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Green Biocidal Nanotechnology Use for Urban Stone-Built Heritage—Case Study from Oradea, Romania

Dorina Camelia Ilies [1], Lucian Blaga [1], Alexandru Ilies [1], Ana Cornelia Pereș [2], Tudor Caciora [1,*], Thowayeb H. Hassan [3,4], Nicolaie Hodor [5], Alexandru Turza [6], Hamid R. Taghiyari [7], Lucian Barbu-Tudoran [6,8], Ranjan Kumar Dahal [9], Paula Dejeu [10], Bahodirhon Safarov [11] and Mallik Akram Hossain [12]

1 Department of Geography, Tourism and Territorial Planning, Faculty of Geography, Tourism and Sport, University of Oradea, 1 Universitatii Street, 410087 Oradea, Romania; dilies@uoradea.ro (D.C.I.); lblaga@uoradea.ro (L.B.); ilies@uoradea.ro (A.I.)
2 Department of Environmental Engineering, Faculty of Environmental Protection, University of Oradea, Magheru Street 26, 410087 Oradea, Romania; peresana35@yahoo.com
3 Department of Social Studies, College of Arts, King Faisal University, Al Ahsa 31982, Saudi Arabia; thassan@kfu.edu.sa
4 Tourism Studies Department, Faculty of Tourism and Hotel Management, Helwan University, Cairo 12612, Egypt
5 Faculty of Geography, "Babes-Bolyai" University, 5-6 Clinicilor Street, 400006 Cluj-Napoca, Romania; nicolaie.hodor@ubbcluj.ro
6 National Institute for R&D of Isotopic and Molecular Technologies, 67-103 Donat Street, 400293 Cluj-Napoca, Romania; alexandru.turza@itim-cj.ro (A.T.); lucian.barbu@itim-cj.ro (L.B.-T.)
7 Faculty of Materials Engineering & New Technologies, Shahid Rajaee Teacher Training University, Tehran 16788-15811, Iran; htaghiyari@sru.ac.ir
8 Electron Microscopy Center "Prof. C. Craciun", Faculty of Biology & Geology, "Babes-Bolyai" University, 5-7 Clinicilor Str., 400006 Cluj-Napoca, Romania
9 Central Department of Geology, Tribhuvan University Kirtipur, Kathmandu 44618, Nepal; ranjan.dahal@cdgl.tu.edu.np
10 Medical Laboratory Service M.D., Bethany Medical Clinic Oradea, Bihor County, 410004 Oradea, Romania; office@betania-centrumedical.ro
11 Department of Digital Economy, Samarkand State University, Samarkand 140105, Uzbekistan; safarovb@rambler.ru
12 Department of Geography and Environment, Jagannath University, Dhaka 1100, Bangladesh; ahmallik@yahoo.com
* Correspondence: caciora.tudoriulian@student.uoradea.ro; Tel.: +40-740-941-144

Abstract: Heritage buildings clad with natural rock endure over time destruction caused by weathering mechanisms, pollution from urban areas, biodeterioration due to organisms, microorganisms, and also the anthropic factor. On the surface of the limestone samples taken from the ornamental natural rock with which the outside of Markovits-Mathéser house, Oradea, Romania, is clad, two species of fungi were inoculated in the laboratory: *Aspergillus* spp. and *Cladosporium* spp. Wollastonite was then applied, and from the imaging analysis (SEM), the inhibition of fungi by it is clearly observed (48 h after its application), which was also confirmed using the image segmentation method. It was also noted that the hydrophilicity of the aqueous suspension of wollastonite resulted in the absorption of water in the substrate, which in turn resulted in the drying out and surface breakage of the specimens. X-ray diffraction analysis showed the presence of the two phases (calcite and quartz) as in the starting sample, and also an additional phase assigned to wollastonite in the later phase of the experiment. An amorphous component, due to the applied gel composition, was also reported. This research highlights the fact that there are good premises for aqueous suspension of wollastonite to have a biocidal character for *Aspegillus* spp. and *Cladosporium* spp., when applied on natural stone used in the construction of heritage buildings located in temperate climates; due to its easy application, green and ecofriendly properties, and also low cost of acquisition and application.

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1. Introduction

Tangible and intangible cultural heritage is a sustainable resource for the whole world. The responsible management of this type of heritage may have a positive effect in solving problems aimed at social inclusion and cohesion, improving the quality of the living environment, and in the industry of tourism [1,2].

Historical buildings and their heritage elements, monuments, and architectural complexes that are part of the tangible cultural heritage, are mostly built of natural rocks, and consequently suffer damage caused by weathering mechanisms [3–6]. Although initially the weathering process was conceived as an association of anical and chemical processes, research performed in recent decades has emphasised the ability of bacteria, fungi, plants, and other living organisms to intervene directly and alone (bioweathering or biodeterioration), or indirectly and in association with other forces in these mechanisms [7]. Their rhythm and intensity depend mainly on the physical and chemical properties of the rocks that make up heritage buildings and on the outdoor climatic conditions of the heritage site. In addition, climatic changes can intervene in accelerating their pace and intensity, the effect consisting of an increase in the rates of decay for buildings [8–10]. According to the IPCC [11], in Eastern Europe, an increase in periods with extremely high temperatures and heavy precipitation has been observed since 1950, and the studies carried out for Romania [12,13] indicate, since 1961, an increase in average temperatures (summer and spring), associated with an increase in extreme temperatures. Moreover, the way in which climate change manifests itself in urban climates, in which more than 70% of the cultural heritage properties inscribed on the World Heritage List are located [14], is different from that in rural climates [15]. Thus, urban climate is characterised by higher air temperature values compared to the countryside by up to 10 °C (in the case of large cities, and especially at night, in anticyclonic mode), and changes in the local atmospheric circulation can increase urban cloud cover and precipitation, with an impact on the increase in humidity over certain time intervals [16,17]. The statistical analyses performed by Liu et al. [18] on satellite data for land surface temperatures (2002–2021) show that the mean surface warming trend in the central part of the more than 2000 urban agglomerations worldwide is 29% higher than in the surrounding countryside. These climatic specificities, also present in Central–Eastern Europe, make mechanical weathering processes, such as thermoclastic (heating and cooling), wetting and drying, associated with chemical ones (solution, hydration, oxidation and reduction, carbonation) and biotics act with increased efficiency, and thus, represent a real danger to the built cultural heritage.

In these conditions, the cultural heritage loses buildings and architectural assemblies containing natural stone year after year due to decay and biodegradation. Artifacts exhibited or stored in inappropriate conditions in museums or private collections are affected by superficial or deep biodegradation processes, due to the impact of the cumulative action of thermohygrometric factors, pollutants, light, etc. [19–22]. In the case of natural rocks used in built cultural heritage monuments, biodegradation is due to organisms that act in isolation or in colonies, affecting the natural rock's composition, even if it is a poor source of nutrients, and supports large differences in humidity, being affected by the mechanical erosion of wind, precipitation, or UV radiation [23], depending on the type of climate. An essential role in the alteration of the rock part of monuments and buildings is played by fungi, the epiphytic type that colonises and develops on the surface, and endolithic microorganisms by penetrating the rocks via intercrystallite spaces through active erosive chemical–mechanical processes [24–26]. When interpreting colonisation, biodegradation of built structures, interrelations between microorganisms, geological processes, and stone, geomicrobiology brings an important contribution and can provide

