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Micromycetes as colonizers of mineral building materials in historic monuments and museums

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ABSTRACT

Complex of microfungi colonizing mineral building materials, i.e. limestone and plaster, in interiors of cultural heritage was characterized. Wide-scale investigation was carried out with fourteen objects studied. We have revealed a specific culturable community. We have analyzed role of obtained microfungi in biodeterioraton process on the basis of our tests (pH and water activity preferences, ability to solubilize $CaCO_3$) and literature data (substrate preferences and enzyme activities). The species most actively developing in mineral materials in indoor environments were *Acremonium charticola*, *Acremonium furcatum*, *Lecanicillium* sp., *Parengyodontium album*, *Purpureocillium lilacinum* and *Sarocladium kiliense*. Considering this fact and their ability to develop successfully at extremely wide range of pH values from slightly acidic to alkaline ones and their high enzymatic activities we conclude that the listed species are of high interest in seeking the cause of biodeterioration. These species can actively develop in materials penetrating for years deep into the substrates and causing their deterioration in conditions of considerably heightened moisture content. In this group, *A. charticola* and *Lecanicillium* sp. were able to solubilize $CaCO_3$.

1. Introduction

Restoration and preservation of historical objects requires understanding the causes and mechanisms involved in the deterioration. Colonization of stone building materials by micromycetes have received a lot of attention in recent years, as fungi are shown to play one of the leading roles in their deterioration (Gadd, 2007, 2017; Gorbushina et al., 2004; Suihko et al., 2007; Warscheid and Braams, 2000). If conditions for fungal growth are suitable, fungal hyphae may quickly penetrate mineral substrates (Warscheid and Braams, 2000) and with time fungi grow in depth (Ciferri, 1999). While developing on mineral materials, fungi affect them both mechanically and chemically. All these processes cause aesthetic damage, such as surface stains, as well as material etching, pitting, sanding, etc (Ciferri, 1999; Gaylarde and Morton, 1999; Warscheid and Braams, 2000).

It was shown that microorganisms (i.e. bacteria, algae, etc.) can form biofilms on stone surfaces (Gaylarde and Morton, 1999; Morton and Surman, 1994). When developing biofilms, the microorganisms significantly increase their tolerance to biological, physical or chemical stresses including resistance to antimicrobial agents (Harding et al., 2009; Morton and Surman, 1994). Fungi are essential members of these complex microbial consortia (Gadd, 2007; Morton and Surman, 1994; Suihko et al., 2007).

Many researchers have analyzed mycobiota of stone surfaces in interiors of one (eg. Gorbushina et al., 2004; Karpovich-Tate and Rebrikova, 1990; Šimonovičová et al., 2004b) or several objects of cultural heritage (eg. Berner et al., 1997; Suihko et al., 2007). Meanwhile, summarizing of data on the species development on indoor mineral substrates and their role in deterioration is required. Abundance and frequency of occurrence of isolated fungal species reflect their development in material and thus their deteriorative

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potential (Gómez-Cornelio et al., 2012), which is necessary for carrying out effective biocontrol procedures (Suihko et al., 2007). Moreover, these parameters shed some light on specific characters of the community of colonizers of stone building materials.

We believe that data on abundance and occurrence, together with the physiological parameters such as pH and water activity preferences, as well as calcite dissolution activity, will allow to evaluate the role of cultivated species in the process of colonization of mineral building materials in historic monuments and museums.

We have attempted to characterize the main community groups of culturable micromycetes of stone building materials, i.e. limestone and plaster, in interiors of cultural heritage from the point of view of possible participation in the biodeterioration process using both literature data and the results of the experiments presented here.

2. Material and methods

2.1. Sampling and isolation of fungi

Samples of mineral building materials (limestone and plaster) were taken from the indoor surfaces, i.e. walls and columns, of fourteen buildings (six cultural monuments and eight museums) located in Russian cities and villages in the period from Oct 2011 to Nov 2014. Locations of the studied objects are presented at Table 1 and are shown on the map in Supplementary Fig. S1. Samples were taken from areas of loose, powdered and flacking mineral materials with a sterile scalpel (Berner et al., 1997) at the depth of approximately 5–30 mm depending on material weakness. Each sample consisted of material from three points of area of about 5 cm^2, which were mixed to obtain a complex sample. As a control, samples from surfaces without evidence of alteration were taken. Moisture content of sampling sites was measured as a weight percentage compared to the dry weight by the contact microsensor Testo 616 (Testo, Germany) with measuring ranges up to 20 %, and additionally parameters of temperature and relative humidity (RH) in interiors were measured by thermohygrometer IVTM-7K (Eksis, Russia). Detailed descriptions of the sampling sites are provided in Table 1 and appearance of deterioration elements is presented in Supplementary Table S1. Obtained samples were transferred to sterile Petri dishes and directly transported to the laboratory, where sample material was aseptically crushed with a mortar. Altogether 85 plaster samples and 32 limestone samples were analyzed.

For isolation of fungi, the soil-plate method was used (Nikolskaya, 1982; Warcup, 1950). Warcup (1950) stated that this method allows to yield a wider range of species from samples than dilution one, because not all fungi in sample may pass into suspension containing sample particles diluted in water. According to this method a sterile water drop was placed into the sterile Petri plate; 10 mg of powdery sample was aseptically placed into this drop. After that 15–20 ml of warm (45 °C) medium was placed in this plate, and the plate was accurately rotated to mix sample particles in medium. As a result, 10 mg of sample was diluted in 15–20 ml of medium and for our limestone and plaster samples subsequent dilutions were not required. Two agar media, Czapek and Starch Czapek, were used. Czapek was chosen as traditional medium for the isolation and cultivation of fungi. Starch Czapek was shown to be preferable for isolation of certain fungal genera inhabiting interior surfaces, i.e. *Acremonium*, *Gliocladium*, *Scopulariopsis*, etc., as their growth on sucrose-rich Czapek medium can be inhibited by fast growing 'sugar fungi', i.e., *Aspergillus*, *Penicillium*, etc (Rebrikova, 1999). Moreover, Starch Czapek is suitable for isolation of alkalitolerant and alkaliphilic fungal species due to its neutral or slightly alkaline pH values. The composition of Starch Czapek was the following (g/l): starch, 30; $NaNO_3$, 2; K_2HPO_4, 1; $MgSO_4 \times 7H_2O$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; agar, 20, pH 7.2–7.4. We used Rifampicin (2 g/l) for the suppression of bacterial growth (Grum-Grzhimaylo et al., 2016). Final fungal counts in samples as colony forming units per gram (CFU g^{-1}) were estimated after 10 d of incubation at 25 °C. All tests were carried out in duplicate for each media (in four replicates per sample).

2.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was used for the direct study of fungal development on deteriorated building materials. For this purpose, flattened pieces of plaster were taken from areas of deteriorated materials (Table S1). Samples were air-dried and sputter-coated with Pt/Pd using an Eiko IB-3 Ion Coater (Eiko Engineering, Japan) and examined using a JSM-6380LA (JEOL, Japan) scanning electron microscope at Electron microscopy laboratory of Moscow State University Biology Faculty.

2.3. Identification

The isolates were identified by morphological and cultural features using diagnostic keys (Bissett, 1982; Crous et al., 2007; Domsch et al., 2007; Gams, 1971; Klich, 2002; Raper and Fennell, 1965; Raper et al., 1968; Samson et al., 2004; Seifert et al., 2011; Zare and Gams, 2001).

Fungal strains were also identified using nucleotide sequences of the regions from the nuclear ribosomal DNA cistron (rDNA) as a DNA barcode, namely, the internal transcribed spacer (ITS) region and/or the D1/D2 domains of the large subunit (26S/28S or LSU). ITS region was chosen as a universal DNA barcode marker for fungi (Schoch et al., 2012), while LSU is suitable for *Acremonium* identification, as phylogenetic analysis of *Acremonium* is based on this region (Summerbell et al., 2011). The protocols used for DNA extraction, amplification of rDNA regions, purification and sequencing were described by Libkind et al. (2003) and Glushakova et al. (2016). For species identification, the nucleotide sequences were compared with sequences of type strains in the NCBI (ncbi.nlm.nih.gov) and MycoID (www.mycobank.org) databases. The obtained nucleotide sequences were deposited in GenBank under the accession numbers given in Table 2.

2.4. Data analysis

The fungal communities were analyzed according to abundance and frequency of occurrence of isolated species. Abundance of a particular species was defined as the percent composition of the CFU g^{-1} of a particular species relative to the total CFU g^{-1} of all species obtained. Frequency of occurrence of a particular species was defined as the percent composition of number of samples in which a particular species have occurred relative to the total number of all samples obtained. Communities isolated from limestone and plaster were compared using Sørensen-Czekanowski similarity coefficient (*Ks'*) that was calculated according to the formula

$$Ks' = \frac{2\sum_{i=1}^{p}\min(x_{Ai}, x_{Bi})}{\sum x_{Ai} + \sum x_{Bi}},$$

where p – number of taxa; x_{Ai} – abundance of species i in samples A (plaster samples); x_{Bi} – abundance of species i in samples B (limestone samples).

Furthermore, fungal communities from building materials were evaluated by Simpson diversity index (D) that was calculated according to the formula

Table 1
List of the studied objects, their locations, characteristics of the sampling sites and their moisture content.

