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ORIGINAL PAPER

Using ZnO nanoparticles in fungal inhibition and self-protection of exposed marble columns in historic sites

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Abstract

The marble columns at many historic sites represent one of the most important and fundamental architectural elements in a building. They are almost always subject to serious damage, whether in the base, middle, or crowns of columns by fungal infection. In most cases, the microbial deterioration affects the physical and mechanical properties of historic marble columns, which have in turn been affected by other damaging factors (e.g., weathering from the elements or mechanical damage), leading to their partial or total collapse. In this current study, researchers are turning to new technologies in order to find the ideal solution to inhibit fungal growth, and, in turn achieve the total protection of exposed historic marble columns. The photocatalytic inorganic nanoparticles of ZnO have been employed for the purpose of long-term protection of exposed marble columns by inhibiting microbial-fungal attack and forming a protective surface layer. ZnO nanoparticles were dispersed in laboratory synthesized acrylic polymer to create a combined biocidal and consolidating coating to be applied on historic marble columns substrate. The synthesized nanocomposite coating was characterized and applied to marble samples collected from various archeological sites in Egypt. The protecting effect of synthesized nanocoating against fungal attack by *Aspergillus niger* and *Penicillium* sp., in addition to RH/Temperature, UV aging, and mechanical deterioration, was studied. The consolidating action of the obtained mixtures was evaluated through microscopic examination and capillary water absorption. Further, colorimetric measurements have been performed to evaluate the optical appearance of the columns. ZnO nanocomposites displayed better performance when compared to the pure synthesized acrylic polymer. The coated ZnO nanoparticles enhanced the durability of stone surface to resist the fungal attack when subjected to inoculums containing *Aspergillus niger* and *Penicillium* sp. and improved the resistance to UV aging, relative humidity, and thermal effect compared to the samples coated with the acrylic polymer without ZnO nanoparticles. Self-protection properties were confirmed without any obvious color changes on marble surfaces.

Keywords ZnO nanocomposites · Biocidal remedy · Fungal inhibition · Historic sites · Marble columns · Self-protection

Introduction

Through the centuries, the exposed marble columns in historic buildings have deteriorated, due to physiochemical and mechanical factors such as rain water, sunlight, wind, frost, and excess loads (Winkler 2002). Moreover, the microbiological deterioration of historic stone buildings and their architectural elements represents a serious threat for their future

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existence (Macedo et al. 2009; Rebricova 1991). The growth of microorganisms such as fungi, algae, and bacteria on the stone surface and internally is usually linked to the ideal environmental conditions in terms of light, food, temperature, and moisture, as well as by the chemical nature of the substratum (Gorbushina et al. 1993). Microbiological deteriogens can play a pivotal role in the deterioration of architectural marble elements of historic buildings by a variety of mechanisms, such as powdering, cracking, and biocorrosion caused by the excretion of corrosive organic and inorganic acids. In addition, the formation of black biofilm on stone surfaces can lead to the partial or full loss of stone monuments and disfigurement of building stone material (Abdelhafez et al. 2012; Farooq et al. 2015; Monte 2003).

The protection and bioconservation of these historic elements is an important target (Ditaranto et al. 2011). In the last five decades, conservation strategies have usually relied upon the use of the synthetic polymeric coatings to protect the stone surface, hence the bioreceptivity (Bracci and Melo 2003; Johnson and Mclntyre 1996), and on the application of biocidal products to inhibit biological activity. Acrylic copolymers in particular Paraloid B-44 and Paraloid B-72 have been widely used for creating self-protection stone coatings that are extremely stable to degradation by ultraviolet, heat, and oxidation. However, these polymeric coatings can be subjected to degradation by microorganism attack, and the fungal growth interfered with the structural fabric of the polymer, modifying their hydrophobic properties (McNeill 1992; La Russa et al. 2012; Ruffolo et al. 2010). Conventional conservation strategies typically rely on the use of water repellents to reduce water absorption, hence the bioreceptivity and upon the application of biocidal products to inhibit biological activity (Nugari and Pietrini 1997; Moreau et al. 2008). Unfortunately, the improper interaction between the biocide and the water-repellent material may occur when separately applied to the substrate. These challenges and drawbacks in polymeric materials have attracted the attention of conservation experts to use the modern techniques of other sciences to increase the efficacy of the conventional methods to achieve higher consolidation and protection efficiency (Malagodi et al. 2000; Urzi and De Leo 2007; Muynck et al. 2009).

Therefore, some recent studies have focused on other innovative strategies in order to overcome some of these drawbacks. Among them, the possibility of embedding the photocatalytic nanoparticles in polymeric matrices to obtain bioactive coating with self-cleaning and consolidating properties aimed at the long-term preservation of marble stone monuments have been pursued (Frank-Kamenetskaya et al. 2010; Pinna et al. 2018). There is an immediate need to develop alternative multifunctional surface protection coating capable of acting as a remedial conservation and to prevent microbial deterioration in one step (Shilova et al. 2009; Monteiro et al. 2009). Recently, in the field of building material and stone conservation, photocatalytic treatments based on photoactive nanomaterials have been set up as an effective strategy to reduce the accumulation of pollutants, biofilm, and particulate matter on architectural surfaces with a decrease in the esthetical and chemical decay over time (Aldosari et al. 2017a, b). The marked biological and self-protection activity of the nanomaterials against a wide range of different living microorganisms using various semiconductors is well known (Dutta et al. 2012). Metals and metal oxide nanomaterials, e.g., TiO_2, Ag, MgO, and CaO are receiving significant scientific attention due to their vast existing, as well as potential newer applications, especially as biocidal and self-protection coatings have hydrophobic and consolidation properties (Nations et al. 2011). These materials have longer life spans as compared to organic antimicrobial agents and are chemically stable in extreme conditions. Moreover, these nanomaterials attack a broad range of targets in microorganisms, thus avoiding the development of resistance (Zhang et al. 2010). The possibility of embedding nanoparticles such as copper nanoparticles, SiO_2, and TiO_2 in inert polymeric matrices to obtain nanostructured bioactive coatings for different applications has been reported by the authors in various studies (Kapridaki and Maravelaki-Kalaitzaki 2013; Cioffi et al. 2004; Cioffi et al. 2005). In another study, the preparation and characterization of antimicrobial surfaces made by combining $Ca(OH)_2$ suspensions with titania or zinc oxide nanoparticles for the conservation of limestone monuments has been reported (Gomez-Ortez et al. 2013; Van der Werf et al. 2015).

In this regard, zinc oxide nanoparticles (ZnO-NPs) have attracted the interest of the scientific community for their multifunctional properties: biocompatibility, bioactivity, and chemical stability. With regard to the bioactivity, ZnO-NPs have been demonstrated to be effective in killing both Gram-positive and Gram-negative bacteria and also in inhibiting the growth of fungi (Lipovsky et al. 2011; Ditaranto et al. 2015). ZnO-NPs are one of the most efficient photocatalyst material; they were found to versatile and considered to be the new potential next-generation material as self-protection, and biocidal or disinfecting agents (Espitia et al. 2012), which are attributed to the role of reactive oxygen species (ROS) generated on the surface of the particles (Golego et al. 2000). These new treatments of zinc ion release (Jayaseelan et al. 2012) and nanoparticle internalization (He et al. 2011) are the subject of this study. Since ZnO has almost the same band gap energy (3.2 eV) as TiO_2, their photocatalytic capacity is anticipated to be similar to that of TiO_2. Moreover, some studies have confirmed that ZnO exhibit a better efficiency than TiO_2 in photocatalytic degradation of some dyes, even in an aqueous solution (Sawai and Yoshikawa 2004). The photocatalyst, ZnO, has been used in the degradation and complete mineralization of environmental pollutants (Sharma et al. 2010). Furthermore, ZnO nanoparticles are able to decompose and mineralize bio-recalcitrant organic pollutants in the form of CO_2 and H_2O (Moafi et al. 2011).

