



Cite this: *New J. Chem.*, 2015, 39, 6836

Characterization and behaviour of ZnO-based nanocomposites designed for the control of biodeterioration of patrimonial stoneworks

Nicoletta Ditaranto,^{*ab} Inez Dorothé van der Werf,^{*a} Rosaria Anna Picca,^a Maria Chiara Sportelli,^a Lorena Carla Giannossa,^a Elisabetta Bonerba,^c Giuseppina Tantillo^c and Luigia Sabbatini^{ab}

In this study, a preventive method for fighting bio-deterioration of stone substrates is proposed. This is based on the use of bioactive zinc oxide nanoparticles (ZnO-NPs), which are able to exert a marked biological activity over a long period of time due to their peculiar structure. ZnO-NPs are synthesised by a simple and reproducible electrochemical procedure. The nanomaterials are embedded in consolidant/water-repellent matrices to obtain nanostructured coatings. Commonly used products based on tetraethoxysilane and/or polysiloxanes were tested. The resulting nanomaterials were fully characterised by X-ray photoelectron spectroscopy (XPS) to investigate the amount and composition of the NPs and the behaviour of the nanocomposites. Inductively coupled plasma mass spectrometry (ICP-MS) was used for the study of the release of metal from the composites when put in contact with artificial rainwater. The nanocomposites were applied to specimens composed of three different types of stone and chromatic changes upon curing were measured by spectrophotocolorimetry. Finally, morphological characterization by scanning electron microscopy (SEM) was performed. The bioactivity of ZnO-NPs nanocomposites was also assessed in preliminary tests against *Aspergillus niger* fungus.

Received (in Montpellier, France)
3rd March 2015,
Accepted 25th May 2015

DOI: 10.1039/c5nj00527b

www.rsc.org/njc

Introduction

Biodeterioration of stone substrates is of great concern due to chemical/physical and aesthetic damage. The predominantly outdoor location of the majority of this type of artefact requires continuous maintenance, which mainly consists of periodical cleaning treatments. This implies high costs and stress for the constituent materials. So far, conservation strategies usually rely on the use of water repellents to reduce bioreceptivity and on the application of biocidal products to remove biofilm and inhibit biological activity for some time. Recently, metal oxide nanoparticles have received increased attention as antimicrobial agents.^{1–5} These materials have longer lifetimes compared to organic antimicrobial agents and are chemically stable in extreme conditions. Moreover, these nanomaterials attack a broad range of target microorganisms, thus avoiding the development

of resistance.⁶ Among these materials, zinc oxide nanoparticles (ZnO-NPs) have attracted the interest of the scientific community due to their multifunctional properties: biocompatibility, bioactivity, and chemical stability. With regard to 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.^{7,8} Although there are many studies on the antibacterial properties of zinc oxide nanoparticles, the mechanism of their antibacterial action is still not completely understood. In some papers it has been ascribed to the action of reactive oxygen species that are formed on the ZnO-NPs surface by a photocatalytic process.^{9,10} Other authors report that the antibacterial effect is related to the abrasiveness of ZnO-NPs due to surface defects¹¹ or that the toxicity of ZnO should be attributed to release of Zn²⁺ from nanoparticles.^{12,13} A very recent study demonstrated the combined effect of oxidative stress caused by both ZnO-NPs and the concentration of Zn²⁺ that accumulated in a cell wall.¹⁴ Although their mechanism of action needs to be thoroughly investigated, ZnO-NPs have found applications as antimicrobial agents in many fields. To the best of our knowledge, only one recent paper reports their use in the preservation of cultural heritage; specifically, ZnO-NPs are employed in the surface treatment of oil paintings to fight the proliferation of fungi.¹⁵ The aim of this study is to investigate the use of

^a Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", via Orabona 4, 70125 Bari, Italy. E-mail: nicoletta.ditaranto@uniba.it, inezdoroth.vanderwerf@uniba.it

^b Centro Interdipartimentale "Laboratorio di ricerca per la diagnostica dei Beni Culturali", Università degli Studi di Bari "Aldo Moro", via Orabona 4, 70125 Bari, Italy

^c Dipartimento di Medicina Veterinaria, Università degli Studi di Bari "Aldo Moro", Strada prov.le per Casamassima km. 3, Valenzano (BA), Italy

ZnO-NPs mixed with state-of-the-art consolidant/water-repellent materials. The idea is to exploit the combined action of the bioactivity of zinc oxide nanoparticles and the conservation properties of the matrices without affecting the aesthetics or being harmful to the stone substrates.

Concerning the consolidants/water repellents that were employed in this study, we chose commercial silicon-based products that have been widely used for about thirty years as protective and consolidating agents to preserve stone materials from decay. The advantages of this type of material are related to good chemical stability, low surface tension, good elasticity, and resistance to thermal stress.¹⁶ As for the influence of these materials, in particular tetraethoxysilane, on the porosity of treated stones, several studies are dedicated to this problem.^{17–19} Indeed, restorers should be well aware of all possible advantages and drawbacks related to the use of these materials in conservation treatments, taking into account the specific properties and characteristics (composition, type of porosity, degree and type of decay, *etc.*) of the stone substrate to be treated. In particular, the pore structure may strongly influence the movement of water and therefore decay phenomena such as salt crystallization, biodeterioration, freeze–thaw processes, *etc.*²⁰

