REZISTENȚA UNOR NOI MORTARE LA BIODETERIORARE
RESITANCE OF NEW MORTARS TO BIODETERIORATION

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The fungal resistance of 4 new mortars was studied according to SREN 847:2000 (ISO 847:1997). Spore suspensions of filamentous fungi belonging to Aspergillus niger, Cladosporium herbarum, Ulocladium chartarum and Penicillium sp., isolated from the contaminated frescoes of Amărăşti and Ioneşti churches from Vâlcea County, were inoculated onto the surface of mortars with and without glucose and incubated in humid chambers (RH = 90-95%, temperature = 22-26^{0}C); fungal growth was analyzed at 1, 3, 6 and 9 months by visual inspection, optical microscope and Scanning Electron Microscope. The fungal growth on the surface of mortars with and without glucose, the toxic effect of mortars as well as mortars resistance to biodeterioration were marked with numbers 0-5. Due to the fact that all mortars had been sensitive to biodeterioration, chemical treatments with 10% Preventol RI 50, 8% Biotin R and 3% Biotin T had been performed. Assessment of the efficiency of biocides for decontamination allowed suggesting a procedure to be followed in order to obtain long term resistant mortars and to suggest the procedure of chemical treatment in case that new fungal growth will be detected.

Rezistenţa la fungi a unui număr de 4 noi mortare a fost studiată în conformitate cu SREN 847:2000 (ISO 847: 1997). Suspensii de spori obţinute de la fungi filamentoşi aparţinând speciilor Aspergillus niger, Cladosporium herbarum, Ulocladium chartarum şi Penicilium sp. izolate de pe frescele contaminate de la biserica Amărăşti din judeţul Vâlcea au fost inoculate pe suprafaţa mortarelor în prezenţa şi în absenţa glucozei. După incubarea în camere umede (RH = 90-95%, temperatura = 22-26^{0}C), creşterea fungilor a fost evaluată la 1, 3, 6 şi 9 luni, prin analiza vizuală, la microscopul optic şi la cel cu baleiaj. Creşterea fungică pe suprafaţa mortarului în prezenţa şi în absenţa glucozei, efectul toxic al mortarelor şi rezistenţa acestora la biodeteriorare a fost exprimată valoric (0-5). Ca urmare a faptului că mortarele au fost sensibile la biodeteriorare s-a studiat sensibilitatea fungilor la următorii biocizi: Preventol RI 50 10%; Biotin R 8%; Biotin T 3%. Stabilirea eficienţei biocizilor în activitatea de decontaminare a permis elaborarea procedeului adecvat de obţinere a mortarelor precum şi a metodologiei de decontaminare in situ.

Keywords: *mortar, biodeterioration, spore suspensions, fungal growth, biocides, mortar toxicity*

1. Introduction

Biodeterioration of cultural heritage is a complex of undesirable changes of the surface produced by living organisms which interact with mineral materials, organic deposits and environment. They colonize the material surface, its pores and microcracks causing aesthetical, functional and structural changes [1].

The main microorganisms which are able to deteriorate historical monuments and materials used for restoration belong to bacteria (*Halobacillus* sp., *Halobacillus naozuensis*, *Rubrobacter* sp. [2], lichens (*Lecanora dispersa*, *Lecanora albescens*, *Xanthoria parietina*, *Caloplaca* sp. [3] and fungi (*Alternaria alternata*, *Aspergillus niger*, *Cladosporium sphaerospermum*, *Penicillium* sp., *Ulocladium tuberculatum*, *Stachybotrys chartarum*, *Trichoderma* sp. [4, 5]. There are not microorganisms able to grow below the equilibrium relative humidity of the substrate (pictorial layers, mortars, bricks). Xerophilic fungi are able to grow below 70%; opposite, hydrophilic microorganisms (bacteria and fungi) require 90-95%. Pink biopigmentation was found on wall paintings [2, 4, 6-8] and archaeological monuments [8].