Therefore, the aim of this current study is to investigate the photocatalytic efficiency of ZnO nanoparticles dispersed in synthesized polymeric matrix as biocidal and self-protection coatings applied to deteriorated marble stone surfaces. This work presents a novel experimental laboratory study concerning the possibility of the use of a lab-synthesized acrylic polymer embedded by the nanoparticles to obtain a new nanocomposite by in situ emulsion polymerization method and the possibility for its application for the conservation of historic marble stone. In order to evaluate the efficiency of the coating, isolation of fungal species from deteriorated surface was carried out using a sterilized sticking tape and analyzed to understand the corrosive effects of microbial colonization on the stone material. After that, a semi-quantitative method assessed the biocidal efficiency of the pure polymer and nanocomposite coatings, detecting the growth inhibition of *Penicillium* sp. and *Aspergillus niger* colonies on the stone surface. Surface morphology, before and after treatment, and the penetration of nanoparticles within the stone materials were examined by scanning electron microscopy (SEM), and changes in the molecular structure occurring in treated samples before and after aging were studied using Fourier-transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR). Capillary water absorption and colorimetric measurements have also been conducted.

Materials and methods

Materials

Laboratory synthesis of coating materials

An acrylic resin, similar to Paraloid B-44, was synthesized. Paraloid B-44 is an acrylic co-polymer based on methyl methacrylate and ethyl acrylate. It was chosen as a dispersing medium of ZnO nanoparticles; it is one of the major materials used to form hard coating for protecting art works. ZnO nanopowder (with particle mean diameter of 40 nm) was obtained from Sigma-Aldrich, Munich, Germany and used as received.

The acrylic resin used in this study was synthesized at concentration (*2% w/v*) and ZnO nanopowder was dispersed in synthesized Paraloid B-44 (Polymer 2% *w/v*, ZnO 0.04 g) (Ruffolo et al. 2010; La Russa et al. 2012). The synthesis process of ZnO nanoparticles/polymer nanocomposite has been prepared by in situ emulsion polymerization system, which was the first method used to synthesize polymer/nanocomposites based on polyamide 66 (Feng and Leonardo 2006; Mohamed et al. 2009) (Fig. 1). The procedure consisted of synthesis of the acrylic polymer with fixed concentration 2% *w/v* (solid content 2 g/100 mL), then 0.04 g of ZnO nanoparticles was added during the synthesis of the polymer to make the concentration of the nanoparticles in nanocomposites equal to 2% *w/v*. The ZnO nanoparticle concentration depends on polymer solid content (Aldosari et al. 2017a, b) (see Table 1).

On completion of the mixture process, the obtained nanocomposites were characterized by transmission electron microscopy (TEM); Tecnai G20, Super twin, double tilt, electron accelerating voltage 200 kV using lanthanum hexaboride (LaB6) electron source gun, and the diffraction pattern imaging (the examination was carried out in TEM lab, Agriculture Research Center, Cairo University, Cairo, Egypt).

Preparation of experimental marble samples and application of nanocoatings

5 cm × 5 cm × 5 cm of cuboid Carrara marble samples were used. The samples were polished, washed with distilled water, and dried in an oven at 105 °C for at least 24 h to reach a constant weight. Then, the samples were left to cool at room temperature and controlled relative humidity (RH) 50%, and then weighed again (Licciulli et al. 2011).

The application of nanocoatings on marble samples has been carried out by brushing them at room pressure and temperature. The operation was repeated three times within 2 h between each application (Aldosari et al. 2017a, b). After that, the samples were left to dry off for 1 month at room temperature and a controlled relative humidity (RH) of 50% (Bakr 2011). Some samples were submitted for microbiological tests and the others were submitted to the artificial aging and investigation methods.

Methods

Isolation of fungi colonies from the deteriorated historic marble surface

The fungal isolation process was carried out on fungi samples collected from microbial infected marble surfaces of archeological marble columns in Amr ibn al-As mosque (641–642 AD), Al-Mansur Qalawun complex (1284–1285 AD), and Al-Tunbugha Al-Maridani mosque (1338–1339 AD) in the historic Cairo, Egypt (Fig. 2).

Two types of samples were collected from the infected marble: (1) Sterilized cotton swabs were used for isolation of fungi from the surface of infected marble. Then, they were kept in a sterile bag until laboratory inoculation. The collected samples were then transferred right onto two prepared media {DG18 & Potato Dextrose Agar (PDA)}. Plates were then incubated at 28–30 °C for 1–7 days, depending on the microorganism (Strzelczyk 2004; Gilman 1974). (2) Carefully, small hard crusts were scratched from the infected marble surfaces by a sterile spatula, kept in sterile bag, and used as collected for microscopic investigation to understand the effect of microbial growth on stone structures.

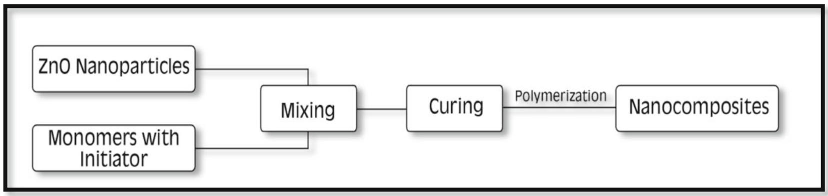

Fig. 1 The different steps of nanocomposite preparation by in situ polymerization technique

Identification and examination of isolated fungi

Plates of DG18 and Potato Dextrose Agar (PDA) were inoculated with each isolated sample and incubated for 1–7 days at 28 °C. The grown colonies were purified on the same medium and each single colony incubated was picked for identification (Domsch et al. 2008). A light microscope (Ziess 2010 Microscope with analysis unit) was used for studying the macro morphological and micro morphological features of taxonomic. The identification of isolated fungi was done with the help of reference standard and work (Cooke 1963). SEM microscope was used for investigation of the infected marble hard crust samples to evaluate the role of microorganism activity in promoting stone deterioration.

Inoculation of the experimental samples with *A. niger* and *Penicillium* sp. fungi

Untreated and treated experimental marble surfaces were inoculated with fungi colonies to evaluate the biocidal efficiency of the coating materials by observing the microbial growth on the surface. *Aspergillus niger* and *Penicillium* sp. fungi were chosen because they are among the most common airborne fungi, and the most common microorganisms living in rocks, and stone works, in addition to their ability to degrade the stone monuments (Nilsson 1983). The tested fungal strains were inoculated in Dox's agar plates, putted inside an incubation chamber at 28 °C for 7 days. Spore suspension was prepared by adding 10 mL of sterilized saline solution (8.5 g NaCl/L H_2O) to each plate and then spores were scraped with a brush and used (Diakumaku et al. 1994; Matysik et al. 2008).

Once the fungi series had developed, 500 L of suspension was put on experimental marble surface. Series 1 (uncoated marble samples and samples coated with the polymer with or without ZnO nanoparticles) was inoculated with *A. niger* culture. Series 2 (uncoated marble samples and samples coated with the polymer with or without ZnO nanoparticles) was inoculated with *Penicillium* sp. culture. And then, all the samples were put in incubated chamber at 28 °C during 4 weeks, for greater accuracy (Lopez and Gomez 1996). Experiments for each fungi colony were performed in triplicate before and after coating and checked each 3 days to observe the inhibition ratio.

Morphological characterization by SEM

SEM observation of uncoated, coated, and coated aged experimental marble samples was performed through scanning electron, Philips (XL30), (the examination was carried out in SEM lab, Housing and Building National Research Center, Cairo, Egypt). The characterization was performed to evaluate the distribution behavior of coating materials on stone surfaces and surface morphology. Images were acquired in backscattered mode (BSE).

