

# Microbiological Hazards Associated with Archaeological Works, Illustrated with an Example of Fredro Crypt (Przemyśl, Poland)

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## ABSTRACT

*The human remains and other materials found in crypts can be highly contaminated with microorganisms. Archaeologists are exposed to microorganisms in many ways (e.g by inhaling dust, contaminating scratches or cuts). We aimed at evaluating microbial hazards associated with human remains and bioaerosols formed during archaeological works in burial crypts. The samples of the human remains, bioaerosols and personal protective equipment (dust respirators, disposable coveralls) were collected during archaeological works in the vault of the Cathedral Basilica of St. John the Baptist and the Assumption of the Blessed Virgin Mary in Przemyśl (Fredro crypt). The microbiological examination involved determining the number of spores of aerobic and anaerobic bacteria, the number of mesophilic and hemolytic bacteria, and the number of xerophilic, non-xerophilic and keratinolytic fungi. The air as well as objects and materials found in crypts are highly contaminated with bacteria and fungi. The xerophilic fungi were the most numerous in all samples of human remains 1–230·10<sup>3</sup> cfu(colony forming units/g). The non-xerophilic fungi were predominant in bioaerosols (>10<sup>4</sup> cfu/m<sup>3</sup> during archaeological works). The majority of bacterial strains (81.3%) belonged to the genus *Bacillus*. Fungi belonging to the genera *Penicillium* (65.2%) and *Aspergillus* (28.6%) dominated among the isolated fungi. Fogging sterilization of the crypt turned out to be ineffective. The high number of microorganisms both in the air and on human remains indicates that there is a need for particular caution during archaeological works which cause dust emission. In order to reduce exposure to harmful biological factors, the use of disposable personal protective equipment seems necessary.*

**Key words:** archaeological works, health risk, bioaerosol, microorganisms

## Introduction

Archeology, the study of human activity in the past, investigates material remnant of people including garbage dumps, cesspits, graves and tombs. It must be emphasized that neither human remains nor other objects and materials found in crypts (coffins, soil) are sterile<sup>1-3</sup>. Archaeologists can be invaded by microorganisms in many ways: by inhaling dust during excavation work, contaminating scratches and cuts or eating with dirty hands. Microbes “transported” on clothes from the excavation site can also pose a health hazard. There is little likelihood that pathogenic species, responsible for the majority of serious diseases (plague, cholera, typhus, and tuberculosis) could survive in human remains dating to

100 or 200 years ago. However, the risk of an infection with anthrax bacterium or poxvirus is much more real<sup>4</sup>.

Occupational risk factors include not only infectious agents (viruses, bacteria, fungi, parasites), but also allergenic and toxic ones, the main cause of diseases in many occupational groups<sup>5-7</sup>. Many of these factors are found in bioaerosols that form during excavation and renovation works at archaeological sites<sup>2,3</sup>.

Many archaeologists seem to be unaware of microbial health risks. It is a common practice to handle human remains without proper equipment and protecting clothing. Keeping that in mind, we aimed at evaluating microbial hazards associated with human remains and bioaerosols formed during archaeological works in burial crypts.

## Material and Methods

### Research site

Material for the study was collected in the vault of the Cathedral Basilica of St. John the Baptist and the Assumption of the Blessed Virgin Mary in Przemyśl, Poland. All samples were collected in the crypt of the Chapel of St. Cross (Fredro crypt), which was built in the mid-eighteenth century (Figures 1a and b). The crypt has plastered brick walls, is dry and ventilated through two small windows located under the ceiling. It has the surface area of approx. 40 square meters, and the height at the highest point of approx. 2 meters. 11 coffins with human remains were found in the crypt. Four coffins contained mummified bodies and the remaining seven, human skeletons. Fragments of dried tissue were found on several skeletons. Coffins lied on a two-level shelf, which was supported by pillars joined with metal rails, parts of a narrow-gauge railway. The construction was built during clean up works, probably at the end of the nineteenth century (Figure 1c).

The samples were collected within 2 working days, on 24 and 25 February 2014. During sampling there were five people in the crypt. They were engaged in the following: archaeological works (3 people), supervision (1 person), sampling (1 person).

### Microbiological analysis of human remains

Samples (weight of 3–5 g) were collected from the surface of the bones (using sterile metal scrapers), skin and hair (using sterile tweezers or scissors), placed in sterile plastic containers and transported to the laboratory at the temperature of 10–15 °C (similar to the temperature in the crypt). Time between sample collection and microbiological analysis never exceeded 48 hours. From subsamples (weight of 1 g) 10-fold dilutions were prepared using sterile saline solution (0.85%) supplemented with peptone (0.1%). In order to eliminate vegetative forms of microorganisms and to stimulate spore germination, diluted samples were pasteurized for 30 minutes at 80 °C before inoculation on a suitable substrate.

