European Journal of Biology and Medical Science Research

Vol.3, No.4, pp.17-23, August 2015

Published by European Centre for Research Training and Development UK (www.eajournals.org)

STUDY OF BIOFOULING IN BOOKS STORED AT THE ARCHIVE OF THE LIBRARY OF SHUMEN UNIVERSITY

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ABSTRACT: *The aims of this paper were to study the biofouling and biodeterioration of books stored at the Archive of the library of Shumen University, Bulgaria, and to carry out the physiological characterization of isolated fungi and bacteria. Also the role of the environmental microbiota in the biofouling formation was studied. Microbial assemblages on documents were sampled by sedimentation method as described by Omeliansky. Biofouling were monitored by microscope and stereomicroscope OPTIKA (Italy). Large microbial assemblages were found at archive with the prevalence of genera Aspergillus, Cladosporium and Penicillium. Most of the fungi degraded cellulose and produced pigments and acids, and all of the isolated bacteria had proteolytic and/or cellulolytic activity. In shed a higher concentration of viable bacteria than of fungi was isolated. The existence of this bacterial genus in the Library of Shumen University indicates that the faulty ventilation system needs to be repaired and suitable anti-dust filters to be set.*

KEYWORDS: Archives, Biodeterioration, Environment, Microorganisms, Paper

INTRODUCTION

Highly significant evidence of the intellectual and cultural efforts of the human race is contained in documents. They take many forms, from papyri through paper to modern magnetic media and optical records. These items are mainly made of organic materials many of which contain polymers, which span from cellulose and its derivatives to synthetic resins. As with other manmade objects, however, documentary heritage is susceptible to chemical, physical, and biological damage. For the colonization and establishment of any biological community, the composition of materials used, their status of conservation, and environmental and climatic factors, such as temperature and humidity, are important elements to take into account [9].

A large number of particles of different origin, shape and size are suspended in the air in outdoor and indoor environments and they constitute the atmospheric aerosol. They can be classified in different ways, taking into account the origin (biological, organic and inorganic), the location (marine, continental, rural, industrial and urban) and the effect they cause on surfaces where they may be deposited (chemical, toxic, pathogenic, degrading) [21]. Among the particles of biological origin are included bacteria, fungal spores, algae, viruses, protozoa and pollen grains. Microorganisms can be carried indoors on dust particles present in outdoor air through ventilation and visitors [12; 28]. Their colonization and growth on the surface of objects within the building can be both an important source of further indoor air contamination [5;23]. Microorganisms participate actively by adhering to surface of different documentary support through biofilms formation. Biodeterioration and biofouling are due to microbiological,

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

biological and physicochemical processes [4]. Biodeterioration can be defined as a change in material properties due to the vital activities of the organisms [16] and biofouling is the accumulation of biological deposit on a surface [4]. Many of the bacteria present in these materials, and particularly in archive materials, grow using very low concentrations of nutrients and may cause chemical and esthetic biodeterioration, as well as facilitating the last development of other microorganisms such as fungi, which are well-known deteriogens [18]. Their initial growth on materials is usually due to other organic material present in the dust, not to the nutrients in the supports [15]. When the colonized substrates are heritagematerials, the biodeterioration becomes a serious social and economic problem [13]. The aims of this paper were to study the biofouling and biodeterioration of books stored at the Archive of the library of Shumen University, Bulgaria, and to carry out the physiological characterization of isolated fungi and bacteria.

MATERIALS AND METHODS

Characteristics of the environments studied

Microbial studies were performed at the Archive of the library of Shumen University, Bulgaria. The physicochemical parameters of of the library of Shumen University, temperature 21.4 ◦C, 64,8% RH. All rooms were protected areas with artificial lighting, and also air conditioned.

Microbiological sampling of air

Microbiological sampling was carried out by the sedimentation method as described by Omeliansky [6;8]. Hence, Petri dishes containing YGC Agar (yeast extract, glucose and chloramphenicol) for the isolation of fungi and Nutrient Agar for bacteria, were placed open at approximately 2 m above the floor and were exposed for 5 min. Five different points of Library of were sampled – 1 points (discotheque); 2 points (reading room); 3 points (repository); 4 (passage to reading room) and 5 (shed). All samples were collected by triplicates. Afterwards, plates with YGC were incubated for 7 days at 28 ◦C and those containing Nutrient Agar were kept at 32 ◦C for 72 h. The CFU number per cubic meters of air was estimated according to Omelianskyıs [14].

Identification of isolated organisms

Cultural and morphological characteristics of fungal colonies were observed and the identification was performed according to Barnett and Hunter [3]. Bacteria were grouped on the basis of the Gram staining.