Object number	Objects	Object location	Sampling material	Sampling sites description	Moisture content of sampling areas, %
Cultural monuments					
1	Bogolyubskiy Cathedral (XIX century)	Bogolyubovo rural locality, Vladimir region	Limestone	Wall areas of loose limestone with high moisture content	8.0, 8.1, 9.0, 12
2	Church of Boris and Gleb (XII century)	Kideksha village, Vladimir region	Limestone, plaster	Wall areas of loose limestone with high moisture content, colonized by algae, with flaking wall areas and with precipitation of salts at the surface; loose plaster arcosolium area	7.8, 8.0, 14.0, 17.4
3	Dormition Cathedral (XII century)	Vladimir city	Limestone	Walls without evidence of deterioration (control)	2.2, 3.7
4	Rozhdestvenskiy Cathedral (XIII century)	Suzdal city	Limestone	Wall areas of loose limestone with high moisture content	6.0, 10.0, 12.2, 14.0
5	Saint George Cathedral (XIII century)	Yuryev-Polsky city	Limestone	Wall areas of loose limestone with precipitation of salts at the surface, sites of columns of loose stone material colonized by algae	4.5, 10.0, 13.3
6	Scientific Research Institute for Restoration (former palace of Elizabeth Petrovna, the Empress of Russia) (XVIII–XIX century), basement	Moscow city	Plaster	Wall areas of loose plaster with cracking and flaking of paint; wall area without evidence of deterioration (control)	4.0 (control area), 4.2, 4.7, 5.2, 5.3
Museums					
7	Kuskovo Manor (XVIII century), the depositary of furniture, semibasement	Moscow city	Plaster	Flaking of paint and plaster wall areas	4.5, 5.1, 6.3, 13.0, 19.0
8	Novgorod State Museum-Reserve, Nikita body Novgorod Kremlin (XV–XVII century)	Velikiy Novgorod city	Plaster	Wall areas with cracking and flaking of paint and plaster and with high moisture content	6.2, 8.5
9	Ostankino Museum-Estate (XVII–XVIII centuries), the Egyptian pavilion	Moscow city	Plaster	Wall areas of loose plaster with cracking and flaking of the paint layer	0.4, 3.5, 3.6, 4.2, 7.0, 9.0
10	The State Tretyakov Gallery on Lavrushinsky Lane (XX century), exhibition halls	Moscow city	Plaster	Wall areas of loose plaster with high moisture content and blistering, cracking and flaking of paint near windows	11.8, 12.0
11	The State Tretyakov Gallery's Archive, building 1, basement (XVII–XIX century)	Moscow city	Limestone, plaster	Facade wall areas of loose plaster with cracking and flaking of paint layer, loose limestone area of the interior wall	6.5, 11.7
12	The State Tretyakov Gallery's Archive, building 2, basement (XVII–XIX century)	Moscow city	Plaster	Facade wall areas of loose plaster with cracking and flaking of paint	9.0
13	The State Tretyakov Gallery's Collection Storage (XX century)	Moscow city	Plaster	Wall areas of loose plaster with brown surface stains, etchings and a hole in the facade wall, cracking and flaking of the paint layer, precipitation of salts at the wall surface in the area contacting with ground; wall surfaces without evidence of deterioration (control)	3.4–4.0 (control area), 6.7, 10.8
14	The Tver Regional Art Gallery (XVIII–XIX century) basement and the first floor of the outbuilding	Tver city	Limestone, plaster	Facade wall areas in basement of loose limestone with extremely high moisture content, brown surface stains, cracking and flaking of the paint and precipitation of salts at the wall surface; blistering and flaking wall areas of loose limestone and plaster at the first floor; the lower side of the walls of loose plaster with high moisture content at the first floor; wall surfaces without evidence of deterioration (control) at the first floor	3.8 (control area), 9.0, 9.5 (first floor), 19.0, >20 (basement)

$$D = 1 - \sum_{i=1}^{S} x_i^2,$$

where x_i is the relative abundance of the ith species; S - species richness. Simpson diversity index near to one indicates high diversity (Kiel and Gaylarde, 2007).

2.5. Growth at different pH values

We have carried out growth tests with all species isolated in essential amounts (10^4 CFU g^{-1} and above). Among species that were detected in materials in amount of 10^3 CFU g^{-1}, we have randomly chosen four of them which abundance was 0.1 % and above. If it was possible, three strains from every tested species were randomly chosen for this experiment. Altogether 38 isolates from 18 species were tested. To elucidate the pH growth optimum of the strains malt yeast agar-based media buffered at five pH levels, i.e. 4, 5, 7, 9 and 10 were used. Media were prepared as described in Grum-Grzhimaylo et al. (2016). Petri dishes contained media with given pH values were inoculated into the center in triplicate for each strain. Petri dishes were sealed thoroughly with Parafilm to avoid desiccation and put at 25 °C in the dark. The growth expansion was followed for 2–4 weeks depending on the strain. Every other day we measured diameter of fungal colonies in two perpendicular directions. To determine linear growth of the strains, the linear regression model was used. The regression coefficient corresponded numerically to the linear growth rate of the colony (Baranova et al., 2017).

2.6. Influence of the water activity of a solid substrate on the fungal growth rate

We have carried out this test with all species isolated in essential amounts (10^4 CFU g^{-1} and above). The basic medium used was malt extract agar (MEA) with pH of 5.46. The water activity of this medium was 0.99. The a_w was modified by the addition of the non-ionic compatible solute, glycerol (Gervats et al., 1988; Marin et al., 1995), in order to obtain a_w levels of 0.95, 0.90, 0.85, 0.80 and 0.75. The required amount of glycerol for the preparation of the solutions with the listed a_w values was calculated according to the formula proposed by Gervats et al. (1988):

$$a_w = (1 - X)e^{-KX^2},$$

Table 2
GenBank accession numbers of strains isolated from deteriorated limestone and plaster in interiors.

Species	Voucher	ITS1-5.8S-ITS2			D1-D2 LSU		
		Top BLAST search results (similarity, %)	Query coverage (%)	NCBI gene accession no.	Top BLAST search results (similarity, %)	Query coverage (%)	NCBI gene accession no.
Acremonium camptosporum		_	_	_	*Acremonium camptosporum* CBS 756.69 (100)	91	LT549085
Acremonium charticola		*Acremonium charticola* CBS 221.70 (99)	100	LT598640	*Acremonium charticola* CBS 221.70 (100)	100	LT598640
Acremonium charticola		*Acremonium charticola* CBS 221.70 (100)	70	LT598639	_	_	_
Acremonium charticola	VKM FW-1539	*Acremonium charticola* CBS 117.25 (100)	100	LT598642	_	_	_
Acremonium charticola	VKM F-4769	*Acremonium charticola* CBS 117.25 (100)	64	LT598643	_	_	_
Acremonium furcatum	CBS 142822	*Acremonium furcatum* P266 D2 27 (99)	100	KY995576	_	_	_
Acremonium furcatum	VTT D-151581	*Acremonium furcatum* P266 D2 27 (99)	100	KY995577	_	_	_
Acremonium furcatum	VTT D-151582	*Acremonium furcatum* P266 D2 27 (99)	100	KY995578	_	_	_
Acremonium furcatum	VTT D-151583	*Acremonium furcatum* P266 D2 27 (99)	100	KY995579	_	_	_
Acremonium furcatum	VTT D-151584	*Acremonium furcatum* P266 D2 27 (99)	100	KY995580	_	_	_
Acremonium sp.1	CBS 142823	*Acremonium* sp. G246 (91)	100	LT549084	_	_	_
Acremonium sp.2	CBS 142819	*Sarocladium kiliense* CBS 400.52 (100)	100	LT598644	*Acremonium charticola* CBS 117.25 (99)	100	LT598644
Acremonium sp.3		*Acremonium charticola* CBS 221.70 (99)	100	LT598641	*Acremonium sordidulum* CBS 385.73 (100)	100	LT598641
Botrytis sp.		*Botrytis elliptica* CBS 204.64 (100)/*Botrytis tulipae* CBS 130.37 (100)	77/76	LT549079	_	_	_
Chordomyces antarcticus	VKM F-4770	*Chordomyces antarcticus* CBS 987.87 (100)	88	LT549083	_	_	_
Cladosporium langeronii	VKPM F-1427	*Cladosporium sphaerospermum* CBS 797.97 (100)/ *Cladosporium langeronii* CBS 189.54 (100)/*Cladosporium psychrotolerans* CBS 119412 (100)	96/97/83	MG589512			
Emericellopsis sp.		_	_	_	*Emericellopsis stolkiae* CBS 159.71 (100)/*Emericellopsis maritima* CBS 491.71 (100)		LT598651
Fusarium sp.	CBS 142825	*Fusarium lichenicola* CBS 115.40 (100)/*Fusarium javanicum* CBS 420.76 (100)	99/100	LT549086	_	_	_
Lecanicillium sp.1	CBS 142816	*Lecanicillium kalimantanense* BTCC-F23 (95)	100	LT598647	*Lecanicillium kalimantanense* BTCC-F23 (98)	100	LT598647
Lecanicillium sp.1	VKPM F-1421	*Lecanicillium kalimantanense* BTCC-F23 (95)	100	MF682449	_	_	_
Lecanicillium sp.1	CBS 142821	*Lecanicillium kalimantanense* BTCC-F23 (95)	97	MF682448	*Lecanicillium kalimantanense* BTCC-F23 (98)	98	LT598650
Lecanicillium sp.2	CBS 142817	*Lecanicillium kalimantanense* BTCC-F23 (95)	99	LT598645	*Lecanicillium kalimantanense* BTCC-F23 (99)	99	LT598648
Lecanicillium sp.2	CBS 142818	*Lecanicillium kalimantanense* BTCC-F23 (95)	99	LT598646	*Lecanicillium kalimantanense* BTCC-F23 (99)	99	LT598650
Mortierella alpina		*Mortierella alpina* MSCL 1314 (100)	98	LT549087	_	_	_
Parengyodontium album	VKPM F-1422	*Parengyodontium album* CBS 368.72 (100)	76	LT549073	_	_	_
Parengyodontium album		*Parengyodontium album* CBS 368.72 (100)	77	LT549074	_	_	_
Parengyodontium album	VKPM F-1386	*Parengyodontium album* CBS 368.72 (100)	73	LT549075	_	_	_
Parengyodontium album	CBS 142824	*Parengyodontium album* CBS 368.72 (100)	72	LT549076	_	_	_
Pseudogymnoascus pannorum	VKPM F-1402	*Geomyces pannorum* CBS 105.53 (100)	80	LT549077	_	_	_
Pseudogymnoascus pannorum		*Geomyces pannorum* CBS 105.53 (100)	79	LT549078	_	_	_
Purpureocillium lilacinum	VKPM F-1387	*Purpureocillium lilacinum* CBS 284.36 (100)	96	MH818848		_	_
Sarocladium kiliense	VKPM F-1403	_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549064
Sarocladium kiliense	VKM FW-1540, CBS 142820	*Sarocladium kiliense* CBS 400.52 (99)	100	LT549088	_	_	_
Sarocladium kiliense	VKPM F-1362	*Sarocladium kiliense* CBS 400.52 (99)	100	LT549089	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549089

(*continued on next page*)

Table 2 (*continued*)

Species	Voucher	ITS1-5.8S-ITS2			D1-D2 LSU		
		Top BLAST search results (similarity, %)	Query coverage (%)	NCBI gene accession no.	Top BLAST search results (similarity, %)	Query coverage (%)	NCBI gene accession no.
Sarocladium kiliense	VKPM F-1363	*Sarocladium kiliense* CBS 400.52 (100)	100	LT549090	_	_	_
Sarocladium kiliense	VKPM F-1404	_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549068
Sarocladium kiliense	VKPM F-1364	*Sarocladium kiliense* CBS 400.52 (99)	99	MF682451	_	_	_
Sarocladium kiliense		_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549065
Sarocladium kiliense	VKM F-4771	_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549067
Sarocladium kiliense	VKPM F-1368	*Sarocladium kiliense* CBS 400.52 (99)	97	MF682447			
Sarocladium kiliense		_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549071
Sarocladium kiliense		_	_	_	*Sarocladium kiliense* CBS 122.29 (99)	100	LT549070
Sarocladium kiliense	VKPM F-1366	_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549066
Sarocladium kiliense	VKPM F-1367	_	_	_	*Sarocladium kiliense* CBS 122.29 (100)	100	LT549072
Sarocladium kiliense		_	_	_	*Sarocladium kiliense* CBS 122.29 (100)		LT549063
Verticillium zaregamsianum	VKPM F-1361	*Verticillium zaregamsianum* CBS 130342 (100)		LT549080	_	_	_

where a_w is the water activity value; X – the solute molar fraction; K = 1.16 at 25 °C for glycerol. Petri dishes contained media with given a_w values were inoculated into the center in duplicate for each strain. To avoid desiccation, Petri dishes were sealed thoroughly with Parafilm and were put into desiccators containing glycerol-water solutions with the same glycerol content as in solid substrate with appropriate a_w. The growth expansion was followed for 4 weeks at room temperature (24 °C). The Petri dishes were examined every other day, and the diameter of fungal colonies was measured in two perpendicular directions (Marin et al., 1996). To determine linear growth of the strains, the linear regression model was used. The regression coefficient corresponded numerically to the linear growth rate of the colony (Baranova et al., 2017).