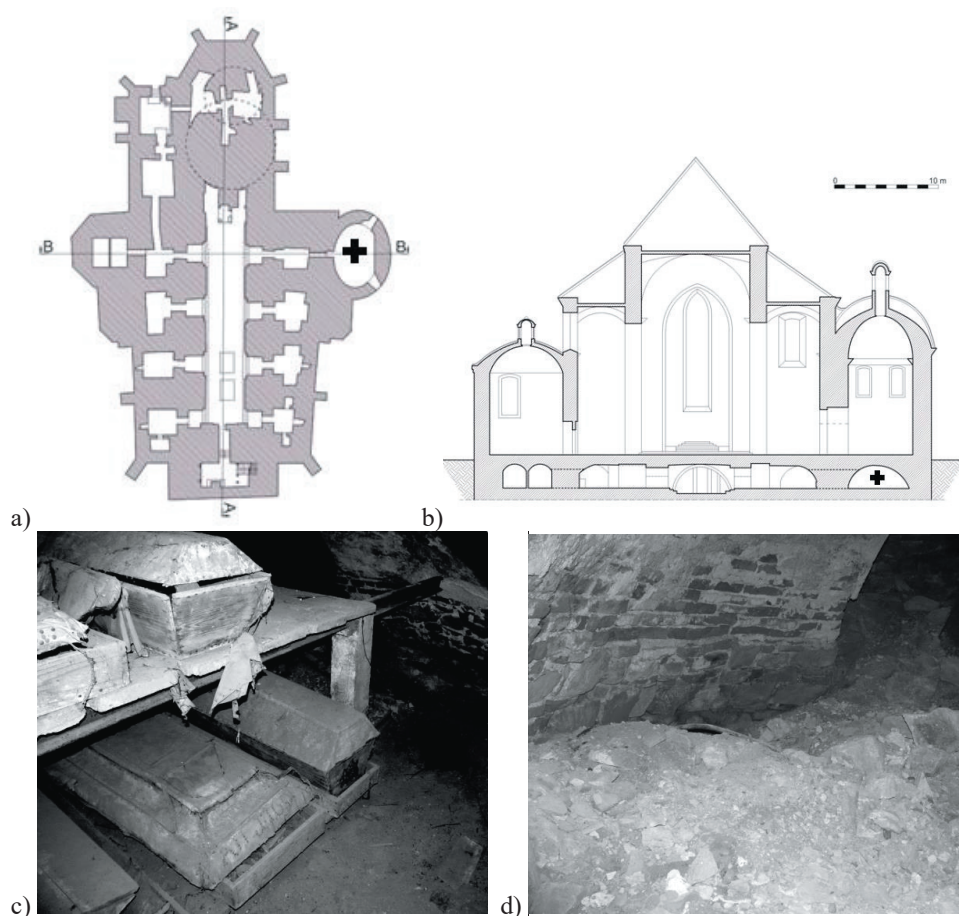


Fig. 1. The Fredro crypt (+) in the Cathedral Basilica of St. John the Baptist and the Assumption of the Blessed Virgin Mary in Przemyśl a) Location in the horizontal section b) Location in the vertical section c) Coffins inside of the Fredro crypt (Fot. A. Drązkowska) d) Rubble and soil material sifting during archaeological work (Fot. A. Burkowska-But).

We determined the number of the following groups of microorganisms: spores of aerobic and anaerobic, non-xerophilic fungi; xerophilic fungi and *keratinolytic* fungi.

### ***Microbiological analysis of bioaerosols in the crypt***

Air samples were collected directly on the appropriate substrate by the impaction method, using MAS–100 (Merck KGaA) microbial air sampler. The airflow rate was approx. 100 L/min. The collection time for each sample was 30 seconds. Bioaerosol samples were collected from the central part of the crypt, 1.2 m above the floor. Each sample collection was repeated three times for each substrate. This guaranteed a minimal error related to the randomness in sampling. The results were expressed as colony forming units (cfu) per m<sup>3</sup> of air.

Samples were collected 4 times:

- before sterilization and archaeological works;
- before sterilization, while sifting rubble and soil (Figure 1d), which also contained human bones, fragments of coffins (wood, nails, hardware fittings, upholstery), and fragments of clothing;
- immediately after fogging sterilization of the crypt using Nocospray (OXY'PHARM), before resuming work. Nocospray system generates dry vapor (particles of approx. 5 microns in diameter). It uses Nocolyse (OXY'PHARM) disinfecting solution containing H<sub>2</sub>O<sub>2</sub> i Ag<sup>+</sup> ions. Sterilization time, determined according to the manufacturer's instructions for the given volume, was 10 minutes. During the sterilization and 30 minutes after this procedure there were no people in the crypt.
- after sterilizing the crypt and resuming work. Work was resumed after air sampling, 60 minutes after sterilization.