RESULTS

Microbial contamination in air

The fungal and bacterial concentrations in five different points are shown in Table 1.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

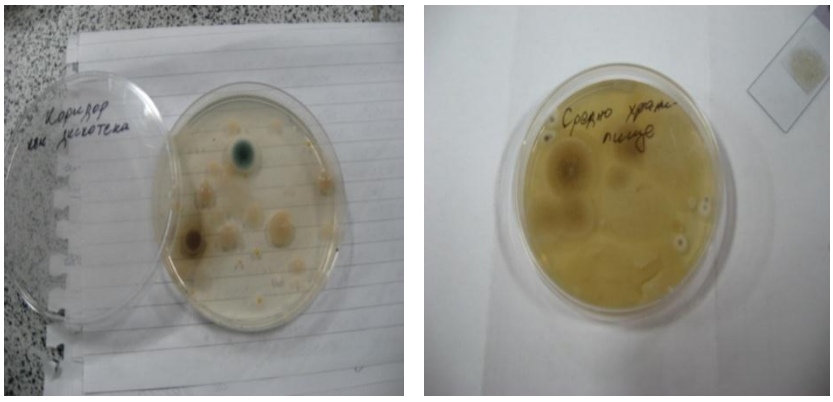
Table 1. Microbial prevalence in the air at the Library.

Position/ parameters	Fungi	Bacteria	Total Microorganisms ($CFUm^{-3}$)	T (^0C)	RH (%)
1	2	-	2000	19.1	68,8%
2	7	5	6000	23.4	71,3%
3	26	2	14000	21.4	64,8%
4	45	4	24500	24.3	70,8%
5	3	21	12000	22.4	67,6%

[a]Results are mean ± SEM of three separate trails.

Microorganisms concentrations were higher in the passage to reading room where the temperature and relative humidity was higher. At discotheque both bacteria and fungi concentrations were low, in this case the environment can be considered not to be contaminated. On the other hand, in repository, shed and passage to reading room bacterial concentration is very high, so the environment may be considered as highly contaminated. In the discotheque air the only detected microorganisms was the fungal. Fungi genera were also found in the air predominantly *Penicillium* genus (figure 3), *Aspergillus sp.* (figure 2) and *Cladosporum sp.* (figure 4). With relation to bacteria it can be appreciated that Gram-positive isolated genera *Streptococcus*, *Bacillus* and *Streptomyces* genera (figure 1 and table 1). *Enterobacter sp.* could be identified among the Gram negative species (figure 1 and table 1).

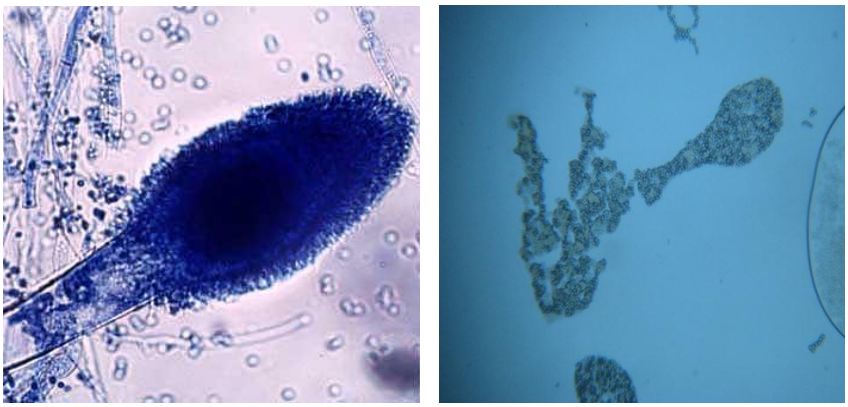
The microscopic pictures also confirmed the presence of high microbial species diversity in the samples (Figure 1 and 2).



A B

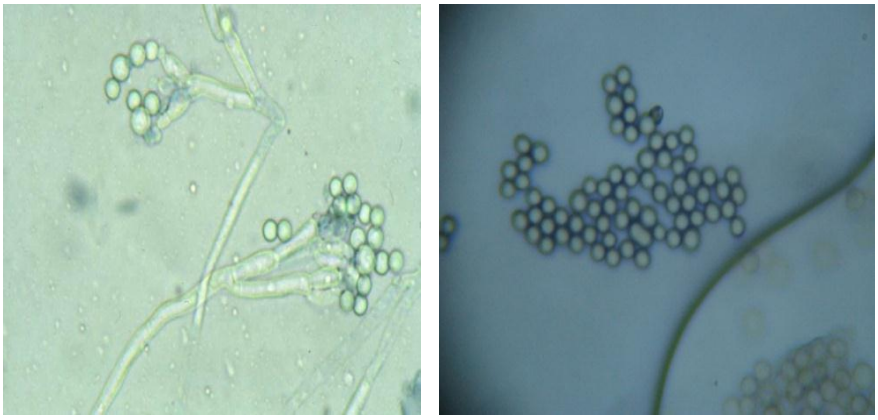
Fig. 1.Pictures of the colonies of isolated species: A) passage to reading room B) repository. The pictures were taken using stereomicroscope OPTIKA (Italy).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)



