ibiod.2021.105282>.
- [122] A. Makhmetova, E.-S. Negim, D. Ainakulova, G. Yeligbayeva, J. Khatib, An Overview of Epoxy Resins as coating to protect metals from corrosion, *Kompleksnoe Ispol'zovanie Mineral'nogo Syr'â/Complex Use of Mineral Resources/Mineraldik Shikisattardy Keshendi Paidalanu* 328 (2024) 20–32. <https://doi.org/10.31643/2024/6445.03>.
- [123] S. Centenaro, G. Franceschin, E. Cattaruzza, A. Traviglia, Consolidation and coating treatments for glass in the cultural heritage field: A review, *J Cult Herit* 64 (2023) 132–143. <https://doi.org/10.1016/j.culher.2023.09.006>.
- [124] S. Aksu, O. Kelleci, D. Aydemir, A. Istek, Application of acrylic-based varnishes reinforced with nano fillers for conservation of weathered and worn surfaces of the historical and cultural wooden buildings, *J Cult Herit* 54 (2022) 1–11. <https://doi.org/10.1016/j.culher.2022.01.003>.
- [125] G. Cappelletti, P. Fermo, Hydrophobic and superhydrophobic coatings for limestone and marble conservation, in: *Smart Composite Coatings and Membranes*, Elsevier, 2016: pp. 421–452. <https://doi.org/10.1016/B978-1-78242-283-9.00015-4>.
- [126] D.K. Chattopadhyay, K.V.S.N. Raju, Structural engineering of polyurethane coatings for high performance applications, *Prog Polym Sci* 32 (2007) 352–418. <https://doi.org/10.1016/j.progpolymsci.2006.05.003>.
- [127] F. Cappitelli, C. Sorlini, Microorganisms Attack Synthetic Polymers in Items Representing Our Cultural Heritage, *Appl Environ Microbiol* 74 (2008) 564–569. <https://doi.org/10.1128/AEM.01768-07>.
- [128] C. Defeyt, J. Langenbacher, R. Rivenc, Polyurethane coatings used in twentieth century outdoor painted sculptures. Part I: comparative study of various systems by means of ATR-FTIR spectroscopy, *Herit Sci* 5 (2017) 11. <https://doi.org/10.1186/s40494-017-0124-7>.
- [129] A. Chatzigrigoriou, I. Karapanagiotis, I. Poullos, Superhydrophobic Coatings Based on Siloxane Resin and Calcium Hydroxide Nanoparticles for Marble Protection, *Coatings* 10 (2020) 334. <https://doi.org/10.3390/coatings10040334>.
- [130] C.B. Sims, J.C. Furgal, Overview of the challenges and current solutions in organic and hybrid coatings for historical monuments and architecture, *Polym Int* 73 (2024) 591–602. <https://doi.org/10.1002/pi.6626>.
- [131] F.G. Adamopoulos, E.C. Vouvoudi, E. Pavlidou, D.S. Achilias, I. Karapanagiotis, TEOS-Based Superhydrophobic Coating for the Protection of Stone-Built Cultural Heritage, *Coatings* 11 (2021) 135. <https://doi.org/10.3390/coatings11020135>.
- [132] G. Zhao, X. Ma, Z. Shao, X. Huang, J. Huang, H. Luo, Breathable hyperbranched polysiloxane for the conservation of silicate cultural heritages, *J Solgel Sci Technol* 106 (2023) 518–529. <https://doi.org/10.1007/s10971-022-05932-8>.

- [133] P. Baglioni, E. Carretti, D. Chelazzi, Nanomaterials in art conservation, *Nat Nanotechnol* 10 (2015) 287–290. <https://doi.org/10.1038/nnano.2015.38>.
- [134] F. Gherardi, P.N. Maravelaki, Advances in the application of nanomaterials for natural stone conservation, *RILEM Technical Letters* 7 (2022) 20–29. <https://doi.org/10.21809/rilemtechlett.2022.159>.
- [135] H. Zhou, Q. Xu, J. Zhao, H. Luo, X. Huang, J. Huang, Biomimetic super slippery surface with excellent and durable anti-icing property for immovable heritage conservation, *Prog Org Coat* 184 (2023) 107818. <https://doi.org/10.1016/j.porgcoat.2023.107818>.
- [136] I. Karapanagiotis, A. Chatzigrigoriou, P.N. Manoudis, Waterborne superhydrophobic coatings for the conservation of the cultural heritage, in: *Handbook of Waterborne Coatings*, Elsevier, 2020: pp. 229–247. <https://doi.org/10.1016/B978-0-12-814201-1.00010-X>.
- [137] A. Sierra-Fernandez, S.C. De la Rosa-García, L.S. Gomez-Villalba, S. Gómez-Cornelio, M.E. Rabanal, R. Fort, P. Quintana, Synthesis, Photocatalytic, and Antifungal Properties of MgO, ZnO and Zn/Mg Oxide Nanoparticles for the Protection of Calcareous Stone Heritage, *ACS Appl Mater Interfaces* 9 (2017) 24873–24886. <https://doi.org/10.1021/acsami.7b06130>.
- [138] N. Gómez-Ortíz, S. De la Rosa-García, W. González-Gómez, M. Soria-Castro, P. Quintana, G. Oskam, B. Ortega-Morales, Antifungal Coatings Based on Ca(OH)₂ Mixed with ZnO/TiO₂ Nanomaterials for Protection of Limestone Monuments, *ACS Appl Mater Interfaces* 5 (2013) 1556–1565. <https://doi.org/10.1021/am302783h>.
- [139] S. Lázaro-Mass, S. De la Rosa-García, C. García-Solis, J. Reyes-Trujeque, M. Soria-Castro, A.F. Fuentes, P. Quintana, S. Gómez-Cornelio, Controlling growth of phototrophic biofilms on limestone using CaZn₂(OH)₆ · 2H₂O and ZnO nanoparticles, *Journal of Chemical Technology & Biotechnology* 97 (2022) 3011–3023. <https://doi.org/10.1002/jctb.7065>.
- [140] S. Kumar-Krishnan, E. Prokhorov, M. Hernández-Iturriaga, J.D. Mota-Morales, M. Vázquez-Lepe, Y. Kovalenko, I.C. Sanchez, G. Luna-Bárcenas, Chitosan/silver nanocomposites: Synergistic antibacterial action of silver nanoparticles and silver ions, *Eur Polym J* 67 (2015) 242–251. <https://doi.org/10.1016/j.eurpolymj.2015.03.066>.
- [141] J.S. Kim, E. Kuk, K.N. Yu, J.-H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.-Y. Hwang, Y.-K. Kim, Y.-S. Lee, D.H. Jeong, M.-H. Cho, Antimicrobial effects of silver nanoparticles, *Nanomedicine* 3 (2007) 95–101. <https://doi.org/10.1016/j.nano.2006.12.001>.
- [142] D.K. Božanić, L. V. Trandafilović, A.S. Luyt, V. Djoković, ‘Green’ synthesis and optical properties of silver–chitosan complexes and nanocomposites, *React Funct Polym* 70 (2010) 869–873. <https://doi.org/10.1016/j.reactfunctpolym.2010.08.001>.
- [143] A. Panáček, M. Kolář, R. Večeřová, R. Pucek, J. Soukupová, V. Kryštof, P. Hamal, R. Zbořil, L. Kvítek, Antifungal activity of silver nanoparticles against *Candida* spp., *Biomaterials* 30 (2009) 6333–6340. <https://doi.org/10.1016/j.biomaterials.2009.07.065>.
- [144] C. Giuliani, M. Pascucci, C. Riccucci, E. Messina, M. Salzano de Luna, M. Lavorgna, G.M. Ingo, G. Di Carlo, Chitosan-based coatings for corrosion protection of copper-based alloys: A promising more sustainable approach for cultural heritage applications, *Prog Org Coat* 122 (2018) 138–146. <https://doi.org/10.1016/j.porgcoat.2018.05.002>.
- [145] A. Bakhshian Nik, H. Zare, S. Razavi, H. Mohammadi, P. Torab Ahmadi, N. Yazdani, M. Bayandori, N. Rabiee, J. Izadi Mobarakeh, Smart drug delivery: Capping strategies for

