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Methods of evaluating biocides for the conservation of porous building materials

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Riassunto

La valutazione dell'efficacia di un biocida non è un'operazione facile a causa dei numerosi fattori che possono interferire quando si eseguono trattamenti di questo tipo. La composizione mineralogica del substrato ed eventuale materiale «estraneo» che può accumularsi sulla pietra possono ad esempio aumentare o diminuire l'efficacia di un certo prodotto. Il primo fattore da prendere in considerazione è il tipo di colonizzazione biologica tenendo conto che solitamente sono presenti comunità complesse di microrganismi ed organismi diversi, quali batteri, alghe, funghi, lieviti, licheni. Molti di essi secernono metaboliti extracellulari (generalmente polimeri) che spesso si stratificano intorno alle comunità conferendo un effetto protettivo. Probabilmente sono in parte ascrivibili a ciò i risultati contraddittori spesso ottenuti tra le sperimentazioni di laboratorio e quelle in situ.

Deve essere definito chiaramente anche l'obiettivo che si vuole raggiungere: l'eliminazione di microrganismi causa di un disturbo «estetico», dei microrganismi deteriogeni o di tutti i microrganismi presenti? Parallelamente alle specie da eliminare, per la scelta del biocida più idoneo, dobbiamo considerare il tipo di substrato da trattare: l'efficacia di un biocida può essere infatti condizionata dalla composizione mineralogica della pietra. Nel corso del Congresso è stato illustrato come ad esempio la presenza di argille possa prolungare l'efficacia di un biocida. Ne deriva che la concentrazione più idonea dovrebbe essere scelta non solo a seconda degli organismi bersaglio ma anche della composizione mineralogica del substrato.

Talvolta il biocida potrebbe indurre una maggior produzione di polimeri extracellulari, da parte dei microrganismi associati a quelli bersaglio, che potrebbero conferire un certo effetto protettivo a tutta la componente biotica diminuendone conseguentemente l'efficacia. Le metodologia da utilizzare per la valutazione dell'efficacia dei biocidi dovrebbero tener conto di tutti questi fattori.

Nel campo della microbiologia esistono disparate tecniche per valutare la carica microbica che potrebbero essere applicate a questo tipo di studi ma sino ad oggi non è stato fissato un protocollo comune di indagini, per cui di volta in volta i diversi autori ne applicano alcune o altre. Un altro aspetto indispensabile da indagare preventivamente all'applicazione di biocidi è l'interazione che questi possono avere sul substrato. La scelta del biocida da adottare non deve infatti tener conto solo degli organismi bersaglio da eliminare ma contemporaneamente non deve indurre alcuna variazione fisico-chimica nel substrato; va inoltre ricordato che va attentamente valutata la compatibilità con gli altri prodotti utilizzati nel corso della pulitura, del consolidamento o della protezione finale poiché talvolta potrebbero verificarsi delle interazioni negative.

È noto che molte resine utilizzate come consolidanti o protettivi possono rappresentare una fonte nutrizionale addizionale per i microrganismi e quindi determinare, in condizioni ambientali favorevoli, una crescita microbica. Allo scopo di prevenire tale possibilità sono state condotte recentemente alcune sperimentazioni che prevedono la miscelazione di biocidi con consolidanti e/o protettivi. Sono comunque necessarie ulteriori ricerche per stabilire l'efficacia di tali procedure, i risultati sinora emersi sono infatti contradditori.

L'Italia è stato uno dei primi paesi che ha sentito la necessità di stabilire metodologie analitiche comuni per lo studio delle alterazioni dei materiali lapidei e la valutazione dell'efficacia dei trattamenti mediante la creazione delle commissioni Normal. In questi ultimi anni l'attività del Gruppo biologico (Normal B) si è focalizzata sui metodi di controllo del biodeterioramento, settore affrontato in modo largamente empirico sino a pochissimo tempo fa. Sono stati pubblicati quattro documenti (NORMAL 30/89 «Metodi di controllo del biodeterioramento», NORMAL 35/91 «Caratterizzazione di biocidi e relativi principi attivi: schema di scheda», NORMAL 37/92 «Trattamenti biocidi: schema di scheda per archiviazione dati», NORMAL 38/93 «Valutazione sperimentale dell'efficacia dei biocidi») ed è stato creato il Sottogruppo operativo «Interferenza biocidi-materiali lapidei», a carattere interdisciplinare, allo scopo di individuare i parameteri più idonei e le relative metodologie di indagine per la valutazione delle interazioni dei prodotti biocidi con il substrato. Sarebbe comunque auspicabile la creazione di un gruppo di lavoro internazionale per la messa a punto di metodologie analitiche comuni riguardanti questo settore.