In this study, three commercially available consolidants/water repellents were selected (Estel 1000, Silo 111, and Estel 1100) for the development of a bioactive nanocomposite system capable of preventing biological decay. The innovative composite materials are designed to provide controlled release of the active component in order to ensure long-lasting efficacy against recolonization of stone substrates. Previously, the authors reported on the development of nanocomposites that were obtained by embedding copper nanoparticles (Cu-NPs) in commercially available silicon-based consolidants/water repellents and on their application.²¹ The nanomaterials have been successfully used as antimicrobials for *in situ* experimentation on different stone substrates.²² Despite the very encouraging results obtained with these nanomaterials, the dark colour of copper may restrict their potential applications due to possible aesthetic alteration: only small amounts could be used or dark substrates could be treated. The preparation of white-coloured ZnO-NPs and development of nanostructured films reported here represent an interesting improvement for different reasons. First of all, these coatings are able to meet the aesthetic requirements for application in the field of cultural heritage. Moreover, their efficacy could vary from metal to metal as a function both of the size and shape of the designed nanomaterial and of the resistance of specific target microorganisms to the antimicrobial agent.²³ Despite the well-known efficacy of both copper and zinc nanomaterials in reducing/blocking the growth of a wide range of microorganisms, it has been proved that the antibacterial effect of ZnO-NPs is significantly more pronounced on Gram-positive bacteria than on Gram-negative strains, which is probably due to their different cell membrane structure.²⁴

In this study, specific amounts of white ZnO-NPs powder were dispersed in consolidant/water repellent solutions and the resulting dispersions were then spin-coated on glass slides or applied to stone substrates. All the nanocoatings were characterised

from spectroscopic and morphological points of view. Preliminary experiments assessing the ability of the nanomaterials to release zinc were carried out, along with evaluation of their antimicrobial properties.

Experimental

Nanomaterial preparation

Zinc oxide nanoparticles were prepared by an electrochemical technique. The preparation was carried out in a three-electrode cell configuration with two zinc plates as working and counter electrodes and Ag/AgCl (KCl sat.) as reference electrode, all immersed in an alkaline solution of NaHCO₃ (30 mM) and polystyrene sulphonate (1 g L^{−1}).^{25,26} Electrosynthesis was performed in galvanostatic mode at 10 mA cm^{−2} using a PAR 263A potentiostat–galvanostat. After centrifugation, the resulting precipitate was dried at 70 °C overnight. The obtained powder was calcined at 600 °C in a muffle furnace in air for 1 h. The calcined samples were morphologically characterised by transmission electron microscopy (TEM) using a FEI Tecnai 12 system operating at HV (120 kV).

Estel 1000, Estel 1100 and Silo 111 are ready-to-use silicon-based consolidant/water-repellent materials provided by CTS (Altavilla Vicentina, Italy). According to the technical data sheet, Silo 111 is a water repellent composed of a mixture of low-molecular-weight organosiloxane oligomers, Estel 1000 is a consolidant composed of tetraethyl orthosilicate (TEOS), whereas Estel 1100 contains both TEOS and siloxane oligomers and is therefore considered to possess both consolidant and water-repellent properties. All materials are dissolved in white spirit D40 (a mixture of hydrocarbons distilled from oil with a boiling point in the range 150–200 °C). The siloxane oligomers are typically composed of dimethylsiloxanes.^{27,28} TEOS is well known to undergo chemical modifications upon exposure to air, leading to the loss of ethanol moieties and to various polymerisation phases. In the first step, hydrolysis of ethyl silicate occurs: it reacts with atmospheric/substrate water to produce ethanol and siloxane moieties. The former evaporates while the latter starts to polymerise, leading to the formation of silica gel, which eventually binds to the stone, thus consolidating its structure.²⁹ ZnO nanopowders were dispersed in Estel 1000, Silo 111, and Estel 1100 to prepare nanostructured thin films. The procedure consisted of mixing ZnO-NPs white powder with the consolidant/water-repellent materials in various %w/w concentrations. The resulting dispersions were then spin-coated on glass slides or applied to stone specimens (see below). Nanostructured films loaded with variable amounts of NPs were prepared by simply modifying the mixing ratio of ZnO powder and consolidant/water repellent solutions.^{21,30,31}

XPS characterization

Films that were deposited on glass slides, consisting of pure Estel 1000, pure Silo 111, pure Estel 1100, Estel 1000/ZnO-NPs, Silo 111/ZnO-NPs, and Estel 1100/ZnO-NPs nanocomposites, were characterised by X-ray photoelectron spectroscopy (XPS), using a Theta Probe VG Scientific spectrometer equipped with a

monochromatised Al K α source (spot = 300 μ m). Survey spectra were recorded in constant analyser energy mode (CAE) at a pass energy of 150 eV, whereas high-resolution regions (C 1s, O 1s, Si 2p, Zn 2p) were acquired in CAE mode at a pass energy of 100 eV. Calibration of the binding energy (BE) scale was performed by fixing the aliphatic component of the C 1s signal at BE values of 284.8 ± 0.1 eV.

Zinc release experiments

Determination of the amount of zinc that was released from the nanostructured films into an aqueous contact solution of nitric acid (2% w/w), used to mimic rainwater, was carried out by inductively coupled plasma mass spectrometry (ICP-MS) analyses using a Perkin Elmer Elan 9000 spectrometer. 1 mL solution was deposited onto each modified glass slide; the solution was sampled after a 24 hour contact period and analysed. Zinc was measured on the basis of its isotope ^{64}Zn . Quantification was performed using indium (25 ppb) as internal standard.