- mesoporous silica nanoparticles, *Microporous and Mesoporous Materials* 299 (2020) 110115. <https://doi.org/10.1016/j.micromeso.2020.110115>.
- [146] G. Infurna, G. Cavallaro, G. Lazzara, S. Milioto, N.T. Dintcheva, Bionanocomposite Films Containing Halloysite Nanotubes and Natural Antioxidants with Enhanced Performance and Durability as Promising Materials for Cultural Heritage Protection, *Polymers (Basel)* 12 (2020) 1973. <https://doi.org/10.3390/polym12091973>.
- [147] F. Sbardella, M.P. Bracciale, M.L. Santarelli, J.M. Asua, Waterborne modified-silica/acrylates hybrid nanocomposites as surface protective coatings for stone monuments, *Prog Org Coat* 149 (2020) 105897. <https://doi.org/10.1016/j.porgcoat.2020.105897>.
- [148] M. Frigione, M. Lettieri, Novel Attribute of Organic–Inorganic Hybrid Coatings for Protection and Preservation of Materials (Stone and Wood) Belonging to Cultural Heritage, *Coatings* 8 (2018) 319. <https://doi.org/10.3390/coatings8090319>.
- [149] A. Fujishima, X. Zhang, Titanium dioxide photocatalysis: present situation and future approaches, *Comptes Rendus. Chimie* 9 (2005) 750–760. <https://doi.org/10.1016/j.crci.2005.02.055>.
- [150] Y. Wei, Q. Wu, H. Meng, Y. Zhang, C. Cao, Recent advances in photocatalytic self-cleaning performances of TiO₂-based building materials, *RSC Adv* 13 (2023) 20584–20597. <https://doi.org/10.1039/D2RA07839B>.
- [151] X. Chen, S.S. Mao, Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications, and Applications, *Chem Rev* 107 (2007) 2891–2959. <https://doi.org/10.1021/cr0500535>.
- [152] A. Kubiak, S. Żółtowska, A. Bartkowiak, E. Gabała, N. Sacharczuk, M. Zalas, K. Siwińska-Ciesielczyk, T. Jesionowski, The TiO₂-ZnO Systems with Multifunctional Applications in Photoactive Processes—Efficient Photocatalyst under UV-LED Light and Electrode Materials in DSSCs, *Materials* 14 (2021) 6063. <https://doi.org/10.3390/ma14206063>.
- [153] F. Pinzari, Synthesis, Photocatalytic and Bio Activity of ZnO-TiO₂ Nanocomposites: A Review Study, *Reactions* 5 (2024) 680–739. <https://doi.org/10.3390/reactions5040035>.
- [154] B. Yang, Z. Ma, Q. Wang, J. Yang, Synthesis and Photoelectrocatalytic Applications of TiO₂/ZnO/Diatomite Composites, *Catalysts* 12 (2022) 268. <https://doi.org/10.3390/catal12030268>.
- [155] R. Nawaz, H. Ullah, A.A.J. Ghanim, M. Irfan, M. Anjum, S. Rahman, S. Ullah, Z. Abdel Baki, V. Kumar Oad, Green Synthesis of ZnO and Black TiO₂ Materials and Their Application in Photodegradation of Organic Pollutants, *ACS Omega* 8 (2023) 36076–36087. <https://doi.org/10.1021/acsomega.3c04229>.
- [156] L. Cassar, Photocatalysis of Cementitious Materials: Clean Buildings and Clean Air, *MRS Bull* 29 (2004) 328–331. <https://doi.org/10.1557/mrs2004.99>.
- [157] I. Abdelfattah, A.M. El-Shamy, A comparative study for optimizing photocatalytic activity of TiO₂-based composites with ZrO₂, ZnO, Ta₂O₅, SnO, Fe₂O₃, and CuO additives, *Sci Rep* 14 (2024) 27175. <https://doi.org/10.1038/s41598-024-77752-5>.
- [158] L. Zhang, Q. Lu, R. Shan, F. Zhang, Y. Muhammad, K. Huang, Photocatalytic degradation of vehicular exhaust by nitrogen-doped titanium dioxide modified pavement material, *Transp Res D Transp Environ* 91 (2021) 102690. <https://doi.org/10.1016/j.trd.2020.102690>.

- [159] A. Folli, M. Strøm, T.P. Madsen, T. Henriksen, J. Lang, J. Emenius, T. Klevebrant, Å. Nilsson, Field study of air purifying paving elements containing TiO₂, *Atmos Environ* 107 (2015) 44–51. <https://doi.org/10.1016/j.atmosenv.2015.02.025>.
- [160] J. Hot, C. Fériot, E. Lenard, E. Ringot, NO_x Abatement by a TiO₂-Based Coating under Real-Life Conditions and Laboratory-Scale Durability Assessment, *Environments* 11 (2024) 166. <https://doi.org/10.3390/environments11080166>.
- [161] M. Aldoasri, S. Darwish, M. Adam, N. Elmarzugi, S. Ahmed, Protecting of Marble Stone Facades of Historic Buildings Using Multifunctional TiO₂ Nanocoatings, *Sustainability* 9 (2017) 2002. <https://doi.org/10.3390/su9112002>.
- [162] R. Ghamarpoor, A. Fallah, M. Jamshidi, A Review of Synthesis Methods, Modifications, and Mechanisms of ZnO/TiO₂-Based Photocatalysts for Photodegradation of Contaminants, *ACS Omega* 9 (2024) 25457–25492. <https://doi.org/10.1021/acsomega.3c08717>.
- [163] O.M. Ishchenko, V. Rogé, G. Lamblin, D. Lenoble, TiO₂- and ZnO-Based Materials for Photocatalysis: Material Properties, Device Architecture and Emerging Concepts, in: *Semiconductor Photocatalysis - Materials, Mechanisms and Applications*, InTech, 2016. <https://doi.org/10.5772/62774>.
- [164] H. Si, M. Zhou, Y. Fang, J. He, L. Yang, F. Wang, Photocatalytic concrete for NO_x degradation: Influence factors and durability, *Constr Build Mater* 298 (2021) 123835. <https://doi.org/10.1016/j.conbuildmat.2021.123835>.
- [165] Q. Liu, Q. Liu, Z. Zhu, J. Zhang, B. Zhang, Application of TiO₂ photocatalyst to the stone conservation, *Materials Research Innovations* 19 (2015) S8-51-S8-54. <https://doi.org/10.1179/1432891715Z.0000000001618>.
- [166] S. Haffad, K. Korir Kiprono, Interfacial structure and electronic properties of TiO₂/ZnO/TiO₂ for photocatalytic and photovoltaic applications: A theoretical study, *Surf Sci* 686 (2019) 10–16. <https://doi.org/10.1016/j.susc.2019.03.006>.
- [167] K. Januszkiewicz, K.G. Kowalski, Air Purification in Highly-Urbanized Areas with Use TiO₂: New Approach to Design the Urban Public Space to Benefit Human Condition, *IOP Conf Ser Mater Sci Eng* 603 (2019) 052071. <https://doi.org/10.1088/1757-899X/603/5/052071>.
- [168] M.V. Diamanti, M. Pedferri, Photocatalytic performance of mortars with nanoparticles exposed to the urban environment, in: *Nanotechnology in Eco-Efficient Construction*, Elsevier, 2019: pp. 527–555. <https://doi.org/10.1016/B978-0-08-102641-0.00022-0>.
- [169] D. Colangiuli, A. Calia, N. Bianco, Novel multifunctional coatings with photocatalytic and hydrophobic properties for the preservation of the stone building heritage, *Constr Build Mater* 93 (2015) 189–196. <https://doi.org/10.1016/j.conbuildmat.2015.05.100>.
- [170] M.L. Coutinho, J.P. Veiga, M.F. Macedo, A.Z. Miller, Testing the Feasibility of Titanium Dioxide Sol-Gel Coatings on Portuguese Glazed Tiles to Prevent Biological Colonization, *Coatings* 10 (2020) 1169. <https://doi.org/10.3390/coatings10121169>.
- [171] A. Calia, M. Lettieri, M. Masieri, S. Pal, A. Licciulli, V. Arima, Limestones coated with photocatalytic TiO₂ to enhance building surface with self-cleaning and depolluting abilities, *J Clean Prod* 165 (2017) 1036–1047. <https://doi.org/10.1016/j.jclepro.2017.07.193>.

- [172] T. de Caro, R.G. Toro, L. Cassone, F.I. Barbaccia, C. Zaratti, I.A. Colasanti, M.F. La Russa, A. Macchia, Functionalization of Artwork Packaging Materials Utilizing Ag-Doped TiO₂ and ZnO Nanoparticles, *Molecules* 29 (2024) 3712. <https://doi.org/10.3390/molecules29153712>.
- [173] D.C. Ilies, L. Blaga, A. Ilies, A.C. Pereş, T. Caciora, T.H. Hassan, N. Hodor, A. Turza, H.R. Taghiyari, L. Barbu-Tudoran, R.K. Dahal, P. Dejeu, B. Safarov, M.A. Hossain, Green Biocidal Nanotechnology Use for Urban Stone-Built Heritage—Case Study from Oradea, Romania, *Minerals* 13 (2023) 1170. <https://doi.org/10.3390/min13091170>.
- [174] P. Munafò, G.B. Goffredo, E. Quagliarini, TiO₂-based nanocoatings for preserving architectural stone surfaces: An overview, *Constr Build Mater* 84 (2015) 201–218. <https://doi.org/10.1016/j.conbuildmat.2015.02.083>.
- [175] A.M. Castro-Hoyos, M.A. Rojas Manzano, A. Maury-Ramírez, Challenges and Opportunities of Using Titanium Dioxide Photocatalysis on Cement-Based Materials, *Coatings* 12 (2022) 968. <https://doi.org/10.3390/coatings12070968>.
- [176] E. Franzoni, A. Fregni, R. Gabrielli, G. Graziani, E. Sassoni, Compatibility of photocatalytic TiO₂-based finishing for renders in architectural restoration: A preliminary study, *Build Environ* 80 (2014) 125–135. <https://doi.org/10.1016/j.buildenv.2014.05.027>.
- [177] A.-I. Gopalan, J.-C. Lee, G. Saianand, K.-P. Lee, P. Sonar, R. Dharmarajan, Y. Hou, K.-Y. Ann, V. Kannan, W.-J. Kim, Recent Progress in the Abatement of Hazardous Pollutants Using Photocatalytic TiO₂-Based Building Materials, *Nanomaterials* 10 (2020) 1854. <https://doi.org/10.3390/nano10091854>.
- [178] F. Ubaldi, F. Valeriani, V. Volpini, G. Lofrano, V. Romano Spica, Antimicrobial Activity of Photocatalytic Coatings on Surfaces: A Systematic Review and Meta-Analysis, *Coatings* 14 (2024) 92. <https://doi.org/10.3390/coatings14010092>.
- [179] M.-C. Le, T.-H. Le, T.-H. Bui Thi, Q.-D. Nguyen, T.-H. Do Thi, M.-N. Tran Thi, Synthesizing and Evaluating the Photocatalytic and Antibacterial Ability of TiO₂/SiO₂ Nanocomposite for Silicate Coating, *Front Chem* 9 (2021). <https://doi.org/10.3389/fchem.2021.738969>.
- [180] F. Izzo, A. Arizzi, P. Cappelletti, G. Cultrone, A. De Bonis, C. Germinario, S.F. Graziano, C. Grifa, V. Guarino, M. Mercurio, V. Morra, A. Langella, The art of building in the Roman period (89 B.C. – 79 A.D.): Mortars, plasters and mosaic floors from ancient Stabiae (Naples, Italy), *Constr Build Mater* 117 (2016) 129–143. <https://doi.org/10.1016/J.CONBUILDMAT.2016.04.101>.
- [181] M. Mogetta, *The Origins of Concrete Construction in Roman Architecture*, Cambridge University Press, 2021. <https://doi.org/10.1017/9781108990516>.
- [182] R. Berto, A. Prà, S.P. Maffei, P. Rosato, The appraisal of reproduction costs of historic properties: the case of Venetian Villas / La stima del costo di riproduzione delle dimore storiche: il caso delle Ville Venete, *Valori e Valutazioni* 30 (2022) 45–58. <https://doi.org/10.48264/VVSIEV-20223003>.
- [183] D. Howard, Four Centuries of Literature on Palladio, *Journal of the Society of Architectural Historians* 39 (1980) 224–241. <https://doi.org/10.2307/989568>.
- [184] M. Caroselli, S.A. Ruffolo, F. Piqué, Mortars and plasters—how to manage mortars and plasters conservation, *Archaeol Anthropol Sci* 13 (2021) 188. <https://doi.org/10.1007/s12520-021-01409-x>.

