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Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles

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ABSTRACT

The results of microbial contamination analysis of air and surfaces in six different museums and archives in Poland are presented. It was shown that the level of microbial contamination of the air ranged from 1.5×10^2 to 7.0×10^3 cfu m^{-3}; contamination of the surfaces was 1.4×10^2 to 1.7×10^4 cfu 100 cm^{-2}. The most common fungi contaminating museums and archives were *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Mucor*, *Rhizopus*, *Trichoderma*, *Paeciliomyces*, *Aureobasidium*, *Botrytis* and *Chrysonila*; and from bacteria *Bacillus*. Some potentially allergenic and toxic species were diagnosed: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Aspergillus versicolor*, *Staphylococcus aureus*. For 32 strains of bacteria and fungi with the highest occurring frequency in the analyzed areas and for 6 strains of ATCC collection, MIC and MBC for the preparation of nanosilver were determined. A concentration of nanosilver with a particle size 10–100 nm at the level of 90 ppm is effective in removing the microorganisms present on the surface of objects, while a concentration of 45 ppm removed 94% of all microorganisms except for the resistant *Bacillus subtilis* and *Staphylococcus xylosus*. It was shown that a preparation of silver nanoparticles can be used as a disinfectant for the surface of historical objects and archival documents.

1. Introduction

Museums, archives and libraries are institutions which collect and provide objects of historical and cultural value, and protect them against destruction. Libraries and archives store mostly paper products, while museums keep collections of leather, wood, metal, fabrics, plastics, rubber, paint or stone. All of these materials, in certain conditions of air humidity and temperature, may undergo a biodeterioration process, due to the action of the microorganisms present in the air (Strzelczyk, 2004; Karbowska-Berent et al., 2011).

Microorganisms are the causes of serious forms of damage, such as weakening or loosening of paper, books overgrown with fungi and foxing, mostly appearing in the form of loss of structure, discoloration on the surface and the presence of filamentous fungi and slimy substances (Strzelczyk, 2004; Michaelsen et al., 2006). Fungi with cellulolytic properties attack wood, creating a white deposit on the surface, causing its brittleness and producing a specific odor (Blanchette, 2000). During fabric biodeterioration, fungal mycelia overgrow and stick to the material layers (Zyska and Zakowska, 2005; Abdel-Kareem, 2010; Borrego et al., 2010; Zotti et al., 2011). In other cases, microbial contamination can lead to peeling or spalling off of paint layers, the formation of cavities and a rigid crust on the surface of stone building facades, sculptures and monuments, and in consequence destroying their aesthetic value and structure (Crispim and Gaylarde, 2005; Sarro et al., 2006).

Furthermore, many toxic and allergenic fungi can cause serious threats to the health of workers in museums, archives or libraries. Health risks caused by excessive microbial contamination occur in indoor air, among others, during work with moldy technical material, e.g., during restoration and cleaning of museum, archive and library objects, as well as by inhaling air heavily contaminated by microbes – of particular importance are airborne molds (Florian, 1997; Flannigan et al., 2001). The mold spores present in these types of facilities result in a serious threat to human health. They can cause superficial and organ mycoses, mycotoxicosis and allergic diseases: rhinitis, conjunctivitis and asthma resulting from harmful effects of toxic substances produced by fungi (Nielsen, 2003; Mesquita et al., 2009; Gutarowska et al., 2010, 2011; Lopez-Aparicio et al., 2011).

The best way to combat all kinds of contamination is to keep the rooms clean and maintain appropriate conditions of temperature and humidity (it is especially important to maintain the humidity level below 55%) (Sterflinger, 2010). The most effective method is disinfection (the destruction of pathogenic and saprophytic microorganisms and their spores by physical or chemical agents in order

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to prevent infection). Chemical agents used in disinfection include quaternary ammonium salts, ethylene oxide, alcohols, aldehydes e.g., formaldehyde, glutaraldehyde, phenolic compounds, biguanides, halogen compounds, dyes, peroxides, surfactants, heavy metals (Rutala and Weber, 1999). The most common method for disinfection of historical objects is ethylene oxide; its high effectiveness is incomparable with any other fumigant. Unfortunately it is a carcinogenic, mutagenic gas, causing allergic reactions and having an irritating action on the respiratory tract (Rakotonirainy et al., 1999; Rakotonirainy and Lavedrine, 2005).