information about remedies, and preventive or restorative treatments [25]. In the interaction of physical–chemical–mechanical processes and rock, damage to rock caused by microorganisms (fungi) consists in the penetration of rock by hyphae, which can lead to mechanical abrasions, fractures, and dislocations, resulting in chemically identical fragments with the original lithic support. The biocorrosive activity is carried out by the action of organic acids resulting from excretion processes, or due to oxidation processes of mineral-forming cations [19,27–29]. Aesthetic changes to the rock surface occur due to the synthesis and excretion of extracellular pigments of microcolonial fungi, highlighting colourations due to melanin in the mycelium and conidia, discolourations, dark stains (in the case of dematiaceous fungi), biogenic patina, black staining, black/grey spots, and biopitting phenomena on limestone and marble artworks [24,26,30,31]. The biogeochemical cycles that can take place, due to metabolic products (metabolic acids, sometimes very aggressive) of organisms, resulting from assimilation or lack of assimilation processes, can generate changes in the support rock in terms of minerals, with consequences for the degradation of minerals and rocks (biocorrosion—dissolution of the mineral substrate) or with the formation of secondary minerals (mycogenic minerals), activities, and processes based on the assimilation of mineral nutrient constituents and the metabolites of some anthropogenic pollutants [18,32–34]. Degradation of the natural stone from monuments and heritage buildings occurs under the effect of atmospheric pollution, humidity and its variation, temperature, freeze–thaw processes, air impurities (dust, smoke, etc.), soluble efflorescence, poorly soluble or insoluble salts whose action leads to physical erosion, chemical corrosion, and biological pollution and implicitly leads to diminishing the internal resistance of stone due to some harmful materials applied during previous restoration interventions [35,36]. Antibacterial agents for the protection and preservation of cultural heritage in natural stone do not fully succeed in annihilating the action of fungi resistant to chemical substances due to their thick and melanised cell walls [37]. Thus, lately, nanotechnology has taken off. It is a green technology and an efficient means to ensure sustainable development due to its ability to control air pollutants [38,39] and nanomaterials; in the case of the latter, conventional ones that are based on polymers [1]. Nanoscience promotes the engineering of nanomaterials that are compatible with the support material to preserve and protect the original characteristics, e.g., aesthetic, physical, chemical, mechanical; creating and maintaining a self-cleaning system, having thermal and biochemical stability, not being toxic, and with a low impact on the environment, having affordable prices, and good adaptability to different environments [20,22,40].

In this context, the current study aims to test the biocidal and antistatic nature of an aqueous suspension of wollastonite, in order to better preserve the natural stone used in the construction of buildings belonging to the cultural heritage. The rock was, thus, collected from the facade of a house built in the Art Nouveau style, in 1911, in the Municipality of Oradea, Romania.

2. Literature Review

Unwanted microorganisms on heritage stone objects can be removed or inhibited by techniques that include chemical methods (traditional biocides and nanoparticles), physical methods (mechanical removal, UV irradiation, gamma radiation, laser cleaning, heat shocking, microwaves, and dry ice treatments) and biological methods (natural molecules with biocidal activity, enzymes, and even ones based on microorganisms) [41,42]. Studies such as those conducted by Hajipour [43], Baglioni et al. [1], David et al. [20], Caneva et al. [44], and Jurca et al. [45] show that for obtaining active surfaces against pollution and microbial contamination, hard (inorganic nanocrystals) and soft (built from molecular blocks) compounds can be successfully used, e.g., inorganic compounds such as metallic nanoparticles (gold, silver, copper) and some metal oxides (zinc, titanium, iron, and aluminium), respectively. Research has been conducted for various treatments by applying nanoparticles to various types of materials, so the paper of some old books that was impregnated with an aqueous suspension of wollastonite (30–110 nm, in gel form)

led to observations underlying the inhibition of *Aspergillus niger* development and a decrease in the permeability [45–47]. Cotton strips exposed to fungi (commonly found on ancient textiles) immersed in wollastonite (30–110 nm, gel—20%) emphasise the significant limitation of *Aspergillus niger* activity on cotton. Taghiyari et al. [48–54], Terzi et al. [55], Tichi et al. [56], and Weththimuni et al. [57] have investigated the effects of heat treatment on solid nano-silver-impregnated wood species with a good perspective to apply and test the methodology for cultural heritage wood objects.

Wollastonite is environmentally known to be of no or little hazard to both humans and wildlife [58–60]. It has even been reported to improve the growth in some plants, and to reduce the effects of special kinds of pathogens (such as some fungi species) [61]. Epidemiological studies have long shown that there was no or little evidence of wollastonite having hazardous side effects; although, long-term exposure of workers to wollastonite dust has been shown to have some slight health implications [58,60]. No correlation of serum angiotensin-covering enzymes was found between long-term inhalation of wollastonite dust and slight pulmonary fibrosis in wollastonite workers [59].

Wollastonite applications have proven remarkable in the biomedical field as bioactivators in bone mass recovery, being easily biodegradable and insoluble in substances which help bone cells' development and function [62]. The biocidal effects of nano-wollastonite have also been reported on other types of materials (e.g., textiles, paper, wood, etc.) [46,47,56]. In the geoconservation domain, there are studies which emphasise the efficiency of silica nanosystems as biocidal agents for the conservation of stone monuments, using the advanced biotechnology of microbiological systems for the biological cleaning of cultural heritage (CH) based on sepiolite [63].

The inhibiting effects of wollastonite on fungi have been investigated in previous studies [46,47,61,64]. However, the mechanism with which wollastonite inhibits the growth of fungi species, or at least limits the rate of mycelium growth on the substrates, is yet to be explored in further studies with an emphasis on the type and degree of enzyme secretion (Table 1).

Table 1. Literature review on applications of nanoparticles in treatments for heritage objects made of stone or which have natural stone as ornaments.

Paper	Location	Methods and Tests	Results
Dei and Salvadori [65]	Limestones	NNPs calcium hydroxide treatments	Innovative and compatible with base material, consolidation process
D'Arienzo et al. [66]	Neapolitan yellow tuff	Nanocomposite systems based on Cloisite	Protective and consolidative
Pinna et al. [41]	Archaeological area of Fiesole, Italy	Tested on three stone substrates with different bioreceptivity, traditional way (tetraethylorthosilicate, methylethoxy polysiloxane, Paraloid B72, tributyltin oxide, dibutyltin dilaurate) and nanotechnology (copper nanoparticles)	Prevention in biological growth; controlling recolonisation on stone after a conservation
Licchelli et al. [67]	Lecce stone, Italy	Ca $(OH)_2$ and Sr $(OH)_2$ nanoparticles applied into the stone substrate. The chemical weathering effect of salt crystallisation of the treated samples which was evaluated through dry weight loss (DWL) test	Good results as consolidating agents

Table 1. *Cont.*

Paper	Location	Methods and Tests	Results
Aldoasri et al. [68]	Marble stone facades of historic buildings, Cairo, Egypt	Nanometric film over the stone surface, TiO_2 nanoparticles, in an aqueous colloidal suspension, applied by spray-coating	Self-cleaning photo-induced effects are obvious in the experiment time and 6 months later
Becerra et al. [40]	Heritage stone from south of Spain	Two AgNPs syntheses have been studied;	Cleaning the limestone due to the biopatina formation reduction using (Ag/TiO_2) nanocomposite treatments
Bruno et al. [69]	Catacombs of SS. Marcellino and Pietro (Rome, Italy)	essential oils (from *L. angustifolia* and *T. vulgaris*) biofilm photosynthetic activity on frescoes stone	Chemical modifications and discolouration, good results
Zarzuela et al. [70]	Cultural heritage stone	CuO/SiO_2 nanocomposites: a multifunctional coating for application on building stone	
Ion et al. [71]	Basarabi chalk monument, Romania	Hydroxyapatite nanoparticles (HAp)	Physical–chemical and mechanical rocks properties improvements
Gallo et al. [72]	Stone in buildings and monuments	Disinfection procedures on natural stone using smectite and ammonium salt	Antimicrobial and long-term biostatic effects
Aldosari et al. [73]	Historic marble columns, Egypt	Nanoparticles of ZnO, dispersed in laboratory synthesised acrylic polymer	Biocidal against *Aspergillus niger* and *Penicillium* sp. Studies for RH/temperature, UV aging, and mechanical deterioration
Capitelli et al. [42]	Conservation of stone monuments of cultural heritage	Functional nano-hydroxyapatite methodological approach against acidic rain corrosion	HAp nanoparticles and their application on stony substrates has been investigated with good results
Caneva et al. [44]	Caestia Pyramid (Rome), Italy	Allelopathic properties of lichen use for stone restoration	Results of the tests emphasise natural product substances are a useful in control of bio-colonisation
Xie et al. [74]	Heritage object marble made	Colloidal protectants based on Al_2O_3 and SiO_2 nano-powder	Self-cleaning stone effects
Weththimuni et al. [75]	Lecce stone, bricks, and marble	For this purpose, ZrO_2-doped-ZnO-PDMS nanocomposites were synthesised by in situ reaction	Self-cleaning effects
Ruffolo et al. [76]	Stone heritage	NNPs calcium, magnesium hydroxide and nano-silica	Superhydrophobic properties of coatings, dirt, pollutants, and microorganisms, etc., washed out by water flowing. The combination of light and photocatalyst generated photocatalytic effects
Weththimuni et al. [77]	Three different stones (Lecce stone, Carrara marble, and brick)	ZnONNPs doped with ZrO_2 sol–gel, to reduce the biodeterioration of cultural heritage stone buildings	Photocatalytic properties and ZnO antibacterial activity