1. BACKGROUND

Evaluating the effectiveness of biocides on porous building materials is complicated by the large number of factors impinging upon the interpretation process. There are many different microenvironments within one stone type and between different porous building materials, all of which may alter the apparent effectiveness of a particular biocide. In addition to variable stone compositions, there is a wide range of adventitious material from the environment that can accumulate on and in the stone, some of which may enhance or impede the effectiveness of any biocide applied to the surface. The prime complication for biocidal effect is the ecological community of biological organisms that may colonize a surface or the inside of the stone. In this community a succession of different micro- and macro-organisms and their associated secretory products (e.g., biopolymers) may be found. Organisms rarely exist in isolation, they usually rather exist in an often complex community of mixed types of organisms, such as fungi, algae, bacteria, yeasts, and lichens. The biological activity of these organisms involves secretion of biomolecules into the environment that often results in layers of biopolymers surrounding and essentially protecting the community from harmful components of the environment, including the effects of biocides. The contradictory results sometimes obtained between laboratory tests and applications in the field could be ascribed to these factors.

With all of this in mind, how can a standard for evaluating the effectiveness of biological products for use on porous building materials be established? A workshop was held in Rome, 19-21 June 1995, entitled «Methods of evaluating products of the conservation of porous builind materials in monuments». This workshop attempted to at least define the problems associated with evaluation of products for these types of materials. During the course of the discussions a number of suggestions and ideas were put forth concerning standardization, these are discussed below.

2. CONSIDERATIONS FOR APPLICATION OF BIOCIDES

Before applying any biocide to a surface, it is necessary to understand how biocides function in general, their toxicological characteristics and how the specific product under consideration is likely to react with the materials to be treated and to any non-target organisms present. A clear definition of the desired goals of the biocide application should be understood. For example, is the main object to remove the visible discoloration of algae, or is the object to remove all of the potentially damaging microorganisms? If the goal is just to remove the microbe(s) causing visual impairment of the surface, then a single biocide product may be sufficient. If the goal is to remove all microbes, then a series of different biocides or a biocide with larger spectrum of action may have to be applied. In either case, a testing procedure has to be devised to assess the effect of the biocides on the material itself, not just on their ability to remove the target organisms.

Another consideration in biocide application is the formulation and concentration necessary to achieve the desired results. The actual concentration needed will vary from stone type to stone type, and depend upon the quantity and diversity of microbes present. Other complications may arise from the presence of biofilms (these are essentially layers of biomolecules secreted by the extant microbes) and pollutants trapped in the biofilm. The type of builing material will also affect the biocide. It has been demonstrated that the effectiveness of the biocide may last significantly longer when clay is present, presumably due to the absorption of the biocide into the clay (Young et al., 1995). Therefore lower concentrations may be suitable for these kinds of materials. On the other hand, a biocide may induce non-target organisms to secrete more biopolymers into the surroundings, thus providing protection for the target and non-target organisms alike from the effects of the biocide. In this latter case, it would appear then during testing that a higher concentration of biocide would have to be em-

ployed to achieve the same effects on different areas of the same stone. A further possibility is that the biocide may interact with components of the biofilm layers itself or with pollutants trapped within the biofilm producing unexpected results (Prof. Ralph Mitchell, oral communication).

Standards for assessing the effectiveness of biocides must take into account the above factors if a realiable assessment of the biocide's effectiveness is to be achieved.

3. BIOLOGICAL QUANTITATIVE ASSESSMENT METHODS

There are numerous methods available in the field of microbiology for quantitative assessment of microbes. These include the standard procedures of MPN (most probable number) approaches using slide and grid counting methods, fluorescent staining and microscopy procedures, nephelometry (density measurements), laser particle counting techniques, visual statistical sampling procedures, and some of the newer procedures, employed in the senior authors laboratory, of ATP-ATPase Luminometer techniques, and Fourier transform infrared spectroscopic techniques for the measurement of respiration by products from living organisms. Any of these procedures can be adapted to assess for the effect and the effectiveness of biocidal agents. For example, Koestler & Santoro (1988) developed and tested a statistical assessment regime for measuring the growth of fungi on stone consolidating materials. In this procedure, a result from a combination of sporulation scale index (Klausmeier, 1971), the percent coverage, and weight-loss measurements were combined using Duncan's Multiple Range test and Student-Newman-Keuls test to statistically rank a series of conservation materials for their susceptibility to begin a food source for fungi.