The search is therefore continuing for a disinfectant offering a high level of activity and prolonged antimicrobial action, which would be safe for both human health and for museum, archive and library collections.

In the past few years, there has been increasing interest in silver nanoparticles, because of their high antimicrobial properties (Kim et al., 2007; Rai et al., 2009). Silver nanoparticles can be synthesized by several physical, chemical, and biological methods (Vaidyanathan et al., 2009; Bankar et al., 2010; Zhang et al., 2011). The most common method for synthesis of Ag nanoparticles (AgNPs) is the chemical reduction of silver ions in different stabilizers such as polymers and surfactants. The mechanism of action of silver in the cells of microorganisms is multidirectional; silver inhibits the replication of DNA, disturbs the electrical potential and functioning of the cytoplasmic membranes, which leads to the outflow of many metabolites from the cells, causes loss of bioactivity of amino acids and disrupts respiratory processes (Feng et al., 2000; Sondi and Salopek-Sondi, 2004; Yamanaka et al., 2005; Rai et al., 2009). Silver's multidirectional action mechanisms give it an advantage over other biocides. AgNPs have been used as an antibacterial agent for a long time because of their stability, their prolonged and broad-spectrum antibacterial activity, and the unlikelihood of development of resistant microorganisms (Zhang et al., 2011). The novel properties of AgNPs have been exploited widely for use in medicine – dressings, surgical masks, implants, creams, textiles, surfaces of polymers, steel, cosmetics, food packaging, filtration of water, renewable energies, environmental remediation, and electronic devices (Gupta and Silver, 1998; Feng et al., 2000; Klasen, 2000; Appendini and Hotchkiss, 2002; Silver, 2003; Furno et al., 2004; Tian et al., 2007; Gong et al., 2007; Rai et al., 2009; Monteiro et al., 2009; Chen et al., 2010; Chaloupka et al., 2010). In view of the common use of silver nanoparticles in many fields of life, much research has been done in recent years into the toxicity of AgNPs to cells of mammals and other animals, and in particular its action in minimal doses for long periods (Braydich-Stolle et al., 2005; Burd et al., 2007; Ji et al., 2007; Kim et al., 2010). Attention is drawn to the risk relating to the toxic action of silver on cells, as well as the possibility of accumulation in the organs of mammals (Tang et al., 2009; Park et al., 2010; Loeschner et al., 2011). Mechanisms of the toxic action of silver on cells of higher animals are already known, involving damage to mitochondria and deactivation of enzymes, which leads to the creation of reactive forms of oxygen and cell apoptosis (Hsina et al., 2008; Lee et al., 2009; Rahman et al., 2009). Similarly, attention is drawn to the effects of nanosilver involving disturbance to the ecological balance in natural systems – air, water, sewage sludge, ash from burnt waste, and soil (Blaser et al., 2008; Liang et al., 2010). For the reasons given above it is important that AgNPs be used in applications where doing so is necessary and justified. Nanosilver has not been used for disinfection of historical objects, and no previous studies had been conducted on the susceptibility of microorganisms isolated from the natural environment (museums) to preparations containing nanosilver. The use of silver nanoparticles to protect historical objects from microbiological biodegradation is justified in view of the long-lasting effect (the nanoparticles that settle on the object remain there and continue to act on microorganisms for a long time). When nanosilver is applied to historical objects, in contrast to other materials such as household products, there is no risk of silver being introduced to the natural environment in the form of waste. The disinfection of historical objects (paintings, archive paper, textiles, sculptures and others) using preparations made with silver nanoparticles will take place in special disinfection chambers using a misting method. The disinfected objects will have limited toxic effect. Contact with staff working on the disinfection and conservation of historical objects will involve only the skin. This type of contact can be reduced by the use of individual protection measures, as in the case of other biocides.

The aim of this work was to assess the level of microbiological contamination of museum, archive and library rooms and the sensitivity of microorganisms isolated from these rooms to silver nanoparticles. The aim was to determine whether the microorganisms present in archive and museum buildings would be effectively reduced by disinfection using nanosilver particles.