We determined the number of the following groups of microorganisms: mesophilic bacteria,

hemolytic bacteria, non-xerophilic fungi and xerophilic fungi.

### ***Microbiological analysis of personal protective equipment***

The following PPE items (personal protective equipment) were examined during archaeological works: disposable FFP2 dust respirators (dust masks) (Filter Service) and disposable coveralls made of flash-spun, high-density polyethylene (DuPont). Used PPE was placed in sterile bags, stored and transported to the laboratory at the temperature of 7 °C. Time between the use of PPE and microbiological analysis never exceeded 48 hours.

Face masks were evaluated for the presence of microorganisms by making an imprint of their outer sides (which did not touch the face) on a suitable substrate. We determined the number of mesophilic and hemolytic bacteria as well as xerophilic and non-xerophilic fungi on the

surface of disposable FFP2 dust respirators. The number of microorganisms was expressed as cfu/100 cm<sup>2</sup>.

Fragments from different areas (arms, legs and shoulders) of the coveralls (total surface area of 500 cm<sup>2</sup>) were also examined. Microorganisms rinsed off the samples using 100 mL of diluent (peptone water + 1% Tween) were inoculated (the amount of 1 µL) on a suitable substrate (using spread plate technique). We determined the number of mesophilic and hemolytic bacteria as well as xerophilic and non-xerophilic fungi on the surface of disposable coveralls. The number of microorganisms was expressed as cfu/100 cm<sup>2</sup>.

### ***The conditions of the incubation and the substrates***

Incubation of the investigated microorganisms was carried out under the following conditions:

- a) spores of aerobic bacteria – pour plate technique, on Plate Count Agar (Difco™), incubated for 24 h at the temperature of 37 °C;
- b) spores of anaerobic bacteria – pour plate technique, on Wilson-Blair Agar for anaerobic bacteria (BTL), incubated anaerobically for 24 hours in AnaeroPack (Mitsubishi) with GENbox anaer (bioMerieux) at the temperature of 37 °C;
- c) mesophilic bacteria – on Plate Count Agar (Difco™), incubated for 24 h at the temperature of 37°C;
- d) hemolytic bacteria – on Columbia agar (Oxoid) with sheep blood, incubated for 24 h at the temperature of 37 °C. We counted colonies of α-hemolytic bacteria (surrounded by a zone of partial hemolysis) and β-hemolytic bacteria (surrounded by a zone of complete hemolysis).
- e) non-xerophilic fungi – pour plate technique, on Czapek-Dox LAB-AGAR (Biocorp), incubated for 5 days at the temperature of 26 °C;
- f) xerophilic fungi – pour plate technique, on Dichloran Glycerol DG18 LAB-AGAR (Biocorp), incubated for 5 days at the temperature of 26°C;
- g) keratinolytic fungi – using bait from defatted children's hair (approx. 1–2 cm long) submerged in aqueous agar<sup>8</sup> (Ulfing, 2003). The amounts of 0.1 mL of 10-fold diluted samples were poured on the surface of hair traps and incubated for 21 days at 20 °C. Hair traps were observed using a binocular magnifying glass. Fungal growth on the surface of the hair was considered a positive result (+).

### ***Identification of bacterial strains***

Isolated bacterial strains were identified using MALDI TOF MS (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) method, based on an analysis of individual protein profiles (different for each bacterial species). Spectra obtained using MALDI Biotyper (Bruker Daltonik) were compared with reference profiles of microorganisms in MBT DB–5627 (Bruker Daltonik) database.

### Identification of fungal strains

Molecular identification of fungi was prepared using ITS1 and ITS2 polymorphic sequences which separate genes encoding 18S, 5.8S and 28S rRNA. Sequencing read was prepared by Oligo IBB DNA Sequencing and Oligonucleotide Synthesis Laboratory in Warsaw. Taxonomic identification was prepared using EzFungi and Genbank databases (<http://www.ezbiocloud.net/ezfungi>) using BLASTN algorithm, assuming sequence similarity threshold of 98%.

## Results

### Microbiological analysis of human remains

Our results indicate that even if stored in very dry and old crypts, human remains are heavily colonized by microorganisms (Table 1). The analysis show that xerophilic fungi were the most numerous in all samples. Their number ranged from  $10^3$  cfu/g (recorded on hair from coffin 6) to  $23 \cdot 10^4$  cfu/g (on hair from coffin 3), which indicates that it was not determined by the type of material.

Non-xerophilic fungi were less numerous. Moreover, a high number of xerophilic fungi was usually correlated to a high number of other types of fungi. The highest number of non-xerophilic fungi ( $4.25 \cdot 10^3$  cfu/g) was recorded in hair from coffin 3. In addition, all samples contained keratinolytic fungi.