The mortars used in restoration of mural paintings have a significant importance on the growth of biodeteriogens. Biodeterioration starts with the colonization of the work surface but, over time, undesirable aesthetic changes based on chemical and physical damages could take place [9, 10]. Heterotrophic microorganisms (bacteria and fungi) use mortars (original and infilling) as well as pictorial layer both as niche and nutrients [11]. The roughness, porosity, and composition of the mortar, the availability of moisture and nutrients stimulate and maintain colonization and biodeterioration. Over time, biodeterioration cause aesthetical, structural and chemical damages as a result of biodeteriogens ability to produce enzymes, organic acids and pigments and to adapt in various environments with poor carbon source, low or high pH and temperature [4, 12]. The cellulose content

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from vegetable debris of mortars is favorable for fungi which can start colonization without any other organic compounds [13]. Generally, the presence in mortars of chemical compounds like $CaSO_4$, oxides of Ca, Al, Si, Fe, Mn, K, Na, S and biocides are impeding synthesis of cellular components as well as enzymes involved in biodeterioration like cellulases. In case of infilling mortars, organic deposits accumulated in time stimulate producing of organic acids (citric, succinic, malic, oxalic) by fungi causing dissolution of the mineral components of mortars in particular of cations (Ca, Mg, Al, Mn, Fe, Si, K). As a consequence, calcium losses of material mass take place. Meristematic fungi cause intercrystalline growth and so, physical disruption of the weakest structural components of the crystals takes place, resulting biopitting and formation of cracks and fissures [14]. Mycelium has been also found on the surface, in the matrix of mortars and in cracks [15]. Biological activity increases mortar porosity which in turn changes the diffusivity of the mortar.

The characterization of microbiota is performed by traditional cultivation methods for enrichment and isolation and by molecular techniques like 16S rRNA gene sequence denaturating gradient gel electrophoresis (DGGE) and fluorescence *in situ* hybridization [15].

One of the widely used ways to investigate the resistance of the new materials including mortars used in restoration is to carry out accelerated tests in laboratory. Protection of mortars from microbial degradation can be enhanced by treatments with biocides [16], adding of protective coatings [17] and reducing the pores size, but keeping them large enough to assure water evaporation [18, 19]. The efficiency of biocides, found as their ability to provide lethal action, depends on concentration, pH, temperature, time of contact, microorganisms' resistance, microbial biofilm, migration of biocide in the depth of the substrate or its leaching rate.

The aim of this study is to analyze the resistance of different new mortars prepared for restoration of mural painting and to suggest a procedure to be followed in order to obtain long term resistant mortars. The chemical treatment is also recommended in case new fungal growth will be detected.

2. Materials and Methods

2.1. Mortars

A number of 4 different mortars (M4; M5; M6, M7) had been prepared to be tested for their possible application in case of wooden churches to restore adherence between mural painting and support. They had different lime: river sand ratio, hemp tow and Acril 33 (Table 1 [5]). River sand had different grain sizes: 0.01-0.500 mm or 0.01-0.710 mm. After 28 days of hardening, all mortars have low apparent density, higher water absorption and moderate mechanical strength at compression (Table 2) [5]. All of them have rough surface.

2.2. The fungal growth on the mortars and mortar resistance

Resistance of mortars to biodeterioration had been evaluated according to SR EN 846 [20] based on ISO 846 [21]. We brought a change regarding the fungal strains using as inoculum strains isolated from the mural paintings of some wooden churches (Amăraşti and Ioneşti from Vâlcea County, Romania). After inoculation, samples had been placed in humid chambers (RH=90-95%, temperature=22-26^{0}C). Colonization of mortars, expressed as fungal growth had been evaluated by visual and microscopic examination (Optical and Scanning Electron Microscopes LM; SEM) performed after 1, 3, 6 and 9 months. The fungal growth on mortar versus mortar + glucose and the fungitoxic effect were evaluated according to Table 3 and the assessment of short respectively long term resistance according to Table 4.

Table 1

Compositions of the mortars / *Compoziţia mortarelor*

Mortar code / *Cod mortar*	Dry powder, gravimetric parts / *Pulbere uscată, părţi gravimetrice*			Hemp tow / *Câlţi de cânepă*, %	Acril 33, %
	Hydrated calcium lime / *Var calcic hidratat*	River sand/ *Nisip de râu*			
		<0.710 mm	<0.500 mm	12-15 mm	
M4	1	0.5	-	1	2
M5	1	1	-	1	2
M6	1	2	-	1	2
M7	1	-	0.5	1	2

Table 2

Physico-mechanical characteristics of the mortars / *Caracteristici fizico-mecanice ale mortarelor*