The objective of this work was: 1. to assess microbiological contamination of the air and surfaces of objects in four museums storing different objects and in two institutions storing archives (libraries and archives); 2. to identify the microorganisms isolated; 3. to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC) for a preparation of nanosilver against microorganisms isolated from the air and surface areas, and make a comparison with pure culture collection strains (ATCC); 4. to determine the optimal dose of nanosilver against environmental microorganisms.

2. Materials and methods

2.1. Description of the institutions

Microbiological contamination was studied at six different institutions: the National Archives in Lodz, the Library in Lodz, the Central Museum of Textiles in Lodz, the Museum of Independence Tradition in Lodz, the Museum of Archeology and Ethnography in Lodz, and the National Museum in Warsaw. In the rooms studied the temperature and humidity of the air were measured using a PWT-401 hygrometer (Elmetron, Poland). The characteristics of rooms in the institutions are presented in the Table 1.

Samples of air were collected from various places in the rooms, and samples from different surfaces (shelves, walls and historical objects), were tested. The selection criteria were the visual diversity of contaminated materials and the degree of microorganism infection. Characteristics of the objects tested are presented in Table 1.

2.2. Preparation of silver nanoparticles

The source of silver nanoparticles was aqueous solution of colloidal silver, produced commercially by the Mint – Metale Szlachetne S.A., Poland. Colloidal silver solutions were prepared chemically by the reduction of silver nitrate. As a regulator, sodium citrate was used, and as a protective colloid PVP was used securing the silver particles suspended in the solution against the aggregation.

In the studies, the concentration of the stock solution being 90 ppm and the nanoparticles having a size of 10–100 nm were used. The colloidal silver solution obtained had a biodispersive distribution of silver particles. It was found using SEM that two types of particles were in the solution: small (10–15 nm) constituted 60–70% of silver in the solution and large size ranged from 50 to 80 nm (30–40%).

2.3. Microorganisms

The sensitivity to silver nanoparticles of 32 strains of microorganisms (15 bacteria, 3 yeasts and 14 molds) was analyzed

Table 1
Characteristics of the examined rooms in each institution.

Institution (designation)	Building type (century of construction)	Tested room	Area (m^3)	Average temperature (°C) (SD)	Average humidity (%) (SD)	Descriptions of stored objects	Tested surface areas
National Archives (NA)	Former factory rooms (20th century)	Warehouse no 1	4045	19.3 (1.5)	48.3 (7.2)	Files from 19th century factories	Books 1–3
		Warehouse no 2	176			Court records from 19th–20th centuries.	Books 4–5; files 1–4
		Restoration workshop	108			Room equipped with machines for patching, mending and deacidification	Surfaces not tested
Library (L)	20th century	Warehouse no 1	49	18.5 (0.7)	27.5 (0.7)	Books from 19–20th centuries	Wooden shelf; books 1–3
		Warehouse no 2	55			Books and periodicals from 19–20th centuries	Books 4–5; wall; wooden shelf
Central Museum of Textiles (CMT)	Complex of classical buildings (19th century)	Restoration workshop no 1	300	19.5 (3.8)	29.0 (3.6)	Room for washing, drying, preliminary textile repair	Washing basin, textile prepared to wash, table for textile lamination, laboratory bench
		Restoration workshop no 2	1200			Room for repairing of textiles	Bench for filling gaps, carpet from the 16th century prepared for repair
		Warehouse no 1	1215			Collection of equipment (wooden, steel) for fibre treatment (19th–20th century).	Plastic windowsill, wooden rack, wooden drum-type washing machine, steel uppers automatic machine, loom (textile)
		Warehouse no 2	456			Collection of textiles (19th–20th century)	On wooden wardrobe, Turkmen bound carpet (19th/20th century), jacquard fabric ("Eagles" – L. Kintopf, ca 1926, Poland), woollen kilim Syon (Eastern Malopolska, 19th century)
Museum of Independence Tradition (MIT)	Radogost prison building (20th century)	Warehouse no 1	281	19.0 (1.0)	41.0 (3.6)	Painting on gantries, weapons (20th century)	Painting on gantry (from the wall side), on wooden wardrobe, wooden desk, painting on gantry (from the room side)
		Warehouse no 2	220			Flags, banners, papers (20th century)	Banner attacked by fungi, rucksack attacked by fungi (leather part), on wooden wardrobe, in drawer of cupboard,
		Warehouse no 3	52			Flags, banners, papers (20th century)	Surfaces not tested
Museum of Archeology and Ethnography (MAE)	20th century	Warehouse no 1	310	13.3 (3.1)	39.7 (4.7)	Collection of furniture and folk art	Wooden sculpture ("Beehive" – S. Mucha), wooden chest, wall near the entrance stairs, wall in the farthest part of the warehouse from the entrance
		Warehouse no 2	1362			Collection of materials such as wood, wicker, iron originating mostly from the 2nd half of the 19th and the 20th century	Steel rack, wall in the farthest part of the warehouse from the entrance, wooden rack, front part (fiberboard), wooden washer
		Warehouse no 3	60			Collection of African culture art	Steel rack, chair, wall on the wooden wardrobe
National Museum (NM)	Modernistic New Building (20th century)	Warehouse no 1	222	22.0 (0.8)	35.5 (11.1)	Cabinet of Polish prints and drawings (13th–19th century)	Wooden rack near the window, wooden shelf near the entrance, wooden desk, wall near the window
		Warehouse no 2	725			Store of oil paintings on canvas and on wood (13th–19th century)	gantry, wooden frame, oil painting, wall near the heater
		Warehouse no 3	171			Medieval art restoration workshop	Plastic table, restoration bench (fiberboard), wooden windowsill, wooden shelf,
		Exhibition hall	3055			Gallery of ancient art, sculptures	Pedestal of Apollo Lykejos, pedestal of Togate Statue, collective pedestal in center of gallery, pedestal of Jupiter Capitolinus