Artificial thermal and UV aging tests

The above tests were carried out in order to evaluate the stability of the self-protection and hydrophobic properties of the coating materials. Thermal aging tests were carried out by subjecting the treated samples to 30 cycles of immersion and drying as follows: 18 h of total immersion in distilled water, then 6 h in a temperature-controlled oven at 105 °C (Lazzari and Chiantore 2000). Accelerated UV aging tests were performed through light emitted by a luminaire C.T.S. Art lux 40 with 2 UV fluorescent tubes (5000 K, 45-cm long, 100 W, 220), with plexiglas protection screen, with a UV-A component, whose UV intensity was 2 W/cm^2. The distance between samples and the light source was 20 cm. The samples were left under UV irradiation for 45 days (Malešič et al. 2005).

Fourier-transformed infrared (ATR-FTIR) spectroscopy

The analysis of ATR-FTIR spectra were processed on Attenuated total reflection Fourier Transform Infrared (ATR-

Table 1 Concentrations of used coating materials

Coating material	ZnO nanoparticles concentration (*w/v*)	ZnO solid content (g)	Polymer solid content (g)	The obtained nanocomposite
Paraloid B-44	–	–	2	Zero composite
ZnO nanoparticles	2%	0.04	2	ZnO nanoparticles/polymer nanocomposites (2%)

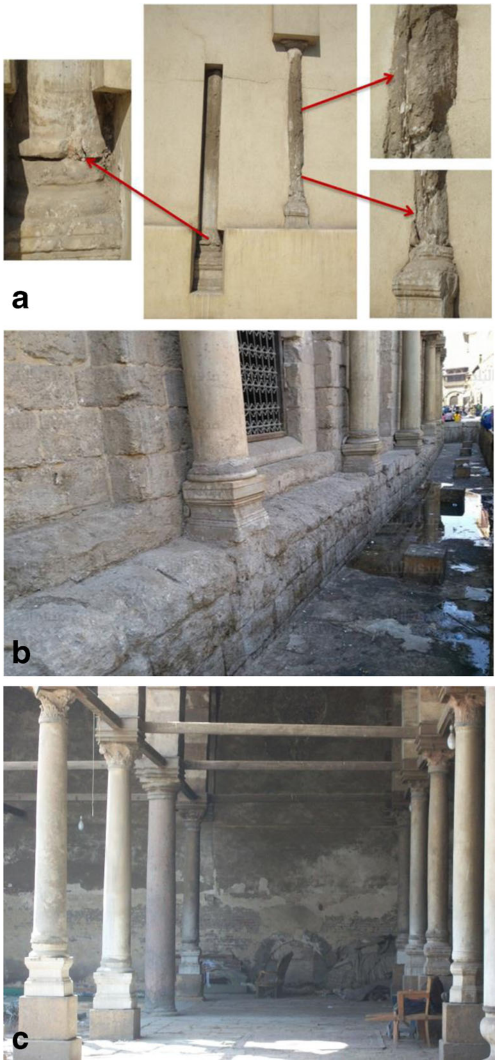

Fig. 2 Exposed marble columns in the historic studied buildings show the microbial infected marble surfaces. **a** Amr ibn al-As mosque, **b** Al-Mansur Qalawun complex, and **c** Al-Tunbugha Al-Maridani mosque

FTIR) Spectroscopy. ATR-FTIR was used for observing the changes in chemical bonding structure of treated samples during accelerated artificial ageing. All measurements were carried out at the same spot within the same samples, so that the results can be comparable. ATR-spectra were processed on BRUKER'S VERTEX 70—Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR spectrometer) in the 650–4000 cm^{-1} range, with a resolution of 4 cm^{-1}. The vibrational bands that appear in the infrared spectra provided information about the chemical functional groups of a sample which led us to study changes in characterization of the materials.

Colorimetric tests

Colorimetric measurements of uncoated, coated, and coated marble samples after artificial aging were carried out using a CM-2600d Kon-ica Minolta spectrophotometer, New York, United States of America (USA), to evaluate the effect of the coating on the optical appearance of stone. Chromatic values are expressed in the CIE L*a*b* space, where L* is the lightness/darkness coordinate, a* the red/green coordinate (+a* indicating red and −a* green), and b* the yellow/blue coordinate (+b* indicating yellow and −b* blue). The instrument was set to automatically give the average value of three measurements for each point. Measurements were performed on the same points before and after coating and aging (CIE Standard 2007).

Physical properties measurements (hydrophobic properties)

Physical properties' values were tested and recorded in order to evaluate the hydrophobic and consolidating activities of coating materials. Two types of measurements, mentioned below, were conducted sequentially as follows.

Static contact angle measurement Contact angle measurements were carried out in order to determine the wettability by means of custom apparatus made in compliance with standard UNI EN 15802 – 2010 (Helmi and Hefni 2014). This test was performed on the marble samples before and after coating. A high-resolution Canon camera with 18–55 lens and equipped with software program to calculate the contact angles values was used to capture the images of water droplets on the marble surface.

Capillarity water absorption test Measurements of water absorption were performed by capillarity which evaluates the amount of water absorbed by a stone specimen per surface unit. This test was performed using the gravimetric method on three samples for each stone species before and after coating and after artificial aging applying the two coatings, according to the following standard (UNI 10859:2000, Cultural Heritage – Natural and artificial stones – determination of water absorption by capillarity (UNI 10859. 2000)). The percentage of absorbed water was calculated using the following equation:

$$\text{Water absorption} = \frac{W2-W1}{W1} \times 100 = \ldots\%.$$

where ($W1$) is the mass of the specimen before immersion. ($W2$) is the mass of the specimen after total immersion in water for 24 h.

Results and discussion

Identification of fungi isolates from the historic marble surface

Three samples were isolated by swabs from three different marble columns at the historic sites studied. Sampling results showed the presence of numbers of fungi; the examination and characterization of the resulted fungal colonies showed nine fungal species belonging to four different genera which were identified as: *Aspergillus*, *Penicillium*, *Cladosporium*, and *Ulocladium* (Table 2). Isolates of each genus were subjected to species characterization based on morphological characteristics. From the results of genera identification and microscopic characterization and according to morphology, it can be seen that genus *Aspergillus niger* was the most predominant organism isolated from all swabs of deteriorated marble followed by *Penicillium* sp. and *Cladosporium* sp., as shown in Fig. 3.

Investigation of the microbial deterioration effect on historic marble columns

The SEM comparison investigations of infected areas in historic marble columns with areas where no fungi are present in the same environmental conditions (Fig. 4) have shown the presence of microorganisms living inside the marble structure and confirmed the role played by microorganisms in promoting stone deterioration through a variety of mechanisms chemically, mechanically, and esthetically as mentioned above (Lamenti et al. 2000; Obuekwe et al. 2005).

Characterization of the synthesized nanocoatings by TEM

Figure 5 shows TEM micrographs of the obtained nanocomposites, to evaluate the mixture process between ZnO nanoparticles and Paraloid B44. The characterization by TEM showed that the nanoparticles were homogenously dispersed and interacted in the nanocomposites without aggregates of nanoparticles in polymer matrix, with the formation of ZnO nanoparticles/polymer nanocomposites (ZnO nanoparticles marked with red circle) (Fig. 5a). Investigation size of ZnO nanoparticles after mixing process indicated that the nanoparticles diameter lies in the range 15 to 50 nm with a spherical morphology, and no aggregates were observed (Fig. 5b).

