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Review

Advances in testing the interference of biocides on stone materials: A comparative analysis and guidelines for a standardised approach

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ABSTRACT

Many biocidal products are applied to stone monuments as a conservative treatment against biodeterioration. However, checking national and international committees for the Conservation of Cultural Heritage and scientific literature, it stands out that most standards define single tests, but in the case of biocides, a well-defined standard methodology to check their interactions with the substrate is not drawn up yet. The present work thus provides comparative evaluations and suggestions to monitor the potential interference of biocides on stone materials. For this purpose, we built a dataset based on fifty scientific papers dealing with interference tests on biocides, and then we compared several methods and the obtained results, considering different biocides, stones and modes of application. The comparative data point out that the most investigated features are: changes in the surface colour, using colourimetry methods; water absorption, using capillary rise; contact sponge; contact angle; and morphology, using Scanning Electron Microscope (SEM). Here, we also provide a guidance standard on these methodologies, considering both *in situ* and in-laboratory analysis.

Introduction

Biodeterioration phenomena on stone monuments are increasingly gaining attention, especially where the environmental conditions(humidity, temperature, lighting), nutrients and edaphic conditions (bioreceptivity) Favour the biological growth on the materials [1–4]. These phenomena can permanently damage the substrates, and to slow down the adverse effects of such processes, which proceed in time, many different biocidal methods and/or products have been used [5,6]. To avoid further damage and frequent interventions, preventive strategies could be useful, intervening in changing some environmental factors to slow down undesirable processes. The ecological assessment of different biodeterioration patterns (BP), i.e. communities showing a certain phenomenology of alteration, could support the intervention plans, reducing at least one limiting factor (Liebig's law of the minimum) under the threshold limits on the occurring species [7,8]. When preventive conservation cannot be easily performed, or if it is not sufficient, different treatments, using physical or chemical methods, come to the rescue. The different choices are also linked to the selection of methods, which can have a lower toxicological potential with respect tobiocides. As a consequence, UV treatment [9], laser cleaning [10,11], microwaves [12,13], and Heat Shock Treatments (HSTs) [14–16] have been employed. Amongst these approaches, nowadays, the research is focusing on novel eco-friendly and sustainable biocides. A green biocide is a substance that kills or retards the growth of microorganisms causing minimal or no harm to the environment. For total sustainability, an eco-friendly product can be prepared in a manner that avoids waste formation, consumes minimal energy and produces no by-products [17]. In the Cultural Heritage field, the main requirements of a biocide are that it must be effective against microorganisms, compatible with the environment and it must not cause any alterations or, more technically, "*interference*" to the substrate where it is applied [18,19].

Moreover, a correct conservative approach is also realised by evaluating parameters that define the efficacy of a biocide, such as its chemical nature, the mode of application and the combina-

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tion with other restoration products, the different microorganisms to be removed, and their potential protective barriers (e.g., the EPS matrix presence can reduce applied treatments' effectiveness), the bioreceptivity and nature of substrate, the climatic conditions of the site and the season of application [20,21]. After evaluating the need of applying a biocide, as well as the selection of the most active and the less dangerous compounds, however, the further parameter of a potential interference of the biocide with the substrate should be considered [18]. As stressed out for a long time, the potential adverse effects of biocides on stone surfaces need to be carefully evaluated before their application *in situ*, if the information already possessed results to be insufficient. The chemical composition of a biocide must be compatible with the substrate and not cause any unwanted colour changes or alter the materials' properties [5,21,22,23]. The main problems about the biocide-substrate interference are linked to the stone chromatic variation, the changes in water absorption, the permeability, elements mobilisation, and the variation of rugosity, with the consequently changing of the substrate bioreceptivity [5,21,22].

In this regard, none of the standards describes procedures to perform interference tests of biocidal products on materials. The International Organization for Standardization (ISO) only provides standards for the characterisation of the substrate. All types of artificial stones have their standards: cement, gypsum, lime, and mortar. However, regarding cultural heritage, the significant standard that could be adopted for the interference tests is ISO 787–25:2019 [24], which refers to the comparison of the colour, in full-shade systems, of white, black and coloured pigments, by colourimetric method. Furthermore, ISO 18,314–1:2015 [25], ISO 18,314–2:2015 [26], ISO 18,314–3:2015 [27], ISO 18,314–4 [28], and ISO/CD 18,314–5 [29] provide standards for analytical colourimetry.

The first work on producing recommendations aimed at standardisation in the analysis of stone materials in the field of cultural heritage started way back (in 1977), in Italy, when the National Research Council (CNR) and the Central Institute of Restauration (ICR- which is now the "Istituto Superiore per la Conservazione e Restauro" - ISCR) initiated the works of the NORMAL Commissions (the Italian acronym for NORmalizzazione MAteriali Lapidei) [30]. Amongst such documents, several recommendations suggested some control methods for biodeterioration: characterisation of biocides; sheets for archives; evaluation of efficiency (NORMAL 30/89 [31]; 35/91 [32]; 37/92 [33]; 38/93 [34]); physical characterisation of natural stone materials; contact angles method, water absorption due to capillarity and at low pressure, and colourimetry; (NORMAL 7/81 [35]; 11/85 [36]; 33/89 [37]; 43/93 [38]; 44/93 [39]). In 1996, such commissions became parts of the Italian standardisation body (UNI – Ente Italiano di Normazione), and the Commission became *UNI-NorMaL*, forming nowadays 7 UNI's Technical Commissions (see Appendix A). In such context, the UNI 10921:2001 [40] (efficacy of water repellent treatments), gives interesting information, which can be useful also for evaluating interference.

In 2004, the European Committee for Standardization (CEN), approved the formation of the Committee for the Conservation of Cultural Heritage "CEN/TC 346", that have produced 10 standardised test methods to characterise the material properties and the performance of new products (see Appendix B). Recently, the CEN approved Document EN 17488 (2021) [41], which gives general suggestions for selecting cleaning methods considering their effects on the cultural heritage. However, to date, no standard gives clear information about the test to be performed for avoiding interference between biocide and substratum. Until now, except for the previously cited documents [40,41], no comprehensive standards for evaluating interference have been produced. Indeed, different studies report several standardised measurement methods, but they are limited to a part of the analysis: colourimetric analysis (UNI EN 15886:2010 [42], UNI Normal 43/93:1994 [38]); water absorption measurements (Normal 44/93:1993 [39], UNI EN 15801:2010 [43], UNI 11432:2011 [44]); contact angle measurements (UNI EN 15802:2010 [45]), morphology studies (Normal 8/81 [46]) and molecular spectroscopic analysis. Moreover,this diversification of methods produces differences which do not allow a simple comparison of results.

Research aim

Based on the scientific literature on biocides applied to stone materials, and on the international standards in the field of cultural heritage, this review wants to suggest guidelines to facilitate a standardised methodology to define the most suitable interference tests to be carried out before a biocide application. Starting from a comparison of materials and methods for measuring the interference, we wish also to highlight the substances/compounds which resulted to be potentially dangerous.

Material and methods

The present work of review was realised according to previous works' approaches in elaborating and using databases [18,47,48]. The methodology was developed following the steps described as follows.

Literature search

To establish the extent of the state of art of experimentation regarding interference tests, we searched the literature to compose an overview of the methods of evaluation of the interference of biocidal substances and products when applied to materials of cultural heritage interest. The search was performed amongst peer-reviewed papers, book chapters and proceedings, using the international database Scopus (https://www.scopus.com/), and considering data from 1992 up to December 2022. It was performed amongst papers' titles, abstracts, and keywords, using the 'TITLE-ABS-KEY' tool. Various combinations of keywords were used, implementing 2- or 3-keyword queries. The keywords chosen were: 'biocide(s)/biocidal' (keyword No. 1), in combination with each of the keywords No. 2, namely 'brick(s)', 'mortar(s)', 'mural painting(s)', 'stone(s)'including: 'granite', 'marble', 'sandstone', and 'limestone'. We did not use the 'SUBJECT AREA' tool –to narrow down the field to cultural heritage– since the specific subject areas' cultural heritage' or 'conservation science' are not presented in Scopus. We considered all the derived papers, also including the resulting ones that were written in Italian, Portuguese, Spanish and French languages.

Amongst the document results obtained, we perused all the document' titles' and 'abstracts', and the whole record of the papers dealing with biocides consistent with the field of cultural heritage and construction science. In other words, we excluded from the study only the documents regarding the medical, agronomical sectors and fuel oil extractions.

Dataset building

Starting from the literature search, mentioned above, a dataset of the only papers regarding biocide interference tests was created. The considered descriptors – each one occupying a column of the dataset –were: 'tested material', 'tested method', 'tested biocide', 'biocide concentration', 'conditions of testing', 'observations', 'accepted limit' and 'reference'. The first five listed descriptors were taken from the experimental or "materials and methods" section of each paper, while the observations of descriptors and the accepted limit were extracted from the "results and discussion" section. Its descriptors are detailed as follows:

Tested material

This descriptor refers to the kind of materials upon which the tests are performed.

Tested method

This descriptor refers to the typology of analysis or measurement performed to test a given biocide's possible interference with a given material.

Tested biocide

This descriptor refers to the biocidal substance or product, both as an unknown experimented substance and a commercial and traditional product.

Biocide concentration and conditions of testing

The first descriptor refers to the biocide's concentration applied to the materials for the test, expressed in one of the following: percentage weight/weight (w/w); percentage volume/weight (v/w); percentage volume/volume (v/v); concentration in millilitres per litre; concentration in milligrams per millilitres, or grams per litres; molarity. The second one refers to the details regarding the application of the biocide. It includes the way of application, such as spraying, brushing, rubbing, immersion, pouring, soaking, deposition (the last one in the case of coating), inoculation (in the case of organisms used as biocides); incorporation in one or more polymer(s), consolidant(s) or coating(s), if applicable; spraying distance, if applicable; the number of repetitions of the application; the time lag between repetitions.

Observed interference

This descriptor refers to the test results or measurement and supplementary observations, all of them reported according to the author of the paper considered.

Accepted limit

In some cases, the author(s) tested multiple and increasing concentrations of the biocide to establish the concentration limit that can be accepted in the field of cultural heritage, which is an alteration non-perceivable or not affecting the material. This descriptor refers to the identified limitations.

Bibliographic information

This descriptor refers to the reference (author(s), year).

Results

Literature search

The search realised in the Scopus database, using the tools and keywords described in the "methods" section, gave back 202 document results. The documents appearing in more than one query are counted once. More in detail, the search produced the following products: 11 document results for the query issued by the combination of keywords' biocide(s)/biocidal AND brick(s)'; 27 document results for the query given by the combination of keywords' biocide(s)/biocidal AND mortar(s)'; 10 document results for the query provided by the combination of keywords' biocide(s)/biocidal AND mural AND painting(s)'; 166 document results for the query given by combining keywords' biocide(s)/biocidal AND stone(s)' included also 'granite', 'marble', 'sandstone', and 'limestone'. The papers dealing with biocides and not describing interference tests, considering any undesired effect of the biocide on the material, or not describing in detail the biocide methods were 40 out of 202. On the other hand, the papers dealing with biocides and describing interference tests represent 25% of the total "*literature search*" with 50 documents (see Fig. 1) plus 3 reviews [18,19,49].