2.7. Carbonate dissolution test

Species that were detected in significant quantities, i.e. 10^4 CFU g^{-1} and above were screened for their ability to solubilize $CaCO_3$ (qualitative analysis). For this purpose, $CaCO_3$ glucose agar of the following composition was used (g/l of deionized water): $CaCO_3$, 5; glucose, 10; agar, 15 (Pangallo et al., 2012), pH 7.1–7.3. The mixture was cooled to 45 °C with gentle stirring to resuspend $CaCO_3$ (Unković et al., 2018). Where it was possible, three different strains of every tested species were randomly chosen for this test. Tested strains were inoculated into the center of the Petri dishes in triplicate. Petri dishes were incubated for 21 d at 25 °C. The positive strains on $CaCO_3$ glucose agar displayed a clear zone around them.

3. Results

3.1. Environmental parameters and CFU counts

In the control samples obtained from areas without evidence of deterioration moisture content was low (up to 4 %), and fungal counts never exceeded 10^2 CFU g^{-1}. In all studied objects except the Scientific Research Institute for Restoration, we have detected sites with noticeably heightened moisture content, which was two or more times higher than that of control areas. As for the Scientific Research Institute for Restoration, the wall moisture content in its basement was slightly higher than the control values (Table 1). The reason of the increased moisture content in walls of eight objects, i.e. of two Archive buildings and the Collection Storage of the State Tretyakov Gallery, of Kuskovo Manor, of Nikita body Novgorod Kremlin, of Ostankino Museum-Estate, of Saint George Cathedral and of the Scientific Research Institute for Restoration was a lack of damp proofing of the walls, so water rose up the walls from the groundwater. We have detected fungi in amounts of 10^4 CFU g^{-1} in deteriorated sites of Archive buildings of the State Tretyakov Gallery, Kuskovo Manor and Scientific Research Institute for Restoration and in amounts of 10^3 CFU g^{-1} in sites of the Collection Storage of the State Tretyakov Gallery, Nikita body Novgorod Kremlin, Ostankino Museum-Estate and Saint George Cathedral. Another reason of the building materials' high dampness was heightened RH in interiors that led to water condensation on interior surfaces due to the temperature gradient between the wall surface and the room (Rebrikova, 1999). In particular, in Bogolyubskiy Cathedral and in Church of Boris and Gleb RH was 89 % and 71 % respectively (temperature in interiors of these objects was 16.9 °C and 24 °C respectively), and amount of obtained micromycetes from deteriorated sites reached 10^5 CFU g^{-1} in Bogolyubskiy Cathedral and 10^4 CFU g^{-1} in Church of Boris and Gleb. Then, moisture content of the wall of the Exhibition hall of State Tretyakov Gallery was heightened due to the lack of protection of the wall outer surface from melted water and rainwater. Fungal counts obtained from this site reached 10^4 CFU g^{-1}. Finally, the reason of high wall dampness of two remaining objects, Rozhdestvenskiy Cathedral and Tver Regional Art Gallery, was complex. Indeed, in Rozhdestvenskiy Cathedral not only a lack of damp proofing of the walls, but heightened RH (72 %, temperature 19 °C) was also observed. Total fungal counts obtained from the Cathedral's deteriorated sites reached 10^6 CFU g^{-1}. In Tver Regional Art Gallery, there was also a lack of damp proofing of the walls and in addition the basement floor has been flooded with water for a period of about a month. Total fungal counts obtained from deteriorated sites of this object reached 10^5 CFU g^{-1}.

The dampness of the salt precipitation areas was high and exceeded 9 % in all studied sites. Nevertheless, in 36 % of samples obtained from salt precipitation areas, fungal counts were comparable with those in the control samples and did not exceed 10^2 CFU g^{-1}.

3.2. SEM analysis

Samples of deteriorated plaster observed in SEM revealed copious development of filamentous fungi on them (Fig. 1). SEM observation revealed that fungi were developing in close contact with the mineral material penetrating into it and bounding material grains by hyphae (Fig. 1A,B). Moreover, in some samples fungal hyphae were immersed in well-developed biofilm-associated slime (Fig. 1D) that coated mineral particles of the substrate (Fig. 1C).

3.3. Fungal isolates

3.3.1. Fungal community of mineral building materials

Two hundred and twenty fungal strains were isolated representing 53 species from 25 genera. In addition, six isolates were *Mycelia sterilia*. The isolated fungal species are listed in Tables 2 and 3.

Nearly all detected fungal species (94 %) belong to *Ascomycota*; the remaining species belong to *Basidiomycota* (2 %) and *Mucoromycota* (4 %). Among *Ascomycota*, the recovered species were distributed within *Eurotiomycetes* (36 %), *Sordariomycetes* (36 %), *Dothideomycetes* (16 %), *Ascomycetes* (6 %), *Leotiomycetes* (4 %) and *Saccharomycetes* (2 %).

According to abundance of isolated species and their CFU counts that reflect their development in material (Gómez-Cornelio et al., 2012), the species obtained from deteriorated materials can be divided into four groups (Fig. 2). The *first group* included species that dominated in the studied materials possessing the highest abundance (from 7 % to 28.9 %) and thus the most actively developing in them. They were *Parengyodontium album*, *Sarocladium kiliense*, *Acremonium charticola*, *Lecanicillium* sp.1, *Purpureocillium lilacinum* and *Acremonium furcatum* (species are listed in order of decreasing their abundance). Counts of all these species in the obtained samples were high and reached 10^5 CFU g^{-1}. The frequency of occurrence of *P. album* was the highest among all the isolated species; *P. lilacinum* and *S. kiliense* have occurred frequently, while the frequency of occurrence of *A. charticola*, *A. furcatum* and *Lecanicillium* sp.1 was low (Table 3, Fig. 2). The *second group* contained species with significantly lower abundance than that of the previous group of species (from 0.4 % to 3.6 %), though with high CFU counts (10^4 CFU g^{-1}). These species were *Penicillium chrysogenum*, *Cladosporium langeronii*, *Lecanicillium* sp.2, *Mortierella alpina*, *Verticillium zaregamsianum*, *Pseudogymnoascus pannorum*, *Aspergillus versicolor*, *Aspergillus flavus*, *Penicillium* sp.1 and *Fusarium* sp. (species are listed in order of decreasing their abundance) (Table 3, Fig. 2). The *third group* included species with very low abundance (from 0.03 % to 0.4 %) and low CFU counts, i.e. 10^3 CFU g^{-1}. They were *Chordomyces antarcticus*, *Penicillium aurantiogriseum*, *Talaromyces funiculosus*, *Acremonium* sp.1, *Acremonium* sp.2, *Aspergillus sclerotiorum*, *Emericellopsis* sp., *Penicillium glabrum*, *Penicillium* sp.2, *Umbelopsis nana*, *Tritirachium oryzae* (species are listed in order of decreasing their abundance) (Table 3, Fig. 2). Finally, the remaining 27 species (Table 3) constituted the *fourth group*. It included species isolated in insignificant amounts (10^2 CFU g^{-1}) from deteriorated materials, which fungal counts had not differed from those in the control samples and which abundance was very low (from 0.03 % to 0.4 %), thus these species had not developed in materials at all.

It is interesting to note that although the copious fungal development in the sites with salt efflorescence generally was not observed, three species, namely *Lecanicillium* sp.1, *P. album* and *P. chrysogenum*, were obtained in significant amounts (10^4 CFU g^{-1}) from them. Other fungal species isolated from salt precipitation sites were detected in low amounts not exceeding 10^3 CFU g^{-1}, i.e. *A. furcatum*, *Acremonium* sp.1, *S. kiliense* and *U. nana*, or in insignificant amounts not exceeding 10^2 CFU g^{-1}, i.e., *Aspergillus nidulans*, *Aspergillus repens*, *A. versicolor*, *P. aurantiogriseum* and *T. funiculosus*. The Simpson diversity index of the community obtained from salt precipitation sites was 0.68, while of community obtained from deteriorated areas without salt efflorescence was 0.84.

From the control samples, we have obtained airborne species only in very low amounts, i.e. *Cladosporium cladosporioides* (10^2 CFU g^{-1}), *A. flavus*, *Aspergillus niger* and *T. funiculosus* (3×10 CFU g^{-1}).

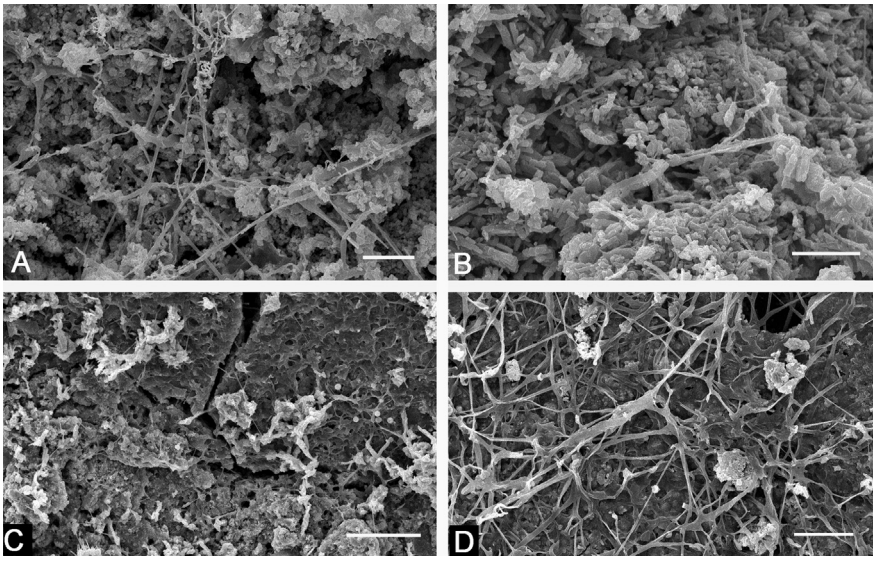


Fig. 1. Scanning electron microscopic images of deteriorated plaster samples. (A) and (B) the presence of abundant fungal mycelium penetrating into mineral substrate; (C) and (D) biofilm-associated slime or extracellular polymeric substances (EPS) developing on mineral particles of the substrate. *Scale bars*: A, B = 10 μm; C = 50 μm; D = 20 μm.