Biological experiments

The nanocomposites were subjected to bioactivity tests using *Aspergillus niger* as target microorganism. This is a very common fungus, which is ubiquitous in soil and commonly reported from indoor and outdoor environments. It was grown on Nutrient Agar (NA) CM 0003 culture medium incubated at 37 °C for 48 hours. Different dilutions (from 10^{-1} to 10^{-9}) were prepared and were then evaluated in biological tests in order to choose the best experimental conditions. Glass slides, which were modified with nanocomposites, were immersed in bottles containing 20 mL 10^{-5} dilution and incubated at 35 °C for 24 hours. Some 1 mL of this solution was then plated and after a further period of 24 hours colonies, if any were present, were counted.

Application step

Stone samples. Three calcareous building stones from Apulia were selected. The first was Calcare di Altamura (C), which is a fine-grained biomicrite packstone-wackestone with rather low porosity, estimated to be around 3%. The other stones were two varieties of Calcare di Gravina: a light-yellow, biolithoclastic fine-grained wackestone-packstone from a quarry district in Massafra (M) and a whitish bioclastic medium-grained packstone from Gravina (G). These materials exhibit a certain similarity in terms of porosity (porosity ranges from ca. 40% (M) to 50% (G)), dry density and uniaxial compressive strength, but have different textural features and pore size distributions. Pore size diameters of 10–30 μ m and 1–10 μ m are prevalent in M, whereas in G pore size diameters >30 μ m were shown to be the most abundant. Detailed information on the petrophysical and mechanical properties of both calcarenites, including porosity, pore size distribution, density, water absorption, degree of saturation, permeability, and thermal properties, as well as compressive strength and flexural strength, have been reported in the literature.^{32,33}

Specimens were prepared by cutting square blocks with dimensions of $2.5 \times 2.5 \times 1$ cm for Calcare di Altamura and quarters of a circle with $r = 2.5$ cm and thickness of 1 cm for both Calcare di Gravina stone types.

The specimens were treated with Estel 1000 and Silo 111 and also with these consolidants/water repellents loaded with 0.5% w/w ZnO-NPs. Application of the materials was carried out by a brush until complete absorption by the specimens, which were then kept at room temperature in order to allow evaporation of the solvent and curing of the coating.

Colorimetric measurements. Evaluation of colour changes of the treated stone samples was carried out by colorimetric measurements using a Konica-Minolta Chroma Meter (CM-2600d). The operating conditions were based on the UNI Normal 43/93 recommendations.³⁴ The results were reported in the CIE $L^*a^*b^*$ system. For each stone specimen, four (samples C) or three (samples M and G) points of analysis were selected; the instrument was set to automatically give the average value of the colorimetric coordinates (L^* , a^* , b^*) of three measurements for each point. The resultant chromatic change ΔE was determined by the following equation: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$. Then, for each specimen the ΔE values were averaged out to obtain a single value and standard deviation. Measurements were performed at the same points of the specimens by means of a suitable mask before and after application of the remedial product.

SEM-EDS analyses. Fragments of all treated and untreated stone specimens were observed with an S360 SEM instrument (Cambridge Instruments) to evaluate the morphological modifications after application of the nanocomposite. The semi-quantitative elemental composition was obtained using an X-ray energy-dispersive spectrometer (EDS) with an Oxford-Link Ge detector equipped with a Super Atmosphere Thin Window (ATW) with a thickness of 0.4 mm. An accelerating voltage of 15 kV and a beam current of 500 pA were used. Before SEM-EDS investigations, specimens were coated with a 30 nm conductive graphite film.

Results and discussion

Nanocomposite preparation

ZnO-NPs were prepared according to an electrochemical procedure reported in the literature²⁵ and modified by adding sodium polystyrene sulphonate as stabilizing agent to the electrolytic medium, which resulted in improved morphology and control of NP growth.²⁶ Final thermal treatment of the nanocolloid at $T = 600$ °C allowed complete conversion into nanostructures with ZnO stoichiometry. A TEM image is shown in Fig. 1, where it is possible to observe the presence of nanostructures. Selected-area electron diffraction analysis (data not reported) showed ZnO faceted wurtzite crystallites with an average size of 50 nm.

Estel 1000, Silo 111, and Estel 1100 were chosen as dispersing media for ZnO-NPs, owing to their consolidant and water-repellent properties. As already stated in the Experimental section, ethyl silicate is well known to undergo chemical modifications upon exposure to air, leading to the loss of ethanol moieties and various polymerization phases. This behaviour was taken into consideration by allowing a period of at least 40 days curing time before any characterization and/or use of the nanocomposites.²¹

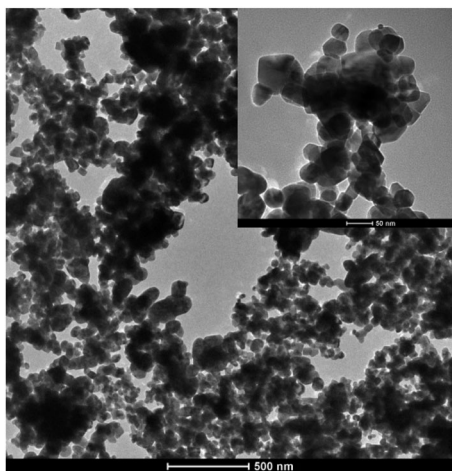


Fig. 1 TEM images of electrosynthesised ZnO after calcination at $T = 600\text{ }^{\circ}\text{C}$. A magnification is shown in the inset.

Specific amounts of ZnO-NPs powder were dispersed into the consolidant/water repellent solution to obtain dispersions with different %w/w of ZnO-NPs.