SD – standard deviation.

(Table 2). The microorganisms were chosen on the basis of isolation frequency in the subject rooms (higher than 20% in the case of bacteria and 15% for fungi). Additionally the sensitivity was determined for collection cultures: 3 bacteria species – *Bacillus subtilis* (NCAIM 01644), *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 10536); 2 fungi species – *Aspergillus niger* (ATCC 16404), *Penicillium chrysogenum* (LOCK CPC 0531); and 1 yeast – *Candida albicans* (ATCC 10231). The microorganisms were stored on agar slants: bacteria on TSA (Tryptic Soy Agar, *Merck*, Germany), molds and yeasts on MEA (Malt Extract Agar, *Merck*, Germany), at a temperature of 4 °C. Bacteria were activated before tests on liquid TSB medium (Tryptic Soy Broth, *Merck*, Germany), fungi on MEB medium (Malt Extract Broth, *Merck*, Germany), and were incubated at a temperature of 30 °C for 24–48 h (bacteria) and at 27 °C for 24 h (yeasts) or 72 h (molds).

2.4. Sampling methods for estimation of microbiological contamination of the air and surfaces

The air samples were collected using an MAS-100 Eco Air Sampler (*Merck*, Germany). The samples were gathered in volumes of 50 l or 100 l on the MEA medium (Malt Extract Agar, *Merck*, Germany) with chloramphenicol (0.1%) for fungi and on the TSA medium (Tryptic Soy Agar, *Merck*, Germany) with nystatin (0.2%) for bacteria. Air samples were taken in 3–4 places in each room in two repetitions. Surface samples were collected by a contact method using RODAC Envirocheck® Contact Plates (Replicate Organism Detection And Counting, *Merck*, Germany) with TSA medium with neutralizers (bacteria) or Sabouraud medium (*Merck*, Germany) (fungi). A traditional swab method was also performed for surfaces, using a sterile 0.85% saline, tampons and metal templates with an area of 25 cm^2. Surface samples were taken in 3–5 places in each room in two repetitions.

2.5. Determination of number of microorganisms in the air and on surfaces

Samples were incubated at 30 °C for 48 h (bacteria) and at 27 °C for 5 days (fungi). After incubation, colonies were counted and expressed as cfu m^{-3} (air) or cfu 100 cm^{-2} (surfaces). The final number is given by the arithmetic mean of all repetitions for each place.