There have been many approaches to assessment of the effectiveness of a biocide. For example, Grant & Bravery (1985) have presented a simple laboratory technique to evaluate biocides and coating on building materials. This technique uses a vermiculite bed to maintain a moist environment around the stones to be tested. Tiano et al. (1995), on the other hand, have demonstrated a more involved protocol for assessing the behavior of selected biocide formulates *in vitro* and *in vivo* using HPLC chromatography and FTIR spectroscopy combined with water capillary and MIC determinations.

The effect of a biocide on a material's surface appearance has been assessed in a binary statistical procedure by Koestler et al. (1993). The procedure proved to be a rapid visual assessment method easily employed by conservators and restorers to screen out the most harmful biocides before they were applied to the surface. One key to the success of this method is agreement on the types of changes to be assessed for. For example, issues like change in color, change in roughness, blanching effects, or other type of visual indicators may be useful in gauging the effect of a biocide on the appearance of the stone. This does not necessarily indicate the effectiveness of the biocide in eliminating a target organism, but may keep one from turning a stone yellow when it should be white! Other Authors measured the effects of some biocides applied on marbles with different techniques (Nugari et al., 1993a,b).

Many quantitative measures for the presence of microbes have been conducted. Nieto et al., (in preparation) utilized the ATP-ATPase reaction with luciferin in a Luminometer to determine the utility of technique for measuring biomass and the activity level (spore to rapid growth state) present within fine art objects composed of wood. The technique has worked well in a defined system with three known species of wood destroying fungi. It may be applicable to stone problems but would require a significant research effort to define the conditions under which the procedure would provide useful information. Koestler (1993) has demonstrated a procedure for measuring gas byproducts from organisms, insect or microbial, living within or on top of fine art objects. This procedure, using a Fourier transform infrared

spectrometer, is sensitive to the ppm range, and does not require sampling from the object. It has been successfully used to quantitatively measure biological activity before and after biocidal treatments. This procedure is usable for objects that can be accomodated in a laboratory setting, but not for entire buildings.

Many other techniques could be adapted from standard microbiological methods.

4. BIOCIDES AND RESINS

During restoration projects, it is often recommended that a water repellent with or without a consolidant be applied to the stone. Application of such a product runs the risk of adding a new nutrient source for microbes to the stone (Nugari & Priori, 1985; Koestler & Santoro, 1988; Salvadori & Nugari, 1988; Sorlini et al., 1991). A series of test carried out by Santoro & Koestler (1991) has devised a method for evaluating the ability of a consolidant or water proofing agent to support fungal growth. In these tests, it was found that some seemingly similar products had opposite effects: for example one permitted fungi to grow easily while the other did not. The procedure in particular evaluated sporulation, percent coverage, and weight loss in a statistical design procedure. The implications of this work are that polymers added to a material may lead to increase microbial growth. So, while trying to control one set of stone deterioration problems, we may inadvertently be encouraging others. The mixed microbial community in the biofilms on stone produce many different biopolymers that no doubt are a nutrition source for other microbes. Adding a polymer during a conservation treatment may be just another nutrient to some microbes.

Attempts to control a good nutrient consolidant/water proofing material using a biocide mixed in with the product were performed by various Authors (Grant & Bravery, 1985; Rasnowsky et al., 1988; Tudor et al., 1990; Leznika, 1992; Mamonova et al., 1992; Tiano et al., 1995). The results of these experiments do not yet permit to evaluate the real effectiveness of these mixtures, and further researches are necessary. Moreover it is necessary to consider the methods of cleaning and the substances used in the other phases of restoration: biocides can react with these treatments to turn the fresco or stone colour (Bettini et al., 1988; Tudor et al., 1990).

5. NORMAL COMMISSION STANDARDS

Italy was one of the first countries in which the necessity to establish common methods of studying stone decay and treatments' effectiveness developed. In 1978 the NORMAL Commission was created with the aim to define a series of «NORMAL Recommendations» dealing with this topic. Recently the Biological Group focused its activity on the control methods of biodeterioration to try to establish some guidelines in a field largerly empiric until that time.