Nanocomposite surface characterization

Materials that were deposited on glass slides were analysed by XPS. Bare Estel 1000, Silo 111, and Estel 1100, along with nanostructured films with different bulk concentrations of ZnO-NPs,

were investigated. The aim was to determine the best experimental conditions for yielding a viable concentration of zinc on the surface of the nanomaterials without affecting the properties of the consolidant/water-repellent materials.

XPS analyses of the different materials revealed the presence of carbon, oxygen, and silicon, as expected on the basis of their chemical composition. Small amounts of zinc were also detected on the surface of nanocomposite materials based on Estel 1000 and Estel 1100. In Fig. 2 XPS spectra of all spectral regions are shown. The first row of Fig. 2 gives a comparison of C 1s, O 1s, and Si 2p spectra for materials based on Estel 1000 with (green line) and without (red line) ZnO-NPs. The second and third rows give analogous comparisons for nanomaterials based on Silo 111 and Estel 1100. In all cases, no differences were found upon inclusion of ZnO-NPs and therefore it can be stated that embedding nanoparticles does not significantly change the surface chemistry of the consolidants/water repellents.

As regards the surface availability of ZnO, no zinc could be detected on the top surface of Silo 111 nanocomposite films, regardless of the curing time and/or bulk loading of ZnO-NPs. A possible explanation for this behaviour might be found in the high density of the polysiloxane matrix which may not allow the nanoparticles to emerge. On the contrary, XPS analyses that were performed on nanocomposites based on Estel 1000 and Estel 1100 revealed some zinc on the surface, especially after curing. In Fig. 3 Zn 2p XPS spectra recorded on an Estel 1100/ZnO-NPs composite are reported. This figure clearly shows the surface

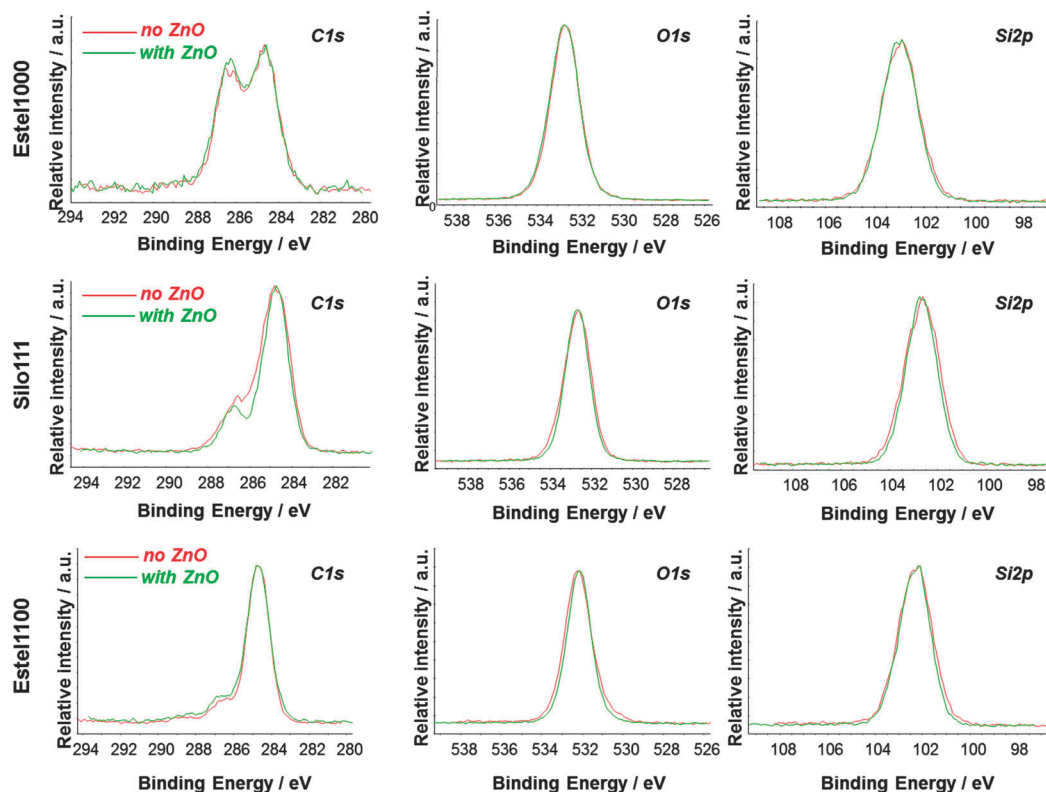


Fig. 2 XP spectra of carbon, oxygen, and silicon regions recorded for Estel 1000, Silo 111, and Estel 1100-based coatings, with (green lines) and without (red lines) ZnO-NPs.

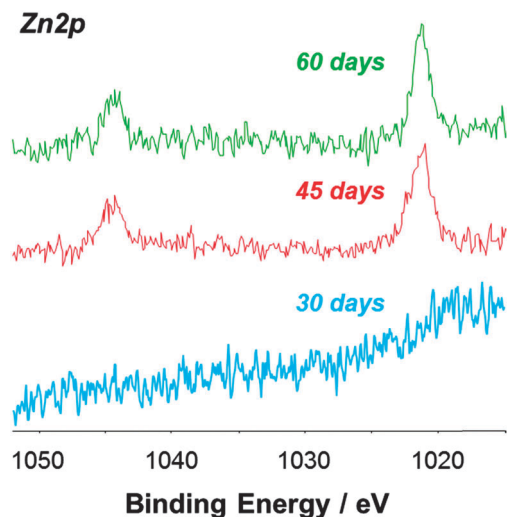


Fig. 3 Zn2p XPS spectral region recorded on Estel 1100/ZnO-NPs nanocomposite after different curing times.