2.6. Identification of microorganisms

After isolation the microorganisms were identified. Identification of bacteria was based on the observation of macroscopic features (color, texture, size, dyes), Gram staining, catalase test and cytochrome oxidase test (Microbiologie Bactident Oxydase, *Merck*, Germany). Next, the bacteria for which the frequency was higher than 25% were chosen, and the identification was performed using API tests (*bioMérieux,* France): the API 50 CH test, API STAPH test and API 20 NE test. Yeasts were identified using the API C AUX test.

After incubation of the molds, identification was performed on the Czapek–Dox Agar medium (*Difco,* USA) and YES (Yeast Extract with Supplements) (yeast extract 20.0 g; sucrose 150.0 g; $MgSO_4 \cdot 7H_20$ 0.5 g; YES salts ($ZnSO_4 \cdot 5H_20$ 0.1 g; $CuSO_4 \cdot 5H_2O$ 0.005 g; distilled water up to 100 ml); agar 20.0 g; distilled water up to 1000 ml; pH 5.8 ± 0.2) based on Samson et al. (1996) and Flannigan et al. (2001).

2.7. Evaluation of the microorganisms' sensitivity to nanosilver preparation

2.7.1. Inoculum preparation

A bacteria culture on 30 ml of liquid TSB medium (Tryptic Soy Broth, *Merck*, Germany) after 24 h of incubation was centrifuged (6000×*g*, 10 min). The supernatant was decanted and the remaining sediment of bacterial biomass was suspended in 30 ml of saline (0.85% NaCl), next 0.1 ml of solution was poured into 9.9 ml of saline.

After the yeasts' incubation on agar slants with MEB medium (Malt Extract Broth, *Merck*, Germany), colonies were transferred into 9.9 ml of saline (0.85% NaCl). In the case of molds, colonies from MEA slants were washed using 25 ml of distilled water with 0.01% of Tween® 80.

The number of microorganisms in the inoculum was determined using a count plate method and Thom chamber. The average density of the suspension of microorganisms was about 10^7 cfu ml^{-1} (bacteria) or 10^6 cfu ml^{-1} (fungi).

Table 2
Microorganisms (origin).

Bacteria	Percentage of API identification	Yeasts	percentage of API identification	Molds
Bacillus megaterium (MAE)	92.8	*Rhodotorula mucilaginosa* (CMT)	98.8	*Alternaria alternata* (NM)
Bacillus pumilus (CMT)	99.9	*Rhodotorula* sp. (NA)	99.1	*Aspergillus niger* (CMT)
Bacillus pumilus (NA)	99.8	*Candida sphaerica* (L)	99.3	*Aspergillus niger* (ATCC 16404)
Bacillus subtilis (CMT)	99.9	*Candida albicans* (ATCC 10231)	–	*Aspergillus versicolor* (MIT)
Bacillus subtilis (NA)	98.1			*Aspergillus versicolor* (L)
Bacillus subtilis (NCAIM 01644)	–			*Cladosporium cladosporoides* (CMT)
Bacillus licheniformis (L)	97.3			*Cladosporium macrocarpum* (CMT)
Brevibacillus laterosporus (CMT)	99.9			*Cladosporium herbarum* (L)
Aneurinibacillus aneurinilyticus (L)	91.3			*Mucor racemosus* (MAE)
Micrococcus sp. (MAE)	99.9			*Penicillium digitatum* (NM)
Micrococcus flavus (L)	98.8			*Penicillium radicola* (CMT)
Staphylococcus aureus (MAE)	99.1			*Pennicillium carneum* (L)
Staphylococcus aureus (ATCC 6538)	–			*Pennicillium crustosum* (L)
Staphylococcus lentus (L)	99.8			*Penicillium chrysogenum* (Lock CPC 0531)
Staphylococcus xylosus (NA)	95.1			*Rhizopus nigricans* (MIT)
Sphingomonas paucimobilis (MIT)	99.1			*Rhizopus nigricans* (NA)
Escherichia coli (ATCC 10536)	–			
Nocardia sp. (L)	99.1			

NA – National Archives in Lodz; L – Library in Lodz; CMT – Central Museum of Textiles in Lodz; MIT – Museum of Independence Tradition in Lodz; MAE – Museum of Archeology and Ethnography in Lodz; NM – National Museum in Warsaw; Lock CPC – The Lock Collection of Pure Culture at the Institute of Fermentation Technology and Microbiology in Technical University of Lodz, Poland; ATTC – American Type Culture Collection; NCAIM – National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary - collection strain.