In the case of the sandstone and limestone blocks' external surfaces, Rai et al. [78] and Essa and Khallaf [79] used AgNPs mixed with two types of consolidation polymers. The stones treated with silicon polymer loaded with AgNPs showed antimicrobial potential against *Aspergillus niger* and *Streptomyces parvulus*. Baglioni et al. [1], Munafo et al. [80,81], Quagliarini et al. [82,83], and De Filpo et al. [84] assessed the durability and sustainability of TiO_2 nanoparticles on stone that made up cultural heritage buildings in Italy and Portugal and highlighted their photocatalytic, biocidal, and self-cleaning performance, and low environmental impact. Vasanelli et al. [85] and Zornoza-Indart and Lopez-Arce [86] have tested the stone consolidation with SiO_2 nanoparticle uses. The authors point out that further studies and tests are needed regarding the risks of nanoparticles regarding their effects on human health as well as on paint materials, especially through the spreading of the particles in the atmosphere after a while. Taghiyari et al. [48,49] and Esmailpour et al. [87] studied the diverse effects in the case of wollastonite-treated medium-density fibreboard.

Research on the effectiveness of different product mixtures in slowing natural biological recolonisation has been carried out for natural stone in buildings or heritage objects made of natural stone in the long term. The study carried out by Bartoli et al. [88], demonstrates that the application of three different biocides (Algophase, Biotin R, and Preventol R80) and two water-repellent substances (hydrophase surfaces and Silo 111), can prevent microbial growth for 3 years.

Regarding the short-term biodeterioration due to algae, cyanobacteria and most bacteria nanoparticles are effective, but are not satisfactory against black fungi, for which physical methods are more effective (mechanical removal and UV, heat shock treatments); TiO_2 based coatings showed efficiency limited to the short/medium term after application [89,90]. Regarding chemical methods, in laboratory conditions, classical biocides (e.g., Preventol RI 50, Biotin R, Rocima^{TM} 103) are still the most effective [91,92], producing efficient results during cleaning procedures. Plant-based extracts show limited effectiveness against fungi [89]. In laboratory conditions, cholinium@Il based coatings have shown that the use of Il's with 12 C chains and DBS as the anion in combination with nanosilica coatings (e.g., Nano Estel) could be effective against the colonisation of black fungi for a period of time of 30 months [90].

3. Materials and Methods

The three-façade, Secession-style building known as the Markovits-Mathéser house, was designed by the architect Frigyes Spiegel in 1911 and commissioned by Markovits Sándor and Mathéser Sámuel [93–95]. Located at the intersection of two streets on a trapezoidal plot, it is one of the most interesting buildings in Oradea Municipality (Figure 1). The construction, that surrounds a small courtyard, stands out for the quality of the execution of the façades, for its elegance, size, volume, and for the use of different textures and materials. The access from Aurel Lazăr street is made through a spacious semicircular dome, strongly outlined by a carved wooden roof covered with shingle [94,95].

The ornamental stone is a fossiliferous limestone, having a real value for the present experiment. Thus, the collection of samples took place after the start of the renovation works of the house, they were collected from two distinct points on the main facade of the monument (Figure 2).

Raw limestone is found on the façade of the basement and second floor of the building, while on the ground floor, exposed brick alternates with plastered sections, and on the upper floors there are exposed brick, wooden elements, stucco mouldings and plastered areas [94]. The rock samples were taken during the renovation works of the building, using a scalpel, gently detaching, from crack lines, small pieces of rock from the basal part of the building. The operation was carried out so that the process was minimally invasive. Next, they were cut to the following size (L × W × H (mm)): R1 23/22/7 mm; R2 22/21/6 mm; sample 1: P1 20/20/5 mm; and sample 2: P2 21/20/5 mm. The samples had the following weights (g): R1: 9.34 g; R2: 8.42 g; P1: 6.69 g; and P2: 7.44 g (Table 2).

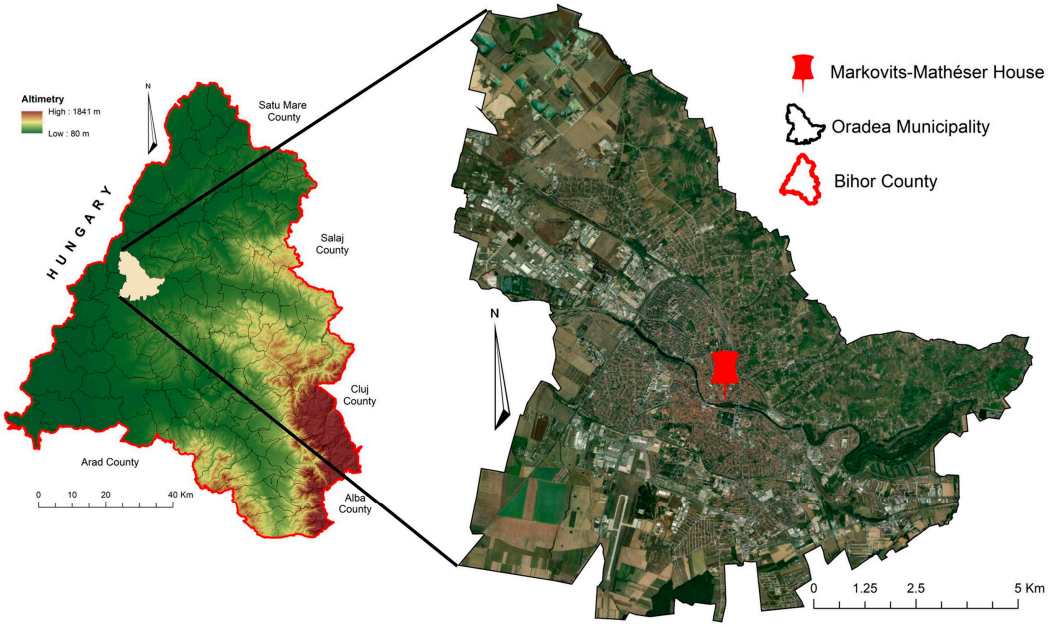


Figure 1. The location of Markovits-Mathéser house within Bihor County and Oradea Municipality.