Mortar code / *Cod mortar*	Apparent density *Densitate aparentă (g/cm^3)*	Water absrorption *Absorbţie apă (%)*	Mechanical strength / *Rezistenţa mecanică la* (MPa)	
			flexural încovoiere	*compressive* compresiune
M4	1.47	24.09	1.4	3.0
M5	1.58	19.59	1.5	3.2
M6	1.67	17.23	1.2	3.1
M7	1.45	24.67	1.5	3.2

Table 3

The fungal growth and fungitoxic effect of mortars / *Creşterea fungică şi a efectului toxic al mortarelor*

Note	Fungal growth / *Creşterea fungilor*	Fungitoxic effect / *Efectul fungitoxic*
0	no fungal growth / *nu are loc creşterea fungilor*	high fungitoxic effect / *efect fungitoxic mare*
1	microscopically visible growth / *creşterea fungilor vizibilă la microscop*	low fungitoxic effect: germinated spores / *efect fungitoxic redus: are loc germinarea sporilor*
2	visible fungal growth: 25% surface covered by mycelium / *creşterea fungilor vizibilă: 25% din suprafaţa mortarului acoperită de miceliu*	partial fungitoxic effect: germination, few and short hyphal branches / *efect fungitoxic parțial, câteva hife cu ramificaţii scurte*
3	50% surface covered by mycelium / *50% din suprafaţa mortarului acoperită de miceliu*	reduced fungitoxic effect: visible colonization on 25% mortar surface / *efect fungitoxic redus: colonizarea vizibilă pe 25% din suprafaţa mortarului*
4	more than 50% surface covered by mycelium / *mai mult de 50% din suprafaţa mortarului acoperită de miceliu*	reduced fungitoxic effect: visible colonization on 50-75% mortar surface/ *efect fungitoxic redus: colonizarea vizibilă pe 50-75% din suprafaţa mortarului*
5	all surface colonized / *toate suprafețele colonizate*	no fungitoxic effect: entire mortar surface colonized / *efect fungitoxic absent: întreaga suprafaţă a mortarului colonizată*

Table 4

Assessment of short respectively long term resistance of mortars / *Evaluarea rezistenţei mortarelor pe termen scurt şi lung*

Note	Short term resistance / *Rezistenţa pe termen scurt*
0	highly resistant – fungitoxic effect (it does not contain nutrients for fungi) / *rezistenţă mare – efect fungitoxic (nu conţine nutrienţi pentru fungi)*
1 - 2	moderately resistant – partial fungistatic effect (it contains low quantities of nutrients allowing germination of spores: 1-2 inoculated species) / *rezistenţă medie – efect fungistatic parţial (conţine cantităţi mici de nutrienţi care permit germinarea sporilor aparţinând la 1-2 specii*
3	sensitive (it contains nutrients allowing colonization lower than 25%) / *rezistenţă scăzută (conţine nutrienţi care permit colonizarea a mai puţin de 25% din suprafaţă)*
4 - 5	very sensitive (it contains nutrients allowing 25-100 % colonization) / *rezistenţă foarte scăzută (conţine nutrienţi care permit colonizarea a 25-100 % din suprafaţă*

Table 5

Assessment of fungal growth and mortar resistance to biodeterioration (9 months)
Evaluarea creşterii fungice şi rezistenţa mortarelor la biodeteriorare

Mortar code *Codul mortarului*	Fungal growth on mortar / *Creşterea fungilor pe mortar*	Fungal growth on mortar + glucose *Creşterea fungilor pe mortar+glucoză*	Short –term mortar resistance/ *Rezistenţa mortarului pe termen scurt*	Long-term mortar resistance / *Rezistenţa mortarului pe termen lung*
M4	2	3	moderately resistant / *rezistenţă moderată*	sensitive / *sensibil*
M5	3	5	sensitive / *sensibil*	very sensitive / *foarte sensibil*
M6	5	5	very sensitive / *foarte sensibil*	very sensitive / *foarte sensibil*
M7	2	2	moderately resistant / *rezistenţă moderată*	moderately resistant *rezistenţă moderată*

2.3. Inoculation of mortars

All mortars, after 28 days of hardening, had been inoculated with aqueous mixed spore suspension belonging to *Aspergillus niger, Cladosporium herbarum, Ulocladium chartarum* and *Penicillium sp.*). These fungal species had been grown on yeast extract-glucose-chloramphenicol-agar -YGCA. Mixt inoculum had been placed on the mortar surface with and without glucose (1%).