Effect of nanocoating on microbial growth

The results of treating the inoculated experimental marble samples with the polymer with or without ZnO nanoparticles were studied to evaluate the ability of ZnO nanoparticles to effect of growth of the two fungal strains *A. niger and Penicillium* sp. on marble stone surfaces. Stereo microscopy of the inoculated marble samples with *A. niger and Penicillium* sp. and incubated at 28 °C for 4 weeks is shown in Figs. 6 and 7. From the results, it was clearly observed that there is a very high rate of a diffuse growth of the two fungal strains *A. niger* and *Penicillium* sp. on untreated marble specimens (Fig. 6a and b and Fig. 7a and b). This is probably due to the chemical composition of marble stone, the greater roughness of stone surface, initial porosity, and mineralogical characteristics that make stone more susceptible to microbiological attack (Karaca et al. 2015). In addition to the high ratio of calcite as a major constituent of marble stone, it is composed primarily of the mineral calcite ($CaCO_3$), and some other mineral impurities, such as clay minerals, quartz, pyrite, iron oxides, and graphite, which provides an appropriate environment of microbial growth (Tiano 2002; Macedo et al. 2009).

The micrographs of marble samples treated with acrylic polymer without ZnO nanoparticles (Fig. 6c and d and Fig. 7c and d) showed the growth of *A. niger* and *Penicillium* sp. in some areas on the surface, although the microbial growth seem less than untreated samples, but the results revealed that the pure polymer could not completely prevent the growth of the fungus and still the spores and hyphae could still be seen. This is due to the fact that, although the acrylic polymer plays an effective role in stabilizing historic stone structures, but as this polymer is an organic polymer, it can not only be degraded by microbial attack, but also, it could conceivably stimulate growth of some other organisms (Flemming 1998; Cappitelli and Sorlini 2008). On the other hand, the micrographs of the marble samples treated with acrylic polymer containing ZnO nanoparticles (Fig. 6e and f Fig. 7e and f) clearly showed that the coating induced an inhibition and prevented fungal growth, and the nanoparticles improved the efficiency of this biocidal feature. This is due to the high photocatalytic

Table 2 Identification of fungal isolates detected in infected marble samples

Serial no.	Physical observation	Fungal genera	Fungal species
1	Brown spots	*Aspergillus*	*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus sydowii*
2	Brown spots	*Penicillium*	*Penicillium* sp., *Penicillium chrysogenum*, *Penicillium* sp. 1
3	Brown spots	*Cladosporium*	*Cladosporium* sp., *Cladosporium herbarum*
4	Black spots	*Ulocladium*	*Ulocladium alternaria*
Total		4	9

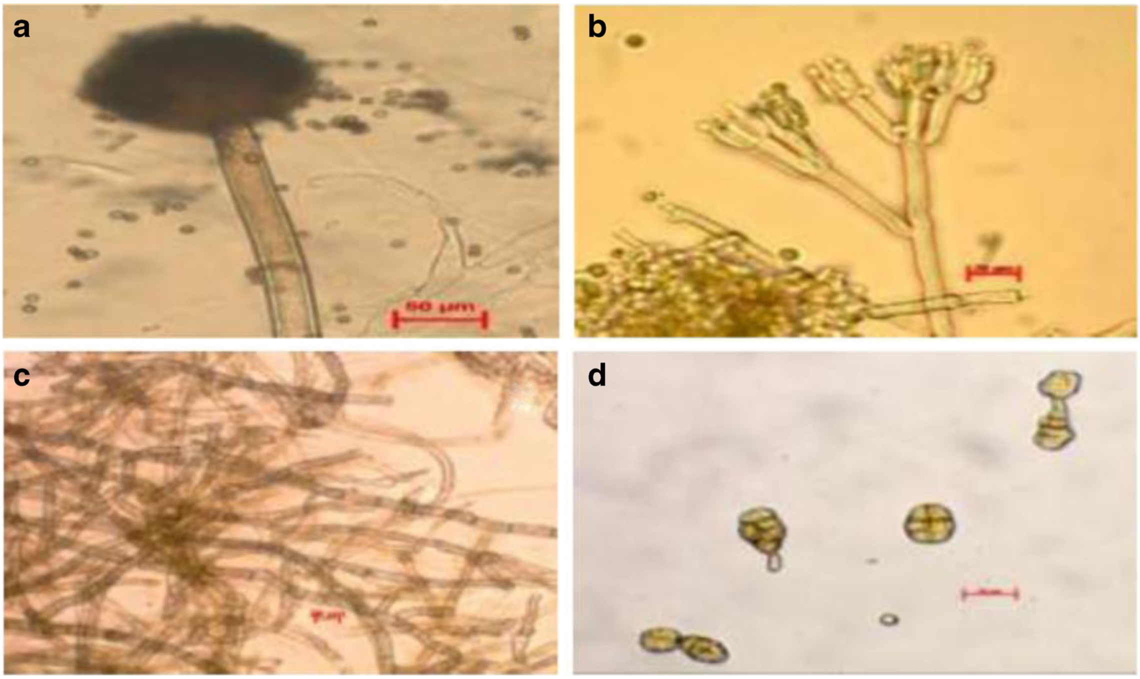

Fig. 3 Microscopic examination of isolated fungi. **a** *Aspergillus niger*, **b** *Penicillium* sp., **c** *Cladosporium herbarum*, and **d** *Ulocladium alternaria*

properties of ZnO as mentioned above, and the ability of ZnO as a semiconducting material, the band gap between conduction and valence electrons played a vital role in the generation of reactive oxygen species (ROS), which causes deterioration of microbial cell membrane.

Evaluation of stone surface morphology by SEM

SEM examination of uncoated, coated, and coated sample post thermal aging clearly showed that adding the photocatalytic ZnO nanoparticles to the polymer matrix plays an effective role in obtaining homogenous and compact coatings on marble surfaces. SEM micrographs of untreated marble samples (Fig. 8a) showed many voids and cracks in stone structures as a result of the disintegration of binding materials. SEM micrograph of coated samples (Fig. 8b and c) revealed that the polymer containing ZnO nanoparticles showed a homogeneous coating of the particles and that they uniformly cover the surface without causing cracks and segregation compared to uncoated samples and the samples coated with the acrylic polymer without ZnO nanoparticles. After artificial thermal aging, some changes were observed in samples

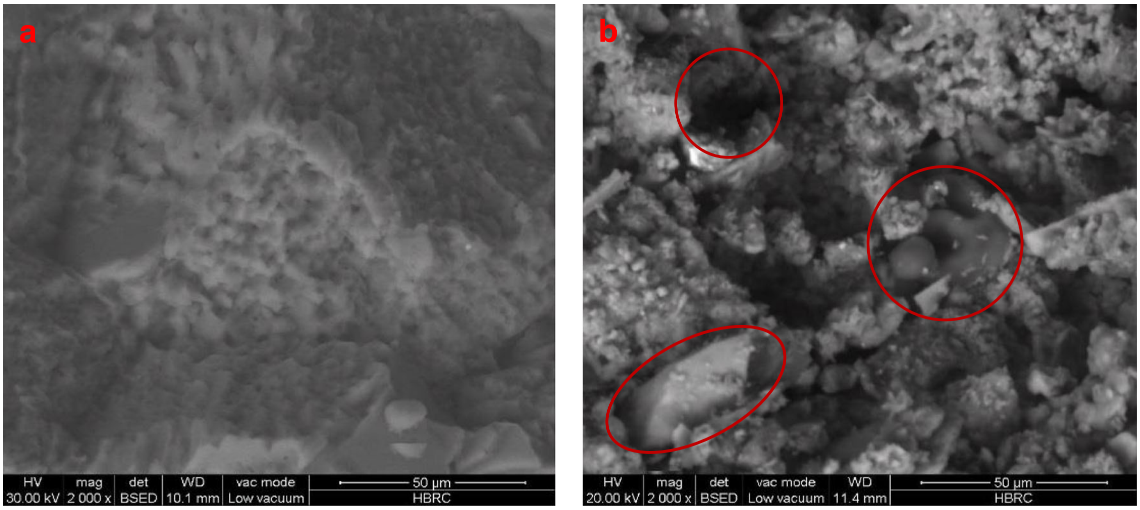

Fig. 4 SEM micrographs show the comparison between infected and uninfected marble samples. **a** Uninfected historic marble sample and **b** the microbial infected historic marble sample showing how mechanical properties of stone are affected due to microbial infection