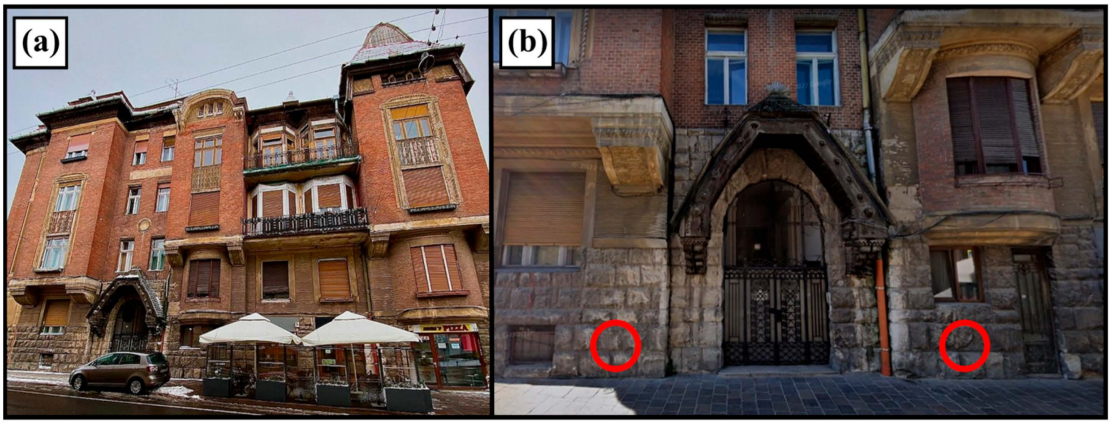


Figure 2. (**a**) Overview of the Markovits-Mathéser house; (**b**) the collection points of rock samples for carrying out the experiment.

Two types of fungi were applied to the samples taken, namely, *Aspergillus* spp. and *Cladosporium* spp. The reason for choosing the two types of inoculated fungi was that they are within the fungal group dominated by hyphomycetes (Alternaria, Cladosporium, Ulocladium, Epicoccum, Aureobasidium), which in temperate and humid environments are the main colonisers of stone, contributing to stone erosion and disintegration, discolourations, etc. At the same time, *Cladosporium* spp. is the main fungi found on rock supports of stone monuments made of granite, calcarenite, limestone, sandstone, marble, etc. [24,26,37,95–100] (Figure 3).

Table 2. Weight of rock samples before and after applying aqueous suspension of wollastonite and 48 h after applying it, respectively, after drying.

Weight (g)	Initial	After Wetting (Distilled Water) (Day 1)	After Fungal Inoculation (Day 1)	Before Applying Aqueous Suspension of Wollastonite (Day 4)	After Applying Nano Aqueous Suspension of Wollastonite (Damp) (Day 4)	After Gel Dried (48 h Post-Application) (Day 6)	Size L × W × H (mm)
Milestone R1	9.34	-	-	-	-	-	23/22/7
Milestone R2	8.42	-	-	-	-	-	22/21/6
Sample 1 A	6.69	6.81	6.82	6.95	7.01 (+ 0.06 g gel)	6.97	20/20/5
Sample 2 E	7.44	7.65	7.70	7.70	7.84 (+ 0.14 g gel)	7.66	21/20/5

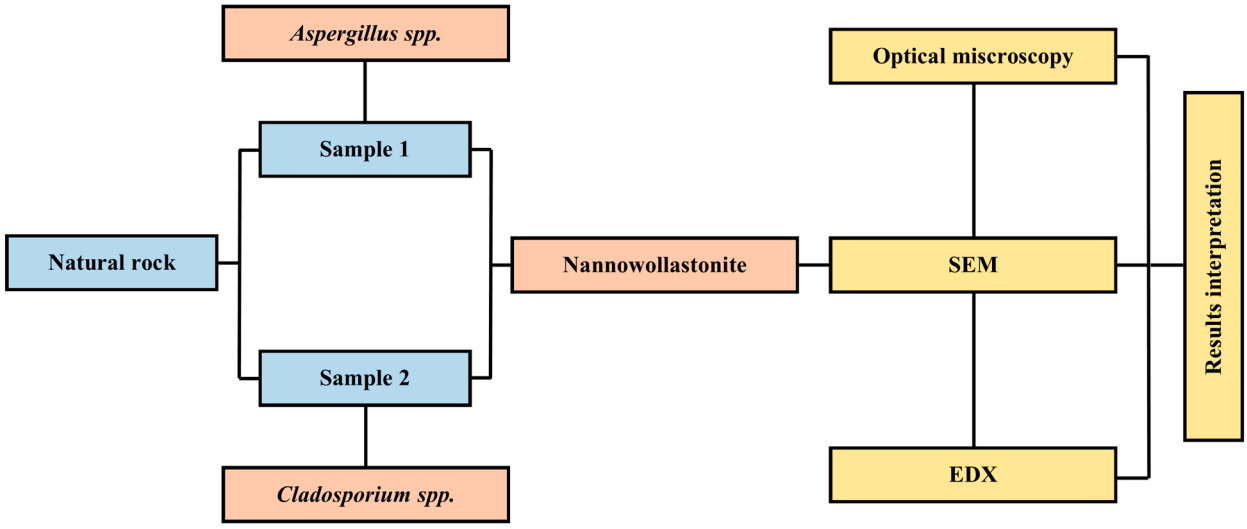


Figure 3. The work flowchart for carrying out the research.

Chemoorganotrophic fungi such as *Aspergillus* spp. can survive on the surfaces of a wide range of stones from stone artifacts and buildings, such as limestone, granite, marble, sandstone, andesite, gneiss, and quartz in different types of climates [101], especially on the stones' surfaces, but because of the hyphal growth can also penetrate into the stone. *Aspergillus* spp. are dominant on the surfaces of buildings and stone artifacts from temperate climates: Crimea [28], United Kingdom [102], Slovakia [103], Poland [104], Serbia [105], Mediterranean Palermo, Italy [106], and from temples in humid climates such as in Cambodia [107,108].

The aqueous suspension of wollastonite used in this study was prepared by the Vard Manufacturing Company, Birjand, Iran. Based on the producer, it was a mixture of nano- (30%) and micro-sized (70%) wollastonite needles. This mixture was chosen to decrease the production costs and increase commercialisation of the compound, in case positive results were achieved [50,87]. The composition of the aqueous suspension of wollastonite is presented in Table 3. Samples without wollastonite gel content were also used for comparison purposes.

Table 3. Composition of aqueous suspension of wollastonite (after Taghiyari et al. [46,47]).

Component	Proportion (%*w*/*w*)
SiO_2	46.96
CaO	39.77
Water	4.67
Al_2O_3	3.95
Fe_2O_3	2.79
MgO	1.39
TiO_2	0.22
Na_2O	0.16
SO_3	0.05
K_2O	0.04

Fungal inoculation on day 1 was performed according to the data above, after immersing the upper surface of the stone in sterile distilled water. This stage was carried out to favour both the inoculation and the optimal development of the selected fungi. The colonies of *Cladosporium* spp. and *Aspergillus* spp. were previously isolated on Sabouraud and Agar Csapek culture media as subcultures, respectively.

Samples inoculated with *Cladosporium* spp. and *Aspergillus* spp. were isolated separately in two special incubation boxes equipped with mini-wells for distilled water, to

create an environment favourable to fungal development, but also to reduce the risk of contamination with spores of other moulds possibly existing in the air during the handling of working materials (Figures 4 and 5).

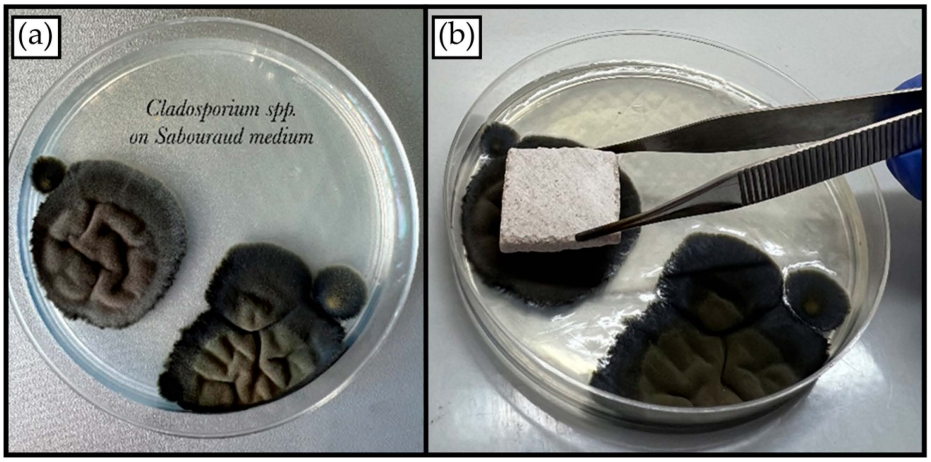


Figure 4. Rock specimens inoculated with *Cladosporium spp.* (**a**—*petri plate seeded with Cladosporium spp.*; **b**—*the seeding method of the rock samples*).