2.4. Decontamination and cleaning of the mortars

Decontamination of mortars had been performed with 10% PREVENTOL RI 50, 8% BIOTIN R and 3% BIOTIN T. The chemical treatment efficiency was assessed by inoculation of samples on nutrient placed in Petri dishes. Also the surface of the mortars has been analyzed by microscopy (LM and SEM). In the end, all mortars were mechanically cleaned.

2.5. Viability

Viability of spores from the surface of colonized mortar, as well as samples from the decontaminated mortars was assessed by inoculation on nutrient (YGCA) and incubation at 28^0C for 15 days.

3. Results and Discussion

3.1. Fungal growth and resistance of mortars to biodeterioration

Germination of spores took place on all mortars, but this process went on to produce either small, white, nonsporulated mycelia (M4, M7), or sporulated mycelia covering 25-100% surface (M5, M6). Taking into account that M4 and M7 mortars have a low content of organic compounds and a high lime: river sand ratio, these mortars were considered moderately resistant on short term (Table 5).

The same mortars with 1% glucose added have low resistance on long term (Table 5). It is considered that glucose can substitute the organic compounds accumulated in time on the surface of mortars applied *in situ*. From this perspective, only M7 mortar can be considered as moderately resistant. All the others are sensitive (M4) or very sensitive (M5, M6).

Visual examination revealed the appearance of mycelium from day 10 after inoculation on the mortar+glucose and from day 15 on the mortar without glucose. Between 30-90 days, an increased colonized surface of all samples had been noticed and till 9 months, the rhythm of surface colonization decreased very much (Table 6). After 90 days of incubation, the crystallization of salts was more visible on M6 and M7 mortar. Biodeteriogens grew better on the mortars with glucose, producing significant biomass and a large surface of colonization.

Colonization of the mortars begins with the intimate contact with spores which is depending on the following chemical and physical characteristics: surface roughness, porosity, water absorption, hydrophobicity, ionic properties, surface properties of biodeteriogens cell wall, toxic compounds. These characteristics have as result the biocompatibility between the biodeteriogens and the type of mortar that will be colonized. Mortar surface roughness allows fungal spores to attach. There is a strong correlation between the surface roughness, the extent of colonization and the environmental conditions (microclimate). The porosity of mortars is also favorable for colonization because pores are involved in water absorption and retention; porous structure allows fixing of hyphae and spores. The bioreceptivity of mortar is assured by surface roughness, porosity, water availability and water absorption. The hydrophobicity of the substrate is very important for the microbial adhesion and attachment. The cells are not able to deeply penetrate the porous substrate because of their need for oxygen. The hydrophobicity/hydrophilic and the ionic properties of the mortars may change over time due to chemical reactions, as well as to metabolic products of microorganisms and in consequence, the bioavailability can be modified or replaced by biostability [3].

Table 6

Visual examination of fungal growth on mortar and mortar+glucose (9 months)
Examinarea vizuală a creşterii fungilor pe mortar şi mortar+glucoză (9 luni)

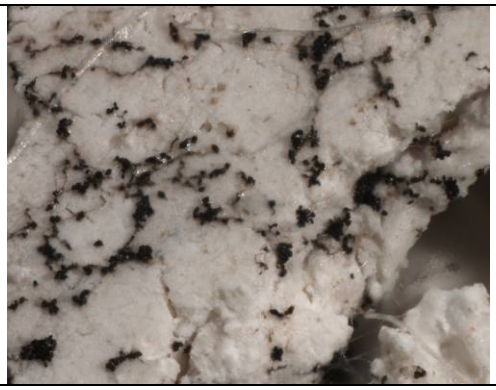
Mortar code *Codul mortarului*	Fungal growth on mortar / *Creşterea fungilor pe mortar*	Fungal growth on mortar+glucose / *Creşterea fungilor pe mortar+glucoză*
M4		
M5		