Table 1 Surface atomic percentages of Zn vs. bulk amount determined by XPS measurements on composites after 40 days of curing

% ZnO-NPs w/w	% Zn _{atomic}		
	Estel 1000 ZnO-NPs	Silo 111 ZnO-NPs	Estel 1100 ZnO-NPs
0.03%	n.d.	n.d.	n.d.
0.15%	n.d.	n.d.	0.3 ± 0.2
0.20%	0.1 ± 0.2	n.d.	0.3 ± 0.2
0.25%	0.4 ± 0.2	n.d.	0.3 ± 0.2

availability of zinc after a 45 day curing period of the film. The spectral region exhibits typical doublet peaks: Zn 2p_{3/2} (at lower BE value) and Zn 2p_{1/2} (at higher BE value) components. Zinc is present in a single oxidation state as zinc oxide and therefore retains the chemical composition of the thermally treated ZnO-NPs.²⁶

A possible dependence of the surface percentage of Zn on the bulk amount was also investigated. The results are reported in Table 1. These data suggest that the bulk concentration does not significantly influence the surface atomic percentage.

Zinc release experiments

ICP-MS analyses were used to determine the release of zinc from the prepared nanomaterials into an appropriate contact solution. An aqueous solution of acid was used to mimic the pH conditions of rainwater to which stone monuments are usually exposed. Glass slides modified with Estel 1000/ZnO-NPs, Silo 111/ZnO-NPs, and Estel 1100/ZnO-NPs nanocoatings at two different %w/w were covered with the contact solution for 24 hours; after this period, the solution was sampled and analysed. The results are reported in Table 2.

The data suggest that the nanomaterials are able to release amounts of zinc in the range of ppm concentrations. Moreover, the amount of zinc released seems to be dependent on the bulk concentration for all matrices. Therefore, apparently the process

Table 2 Amount of zinc released by nanocomposites (curing time 40 days) in aqueous solution of nitric acid after 24 hours contact period

	Zinc release/ppm	
	ZnO 0.15% w/w	ZnO 0.25% w/w
Estel 1000	4.58 ± 0.09	31.6 ± 0.3
Silo 111	2.89 ± 0.08	3.60 ± 0.08
Estel 1100	0.86 ± 0.06	0.98 ± 0.04

of metal release is not strictly influenced by surface availability but by bulk concentration; in fact, Silo 111 materials exhibit a certain release of zinc, although no zinc was found on their surfaces by XPS investigation.

Biological experiments

Preliminary biological experiments were performed on Estel 1100 nanocomposites in order to study the bioactive properties of ZnO-NPs. *Aspergillus niger* was chosen as target microorganism due to its presence in outdoor environments.

The measurements were performed following the protocol reported in the experimental section. The results are shown in Fig. 4.

This figure shows images of Petri dishes containing plated culture broth after 24 hours of contact with the nanocomposites. As can be observed, only the control and bare Estel 1100 dishes show the growth of *Aspergillus* colonies, more or less in the same amounts. All the other plates are completely disinfected, regardless of the ZnO-NPs weight percentage.

Application step

After chemical characterization, release studies, and biological tests, which were performed on nanocomposites applied to glass slides, an application step was undertaken. This step consisted of the study of stone specimens treated with consolidants/water repellents and the respective nanocomposite coatings.

In the field of restoration and conservation of cultural heritage, treatments have to fulfil requirements such as reversibility and compatibility, also from an aesthetic point of view. This means that no significant colour change should be observed upon application of a remedy. In this study, whitish zinc oxide nanoparticles were

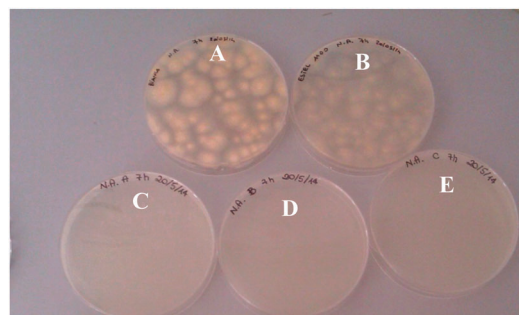


Fig. 4 *Aspergillus niger* colonies after 24 hours of contact with glass slides covered with nanocomposites. (A) control plate; (B) Estel 1100; (C) Estel 1100/ZnO-NPs 0.15% w/w; (D) Estel 1100/ZnO-NPs 0.30% w/w; and (E) Estel 1100/ZnO-NPs 0.60% w/w.

dispersed in colourless consolidants/water repellents, and therefore no significant chromatic changes are to be expected. However, the proper amount of ZnO-NPs that is able to accomplish its bioactive action without affecting the aesthetics should be determined. For this purpose, colorimetric measurements were performed. Moreover, SEM-EDS analyses were carried out to fully understand the behaviour of the nanocomposite-stone specimen system.

Colorimetric measurements were performed on selected stone samples with different porosity: Calcare di Altamura and two types of Calcarene di Gravina (from caves in Massafra and Gravina).

Evaluation of chromatic changes in stone specimens upon application of Estel 1000, Silo 111, and their respective nanocomposites containing 0.5% w/w ZnO-NPs was carried out. The ΔE values that were obtained immediately after application (at 2 hours) show a consistent decrease after 14 days (data not shown) and diminish substantially after 40 days. This trend was expected, as the solvent gradually evaporates and the consolidants/water repellents should have completed curing in that time. The graphs in Fig. 5 show colorimetric data for all treated stone samples at 40 days after application.