Figure 5. Rock specimens inoculated with both *Cladosporium* spp. and *Aspergillus* spp.

The inoculation of the two species of fungi on the natural rock samples was followed by a 72 h incubation period at a temperature of 23 ± 2 °C and humidity of 60 ± 2%, monitored using a calibrated thermohygrometer (Figure 6).

On day 4, a thin layer of the aqueous suspension of wollastonite was applied to the fungal-inoculated surface of the samples using brushes (72 h after fungal inoculation), and the samples were weighed before and after applying the gel. The amount of gel applied was different (0.06 g gel was applied on sample no. 1 and 0.14 g on sample no. 2) depending on the shape, size, and unevenness of the tested surface.

The last weighing took place after the drying of the gel (48 h after applying it). The samples were handed over under appropriate transport conditions on day 6 for additional study procedures.

Figure 6. 72 h incubation period at a temperature of 23 ± 2 °C and humidity of 60 ± 2%.

In order to quantify the inhibitory effect of the aqueous suspension of wollastonite on the fungi, at the level of the sample surfaces, two sets of SEM grayscale 300× magnifier images were used in the segmentation procedure, with dimensions of 417.97 µm × 292.8 µm (1279 × 896 pixels), and with pixel sizes of 0.3268 × 0.3268 µm^2. Each set contained two images: one related to the samples from the third day, after inoculation with the fungi, and one corresponding to the samples after applying the gel. The scanning electron microscope used was a Hitachi SU8230 cold field emission gun STEM (Chiyoda, Tokyo, Japan).

The trainable WEKA segmentation (TWS) plugin based on the Waikato Environment for Knowledge Analysis (WEKA) toolsets was used for image segmentation, implemented in ImageJ, an open source application, considered a standard work tool in biological studies [109]. TWS, as shown by Arganda-Carreras et al. [110], is a tool that practically transforms classic segmentation into a pixel-level classification, based on training samples used by a selected classifier. We used random forest, a machine learning classifier [111], in the fast random forest variant, with default settings for training features (Gaussian blur, Hessian, membrane projections, Sobel filter, difference of Gaussians), plus one filter for texture (median filter) and one filter for noise reduction (Kuwahara filter). A training dataset was built for each image, with two to three segmentation classes for *Aspergillus* spp. and three to four classes for *Cladosporium* spp., depending on their complexity. Before classification, the images were subjected to some initial processing operations (set scale, cropping, contrast enhancement), according to the workflows presented in the literature [112–115]. Accuracy assessment of the models obtained after training the classifier was performed in WEKA with a 10-fold stratified cross-validation test; the following performance measures being selected: correctly classified instances (CCI), kappa coefficient (k), precision (P), and recall (R) (Table 4). In addition, in order to obtain a comparative table regarding the accuracy of the classified images, new sets of training samples were built in the ENVI 5.3 software, independent of the first ones, being used to generate performance metrics similar to those in WEKA using the confusion matrix method: overall accuracy (OA), producer accuracy (PA), user accuracy (UA), and kappa coefficient (k). Previously, the basic and classified images were transferred to ENVI based on the plane coordinates (x–y), and those classified with TWS were reconverted into the ENVI classification.

In addition to the previously mentioned analyses, the X-ray diffraction technique was also applied to the rock samples collected from the facade of the Markovits-Mathéser house. This is an analytical technique for characterising solid materials in order to extract and characterise the crystalline structure of the analysed samples. Powder X-ray diffraction patterns

were obtained using a Bruker D8 Advance diffractometer with CuKα1 monochromatic radiation (λ = 1.5405980 Å) filtered with a germanium monochromator. The diffractometer is equipped with a LINXEYE detector and X-ray tube operating at 40 kV and 40 mA. A scanning rate of 0.05°/s was employed for data collection with the DIFFRAC plus XRD Commander programs' package, at room temperature.

Table 4. Performance metrics used in this study.

Accuracy Measures from WEKA	Accuracy Measures from ENVI	Definition, Notes
Correctly Classified Instances (CCI)	Overall Accuracy (OA)	Proportion of pixels correctly classified [116].
Kappa Coefficient (k)	Kappa Coefficient (k)	It expresses the agreement (correlation) between the performed classification (pre-dictated classes) and the dataset with the true value. It is the proportion of agreement after chance agreement is removed from consideration [117].
Recall (R)	Producer Accuracy (PA)	Ratio between the number of pixels correctly identified as belonging to a class and the total number of pixels of the respective class in the training dataset [118].
Precision (P)	User Accuracy (UA)	Ratio between the number of pixels correctly identified as belonging to a class and the total number of pixels labeled in the classification as belonging to the respective class [118].