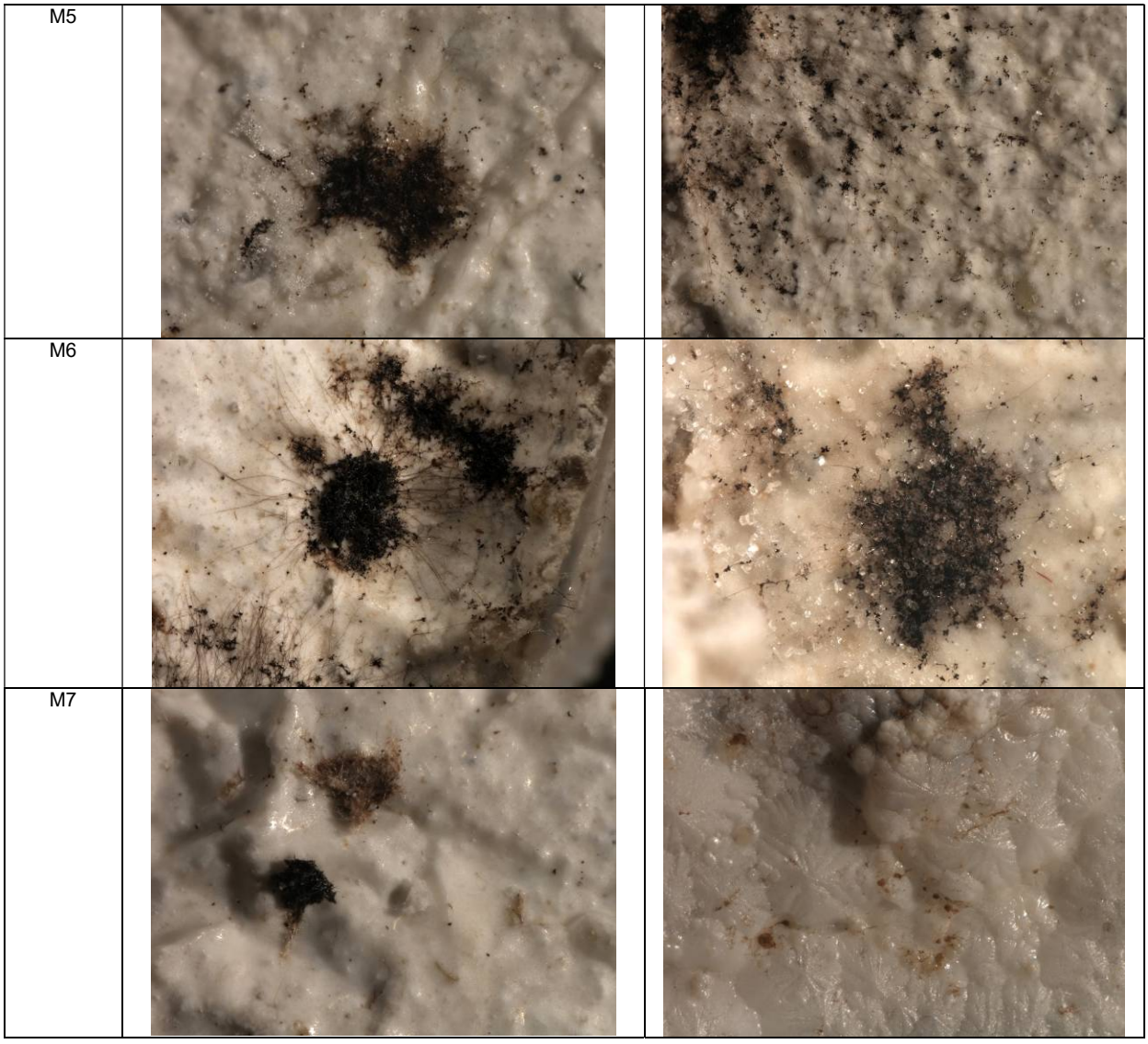
M6		
M7		

Optical observations confirmed the presence of germination spores, branched hyphae, white non sporulated mycelium, sporulated mycelium belonging to all inoculated species (table 7). The addition of glucose stimulated spores germination. This process was identified by microscopical examination starting with the third day after inoculation. Crystallization of salts took place on the surface of all types of mortar. Microscopical observations confirmed an extensive crystallization of salts on M5 and M7 mortars. The fact that fungi are able to grow also on the mortars covered by salts reveals that all inoculated strains are resistant to high concentration of salts. In case of M7 mortar+glucose, it was noticed both by visual and microscopical examination that the surface was completely covered by a crust of salts developed after colonization. This process may have occurred *in situ* and thus, only through visual examination, the sporulated mycelium located beneath the crust could not be identified. Only when the crust will fall or be removed, the hyphae and spores will be detached and will contaminate surrounding areas.