- [185] A. Squassina, Construction Wisdom: Preserving Venice with Both Tradition and Innovation, *Studies in Conservation* 67 (2022) 253–259. <https://doi.org/10.1080/00393630.2022.2046413>.
- [186] A. Ferrighi, The plaster catalogue: comments on the result of the research, *Catalogue of Venetian Plasters and Historical Interventions for High Water Defense* (2006).
- [187] R. Piovesan, R. Siddall, C. Mazzoli, L. Nodari, The Temple of Venus (Pompeii): a study of the pigments and painting techniques, *J Archaeol Sci* 38 (2011) 2633–2643. <https://doi.org/10.1016/J.JAS.2011.05.021>.
- [188] A. Remotto, E. Balliana, F.C. Izzo, G. Biscontin, E. Zendri, Regalzier: study of a typical historical plaster finish in Venice, *Conservation Science in Cultural Heritage* 12 (2012) 309–327. <https://doi.org/10.6092/ISSN.1973-9494/3402>.
- [189] F. Trovo', E. Vettore, The role of craftsmanship in the conservation of Venice. State of the art and perspective, in: *Proceedings HERITAGE 2022 - International Conference on Vernacular Heritage: Culture, People and Sustainability*, Universitat Politècnica de València, Valencia, 2022: pp. 1–9. <https://doi.org/10.4995/HERITAGE2022.2022.15690>.
- [190] N. Tsui, R.J. Flatt, G.W. Scherer, Crystallization damage by sodium sulfate, *J Cult Herit* 4 (2003) 109–115. [https://doi.org/10.1016/S1296-2074\(03\)00022-0](https://doi.org/10.1016/S1296-2074(03)00022-0).
- [191] D. De la Fuente, J.M. Vega, F. Viejo, I. Díaz, M. Morcillo, Mapping air pollution effects on atmospheric degradation of cultural heritage, *J Cult Herit* 14 (2013) 138–145. <https://doi.org/10.1016/J.CULHER.2012.05.002>.
- [192] P. Maravelaki-Kalaitzaki, G. Biscontin, Origin, characteristics and morphology of weathering crusts on Istria stone in Venice, *Atmos Environ* 33 (1999) 1699–1709. [https://doi.org/10.1016/S1352-2310\(98\)00263-5](https://doi.org/10.1016/S1352-2310(98)00263-5).
- [193] I. Costantini, J. Aramendia, E. Tomasini, K. Castro, J. Manuel Madariaga, G. Arana, Detection of unexpected copper sulfate decay compounds on late Gothic mural paintings: Assessing the threat of environmental impact, *Microchemical Journal* 169 (2021) 106542. <https://doi.org/10.1016/J.MICROC.2021.106542>.
- [194] V. Fassina, M. Favaro, A. Naccari, M. Pigo, Evaluation of compatibility and durability of a hydraulic lime-based plaster applied on brick wall masonry of historical buildings affected by rising damp phenomena, *J Cult Herit* 3 (2002) 45–51. [https://doi.org/10.1016/S1296-2074\(02\)01158-5](https://doi.org/10.1016/S1296-2074(02)01158-5).
- [195] G. Leone, A. De Vita, A. Magnani, C. Rossi, Thermal and Petrographic Characterization of Herculaneum Wall Plasters, *Archaeometry* 59 (2017) 747–761. <https://doi.org/10.1111/arc.12275>.
- [196] D. Ergenç, R. Fort, M.J. Varas-Muriel, M. Alvarez de Buergo, Mortars and plasters—How to characterize aerial mortars and plasters, *Archaeol Anthropol Sci* 13 (2021) 197. <https://doi.org/10.1007/s12520-021-01398-x>.
- [197] E. Guolo, P. Romagnoni, F. Peron, Capillary rising damp in Venetian context: state of the art and numerical simulation, *J Phys Conf Ser* 2069 (2021) 012046. <https://doi.org/10.1088/1742-6596/2069/1/012046>.

- [198] E. Sesana, A.S. Gagnon, C. Ciantelli, J. Cassar, J.J. Hughes, Climate change impacts on cultural heritage: A literature review, *WIREs Climate Change* 12 (2021). <https://doi.org/10.1002/wcc.710>.
- [199] L. Falchi, E. Orio, E. Balliana, F.C. Izzo, E. Zendri, Investigation on the relationship between the environment and istria stone surfaces in Venice, *Atmos Environ* 210 (2019) 76–85. <https://doi.org/10.1016/J.ATMOSENV.2019.04.044>.
- [200] 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 (1991) 187–207. [https://doi.org/10.1016/0265-3036\(91\)90042-P](https://doi.org/10.1016/0265-3036(91)90042-P).
- [201] A. Mihajlovski, D. Seyer, H. Benamara, F. Bousta, P. Di Martino, An overview of techniques for the characterization and quantification of microbial colonization on stone monuments, *Ann Microbiol* 65 (2015) 1243–1255. <https://doi.org/10.1007/s13213-014-0956-2>.
- [202] T.C. Dakal, S.S. Cameotra, Microbially induced deterioration of architectural heritages: routes and mechanisms involved, *Environ Sci Eur* 24 (2012) 36. <https://doi.org/10.1186/2190-4715-24-36>.
- [203] C. Sabbioni, A. Bonazza, G. Zappia, Damage on hydraulic mortars: the Venice Arsenal, *J Cult Herit* 3 (2002) 83–88. [https://doi.org/10.1016/S1296-2074\(02\)01163-9](https://doi.org/10.1016/S1296-2074(02)01163-9).
- [204] X. Liu, Y. Qian, F. Wu, Y. Wang, W. Wang, J.D. Gu, Biofilms on stone monuments: biodeterioration or bioprotection?, *Trends Microbiol* 30 (2022) 816–819. <https://doi.org/10.1016/J.TIM.2022.05.012>.
- [205] E. Gioventù, P. Lorenzi, Bio-Removal of Black Crust from Marble Surface: Comparison with Traditional Methodologies and Application on a Sculpture from the Florence’s English Cemetery, *Procedia Chem* 8 (2013) 123–129. <https://doi.org/10.1016/J.PROCHE.2013.03.017>.
- [206] S. Pozo, P. Barreiro, T. Rivas, P. González, M.P. Fiorucci, Effectiveness and harmful effects of removal sulphated black crust from granite using Nd:YAG nanosecond pulsed laser, *Appl Surf Sci* 302 (2014) 309–313. <https://doi.org/10.1016/J.APSUSC.2013.10.129>.
- [207] C. Ricci, F. Gambino, M. Nervo, A. Piccirillo, A. Scarcella, F. Zenuccini, A. Ramil, J.S. Pozo-Antonio, Enhancement of graffiti removal from heritage stone by combining laser ablation and application of a solvent mixture, *Constr Build Mater* 262 (2020) 119934. <https://doi.org/10.1016/J.CONBUILDMAT.2020.119934>.
- [208] S.K. Kirthika, G. Goel, A. Matthews, S. Goel, Review of the untapped potentials of antimicrobial materials in the construction sector, *Prog Mater Sci* 133 (2023). <https://doi.org/10.1016/j.pmatsci.2022.101065>.
- [209] L. Liao, M. Wang, Z. Li, X. Wang, W. Zhou, Recent Advances in Black TiO₂ Nanomaterials for Solar Energy Conversion, *Nanomaterials* 13 (2023) 468. <https://doi.org/10.3390/nano13030468>.
- [210] N. Madkhali, C. Prasad, K. Malkappa, H.Y. Choi, V. Govinda, I. Bahadur, R.A. Abumousa, Recent update on photocatalytic degradation of pollutants in waste water using TiO₂-based heterostructured materials, *Results in Engineering* 17 (2023) 100920. <https://doi.org/10.1016/J.RINENG.2023.100920>.