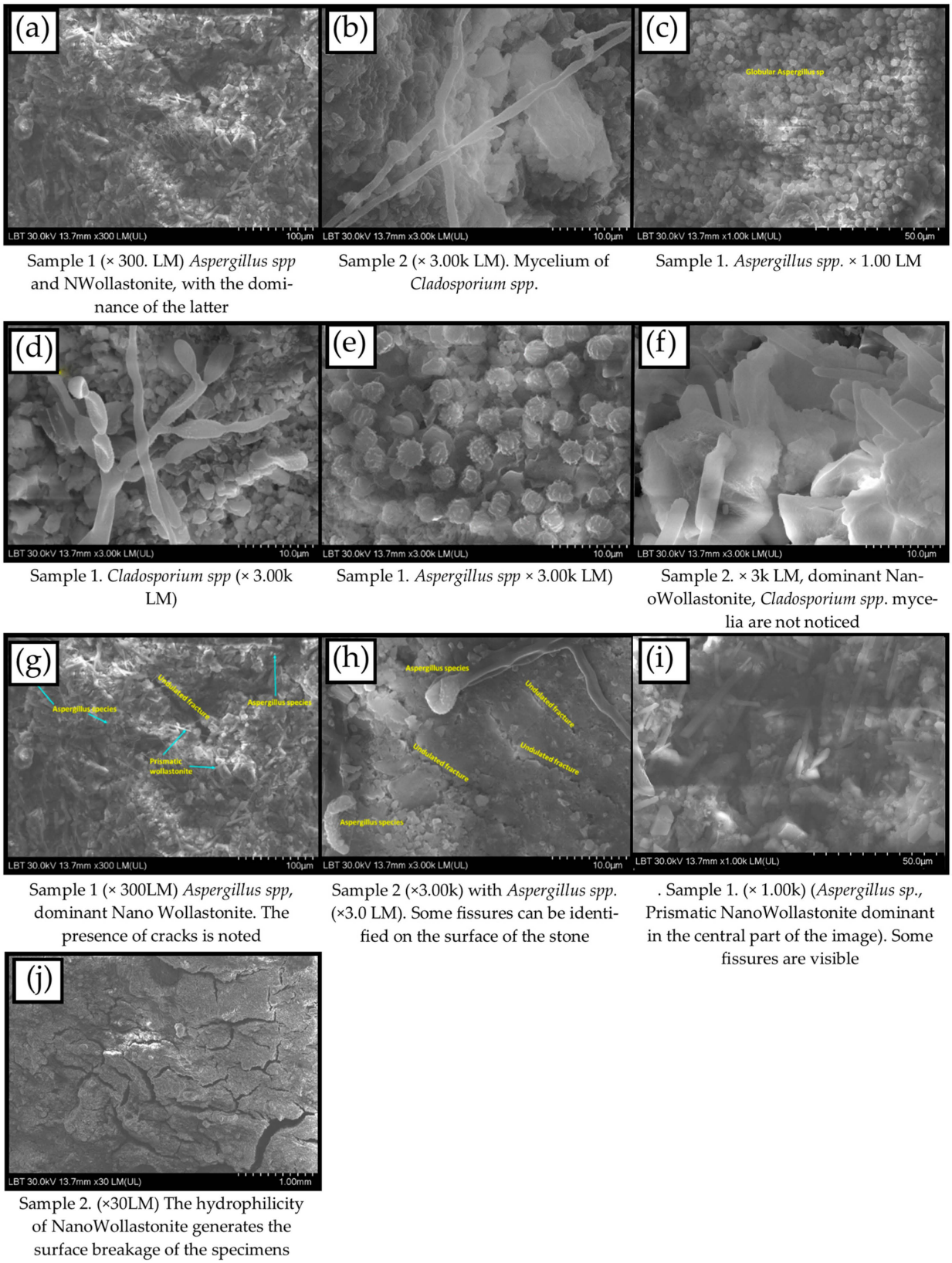
4. Results and Discussions

Following the SEM image analysis, it seems that there are no visible mycelia of *Cladosporium* spp. on the specimens after applying the aqueous suspension of wollastonite (the needle form of wollastonite rods can also seen (Figure 7f,j)). It is hypothesised that the hydrophilicity of the aqueous suspension of wollastonite resulted in the absorption of water in the substrate, which in turn resulted in drying out and surface breakage of the specimens (Figure 7j). *Aspergillus* spp. is still visible (Figure 7i), but the aqueous suspension of wollastonite is dominant (central part of the image in Figure 7i); a few superficial cracks are visible too.

Since rocks, soils, clay, and dust samples have a mineral content with a high crystallinity, powder X-ray diffraction is a suitable analysis method to highlight the component phases. The diffraction pattern of the starting sample shows a high degree of crystallinity and is presented in Figure 8. The mineral composition comprises two distinct phases, namely, calcium carbonate ($CaCO_3$, which is denoted by C) and silicon dioxide (SiO_2, denoted by Q and found in the form of quartz). It can be observed that the calcium carbonate is the dominant component, while quartz displays only three diffraction lines, at 2θ = 20.7°, 26.5°, and 50.0°.

After inoculating the starting material with the two types of fungi and after applying the aqueous suspension of wollastonite layer, a second powder X-ray diffraction analysis was performed, the diagrams being illustrated in Figure 9. The presence of the two phases (calcite and quartz) as in the starting sample can be noted, and also an additional phase assigned to wollastonite ($CaSiO_3$, denoted by W) [119]. There is also an amorphous component that can be distinguished by the halo between 2θ = 17–26°, which appears due to the applied gel.

For the SEM image of the sample inoculated with *Aspergillus* spp. (Figure 10a), the segmentation reveals that 3 days after inoculation, 59.33% (Table 5) of the sample surface is occupied by *Aspergillus*. After applying the gel (Figure 10b), only 2.23% of the surface shows this species of fungi, while the aqueous suspension of wollastonite was identified on 88% of the surface.



Sample 1 (× 300. LM) *Aspergillus spp* and NWollastonite, with the dominance of the latter

Sample 2 (× 3.00k LM). Mycelium of *Cladosporium spp*.

Sample 1. *Aspergillus spp*. × 1.00 LM

Sample 1. *Cladosporium spp* (× 3.00k LM)

Sample 1. *Aspergillus spp* × 3.00k LM)

Sample 2. × 3k LM, dominant NanoWollastonite, *Cladosporium spp*. mycelia are not noticed

Sample 1 (× 300LM) *Aspergillus spp*, dominant Nano Wollastonite. The presence of cracks is noted

Sample 2 (×3.00k) with *Aspergillus spp*. (×3.0 LM). Some fissures can be identified on the surface of the stone

. Sample 1. (× 1.00k) (*Aspergillus sp*., Prismatic NanoWollastonite dominant in the central part of the image). Some fissures are visible

Sample 2. (×30LM) The hydrophilicity of NanoWollastonite generates the surface breakage of the specimens

Figure 7. Scanning electron microscope images of the surfaces loaded with *Aspergillus* spp. and *Cladosporium* spp. and then treated with aqueous suspension of wollastonite.

In the case of the SEM images corresponding to the sample where *Cladosporium* spp. was inoculated (Figure 11a), and then the aqueous suspension of wollastonite was applied

(Figure 11b), the segmentations indicate that after 3 days, 36.6% of the sample surface was occupied by this genus of fungi (Table 6), and after applying the gel, *Cladosporium* spp. disappears almost completely (0.25% weight of the sample surface). The aqueous suspension of wollastonite layer was identified on 85.7% of the sample.

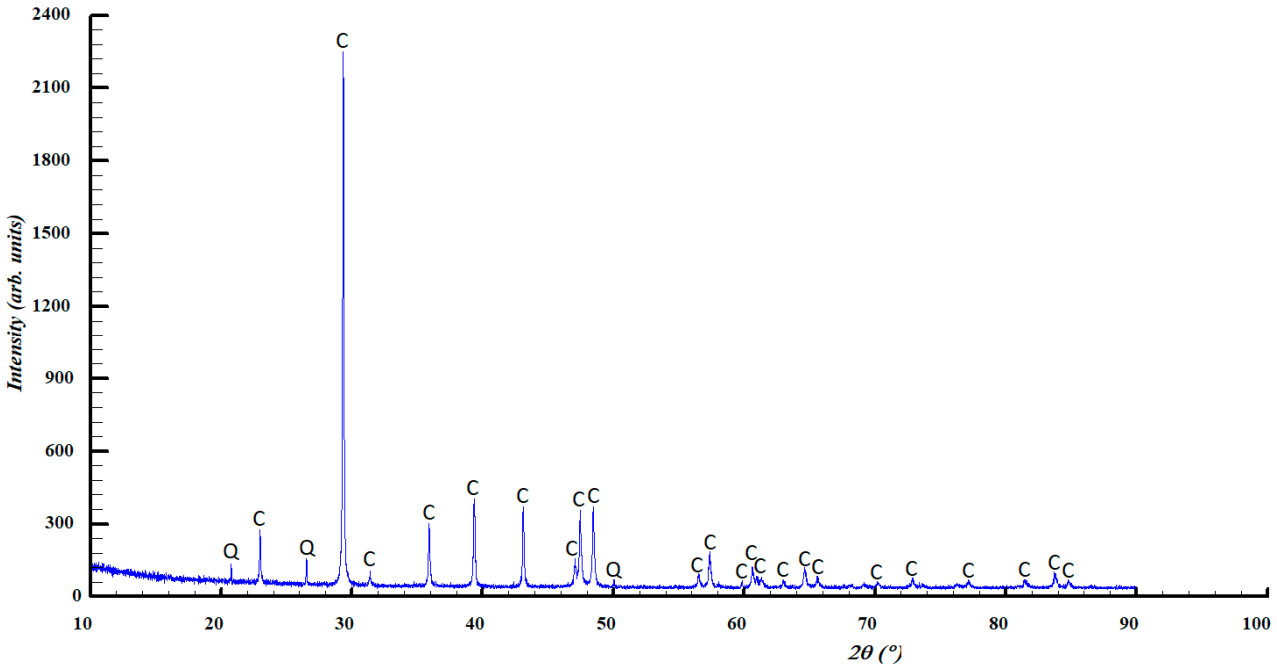


Figure 8. Powder X-ray diffraction pattern of starting sample indicating mineral composition: Q—quartz; C—calcite.