Spores of aerobic bacteria were the second most numerous. Their highest number ( $11.5 \cdot 10^4$  cfu/g) was recorded in a sample collected from the mummified abdomen skin from coffin 5. Hair from coffin 3 also contained a large number of these spores ( $7.6 \cdot 10^4$  cfu/g). On the other hand, the number of anaerobic spores was very low.

All samples except for one (collected from the skull from coffin 2) contained less than 1 cfu/g.

### Microbiological analysis of bioaerosols in the crypt

Non-xerophilic fungi were predominant in bioaerosols, where their number amounted to  $>10^4$  cfu/m<sup>3</sup> during archaeological works. The number of heterotrophic bacteria ranged from 20 cfu/m<sup>3</sup> (before archaeological works) to  $> 5 \cdot 10^3$  cfu/m<sup>3</sup> (while archaeological works were in progress). Microbial air quality deteriorated significantly during archaeological works, both before and after sterilizing the crypt (Table 2). The number of mesophilic bacteria was one hundred times higher during archaeological works than before they started. In addition,  $\beta$  – hemolytic bacteria, which were absent before work in the crypt was initiated, were identified later, when the work progressed.

The number of non-xerophilic and xerophilic fungi did not decrease after crypt sterilization and the number of mesophilic and hemolytic bacteria was only slightly lower (Table 2).

### Microbiological analysis of protective personal equipment

Non-xerophilic and xerophilic fungi predominated among the microorganisms found on the surface of clothes, human remains, and in the air (Table 3 and 4). Considerably higher numbers were recorded on the uniforms than on the masks, probably as the result of applying different methods of analysis. The number of microorganisms on protective clothing depended on both time spent in the crypt and the type of tasks performed by archaeologists. The highest number was recorded on clothes used only for half an hour, but while sifting soil and rubble (Table 4).

TABLE 1

CONCENTRATION OF MICROORGANISMS ON HUMAN REMAINS STORED IN FREDRO CRYPT

			N	Spores of aerobic bacteria	Spores of anaerobic bacteria	Non-xerophilic fungi <sup>a</sup>	Xerophilic fungi <sup>b</sup>	Keratinolytic fungi
			[cfu/g]					
Bone	Skull	Coffin 2, skeleton	4	2317±431*	1.28±0.52	1539±375	14808±850	+
	Thigh bone	Coffin 3, skeleton, partly mummified body	5	3723±517	< 1	266±92	36170±1740	-
	Skull	Coffin 6, skeleton, partly mummified body	5	5086±688	< 1	776±101	4828±809	+
Shoulders		Coffin 4, mummified body	2	420±97	< 1	270±105	8330±752	+
		Coffin 5, mummified body	3	115385±1873	< 1	19.2±7.6	1365±211	+
Hair		Coffin 3, skeleton, partly mummified body	2	76500±1655	< 1	4250±814	233333±2827	+
		Coffin 6, skeleton, partly mummified body	3	33±12	< 1	130±63	1022±349	+

n – number of samples, a – fungi cultivated on Czapek-Dox medium, b – fungi cultivated on Dichloran Glycerol DG18 medium, \* – average± standard deviation, “+” – presence of keratinolytic fungi, “-” – keratinolytic fungi absent

**TABLE 2**  
CONCENTRATION OF MICROORGANISMS IN THE AIR OF FREDRO CRYPT

The air sampling:		N	Mesophilic bacteria	Hemolytic bacteria [cfu/m <sup>3</sup> ]	Non-xerophilic fungi <sup>a</sup> [cfu/m <sup>3</sup> ]	Xerophilic fungi <sup>b</sup> [cfu/m <sup>3</sup> ]
Before sterilization	Before working (background)	6	30±7.1*	α 7±3.8 β 0	420±36	1150±166
	During the work**	6	> 4880	α 5±2.2 β 125±15	>10000	>5000
After fogging sterilization	Before working	6	20±5.3	α 0 β 0	700±91	1630±218
	During the work**	6	3440±971	α 10±5.7 β 67±28	>10000	>5000

*n* – number of samples; <sup>a</sup> – fungi cultivated on Czapek-Dox medium, <sup>b</sup> – fungi cultivated on Dichloran Glycerol DG18 medium, α – number of α-hemolytic bacteria, β – number of β-hemolytic bacteria, \* – average± standard deviation, \*\* – during the sifting rubble and soil contained human bones and fragments of coffins

**TABLE 3**  
CONCENTRATION OF MICROORGANISMS ON THE OUTER SURFACE OF THE DISPOSABLE RESPIRATOR FFP2

Sample	N	Mesophilic bacteria	Hemolytic bacteria [cfu/100 cm <sup>2</sup> ]	Non-xerophilic fungi <sup>a</sup>	Xerophilic fungi <sup>b</sup>
R1	4	49±4.5*	α 4±2.2 β 2±1.8	38±6.9	208±7.9
R2	4	42±4.8	α 5±2.1 β 10±3.6	72±3.7	257±11.3
R3	4	35±4.7	α 3±1.1 β 19±3.9	214±9.1	631±15.8