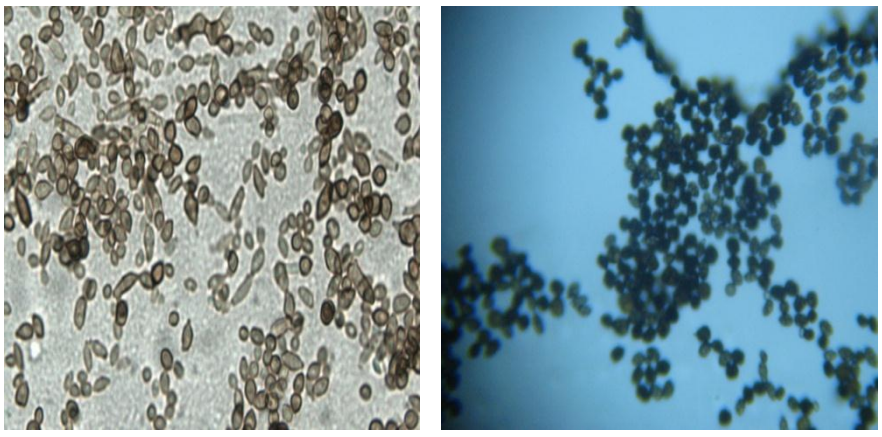
A B

Fig 2. Light microscopic visualizations of preparation imprint: A) picture by www.dehs.umn.edu350 × 350 *Aspergillus sp.*; B) picture made by us (The picture were taken using microscope OPTIKA (Italy) at magnification 1000 by immersion).



A B

Fig 3. Light microscopic visualizations of preparation imprint: A) picture by mycota-crcc.mnhn.fr773 × 512 *Penicillium sp.*; B) picture made by us (The picture were taken using microscope OPTIKA (Italy) at magnification 1000 by immersion).



A B

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Fig 4. Light microscopic visualizations of preparation imprint: A) picture by www.falaboratories.com849 × 518 *Cladosporium sp.;* B) picture made by us (The picture were taken using microscope OPTIKA (Italy) at magnification 1000 by immersion).

DISCUSSION

Microbial concentrations were significantly higher at passage to reading room and repository. Temperature, relative humidity and ventilation have been appropriate for this. This would be related to ventilation of deposits, since it has been reported that ventilation can play an important role in slowing down microbial growth because air movements are considered as a way to avoid high concentration of microbes in the air [14;28]. Fungal levels detected at Library of Shumen University repositories were lower and this is possibly due to the fact that some very light fungal spores do not sediment readily. It has been suggested that spores ≤ 5 μm require winds stronger than $25 mseg^{-1}$ to be deposited [19]. However, bacterial cells are generally carried on dust which makes them heavy enough to be deposited on the Petri dishes. Similar microbial concentrations were detected in Cuba inside homes, archives, libraries and museums that had been sampled with bio collectors [7;24]. In contrast, in previous studies performed in other Library of Shumen University repositories using an aeroscope as biocollector [5;6] significantly lower microbial contamination concentration was found. This proves that in order to know the microbiota variability a systematic sampling is needed. Other authors reported high levels of Gram positive bacteria in indoor environments [8;27]. Apart of the increase of microbial assemblage caused by dust penetration, it should be noted that Gram positive bacteria can also penetrate as a consequence of human activity, since many of them are carried in the skin and in mucous membranes [29]. It has been reported that high levels of *Bacillus* in indoor air are commonly indicative of damage caused by water or a need for building maintenance [17]. The complex enzyme systems of the proteolytic activity of *Bacillus* species can degrade the fibrous and non-fibrous material of paper [11]. A large number of Bacillus species produce endospores that are highly resistant to extreme environmental conditions, to antibiotics, to disinfectants and other chemical substances. Endospores are easily spreadable. *Bacillus*, *Clostridium* and *Streptomyces* genera have intense cellulolytic activity [2;10] and thus can attack paper and degrade it within 24 h at a relative humidity of 90%, which could occur if the relative humidity in the repositories should suddenly increase. This is also in agreement with our research data.

It is notable that the *Aspergillus* and *Penicillium* genera were also isolated from the air of the buildings; these cosmopolitan genera can colonize various surfaces [20]. Fungal teleomorphs are difficult to isolate from the surfaces of art objects and documents. This is the first time that has been reported [14]. This form of *Penicillium* has cellulolytic activity [10;22;26], its presence involves a high risk for document conservation. Similar results have been obtained by other authors in relation to fungal genera isolated from air in buildings, as well as the physiological characteristics of the strains degrading cellulose and producing pigments and acids [7] they not only cause esthetic damages but are also part of a biofouling which in turn is made up of other components (e.g. insect fecal material). The presence of spores and/or vegetative cells of microorganisms on the surfaces of materials indicate a potential source of biodegradation or biodeterioration. Colonization or microbial growth on a material always produces the biodeterioration of that material [1;25]. It is necessary, therefore, to clean the documents before placing them into the cases, in order to minimize the chance of future microbial growth under unfavorable environmental conditions or in the case of water disasters.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

CONCLUSION

The existence of this bacterial genus in the Library of Shumen University indicates that the faulty ventilation system needs to be repaired and suitable anti-dust filters to be set.

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ISSN 2053-406X(Print) ISSN 2053-4078(Online)