- [211] A. Bathla, S.A. Younis, K.-H. Kim, X. Li, TiO₂-based catalytic systems for the treatment of airborne aromatic hydrocarbons, *Mater Horiz* (2023). <https://doi.org/10.1039/D2MH01583H>.
- [212] S. Veltri, A.M. Palermo, G. De Filpo, F. Xu, Subsurface treatment of TiO₂ nanoparticles for limestone: Prolonged surface photocatalytic biocidal activities, *Build Environ* 149 (2019) 655–661. <https://doi.org/10.1016/J.BUILDENV.2018.10.038>.
- [213] I. Alfieri, A. Lorenzi, L. Ranzenigo, L. Lazzarini, G. Predieri, P.P. Lottici, Synthesis and characterization of photocatalytic hydrophobic hybrid TiO₂-SiO₂ coatings for building applications, *Build Environ* 111 (2017) 72–79. <https://doi.org/10.1016/J.BUILDENV.2016.10.019>.
- [214] M.F. La Russa, A. Macchia, S.A. Ruffolo, F. De Leo, M. Barberio, P. Barone, G.M. Crisci, C. Urzì, Testing the antibacterial activity of doped TiO₂ for preventing biodeterioration of cultural heritage building materials, *Int Biodeterior Biodegradation* 96 (2014) 87–96. <https://doi.org/10.1016/J.IBIOD.2014.10.002>.
- [215] D. Zanardo, E. Ghedini, F. Menegazzo, A. Giordana, G. Cerrato, A. Di Michele, G. Cruciani, M. Signoretto, Traditional Venetian marmorino: Effect of zinc-based oxides on self-bleaching properties, *J Cult Herit* 50 (2021) 171–178. <https://doi.org/10.1016/J.CULHER.2021.04.006>.
- [216] M. Dell'Edera, C. Lo Porto, I. De Pasquale, F. Petronella, M.L. Curri, A. Agostiano, R. Comparelli, Photocatalytic TiO₂-based coatings for environmental applications, *Catal Today* 380 (2021) 62–83. <https://doi.org/10.1016/J.CATTOD.2021.04.023>.
- [217] L. Scappin, A. Campostrini, D. Zanardo, E. Ghedini, M. Signoretto, G. Berto, F. Menegazzo, Marmorino and photocatalysts: a meeting between tradition and innovation, *International Journal of Architectural Conservation and Restoration I* (2022) 35–49. <https://doi.org/10.57639/SIRA.INTR0104>.
- [218] H. Shu, M. Yang, Q. Liu, M. Luo, Study of TiO₂-Modified Sol Coating Material in the Protection of Stone-Built Cultural Heritage, *Coatings* 10 (2020) 179. <https://doi.org/10.3390/coatings10020179>.
- [219] F. Gherardi, A. Colombo, M. D'Arienzo, B. Di Credico, S. Goidanich, F. Morazzoni, R. Simonutti, L. Toniolo, Efficient self-cleaning treatments for built heritage based on highly photo-active and well-dispersible TiO₂ nanocrystals, *Microchemical Journal* 126 (2016) 54–62. <https://doi.org/10.1016/j.microc.2015.11.043>.
- [220] N. Ditaranto, I.D. van der Werf, R.A. Picca, M.C. Sportelli, L.C. Giannossa, E. Bonerba, G. Tantillo, L. Sabbatini, Characterization and behaviour of ZnO-based nanocomposites designed for the control of biodeterioration of patrimonial stoneworks, *New Journal of Chemistry* 39 (2015) 6836–6843. <https://doi.org/10.1039/C5NJ00527B>.
- [221] J.D. Bersch, I. Flores-Colen, A.B. Masuero, D.C.C. Dal Molin, Photocatalytic TiO₂-Based Coatings for Mortars on Facades: A Review of Efficiency, Durability, and Sustainability, *Buildings* 13 (2023). <https://doi.org/10.3390/buildings13010186>.
- [222] G.M.C. Gemelli, M. Luna, R. Zarzuela, M.L.A. Gil Montero, M. Carbú, I. Moreno-Garrido, M.J. Mosquera, 4-Year in-situ assessment of a photocatalytic TiO₂/SiO₂ antifouling treatment for historic mortar in a coastal city, *Build Environ* 225 (2022) 109627. <https://doi.org/10.1016/j.buildenv.2022.109627>.

- [223] S. Veltri, A.M. Palermo, G. De Filpo, F. Xu, Subsurface treatment of TiO₂ nanoparticles for limestone: Prolonged surface photocatalytic biocidal activities, *Build Environ* 149 (2019) 655–661. <https://doi.org/10.1016/j.buildenv.2018.10.038>.
- [224] G.B. Goffredo, S. Accoroni, C. Totti, T. Romagnoli, L. Valentini, P. Munafò, Titanium dioxide based nanotreatments to inhibit microalgal fouling on building stone surfaces, *Build Environ* 112 (2017) 209–222. <https://doi.org/10.1016/j.buildenv.2016.11.034>.
- [225] K. Loh, C.C. Gaylarde, M.A. Shirakawa, Photocatalytic activity of ZnO and TiO₂ ‘nanoparticles’ for use in cement mixes, *Constr Build Mater* 167 (2018) 853–859. <https://doi.org/10.1016/j.conbuildmat.2018.02.103>.
- [226] M. Abd Elgaleel Hossin, T. M. Tiama, A. Hussein A. Russlan, The Anti-Bacterial Lime Mortar Used in the Restoration of Ancient Limestone Buildings by Adding Heavy Metal Oxide, *International Journal of Multidisciplinary Studies in Architecture and Cultural Heritage* 5 (2022) 93–122. <https://doi.org/10.21608/ijmsac.2022.276737>.
- [227] M.M. Khan, M.H. Harunsani, A.L. Tan, M. Hojamberdiev, Y.A. Poi, N. Ahmad, Antibacterial Studies of ZnO and Cu-Doped ZnO Nanoparticles Synthesized Using Aqueous Leaf Extract of *Stachytarpheta jamaicensis*, *Bionanoscience* 10 (2020) 1037–1048. <https://doi.org/10.1007/s12668-020-00775-5>.
- [228] S. Gunalan, R. Sivaraj, V. Rajendran, Green synthesized ZnO nanoparticles against bacterial and fungal pathogens, *Progress in Natural Science: Materials International* 22 (2012) 693–700. <https://doi.org/10.1016/j.pnsc.2012.11.015>.
- [229] J. Sawai, Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay, *J Microbiol Methods* 54 (2003) 177–182. [https://doi.org/10.1016/S0167-7012\(03\)00037-X](https://doi.org/10.1016/S0167-7012(03)00037-X).
- [230] N. Jones, B. Ray, K.T. Ranjit, A.C. Manna, Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms, *FEMS Microbiol Lett* 279 (2008) 71–76. <https://doi.org/10.1111/j.1574-6968.2007.01012.x>.
- [231] I. Klapiszewska, A. Kubiak, A. Parus, M. Janczarek, A. Ślosarczyk, The In Situ Hydrothermal and Microwave Syntheses of Zinc Oxides for Functional Cement Composites, *Materials* 15 (2022). <https://doi.org/10.3390/ma15031069>.
- [232] V. Trevisan, M. Signoretto, F. Pinna, G. Cruciani, G. Cerrato, Investigation on Titania Synthesis for Photocatalytic NO_x Abatement, *Chemistry Today* 30 (2012) 25–28.
- [233] D. Zanardo, G. Forghieri, E. Ghedini, F. Menegazzo, A. Giordana, G. Cerrato, E. Cattaruzza, A. Di Michele, G. Cruciani, M. Signoretto, Effect of the Synthetic Parameters over ZnO in the CO₂ Photoreduction, *Molecules* 28 (2023) 4798. <https://doi.org/10.3390/molecules28124798>.
- [234] R.A. Samson, J. Houbaken, U. Thrane, J.C. Frisvad, B. Andersen, *Food and Indoor Fungi*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, 2010.
- [235] D. Sharma, J. Rajput, B.S. Kaith, M. Kaur, S. Sharma, Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties, *Thin Solid Films* 519 (2010) 1224–1229. <https://doi.org/10.1016/j.tsf.2010.08.073>.

- [236] M.A. Shirakawa, I.B. Beech, R. Tapper, M.A. Cincotto, W. Gambale, The development of a method to evaluate bioreceptivity of indoor mortar plastering to fungal growth, *Int Biodeterior Biodegradation* 51 (2003) 83–92. [https://doi.org/10.1016/S0964-8305\(01\)00129-9](https://doi.org/10.1016/S0964-8305(01)00129-9).
- [237] J.E. Spraker, L.M. Sanchez, T.M. Lowe, P.C. Dorrestein, N.P. Keller, *Ralstonia solanacearum* lipopeptide induces chlamydospore development in fungi and facilitates bacterial entry into fungal tissues, *ISME J* 10 (2016) 2317–2330. <https://doi.org/10.1038/ismej.2016.32>.
- [238] A. Micheluz, F. Pinzari, E.G. Rivera-Valentín, S. Manente, J.E. Hallsworth, Biophysical Manipulation of the Extracellular Environment by *Eurotium halophilicum*, *Pathogens* 11 (2022) 1462. <https://doi.org/10.3390/pathogens11121462>.
- [239] T.-C. Lin, M.Y.A. Mollah, R.K. Vempati, D.L. Cocke, Synthesis and Characterization of Calcium Hydroxyzincate Using X-ray Diffraction, FT-IR Spectroscopy, and Scanning Force Microscopy, *Chemistry of Materials* 7 (1995) 1974–1978. <https://doi.org/10.1021/cm00058a031>.
- [240] K. Kobayakawa, Y. Nakazawa, M. Ikeda, Y. Sato, A. Fujishima, ChemInform Abstract: Influence of the Density of Surface Hydroxyl Groups on TiO₂ Photocatalytic Activities., *ChemInform* 22 (1991). <https://doi.org/10.1002/chin.199112023>.
- [241] A. Fouda, M. Abdel-Nasser, A.M. Eid, S.E.-D. Hassan, A. Abdel-Nasser, N.K. Alharbi, A.H. AlRokban, G. Abdel-Maksoud, An Eco-Friendly Approach Utilizing Green Synthesized Titanium Dioxide Nanoparticles for Leather Conservation against a Fungal Strain, *Penicillium expansum* AL1, Involved in the Biodeterioration of a Historical Manuscript, *Biology (Basel)* 12 (2023) 1025. <https://doi.org/10.3390/biology12071025>.
- [242] S. Park, S. Lee, B. Kim, S. Lee, J. Lee, S. Sim, M. Gu, J. Yi, J. Lee, Toxic effects of titanium dioxide nanoparticles on microbial activity and metabolic flux, *Biotechnology and Bioprocess Engineering* 17 (2012) 276–282. <https://doi.org/10.1007/s12257-010-0251-4>.
- [243] L. Canesi, A. Sardella, R. Vogler, A. Kaiser, C. Vaccaro, A. Bonazza, Hazard Analysis and Vulnerability Assessment of Cultural Landscapes Exposed to Climate Change-Related Extreme Events: A Case Study of Wachau (Austria), *Heritage* 7 (2024) 1917–1934. <https://doi.org/10.3390/heritage7040091>.
- [244] A. Michaelsen, F. Pinzari, N. Barbabietola, G. Piñar, Monitoring the effects of different conservation treatments on paper-infecting fungi, *Int Biodeterior Biodegradation* 84 (2013) 333–341. <https://doi.org/10.1016/j.ibiod.2012.08.005>.
- [245] J. Becerra, M. Mateo, P. Ortiz, G. Nicolás, A.P. Zaderenko, Evaluation of the applicability of nano-biocide treatments on limestones used in cultural heritage, *J Cult Herit* 38 (2019) 126–135. <https://doi.org/10.1016/j.culher.2019.02.010>.
- [246] I.A. Jones, L.T. Joshi, Biocide Use in the Antimicrobial Era: A Review, *Molecules* 26 (2021) 2276. <https://doi.org/10.3390/molecules26082276>.
- [247] E. Kampasakali, T. Fardi, E. Pavlidou, D. Christofilos, Towards Sustainable Museum Conservation Practices: A Study on the Surface Cleaning of Contemporary Art and Design Objects with the Use of Biodegradable Agents, *Heritage* 4 (2021) 2023–2043. <https://doi.org/10.3390/heritage4030115>.
- [248] A.J. Fonseca, F. Pina, M.F. Macedo, N. Leal, A. Romanowska-Deskins, L. Laiz, A. Gómez-Bolea, C. Saiz-Jimenez, Anatase as an alternative application for preventing biodeterioration of