Dataset building

The structure of the dataset describing only the biocide interference tests is shown in Tables 1–4, and the data obtained for each descriptor are detailed as follows:

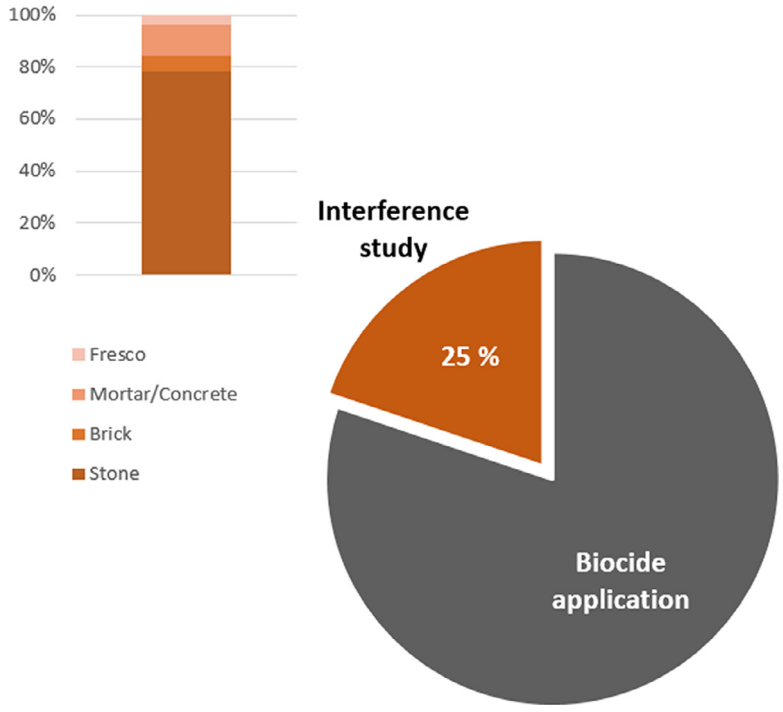

Fig. 1. Amount of papers dealing with biocides and describing interference tests. The histogram reports the percentage of tested materials for interference tests.

Table 1
Commercial and usual biocide: Dataset of all the interference tests performed present in the scientific literature. **T. Bio.** =Tested biocide; **B. Con.** = Biocide concentration; **Con. Tes.** = Conditions of testing; **T. Mat.**= Tested materials; **S. Mat.**= sub material; ***M***= Methods; **W. Abs.**= Water Absorption; Colour; **Morp.** = Morphologic; **A. lim.** = Accepted limit; **Ref.**= Reference. References [21,50–62,64] are cited in this table.

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	M	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
Commercial and usual biocide										
Glyphosate mono-isopropylammonium	10%	CA	Ma	*Carrara marble*	C,CWA, OM, SEM	I	ΔE= 1.96, greyng effect	A	Saturated for 24 h	[50]
Sodium dimethyldithiocarbanates and sodium 2-mercapto-benzo-thiazole	10%					I	ΔE= 3.56, yellowing effect	A		
Hydrogen peroxide	100%					SI	ΔE= 0.31	A		
Glyphosate mono-isopropylammonium	10%		S	*Pietra Serena*		D	ΔE= 1.03	N		
Sodium dimethyldithiocarbanates and sodium 2-mercapto-benzo-thiazole	10%					N	ΔE= 1.09	N		
Hydrogen peroxide	100%					I	ΔE= 1.52 rust-coloured spot	N		
Algophase *(2,3,5,6-tetrachloro-4-methylsulfonyl- pyridine)*	3%	S	Ma	*Carrara marble*	C, CWA, SEM/EDS, SS	D	ΔE 2.04, yellowing effect	A	Saturated for 48 h	[21]
Arsenal *(2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid)*						SI	ΔE= 0.82			
Roundup *(N-(phosphonomethyl) glycine)*						SI	ΔE= 0.61			
Metatin N58-10/101 *(Tributyltin naphtenate + quaternary ammonium salt)*						D	ΔE= 0.87			
Algophase *(2,3,5,6-tetrachloro-4-methylsulfonyl- pyridine)*			S	*Sandstone from Florenzuola*		I	ΔE= 1.17			
Arsenal *(2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid)*						N	ΔE= 0.73			
Roundup *(N-(phosphonomethyl) glycine)*						I	ΔE= 16.12			
Metatin N58-10/101 *(Tributyltin naphtenate + quaternary ammonium salt)*						D	ΔE= 0.41			
Algophase *(2,3,5,6-tetrachloro-4-methylsulfonyl- pyridine)*			L	*Travertine*		I	ΔE= 0.51			
Arsenal *(2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid)*						N	ΔE= 2.29			
Roundup *(N-(phosphonomethyl) glycine)*						I	ΔE= 8.89			
Metatin N58-10/101 *(Tributyltin naphtenate + quaternary ammonium salt)*						D	ΔE= 2.77			
Koretrelr *(Alkyleneoxide (97.6%), alkylaminotriazine (0.98%), N0 -(3,4-dichlorophenyl)-N,Ndimethyl urea (0.98%), denaturated alkyltrihydroxybenzene polyoxide (0.48%))*	Pure	I	S	*Muggia stone*	C, CWA, SEM	D	ΔE = 38,17 / after washing ΔE = 2,10	A	N.D.	[51]
			L	*Alveoline and Nummulite limestone*			ΔE = 29,08/ after washing ΔE = 5,37	N		
			G	/			ΔE = 30,04/ after washing ΔE = 0,94	N		
DTSACl *(Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride)*	/	P - Autoclaved aerated concrete (2 L m^2)	Co	*Autoclaved aerated concrete*	C, AAT	/	ΔE= 0.6, darkening effect	/	/	[52]
DAVICIL *(2,3,5,6-Tetrachloro-4-methylsulfonyl-pyridine)*							ΔE= 0.4, darkening effect			
DAVICIL followed by Alkyl alkoxy silane							ΔE= 0.1, darkening effect			
Alkyl alkoxy silane followed by**DAVICIL**							ΔE= 1.20, darkening effect			
Mixing of alkyl alkoxy silane and**DAVICIL**							ΔE= 7.9, darkening effect			
DTSACl *(Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride)*		B - White concrete (0.25 L m^2)		*White architectural concrete*			ΔE= 4.98, darkening effect			
DAVICIL followed by Alkyl alkoxy silane							ΔE= 3.3, darkening effect			
Rocima 110 ® *(Sn tributylnaphtenate, didecyldimethylammonium chloride)*	2%	B	F	*Matera calcarenite*	C, CWA	*	ΔE =3.82	/	N.D.	[53]
Preventol R80® *(Alkyldimethylbenzylamine chloride and isopropanol)*	2%					*	ΔE = 5.07			
Umonium 38 ® *(Tridecyl-dimethyl ammonium chloride and isopropyl alcohol)*	1%; 2%					D	ΔE = 4.15			
Mixing of Hydrophase and**Biotin R** *(3-iodopropynylbutylcarbamate and 2-octyl-3(2H) Isothiazolone)*	2%	B - (5 g/m^2)	Ma	/	AAT, C, CWA,	D	ΔE =2.84, after ageing ΔE =2.45	/	/	[54]
Mixing of Hydrophase and**Algophase** *(2,3,5,6-tetrachloro-4-methylsulfonyl-pyridine)*						D	ΔE =2.72, after ageing ΔE =1.60			
Mixing of Akeogard P and**Preventol R80®** *(Alkyldimethylbenzylamine chloride and isopropanol)*						D	ΔE =2.61, after ageing ΔE =1.43			
Mixing of Paraloid B72 and**Algophase** *(2,3,5,6-tetrachloro-4-methylsulfonyl-pyridine)*						D	ΔE =1.83, after ageing ΔE =3.60			
Mixing of Akeogard CO and**Algophase** *(2,3,5,6-tetrachloro-4-methylsulfonyl-pyridine)*						SD	ΔE =3.19, after ageing ΔE =3.99			
Mixing of Akeogard CO and**Preventol R80®** *(Alkyldimethylbenzylamine chloride and isopropanol)*						SD	ΔE =6.65, after ageing ΔE =6.83			

(*continued on next page*)

Table 1 (*continued*)

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	M	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
Bioestel *(Tetraethylorthosilicate mixed to Tributyltin oxide+dibutyltin dilaurate)*	<2% p/p	105 g/m^2	P	/	C, CSM	SD	N	/	/	[55]
		63 g/m^2	Ma			N	In the early stages surface whitening			
		74 g/m^2	S			D	In the early stages darkening			
	<2% p/p	105 g/m^2	P	/	CSM	N	/	/	/	[58]
		63 g/m^2	Ma			D				
		74 g/m^2	S			SD				
Benzalkonium chloride	1%	P - (25 ul/cm^2)	Ma	*Carrara marble*	AAT, C	/	ΔE =0.93	/	/	[56]
	5% w/v	Po	Ma	/	C	/	After 14 days ΔE<4, after 60 days ΔE>6	/	/	[60]
	0.1%, 0.3%, and 0.5% diluted with 75% of ethanol	Sp	F	/	C	/	ΔE=1.88	/	/	[62]
Natria *(based on pelargonic acid)*	200mL per liter	Sp	B	/	C,CWA, OM, SEM, SS	SD	ΔE < 4	N	3 applications every 48 hours	[57]
Rocima 103 ® (2-octyl-2H-isothiazolin-3-one and didecyldimethylammonium chloride in propane-2-ole and formic acid)	5% v/v	Po - for 48 h.	Ma, S	/	C, CSM	N	ΔE >10; After 5 years ΔE >5	/	/	[59]
Biotin T plus Silo 112	Silo 112 98%, Biotin T 2%	B- In two layers (4 g/dm2)	T	Neapolitan yellow tuff	C, CA, CWA	D	After 48h ΔE=5.15 After 7 days ΔE=4.80	/	/	[61]
Clay loaded with**Biotin T** plus Silo 112	Silo 112 95%, Biotin T 2%and a clay content of 3 %,						After 48h ΔE=3.43 After 7 days ΔE= 2.58			
1,2-benzisothiazol3(2H)-one **Dichlorophene** **Ooctylisothiazolinone**	0.1%, 0.3%, and 0.5% diluted with 75% of ethanol	Sp	F	/	C	/	ΔE=1.88	/	/	[62]
Nanocapsules and silica mesoporous nanoparticles loaded with**2-mercaptobenzothiaole** plus TiO_2 nanoparticles	0.1% w/w	B - until saturation	Ma,B,L, P	*Carrara marble*	C, CA, CWA, Pr	D	ΔE < 5	N	/	[63]
Propiconazole	1% v/v	Sp	S	/	C, CA, SEM	N	ΔE < 1	N	/	[64]
Copper (Succinate, Glutarate, Adipate)							ΔE > 15			
AW-600 Octhilinone							ΔE < 1			
Naphthylacetic acid							ΔE < 1			
Difenoconazole							ΔE < 1			

Legend. Conditions of testing: (**AG**) Air gun with pressure; (**B**) Brushed; (**CA**) Capillarity absorption; (**D**) Deposition; (**I**) Immersion; (**In**) Inoculated; (**P**) Poured; (**Po**) Poultice; (**S**) Soaked; (**Sp**) Sprayed - ***Tested material***= (**B**) Brick; (**Co**) Contrete; (**D**) Dolostone; (**F**) Fresco; (**G**) Granite; (**L**) Limestone; (**Ma**) Marble; (**P**) Plaster; (**S**) Sandstone; (**T**) Tuff - **M**: (AAT) Accelerated ageing tests; (**C**) Colorimetry; (**CA**) Contact angle meausure; (**CSM**) Contact sponge method; (**CWA**) Capillary water absorption; (**FTIR**) Fourier-transform infrared spectroscopy; (**L**) Lidar fluorosensor scanning); (**OM**) Optical Microscope; (**P**) Porosity; (**Pr**) Profilometer; (**SEM/EDS**) Scanning Electron Microscopy/Microprobe analyses; (**SS**) Soluble salts; (**W**) Change in weight; (**XRD**) X-ray diffraction - ***Observed interference*** = (**A**) Alteration; (**D**) Decrease; (**I**) Increment; (**N**) No Alteration; (**ND**) Nessun dato; (**SC**) smallest changes; (**SD**) Slightly decrease; (**SI**) Slightly increase; (*) The hash mark indicates that the material was not considerable.