Examination of these data leads to the following conclusions. Significant differences can be observed between the three types of stone substrates. In particular, all samples that are composed of Calcare di Altamura (C) show the highest ΔE values, although these are all below 4.5. The more porous Calcarene di Gravina samples (M and G) exhibit smaller chromatic changes, which is probably due to better absorption/penetration of the applied materials. Comparison between the behaviour of pure Estel 1000 and Silo 111 reveals that the former generates more significant color changes than the latter. It should be mentioned, however, that the addition of ZnO-NPs seems to reduce the variations in color produced by application of Estel 1000 and Silo 111, at least on the C substrate. In the other cases, admixing of ZnO-NPs does not substantially change the colour of the coatings. An exception is G stone specimens treated with Estel 1000 and Estel 1000/ZnO-NPs, which is probably due to the non-homogeneous distribution of the composite on the specimen caused by the high surface roughness of this stone substrate.

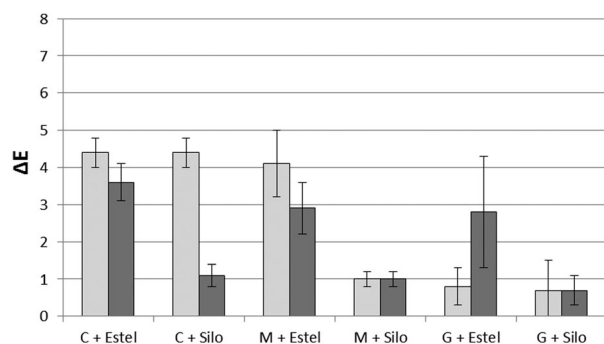


Fig. 5 Colour changes (ΔE) of specimens of Calcare di Altamura (C), Calcarene di Gravina from Massafra (M), and Calcarene di Gravina from Gravina (G) treated with Estel 1000 and Silo 111 alone (light grey bars) and admixed with ZnO-NPs (0.5% w/w) (dark grey bars) measured at 40 days after application.

These results represent a very interesting improvement with respect to the use of copper-based coatings, as the highest values of ΔE in this study are all lower than 5, despite the amount of embedded ZnO-NPs being much higher. Indeed, a fourfold reduction in values of ΔE is observed for all the stone substrates, even when using tenfold %w/w of ZnO-NPs with respect to CuNPs.²¹

SEM-EDS analyses were performed on fragments that were detached from the surfaces of stone specimens treated with Estel 1000, Silo 111, Estel 1000/ZnO-NPs (0.5% w/w), and Silo 111/ZnO-NPs (0.5% w/w). For each sample, three backscattered electron (BSE) images at different magnifications – 150 $\times$, 750 $\times$, and 2000 $\times$ – were acquired, as well as an ED raster spectrum for elemental analysis. Fragments composed of Calcare di Altamura (C) display a homogeneous, compact surface with low porosity. EDS data indicate a prevalence of calcium carbonate with low contents of silicon and magnesium. Treatment with Silo 111 and Estel 1000 seems to produce a more homogeneous surface as compared with untreated samples. Microchemical analyses revealed a higher relative amount of silicon for Estel 1000 as compared with Silo 111, which indicates better absorption of the latter product. When admixed with ZnO-NPs, Estel 1000 shows some cracking, which is clearly visible in the BSE image and which is more pronounced in relation to large grains. Similarly, the compact C stone specimen treated with Silo 111/ZnO-NPs is characterised by homogeneously diffused whitish areas with a very high concentration of 5 μ m clusters rich in zinc and silicon (Fig. 6A). In some interstices, Silo 111 has formed amorphous characteristic structures. The relative amount of zinc with respect to calcium and silicon is lower in the Silo 111 sample as compared to the Estel 1000 sample.

Regarding stone specimens of Calcarene di Gravina from Massafra (M), the acquired BSE images allow confirmation of observations made with optical microscopy. This stone is less compact than the previous limestone and is mainly composed of calcium carbonate with significant amounts of magnesium, aluminium and silicon. Whereas the latter two elements can probably be ascribed to the presence of clay minerals, the occurrence of magnesium is most likely to be attributed to dolomite. BSE images of the treated specimens gave evidence of a homogeneous distribution of Silo 111 and Estel 1000. As for the Calcare samples, ED raster spectra show a higher concentration of silicon on the surface of Estel 1000 samples with respect to those treated with Silo 111. In an SEM-BSE image of an M sample treated with Estel 1000/ZnO-NPs, luminous zinc particles can be clearly observed. As previously remarked for the C specimen, the consolidant tends to concentrate in specific areas that contain zinc oxide aggregates (2–20 μ m) and exhibit cracking (Fig. 6B). In Fig. 6C the ED raster spectrum is shown. Application of Silo 111/ZnO-NPs produces a fairly good distribution of the water repellent as well as of zinc oxide, which is present as single crystals and agglomerates. Again, the relative content of zinc with respect to the other main elements was found to be lower in the Silo 111 sample with respect to the Estel 1000 sample.

SEM analyses confirm the high porosity of Calcarene di Gravina from the Gravina cave (G), which reaches 45%. This should be taken into account when evaluating the penetration capacity of applied materials like the consolidants/water repellents used in this study.