Table 3
Abundance, frequency of occurrence and maximum CFU g^{-1} of species isolated (+) from deteriorated materials.

Fungi	Type of material		Abundance, %	Maximum CFU g^{-1}	Frequency of occurrence, %	Object numbers
	limestone	plaster				
Parengyodontium album	+	+	29.90	10^5	23.0	2, 6, 7, 11, 12
Sarocladium kiliense	+	+	14.85	10^5	14.8	5, 6, 11, 13, 14
Lecanicillium sp.1	+	+	13.39	10^5	4.1	2, 6, 10
Acremonium charticola	+	+	11.60	10^5	1.6	1, 4, 6
Purpureocillium lilacinum		+	7.95	10^5	12.3	6, 10, 13
Acremonium furcatum	+	+	7.28	10^5	4.9	2, 14
Penicillium chrysogenum	+	+	3.70	10^4	18.9	2, 6, 7, 10-13
Cladosporium langeronii	+		3.64	10^4	0.8	1
Lecanicillium sp.2		+	1.09	10^4	1.6	6, 10
Mortierella alpina		+	0.90	10^4	2.5	6
Pseudogymnoascus pannorum	+		0.74	10^4	1.6	1, 5
Verticillium zaregamsianum	+		0.74	10^4	4.9	1
Aspergillus versicolor	+	+	0.61	10^4	13.9	6, 8, 11, 12
Aspergillus flavus	+	+	0.60	10^4	17.2	1, 2, 9- 11
Penicillium sp.1	+	+	0.45	10^4	8.2	14
Fusarium sp.		+	0.42	10^4	1.6	11
Chordomyces antarcticus		+	0.26	10^3	1.6	14
Penicillium aurantiogriseum	+	+	0.26	10^3	13.9	5, 6
Talaromyces funiculosus	+	+	0.24	10^3	10.7	1, 2, 7, 9
Cladosporium cladosporioides	+		0.15	10^3	1.6	5
Trichoderma harzianum	+		0.15	10^3	1.6	5
Aspergillus sclerotiorum		+	0.14	10^3	1.6	11
Umbelopsis nana	+		0.13	10^3	1.6	14
Acremonium sp.1		+	0.11	10^3	1.6	11
Penicillium glabrum	+		0.11	10^3	0.8	11
Emericellopsis sp.	+	+	0.10	10^3	1.6	4
Acremonium sp.2		+	0.09	10^3	0.8	12
Acremonium sp.3	+		0.05	10^3	0.8	1
Tritirachium oryzae		+	0.03	10^3	0.8	8
Alternaria sp.	+		0.03	10^2	0.8	5
Acremonium camptosporum		+	0.02	10^2	1	10
Acrostalagmus luteoalbus	+		0.02	10^2	1.6	2
Alternaria alternata	+	+	0.02	10^2	3.3	5, 6
Geotrichum candidum		+	0.02	10^2	1.6	6
Mycelia sterilia 1	+	+	0.02	10^2	2.5	14
Mycelia sterilia 2		+	0.02	10^2	0.8	6
Cladosporium sp.		+	0.02	10^2	0.8	13
Aspergillus nidulans		+	0.01	10^2	0.8	12
Aspergillus ochraceus		+	0.01	10^2	0.8	8
Epicoccum nigrum	+		0.01	10^2	0.8	5
Mycelia sterilia 3		+	0.01	10^2	0.8	6
Mycelia sterilia 4	+		0.01	10^2	0.8	11
Penicillium verrucosum		+	0.01	10^2	0.8	14
Trichoderma atroviride		+	0.01	10^2	1.6	2
Alternaria botrytis		+	0.005	10^2	0.8	6
Aspergillus niger		+	0.005	10^2	0.8	14
Aspergillus repens		+	0.005	10^2	0.8	12
Aspergillus sp.1		+	0.005	10^2	0.8	8
Aspergillus sp.2		+	0.005	10^2	0.8	14
Aspergillus ustus		+	0.005	10^2	0.8	11
Botrytis sp.	+		0.005	10^2	0.8	1
Chaetomella sp.		+	0.005	10^2	0.8	6
Chaetomium globosum		+	0.005	10^2	0.8	6
Cladosporium oxysporum		+	0.005	10^2	0.8	6
Mycelia sterilia 5		+	0.005	10^2	0.8	11
Mycelia sterilia 6		+	0.005	10^2	0.8	6
Penicillium brevicompactum		+	0.005	10^2	0.8	11
Penicillium sp.2		+	0.005	10^2	0.8	11
Trichoderma sp.	+		0.005	10^2	0.8	5

3.3.2. The comparison of limestone and plaster fungal complexes

The species most actively developing in limestone possessing the highest abundance in this material were *S. kiliense* (40 %), *C. langeronii* (14 %), *P. pannorum* (11 %), *Lecanicillium* sp.2 (10 %), *A. furcatum* (3 %) and *V. zaregamsianum* (3 %). The frequency of occurrence of *V. zaregamsianum* in samples was high (19 %), while the frequency of occurrence of *A. furcatum*, *S. kiliense*, *P. pannorum*, *C. langeronii* and *Lecanicillium* sp.2 was low (9 %, 9 %, 6 %, 3 % and 3 % respectively).

The species possessing the highest abundance in plaster were the following: *P. album* (38 %), *A. charticola* (15 %), *Lecanicillium* sp.1 (12 %), *A. furcatum* (8 %), *P. lilacinum* (7 %), *S. kiliense* (6 %) and *P. chrysogenum* (5 %). The occurrence of *P. album* and *P. chrysogenum* was the highest among all the isolated species from plaster (29 % and 27 % respectively), followed by *S. kiliense* and *P. lilacinum* (21 % and 18 % respectively), while the frequency of occurrence of *A. charticola* and *A. furcatum* was low (4 % for both).

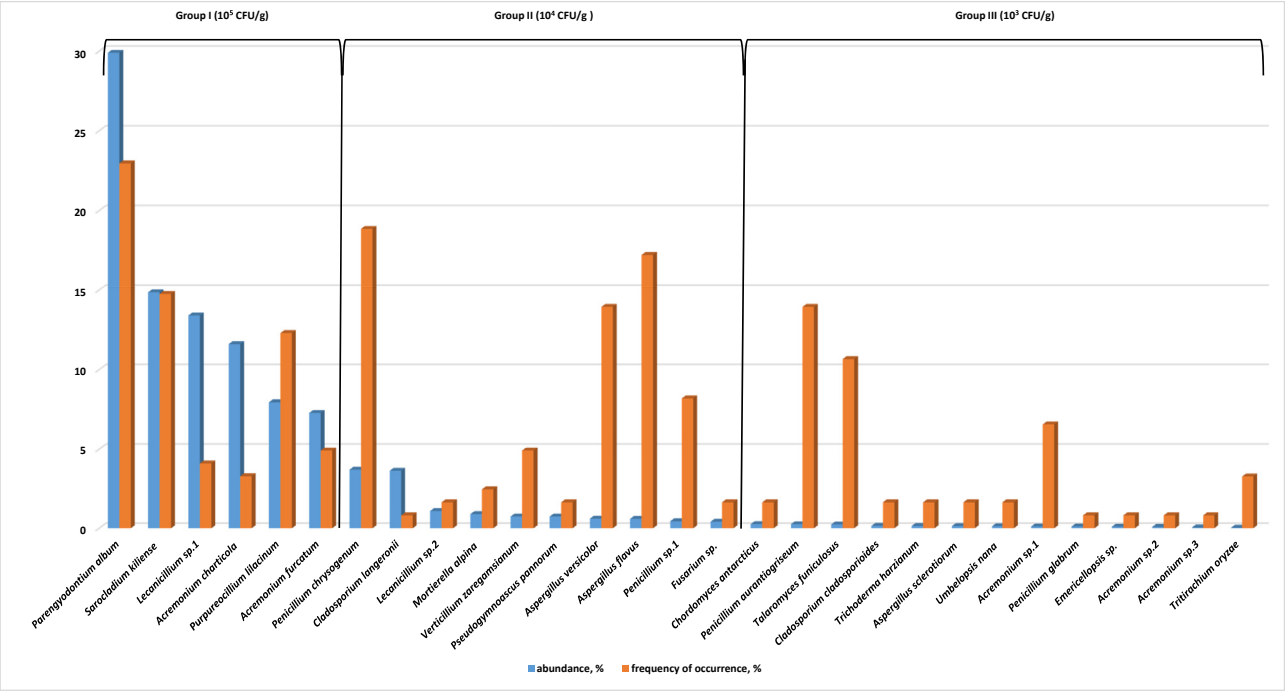


Fig. 2. Abundance, frequency of occurrence and CFU counts of fungal species isolated in amounts of 10^3 CFU g^{-1} and above and their division into groups.

The Simpson diversity index of the community obtained from limestone was 0.79 and of the community obtained from plaster was 0.80.

Sørensen-Czekanowski similarity coefficient between species isolated from limestone and plaster was low (0.16), which indicates high difference between species contents of two studied materials. Nevertheless, species of the genera *Acremonium*, *Lecanicillium* and *Sarocladium* were among the most abundant in both limestone and plaster.