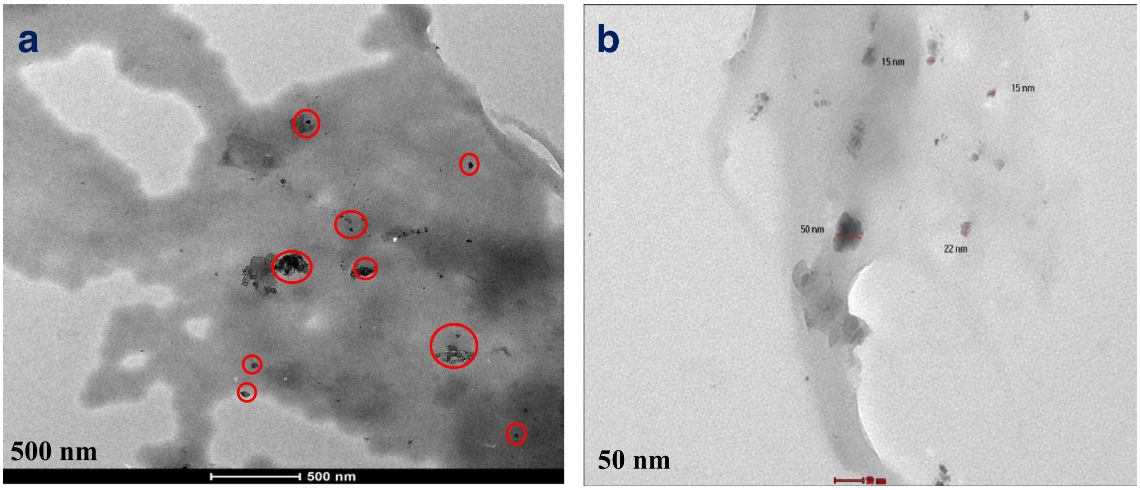

Fig. 5 TEM micrographs of the prepared ZnO nanoparticles/polymer nanocomposites after synthesis process: **a** showing the homogeneous dispersion between ZnO nanoparticles and Paraloid B44 and **b** showing ZnO particle size and spherical morphology

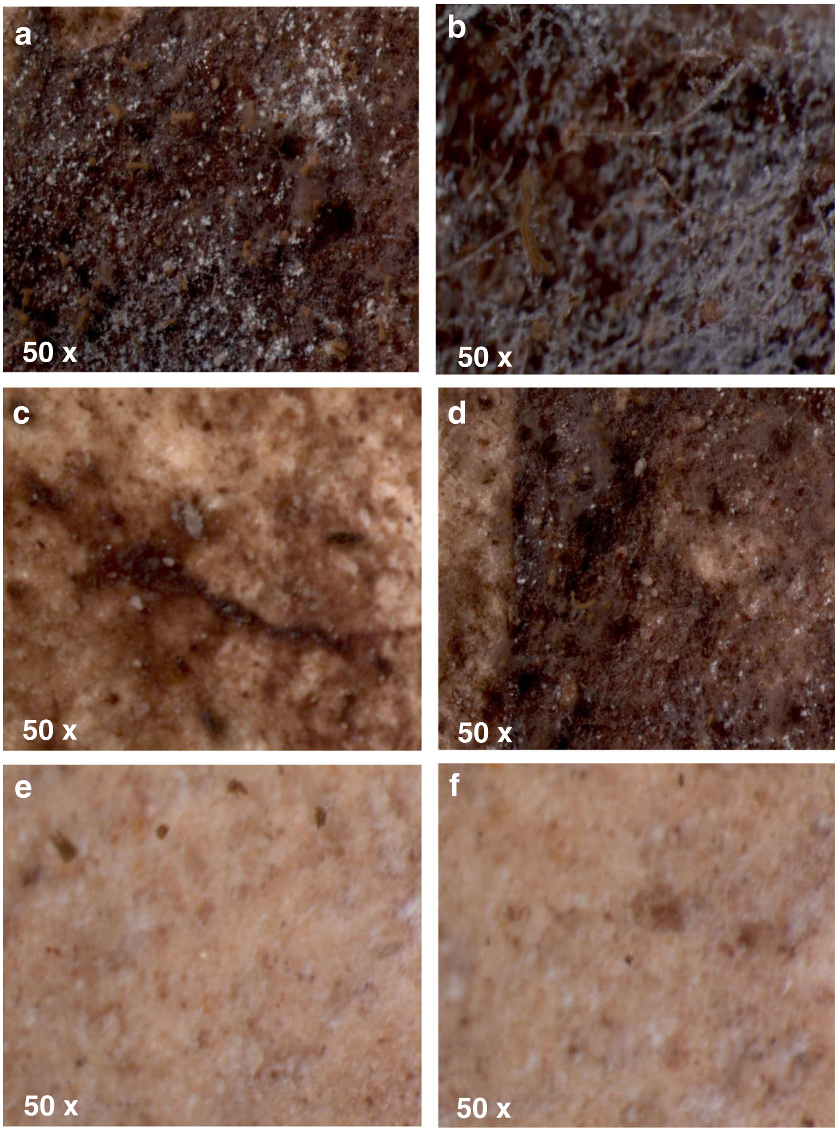

Fig. 6 Stereo microscopy of fungal colonization growth on experimental marble samples after 4 weeks since inoculation by *A. niger* fungi; **a**, **b** Untreated marble samples; **c**, **d** Experimental samples treated with synthesized B44; **e**, **f** Samples treated with synthesized ZnO/polymer nanocomposites

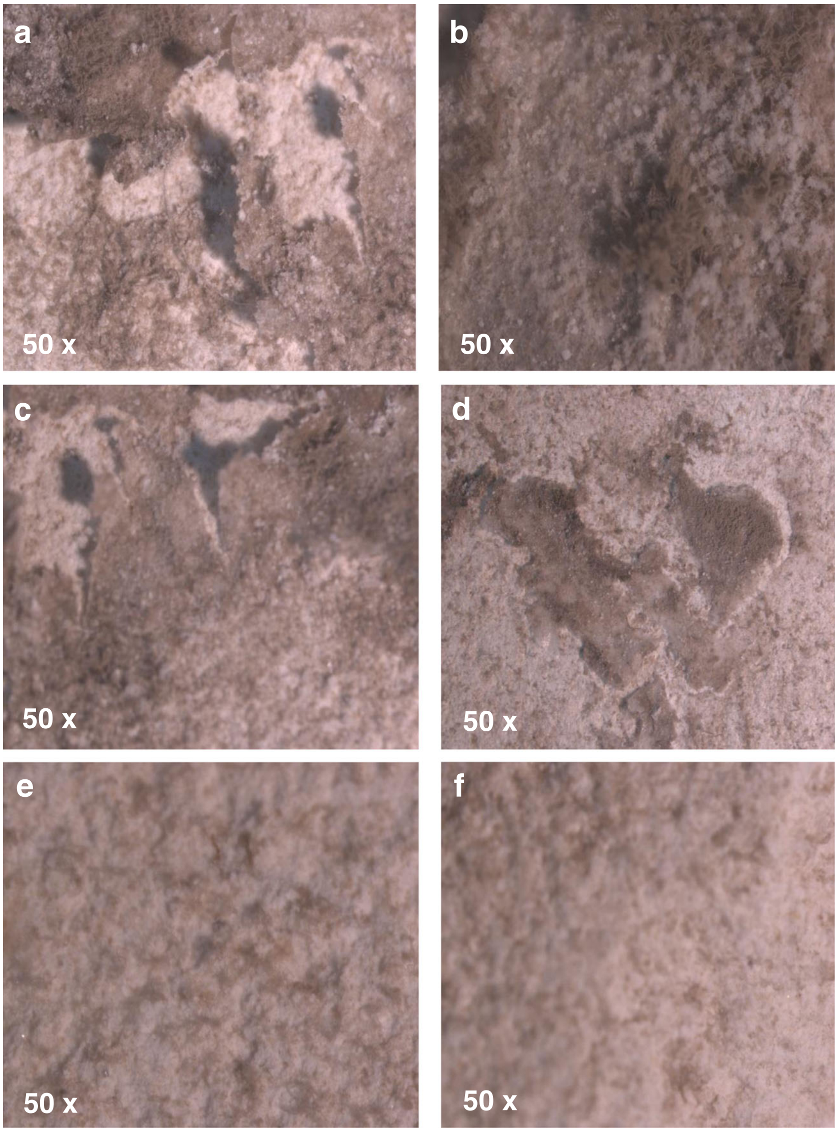

Fig. 7 Stereo microscopy of fungal colonization growth on experimental marble samples after 4 weeks since inoculation by *Penicillium* sp. fungi; **a**, **b** Untreated marble samples; **c**, **d** Experimental samples treated with synthesized B44; **e**, **f** Samples treated with synthesized ZnO/polymer nanocomposites