Tested materials

From the collection of papers for the present dataset describing interference tests, many different stone materials were considered. In the same papers, we can find different materials investigated. The most frequently tested materials were: limestone (**Lim.**)- 22 papers; marble (**Mar.**) - 18 papers; sandstone (**San.**) – 9 papers; calcarenite (**Cal**) – 6; travertine (**Tra.**) - 5 papers; firebrick (**Fir.**) - 5 papers; plaster (**Pla.**) –4 papers; and granite (**Gra.**) - 2 papers; tuff (**Tuf.**)– 2 paper; concrete **(Con.)**– 1 paper; fresco (**Fre.**) – 3 papers (See Fig. 2).

Tested methods

Colourimetry (**C**) methods (44 cases) were the most recurrent (see Tables 1–4) (see Fig. 2), and they were used in almost all the substrata, primarily on limestone and marble. With a lower extent, different indirect methods were used to measure the stone's porosity and hydrophobic proprieties changes, such as water absorption by capillarity (**CWA**) (15 cases), contact angle measurement (**CA**) (9 cases), ^{1}H NMR relaxometry (**NMR**) (2 cases), porosity by porosimeter (**P**) (4 cases), water absorption by contact sponge(**CS**) (4 cases), water vapour diffusion (**WVP**) (1 case), Scanning Electron Microscopy (**SEM**) (15 cases) also resulted frequently applied, mainly for the limestone, along with image observation through optical microscopy, stereomicroscope and digital microscope (**OM**) (3 cases), rugosity measure by profilometer system (**Pr**) (3 cases) and detection of discolouration of the surface by a lidar system (**L**) (1 case). Mechanical methods and physical methods able to detect chemical compound resulted rarely employed, as the weight change (**W**) (1 case); drilling test/peeling test/ Vickers hardness test (**DR**) (1 case), X-ray diffraction (**XRD**) (2 cases), FT-IR analysis (**FTIR**) (1 case) and determination of soluble salts (**SS**) (2 cases). About 30% of the studies applied accelerated ageing tests (e.g. exposure to heat, moisture, UV light, etc.) (**AAT**) after the biocidal treatments to monitor over time the stability of the product and the absence of alteration on the substrate. For the most recurring interference tests, we enhance the presence of standardised methods: Colour measurement UNI EN 15886:2010 [42], Water absorption by capillarity UNI EN 15801:2010 [43]; Contact sponge UNI 11432:2011 [44]; Contact angle UNI EN 15802:2010 [45] (Table 1S - Supplementary material):

Colourimetry. This technique investigates the chromatic alterations on a surface by measuring colourimetric coordinates according to the CIELAB system.

L* indicates lightness (0= absolute black, 100 = absolute white); a* and b* are the chromaticity coordinates. a* is the position between green (a*<0) and red/magenta (a*>0); b* is the position between blue (b*<0) and yellow (b*>0). The global colour variation is underlined by ΔE* [42,99].

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

Table 2
Metal-based nanocoating and metal oxides biocide: Dataset of all the interference tests performed present in the scientific literature. **T. Bio.** =Tested biocide; **B. Con.** = Biocide concentration; **Con. Tes.** = Conditions of testing; **T. Mat.**= Tested materials; **S. Mat.**= sub material; ***M***= Methods; **W. Abs.**= Water Absorption; Colour; **Morp.** = Morphologic; **A. lim.** = Accepted limit; **Ref.**= Reference. References [64–79] are cited in this table.

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	P	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
Metal-based nanocoating and metal oxides										
Ailsesquioxane with methacrylate units and TiO_2 and/or Ag NPs	/	B	L	*Bioclastic oolitic "Repedea"*	FTIR, XRD	/	/	/	/	[65]
TiO_2 nanocoating	1% (w/v)	Sp - (a nozzle of 0.8 mm diameter from a distance of 250 mm)	B	*High porous rough surface* *High porous smoothed surface* *Low porous rough surface* *Low porous smoothed surface*	C, P	/	ΔE= 0.68 ΔE= 2.07 ΔE= 4.02 ΔE= 2.69	N	/	[66]
Nanocoating-TiO_2	/	100%; 10%; 1%	Ma	/	C	/	(100%) ΔE =3.6; (10%)ΔE = 3.4; (1%) ΔE = 3.8	/	/	[67]
Nanocoating Ag-TiO_2	Ag 5%						(100%) ΔE =25.1; (10%)ΔE = 4.9; (1%) ΔE = 3.0			
Nanocoating Ag-Fe-TiO_2	Ag 2.5%; Fe 2.5%						(100%) ΔE =23.8; (10%)ΔE = 7.1; (1%) ΔE = 5.9			
Nanocoating Ag-Sr-TiO_2	Ag 2.5%; Sr 2.5%						(100%) ΔE =38.3; (10%)ΔE = 10.7; (1%) ΔE = 5.1			
Nanocoating Fe-TiO_2	Fe 5%						(100%) ΔE =36.5; (10%)ΔE = 8; (1%) ΔE = 8			
Nanocoating Sr-TiO_2	Sr 5%						(100%) ΔE =5.8; (10%)ΔE = 4.7; (1%) ΔE = 3.1			
Nanocoating-TiO_2	1 wt%	Sp (0.60 g/m^2)	L	/	AAT, C	N.D.	ΔE = 2.36	N.D.	/	[68]
TiO_2 nanocoating	1% (wg/vol)	Sp - (a nozzle of 0.8 mm diameter from a distance of 250 mm)	B	*High porous rough surface* *High porous smoothed surface* *Low porous rough surface* *Low porous smoothed surface*	C, P	/	ΔE= 0.68, whitening effect ΔE= 2.07, whitening effect ΔE= 4.02, whitening effect ΔE= 2.69, whitening effect	/	/	[69]
TiO2 NPs	0.03 mg/mL to 0.66 mg/mL	/	L	*Limestone of "Utrera"*	C	/	ΔE > 20	/	/	[70]
Nanocomposites Ag-TiO_2 and activated carbon							ΔE > 20			
Nanocomposites Ag-TiO_2							ΔE > 20			
Nanocomposites 1/4 Ag-TiO_2							ΔE > 20			
Nano-Ag and trisodium citrate							20> ΔE > 10			
Nanocomposites Ag-TiO_2 and trisodium citrate							ΔE < 10			
TiO_2 NPs (sol-gel)	TiO_2 (1 wt%)	Sp - (34.90 g/m^2)	L	*Travertine*	AAT, C, CSM, SEM	D - after ageing I	ΔE =0.36, after ageing ΔE =3.52	N	/	[71]
Sol-gel TiO_2 and Ag NPs		Sp - (40.14 g/m^2)				D - after ageing SI	ΔE =0.80, after ageing ΔE =2.3			
Sol-gel TiO_2 and Cu particles		Sp - (37.56 g/m^2)					ΔE =1.87, after ageing ΔE =1.19			
TiO_2 NPs (sol-gel)	TiO_2 (1 wt%)	Sp - (1.8 ml per layer, a nozzle of 1.5 mm in diameter from a distance of 30 cm, three overlapping layers per sample)	L	*Travertine*	AAT, C	/	ΔE = 0.3	/	/	[72]
Sol-gel TiO_2 and Ag NPs	TiO_2 (1 wt%), Ag (1 mol%)						ΔE = 0.4, after ageing darkening effect of the coating			
	TiO_2 (1 wt%), Ag (5 mol%)						ΔE = 1.0, after ageing darkening effect of the coating			
Sol-gel TiO_2 and Cu NPs	TiO_2 (1 wt%), Cu (1 wt%)						ΔE = 0.7			
	TiO_2 (1 wt%), Cu (5 wt%)						ΔE = 0.8			
Nanocoating CuO-SiO_2	CuO (0.00 %w/v)	B	L	/	C, CA, CWA, DT, SEM	D	ΔE = 5.86	A	/	[73]
	CuO (0.05 %w/v)						ΔE = 3.88			
	CuO (0.15 %w/v)						ΔE = 2.49			
	CuO (0.35 %w/v)						ΔE = 9.21			
TiO_2 NPs	/	200 µL	L	*Limestone of (Utrera)*	AAT, C,WVP,	/	ΔE= 0.84	/	/	[74]
Nano-Ag and trisodium citrate	/						ΔE= 6.69			
Nanocomposites Ag-TiO_2 and trisodium citrate	0.04 (Ag:TiO_2)						ΔE= 10.12			
	0.08 (Ag:TiO_2)						ΔE= 8.63			
Nanocomposites Ag-TiO_2	0.04 (Ag:TiO_2)						ΔE= 24.24			
	0.08 (Ag:TiO_2)						ΔE= 13.89			
	0.16 (Ag:TiO_2)						ΔE= 5.14			

(*continued on next page*)

Table 2 (*continued*)

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	P	Observed interference			A. lim.	Ref.
						W. Abs.	Colour	Morp.		
TiO_2 NPs	1 % wg/vol	Sp - (0.12 g/m^2, a nozzle of 0.8 mm diameter, from a distance of 25 cm)	L	*Caccamo lake*	C	/	ΔE = 1.59	/	/	[75]
				Furlo gorge			ΔE = 3.16			
				Cingoli			ΔE = 0.79			
			S	*Camerino*			ΔE = 6.92			
				Tennacola torrent			ΔE = 5.33			
			T	*Tuff of Lazio*			ΔE = 5.42			
CuO/SiO_2 (Sol-gel)	Cu (0.00% w/v)	AG	L	/	SEM	D	/	A	Five replicates until apparent saturation	[76]
	Cu (0.05% w/v)									
	Cu (0.15% w/v)									
	Cu (0.35% w/v)									
Nanocomposites Ag-TiO_2	0.1 (mg/mL)	D - (Two applications of 200 µL of an aqueous suspension of nanocomposites)	L	*Calcarenite (Cadiz)*	C	/	ΔE= 0.94	/	/	[77]
Nano-Ag and trisodium citrate	0.015 (mg/mL)						ΔE= 0.70			
Nanocomposites Ag-TiO_2 and trisodium citrate	0.145 (mg/mL)						ΔE= 1.20			
Nanocomposites Ag-TiO_2	0.1 (mg/mL)			*Biosparite (Seville)*			ΔE= 4.53			
Nano-Ag and trisodium citrate	0.015 (mg/mL)						ΔE= 4.17			
Nanocomposites Ag-TiO_2 and trisodium citrate	0.145 (mg/mL)						ΔE= 5.73			
Nanocomposites Ag-TiO_2	0.1 (mg/mL)		L	*Biosparite (Alicante)*			ΔE= 5.69			
Nano-Ag and trisodium citrate	0.015 (mg/mL)						ΔE= 13.00			
Nanocomposites Ag-TiO_2 and trisodium citrate	0.145 (mg/mL)						ΔE= 8.63			
Nano-TiO_2	0.3 (mg/mL)	B - (Four applications of 100 µL of treatment)	L	*Oolitic limestone (Sevilla)*	AAT, C, SEM	/	ΔE= 0.3	/	/	[78]
Nano-Cu	0.3 (mg/mL)						ΔE= 4.7			
Nano-ZnO	0.3 (mg/mL)						ΔE= 0.6			
Nanocomposites-Ag and TEOS	0.03 (mg/mL)						ΔE= 6.8			
Nanocomposites-Ag/TiO_2 and TEOS	0.33 (mg/mL)						ΔE= 8.2			
Nanocomposites-Ag/TiO_2 and trisodium citrate	0.33 (mg/mL)						ΔE= 9.4			
Nano-Ag/TEOS	0.03 (mg/mL)	B	L	*Oolitic limestone (Sevilla)*	C, OM, SEM,	/	ΔE= 6.8	N	/	[79]
CuOH	1% v/v	Sp	S	/	C, CA, SEM	N	ΔE > 15	N	/	[64]

Legend. Conditions of testing: (**AG**) Air gun with pressure; (**B**) Brushed; (**CA**) Capillarity absorption; (**D**) Deposition; (**I**) Immersion; (**In**) Inoculated; (**P**) Poured; (**Po**) Poultice; (**S**) Soaked; (**Sp**) Sprayed - ***Tested material***= (**B**) Brick; (**Co**) Contrete; (**D**) Dolostone; (**F**) Fresco; (**G**) Granite; (**L**) Limestone; (**Ma**) Marble; (**P**) Plaster; (**S**) Sandstone; (**T**) Tuff - **M**: (AAT) Accelerated ageing tests; (**C**) Colorimetry; (**CA**) Contact angle meausure; (**CSM**) Contact sponge method; (**CWA**) Capillary water absorption; (**FTIR**) Fourier-transform infrared spectroscopy; (**L**) Lidar fluorosensor scanning); (**OM**) Optical Microscope; (**P**) Porosity; (**Pr**) Profilometer; (**SEM/EDS**) Scanning Electron Microscopy/Microprobe analyses; (**SS**) Soluble salts; (**W**) Change in weight; (**XRD**) X-ray diffraction - ***Observed interference*** = (**A**) Alteration; (**D**) Decrease; (**I**) Increment; (**N**) No Alteration; (**ND**) Nessun dato; (**SC**) smallest changes; (**SD**) Slightly decrease; (**SI**) Slightly increase; (*) The hash mark indicates that the material was not considerable.