3.4. Growth at different pH values

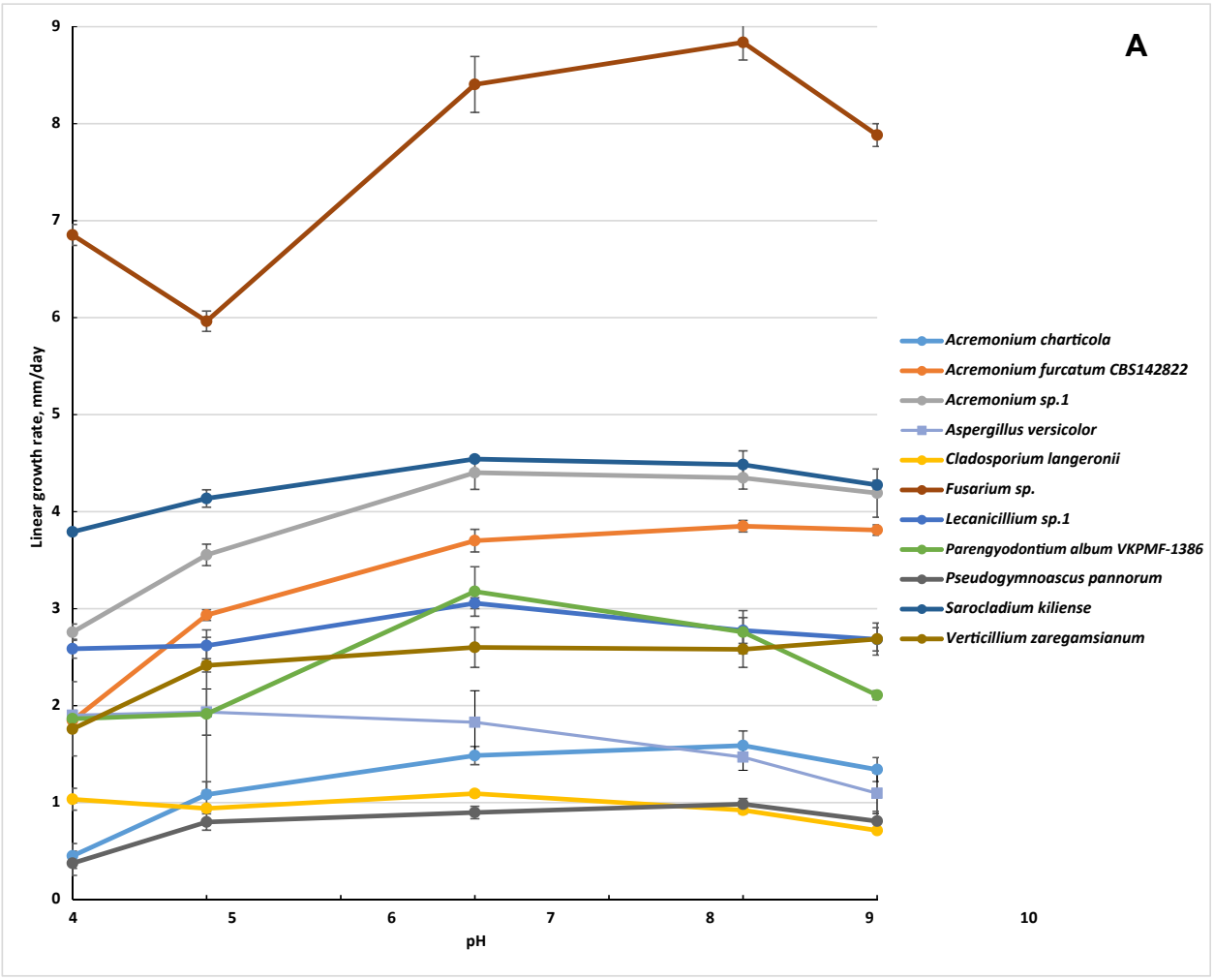
The results of our growth experiments are shown at Fig. 3. When pH preferences of isolates of the same species were the same, we have presented graph for one isolate only. For interpretation of our growth experiments at different pH values, we have used classification proposed by Grum-Grzhimaylo et al. (2016). According to this classification, the overwhelming majority, namely, 14 out of 18 species (78 %) isolated from deteriorated mineral materials in amount of 10^3 and above were alkalitolerants (Fig. 3A,B,D), as they have displayed ability to grow at alkaline pH values and their growth optimum included pH values below 8. Alkaliphilic phenotype with the growth optimum at pH above 8 was revealed for four out of 18 tested species (22 %) (Fig. 3C); two species (11 %) expressed acidophilic phenotype, one of which could not grow at alkaline pH values at all (Fig. 3D).

Almost all the species that were the most abundant in the studied mineral materials (representatives of the first group) were alkalitolerants except two species that revealed alkaliphilic phenotype. In particular, *A. charticola*, one strain of *A. furcatum* (CBS 142822), *Lecanicillium* sp.1, two strains of *P. album* (VKPM F-1386, VKPM F-1422) and *S. kiliense* were strong alkalitolerants. Indeed, they have shown little or no reduction in growth at alkaline pH. Their growth optimum always included neutral pH and was often wide as seen in Fig. 3A. *P. lilacinum* was the only species in this group that belong to moderate alkalitolerants, as its growth rate was reduced at about half at high pH as compared to neutral pH; its growth optimum included pH 7 too (Fig. 3B). Concerning alkaliphilic strains, they belonged to *A. furcatum* (VTT D-151581, VTT D-151584) and to *P. album* (CBS 142824) and they grew best at pH 9–10, though they retained their ability to develop at acidic pH (Fig. 3C).

Species of the second group (with significantly lower abundance than that of the previous group though isolated in high amounts) in general displayed similar pH preferences as representatives of the previous group. Indeed, they were all alkalitolerants except for *Lecanicillium* sp.2 that was an alkaliphile. In particular, five species in this group were strong alkalitolerants, namely, *A. versicolor*, *C. langeronii*, *Fusarium* sp., *P. pannorum* and *V. zaregamsianum*. These species have demonstrated little or no reduction in growth at alkaline pH; they grew best at neutral pH (i.e. *C. langeronii*) or possessed wide growth optimum, such as pH 4–7 (i.e. *A. versicolor*), pH 7–9 (i.e. *Fusarium* sp. and *P. pannorum*) or pH 7–10 (i.e. *V. zaregamsianum*) (Fig. 3A). A single species in this group, *A. flavus*, was a moderate alkalitolerant, as it grew at about half the growth rate at high pH, as compared to neutral pH; its growth optimum was pH 7 (Fig. 3B). In contrast to the representatives of the first group, the growth of *P. chrysogenum* was strongly restricted at pH 7 and above and it was weak alkalitolerant possessing acidophilic phenotype with growth optimum at 4 (Fig. 3D).

Among the representatives of the third group (that included species with very low abundance and with low CFU counts), we have observed species with diverse pH preferences. Indeed, one species, *T. funiculosus*, was acidophile, as it preferred pH 4; it could not grow at alkaline pH values at all as seen in Fig. 3D. Then, *Acremonium* sp.1 turned out to be strong alkalitolerant with growth optimum at pH 7–10 as seen in Fig. 3A. Finally, *C. antarcticus* and *Emericellopsis* sp. were alkaliphiles as they grew best at pH 10 and 9 respectively as seen in Fig. 3C.

Among ten samples of deteriorated mineral materials that were randomly chosen for pH measurements, one sample was slightly acidic (pH 6), two samples were neutral (pH 7) and six samples



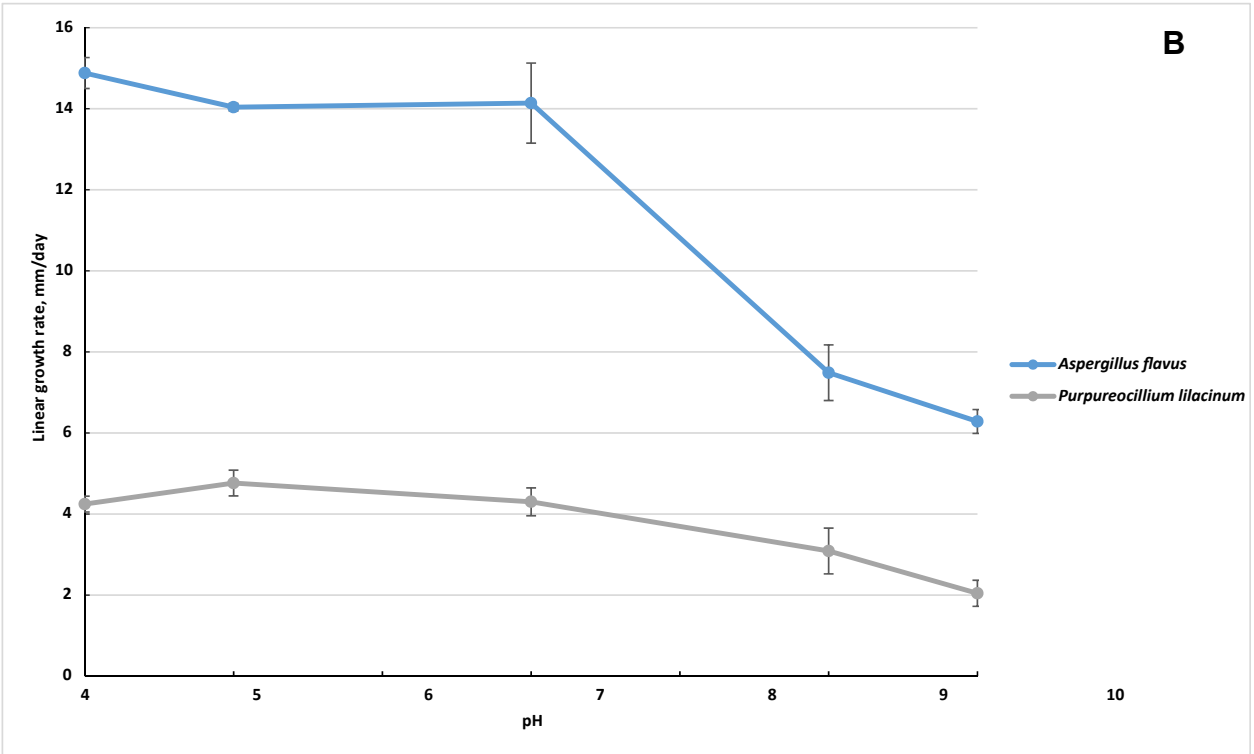
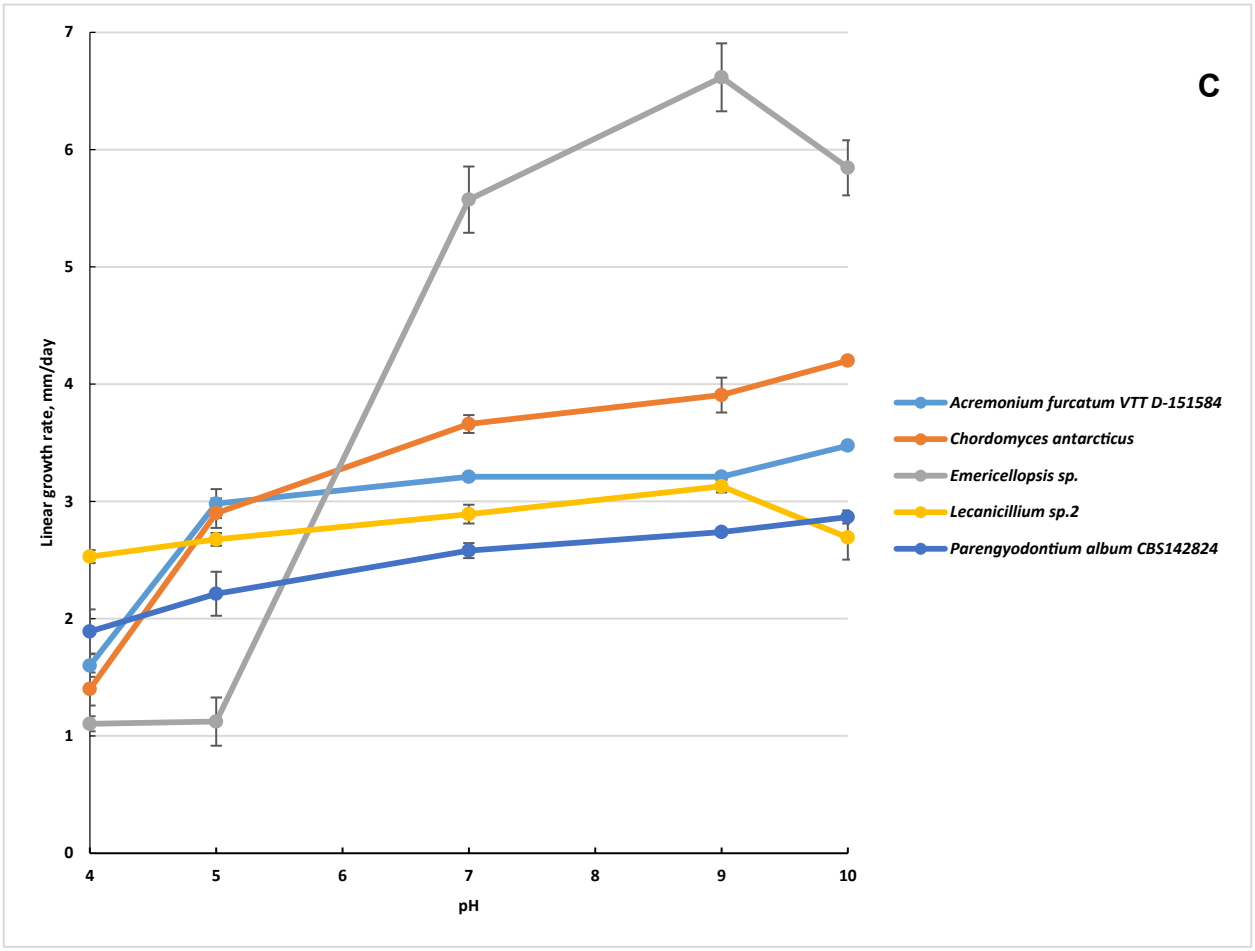