R1 – the disposable respirator FFP2 used for 30 minutes while air sampling (before sterilization of the crypt)

R2 – the disposable respirator FFP2 used for 1 hour, during supervision in the crypt (before sterilization of the crypt)

R3 – the disposable respirator FFP2 used for 30 minutes while sifting rubble and soil (after fogging sterilization of the crypt)

\* – average±standard deviation, *N* – number of samples; <sup>a</sup> – fungi cultivated on Czapek-Dox medium, <sup>b</sup> – fungi cultivated on Dichloran Glycerol DG18 medium, α – number of α-hemolytic bacteria, β – number of β-hemolytic bacteria,

### Identification of microorganisms

All strains isolated from human remains belonged to the genus *Bacillus* (Table 5). The majority of bacterial strains isolated from the air (83.3%) and disposable coveralls (80.0%) belonged to the genus *Bacillus* as well; the remaining to the genera *Pseudomonas*, *Arthrobacter*, *Staphylococcus*, and *Micrococcus* (on disposable dust respirators and in air). *Bacillus pumilus* strain was most frequently identified in both the air (66.7%), disposable coveralls (42.9%) and human remains (29.0%). Fungi belonging to the genera *Penicillium* (65.2%) and *Aspergillus* (28.6%) dominated among the isolated fungi although human remains also contained *Isaria farinosa* (Table 6).

### Discussion

Crypts constitute a specific environment populated with many groups of microorganisms, involved in decomposition of organic matter found in their interiors. Although microorganisms colonizing human remains may pose a health hazard for archaeologists exploring crypts, there is still insufficient amount of information related to this topic<sup>2,3</sup>. Regrettably, earlier microbiological anal-

**TABLE 4**  
CONCENTRATION OF MICROORGANISMS ON THE DISPOSABLE COVERALLS

The time and the type of tasks performed by archeologists	N	Mesophilic bacteria	Hemolytic bacteria	Non-xerophilic fungi <sup>a</sup>	Xerophilic fungi <sup>b</sup>
		[cfu/100 cm <sup>2</sup> ]			
2 hours air sampling	4	7830±560*	α 750±61	39200±3422	42500±3988
			β 500±38		
1 hour supervision	3	4420±291	α 580±57	830±95	7500±612
			β 420±29		
30 minutes sifting soil and rubble before sterilization	7	24100±2191	α 700±72	30000±2973	29000±2165
			β 0		

\* – average±standard deviation, *N* – number of samples, <sup>a</sup> – fungi cultivated on Czapek-Dox medium, <sup>b</sup> – fungi cultivated on Dichloran Glycerol DG18 medium, α – number of α-hemolytic bacteria, β – number of β-hemolytic bacteria

ysis were mainly aimed at investigating organisms colonizing burial garments<sup>9–11</sup>, coffins, and crypt walls<sup>12</sup>.

Our results indicate that human remains kept in the crypt are colonized by high numbers of microorganisms. Xerophilic fungi and other molds are the most numerous,

**TABLE 5**  
IDENTIFIED BACTERIAL SPECIES

The material:	N	Isolated bacteria	% in the material
Human remains	62	<i>Bacillus pumilus</i>	29.0
		<i>Bacillus subtilis</i>	25.8
		<i>Bacillus cereus</i>	16.1
		<i>Bacillus thuringiensis</i>	7.7
		<i>Bacillus muralis</i>	6.5
		<i>Bacillus megaterium</i>	6.5
		<i>Bacillus simplex</i>	3.2
		<i>Bacillus licheniformis</i>	3.2
		<i>Bacillus mycoides</i>	1.6
		Air	48
<i>Bacillus cereus</i>	16.7		
<i>Pseudomonas xanthomarina</i>	8.3		
<i>Arthrobacter oxydans</i>	4.2		
<i>Arthrobacter crystallopoietes</i>	4.2		
<i>Staphylococcus epidermidis</i>	42.6		
Disposable dust respirators	21	<i>Micrococcus luteus</i>	28.6
		<i>Bacillus simplex</i>	14.3
		<i>Bacillus mycoides</i>	9.5
		<i>Lysinibacillus fusiformis</i>	4.8
Disposable coveralls	35	<i>Bacillus pumilus</i>	42.9
		<i>Bacillus muralis</i>	17.1
		<i>Bacillus simplex</i>	11.4
		<i>Bacillus cirulans</i>	8.6
		<i>Curtobacterium sp.</i>	5.7
		<i>Microbacterium sp.</i>	5.7
		<i>Staphylococcus pasteurii</i>	5.7
		<i>Rhodococcus erythropolis</i>	2.9
<i>Achromobacter piechaudii</i>	2.9		