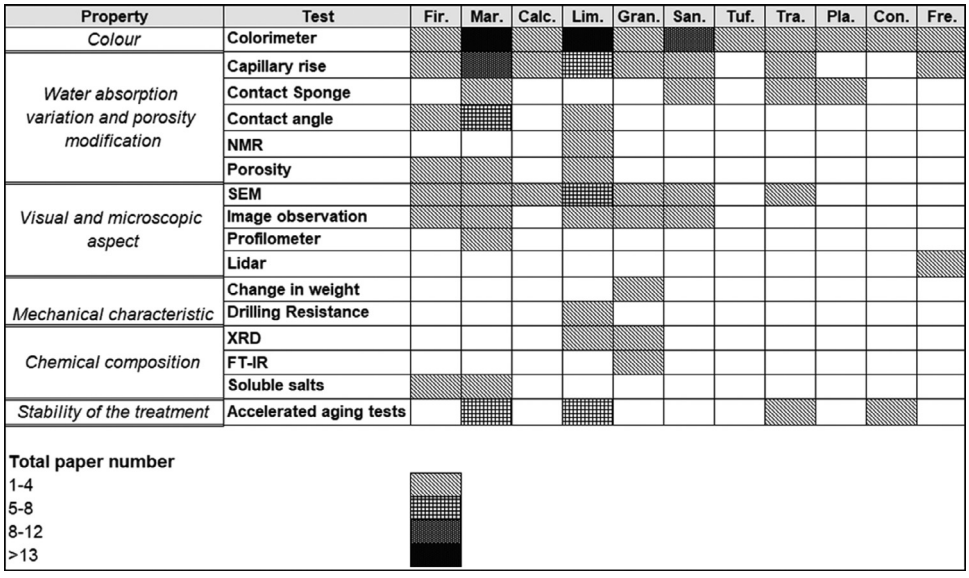

Fig. 2. The frequency, expressed as a number of the scientific paper, of specific interference tests on different substrata: **Lim**. (Limestone); **Mar.** (Marble); **Pla**. (Plaster); **Tra.** (Travertine); **San.** (Sandstone); **Gra.** (Granite); **Cal.** (Calcarenite); **Fir.** (Firebrick); **Tuf.** (tuff); **Con**. (Concrete); **Fre.** (Fresco).

Table 3
Biocides based on metal NPs and oxides with water repellents and consolidants: Dataset of all the interference tests performed present in the scientific literature. **T. Bio.** =Tested biocide; **B. Con.** = Biocide concentration; **Con. Tes.** = Conditions of testing; **T. Mat.**= Tested materials; **S. Mat.**= sub material; ***M***= Methods; **W. Abs.**= Water Absorption; **Colour; Morp.** = Morphologic; **A. lim.** = Accepted limit; **Ref.**= Reference. References [52,55,58,80–87] are cited in this table.

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	P	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
Blend of metal NPs and oxides with water repellents and consolidants										
Zeolites containing Cu and Ag ions	Cu (6.5% (w/w) , Ag (3.5% (w/w))	P- Autoclaved aerated concrete (2 L m^2)		*Autoclaved aerated concrete*	AAT, C	/	ΔE= 3.7, darkening effect	/	/	[52]
Ag NPs	/						ΔE= 8.7, darkening effect			
First application of Zeolites containing Cu and Ag ions followed by silane siloxane mixture	/						ΔE= 6.1, darkening effect			
Ag NPs	/	B - White concrete (0.25 L m^2)		*White architectural concrete*	AAT, C		ΔE= 15.9, darkening effect			
Mixing of acrylic polymers and ZnO	Zn (0.10%)	B - (5 g/m^2)	Ma	/	AAT, C, CA, CWA	D	ΔE =1.43, after ageing ΔE =0.79	/	/	[80]
Mixing of fluorinated polymers and ZnO	Zn (0.50%)						ΔE =0.58, after ageing ΔE =0.43			
Mixing of acrylic polymers and $ZnTiO_3$	$ZnTiO_3$ (0.05%)						ΔE =1.29, after ageing ΔE =0.32			
Mixing of fluorinated polymers and $ZnTiO_3$	$ZnTiO_3$ (0.05%)						ΔE =0.78, after ageing ΔE =0.55			
Mixing of Estel 1100 and nano-Cu	0.02% w/w	B	L	*Calcare di Altamura/C alcarenite di Gravina*	C, SEM	/	after 2 months ΔE<5	A	Until saturation	[81]
	0.05% w/w						after 2 months 5<ΔE<10			
	0,14% w/w						after 2 months ΔE>10			
Mixing of acrylic polymer and TiO_2 NPs	0.3 wt%	B - (2 g/m^2)	Ma	*Lower porosity*	AAT, C, CA, CWA	SD	ΔE =1.1, after ageing ΔE =1.3	/	/	[82]
		B - (4 g/m^2)		*Highter porosity*		SD	ΔE =1.2, after ageing ΔE =1.3			
		B - (20 g/m^2)	L	*Lower porosity*		D	ΔE =1.5, after ageing ΔE =1.4			
		B - (40 g/m^2)		*Highter porosity*		D	ΔE =1.8, after ageing ΔE =2.1			
Mixing of Acrilico 30 and Cu NPs	0.22 mg/ml	139 g/m^2	P	/	C, CSM	SD	Brightness of the substrate	/	/	[55]
Mixing of Estel 1000 and Cu NPs		102 g/m^2				D	N			
Silo 111+ Cu NPs		45 g/m^2	Ma	/		D	N			
Mixing of Estel 1000 and Cu NPs		51 g/m^2				N	In the early stages surface whitening			
Silo 111+ Cu NPs		95 g/m^2	S	/		D	Translucent film over the surface			
Mixing of Estel 1000 and Cu NPs		124 g/m^2				D	Darkening of the surface			
Mixing of TEOS and Chitosan, $AgNO_3$ and Hydro Silica	Higher concentrations of $AgNO_3$	/	L	Bioclastic (Dom stone)	C	/	ΔE = 3.6	/	/	[83]
Mixing of TEOS and Chitosan, $AgNO_3$ and Hydro Silica	Mdium concentrations of $AgNO_3$						ΔE = 1.7			
Mixing of TEOS and Chitosan, $AgNO_3$ and Hydro Silica	Lower concentrations of $AgNO_3$						ΔE = 1.9			
Mixing of TEOS and Chitosan and $AgNO_3$	Lower concentrations of $AgNO_3$						ΔE = 1.8			
Mixing of TEOS and $AgNO_3$ and Hydro Silica	Higher concentrations of $AgNO_3$						ΔE = 8.7			
Mixing of TEOS and $AgNO_3$ and Hydro Silica	Lower concentrations of $AgNO_3$						ΔE = 1.7			
Mixing of TEOS and $AgNO_3$	Higher concentrations of $AgNO_3$						ΔE = 1.5			
Mixing of TEOS and $AgNO_3$	Lower concentrations of $AgNO_3$						ΔE = 1.1			
Mixing of TEOS and Hydro Silica	/						ΔE = 2.2			
Mixing of Estel 1000 and ZnO NPs	0.5% w/w	B	L	Calcare di Altamura /Calcarenite di Gravina	C, SEM	N.D.	(2h) ΔE = 10.4; (14 days) ΔE = 6.8; (38 days) ΔE = 3.6	A	Until saturation	[84]
Mixing of Silo111 and ZnO NPs							(2h) ΔE = 6.3; (14 days) ΔE = 1.2; (38 days) ΔE = 1.1			
Mixing of Estel 1000 and ZnO NPs							(2h) ΔE = 10.1/10.2; (14 days) ΔE = 5.9/5.8; (38 days) ΔE = 2.9/2.8	N		
Mixing of Silo111 and ZnO NPs							(2h) ΔE = 3.4/4; (14 days) ΔE = 1.5/2.5; (38 days) ΔE = 1.0/0.7			

(*continued on next page*)

Table 3 (*continued*)

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	P	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
Siloxane wax with TiO_2	TiO_2 (0.1 %wt)	R - (25 g/m^2)	Ma	/	C, CA	D	ΔE<4	/	/	[85]
Siloxane wax with TiO_2	TiO_2 (1 %wt)					D	ΔE<4			
Siloxane wax with TiO_2 and Ag	TiO_2 (0.1 %wt) and Ag (0.001 %wt)					D	ΔE<5			
Siloxane wax with TiO_2 and Ag	TiO_2 (1 %wt) and Ag (0.01 %wt)					D	ΔE<5			
Siloxane wax with Zn	Zn(0.1 %wt)					SD	ΔE<4			
Siloxane wax with Zn	Zn (1 %wt)					SD	ΔE<5			
Siloxane wax with Ag and Zn	Ag (0.001 %wt) and Zn (0.1 %wt)					SD	ΔE<5			
Siloxane wax with Ag and Zn	Ag (0.001 %wt) and Zn (1 %wt)					SD	ΔE>5			
Tegosivin® HE 328 + Chitosan (Sol-gel)	T (97 g/L), C (1.5 g/L)	Sp - (0.2 L/m^2)	L	*Bioclastic (Dom stone)*	AAT, C, P		ΔE<2, after ageing ΔE <4	/	/	[86]
	T (97 g/L), C (10.1 g/L)	Sp - (0.2 L/m^2)					ΔE=4, after ageing ΔE <4			
	T (97 g/L), C (13.6 g/L)	Sp - (0.2 L/m^2)					ΔE<4, after ageing ΔE <4			
Tegosivin® HE 328 + Chitosan + Silver Nitrate (Sol-gel)	T (97 g/L), C (1.5 g/L), Ag (0.9 g/L)	Sp - (0.2 L/m^2)					ΔE<4 (darken and redden stone), after ageing ΔE <4			
	T (97 g/L), C (1.5 g/L), Ag (0.9 g/L)	Sp - (0.4 L/m^2)					ΔE=6.4 (darken and redden stone), after ageing ΔE = 4.26			
Mixing of Acrilico 30 and Cu NPs	0.22 mg/ml	139 g/m^2	P	/	CSM	N	/	/	/	[58]
Mixing of Estel 1000 and Cu NPs		102 g/m^2				SD				
Silo 111+ Cu NPs		45 g/m^2	Ma			D				
Mixing of Estel 1000 and Cu NPs		51 g/m^2								
Silo 111+ Cu NPs		95 g/m^2	S			SD				
Mixing of Estel 1000 and Cu NPs		124 g/m^2								
Paraloid B-44 + ZnO NPs	Polymer (2% w/v), ZnO (0.04 g)	B - (The operation was repeated three times within 2 h between each application)	Ma	*Carrara marble*	AAT, C, CA, CWA, SEM	D	ΔE= 0.45, after UV ageing ΔE= 2.41, after thermal ageing ΔE= 2.59	A	/	[87]