Fig. 3. Linear growth rate patterns at different pH values of isolated fungi: A - with strong alkalitolerant phenotype; B - with moderate alkalitolerant phenotype; C - with alkaliphilic phenotype; D – with acidophilic phenotype.



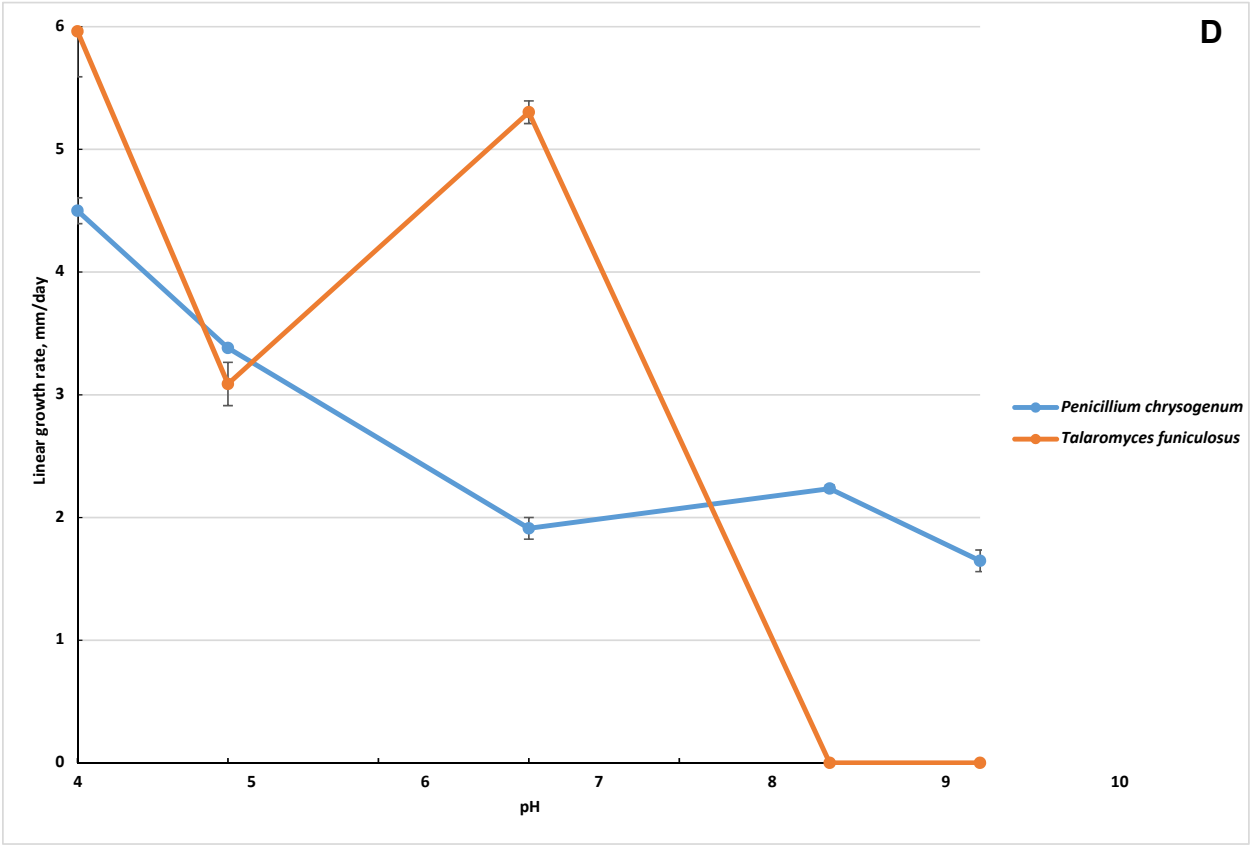


Fig. 3. (*continued*).

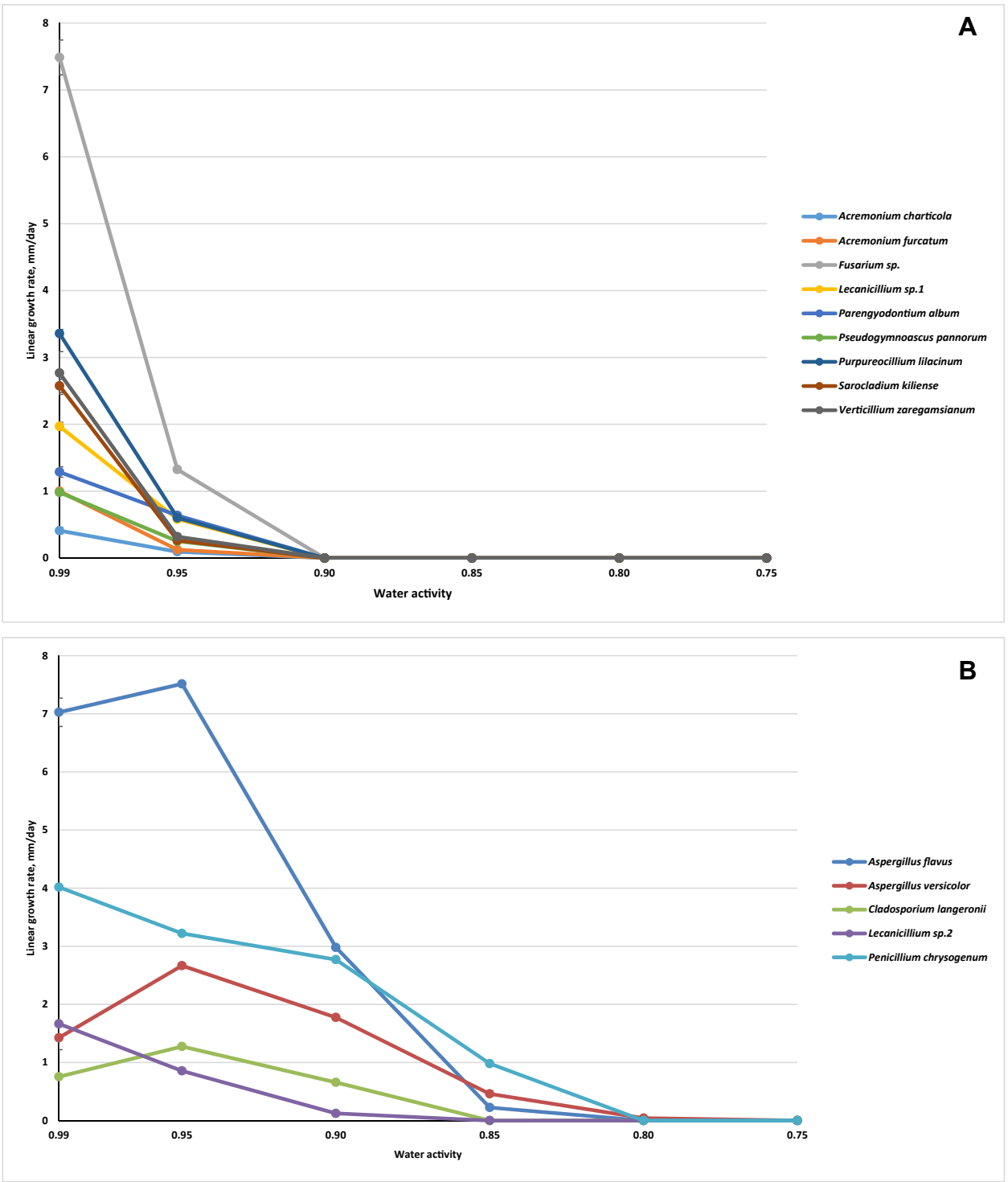


Fig. 4. Effect of water activity on linear growth rate of isolated fungi: A - species with significantly restricted growth at 0.95 a_w; B - species that grew well at 0.95 a_w.

were slightly alkaline (five samples with pH 8 and one sample with pH 9).

3.5. *Influence of the water activity of a solid substrate on the fungal growth rate*

The results of our growth experiments are shown at Fig. 4. Water activity preferences for growth of isolated species differed. Generally, growth rates of species were reduced when a_w decreased. All the species that were the most abundant in the studied mineral materials (representatives of the first group), i.e. *A. charticola*, *A. furcatum*, *Lecanicillium* sp.1, *P. album*, *P. lilacinum* and *S. kiliense*, showed maximum growth at unmodified a_w medium, significantly reduced growth at 0.95 a_w and did not grow at 0.9 a_w (Fig. 4A). Species of the second group have possessed various a_w preferences for growth. Indeed, three species in this group, i.e.