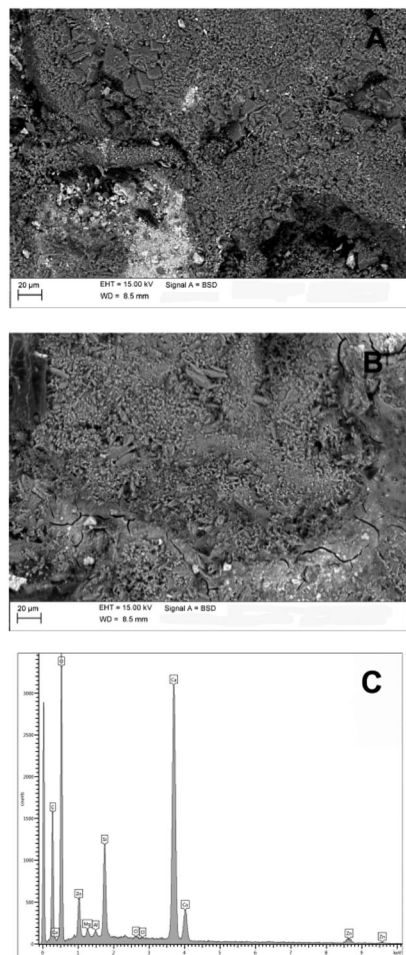


Fig. 6 SEM–EDS analyses of stone specimens. (A) BSE image of Calcare di Altamura treated with Silo 111/ZnO-NPs; (B) BSE image of Calcare di Gravina from Massafra treated with Estel 1000/ZnO-NPs and (C) its respective raster ED spectrum.

Microanalyses indicate the presence of calcium carbonate as well as of clay minerals. Again, SEM investigation provided evidence of a higher amount of silicon on the surface for Estel 1000 samples with respect to Silo 111 samples.

The BSE image of the sample treated with Estel 1000 shows that the consolidant is present in cavities as an amorphous pseudocrystalline material. The ED raster spectrum acquired for the specimen treated with Estel 1000/ZnO-NPs shows a rather high amount of silicon and little zinc, which is, however, well distributed in small aggregates with dimensions ranging from 1 to 20 μm. Concerning the application of Silo 111/ZnO-NPs, SEM-BSE images show an even distribution of 1–10 μm zinc grains on the surface of the calcarenite. In this case, the zinc peak in the ED spectrum is higher than that for silicon, contrary to what was previously observed for C and M samples.

Conclusions

In this study, the ability of zinc oxide nanoparticles to exhibit biocidal activity when embedded in commonly used

consolidant/water-repellent materials has been demonstrated. X-ray photoelectron spectroscopy showed that the presence of nanoparticles did not affect the surface chemistry of the matrices. The surface or sub-surface availability of zinc was also assessed. For materials based on Estel 1000 and Estel 1100, the amount of Zn was shown to increase in the surface region with curing, whereas zinc remained confined within the sub-surface region in composites based on Silo 111. Preliminary release data suggested, however, that zinc was released in acid contact solutions independently of its availability at the top surface. The diffusion of nanoparticles from the bulk to the contact solution is a plausible mechanism. Biological tests with *Aspergillus niger* gave evidence of remarkable biostatic activity of the tested nanocomposites, even at rather low ZnO loadings. Therefore, we can state that the prepared nanomaterials are able to embed zinc oxide nanoparticles and release zinc in order to exhibit interesting bioactive properties. The application of ZnO nanocomposites to calcareous stone specimens was shown to generate acceptable variations in colour. Scanning electron microscopy with X-ray microanalysis measurements, which was performed on single consolidants and water repellents, seems to confirm the surface spectroscopy data, in that the relative amount of zinc is lower on the surface of samples treated with Silo 111 as compared to those treated with Estel 1000. The mixed matrix, Estel 1100, is expected to display mixed behaviour. All the nanocomposites proved to be stable, as morphological and surface chemical analyses performed after more than one year show results similar to those obtained soon after application. Moreover, no detachment of the coatings was observed after such a period. In perspective, kinetic measurements will be designed and performed to evaluate the long-term zinc release ability of the nanocoatings. *In situ* experiments have been started with the application of Estel 1100/ZnO-NPs and Silo 111/ZnO-NPs: after six months, no colonization could be observed either on the treated or on the control areas. This was expected, as biological colonization may take a very long time. Moreover, biocidal activity tests will be extended to different micro-organisms that are usually present in the biological community that colonizes stone substrates.

Acknowledgements

The authors would like to thank Prof. Pasquale Acquafredda (Dipartimento di Scienze della Terra e Geoambientali, Università degli Studi di Bari “Aldo Moro”, Italy) for petrographic characterization and for his precious assistance in the SEM-EDS analyses, and Mr Simoncarlo Giacummo for his valuable support in the XPS analyses. This work was performed in the framework of the PRIN 2010–2011 “Sustainability in cultural heritage: from diagnosis to the development of innovative systems for consolidation, cleaning and protection” code 2010329WPF, funded by MIUR. The authors are grateful to the projects PO Puglia FESR 2007–2013 “RESTAUREO” (3Z3VZ46) and PON03 “MAIND” (PE0004) for partial financial support.