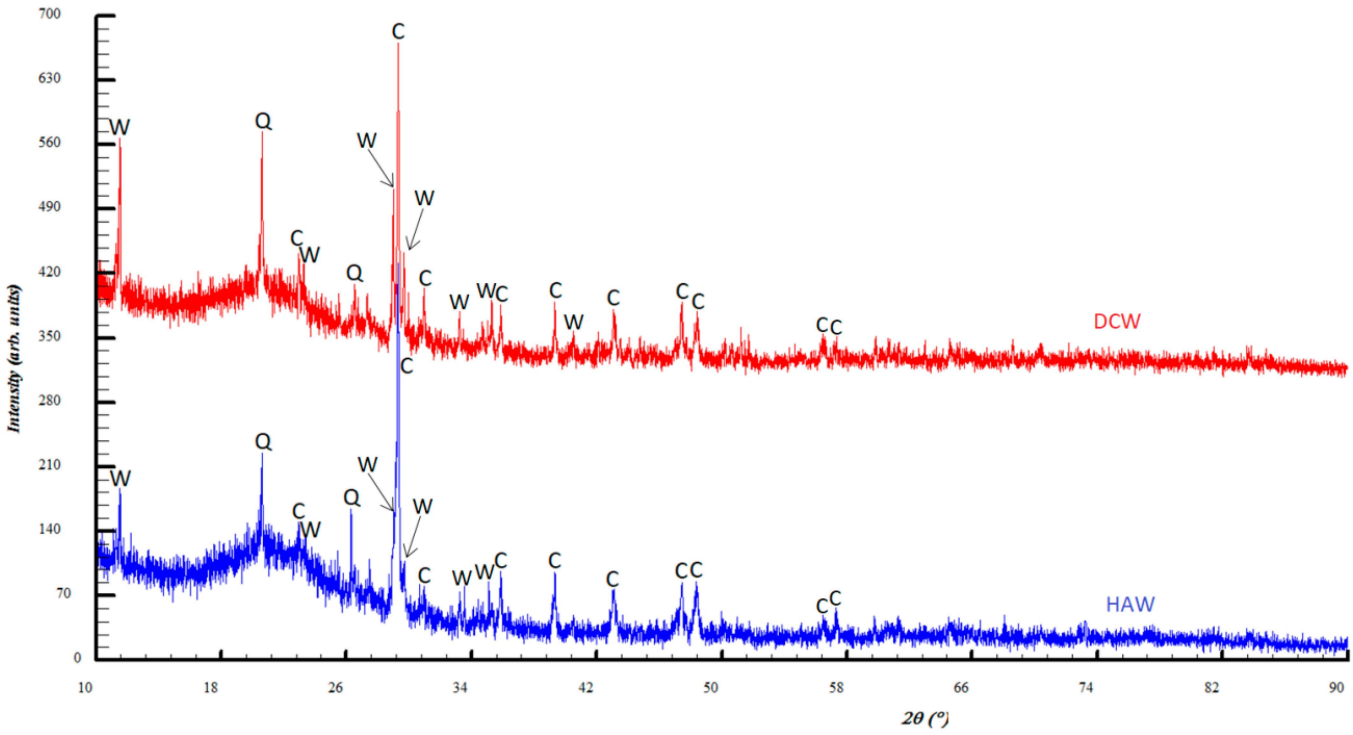


Figure 9. Powder X-ray diffraction patterns treated with aqueous suspension of wollastonite: Q—quartz; C—calcite; W—wollastonite.

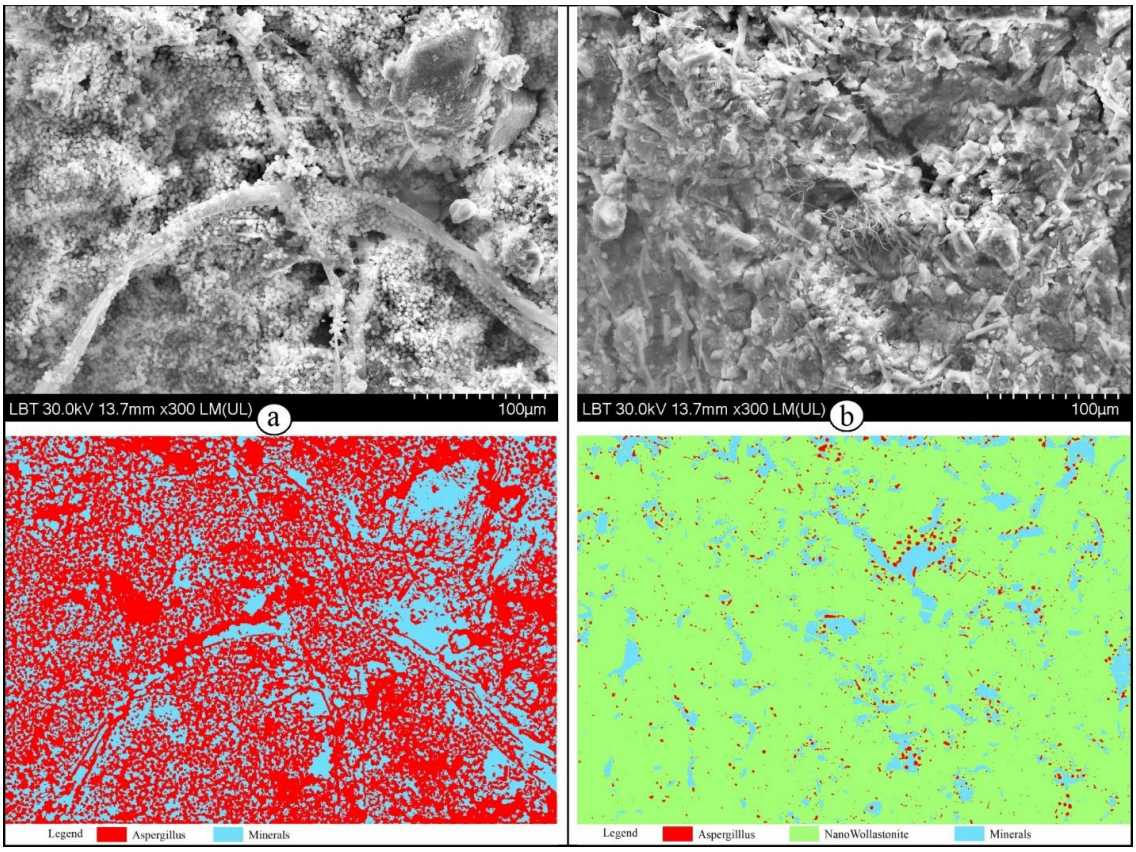


Figure 10. (**a**) Segmented SEM image for the sample with inoculated *Aspergillus* spp.; (**b**) segmented SEM image for the sample with *Aspergillus* spp. inoculated after applying the aqueous suspension of wollastonite.

Table 5. The percentage of the surfaces occupied by *Aspergillus* spp. and *Cladosporium* spp. before and after applying the aqueous suspension of wollastonite.

Sample	Class	Pixel Count	Percent (%)	Total Pixel Count
***Aspergillus* spp.**	***Aspergillus***	679,995	**59.33**	1,145,984
	Minerals	465,989	40.67	
***Aspergillus* spp. aqueous suspension of wollastonite**	***Aspergillus***	25,614	**2.23**	1,145,984
	Minerals	111,056	9.69	
	Aqueous suspension of wollastonite	1,009,314	88.08	
***Cladosporium* spp.**	***Cladosporium***	420,198	**36.67**	1,145,984
	Class 2 (background)	44,860	3.91	
	Class 3 (minerals)	36,917	3.22	
	Class 4 (minerals)	644,009	56.2	
***Cladosporium* spp. aqueous suspension of wollastonite**	***Cladosporium***	2801	**0.25**	1,145,984
	Class 2 (fractures and minerals)	160,966	14.04	
	Aqueous suspension of wollastonite	982,217	**85.71**	