Table 4
Calcite dissolution ability (+) of species that were detected in 10^4 CFU g^{-1}.

Species	Code	Voucher	Calcite solubilization
Acremonium charticola	R7	VKM F-4769	+[a]
Acremonium furcatum	Kd3	CBS 142822	–
Acremonium furcatum	Kd9	VTT D-151581	–
Acremonium furcatum	T10	VTT D-151584	–
Aspergillus flavus	B4		–
Aspergillus flavus	O1		–
Aspergillus flavus	TK1-4		–
Aspergillus versicolor	N6		+[a]
Aspergillus versicolor	T10		–
Cladosporium langeronii	B11	VKPM F-1427	–
Fusarium sp.	TK1-14	CBS 142825	–
Lecanicillium sp.1	57-9-2	CBS 142816	+
Lecanicillium sp.1	G4-5	VKPM F-1421	–
Lecanicillium sp.1	Kd1	CBS 142821	+
Lecanicillium sp.2	G42	CBS 142817	+
Lecanicillium sp.2	GTG12-1	CBS 142818	+
Parengyodontium album	B5-1	VKPM F-1422	–
Parengyodontium album	K8	VKPM F-1386	–
Parengyodontium album	Kd7	CBS 142824	–
Penicillium chrysogenum	GTG1		+
Penicillium chrysogenum	Kd2	VKPM F-1401	+
Penicillium chrysogenum	TK1-7		+
Pseudogymnoascus pannorum	B13	VKPM F-1402	+[a]
Purpureocillium lilacinum	G15		–
Purpureocillium lilacinum	G19		–
Purpureocillium lilacinum	GTG12-2	VKPM F-1387	–
Sarocladium kiliense	G3-6	VKPM F-1362	–
Sarocladium kiliense	To3	VKPM F-1368	–
Sarocladium kiliense	TK1-21	VKPM F-1366	–
Verticillium zaregamsianum	B7-1	VKPM F-1361	–

[a] Weak dissolution activity.

Fusarium sp., *P. pannorum* and *V. zaregamsianum,* similarly with species from the first group grew best at maximum a_w studied and could not tolerate a_w 0.9 (Fig. 4A). Then, *Lecanicillium* sp.2 and *P. chrysogenum* preferred unmodified a_w medium for growth though they tolerated reduced a_w values (Fig. 4B). The remaining three species, i.e. *A. versicolor*, *A. flavus* and *C. langeronii,* grew optimally at 0.95 a_w and tolerated reduced a_w values though they successfully grew on unmodified a_w medium (Fig. 4B).

3.6. Calcium carbonate dissolution potential of fungal isolates

Calcium carbonate dissolution potential of the studied fungal isolates is presented in Table 4. Six species out of 14 (43 %), i.e. *A. charticola*, *A. versicolor*, *Lecanicillium* sp.1, *Lecanicillium* sp.2, *P. pannorum* and *P. chrysogenum,* displayed the ability to dissolve $CaCO_3$. It is worth mentioning that dissolution activity could vary depending on the strain as it was observed in *A. versicolor* and *Lecanicillium* sp.1 strains (Table 4). Solubilization activity of both *Lecanicillium* species and *P. chrysogenum* was high as dissolution transparent zone around their colonies was wide, while *A. charticola*, *A. versicolor* and *P. pannorum* displayed barely noticeable zone that appeared on fourth day of the experiment and disappeared after a week.

4. Discussion

4.1. Specific characters of fungal complex obtained from mineral materials

The development of fungi over a period of time may be determined by a combination of various factors (Dornieden et al., 2000; Gómez-Cornelio et al., 2012, 2016; Warscheid and Braams, 2000). These factors are climatic conditions such as water availability, temperature and others; nutrient sources; bioreceptivity of materials, which is controlled by their surface roughness, porosity, permeability and mineral composition.

It is well known that moisture content of materials is the chief factor determining fungal growth (Gaylarde and Morton, 1999). It is

Table 5
Enzymatic activities of species possessing the highest abundance under our investigation.

Fungal species	Enzymes							References
	cellulase	xylanase	α-amylase	chitinase	protease	esterase	phosphatase	
A. charticola	+							Semenov et al. (1996)
A. furcatum	+	+			+			Błyskal (2009); Palaniswamy et al., (2008); Saleem et al., (2013)
Parengyodontium album			+	+	+	+	+	Ali et al., (2014); Błyskal (2009); Chellappan et al., (2011); Karpovich-Tate and Rebrikova (1990); Šimonovičová et al., (2004a)
Purpureocillium lilacinum	+	+		+	+	+	+	Błyskal (2009); Cavello et al., (2013); Prasad et al., (2015); Wang et al., (2016); Xie et al., (2016)
Sarocladium kiliense	+	+			+	+	+	Błyskal (2009); Chesson et al., (1978); Tarayre et al., 2014; Van Heyningen and Secher (1971)

worth mentioning that heightened water content in materials leads to their physical, chemical and biological deterioration. In buildings with poor protection from groundwater (eight objects under our investigation) or from rainwater (one object under our investigation), a variety of salts, i.e. carbonates, chlorides, nitrates, sulphates, etc. migrates through pores of material; furthermore, the groundwater can transport organic acids that chemically weather minerals. These salts can dry out of the solution, crystallize and thus produce an additional pressure on material that results in material loss and destruction (Papida et al., 2000; Piñar et al., 2009; Rebrikova, 1999). We found that in the areas of salt precipitation the fungal diversity was much lower than in other deteriorated areas as the fungal growth was negatively affected by high osmotic potential of substratum, that fully corresponds to literature data (Piñar et al., 2009; Rebrikova, 1999). Nevertheless, salt efflorescence favors for proliferation of halophilic and halotolerant microorganisms, which have an advantage over other organisms under these conditions (Piñar et al., 2009). This pattern was caused by prosperous development of three species in these sites, namely, *Lecanicillium* sp., *P. album* and *P. chrysogenum*. Halotolerance of *P. album* and *P. chrysogenum* was confirmed in other studies (Gorbushina and Petersen, 2000; Gunde-Cimerman et al., 2009).

Differences between complexes of fungal species obtained from limestone and plaster were predictable as physical and chemical properties of these materials differ. Nevertheless, one can reveal some similar peculiar traits of these materials and of fungal complexes obtained from them as well. Indeed, both limestone and plaster are characterized by lack of organic sources, high salt concentration and slightly acidic to slightly alkaline pH values (pH 6–9 in our samples of deteriorated materials). The fungal diversity in both these materials was equally high that may be connected with site-specificity of mycoflora colonizing them. Considerable part of fungi that actively developed in both limestone and plaster belonged to *Hypocreomycetidae* (*Ascomycota*), and all of them tolerated high pH values. This is in agreement with the fact that *Hypocreomycetidae* is characterized by the presence of alkalitolerant and alkaliphilic fungi (Grum-Grzhimaylo et al., 2016).

Species from the isolated fungal complex possess different physiological and ecological characters and realize various life strategies; below we discuss these features according to groups proposed in our work.

The most abundant and thus the most actively developing (representatives of the *first group*) were *A. charticola*, *A. furcatum*, *Lecanicillium* sp.1, *P. album*, *P. lilacinum* and *S. kiliense*. These species occur in various habitats including stressful ones. Indeed, *A. charticola*, *A. furcatum*, *P. lilacinum* and *S. kiliense* were obtained from calcite mineral substrates in natural habitats (Ellanskaya et al., 2000; Nagai et al., 1998; Wasser et al., 1995), *A. furcatum* and *P. album* were detected in hypersaline environments (Gallardo et al., 2006; Muhsin and Booth, 1987; Tsang et al., 2016), while *A. furcatum* and *P. lilacinum* in alkaline ones (Grum-Grzhimaylo et al., 2016; Nagai et al., 1998). Moreover, *P. album*, *P. lilacinum* and *S. kiliense* are known as opportunistic pathogens of vertebrates (Luangsa-ard et al., 2011; Perdomo et al., 2011; Tsang et al., 2016), while *P. album* and *P. lilacinum* as pathogens of invertebrates (Lopez et al., 2014; Luangsa-ard et al., 2011; Tsang et al., 2016). Then, *A. charticola*, *A. furcatum*, *P. album*, *P. lilacinum* and *S. kiliense* were also isolated from plant material (Gams, 1971; Lou et al., 2013; Luangsa-ard et al., 2011; Tsang et al., 2016). As for species of *Lecanicillium*, they are entomogenous and fungicolous (Zare and Gams, 2001).

Another specific character of the species in this fungal group is that they are known to possess high enzymatic activities (Table 5). It is worth mentioning that in strains of *P. album* and *S. kiliense* alkaline proteases were detected showing maximum activity at pH about 11, but also remaining active over a wide range of pH (from 6 to 12 and from about 5 to 12.5 respectively) (Chellappan et al., 2011; Van Heyningen and Secher, 1971). The keratinolytic serine protease from *P. lilacinum* is also stable over a wide pH range, i.e. from 4 to 9 (Cavello et al., 2013). Thus, these proteases may stay active on limestone and plaster. As can be seen from the table, these species are able to decompose plant material and various cell components due to esterase, phosphatase and chitinaze activities. Therefore, the species in this group do not possess any specific trophic preferences as due to high enzymatic activities they can decompose a wide spectrum of organic matter including plants, bacteria, other fungi, arthropods, etc. (Gallardo et al., 2006; Gams, 1971; Gorbushina and Petersen, 2000; Lou et al., 2013; Luangsa-ard et al., 2011; Srivastava et al., 1981; Tarayre et al., 2015; Tsang et al., 2016), so they can realize various nutritional models. That gives them an opportunity to survive and thrive in oligotrophic conditions of stone substratum.

In interiors of historical objects and museums, in conditions of long-lasting material humidification and microbial deterioration, pH of limestone and plaster may change. Furthermore, heightened moisture content leads to increase of substrate water activity. Based on this, species from the isolated fungal community were tested for their ability of growth in the range of pH and a_w values. Growth tests at different pH values have confirmed pH-tolerant properties of the species listed above. According to the water activity requirements, all species in this group showed a marked dependence and could not grow under 0.95 a_w. Thus, their growth occurs at areas with significantly heightened moisture content and the presence of these species in stone materials in high amounts may be an indicator of a lack of waterproofing of building structures. We believe that their ability to develop successfully at extremely wide range of pH values, from slightly acidic to alkaline, in conjunction with high enzymatic activities give them an advantage in colonization of limestone and plaster and proliferating there for a long period in the conditions of long-lasted material humidification. Therefore, under these specific conditions species from this group begin to prevail with time, occupying competition-free niche. Thereby, the analysis of mycobiota can reveal whether the increased dampness and deterioration process of mineral substrate has lasted for a long time. In our samples, the material was not only from the surface, but also from deeper layers and thus the listed species are presumably the part of endolithic community living inside the material, its pores and fissures. Our SEM observations affirm fungal ability to thrive in subsurface layers of stone substrates, to penetrate into material particles and to form a biofilm inside material.