- mortars: Evaluation and comparison with other biocides, *Int Biodeterior Biodegradation* 64 (2010) 388–396. <https://doi.org/10.1016/j.ibiod.2010.04.006>.
- [249] C. Dresler, M.L. Saladino, C. Demirbag, E. Caponetti, D.F. Chillura Martino, R. Alduina, Development of controlled release systems of biocides for the conservation of cultural heritage, *Int Biodeterior Biodegradation* 125 (2017) 150–156. <https://doi.org/10.1016/j.ibiod.2017.09.007>.
- [250] T. YOSHIMATSU, K.-I. HIYAMA, Mechanism of the Action of Didecyldimethylammonium chloride (DDAC) against *Escherichia coli* and Morphological Changes of the Cells, *Biocontrol Sci* 12 (2007) 93–99. <https://doi.org/10.4265/bio.12.93>.
- [251] J.O. Morley, A.J. Oliver Kapur, M.H. Charlton, Structure–activity relationships in 3-isothiazolones, *Org Biomol Chem* 3 (2005) 3713. <https://doi.org/10.1039/b509529h>.
- [252] S.A. Ruffolo, M.F. La Russa, M. Malagodi, C. Oliviero Rossi, A.M. Palermo, G.M. Crisci, ZnO and ZnTiO₃ nanopowders for antimicrobial stone coating, *Applied Physics A* 100 (2010) 829–834. <https://doi.org/10.1007/s00339-010-5658-4>.
- [253] M. Roveri, F. Gherardi, S. Goidanich, D. Gulotta, V. Castelvetro, R. Fischer, L. Winandy, J. Weber, L. Toniolo, Self-cleaning and antifouling nanocomposites for stone protection: properties and performances of stone-nanomaterial systems, *IOP Conf Ser Mater Sci Eng* 364 (2018) 012070. <https://doi.org/10.1088/1757-899X/364/1/012070>.
- [254] T.A. Debele, S.L. Mekuria, H.-C. Tsai, Polysaccharide based nanogels in the drug delivery system: Application as the carrier of pharmaceutical agents, *Materials Science and Engineering: C* 68 (2016) 964–981. <https://doi.org/10.1016/j.msec.2016.05.121>.
- [255] P. Severino, J.F. Fanguero, M. V. Chaud, J. Cordeiro, A.M. Silva, E.B. Souto, Advances in nanobiomaterials for topical administrations: new galenic and cosmetic formulations, in: *Nanobiomaterials in Galenic Formulations and Cosmetics*, Elsevier, 2016: pp. 1–23. <https://doi.org/10.1016/B978-0-323-42868-2.00001-2>.
- [256] S. Rondinini, S. Ardizzone, G. Cappelletti, A. Minguzzi, A. Vertova, MATERIALS | Sol–Gel Synthesis, in: *Encyclopedia of Electrochemical Power Sources*, Elsevier, 2009: pp. 613–624. <https://doi.org/10.1016/B978-044452745-5.00054-X>.
- [257] M. Hasanzadeh, N. Shadjou, E. Omidinia, M. Eskandani, M. de la Guardia, Mesoporous silica materials for use in electrochemical immunosensing, *TrAC Trends in Analytical Chemistry* 45 (2013) 93–106. <https://doi.org/10.1016/j.trac.2012.12.017>.
- [258] Y. Wang, F. Caruso, Mesoporous Silica Spheres as Supports for Enzyme Immobilization and Encapsulation, *Chemistry of Materials* 17 (2005) 953–961. <https://doi.org/10.1021/cm0483137>.
- [259] K. Möller, T. Bein, Degradable Drug Carriers: Vanishing Mesoporous Silica Nanoparticles, *Chemistry of Materials* 31 (2019) 4364–4378. <https://doi.org/10.1021/acs.chemmater.9b00221>.
- [260] E. Molina, M. Mathonnat, J. Richard, P. Lacroix-Desmazes, M. In, P. Dieudonné, T. Cacciaguerra, C. Gérardin, N. Marcotte, pH-mediated control over the mesostructure of ordered mesoporous materials templated by polyion complex micelles, *Beilstein Journal of Nanotechnology* 10 (2019) 144–156. <https://doi.org/10.3762/bjnano.10.14>.

- [261] L. Contessotto, E. Ghedini, F. Pinna, M. Signoretto, G. Cerrato, V. Crocellà, Hybrid Organic–Inorganic Silica Gel Carriers with Controlled Drug-Delivery Properties, *Chemistry – A European Journal* 15 (2009) 12043–12049. <https://doi.org/10.1002/chem.200900603>.
- [262] E. Ghedini, V. Nichele, M. Signoretto, G. Cerrato, Structure-Directing Agents for the Synthesis of TiO₂-Based Drug-Delivery Systems, *Chemistry – A European Journal* 18 (2012) 10653–10660. <https://doi.org/10.1002/chem.201201355>.
- [263] S.K. Jana, A. Mochizuki, S. Namba, Progress in Pore-Size Control of Mesoporous MCM-41 Molecular Sieve Using Surfactant Having Different Alkyl Chain Lengths and Various Organic Auxiliary Chemicals, *Catalysis Surveys from Asia* 8 (2004) 1–13. <https://doi.org/10.1023/B:CATS.0000015110.85694.d9>.
- [264] V. Gold, ed., *The IUPAC Compendium of Chemical Terminology*, International Union of Pure and Applied Chemistry (IUPAC), Research Triangle Park, NC, 2019. <https://doi.org/10.1351/goldbook>.
- [265] S.-H. Wu, C.-Y. Mou, H.-P. Lin, Synthesis of mesoporous silica nanoparticles, *Chem Soc Rev* 42 (2013) 3862. <https://doi.org/10.1039/c3cs35405a>.
- [266] H.-P. Lin, C.-Y. Mou, Structural and Morphological Control of Cationic Surfactant-Templated Mesoporous Silica, *Acc Chem Res* 35 (2002) 927–935. <https://doi.org/10.1021/ar000074f>.
- [267] R. Narayan, U. Nayak, A. Raichur, S. Garg, Mesoporous Silica Nanoparticles: A Comprehensive Review on Synthesis and Recent Advances, *Pharmaceutics* 10 (2018) 118. <https://doi.org/10.3390/pharmaceutics10030118>.
- [268] Z. Allothman, A Review: Fundamental Aspects of Silicate Mesoporous Materials, *Materials* 5 (2012) 2874–2902. <https://doi.org/10.3390/ma5122874>.
- [269] S. Kumar, M.M. Malik, R. Purohit, Synthesis Methods of Mesoporous Silica Materials, *Mater Today Proc* 4 (2017) 350–357. <https://doi.org/10.1016/j.matpr.2017.01.032>.
- [270] A. Maleki, H. Kettiger, A. Schoubben, J.M. Rosenholm, V. Ambroggi, M. Hamidi, Mesoporous silica materials: From physico-chemical properties to enhanced dissolution of poorly water-soluble drugs, *Journal of Controlled Release* 262 (2017) 329–347. <https://doi.org/10.1016/j.jconrel.2017.07.047>.
- [271] R.K. ILER, The Colloid Chemistry of Silica and Silicates, *Soil Sci* 80 (1955) 86. <https://doi.org/10.1097/00010694-195507000-00014>.
- [272] A. Rimola, D. Costa, M. Sodupe, J.-F. Lambert, P. Ugliengo, Silica Surface Features and Their Role in the Adsorption of Biomolecules: Computational Modeling and Experiments, *Chem Rev* 113 (2013) 4216–4313. <https://doi.org/10.1021/cr3003054>.
- [273] C. Pizzolitto, E. Ghedini, F. Menegazzo, M. Signoretto, A. Giordana, G. Cerrato, G. Cruciani, Effect of grafting solvent in the optimisation of Sba-15 acidity for levulinic acid production, *Catal Today* 345 (2020) 183–189. <https://doi.org/10.1016/j.cattod.2019.11.012>.
- [274] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, *J Pharm Anal* 6 (2016) 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>.
- [275] F.A. Arzani, J.H.Z. dos Santos, Biocides and techniques for their encapsulation: a review, *Soft Matter* 18 (2022) 5340–5358. <https://doi.org/10.1039/D1SM01114F>.