Table 7

Microscopical examination of fungal growth on mortar and mortar+glucose (9 months)
Examinarea microscopică a creşterii fungilor pe mortar şi mortar+glucoză (9 luni)

Mortar code / *Codul mortarului*	Fungal growth on mortar / *Creşterea fungilor pe mortar*	Fungal growth on mortar+glucose / / *Creşterea fungilor pe mortar+glucoză*
M4		



3.2. Decontamination of mortars

Due to the fact that all mortars had been sensitive to biodeterioration, chemical treatments with 10% Preventol RI 50, 8% Biotin R and 3% Biotin T had been performed. Visual examination, as well as optical microscopy performed after treatment showed that new fungal growth did not occurred. Shrunken mycelia, groups of hyphae, germinated and non-germinated spores on the surface and into the cracks were identified on scanning electron photomicrographs (Fig.1 – Fig.4). Similar results were also found by Kamh [22].

Choice of chemical treatment was based on available biocides for restoration of historical monuments. It was not found efflorescence or powdery deterioration after one year of decontamination. The monitoring of new growth and deterioration of mortars is in progress.

The viability test also revealed no viable spores or hyphae. Not all hyphae or spores could be removed by mechanical cleaning. SEM images showed that some hyphae and spores were still attached on the surface of mortars (Fig.2, Fig.3). Because of the melanin from the cell walls and spores, the cleaned surface remained dark colored. On samples treated with Biotin R, groups of hyphae and spores covered by biocide crystals were found (Fig.3, Fig.4).

Biotin R is a complex biocide containing OIT (2-Octyl-2H-isothiazol-3-one) and carbamate (sodium dimethyl dithiocarbamate and disodium ethylene bisthiocarbamate). The first component covalently reacts with the cellular nucleophiles to inactivate enzymes and initiate the formation of intracellular free radicals which contribute to their actions. The second component promotes cell aggregation, inducing changes in the surface electric charge of the cell from negative to positive or neutral [23, 24].

Biotin T is a mixture of OIT and quaternary ammonium salts. Cationic membrane active biocides like quaternary ammonium compounds act on membranes leading to cell lysis [3].

Preventol RI 80 contains also quaternary ammonium salts and lead to cell lysis [25, 3].

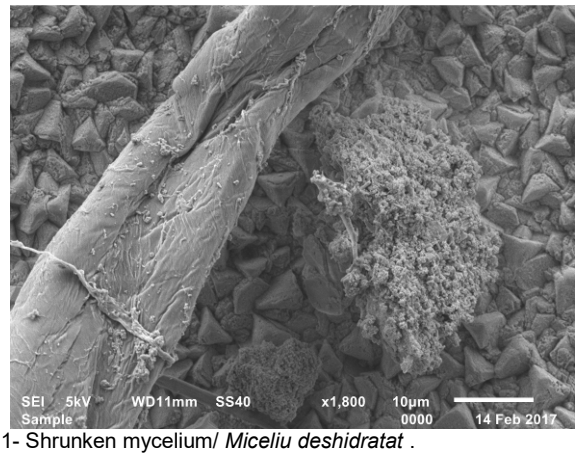


Fig.1- Shrunken mycelium/ *Miceliu deshidratat* .

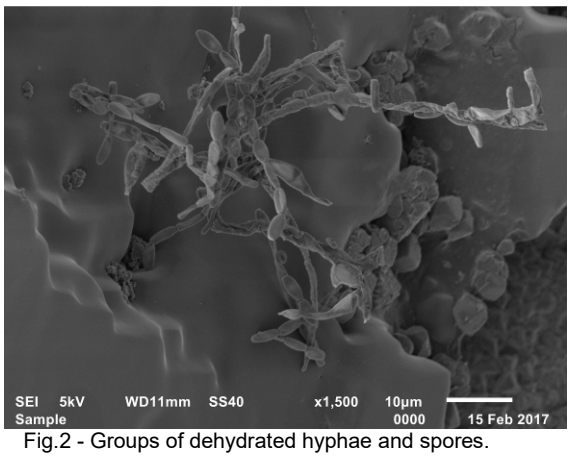


Fig.2 - Groups of dehydrated hyphae and spores.
Grupuri de hife şi spori deshidrataţi.