treated with both products; SEM micrograph of the samples coated with pure polymer without nanoparticles (Fig. 8d) was found to be dried and the temperature affected on the film homogeneity due to solvent removal and subsequent decrease of the film free volume; showing cracks of the resins. On the other hand, in the samples coated with Paraloid B-44 containing ZnO nanoparticles (Fig. 8e), small fine cracks were observed in the coating film in some areas, but without any side effects on the film uniform and homogeneity, no further influence observed on the coating properties of the remedy compared to those coated with Paraloid B-44 without the nanoparticles. The nanoparticles enhanced the durability of the coating to be stable under the effect of the artificial thermal aging. And, due to the very low porosity (less than 1%) of both nanocoatings, they do not easily penetrate in the crystalline porous structure. ZnO/polymer nanocomposites show a slightly better penetration in this substrate.

Fourier-transformed infrared (ATR-FTIR) spectroscopy

The infrared spectrum of the sample treated with pure Paraloid B-44 before artificial aging showed the presence of multiple bands in asymmetric and symmetric C–H stretching region between 3061 and 2872 cm^{-1} and in C–O stretching region between 1158 and 1028 cm^{-1} in addition to C=O stretching band at 1727 cm^{-1} which corresponds to the main functional group of B-44.

After thermal aging, the spectrum showed that the intensity of C=O stretching band at 1727 cm^{-1} sharply decreased. While, all the absorption bands in C–O stretching region and C–H stretching region vanished compared with those of the unaged treated sample. This meant that the typical absorption bands varied and the major chemical groups were affected during thermal degradation. This may be due to the loss of monomers and small fragments

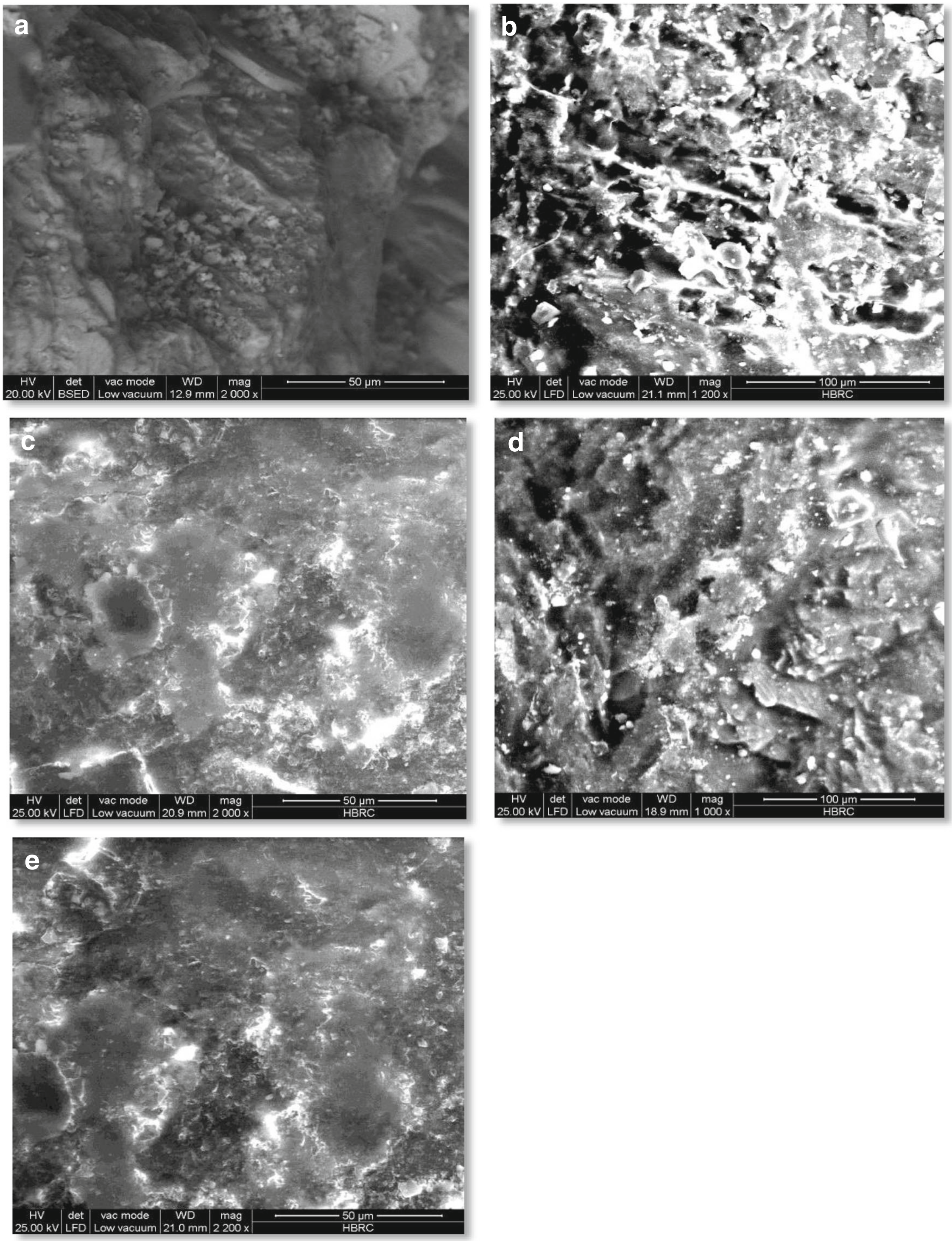

Fig. 8 SEM micrographs of the experimental marble samples. **a** Untreated; **b** coated with Paraloid B44, and **c** coated with Paraloid B44 + ZnO nanoparticles, and **d** coated with Paraloid B44 after thermal aging, and **e** coated with Paraloid B44 + ZnO nanoparticles after thermal aging

formed as a result of the chain scissions. Moreover, no oxygen-containing functional groups were formed indicating the good stability of B-44 towards thermal oxidation. These conclusions are in tandem with those obtained by Lazzari and Chiantore, who concluded that in both acrylic and methacrylic resins where all or the majority of the alkyl side groups are short, chain scissions prevail over cross-linking and the resins showed good stability towards oxidation (Lazzari and Chiantore 2000; Chiantore and Lazzari 2001).