The Normal Commission has published a first set of suggested standards concerning biocides' use on stone materials. *NORMAL 30/89 «Methods to control biodeterioration»* illustrates the direct and indirect methods (manual, physical and chemical) that can be used against biodeterioration and different methodologies of biocide application. *NORMAL 35/91 «Characterization of biocides: scheme of card»* refers to the collection of necessary information about physical, chemical, toxicological, and utilization data of biocides and active ingredients. *NORMAL 37/92 «Treatments with biocides: scheme of card to file data»* established how to gather a series of necessary information about biocide treatments including data about artwork, treated area, alterations, analyses performed, application regime, products, effectiveness of the treatment, and description of other kind of intervention (cleaning, application of consolidant or water repellent). Infact the information about biocidal treatments in literature are abundant but often defective, so it is impossible to compare data

and results or to perform a statistical analysis. *NORMAL 38/93 «Experimental evaluation of biocides' effectiveness»* illustrates some tests useful to evaluate the effectiveness of products in the laboratory and of biocidal treatments in situ.

Moreover in 1991 the Subgroup «Biocides-stone materials interference» (with biologists, chemists, geologists and restorers) was created with the aim to devise and standardize procedures, on the basis of experimental work simultaneously performed in different laboratories, to assess any collateral damage to the porous building material being treated with biocides.

6. RECOMMENDATIONS FOR ACHIEVING PROGRESS IN EVALUATING BIOLOGICAL PRODUCTS FOR THE CONSERVATION OF BUILDING MATERIALS

Based upon the initial success of this first workshop on evaluating the effectiveness of biocides for the conservation of porous building materials, some recommendations are presented.

1. A biocide is not a panacea for all microbial problems and, indeed, a biocide should not be used only for esthetic reasons.

2. The application of a simple biocide to control all organisms is naive.

3. Biofilms on the surface may be exceedingly complex. A biocide may actually encourage the secretion of biopolymers by the organisms present.

4. There is some question as to whether we should be trying to remove the biopolymers rather than the microbes directly, when cleaning of the surface is desired. There are many studies in the biodeterioration and microbiological literature that address aspects of measuring for microbial activity that could be adapted for use in evaluating the effectiveness of biocides. What is required though is an international body to evaluate the literature and different methodological approaches and then to recommend specific guidelines for evaluation procedures. A small working group meeting, perhaps under ICCROM, or a EuroCare Project, should be held to undertake this task.

REFERENCES

Bettini, C., Agarossi, G., Ferrari, R. & Monte, M. (1988): Fenomeni di biodeterioramento in ambienti ipogei dipinti. Esperienze di controllo di alcune specie microbiche. In *«Proceedings of the 2nd International Conference on Non-destructive Testing, Mycroanalytical Methods and Environment Evaluation for Study and Conservation of Works of Art». Perugia. Comas Grafica, Roma*, 1-14.

Caneva, G., Nugari, M. P., Pinna, D. & Salvadori, O. (1996): Il controllo del degrado biologico. I biocidi nel restauro dei materiali lapidei. *Nardini Editore, Firenze*, 208 pp.

Grant, C. & Bravery, A. F. (1985): Laboratory evaluation of algicidal biocides on construction materials. 3. Use of vermiculite bed technique to evaluate toxic washes, surface coatings and surface treatments. *International Biodeterioration*, 21(4), 285-293.

Klausmeier, R. E. (1971): Mix of pure culture inocula for assessing biodeterioration of plastics: an interlaboratory study. In *«Biodeterioration of Materials». A. H. Walters & E. H. Hueck-van der Plas Editors. John Wiley and Sons, New York, Vol. II*, 201-207.

Koestler, R. J. (1993): Inert eradication using controlled atmospheres, and FTIR measurement for insect activity. In *«Proceedings of ICOM, 9th Triennial Meeting». Washington D.C., August 22-27, 1993, Vol. II*, 882-886.

Koestler, R. J. & Mathews, T. F. (1994): Application of anoxic treatment of insect control in manuscripts of the library of Megisti Laura, Mount Athos, Greece. In *«Actes des Journees Internationales d'Etudes de l'ARSAG, Environment et Conservation de l'Ecrit, de l'Image et du Son». Association pour la Recherche Scientific sur les Arts Graphiques, 36, rue Geoffroy St. Hilaire, 75005 Paris*, 59-62.

Koestler, R. J. & Santoro, E. D. (1988): Assessment of the susceptibility to biodeterioration of selected polymers and resins. *Special limited publication of The Getty Conservation Institute, 4503 Glencoe Ave., Marina del Rey, CA*, 98 pp.

Koestler, R. J., Parreira, E., Santoro, E. D. & Noble, P. (1993): Visual effects of selected biocides on easel painting material. *Studies in Conservation*, 38(4), 265-273.