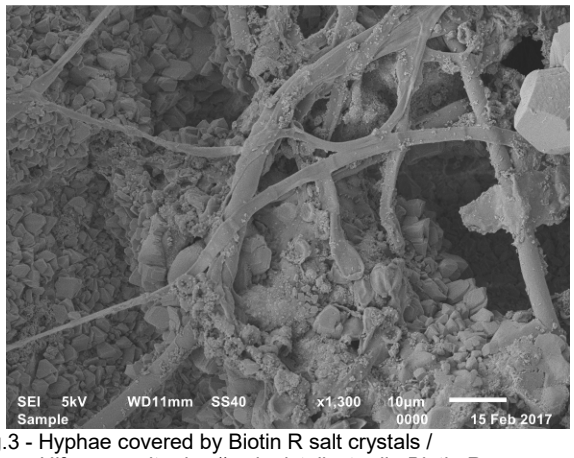


Fig.3 - Hyphae covered by Biotin R salt crystals /
Hife acoperite de săruri cristalizate din Biotin R.

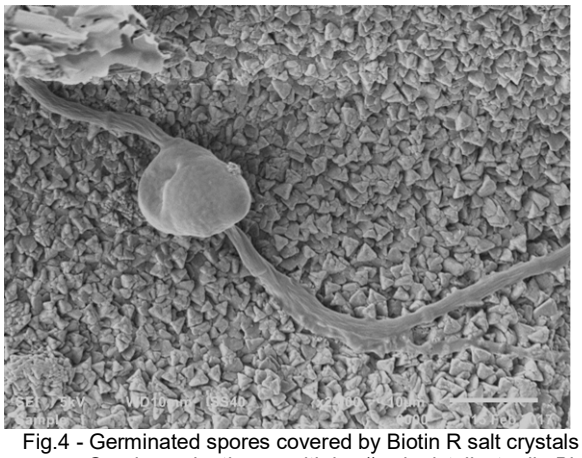


Fig.4 - Germinated spores covered by Biotin R salt crystals
Spori germinaţi acoperiţi de săruri cristalizate din Biotin R.

To prevent fungal growth *in situ* we recommend adding of 8% Biotin R during preparation of mortars.

Some of biocides used for decontamination can be found in other papers. With the aim of mitigating biodeterioration, Coutinho et al [26] applied *in situ* four different biocides: TiO_2 nanoparticles, Biotin T®, Preventol® RI 80 and Albilex Biostat® and monitored their efficiency. They found significant changes in the microbial community composition after 4 months of treatment with Biotin T®, Preventol® RI 80 and after 6 months of treatment with TiO_2 nanoparticles.

The main characteristics of the biocides used in conservation and restoration of historical monuments are: biocidal efficiency on a wild range of microorganisms including those isolated on the artifacts, stability under environmental conditions, stability of the biocide efficiency in case of changes of the substrate (pH, metabolic products provided by other biodeteriogens, increased porosity, changes in water availability including development of efflorescences etc), compatibility with the surface, including protection of the pictorial layer (avoiding color change, staining, discoloration, chemical degradation).

4. Conclusions

New mortars based on lime, river sand, hemp tow and additives had been analyzed for their resistance to biodeteriogens expressed as their bioreceptivity for fungal growth. All of them were colonized by *Aspergillus niger, Cladosporium herbarum, Ulocladium chartarum* and *Penicillium sp.,* but with different rates. The bioreceptivity of mortars is assured by surface roughness, porosity, water availability and water absorption. M4 and M7 mortar samples revealed moderate resistance for short -term and only M7 had moderate resistance for short and long-term.

Resistance of mortars to biodeterioration has to be evaluated not only according to SR EN 846 and ISO 846 but also by optical and electron microscopy observations.

To prevent fungal growth on mortars applied *in situ*, the adding of 8% Biotin R is required during preparation process.

Microscopical and viability of spores analysis proved the efficiency of Biotin R for short and long-term conservation.

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