Legend. Conditions of testing: (**AG**) Air gun with pressure; (**B**) Brushed; (**CA**) Capillarity absorption; (**D**) Deposition; (**I**) Immersion; (**In**) Inoculated; (**P**) Poured; (**Po**) Poultice; (**S**) Soaked; (**Sp**) Sprayed - ***Tested material***= (**B**) Brick; (**Co**) Contrete; (**D**) Dolostone; (**F**) Fresco; (**G**) Granite; (**L**) Limestone; (**Ma**) Marble; (**P**) Plaster; (**S**) Sandstone; (**T**) Tuff - **M**: (AAT) Accelerated ageing tests; (**C**) Colorimetry; (**CA**) Contact angle meausure; (**CSM**) Contact sponge method; (**CWA**) Capillary water absorption; (**FTIR**) Fourier-transform infrared spectroscopy; (**L**) Lidar fluorosensor scanning); (**OM**) Optical Microscope; (**P**) Porosity; (**Pr**) Profilometer; (**SEM/EDS**) Scanning Electron Microscopy/Microprobe analyses; (**SS**) Soluble salts; (**W**) Change in weight; (**XRD**) X-ray diffraction - ***Observed interference*** = (**A**) Alteration; (**D**) Decrease; (**I**) Increment; (**N**) No Alteration; (**ND**) Nessun dato; (**SC**) smallest changes; (**SD**) Slightly decrease; (**SI**) Slightly increase; (*) The hash mark indicates that the material was not considerable.

Amongst the experimental conditions, the standard guidelines (Table 1S - Supplementary material) define 5 five single colourimetric readings on a minimum of 5 specimens, while the conditions reported in the literature appear more varied also on equivalent stone for material and size. Amongst 44 documents dealing with the colourimetric measures, 23 papers contain data about the number of measures performed on the samples and 14 report information about the diameter measurement heads. Few and no clear information is found about the total number of investigated specimens, while often the measured area reported is 25 cm^2, except for a few studies where it is smaller or about 36 cm^2. As summarised in Fig. 3, “9” is the most recurring colourimetric reading number (labelled in the figure with circles) with different diameter measurement heads (3, 8, 11 cm). Later, “3” or “4” readings for each sample are commonly found.

Moreover, the literature review points out the great variability of the threshold limit for the colour variation values, ΔE^*. Over this limit, the treatment is not accepted as tolerable for stone cultural heritage. As reported in literature (Table 2S - Supplementary material), the suggested threshold changes, and, depending on the single study, goes from a value of $\Delta E^* < 2$ to $\Delta E^* < 10$.

Water absorption by capillarity. This technique investigates the amount and rate at which a specimen absorbs water by capillarity through the test surface when in contact with water. Any variation points out a change in the porosity of the materials treated.

The mathematical formula for the measurements of water amount absorbed by the specimen per unit area (Qi) at time t_I (time elapsed from the beginning of the test, in (s)) is calculated as follows [43]:

$$Qi = [(m_i - m_0)/A]$$

Where (m_i) mass of the specimen at time ti, (m_0) mass of the dry specimen, and (A) the area of the sample in contact with water. Amongst the standard, the minimum number of specimens to investigate is three. In literature, to detect only the first initial water uptake, like the contact sponge method, and not the entire water absorption, the coefficient absorption times reported are values of 30, 60, 90 or 120 s [100].

Contact sponge. This method works through a contact sponge positioned on a contact plate. The investigated characteristics detect the initial water absorption of material just below the surface.

The contact plate’s difference in weight gives the amount of water absorbed by the stone before and after the proof. It is essential to perform the analysis before and after the treatment since tests made after some days or more are not reliable.

The mathematical formula for the *in-situ* test to be applied is [44] :

$$Wa\left(\frac{g}{cm^2} * min\right) = \frac{P_i - P_f}{23.76 * t}$$

Where (Wa) is water absorbed; (P_i) is the initial weight of the sponge inside the plate; (P_f) is the weight of the sponge inside the plate after the contact (23.76) is the sponge area; (t) is the total time of contact.

Table 4
Natural biocides and alternative to biocide treatments: Dataset of all the interference tests performed present in the scientific literature. **T. Bio.** =Tested biocide; **B. Con.** = Biocide concentration; **Con. Tes.** = Conditions of testing; **T. Mat.**= Tested materials; **S. Mat.**= sub material; ***M***= Methods; **W. Abs.**= Water Absorption; Colour; **Morp.** = Morphologic; **M. Com.**= Mineral composition; **Por.** = Porosity; **A. lim.** = Accepted limit; **Ref.**= Reference. References [7,56,60,64,88–98] are cited in this table.

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	P	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
Natural compounds: essential oils (EO) and constituents of essential oils (CEO), secondary metabolites of lichens (SM), Microorganism/microbial by-products (M)										
(SM) **Norstictic acid**	0.05 mM	P - (25 ul/cm^2)	Ma	*Carrara Marble*	AAT, C	/	ΔE =0.45	/	/	[56]
(SM) **Parietin**	0.05 mM						ΔE =0.35			
(SM) **Acid usnic**	0.02 mM						ΔE =0.38			
(M) **New FloorCleaner** *(probiotic products (PIP), consisting in spore of Bacillus subtilis, B. megaterium and B. pumilus)*	10mL per liter	Sp	B	/	C,CWA, OM, SEM, SS	N	ΔE < 4	N	3 applications every 48 hours	[7]
(M) ***B. gladioli pv. Agaricicola***	0.2μM	In	L	*White bioclastic (Hontoria)*	C	/	ΔE = 13.88	/	500 ml of every fifteen days (3 applications)	[88]
(M) ***S. nigrum***	500μM						ΔE = 8.28			
(M) ***T. harzianum* T22**	0.2μM						ΔE = 9.06			
(CEO) Mixing water of **Eugenol** + Tween 20 and Span 20	Eugenol (5%), mixing ratio (v/v) of Tween 20 and Span 20 of 7:3.	I - lab test/Sp - in situ test 10 ml	G	/	AAT, OM, XRD, W	N	/	/	Until saturation for lab test	[89]
(EO) ***Lavandula angustifolia*** and ***Thymus vulgaris*** and Tween 20	EO (10% v/v)	Applied twice a week apart	F	/	C, L	/	ΔE= 2.72	/	/	[90]
	EO (1% v/v)						ΔE= 2.36			
(EO) ***Lavandula angustifolia***	EO (10% v/v)						ΔE= 2.00			
	EO (1% v/v)						ΔE= 0.56			
(EO) ***Thymus vulgaris***	EO (10% v/v)						ΔE= 1.34			
	EO (1% v/v)						ΔE= 4.77			
(EO) Nanocapsule loaded with ***Origanum vulgare***	/	100 μL	Ma	*Sant'Agata red marble*	OM, SEM	/	N	N	/	[91]
(EO) Nanocapsule loaded with ***Thymus capitatus***										
(EO) ***Thymus vulgaris***, ***Syzygium aromaticum*** and water essence of ***Coridothymus capitatus***	1.6 (% w/w)	Po	Ma	/	C	/	after 14 days ΔE<2, after 60 days ΔE<3	/	/	[60]
(EO) **Thymus vulgaris**, **Syzygium aromaticum** and water essence of **Coridothymus capitatus**	2.25 (% w/w)						after 14 days ΔE<1, after 60 days ΔE<3			
(EO) ***Origanum hirtum***, ***Syzygium aromaticum***, ***Cinnamomum zeylanicum***	2.25 (% w/w)						after 14 days ΔE<2, after 60 days ΔE<2			
(EO) ***Origanum hirtum***, ***Syzygium aromaticum***, water essence of ***Coridothymus capitatus***	1.6 (% w/w)						after 14 days ΔE<2, after 60 days ΔE<2			
(EO) ***Origanum hirtum***, ***Syzygium aromaticum***, water essence of ***Coridothymus capitatus***	2.25 (% w/w)						after 14 days ΔE<1, after 60 days ΔE<2			
(EO) ***Origanum hirtum***, ***Coridothymus capitatus***, ***Thymus vulgaris***	2.25 (% w/w)						after 14 days ΔE<4, after 60 days ΔE<4			
(EO) ***Coridothymus capitatus***, ***Syzygium aromaticum***, ***Cinnamomum zeylanicum***	2.25 (% w/w)						after 14 days ΔE<2, after 60 days ΔE<2			
(EO) ***Origanum vulgare***	pure	B/ Po	Ma	*Carrara marble*	AAT, C	/	after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2	/	/	[92]
(EO) ***Coridothymus capitatus***							after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
(EO) ***Cinnamomum zeylanicum***							after 1 day ΔE>4, after 30 days ΔE<4, after 60 days ΔE=4			
(EO) **Syzigium aromaticum**							after 1 day ΔE>10, after 30 days ΔE>10, after 60 days ΔE>10			
(EO) ***Origanum vulgare***	0.75% w/w						after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
(EO) ***Coridothymus capitatus***							after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
(EO) ***Cinnamomum zeylanicum***							after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
(EO) **Syzigium aromaticum**							after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
(EO) ***Origanum vulgare***, ***Cinnamomum zeylanicum*** and ***Syzigium aromaticum***	2.25% w/w						after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
(EO) ***Origanum vulgare***, ***Coridothymus capitatus*** and ***Syzigium aromaticum***							after 1 day ΔE<2, after 30 days ΔE<2, after 60 days ΔE<2			
Nanocapsules and silica mesoporous nanoparticles loaded with **zosteric acid plus TiO_2 nanoparticles**	0.1% w/w	B - until saturation	Ma,B,L ,P	*Carrara marble*	C, CA, CWA, Pr	D	ΔE < 5	N	/	[93]
Wood distillate	Pure,	Po- containing ca. 12 mL cm^{-3}	S	*Pietra Serena*	C	/	ΔE = 4.6 After wash ΔE = 2.6	/	/	[94]
	10%						ΔE = 3.5 After wash ΔE = 2.9			

(*continued on next page*)

Table 4 (*continued*)

T. Bio.	B. con.	Con. Tes.	T. Mat.	S. Mat.	P	Observed interference			A. lim.	Ref.
						W. Abs.	*Colour*	*Morp.*		
(EO) ***Cinnamon essential oil*** *(EO)* ***Oregano oil*** *(EO)* ***Oleum Ocimi Gratissimi*** *(CEO)* ***Eugenol***	1% v/v	Sp	S	/	C, CA, SEM	N	ΔE < 1	N	/	[64]
Alternative to biocide treatments										
Solvent gel containing dimethyl sulfoxide (DMSO)	DMSO (89.15% w/w), Ethomeen® C25 (8.41% w/w), Carbopol® 934 (2.44% w/w)	Spreaded and roughly removed after 24 h using dry (2 to 6 times up to a deep cleaning).	Ma	*Carrara marble*	C, CWA, P, Pr	N	ΔE= 2.76	N	/	[95]
Alginate hydrogels	Alginate (5 wt%) and $Ca(ClO)_2$(0.4 wt%) Alginate (5 wt%) and $Ca(ClO)_2$(1 wt%)	B- inserting a cotton gauze and left for about 12 h until it dried	L	*Pietra di Lecce*	C, NMR	N	ΔE< 1.	/	/	[96]
Alginate hydrogels	Alginate 5%, TiO_2 2% and $CaCl_2$ 0.15% Alginate 5%, sodium dichloroisocyanurate 0.4% and $CaCl_2$ 0.3%	B- 0.1 g/cm^2 . Application by a gauze, and after 24 h the dried gels were removed	L	*Pietra di Lecce*	C, CWA, NMR, SEM/EDS	N	ΔE=1.1 ΔE=1.2	N	/	[97]
Alginate hydrogels	Alginate (10 wt.%—500 mL), $Ca(ClO)_2$ (2 wt.%—500 mL) in 1.5 wt.% glacial acetic acid Alginate (10 wt.%—500 mL), sodium dichloroisocyanurate (1.6 wt.%—500 mL) and $CaCl_2$ (0.6 wt.%)	B- (1 kg/m^2) on a cotton gauze. Washing the surface to remove hydrogel residues.	L	/	C	/	ΔE=5 (Area1/2); after 2 years ΔE=5 (Area1), after 2 years (Area 2) ΔE=1 ΔE=2 (Area1); ΔE=5 (Area 2), ΔE=4 (Area 3) .	/	/	[98]