N – number of identified bacterial strains

which is in line with measurements made by other authors<sup>2,3,13</sup>. Fungi of the genera *Penicillium* and *Aspergillus*, predominant among the investigated isolates, also dominated on the mummified body of St. Martin<sup>3</sup> and on mummies in the Archaeological Museum in Zagreb<sup>2</sup>. These fungi can, in certain conditions, produce mycotoxins and a range of allergens which may cause hay fever or asthma in susceptible individuals<sup>13–15</sup>. According to Nielsen<sup>16</sup> *Aspergillus versicolor*, identified on human remains and in the air, produces large amounts of hepatotoxic sterigmatocystin (up to 1% of its biomass), a strong inhibitor of the movement of tracheal cilia. The majority of *A. versicolor* strains isolated from indoor environments have the ability to produce sterigmatocystin. At the same time *A. versicolor* are present in the majority of samples containing this toxin<sup>17,18</sup>.

**TABLE 6**  
IDENTIFIED FUNGAL SPECIES

The material:	N	Isolated molds	% in the material
Human remains	66	<i>Penicillium chrysogenum</i>	36.4
		<i>Aspergillus versicolor</i>	19.7
		<i>Isaria farinosa</i>	15.2
		<i>Aspergillus clavatus</i>	9.1
		<i>Aspergillus conicus</i>	9.1
		<i>Aspergillus tennesseensis</i>	3.0
		<i>Penicillium citreonigrum</i>	3.0
		<i>Penicillium roseopurpureum</i>	1.5
		<i>Aspergillus penicillioides</i>	1.5
		<i>Aspergillus pragensis</i>	1.5
Air	49	<i>Penicillium chrysogenum</i>	40.8
		<i>Aspergillus versicolor</i>	24.5
		<i>Penicillium waksmanii</i>	14.3
		<i>Penicillium commune</i>	12.2
		<i>Penicillium citreonigrum</i>	8.2
Disposable dust respirators	19	<i>Penicillium chrysogenum</i>	47.4
		<i>Penicillium commune</i>	31.6
		<i>Penicillium waksmanii</i>	21.0
Disposable coveralls	27	<i>Penicillium chrysogenum</i>	44.4
		<i>Penicillium commune</i>	37.0
		<i>Aspergillus versicolor</i>	18.6

N – number of identified strains of molds

All examined samples of human remains were contaminated with keratinolytic fungi, capable of decomposing keratin, a protein component of hair, nails, and calloused skin. Many of these species are pathogens or potential pathogens which can cause fungal infections in humans<sup>19,20</sup>.

Samples from Fredro Crypt contained a high number of spores of aerobic bacteria. Other authors also reported that spores of aerobic bacteria were the most numerous microorganisms in burial crypts and sarcophagi<sup>2,3,11</sup>. Some of the studied isolates were identified as bacteria of the genus *Bacillus*. The vast majority of isolated bacteria are saprophytic microorganisms commonly found in soil, water, air, and the human body and do not pose a health risk. The pathogenic *Bacillus cereus*, known to produce toxins which can cause food poisoning and diarrhea<sup>21</sup>, seems to be an exception. In addition, the isolated *B. subtilis* and *B. thuringiensis* now considered allergens<sup>22</sup>.

Bioaerosols, microorganisms suspended in the air on dust particles or liquid droplets, can pose a serious health hazard. They can cause asthma, allergic alveolitis, irritation of mucous membranes, contagious diseases or even cancer<sup>7</sup>. Our results show that significantly increased bioaerosol concentration was recorded during archaeological works in the crypt. No universally applicable standards define acceptable levels of microorganisms in the air<sup>6,23</sup>. It is therefore difficult to accurately assess the exposure of archaeologists to biological agents in the crypt. According to Team of Experts for Biological Factors of the Interministerial Commission for the Threshold Limit Val-

ues of Harmful Factors in the Work Environment the number of fungi  $>5 \cdot 10^3$  cfu/m<sup>3</sup> exceeds the limit values for non-industrial workplaces<sup>24</sup>. Exposure to such high numbers of fungi has been recorded in areas heavily contaminated with organic dust, e.g. in sorting stations at landfill sites<sup>25</sup> or at indoor farms<sup>26</sup>.

People supervising archaeological works are obliged to inform all employees about microbial risk as well as about health and safety standards. Good organization of work in the crypts is equally important. Minimizing exposure to biological agents should be a priority. Dust lifting should be reduced to a minimum, for example by wetting surfaces<sup>27</sup>. It is also important to provide appropriate microclimate in the crypt (e.g. by ensuring proper ventilation). This improves the safety of archaeologists and prevents damage to the studied materials<sup>28</sup>. Moreover, many risks can be easily prevented by following the instructions concerning occupational health (e.g. eating, drinking and using cosmetics during archaeological works should not be permitted).