- [276] A. Michaelsen, F. Pinzari, N. Barbabietola, G. Piñar, Monitoring the effects of different conservation treatments on paper-infecting fungi, *Int Biodeterior Biodegradation* 84 (2013) 333–341. <https://doi.org/10.1016/j.ibiod.2012.08.005>.
- [277] A. Ramirez, J.-M. Clacens, C. Lorentz, Y. Pouilloux, Comparison Between SBA-15 and MCM-41 Structure on the Stability and the Selectivity of Basic Catalysts in Oligomerization of Glycerol, *Curr Org Chem* 16 (2012) 2774–2781. <https://doi.org/10.2174/138527212804546903>.
- [278] M. Thommes, R. Köhn, M. Fröba, Sorption and pore condensation behavior of pure fluids in mesoporous MCM-48 silica, MCM-41 silica, SBA-15 silica and controlled-pore glass at temperatures above and below the bulk triple point, *Appl Surf Sci* 196 (2002) 239–249. [https://doi.org/10.1016/S0169-4332\(02\)00062-4](https://doi.org/10.1016/S0169-4332(02)00062-4).
- [279] I. Díaz, F. Mohino, J. Pérez-Pariente, E. Sastre, Study by TG–MS of the oxidation of SH-MCM-41 to SO₃H-MCM-41, *Thermochim Acta* 413 (2004) 201–207. <https://doi.org/10.1016/j.tca.2003.10.008>.
- [280] D. Pinna, Can we do without biocides to cope with biofilms and lichens on stone heritage?, *Int Biodeterior Biodegradation* 172 (2022) 105437. <https://doi.org/10.1016/j.ibiod.2022.105437>.
- [281] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, *J Pharm Anal* 6 (2016) 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>.
- [282] L. Randazzo, G. Montana, R. Alduina, P. Quatrini, E. Tsantini, B. Salemi, Flos Tectorii degradation of mortars: An example of synergistic action between soluble salts and biodeteriogens, *J Cult Herit* 16 (2015) 838–847. <https://doi.org/10.1016/j.culher.2015.04.002>.
- [283] P. Fernandes, Applied microbiology and biotechnology in the conservation of stone cultural heritage materials, *Appl Microbiol Biotechnol* 73 (2006) 291–296. <https://doi.org/10.1007/s00253-006-0599-8>.
- [284] E. Franzoni, L. Volpi, A. Bonoli, Applicability of life cycle assessment methodology to conservation works in historical building: The case of cleaning, *Energy Build* 214 (2020) 109844. <https://doi.org/10.1016/j.enbuild.2020.109844>.
- [285] F. Gabriele, M. Tortora, L. Bruno, C. Casieri, M. Chiarini, R. Germani, N. Spreti, Alginate-biocide hydrogel for the removal of biofilms from calcareous stone artworks, *J Cult Herit* 49 (2021) 106–114. <https://doi.org/10.1016/j.culher.2021.02.009>.
- [286] A. Sala-Luis, H. Oliveira-Urquiri, P. Bosch-Roig, S. Martín-Rey, Eco-Sustainable Approaches to Prevent and/or Eradicate Fungal Biodeterioration on Easel Painting, *Coatings* 14 (2024) 124. <https://doi.org/10.3390/coatings14010124>.
- [287] G. Granata, S. Stracquadanio, M. Leonardi, E. Napoli, G.M.L. Consoli, V. Cafiso, S. Stefani, C. Geraci, Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation, *Food Chem* 269 (2018) 286–292. <https://doi.org/10.1016/j.foodchem.2018.06.140>.
- [288] M.R. Fidanza, G. Caneva, Natural biocides for the conservation of stone cultural heritage: A review, *J Cult Herit* 38 (2019) 271–286. <https://doi.org/10.1016/j.culher.2019.01.005>.

- [289] M. Zuenä, 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* 11 (2021) 1109. <https://doi.org/10.3390/coatings11091109>.
- [290] C. Dima, S. Dima, Essential oils in foods: extraction, stabilization, and toxicity, *Curr Opin Food Sci* 5 (2015) 29–35. <https://doi.org/10.1016/j.cofs.2015.07.003>.
- [291] M. Llana-Ruiz-Cabello, S. Pichardo, S. Maisanaba, M. Puerto, A.I. Prieto, D. Gutiérrez-Praena, A. Jos, A.M. Cameán, In vitro toxicological evaluation of essential oils and their main compounds used in active food packaging: A review, *Food and Chemical Toxicology* 81 (2015) 9–27. <https://doi.org/10.1016/j.fct.2015.03.030>.
- [292] A. Lucia, E. Guzmán, Emulsions containing essential oils, their components or volatile semiochemicals as promising tools for insect pest and pathogen management, *Adv Colloid Interface Sci* 287 (2021) 102330. <https://doi.org/10.1016/j.cis.2020.102330>.
- [293] C. Fuentes, A. Fuentes, J.M. Barat, M.J. Ruiz, Relevant essential oil components: a minireview on increasing applications and potential toxicity, *Toxicol Mech Methods* 31 (2021) 559–565. <https://doi.org/10.1080/15376516.2021.1940408>.
- [294] L. Ruggiero, M.R. Fidanza, M. Iorio, L. Tortora, G. Caneva, M.A. Ricci, A. Sodo, Synthesis and Characterization of TEOS Coating Added With Innovative Antifouling Silica Nanocontainers and TiO₂ Nanoparticles, *Front Mater* 7 (2020). <https://doi.org/10.3389/fmats.2020.00185>.
- [295] L. Ruggiero, F. Bartoli, M.R. Fidanza, F. Zurlo, E. Marconi, T. Gasperi, S. Tuti, L. Crociani, E. Di Bartolomeo, G. Caneva, M.A. Ricci, A. Sodo, Encapsulation of environmentally-friendly biocides in silica nanosystems for multifunctional coatings, *Appl Surf Sci* 514 (2020) 145908. <https://doi.org/10.1016/j.apsusc.2020.145908>.
- [296] F. Bartoli, L. Corradi, Z. Hosseini, A. Privitera, M. Zuenä, A. Kumbaric, V. Graziani, L. Tortora, A. Sodo, G. Caneva, In Vitro Viability Tests of New Ecofriendly Nanosystems Incorporating Essential Oils for Long-Lasting Conservation of Stone Artworks, *Gels* 10 (2024) 132. <https://doi.org/10.3390/gels10020132>.
- [297] M.M. Gakuubi, A.W. Maina, J.M. Wagacha, Antifungal Activity of Essential Oil of *Eucalyptus camaldulensis* Dehnh. against Selected *Fusarium* spp., *Int J Microbiol* 2017 (2017) 1–7. <https://doi.org/10.1155/2017/8761610>.
- [298] G. D’Agostino, B. Giambra, F. Palla, M. Bruno, N. Badalamenti, The Application of the Essential Oils of *Thymus vulgaris* L. and *Crithmum maritimum* L. as Biocidal on Two Tholu Bommalu Indian Leather Puppets, *Plants* 10 (2021) 1508. <https://doi.org/10.3390/plants10081508>.
- [299] S. Sparacello, G. Gallo, T. Faddetta, B. Megna, G. Nicotra, B. Bruno, B. Giambra, F. Palla, *Thymus vulgaris* Essential Oil and Hydro-Alcoholic Solutions to Counteract Wooden Artwork Microbial Colonization, *Applied Sciences* 11 (2021) 8704. <https://doi.org/10.3390/app11188704>.
- [300] A. Campostrini, E. Ghedini, T. Botrè, S. Manente, A. Giordana, G. Cerrato, G. Cruciani, A.W. Robertson, M. Signoreto, F. Menegazzo, Engineered silica NPs to hold and release the antimicrobial product Biotin T®, *Next Materials* 7 (2025) 100373. <https://doi.org/10.1016/j.nxmte.2024.100373>.

- [301] A. Breda, M. Signoretto, E. Ghedini, F. Pinna, G. Cruciani, Acylation of veratrole over promoted SZ/MCM-41 catalysts: Influence of metal promotion, *Appl Catal A Gen* 308 (2006) 216–222. <https://doi.org/10.1016/j.apcata.2006.04.039>.
- [302] L.B. Eddin, N.K. Jha, M.F.N. Meeran, K.K. Kesari, R. Beiram, S. Ojha, Neuroprotective Potential of Limonene and Limonene Containing Natural Products, *Molecules* 26 (2021) 4535. <https://doi.org/10.3390/molecules26154535>.
- [303] S. Chouhan, K. Sharma, S. Guleria, Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives, *Medicines* 4 (2017) 58. <https://doi.org/10.3390/medicines4030058>.
- [304] S. Oliveira Ribeiro, V. Fontaine, V. Mathieu, A. Zhiri, D. Baudoux, C. Stévigny, F. Souard, Antibacterial and Cytotoxic Activities of Ten Commercially Available Essential Oils, *Antibiotics* 9 (2020) 717. <https://doi.org/10.3390/antibiotics9100717>.
- [305] J. Díaz-Alonso, A. Bernardos, J.L. Regidor-Ros, R. Martínez-Máñez, P. Bosch-Roig, Innovative use of essential oil cold diffusion system for improving air quality on indoor cultural heritage spaces, *Int Biodeterior Biodegradation* 162 (2021) 105251. <https://doi.org/10.1016/j.ibiod.2021.105251>.
- [306] V. López, M. Cascella, G. Benelli, F. Maggi, C. Gómez-Rincón, Green drugs in the fight against *Anisakis simplex*—larvicidal activity and acetylcholinesterase inhibition of *Origanum compactum* essential oil, *Parasitol Res* 117 (2018) 861–867. <https://doi.org/10.1007/s00436-018-5764-3>.
- [307] N.C. Silva, A.R. Madureira, M. Pintado, P.R. Moreira, Biocontamination and diversity of epilithic bacteria and fungi colonising outdoor stone and mortar sculptures, *Appl Microbiol Biotechnol* 106 (2022) 3811–3828. <https://doi.org/10.1007/s00253-022-11957-4>.
- [308] N.L. Glass, G.C. Donaldson, Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes, *Appl Environ Microbiol* 61 (1995) 1323–1330. <https://doi.org/10.1128/aem.61.4.1323-1330.1995>.
- [309] S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res* 25 (1997) 3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
- [310] C.E.H. Maung, H.G. Lee, J.-Y. Cho, K.Y. Kim, Antifungal compound, methyl hippurate from *Bacillus velezensis* CE 100 and its inhibitory effect on growth of *Botrytis cinerea*, *World J Microbiol Biotechnol* 37 (2021) 159. <https://doi.org/10.1007/s11274-021-03046-x>.
- [311] İ. Kahramanoğlu, O. Panfilova, T.G. Kesimci, A.U. Bozhüyük, R. Gürbüz, H. Alptekin, Control of Postharvest Gray Mold at Strawberry Fruits Caused by *Botrytis cinerea* and Improving Fruit Storability through *Origanum onites* L. and *Ziziphora clinopodioides* L. Volatile Essential Oils, *Agronomy* 12 (2022) 389. <https://doi.org/10.3390/agronomy12020389>.
- [312] A. Janatova, A. Bernardos, J. Smid, A. Frankova, M. Lhotka, L. Kourimská, J. Pulkrabek, P. Kloucek, Long-term antifungal activity of volatile essential oil components released from mesoporous silica materials, *Ind Crops Prod* 67 (2015) 216–220. <https://doi.org/10.1016/j.indcrop.2015.01.019>.