Legend. Conditions of testing: (**AG**) Air gun with pressure; (**B**) Brushed; (**CA**) Capillarity absorption; (**D**) Deposition; (**I**) Immersion; (**In**) Inoculated; (**P**) Poured; (**Po**) Poultice; (**S**) Soaked; (**Sp**) Sprayed - ***Tested material***= (**B**) Brick; (**Co**) Contrete; (**D**) Dolostone; (**F**) Fresco; (**G**) Granite; (**L**) Limestone; (**Ma**) Marble; (**P**) Plaster; (**S**) Sandstone; (**T**) Tuff - **M**: (AAT) Accelerated ageing tests; (**C**) Colorimetry; (**CA**) Contact angle meausure; (**CSM**) Contact sponge method; (**CWA**) Capillary water absorption; (**FTIR**) Fourier-transform infrared spectroscopy; (**L**) Lidar fluorosensor scanning); (**NMR**) Measurements of hygroscopic properties through NMR (**OM**) Optical Microscope; (**P**) Porosity; (**Pr**) Profilometer; (**SEM/EDS**) Scanning Electron Microscopy/Microprobe analyses; (**SS**) Soluble salts; (**W**) Change in weight; (**XRD**) X-ray diffraction - ***Observed interference*** = (**A**) Alteration; (**D**) Decrease; (**I**) Increment; (**N**) No Alteration; (**ND**) Nessun dato; (**SC**) smallest changes; (**SD**) Slightly decrease; (**SI**) Slightly increase; (*) The hash mark indicates that the material was not considerable.

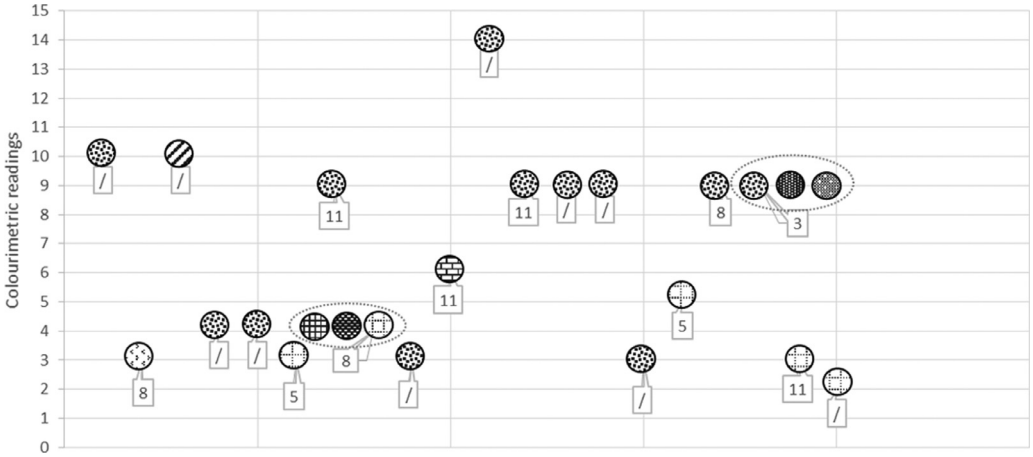

Fig. 3. Number of colourimetric readings for each sample reported in the literature. The single readings are labelled in the figure with circles. The different trama underlines specific substrata. The numbers in the squares indicate the diameter expressed in cm of the measuring head. The dashed circle encloses more results reported in a single article.

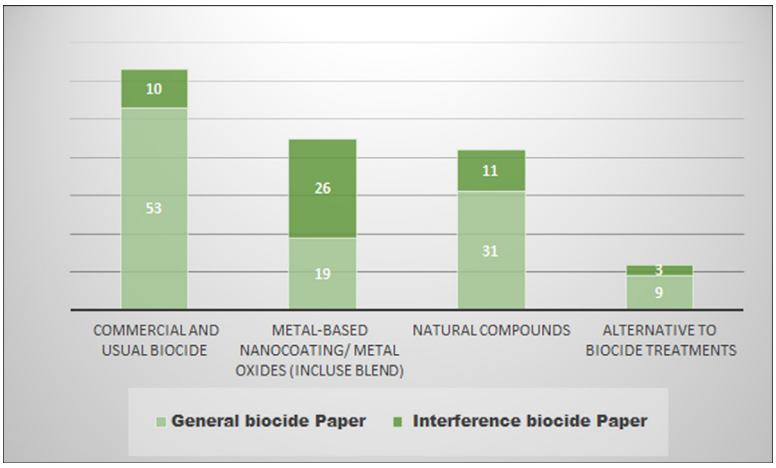

Fig. 4. The ratio between the documents concerning the interference tests. For each kind of biocide, the ratio is between the documents concerning the interference tests following the biocide application, and the paper dealing with biocides but not considering any effect on the substrate.

The standard guidelines (Table 1S - Supplementary material) suggest for the application method, 4 different measures on treated/untreated areas for each sample with a sponge diameter of 5.8 cm or 2.7 cm, with a time, starting from 30 s until 3 min. In the literature, the experimental conditions changed from two zones for each treated/untreated area [55,58] by a sponge diameter of 5.8 cm for a time of 1 min, to three areas per treatment by a sponge diameter of 8 cm for a time of 30 s [71]. The test frequency suggested in the scientific literature is four times a year, one for each season [55]. Cause the ability to absorb water from the content of manufacture humidity becomes crucial to detect temperature and relative humidity during the measurement [101]. A positive evaluation of the material is represented by no variation between the treated and untreated areas.

Contact angle. This technique investigates the static contact angle between a water drop and the test surface of the specimen. Any contact angle variation underlines a change in the hydrophobic materials' proprieties. The mathematical formula for the measurements is calculated as follows [45]:

$$\theta = 2arctg\frac{2h}{d}$$

Where (θ) is the static contact angle, (h) is the drop's height and (d) is the diameter of the contact surface. A positive evaluation of the material is represented by no variation in the contact angle.

Tested biocide

Several biocidal substances and products were subjects to investigation in the dataset's papers; the commercial names/substances are summarised in Tables 1–4.

In detail, the biocidal compounds based on metal oxides mixed with other metal elements or alone are the major investigated products for their potential harm on the substrate (57% of the total papers) (Fig. 4). On the contrary only 11 papers on a total of 42 documents regarding natural biocides on cultural substrata report the interference test. However, the worse ratio is for commercial and usual biocide where only 16% of interference tests are adopted in the biocide studies (see Fig. 4).

Biocide concentration and conditions of testing

Authors often used different concentrations of biocides, which were expressed in various measurement units, according to each experiment's requirements (see Tables 1–4). The testing conditions were different, according to the origin of the substance (commercial product, nanoparticles, organisms, etc.) and the material's requirements (see Tables 1–4). In 15 cases the biocides were applied by brushing (**B**). In 13 cases, spraying (**Sp**), in a specific case, by air gun with pressure (**AG**) was the application mode. In most cases (15 cases), the biocide was applied through nanocoating, especially for those based on metal oxides. The lichen secondary metabolites investigated by Gazzano et al. [56] were poured (**P**) onto the marble slabs. The same application method was used by De Muynck et al. [52] for biocide based on metal oxides. After incorporating the biocides, Barriuso et al. [102], Spada et al. [60] and Bianchi et al. [94] used cellulose poultices (**Po**) to let the material absorb the product by capillarity. Romano et al. [91]. inoculate (In) the limestone slabs with the cell-free filtrate of the bacterium Burkholderia gladioli pv. agaricicola ICMP 11096 strain. Jeong et al. [89]. Tretiach et al. [51], and Altieri et al. [21] immersed (**I**) the stone samples into the biocidal solution. Becerra et al. [74,77] applied the nanocomposites of silver and titanium dioxide via deposition (**D**) onto the limestone slabs. Whereas Nugari et al. [50] applied the biocide using capillarity absorption (**CA**).

Observed interference

The variation observed about the water absorption was reported as (**D**) decrement; (**SD**) slightly decrement; (**I**) increment; (**SI**) slightly increment; (**N**) no alteration, (**ND**) no data reported; (*) not considered for the authors. An increment in the water absorption put evidence of a change in the stone's intrinsic characteristics as the porosity value. On the other hand, a reduction of the absorption gives to the stone's hydrophobic characteristic. To point out the changes in the surface morphologies and the mineral composition in Tables 1–4 are labelled with (**SC**) any smallest changes, (**A**) alteration, and (**N**) no alteration.

Accepted limit

Few authors tested multiple concentrations of biocides to identify the endured concentration limit, that is, the maximum concentration of the biocidal substance that can be used without altering the surface or causing negligible and acceptable alteration for the field of cultural heritage. Indeed, Quagliarini et al. [75] tested different amounts of TiO_2 until identified the maximum percentage concentration causing minor alteration. In this case, the concentration was 1% weight/volume [75]. Goffredo et al. [68] directly tested titanium dioxide nanoparticles, titanium dioxide and silver nanoparticles, and titanium dioxide and copper nanoparticles, in all three cases at a concentration of 1% weight/weight,

to avoid unacceptable chromatic changes or other incompatibilities, basing their assumption on previous studies. Zarzuela et al. [73] tested multiple percentage concentrations of a nanocomposite based on copper oxide and silica, identifying the value of 0.15% weight/volume as the accepted limit to avoid significant chromatic alterations or mechanical modifications. Becerra et al. [70] tested a different kind of nanocomposite to identify that only the one based on silver and titanium dioxide stabilised with trisodium citrate, and containing 0.02 mg/ml of silver and 0.12 mg/ml of titanium dioxide, could be considered for applications on limestone.

Discussion and suggested guidelines

Tested materials

The materials investigated by the interference tests were different but most data regard limestone, marble and sandstone. Only 35 papers (see Tables 1–4) give detailed information on stone characteristics, mineralogy, chemical composition, porosity, and roughness. This lack seems relevant since, as underlined previously stressed, different porosity and roughness values could influence the final interference test, driving to different results. Therefore, such information is essential, before applying a biocide, to avoid the undesirable effect of a product, and help in a better selection.

Tested methods

Results showed the heterogeneity of the methods of measurements, carried out in the assessment of biocides interferences. Indeed, adopting only one method can be insufficient, since each analytical method can study only some properties, but not all the needed evaluations in the field of cultural heritage protection. Most techniques were focused on the colourimetric analysis and the water absorption surveys, and a few on the solubles salts, monitoring or characterising new mineralogic phases.