Notes and references

- 1 N. C. T. Martins, C. S. R. Freire, C. P. Neto, A. J. D. Silvestre, J. Causio, G. Baldi, P. Sadocco and T. Trindade, *Colloids Surf., A*, 2013, **417**, 111.
- 2 T. Gordon, B. Perlstein, O. Houbara, I. Felner, E. Banin and S. Margel, *Colloids Surf., A*, 2011, **374**, 1.
- 3 N. Jones, B. Ray, K. T. Ranjit and A. C. Manna, *FEMS Microbiol. Lett.*, 2008, **279**, 71.
- 4 L. Huang, D. Q. Li, Y. J. Lin, M. Wei, D. G. Evans and X. Duan, *J. Inorg. Biochem.*, 2005, **99**, 986.
- 5 F. R. Marciano, D. A. Lima-Oliveira, N. S. Da-Silva, A. V. Diniz, E. J. Corat and V. J. Trava Airoldi, *J. Colloid Interface Sci.*, 2009, **340**, 87.
- 6 L. L. Zhang, Y. H. Jiang, Y. L. Ding, N. Daskalakis, L. Jeuken, M. Povey, A. J. O'Neill and D. W. York, *J. Nanopart. Res.*, 2010, **12**, 1625.
- 7 R. Wahab, A. Mishra, S. I. Yun, Y. S. Kim and H. S. Shin, *Appl. Microbiol. Biotechnol.*, 2010, **87**, 1917.
- 8 A. Lipovsky, Y. Nitzan, A. Gedanken and R. Lubart, *Nanotechnology*, 2011, **22**, 105101.
- 9 S. Selvam and M. Sundarajan, *Carbohydr. Polym.*, 2012, **87**, 1419.
- 10 C. Karunakaran, V. Rajeswari and P. Gomathisankar, *J. Alloys Compd.*, 2010, **508**, 587.
- 11 N. Padmavathy and R. Vijayaraghavan, *Sci. Technol. Adv. Mater.*, 2008, **9**, 035004.
- 12 T. J. McCarthy, J. J. Zeelie and D. J. Krause, *J. Clin. Pharm. Ther.*, 1992, **17**, 51.
- 13 J. J. Zeelie and T. J. McCarthy, *Analyst*, 1998, **123**, 503.
- 14 H. Zhou, X. Wang, Y. Zhou, H. Yao and F. Ahmad, *Anal. Bioanal. Chem.*, 2014, **406**, 3689.
- 15 O. M. El-Feky, E. A. Hassan, S. M. Fadel and M. L. Hassan, *J. Cult. Herit.*, 2013, **15**, 165.
- 16 L. Lazzarini and M. Laurenzi Tabasso, *Il restauro della pietra*, CEDAM, Padova, 1994.
- 17 J. Delgado-Rodrigues, Consolidation of decayed stones: a delicate problem with few practical solutions, in *Proceedings of 3rd International Seminar on Historical Constructions*, ed. P. B. Lourenço and P. Roca, Universidade do Minho, Guimarães, 2001, pp. 3–14.
- 18 R. Zárraga, J. Cervantes, C. Salazar-Hernandez and G. Wheeler, *J. Cult. Herit.*, 2010, **11**, 138.
- 19 G. Wheeler, *Alkoxysilanes and the consolidation of stone*, The Getty Conservation Institute, Research in Conservation, Los Angeles, 2005.
- 20 C. Rodriguez-Navarro and E. Doehne, *Earth Surf. Processes Landforms*, 1999, **24**, 191.
- 21 N. Ditaranto, S. Loperfido, I. D. van der Werf, A. Mangone, N. Cioffi and L. Sabbatini, *Anal. Bioanal. Chem.*, 2011, **399**, 473.
- 22 D. Pinna, B. Salvadori and M. Galeotti, *Sci. Total Environ.*, 2012, **423**, 132.
- 23 D. Longano, N. Ditaranto, L. Sabbatini, L. Torsi and N. Cioffi, in *Nano-Antimicrobials. Progress and Prospects*, ed. N. Cioffi and M. Rai, Springer, 2012.
- 24 A. C. Manna, in *Nano-Antimicrobials. Progress and Prospects*, ed. N. Cioffi and M. Rai, Springer, 2012.
- 25 K. G. Chandrappa, T. V. Venkatesha, K. Vathsala and C. Shivakumara, *J. Nanopart. Res.*, 2010, **12**, 2667.
- 26 M. C. Sportelli, D. Hötger, R. A. Picca, K. Manoli, C. Kranz, B. Mizaikoff, L. Torsi and N. Cioffi, *MRS Online Proc. Libr.*, 2014, **1675**, mrss14-1675-k05-04, DOI: 10.1557/opl.2014.847.
- 27 E. Tesser, F. Antonelli, L. Sperti, R. Ganzerla and N. P. Maravelaki, *Polym. Degrad. Stab.*, 2014, **110**, 232.
- 28 L. Téllez, J. Rubio, F. Rubio, E. Morales and J. L. Oteo, *Spectrosc. Lett.*, 2004, **37**, 11.
- 29 F. Rubio, J. Rubio and J. L. Oteo, *Spectrosc. Lett.*, 1998, **31**, 199.
- 30 N. Cioffi, L. Torsi, N. Ditaranto, L. Sabbatini, P. G. Zambonin, G. Tantillo, L. Ghibelli, M. D'Alessio, T. Bleve-Zacheo and E. Traversa, *Appl. Phys. Lett.*, 2004, **85**, 2417.
- 31 N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, L. Sabbatini, T. Bleve-Zacheo, M. D'Alessio, P. G. Zambonin and E. Traversa, *Chem. Mater.*, 2005, **17**, 5255.
- 32 G. F. Andriani and N. Walsh, in *Natural Stone Resources for Historical Monuments*, ed. R. Přikryl and Á. Török, Geological Society, London, Special Publications, 2010, vol. 333, p. 129.
- 33 G. F. Andriani and N. Walsh, in *Building Stone Decay: From Diagnosis to Conservation*, ed. R. Přikryl and B. J. Smith, Geological Society, London, Special Publications, 2007, vol. 271, p. 179.
- 34 Raccomandazioni NorMal 43/93, 1993, Rome. CNR-ICR.