Leznicka, S. (1992): Antimicrobial protection of stone monuments with phydroxybenzoic acid esters and silicone resins. In *«Proceedings of the 7th International Congress on Deterioration and Conservation of Stone», Lisbon*, 481-490.

Mamonova, I. V., Kurochkin, V. E., Paramonov, G. A., Petrjakov, O. & Panina, L. K. (1992): The determination of threshold concentrations of biocides for

museum exhibits by a photometric methods. *International Biodeterioration*, 30, 303-312.

Nieto, F., Lee J. J. & Koestler, R. J.: Changes in nucleotide content and energy charge of the mycelia of three species of wood decaying fungi during growth in batch cultures. *In preparation.*

Nieto, F., Lee, J. J. & Koestler, R. J.: Nucleotide extraction from the mycelium of the wood decaying fungus *Phanerochaete chysosporium* growth in liquid batch cultures and in wood blocks. *In preparation.*

NORMAL (1991): Metodo di controllo del biodeterioramento, *NORMAL-30/89, CNR-ICR*, 1-11.

NORMAL (1994): Caratterizzazione di biocidi: schema di scheda, *NORMAL-35/91, CNR-ICR*, 1-5.

NORMAL (1994): Trattamenti biocidi: schema di scheda per archiviazione dati, *NORMAL-37/92, CNR-ICR*, 1-8.

NORMAL (1994): Valutazione sperimentale dell'efficacia dei biocidi, *NORMAL-38/93, CNR-ICR*, 1-6.

Nugari, M. P. & Priori, G. F. (1985): Resistance of acrylic polymers (Paraloid B72, Primal AC33) to microorganisms. – First part. In *«Proceedings of the 5th International Congress on Deterioration and Conservation of Stone». Presses Polytechniques Romandes, Lausanne*, 685-693.

Nugari, M. P., D'Urbano, M. S. & Salvadori, O. (1993a): Test methods for comparative evaluation of biocide treatments. In *«Proceedings of the International UNESCO/RILEM Congress on Conservation of Stone and Other Materials». M. J. Thiel Editor. E & FN Spon, London*, 565-572.

Nugari, M. P., Pallecchi, P. & Pinna, D. (1993b): Methodological evaluation of biocidal interference with stone materials - preliminary laboratory tests. In *«Proceedings of the International UNESCO/RILEM Congress on Conservation of Stone and Other Materials». M. J. Thiel Editor. E & FN Spon, London*, 295-302.

Rasnowsky, W., Matraka, W. & Uhacz, K. (1988): The conservation of monumental stone fountains on the example of Neptun fountain in Gdansk. In *«Proceedings of the 6th International Congress on Deterioration and Conservation of Stone», Nicholas Copernicus University, Torun*, 740-747.

Salvadori, O. & Nugari, M. P. (1988): The effects of microbial growth on synthetic polymers used on works of art. In *«Biodeterioration 7». D. R. Houghton, R. N. Smith & H. O. W. Eggins Editors. Elsevier Applied Science, London and New York*, 424-427.

Santoro, E. D. & Koestler, R. J. (1991): A methodology for biodeterioration testing of polymers and resins. In *«Biodeterioration of Cultural Property». R. J. Koestler Editor. Elsevier Applied Science, New York*, 81-90.

Sorlini, C., Falappi, D. & Ranalli, G. (1991): Biodeterioration preliminary tests on samples of Serena stone treated with resins. *Annales des Microbiologie*, 41, 71-79.

Tiano, P. Camaiti, M. & Accolla, P. (1995): Methods for the evaluation of products against algal biocoenosis of monumental fountains. In *«Preprints of ICCROM, International Colloquium on Methods of evaluating products for the conservation of porous building materials in monuments», Rome, June 19-21, 1995*, 75-86.

Tudor, P. B., Matero, F. G. & Koestler, R. J. (1990): Restoration of an outdoor marble sculpture in New Orleans: a case study of the compatibility of biocidal cleaning and consolidation. In *«Biodeterioration Research 3». G. C. Llewellyn & C. E. O'Rear Editors. Plenum Press, New York*, 525-533.

Young, M. E., Wakefield, R., Urquhart, D. C. M., Nicholson, K. & Tonge, K. (1995): Assessment in a field setting of various biocides on sandstone. In *«Preprints of ICCROM, International Colloquium on Methods of evaluating products for the conservation of porous building materials in monuments», Rome, June 19-21, 1995*, 93-99.

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