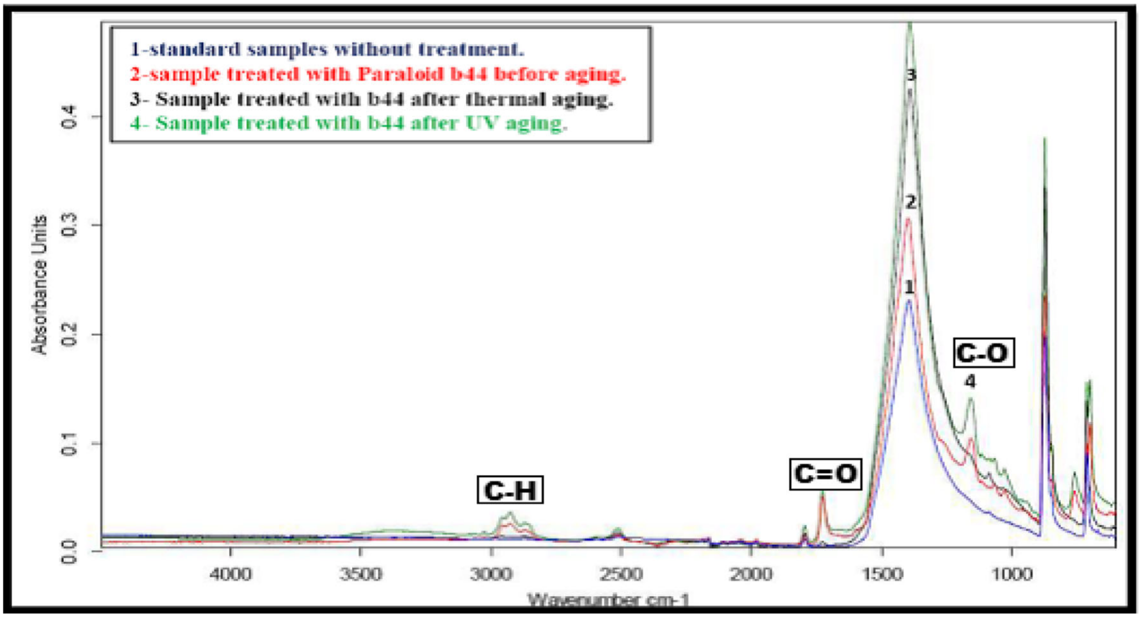

Fig. 9 FTIR-ATR spectra of the sample treated with Paraloid B-44. (1) Untreated surface; (2) treated surface before aging; (3) surface after thermal aging; (4) surface after UV aging

On the other hand, UV aging showed slight variations in all the absorption bands representing B-44. The same results were obtained by Chiantore and Lazzari, who found that the resins containing only ethyl and methyl esters displayed a good stability towards oxidation, reaching an equilibrium between scission reactions and macromolecular coupling which permit them to maintain their molecular characteristics during artificial light aging. The changes that happened at the main absorption peaks of B-44 during thermal and UV aging are shown in (Fig. 9).

The infrared spectra of the samples treated with the ZnO nanoparticles/polymer nanocomposites before and after artificial thermal aging (Fig. 10) showed slight variations in the main absorption bands of B-44 suggesting the stability of

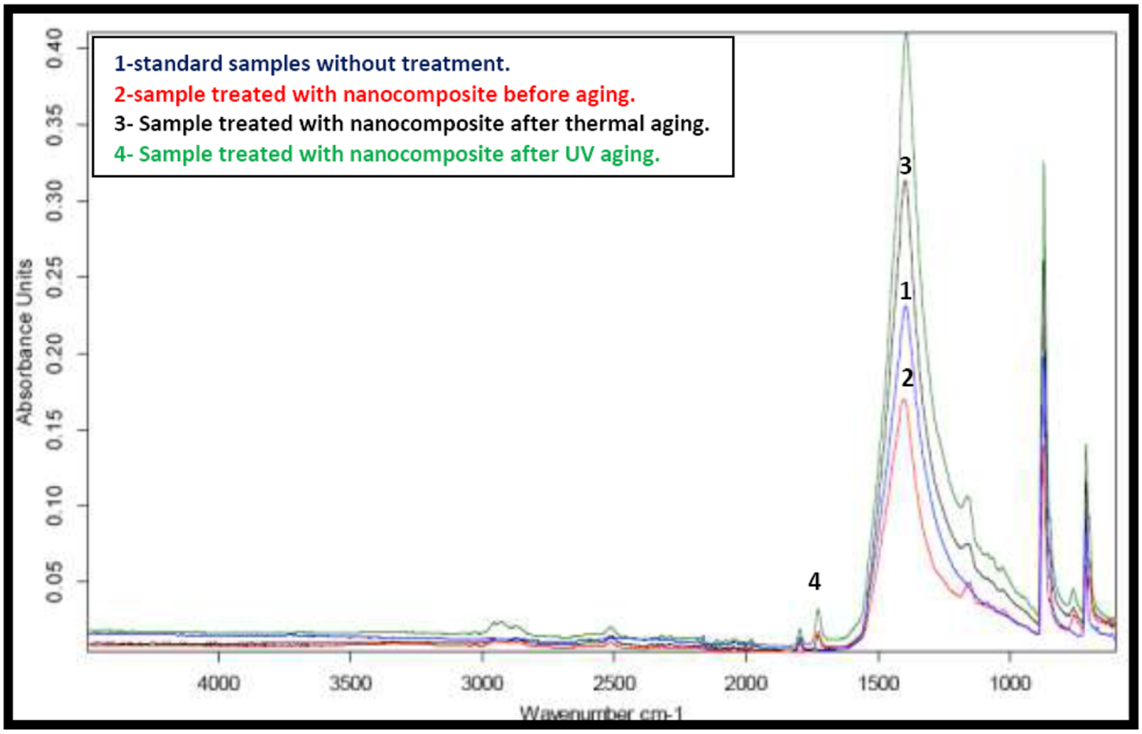

Fig. 10 FTIR-ATR spectra of the sample treated with ZnO nanoparticles/polymer nanocomposites. (1) Untreated surface; (2) treated surface before aging; (3) surface after thermal aging; (4) surface after UV aging

Table 3 Color measurements on untreated, treated, and aged samples

Applied protective materials	Δ (treated and untreated samples)				Δ (UV aged and untreated samples)				Δ (thermal aged and untreated samples)			
	Δ*L**	Δ*a**	Δ*b**	Δ*E*	Δ*L**	Δ*a**	Δ*b**	Δ*E*	Δ*L**	Δ*a**	Δ*b**	Δ*E*
Paraloid B44	0.86	0.09	0.47	*0.98*	0.63	0.53	2.30	*2.44*	2.06	0.61	2.97	*3.67*
ZnO nanoparticles/polymer nanocomposites	0.11	0.28	0.34	*0.45*	−1.56	−0.48	1.77	*2.41*	−2.17	0.40	1.36	*2.59*

the corresponding functional groups during thermal aging process compared with those of the samples treated with acrylic polymer without ZnO nanoparticles. No remarkable changes were observed in bands intensities of the sample treated with the ZnO nanoparticles/polymer nanocomposites after exposure to UV radiations, suggesting the success of ZnO nanoparticles in enhancing the physical and mechanical properties of acrylic polymers.

In general, the ZnO nanoparticles/polymer nanocomposites showed good stability towards the UV aging compared to the pure B-44. Also, the treatment with ZnO nanoparticles/polymer nanocomposites appears to be better than that with pure B-44 towards the thermal aging. This is attributed to not only the high chemical and physicomechanical properties of nanoparticles which play an effective role in increasing the hydrophobic character of the polymer, but also due to the superior properties of Paraloid B-44 which forms a hard coating, that is extremely stable to degradation by heat and oxidation, even though there were small effects in bands intensities, but better than that of the samples treated with pure B-44 only.