Such methods can have some critical points to be considered in the final assessment, such as downside effects. For example, in the case of the colourimetry methods, the critical element is given by possible differences in relative humidity between the readings. The colour changes to yellow and darkens under high humidity conditions. In the laboratory, this difficulty could be overcome by using drying systems. Then, in the field, it is important to register the microclimatic parameters before starting tests [103]. Moreover, the colourimetric values vary from type to stone. Stones can have irregularity due to great cavities (travertine), different grains colour (granite) or material included (bioclastic stone). These irregularities should be evaluated to define accurately both the number of readings and the measurement head size. As reported in the study of Prieto et al. [104], concerning a proposed procedure for the colour measuring of granite rocks (excluded by the dataset because not allude to biocidal use), in inhomogeneous samples like granite stones, the number of colourimetric readings should increase. In detail, in granite stone, the recommendations are 6 readings for a 36 cm^2 surface area using a 50 mm diameter measurement head, 14 readings for a 36 cm^2 surface area for 8 and 10 mm diameter measurement heads, and 17 for a 36 cm^2 surface area for a 5 mm measurement head [104]. This approach has been applied by He et al. [62] on a wall painting, where a total of 9 measurements were applied using a 4-mm measurement head within 4 cm^2 of surface area.

In addition, the great critical point concerns the different colour variation values (ΔE^*), considered acceptable for historical or monumental surfaces. In this review, the lack of an univocal limit value (ΔE^*) above it emerges and the chromatic variation cannot be considered suitable for the cultural heritage. This aspect implies that the same product could be believed adequate in some studies and not suitable for others. The absence of uniformity raises from the lack of rules in the standards of Cultural Heritage, and the indicated threshold limit came from analysis and visual observations performed in other research studies. For example, certain papers [68,70,74,78] follow the classification suggested by García and Malaga [105] for antigraffiti products. These authors state that a total colour change cannot be seen by a human eye only when ΔE^* values are < 5 units, but following this classification, ΔE^* values range between 5 and 10 units and are still considered to be acceptable, even if slightly visible. Other reports that the colour variation is very distinct when $\Delta E^* > 3.0$, small differences when $1.5 < \Delta E^* < 3.0$ and cannot be distinguished by naked eyes when $\Delta E^* < 1.5$ [62]. On the other hand, most studies [6,19,86,95,106], indicate ΔE^* values below 4 as acceptable in the field. Therefore, it comes to light that is necessary to draw a common value to follow. Concerning the water absorption interference tests, the contact sponge test can be applied with high precision for less porous materials, but it is unfit for more porous stones [107]. In this case, it is possible to replace the water absorption method, using the Karsten pipe method [108]. Both these methods, the contact sponge and Kasten pipe technique, are specific for *in situ* conditions. Furthermore, the availability of instruments for the *in situ* measurements is essential to monitor biocide-substratum interaction in the original environmental condition. Again, this implicates the possibility of periodic monitoring by non-destructive methods, relatively handy and straight forward, that permit to keep preventive maintenance.

Then, such comparative evaluation underlines the importance of carrying out several evaluations of the interference, before and after treatment, such as colour, water absorption, water vapour permeability, contact angle, visual and microscopic analysis, mechanical tests, and chemical and mineralogical analysis.

Tested biocide

Concerning biocidal products used in stone conservation and investigated for their interference with the substrate, the predominance is represented by the metals and metal-based compounds (see Fig. 4). Although a growing number of studies proposes essential oils and components of EOs as alternatives to conventional biocides, data about their stone interference are often lacking, thus excluding a limited number of papers (see Table 4).

It is decisive, for further research related to the natural and innovative biocides in stone restoration, including the tests about their potential interference with the materials (changes in colour, water absorption, morphological aspect variation, mobilisation of the solubles salt, and accelerated ageing tests).

Biocide concentration and conditions of testing

Concentrations and applications of biocide greatly varied. Only in a few studies, detailed information about the biocide is described. For example, not always the solvent (e.g. water, alcohol, acetone, or mixing) or the additive (e.g. surfactant as Tween 20/80 or Span 20) adopted are specificated. Data about methodology detail are also lacking: e.g. the distance and the diameter of the spray nozzle used for the biocide application. Instead, these data could be useful for the correct reproducibility of the experiment.

In addition, the information about the physical characteristics of the biocide adopted, such as pH, density and viscosity values are rarely reported in the studies. These features are extremely important to understand the interaction of the products with the stone: How much can it penetrate the substrate? Can it activate

an acid dissolution of the stone? Is it so viscose that could remain on the surface? The solvent or the additive could affect negatively the substrate? This is to conduct a selection of the suitable biocide product concerning the stone characteristic (e.g. carbonatic or siliceous stone, high or low porosity, etc.). Thus, a complete analysis report information about the biocide is advisable, such as [18]: a) substance: scientific name, origin; b) application (in vitro and situ); c) concentration; d) material: lithotype, chemical, and mineral characterization; d) target organism: referring to potential biodeteriogens; e) efficacy: ranking of the level of activity for each substance and f) the physical/chemical characteristic of the biocide, its solvent and/or additive.

Observed interference

The data highlighted that the chromatic final results strongly depend on the biocide concentration, stone material characteristicssuch as porosity, roughness, mineral and chemical composition and water amount.

Graziani et al. [66,69] reported that the same product (TiO_2 nanocoating) applied to stones with different porosity and roughness gives different chromatic variations: clay bricks with highly porous and rough surfaces showed no relevant colour changes concerning those with low porous smoothed surface. A high porosity in the substrata allows the biocide to penetrate almost totally inside the stone avoiding the depositing of the product on the surface causing a visual variation [19]. In the study by Altieri et al. [21], Algophase on the marble stone produced a yellowing effect, which was not registered in the sandstone or travertine samples. Similarly, the application of the hydrogen peroxide on marble stone did not cause any visual change, but stains to a rust-coloured spot were registered on sandstone "Pietra Serena" [50], probably due to the interaction between hydrogen peroxide and the iron presence inside the stone.

Some products (DTSACl, DAVICIL, Glyphosate mono-isopropylammonium, Bioestel) (see Table 1) caused darkening on concrete, marble and sandstone surface [50,52,55], while other biocides (sodium dimethyldithiocarbanates and sodium 2-mercapto-benzo-thiazole) produced a yellowing effect on the surface or a whitening effect (Bioestel) on Carrara marble [21,50,55].

Furthermore, the chromatic change due to the application of TiO_2-based coatings and nanocoatings showed a great variability in the final results depending on concentration, stone material and the kind of nanoparticles metal mixture. The variation seems to be strongly dependant on the amount of TiO_2, in fact, in the research by La Russa et al. [67] colour variation decreases as TiO_2 content decreases, except for the pure TiO_2. The whitening effect is more evident on dark stones and could be underrated on light colour stones [66,69]. The metal particles that have registered a significant chromatic alteration ($\Delta E > 5$) are Ag/TiO_2 nanocomposites, ¼ Ag/TiO_2 nanocomposites, Ag nanoparticles (with trisodium citrate), and Nano-Ag plus TEOS (tetraethyl orthosilicate) applied on limestone materials [70,74,77,78,79]. In general, the presence of Ag-nanoparticles is responsible for the higher alteration of the final colour as underlined in the study of De Muynck et al. [52], where Ag-nanoparticles at the concentration of 250 and 100 mg/m^2 induce a darkening with ΔE^* values of 8.7 and 15.9. Amongst the biocidal natural compounds, the products based on essential oils (**EO**) and constituents of essential oils (**CEO**) have given a good result with ΔE^* value <4 [90,91,60], except for the pure application of *Syzigiumaromaticum* on marble registered in the study of Spada et al. [92], where the ΔE^* is >12 after artificial ageing of 60 days. Also, the biocides as the New FloorCleaner and the biocides based on lichen secondary metabolites (**SM**) (see Table 4) appeared to be suitable, not showing any kind of chromatic alteration respectively on materials such as firebrick and marble [56,57].

On the other hand, high ΔE^* values have been registered for the biocides constituted by microorganisms or microbial by-products (**M**). In the study of Sasso et al. [88], after the application of *B. gladioli pv. Agaricicola, S. nigrum,* and *T. harzianum* T22 a critical chromatic variation with a ΔE^* value > 8 have been highlighted.

Interestingly, some studies have also taken into account the stability of the applied biocide, monitoring its effect over time. As reported by Van der Werf et al. [84], two hours after the application of a hydrorepellent mixing with a nano-ZnO on limestone the ΔE^* was about 10.1/10.2, passed 14 days the value decreased with a $\Delta E^* = 5.9/5.8$, up to a negligible chromatic effect after 38 days with a value $\Delta E^* = 2.9/2.8$ [84]. Moreover, in Aldosari et al. [87] a slight variation after two different ageing processes has been highlighted: without ageing a $\Delta E^* = 0.45$, after UV ageing $\Delta E^* = 2.41$, and after thermal ageing $\Delta E^* = 2.59$.

However, other studies have noted the opposite effect, with a worsening of the chromatic values with ageing. In the studies of Spada et al. [60], firstly (after 14 days) the benzalkonium chloride application gives a good result with a $\Delta E^* < 4$, but after 60 days the chromatic variation increased until a value of $\Delta E^* > 6$. On the other hand, in the same study, the natural biocide (**EO**) did not undergo any change in chromatic values. Moreover, In Goffredo et al. [71] after ageing, a darkening effect of the coating has been observed. Concerning the change in water absorption after biocide application, a decrease in water amount absorption is observed when the biocide is mixed with a hydrorepellent/consolidant product [54,55,58,80,82,85,87]. As for the chromatic results, also for water absorption, the porosity of the stone influences the results. In Altieri et al. [21], four commercial products (Algophase, Arsenal, Roundup, and Metatin N58–10/101) were applied on three different lithotypes: Carrara marble, sandstone from Fiorenzuola, and travertine. Results showed that Algophase reduces the water absorption in the marble stone, but on the contrary, it increases in the sandstone, while its effect is negligible on travertine. This different behaviour is probably due to a larger deposition on the sandstone surface. Roundup was the most aggressive product, inducing a stone dissolution of all four substrates and consequently increasing water absorption [21]. In the study by Nugari et al. [50] marble appeared to be more affected by an increment of water absorption to limestone samples, probably as a consequence of an alteration of the microporosity inside the stone [50]. Regarding the biocides made up of metal oxide, the results underlined a hydrophobic behaviour. In almost every case, it was registered a decrement in water absorption after the applicationof nano-metals coating with a biocidal effect [72,73,76]. All the data about water absorption for natural biocide have not revealed any alteration of the water absorption proprieties [57,64,89] ad exception in Zuena et al. [63] where a hydrophobic behaviour, due to the nanocapsules and silica mesoporousnanoparticles, has been registered. In the database (Tables 1–4) the morphological alterations, labelled as "**A**", are considered "any change in the morphological structure of the stone," although some superficial modification can implicate an improvement as a better superficial cohesion. In fact, in biocides based on $CuO\text{-}SiO_2$ [73,76] the coating reduces the surface roughness forming a more compact film on stone. The same surface modification was detected on the sample treated by mixing Estel 1000 and nano-ZnO [81,84], where the homogeneity of the stone surface was improved. A similar study is reported by Aldosari et al. [87] where the biocide evens the surface without creating fractures. In Nugari et al. [50], three commercial biocides, Glyphosate mono-isopropylammonium, sodium dimethyldithiocarbanates and sodium 2-mercapto-benzo-thiazole, and hydrogen per-