- [313] R. Ribeiro-Santos, M. Andrade, A. Sanches-Silva, Application of encapsulated essential oils as antimicrobial agents in food packaging, *Curr Opin Food Sci* 14 (2017) 78–84. <https://doi.org/10.1016/j.cofs.2017.01.012>.
- [314] J. Yammine, N.-E. Chihib, A. Gharsallaoui, A. Ismail, L. Karam, Advances in essential oils encapsulation: development, characterization and release mechanisms, *Polymer Bulletin* 81 (2024) 3837–3882. <https://doi.org/10.1007/s00289-023-04916-0>.
- [315] S. Beirão da Costa, C. Duarte, A.I. Bourbon, A.C. Pinheiro, A.T. Serra, M. Moldão Martins, M.I. Nunes Januário, A.A. Vicente, I. Delgadillo, C. Duarte, M.L. Beirão da Costa, Effect of the matrix system in the delivery and in vitro bioactivity of microencapsulated Oregano essential oil, *J Food Eng* 110 (2012) 190–199. <https://doi.org/10.1016/j.jfoodeng.2011.05.043>.
- [316] A.R. Mukurumbira, R.A. Shellie, R. Keast, E.A. Palombo, S.R. Jadhav, Encapsulation of essential oils and their application in antimicrobial active packaging, *Food Control* 136 (2022) 108883. <https://doi.org/10.1016/j.foodcont.2022.108883>.
- [317] S. Ramadan, S.A. El-Kholy, A.F. ElHagrassy, Comparative study on the effect of chitosan, chitosan nanoparticles, and SiO₂ loaded chitosan for the consolidation of the painted stone, *J Cult Herit* 70 (2024) 312–322. <https://doi.org/10.1016/j.culher.2024.10.006>.
- [318] M.H. Alyami, A.A. Musallam, T.M. Ibrahim, M.A. Mahdy, H.M. Elnahas, R.A. Aldeeb, The Exploitation of pH-Responsive Eudragit-Coated Mesoporous Silica Nanostructures in the Repurposing of Terbinafine Hydrochloride for Targeted Colon Cancer Inhibition: Design Optimization, In Vitro Characterization, and Cytotoxicity Assessment, *Pharmaceutics* 15 (2023) 2677. <https://doi.org/10.3390/pharmaceutics15122677>.
- [319] M. Rani, K. Parekh, T. Mehta, A. Omri, Formulation development and characterization of luliconazole loaded–mesoporous silica nanoparticles (MCM–48) as topical hydrogel for the treatment of cutaneous candidiasis, *J Drug Deliv Sci Technol* 91 (2024) 105250. <https://doi.org/10.1016/j.jddst.2023.105250>.
- [320] A.A. Monfared, F. Karami, A. Shokrollahi, S. Yazdanpanah, T. Sepehr, K. Zomorodian, Design and Antifungal Activity of Fluconazole and Nystatin Loaded Onto Silica Mesoporous, *Pharm Chem J* 57 (2023) 965–974. <https://doi.org/10.1007/s11094-023-02972-4>.
- [321] M. Kapustová, G. Granata, E. Napoli, A. Puškárová, M. Bučková, D. Pangallo, C. Geraci, Nanoencapsulated Essential Oils with Enhanced Antifungal Activity for Potential Application on Agri-Food, Material and Environmental Fields, *Antibiotics* 10 (2021) 31. <https://doi.org/10.3390/antibiotics10010031>.
- [322] E. Sesana, A.S. Gagnon, C. Ciantelli, J. Cassar, J.J. Hughes, Climate change impacts on cultural heritage: A literature review, *WIREs Climate Change* 12 (2021). <https://doi.org/10.1002/wcc.710>.
- [323] A. Campostrini, S. Manente, E. Ghedini, A. Di Michele, F. Menegazzo, Enhancing Venetian traditional marmorino with TiO₂ and ZnO for antimicrobial protection – A case study, *Next Materials* 7 (2025) 100384. <https://doi.org/10.1016/j.nxmate.2024.100384>.
- [324] A. Breda, M. Signoretto, E. Ghedini, F. Pinna, G. Cruciani, Acylation of veratrole over promoted SZ/MCM-41 catalysts: Influence of metal promotion, *Appl Catal A Gen* 308 (2006) 216–222. <https://doi.org/10.1016/j.apcata.2006.04.039>.

- [325] W. Zeng, X.-F. Qian, J. Yin, Z.-K. Zhu, The drug delivery system of MCM-41 materials via co-condensation synthesis, *Mater Chem Phys* 97 (2006) 437–441. <https://doi.org/10.1016/j.matchemphys.2005.08.040>.
- [326] T. Vrålstad, W.R. Glomm, G. Øye, J. Sjöblom, M. Stöcker, Cobalt functionalization of mesoporous silica by incipient wetness impregnation and co-precipitation, *J Dispers Sci Technol* 26 (2005) 87–94. <https://doi.org/10.1081/DIS-200040230>.
- [327] T.M. Albayati, G.M. Alwan, O.S. Mahdy, High performance methyl orange capture on magnetic nanoporous MCM-41 prepared by incipient wetness impregnation method, *Korean Journal of Chemical Engineering* 34 (2017) 259–265. <https://doi.org/10.1007/s11814-016-0231-2>.
- [328] A. Das, M. Patra, R.R. Wary, P. Gupta, R.G. Nair, Photocatalytic performance analysis of Degussa P25 under various laboratory conditions, in: *IOP Conf Ser Mater Sci Eng*, Institute of Physics Publishing, 2018. <https://doi.org/10.1088/1757-899X/377/1/012101>.
- [329] E. Ghedini, F. Menegazzo, M. Manzoli, A. Di Michele, D. Puglia, M. Signoretto, Multifunctional and environmentally friendly TiO₂-SiO₂ mesoporous materials for sustainable green buildings, *Molecules* 24 (2019). <https://doi.org/10.3390/molecules24234226>.
- [330] A.S. Doost, C. V. Stevens, M. Claeys, P. Van Der Meeren, Fundamental Study on the Salt Tolerance of Oregano Essential Oil-in-Water Nanoemulsions Containing Tween 80, *Langmuir* 35 (2019) 10572–10581. <https://doi.org/10.1021/acs.langmuir.9b01620>.
- [331] H. Mith, R. Duré, V. Delcenserie, A. Zhiri, G. Daube, A. Clinquart, Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria, *Food Sci Nutr* 2 (2014) 403–416. <https://doi.org/10.1002/fsn3.116>.
- [332] H. Mith, A. Clinquart, A. Zhiri, G. Daube, V. Delcenserie, The impact of oregano (*Origanum heracleoticum*) essential oil and carvacrol on virulence gene transcription by *Escherichia coli* O157: H7, *FEMS Microbiol Lett* 362 (2015). <https://doi.org/10.1093/femsle/fnu021>.
- [333] S.R. Lakshmi Renganathan, Study on TiO₂-mediated photocatalytic degradation of methylene blue, 1995.
- [334] C.H. Kwon, H. Shin, J.H. Kim, W.S. Choi, K.H. Yoon, Degradation of methylene blue via photocatalysis of titanium dioxide, *Mater Chem Phys* 86 (2004) 78–82. <https://doi.org/10.1016/j.matchemphys.2004.02.024>.
- [335] A. Houas, Photocatalytic degradation pathway of methylene blue in water, *Appl Catal B* 31 (2001) 145–157. [https://doi.org/10.1016/S0926-3373\(00\)00276-9](https://doi.org/10.1016/S0926-3373(00)00276-9).
- [336] F.S. Rocha, A.J. Gomes, C.N. Lunardi, S. Kaliaguine, G.S. Patience, *Experimental Methods in Chemical Engineering: Ultraviolet Visible Spectroscopy-UV-Vis*, (2018). <https://doi.org/10.1002/cjce.23344>.
- [337] C.S. Uyguner, M. Bekbolet, Evaluation of humic acid photocatalytic degradation by UV-vis and fluorescence spectroscopy, (2005). <https://doi.org/10.1016/j.cattod.2005.03.011>.
- [338] G. Ferraro, A. Di Vera, E. Ghedini, M. Marchiori, G. Forghieri, P. Canton, M. Signoretto, Carbon-dots-conjugated semiconductors for enhanced solar-driven photocatalysis, *Applied Catalysis O: Open* 192 (2024) 206942. <https://doi.org/10.1016/j.apcato.2024.206942>.

- [339] Home - UNI Ente Italiano di Normazione, (n.d.). <https://store.uni.com/> (accessed June 5, 2024).
- [340] Home - UNI Ente Italiano di Normazione, (n.d.). <https://store.uni.com/> (accessed June 4, 2024).
- [341] A. Bhaumik, S. Samanta, N.K. Mal, Iron oxide nanoparticles stabilized inside highly ordered mesoporous silica, *Pramana* 65 (2005) 855–862. <https://doi.org/10.1007/BF02704085>.
- [342] J. Zhang, Y. Zhang, Y. Lei, C. Pan, Photocatalytic and degradation mechanisms of anatase TiO₂: a HRTEM study, *Catal. Sci. Technol* 1 (2011) 273–278. <https://doi.org/10.1039/c0cy00051e>.
- [343] R.R. Castillo, L. de la Torre, F. García-Ochoa, M. Ladero, M. Vallet-Regí, Production of MCM-41 Nanoparticles with Control of Particle Size and Structural Properties: Optimizing Operational Conditions during Scale-Up, *Int J Mol Sci* 21 (2020) 7899. <https://doi.org/10.3390/ijms21217899>.
- [344] C.G. Sonwane, C.W. Jones, P.J. Ludovice, A Model for the Structure of MCM-41 Incorporating Surface Roughness, *J Phys Chem B* 109 (2005) 23395–23404. <https://doi.org/10.1021/jp051713c>.
- [345] M.J. Mosquera, L.A.M. Carrascosa, N. Badreldin, Producing superhydrophobic/oleophobic coatings on Cultural Heritage building materials, *Pure and Applied Chemistry* 90 (2018) 551–561. <https://doi.org/10.1515/pac-2017-0404>.
- [346] E. Pargoletti, V. Comite, P. Fermo, V. Sabatini, L. Annunziata, M.A. Ortenzi, H. Farina, G. Cappelletti, Calcitic-based stones protection by a low-fluorine modified methacrylic coating, *Environmental Science and Pollution Research* 29 (2022) 29455–29466. <https://doi.org/10.1007/s11356-021-15515-9>.
- [347] E. Sassoni, E. Franzoni, Influence of porosity on artificial deterioration of marble and limestone by heating, *Applied Physics A* 115 (2014) 809–816. <https://doi.org/10.1007/s00339-013-7863-4>.
- [348] E.S.R. Lasheen, M.A. Rashwan, M.K. Azer, Effect of mineralogical variations on physico-mechanical and thermal properties of granitic rocks, *Sci Rep* 13 (2023) 10320. <https://doi.org/10.1038/s41598-023-36459-9>.
- [349] C. Lian, Y. Zhuge, S. Beecham, The relationship between porosity and strength for porous concrete, *Constr Build Mater* 25 (2011) 4294–4298. <https://doi.org/10.1016/j.conbuildmat.2011.05.005>.
- [350] A. Viggiano, O. Salo, H. Ali, W. Szymanski, P.P. Lankhorst, Y. Nygård, R.A.L. Bovenberg, A.J.M. Driessen, Pathway for the Biosynthesis of the Pigment Chrysogine by *Penicillium chrysogenum*, *Appl Environ Microbiol* 84 (2018). <https://doi.org/10.1128/AEM.02246-17>.
- [351] F. Fierro, I. Vaca, N.I. Castillo, R.O. García-Rico, R. Chávez, *Penicillium chrysogenum*, a Vintage Model with a Cutting-Edge Profile in Biotechnology, *Microorganisms* 10 (2022) 573. <https://doi.org/10.3390/microorganisms10030573>.
- [352] Y. Qin, Y. Xia, Melanin in fungi: advances in structure, biosynthesis, regulation, and metabolic engineering, *Microb Cell Fact* 23 (2024) 334. <https://doi.org/10.1186/s12934-024-02614-8>.
- [353] S.M. Koch, C. Freidank-Pohl, O. Siontas, M. Cortesao, A. Mota, K. Runzheimer, S. Jung, K. Rebrosova, M. Siler, R. Moeller, V. Meyer, *Aspergillus niger* as a cell factory for the production