In addition, supervisors have to provide employees with protective clothing<sup>29</sup>. Cox and Kneller<sup>1</sup> argue that adequate protection against biological agents involves wearing disposable protective clothing including footwear, uniforms, gloves, goggles, and masks. Our study confirms this necessity because all items of personal protective equipment were contaminated with numerous microorganisms of all investigated groups, including various allergenic fungi. Our results indicate the need for decontaminating or destroying protective clothes to avoid transferring microorganisms beyond the archaeological premises.

## REFERENCES

1. COX M, KNELLER P, Crypt Archaeology: an approach (IFA, Reading, 2001). — 2. ČAVKA M, GLASNOVIĆ A, JANKOVIĆ I, RAJIĆ ŠKANJIC P, PERIĆ B, BRKLJAČIĆ B, MLINARIĆ-MISSONI E, ŠKRILIN J, Coll Antropol, 43(3) (2010) 803. — 3. ŠKRILIN J, KAVUR L, MLINARIĆ EM, JANKOVIĆ I, PETAROS A, BRKLJAČIĆ B, ČAVKA M, Coll Antropol, 35(3) (2011) 923. — 4. Baxter PJ, Brazier AM, Young SE, Brit J Ind Med, 45(5) (1988) 359. — 5. GÓRNY RL, DUDKIEWICZ J, Ann Agric Environ Med, 9(2) (2002) 17. — 6. GÓRNY RL, CYPROWSKI M, ŁAWNICZEK-WAŁCZYK A, GOŁOFIT-SZYMCZAK M, ZAPÓR L, Biohazards in the indoor environment – a role of threshold limit values in exposure assessment. In: DUDZIŃSKA MR (Ed) Management of Indoor Air Quality (CRC Press/Balkema, Leiden, 2011). — 7. EDUARD W, HEEDERIK D, DUCHAINE C, GREEN BJ, J Environ Monit, 14(2) (2012) 334. DOI: 10.1039/C2EM10717A. — 8. ULFIG K, Pol J Environ Stud, 12(4) (2003) 461. — 9. ABDEL-KAREEM OMA, Polym Degrad Stab, 87(1) (2005) 121. DOI: 10.1016/j.polymdegradstab.2004.07.014. — 10. KAVKLER K, GUNDE-CIMERMAN N, ZALAR P, DEMŠAR A, Polym Degrad Stab, 96(4) (2011) 574. DOI: 10.1016/j.polymdegradstab.2010.12.016. — 11. PANGALLO D, KRAKOVA L, CHOVAANOVA K, BUČKOVA, M, PUŠKAROVA A, ŠIMONOVICHOVA A. Microbiol Res, 168(5) (2013) 289. DOI: 10.1016/j.micres.2012.12.001. — 12. NUGARI MP, PIETRINI AM, CANEVA G, IMPERI F, VISCA P, Int Biodeter Biodegr, 63(6) (2009) 705. DOI: 10.1016/j.ibiod.2009.03.013. — 13. EBRAHIMI A, KARIMI S, LOTFALIAN S, MAJIDI F, Jundishapur J Microbiol, 4(4) (2011) 261. — 14. KNUTSEN AP, BUSH RK, DEMAİN JG, DENNING DW, DIXIT A, FAIRS A, GREENBERGER PA, KARIUKI B, KITA H, KURUP VP, MOSS RB, NIVEN RM, PASHLEY CH, SLAVIN RG, VIJAY HM, WARDLAW AJ, J Allergy Clin Immunol, 129(2) (2012) 280. DOI: 10.1016/j.jaci.2011.12.970. — 15. CRAMERI R, GARBANI M, RHYNER C, HUITEMA C, Allergy, 69(2) (2014) 176. DOI:

## Conclusions

The air as well as objects and materials found in crypts are highly contaminated with bacteria and fungi. Samples collected in Fredro crypt contained only a small number of pathogens. However, a high number of microorganisms both in the air and on human remains indicates that there is a need for particular caution during archaeological works causing dust emission (bioaerosol). Good organization of archaeological works in the crypts is necessary for the maximum protection of archaeologists against harmful biological agents. Minimizing exposure to these agents should be a priority. This can be achieved by ensuring proper ventilation and minimizing dust lifting as well as by following instructions concerning occupational health. Moreover, the use of disposable personal protective equipment seems necessary. It should be emphasized that a high number of fungi can trigger an allergic reaction or intolerance with a range of symptoms including the irritation of skin, eyes or the respiratory system in people working in burial crypts.