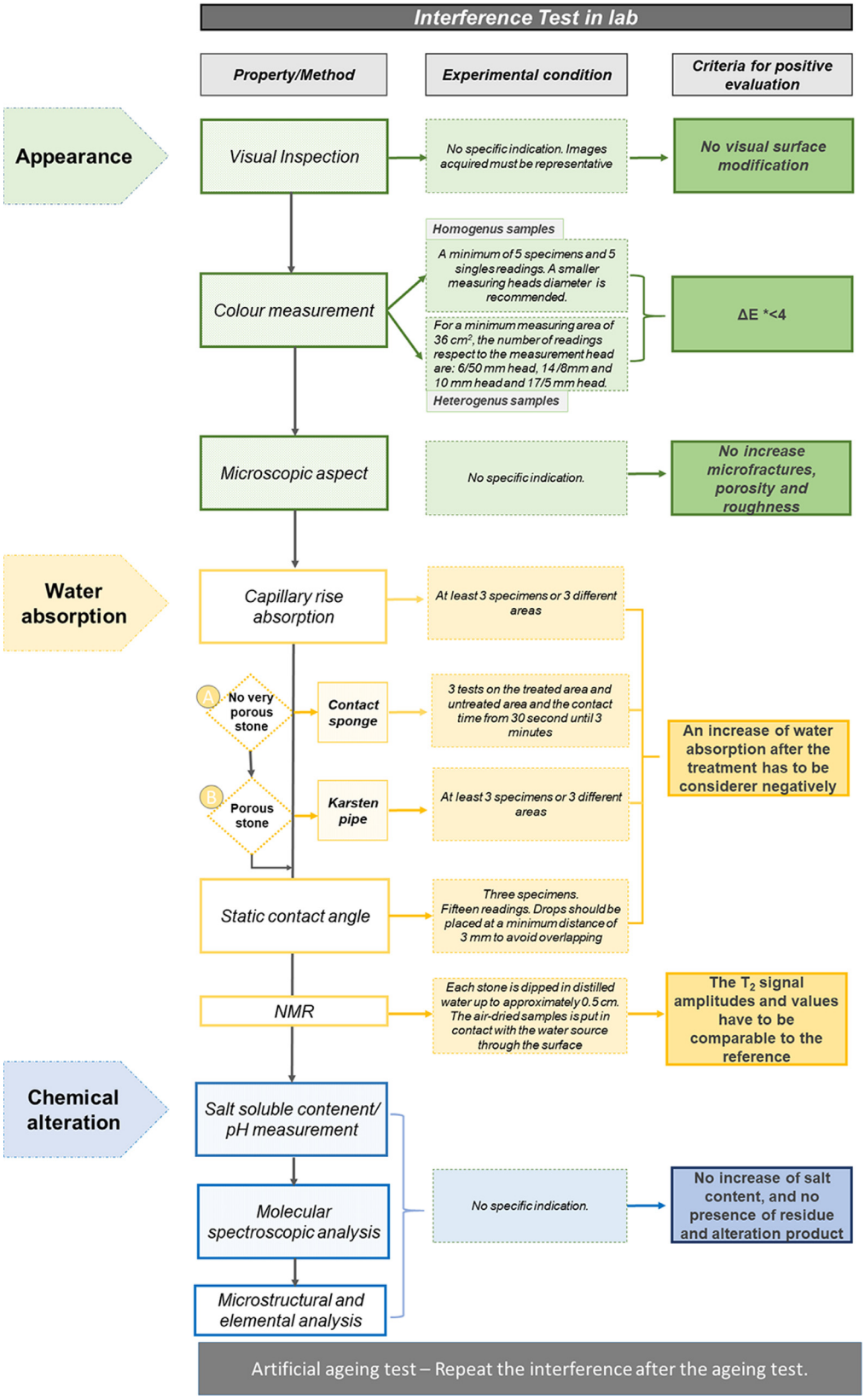

Fig. 5. Proposed method to test the biocide interference on the stone for laboratory tests.

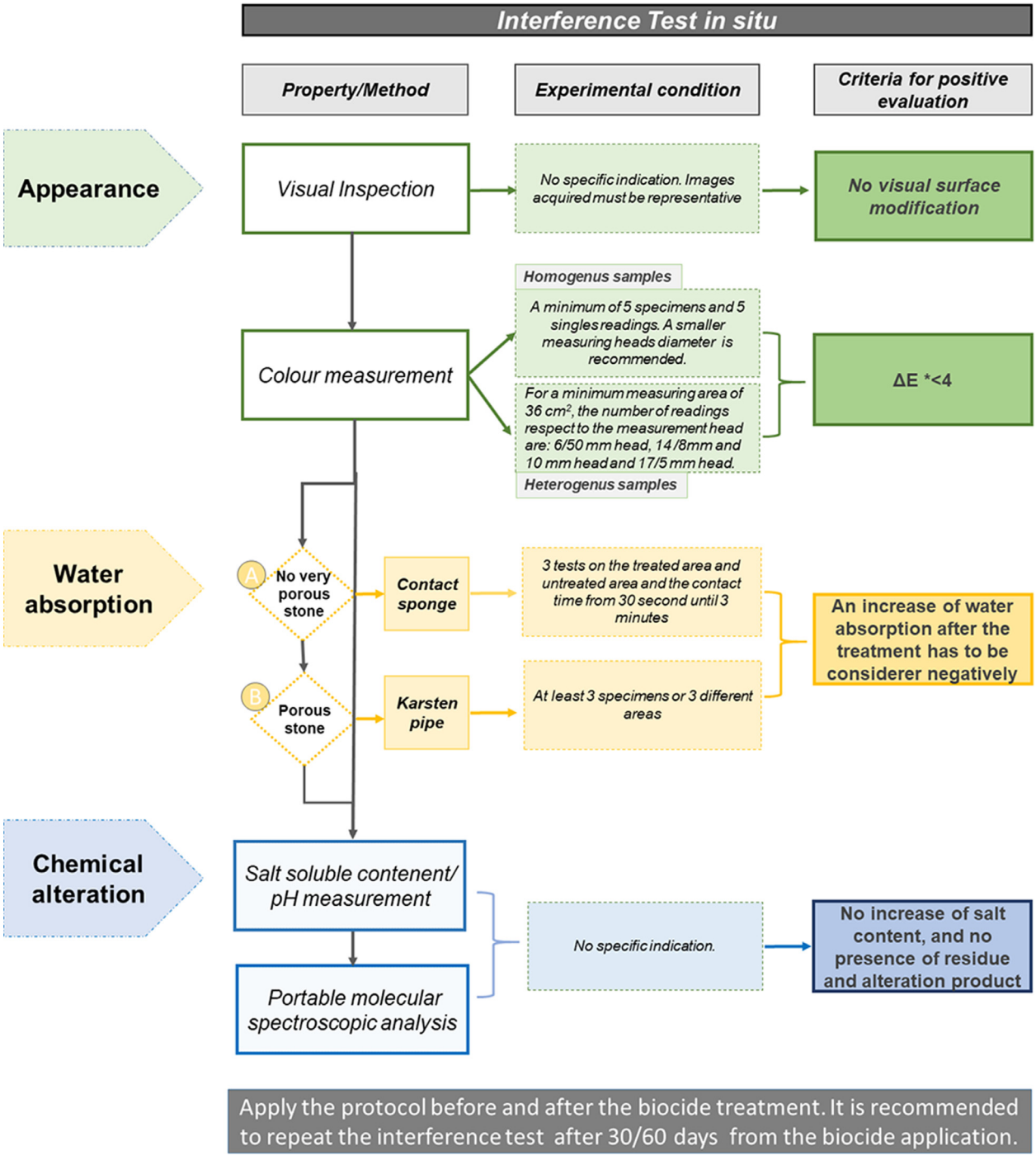

Fig. 6. Proposed method to test the biocide interference on the stone *in situ*.

oxide, resulted to be more aggressive marble stone than on sandstone.

No morphological alteration was reported for natural biocide application on substrates [64,91,93]. The only two porosity analysis applied for the interference test [66,95] reported no variation. Moreover, the observed interferences are limited to a few parameters: water absorption variation, colourimetric modification, and variation in porosity and surface roughness. Other equally important parameters are rarely considered, such as the soluble salt detection (e.g. by specificity conductivity, pH and ions content measurement), and the analysis of residues due to additive (e.g. surfactant) or interaction with the solvent. A biocide based on a water solvent could affect variations in the distribution and concentration of soluble salts, especially in calcareous stones [109,110]. Furthermore, specific chemical compounds present in the biocide formulation can affect pH variation, inducing dissolution phenomena, and changing the concentration of salts soluble. The standard EN 17488:2021 [41]: “Procedure for the analytical evaluation and selection of cleaning methods for porous inorganic materials used in cultural heritage" relating to the methodologies to evaluate the harmful effect and the efficiency of cleaning methods, in addition to optical observation, colour analysis, water absorption assessment, suggests also methodologies as wet chemical analysis on aqueous extract, vibrational spectroscopy investigation, and surface ion analysis and elemental analysis. For a whole analytical evaluation, these parameters should always be taken into consideration

in the study of biocidal interference. Also, accelerated ageing tests (e.g. exposure to heat, moisture, UV light, etc.) are necessary, especially for new products.

Accepted limit

In general, to simulate a real conservative treatment, the biocide application doses should be higher than the dose that has been established as effective for the removal of biodeteriogens. However, few authors tested multiple concentrations of biocides to identify the limited concentration of treatment regarding the interference tests. For this reason, it is relevant to increase the biocide concentrations to establish the concentration limit that can be used without altering the surface or causing negligible and acceptable alteration for the field of cultural heritage. The missing of this parameter is serious because, in a real situation, the exact amount of the applied biocide is not always controllable.

Suggested guidelines

Based on the standard methods (UNI 10921:2001 [40]; UNI EN15886:2010 [42]; UNI EN15801:2010 [43]; UNI EN 15802:2010 [45]; UNI EN 11432.2011 [44];EN 16302:2014 [108];EN 16455:2014 [111]; EN17488:2021 [41]) and guidelines presented in other research studies [104,105,106,112,113,114] in following flow charts (Figs. 5-6), we propose a way to test the possible interference on stone due to the biocidal treatment (Table 3S - Supplementary material). In detail, in Fig. 1S we report a preliminary assessment to plan the correct interference tests based mainly on if the biocide is a new product; if there is data about its interference test; if the conditions for the test are the same and if the substrate has the same chemical and petrography characteristics. By answering these questions both the application of the biocide and the evaluation of the interference tests are more aware. The two flow charts (Figs. 5 and 6) are focusing in detail on the kind and the modality of the tests to apply *in situ* and laboratory. For *in situ* test a representative reference area on site is necessary to compare the interference data obtained with which from the treated area by the biocide. In this way, it is possible to point out any unwanted changes. When no data occurs, the interference tests should be conducted before the biocidal treatment, after one/two months from the product application and after the accelerated ageing cycle. These steps appear to be essential for monitoring product stability.

For each propriety, it is possible to use one or more methods, based on the available technologies and the specific case of studies (Table 3S - Supplementary material).

The biocide application should be consistently overestimated to simulate a real conservative treatment. The proposed method considers checking property as *appearance, water absorption* and *chemical alteration*.

The sequence of application of different tests can vary with the specific case. Concerning the water absorption tests, we suggest focusing on the superficial surface of the stone, because it is the most exposed to variations due to the application of biocides. Usually, the alteration and deterioration processes are concentrated in this area. Furthermore, it is essential to report for each method the corresponding information of the reference standard, especially for the colourimetric measure and the water absorption method, where the temperature and humidity data are necessary.

Conclusions

Data obtained from the current literature confirmed a certain variability in methods for the analysis of stone monuments' alteration "interference" caused by the application of biocides. The main usually applied interference tests are the colourimetric analysis and the water absorption tests. However, diversification of methodological approaches occurred, in measuring field observations and readings, giving rise to the high heterogeneity of acquired final data. This makes it complicated to compare interference tests conducted in different research. Moreover, sometimes the literature interference data are not very useful to a final real application, because the biocide concentration applied during the tests could be distant from a real new situation, where frequently a higher dose occurs. Therefore, based on the national/international standards and the existing literature, we suggest a guideline for interference tests both in the lab and *in situ*, which could help in monitoring any unwanted changes on the stone after the biocide application, especially to detect the great multiplicity of the novel biocides put on the market. Establishing a standardized shared methodology for interference evaluation is crucial to compare the results obtained accurately. This will help identify the application of a particular biocidal product while ensuring the safety of the monument.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.culher.2023.08.001.

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