Colorimetric measurements

As esthetics and historic values are very important issues in conservation science, the color variations were recorded before, after coating and after aging in order to preserve the original color of surfaces. Color alterations are expressed by the ΔE parameter, which indicates the difference between each chromatic coordinate (ΔL*, Δa*, and Δb*) in uncoated, coated, and coated aged samples. The color modification (ΔE) was calculated using the following relation:

$$\Delta E^* = \left[(L_2{}^*{-}L_1{}^*)^2 + (a_2{}^*{-}a_1{}^*)^2 + (b_2{}^*{-}b_1{}^*)^2 \right]^{1/2}.$$

where ΔL*, Δa*, and Δb* represent the difference between the value of each chromatic coordinate in treated samples and the value in untreated ones. According to Italian guidelines for the restoration of stone monuments, the ΔE value must be < 5 (Normal – 43/93. 1993). The results of ΔE values were recorded after coating application and artificial U. V and thermal aging, samples treated with Paraloid B-44 have ΔE values between 0.98, 2.44, and 3.67, but those treated with ZnO nanoparticles/polymer nanocomposites have ΔE values between 0.45, 2.41, and 2.59, it is notified that after treatment and aging, negligible color variations were observed, and all values in the acceptable limit (ΔE value < 5) thus confirming the suitability of the product for conservation purposes. Nevertheless, it is notified that the ΔE values of samples treated with nanocomposites are still more acceptable than those treated with Paraloid B44 without ZnO nanoparticles since they are near to 5. The completed data is listed in (Table 3).

Table 4 Values of static water contact angle θ (°) for uncoated and coated marble samples

Samples	Contact angle measurement for uncoated and coated samples θ (± 3°)	Standard deviation
Uncoated samples	112°	1.24
Samples coated with Paraloid B44	125°	0.82
Samples coated with ZnO/polymer nanocomposites	140°	0.47

Measurements of water contact angle

The equilibrium water contact angle measurements (θ) for uncoated and coated samples were calculated as a reference point. The aim is to characterize the behavior of photoactive nanoparticle in terms of water resistance and decrease in wettability. The “θ” measurement is an average rate by measurements on three drops (UNI 11207. 2007). The results in Table 4 and Fig. 11 showed that the coating containing ZnO nanoparticles achieved the best results in the test of hydrophobicity compared to uncoated samples or those coated with Pure Paraloid B-44. The hydrophobic action mainly attributes to the polymer not nanoparticles; although, the nanoparticles help in increasing the hydrophobic character of the coating and also play an effective role in enhancing the polymer with self-protection properties which are a very important aim to prevent further microbial deterioration. But the highest hydrophobic action depends on the chemical and physical properties of the polymer and the nanoscale roughness of the surface that

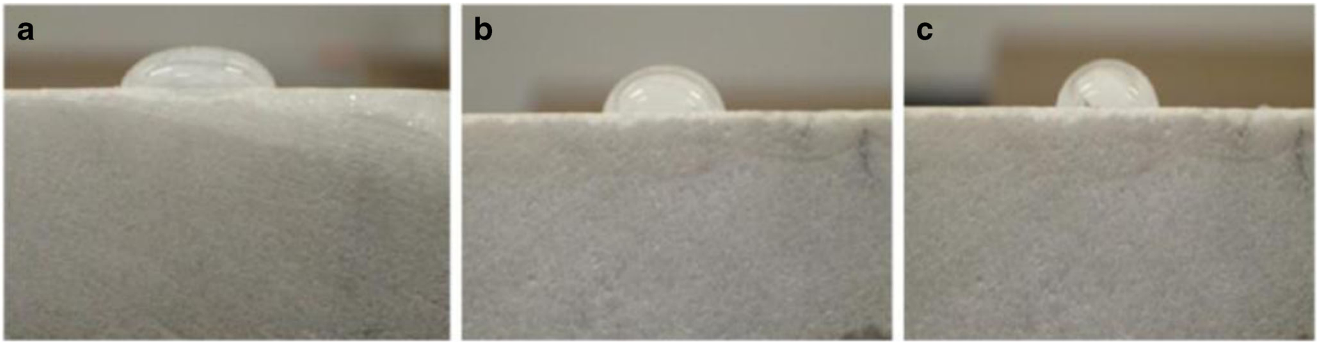

Fig. 11 Drops of distilled water on the surface of the experimental marble samples for static contact angle measurement. **a** Uncoated sample, **b** sample coated with pure Paraloid B44, and **c** sample coated with ZnO nanoparticles/polymer nanocomposites

leads to the trapping of air between the water droplet and the rough surface. Moreover, the very low porosity of marble stone helps to make the surface naturally more hydrophobic.

Evaluation of capillary water absorption

Water plays an effective role in the chemical and microbiological deterioration processes of stone monuments; therefore, conservators will always seek to apply coatings and consolidation materials with hydrophobic properties that are able to reduce water penetration into the stone bulk. From the results, good water repellency was observed in the samples after treatment by pure B44 and after adding the ZnO nanoparticles. In particular, the coating with ZnO nanoparticles reveals much better results. Analyses were carried out on both untreated and freshly treated samples as well as after artificial aging. After UV irradiation, samples coated with pure B44 and blends containing zinc nanoparticles preserve good water repellency, even where there were slight variations observed, but treated surfaces seem to be unaffected by solar radiation, with no significant difference found between the two different amounts of product. After artificial thermal aging, the behavior was slightly different; there is the effect of the polymer degradation that can lead to small alteration of the original features, especially in the case of pure B44, thus revealing a loss of the hydrophobic features of protective coatings, but we can note that the decrease of hydrophobic performance has been revealed in the case of pure acrylic polymer more than polymer containing ZnO nanoparticles. Furthermore, the low porosity of marble stone plays a major role in the water absorption process, so the coating materials cannot penetrate deeply in the stone bulk. Complete data are listed in Table 5.

Conclusion

In this study, the photocatalyst metal oxide ZnO nanoparticles were dispersed in synthesized acrylic polymer (Paraloid B44) to obtain a new nanocoating with effective biocidal, self-protection, and hydrophobic features, to be used in the protection of deteriorated exposed marble columns at historic sites. TEM images showed that ZnO/polymer nanocomposites were successfully prepared by emulsion polymerization. Moreover, the biocidal effectiveness of the ZnO nanoparticles against *Penicillium* sp. and *Aspergillus niger* were also assessed; a diffuse growth of colonies was observed on untreated specimens and on those treated with pure polymer, while growth inhibition was observed on coatings containing ZnO nanoparticles, suggesting that these have effective biocidal properties. Self-protection, consolidation, and hydrophobic features of the nanocoating have been performed before, after coating, and after artificial UV and thermal aging. The results have shown that coatings containing ZnO nanoparticles induce a remarkable increase of contact angle and water capillary absorption. Coatings containing ZnO enhanced the durability of stone surfaces against UV aging and improved their resistance to RH and temperature fluctuations in comparison with samples coated with only acrylic polymer. Fungal inhibition and

Table 5 Physical properties of coated and coated aged samples

Applied protective materials	After coating			After UV aging			After thermal aging		
	Density gm/cm^3	Porosity %	Water absorption %	Density gm/cm^3	Porosity %	Water absorption %	Density gm/cm^3	Porosity %	Water absorption %
Paraloid B44	2.84	0.19%	0.08%	2.81	0.19	0.09	2.75	0.21	0.101
ZnO nanoparticles/polymer nanocomposites	2.87	0.13%	0.05%	2.85	0.14	0.05	2.82	0.16	0.071

self-protection properties were confirmed without any observed color change on the surface. In terms of multifunctional features, ZnO nanocoating is particularly suitable as a surface coating for historic marble stones, with protective properties. This work presented a novel study about improvement of coating materials to obtain a long-term protection of deteriorated historic marble columns at historic sites. The study confirmed the success of the preparation method of nanocomposites by in situ emulsion polymerization and the possibility for the application in conservation of stone monuments.

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Author contributions Sawsan S. Darwish, Sayed M. Ahmed, and Mohammad A. Aldosari conceived and designed the experiments; Sayed M. Ahmed and Mahmoud A. Adam performed the experiments; Sawsan S. Darwish, Nagib A. Elmarzugi, and Mahmoud A. Adam analyzed the data; Mohamed A. Aldosari and Nagib A. Elmarzugi contributed reagents/materials/analysis tools; Sawsan S. Darwish and Sayed M. Ahmed wrote the paper.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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