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- 10.1111/all.12325. — 16. NIELSEN KF, Fungal Genet Biol, 39(2) (2003) 103. DOI: 10.1016/S1087-1845(03)00026-4. — 17. PIONTEK M, ŁUSZCZYŃSKA K, LECHÓW H, Int J Environ Res Public Health, 13(9) (2016) 862. DOI: 10.3390/ijerph13090862. — 18. JURJEVIĆ Ž, PETERSON SW, SOLFRIZZO M, PERAICA M, Mycotoxin Res, 29 (2013) 141. DOI: 10.1007/s12550-013-0160-4. — 19. FILIPELLO-MARCHISIO V, Keratinophilic fungi: Their role in nature and degradation of keratinic substrates. In: KUSHWAHA RKS, GUARRO J (Eds), Biology of dermatophytes and other keratinophilic fungi. (Revista Iberoamericana de Micología, Bilbao, 2000). — 20. DE HOOG GS, GUARRO J, GENÉ J, FIGUERAS MJ, Atlas of clinical fungi (Centraalbureau voor Schimmeltcultures, Utrecht, 2000). — 21. BOTTONE EJ, Clin Microbiol Rev, 23(2) (2010) 382. DOI: 10.1128/CMR.00073-09. — 22. MAPP CE, Occup Environ Med, 58(5) (2001) 354. DOI: 10.1136/oem.58.5.354. — 23. ŁAWNICZEK-WAŁCZYK A, CYPROWSKI M, GOŁOFIT-SZYMCZAK M, WÓJCIK-FATLA A, ZAJĄC V, GÓRNY RL, Med Pr, 69(3) (2018) 269. — 24. AUGUSTYŃSKA D., POŚNIAK M, Czynniki szkodliwe w środowisku pracy: wartości dopuszczalne (CIOP-PIB, Warszawa, 2014). — 25. KALWASIŃSKA A, BURKOWSKA A, SWIONTEK BRZEZIŃSKA M, Clean – Soli, Air, Water, 42(10) (2014) 1337. DOI: 10.1002/clean.201300385. — 26. SOWIAK M, BRÓDKA K, BUCZYŃSKA A, CYPROWSKI M, KOZAJDA A, SOBALA, W., SZADKOWSKA-STANČZYK I, Aerobiologia, 28(2) (2012) 121. DOI: 10.1007/s10453-011-9216-0. — 27. PETERSEN LR, MARSHALL SL, BARTON C, HAJJEH RA, LINDSLEY MD, WARNOCK DW, PANACKAL AA, SHAFFER JB, HADDAD MB, FISHER FS, DENNIS DT, MORGAN J, Emerg Infect Dis, 10(4) (2004) 637. DOI: 10.3201/eid1004.030446. — 28. HAYEN R, DE CLERCQ H, GODTS S, CMAS, 16(1) (2014) 16. — 29. LANGLEY LR, ABBOTT LEJR, NC Archaeol, 49 (2000) 23. DOI: 10.1179/1350503314Z.00000000068.

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## **MIKROBIOLOŠKI RIZICI TIJEKOM ARHEOLOŠKIH RADOVA: PRIMJER KRIPTA FREDRO (Przemyśl, Poljska)**

### **SAŽETAK**

Ljudski ostaci i drugi predmeti koji se nalaze u kriptama često su znatno kontaminirani mikroorganizmima. Arheolozi su izloženi mikroorganizmima na razne načine (udisanjem prašine, zagađenjem ogrebotina ili porezotina. Cilj rada je procijeniti mikrobiološke rizike povezane s ljudskim ostacima i bioaerosolima koji nastaju tijekom arheoloških radova u ukopnim kriptama. Tijekom arheoloških radova na svodu Bazilike Sv. Ivana Krstitelja i Uznesenja Blažene Djevice Marije u Przemyśl-u (Kripta Fredro), prikupljeni su uzorci ljudskih ostataka, bioaerosola i osobne zaštitne odjeće (maske za prašinu, jednokratni radni ogrtači). Mikrobiološko ispitivanje uključilo je određivanje broja spora aerobnih i anaerobnih bakterija, broj mezofilnih i hemolitičnih bakterija te broj kserofilnih, nekserofilnih i keratolitičkih gljivica. Zrak, kao i predmeti i materijali nađeni u kriptama jako su zagađeni bakterijama i gljivicama. Kserofilne gljivice bile su najzastupljenije u ljudskim ostacima 1-230:103 CFU/g, nekserofilne gljivice u bioaerosolima ( $>104$  cfu/m<sup>3</sup> za vrijeme arheoloških radova). Većina bakterija pripadala je rodu *Bacillus* (81.3%). Gljivice rodova *Penicillium* (65,2%) i *Aspergillus* (28,6%) prevladavale su među izoliranim gljivicama. Sterilizacija zamagljivanjem prostora kripote pokazala se neučinkovitom. Veliki broj mikroorganizama u zraku i ljudskim ostacima ukazuje na potrebu posebnog opreza za vrijeme arheoloških radova koji stvaraju prašinu te nužnost upotrebe jednokratne zaštitne odjeće kako bi se smanjila izloženost štetnim biološkim faktorima.