Fungi of the genera *Acremonium* and *Sarocladium* colonize building stone surfaces in interiors (Berner et al., 1997; Gorbushina and Petersen, 2000; Karpovich-Tate and Rebrikova, 1990; Rebrikova, 1999; Suihko et al., 2007) and may occur on outdoor mineral building surfaces (Gómez-Cornelio et al., 2016; Gorbushina et al., 2002). In particular, in agreement with our investigation, the development of *A. charticola*, *A. furcatum* and *S. kiliense* on indoor mineral surfaces was detected (Berner et al., 1997; Gorbushina and Petersen, 2000; Rebrikova, 1999; Suihko et al., 2007). In addition, *S. kiliense* was isolated from outdoor building stone surfaces, i.e. marble monuments (Gorbushina et al., 2002) and limestone walls (Gómez-Cornelio et al., 2016). *P. album* was the most abundant and the most frequently occurred species in our study, many authors observed it in high amounts on mineral substrates inside buildings (Berner et al., 1997; Gorbushina and Petersen, 2000; Jeffries, 1986; Karpovich-Tate and Rebrikova, 1990; Rebrikova, 1999; Šimonovičová et al., 2004a). It is worth mentioning that *A. charticola* and *P. album* were isolated from limestone and plaster of the Old Russian monuments (Karpovich-Tate and Rebrikova, 1990; Rebrikova, 1999). Then, to our knowledge, there are no reports of

the detection of *P. lilacinum* on indoor stone materials. Nevertheless, this species is known as indoor air contaminant (Luangsa-ard et al., 2011) and may inhabit natural limestone formations (Ellanskaya et al., 2000; Wasser et al., 1995), which explains the possibility of its development on limestone and plaster inside buildings. Concerning the genus *Lecanicillium*, there are reports of frequent isolation of *L. lecanii* (*Verticillium lecanii*) from wall surfaces of the Austrian, German and Russian cultural monuments (Berner et al., 1997; Gorbushina and Petersen, 2000; Rebrikova, 1999). However, in the works cited above fungi of this genus were identified by morphological characters only. Meanwhile, species of *Lecanicillium* obtained under our investigation were not phylogenetically close to *L. lecanii*.

Species from the *second group* which were less (twice or more) abundant than those of the previous group, but were obtained in high amounts (10^4 CFU g^{-1}) were generally plant-associated pathogenic fungi, namely, *Fusarium* sp., *P. pannorum* and *V. zaregamsianum* (Inderbitzin et al., 2011; Poole and Price, 1971; Watanabe et al., 2011) or airborne soil species, i.e., *A. flavus*, *A. versicolor* and *P. chrysogenum*. Spores of the plant-associated micromycetes could be carried inside buildings from outdoor vegetation. In our work, *C. langeronii*, *P. pannorum* and *V. zaregamsianum* have occurred in the limestone samples with heavy algal coating. Perhaps, these species used algae for nutrition. Although *Aspergillus* spp. and *Penicillium* spp. were frequent on indoor mineral building materials they were not indigenous to studied substrates and have got into samples mostly with surface contamination. Indeed, species of *Aspergillus* and *Penicillium* have occurred not only on the deteriorated materials, but in the control samples as well. Moreover, it is known that species of the above genera dominate in indoor air and in house dust (Antropova et al., 2003; Petrova-Nikitina et al., 2000) and their spores accumulate in dust particles and on surfaces. Species of *Aspergillus* and *Penicillium* genera may develop rapidly in dust particles on the mineral surfaces with heightened moisture content, as dust is suitable for fungal growth and nutrition (Ponizovskaya et al., 2011, 2014). When organic sources are expended, these species disappear; thus, they presumably cannot stay on mineral substrates for a long period. Our pH growth tests revealed that the majority of species from this group were alkalitolerants and similarly with the species from the first group they could develop over a wide range of pH values. Water activity tests revealed that the vast majority of species in this group are able to develop in more dry conditions than species from the first group. In particular, *A. flavus*, *A. versicolor*, *C. langeronii*, *Lecanicillium* sp.2 and *P. chrysogenum* are able to grow at nonstationary humidity. Under these conditions, they get an advantage over species developing in materials with high water content only, namely, representatives of the first group in our study. Our results on a_w requirements for growth of tested species are with accordance with earlier data (Nielsen and Frisvad, 2011; Pitt and Hocking, 1977; Ponizovskaya et al., 2011, 2014; Rebrikova, 1999). According to Dornieden et al. (2000), airborne soil fungi and plant-derived mycobiota are the first inhabitants on surfaces of stone walls.

While *A. versicolor*, *P. chrysogenum* and *P. pannorum* were isolated from mineral building materials (Berner et al., 1997; Karpovich-Tate and Rebrikova, 1990; Nielsen and Frisvad, 2011), we have observed the development of *V. zaregamsianum* in stone materials for the first time. As for *C. langeronii*, there is little information about its biodeteriorative properties on indoor stone materials. It is known only that this species was isolated from indoor surfaces (Segers et al., 2015) and from mortar (Zalar et al., 2007). Notably, *C. langeronii*, similarly to species from the previous group, occurs in various habitats and does not show any predilection for a particular habitat. Indeed, it was obtained from plant material, polar ice and biomats and also from human (Zalar et al., 2007). Bensch et al. (2012) propose that this species is much more widespread than indicated by the present collections. *C. langeronii* belongs to *Cladosporium sphaerospermum* species complex (Bensch et al., 2012). It is morphologically similar to *C. sphaerospermum* and thus molecular as well as physiological analyzes are required for its proper identification. In earlier works, where molecular approach for identification of fungal strains was not applied (Berner et al., 1997; Gorbushina and Petersen, 2000; Karpovich-Tate and Rebrikova, 1990), *C. sphaerospermum* was frequently reported to be isolated. Perhaps, in these works *C. langeronii* strains could be obtained and identified as *C. sphaerospermum*.

The species from the *third group* (isolated in low amounts) have shown various pH preferences, from acidophilic to alkaliphilic. The species from the *fourth group* (isolated in low or insignificant amounts) and from the third group did not develop in stone materials under our investigation and thus they could not cause any material deterioration. Nevertheless, they are perhaps able to proliferate in substrate under favorable conditions.

4.2. Deteriorative potential of isolated species

While fungi develop on stone materials, mycelium penetration into the substrate causes mechanical destabilization of the mineral structure and in addition allows the transport of water and nutrients through material that is favorable for bacteria growth and can lead to biochemical deterioration (Warscheid and Braams, 2000; Fernandes, 2006). In this way, in consideration of high abundance and listed physiological characters of *A. charticola*, *A. furcatum*, *Lecanicillium* sp.1, *P. album*, *P. lilacinum* and *S. kiliense*, we conclude that they can play an important role in deterioration of stone materials. In this fungal group, *Acremonium* and *Sarocladium* species deserve extra attention during restauration works. Indeed, these species possess compact conidia heads glued by slime and hyphae aggregating in chords also glued by slime (Grum-Grzhimaylo et al., 2016). In our work, *Acremonium* and *Sarocladium* isolates were closely associated with bacteria and we had to use high antibiotic doses to obtain pure fungal cultures. These species form biofilms in association with bacteria on mineral surfaces (Gorbushina et al., 2004; Suihko et al., 2007). Biofilm formation and slime excretion protect these species from adverse environmental factors such as osmotic shock, high pH values and others (Grum-Grzhimaylo et al., 2016; Kozlova et al., 2019) and can make biocidal treatment ineffective (Sand, 1997). Biofilms and their exopolymers clog the pores of material reducing water evaporation and thus increasing material water content (Sand, 1997). Moreover, shrinking and swelling cycles of exopolymers mechanically attack the substrate (Gadd, 2007).

In order to understand the biodeteriorative potential of species from the isolated fungal complex more completely, we have evaluated their carbonate dissolution activity. Fungal dissolution activity of calcium carbonate-amended plates is mainly a result of organic acid production by fungi (Ortega-Morales et al., 2016), although some other mechanisms of calcite carbonate weathering are proposed, for example, by means of enzymes (Unković et al., 2018). According to the literature (Sazanova et al., 2016), Ca^{2+} and CO_3^{2-} stimulate oxalic acid production. It is important considering that limestone and plaster contain $CaCO_3$. It is well known that organic acid production by fungi is one of the most significant biodeteriorative factor as these acids dissolve the binding matrix of material (Boniek et al., 2017; Gadd, 2007; Sazanova et al., 2016; Sterflinger, 2000; Warscheid and Braams, 2000). In our research, only a small number of tested fungal isolates were able to solubilize $CaCO_3$ that corresponds to the previous studies (Pangallo et al.,

2012; Unković et al., 2018). Among species possessed the highest abundance in our work, *A. charticola* and *Lecanicillium* sp.1 were tested positive that may imply additional risk in their development in stone materials.

Some species from the second group also solubilized $CaCO_3$. It was noticed (Unković et al., 2018) that mainly micromycetes from the genera *Aspergillus* and *Penicillium* possess calcite solubilization activity. Three tested strains of *P. chrysogenum* were positive and strains of *A. flavus* were tested negative that corresponds to the results of Pangallo et al. (2009), while dissolution activity of *A. versicolor* varied depending on strain. Moreover, it was shown that *A. versicolor* is able to produce organic acids, in particular, the oxalic acid (Sazanova et al., 2016), though in small amount that fully corroborates our research as *A. versicolor* displayed weak dissolution activity. The same result was obtained for *P. pannorum* as it also displayed weak transparent zone in our test and produced organic acids (in particular, oxalic and glyceric ones) in low amounts (Sazanova et al., 2016). Thus taking into account ability of fungi from the second group to develop in mineral materials and to solubilize $CaCO_3$, we conclude that they may take part in deterioration. It is important to take into consideration that calcite dissolution activity depends on various factors, primarily, on available sources of carbon and nitrogen (Unković et al., 2018), and thus we cannot predict dissolution activity of isolated species *in vivo*.

5. Conclusions

In summary, we isolated and characterized the culturable fungal community colonizing mineral materials in interiors of objects of cultural heritage using parameters of its taxonomic structure and fungal ecophysiology. We have revealed pH and a_w preferences of fungal dwellers of stone materials inside buildings and have confirmed their ability to thrive in conditions of pH change and heightened moisture content, which are characteristic of biodeteriorated objects. In general, isolated fungal species participated in different stages of colonization and played various roles in biodeterioraton process of mineral building materials. We conclude that in the isolated community *A. charticola*, *A. furcatum*, *Lecanicillium* sp.1, *P. album*, *P. lilacinum* and *S. kiliense* played the most important role in deterioration of stone materials in interiors of cultural monuments and museums.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.01.002.

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