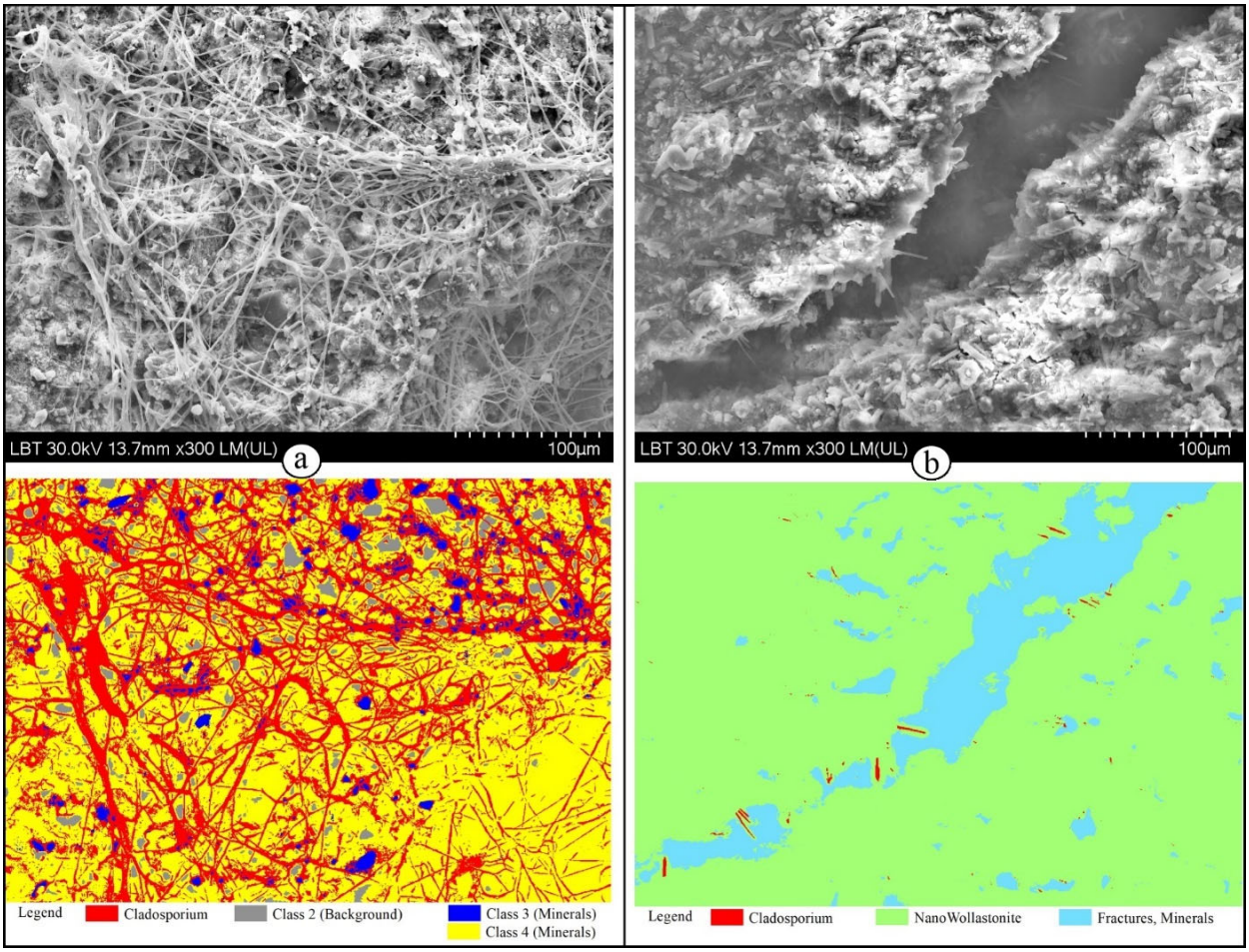


Figure 11. (**a**) Segmented SEM image for the sample with inoculated *Cladosporium* spp.; (**b**) segmented SEM image for the sample with *Cladosporium* spp. inoculated after applying nano-wollastonite.

Table 6. Results for accuracy measures used. R—recall; P—precision; CCI—correctly classified instances; k—kappa coefficient; PA—producer accuracy; UA—user accuracy; OA—overall accuracy.

		Accuracy Measures from WEKA				Accuracy Measures from ENVI			
Sample	**Class**	**R (%)**	**P (%)**	**CCI (%)**	**k**	**PA (%)**	**UA (%)**	**OA (%)**	**k**
Aspergillus spp.	*Aspergillus*	99.5	97.9	98.2	0.96	98.27	69.98	89.5	0.74
	Minerals	95.8	98.9			86.77	99.38		
Aspergillus spp. aqueous suspension of wollastonite	*Aspergillus*	98.8	71.7	98.9	0.94	92.84	76.44	98.5	0.92
	Minerals	99.7	100			99.61	93.15		
	Aqueous suspension of wollastonite	100	98.9			98.57	99.78		
Cladosporium spp.	*Cladosporium*	95.6	93	94.5	0.91	94.92	85.16	87.2	0.79
	Class 2 (background)	99.8	99.8			100	100		
	Class 3 (minerals)	87.7	96.4			82.71	84.11		
	Class 4 (minerals)	95.7	95.2			75.62	92.23		
Cladosporium spp. aqueous suspension of wollastonite	*Cladosporium*	65.9	82.8	98.6	0.96	74.27	94.11	97.1	0.94
	Minerals and fractures	98.2	96.9			94.69	99.8		
	Aqueous suspension of wollastonite	99.1	99.3			99.9	95.63		

Practically, the data extracted from the segmented images provide information related to the effect on the surface that the aqueous suspension of wollastonite has on the fungi taken in the experiment.

Regarding the accuracy of the achieved segmentations, the values for the accuracy measures presented comparatively in Table 6 are an argument for their quality and for the robustness of the models generated by the random forest classifier. The average values for the accuracy indicators used for these segmentations exceed 90%, which means a very high accuracy [120].

Although promising results were achieved in hindering the growth of the two troublesome fungi on structural stones in ancient monuments, further studies should be carried out before coming to a firm and concrete conclusion as to the effectiveness of wollastonite gel. The enzyme content should be measured in the fungi, and the substrate brushed with different levels of wollastonite gel to see if there is a significant decreasing trend correlated with the increasing wollastonite content. The measurement of the enzyme content can also clarify, or at least give us some clues, on the mechanism of action of the aqueous suspension of wollastonite against fungi. Moreover, visual observations of the growth of the fungi should be made at different intervals to find out if the hindering effects of the aqueous suspension of wollastonite can also be effective over longer durations. In addition to the above mentioned further studies, a suitable fixation component should be used to prevent the wollastonite nano- and micro-needles from being washed out or wiped out if used in situ, and not to interfere with its desirable antifungal function as well.

5. Conclusions

Scanning electron microscope (SEM) and electron dispersive X-ray spectroscopy (EDX) analyses of treated stone demonstrated the existence of nanocomposite structures containing elements. Polymers functionalised with an aqueous suspension of wollastonite can be used not only as potent biocides, but also for the consolidation of historic monuments and artifacts [79]. The hydrophilicity of the aqueous suspension of wollastonite resulted in the absorption of water in the substrate, which in turn resulted in the drying out and surface breakage of the specimens. During the 48 h after the application of the gel, the research underlines that there are good pre-requisites for the aqueous suspension of wollastonite to have a biocidal effect on *Aspegillus* spp. and *Cladosporium* spp. when applied to natural stone in the construction of heritage buildings in temperate climates. Its application is easy using a brush, in a thin layer, being at the same time a green and ecofriendly potent biocide, with a price that is not very high.

The performance and protection of natural stone materials can be improved by nanotechnology, with its superhydrophobic and photocatalytic effects being emphasised especially in recent studies. Experiments should be continued over a long period of time, with intensified testing on the long-term impact of such applied materials (nanoparticles) and the protective properties imparted to natural ornamental rock in buildings, but also on the impact of nanoparticles on the environment and human health [76,79]. The aging of works of art sometimes leads to the release of nanoparticles into the environment, therefore, future experiments should consider stabilising the nanoparticles on treated works of art, without influencing their integrity.

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