of pyomelanin, a molecule with UV-C radiation shielding activity, *Front Microbiol* 14 (2023).
<https://doi.org/10.3389/fmicb.2023.1233740>.

Acknowledgments

Firstly, I would like to thank *PON R&I 2014–2020*, the Italian National Operational Program (PON) Research and Innovation 2014-2020, funded by REACT-EU ESF, and the restoration company Uni.S.Ve. Srl for financing this PhD scholarship.

I am deeply grateful to Prof. Federica Menegazzo and Prof. Elena Ghedini for supervising my thesis and for their guidance throughout the past three years. I would also like to thank Prof. Michela Signoretto and the entire CATMAT research group for their support.

Additionally, I extend my sincere thanks to Prof. Pilar Bosch-Roig from *the Instituto Universitario de Restauración del Patrimonio* at the *Universitat Politècnica de València*, who supervised and assisted me during my six-month stay in Spain.

This work would not have been possible without the contributions of my friends and fellow students: Ms. Teresa Botrè, Mr. Nicola Calderaro, Ms. Valentina A. Rizzolo, Ms. Emma De Paoli, and Ms. Alessia Boscolo Meneguolo. I would also like to express my gratitude to other colleagues who collaborated with me: Dr. Sabrina Manente, Dr. Alessandro di Michele, Dr. Alex W. Robertson, Prof. Giuseppe Cruciani, Prof. Giuseppina Cerrato, Dr. Alessia Giordana, and Mr. Agustí Sala Luis.

GLOSSARY

Here, all the sample labels and abbreviations used throughout the text are listed in alphabetical order:

ATR	Attenuated Total Reflectance
BET	Brunauer-Emmett-Teller Theory
BJH	Barrett-Joyner-Halenda Method
BLAST	<i>Basic Local Alignment Search Tool</i>
C	Hybrid hydrogel with chitosan/MTES-TEOS
CATMAT LAB	<i>Laboratorio di Catalisi e Materiali</i> (Catalisys and Materials laboratory)
CECT	Spanish Type Culture Collection
C-MO	Hybrid hydrogel with chitosan/MTES-TEOS and MCM-41 + Oregano EO
C-MOT	Hybrid hydrogel with chitosan/MTES-TEOS and MCM-41 + Oregano EO + Evonik P25 TiO ₂
C-MP	Hybrid hydrogel with chitosan/MTES-TEOS and MCM-41 + Evonik P25 TiO ₂
COT-01	Coating formulation base with 0,1% vegetal extract and SiO ₂ MCM-41, 17 mg/mL
COT-1	Coating formulation base with 1% vegetal extract and SiO ₂ MCM-41, 170 mg/mL
CTAB	Hexadecyltrimethylammonium bromide
DCOIT	2-octyl-4-isothiazolin-3-one (OIT) and 4,5-dichloro-2-octyl-4-isothiazolin-3-one
DDAC	Didecyltrimethylammonium chloride
DDS	Drug Delivery System
DTA	Differential Thermal Analysis
EDS	Energy Dispersive X-ray Spectroscopy
EMR-CPA	Estudios y Métodos de Restauración restoration company
EO	Essential oil
FE-SEM	Field Emission Scanning Electron Microscopy
FNA	Fluorescent Nucleic Acid
HR-TEM	High resolution Transmittance Electron Microscopy
IPBC	3-iodo-2-propynyl N-butyl carbamate
IR	Infrared

ITS	Internal Transcribed Spacer
IWI	Incipient Wetness Impregnation
MCM-41	Mobil Composition of Matter No. 41
MCM-41-BT	Mobil Composition of Matter No. 41 impregnated with the Biotin T ® biocide
MCM-OEO	Mobil Composition of Matter No. 41 impregnated with <i>Oreganum compactum</i> essential oil
MCM-SH	Mobil Composition of Matter No. 41 functionalized with -SH groups
MCM-SO ₃ H	Mobil Composition of Matter No. 41 functionalized with -SO ₃ H groups
MCM-SO ₃ H-BT	Mobil Composition of Matter No. 41 functionalized with -SO ₃ H groups and impregnated with the Biotin T ® biocide
MEA	Malt extrose Agar
MIC	Minimal Inhibitory Concentration
MK	MCM-41 with Kronos VLP TiO ₂
MM	MCM-41 with Mirkat 211 TiO ₂
MO	MCM-41 + Oregano EO
MOT	MCM-41 + Oregano EO + Evonik P25 TiO ₂
MP	MCM-41 with Evonik P25 TiO ₂
MPTMS	(3-Mercaptopropyl)trimethoxysilane
MTES	Methyltriethoxysilane
MUCAES- UPV	Museum of the <i>Universitat Politècnica de València, Vera Campus</i>
NB	Nutrient Broth
NO _x	Nitrogen oxides
NPs	Nanoparticles
Ø	Diameter
OEO	Origanum compactum essential oil
OIT	2-octyl-4-isothiazolin-3-one
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PON	National Operational Program
RH	Relative Humidity

ROS	Oxygen reactive species
rpm	Rate per minute
RT	Room Temperature
SBA-15	Santa Barbara Amorphous-15
SCI	Specular Component Included
SDA	Sabouraud Dextrose Agar
SEM	Scanning Electron Microscopy
SO _x	Sulphur oxides
TCMSP	2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine
TEO	Thymus vulgaris essential oil
TEOS	Tetraethyl orthosilicate
TG	Thermogravimetric Analysis
Ti-COM	Commercial Evonik P25 Titanium Dioxide
Ti-LAB	Labmade Titanium dioxide
TiZn-LAB	Labmade Titanium dioxide - zinc oxide composite material
TPO	Temperature Programmed Oxidation
UNISVE	Unione Stuccatori Veneziani Srl.
UNIVE	Università Ca' Foscari of Venice
UPV	Universitat Politècnica de València
UV	Ultraviolet
VOC	Volatile Organic Compounds
WDCM	World Data Center for Microorganisms
XRD	X-ray powder diffraction
Zn-COM	Commercial Carlo Erba Zinc Oxide
Zn-LAB	Labmade Zinc Oxide

ESTRATTO PER RIASSUNTO DELLA TESI DI DOTTORATO

Studente: Andrea Campostrini matricola: 858327
Dottorato: Science and Technology of Bio and Nanomaterials
Ciclo: XXXVII

Titolo della tesi: New green and multifunctional materials for the protection of cultural heritage

Abstract:

Questa tesi di dottorato (progetto PON – partnerships tra UniVE e UniSve) è focalizzata sull'ottimizzazione di un approccio multidisciplinare per lo sviluppo di un coating *green* multifunzionale per la protezione delle superfici lapidee del patrimonio culturale dal biodegrado.

In primis, NPs fotocatalitiche di TiO_2 e ZnO sono state studiate per i loro effetti antifungini su intonaci storici (Cap. II). Poi, nanocapsule di silice mesoporosa funzionalizzata sono state usate per veicolare il rilascio di un biocida commerciale (Cap. III). Per una migliore sostenibilità, estratti antimicrobici *plant-based* sono stati incapsulati nella silice e testati, dimostrando un'efficace e prolungata attività antimicrobica favorita dalle nanocapsule (Cap. IV). Infine, l'efficacia dei fotocatalizzatori e dell'olio essenziale è stata massimizzata grazie allo sviluppo di un protettivo ibrido con nanocapsule di silice impregnate con olio essenziale e TiO_2 , offrendo proprietà protettive multifunzionali (Cap. V).

This PhD thesis (PON project – partnership UniVE and UniSve) is focused on optimizing a multidisciplinary approach for the development of a green multifunctional coating to protect cultural heritage stone surfaces from biodeterioration.

Firstly, photocatalytic NPs of TiO_2 and ZnO were evaluated for their antifungal effects on historical plasters (Chapter II). Then, functionalized mesoporous silica nanocontainers were studied to control the release of a commercial biocide (Chapter III). To improve sustainability, plant-based antimicrobial extracts were then encapsulated in the silica and tested, demonstrating prolonged and effective antimicrobial activity favored by the nanocontainers (Chapter IV). Finally, the efficacy of photocatalysts and the essential oil was maximized through the development of a hybrid coating with essential oil-impregnated silica nanocontainers and TiO_2 , offering multifunctional protective features (Chapter V).

Firma dello studente