2.7.2. Determination of MIC and MBC of nanosilver preparation

To determine the influence of nanosilver on microorganisms, a serial dilutions method with sterile microtiter plates (*Bioster*, Italy) was used. A series of dilutions of nanosilver preparation in the appropriate medium (TSB for bacteria, MEB for fungi) were made, from 1:2 to 1:16, and then the prepared inoculum was added. The control sample consisted of the medium and inoculum. Next the microplate was incubated at a temperature of 30 °C for 24 h for bacteria and at 27 °C for fungi. After the incubation, the number of microorganisms was determined using a count plate method. Bacteria were incubated at 30 °C for 24–48 h, fungi at 27 °C for 3–5 days, and the number of microorganisms was determined.

Based on the results, Minimal Inhibition Concentration (MIC) and Minimal Biocidal Concentration (MBC) were established for each microorganism. It was assumed that MIC is the nanosilver concentration used when the number of microorganisms is 10^3 times lower, and MBC is the nanosilver concentration used when the number of microorganisms is 10^4 times lower (according to the ASTM E2149-01 standard).

2.8. Mathematical calculations

The frequency of microorganism isolation (f) in the air and on the surfaces in analyzed museums, library and archives was calculated according to the formula:

$$f = \frac{a}{n} \cdot 100\%$$

where: a is the number of samples where the particular strain occurred n is the total number of samples.

The arithmetic mean and standard deviation for number of microorganisms in the air and on the surfaces were also calculated. All mathematical calculations were made using the Excel program.

3. Results

The level of microbiological contamination in the museums, archives and library was varied (Table 3). The level of microbial contamination of the air was low and ranged from 1.5×10^2 to 7.0×10^3 cfu m^{-3}; contamination of the surfaces was 1.4×10^2 to 1.7×10^4 cfu 100 cm^{-2}, depending on the microorganisms analyzed. The least contamination was found at the National Archives in Lodz and the National Museum in Warsaw (air: 1.5×10^2–2.1×10^2 cfu m^{-3}, surfaces 9.0×10^2–1.4×10^2 cfu 100 cm^{-2}). In these institutions the temperature and humidity of the air (14.5–22.0 °C; 26.5–39.0% RH) were at levels not conducive for microbial active growth (Table 1).

The most contaminated was the Museum of Independence Tradition in Lodz (air: 7.0×10^3 cfu m^{-3}, surfaces 1.7×10^4 cfu 100 cm^{-2}). The dominant microorganisms in the air were fungi, which accounted for 98% of the isolated microorganisms. Art objects in this museum were often inhabited by fungi rather than by bacteria; on the surfaces of exhibits, furniture and walls the percentage of fungi was found to be 99.9% (Table 3). It is worth noting that this Museum has the highest value of relative humidity of the air (41%), which may stimulate the growth of fungi. Also in the Library and the Museum of Archeology and Ethnography the percentage of fungi on the analyzed surfaces was found to be 86–91%, which indicates that the fungi are a major contaminator of these objects and are a serious threat to the objects and to the people who work in these institutions.

The most frequently isolated microorganisms were molds and bacteria (Table 4): Gram-positive endospore-forming rods and Gram-positive cocci. The most frequently found molds belong to the genera *Alternaria* (e.g., *Alternaria alternata*), *Aspergillus* (e.g., *Aspergillus versicolor*), *Cladosporium* (e.g., *Cladosporium herbarum*), *Penicillium* (e.g., *Pennicillium carneum*) and *Rhizopus nigricans*. Of Gram-positive rods, the most common were *Bacillus* sp. (e.g., *B. subtilis*). As concerns Gram-positive cocci, the most frequent were *Micrococcus* sp. and *Staphylococcus* sp. (e.g., *Staphylococcus lentus* and *Staphylococcus xylosus*). Among all isolated bacteria, Gram-negative rods, like *Sphingomonas paucimobilis* and *Pseudomonas alcaligenes*, were noted. In the National Archives and the Library actinomycetes from the *Nocardia* genus were also found. All identified microorganisms were isolated from many different materials, like leather or paintings. However the most often populated were wood and walls.

Analyzing Table 5, we can form a list of microorganisms according to their sensitivity to silver nanoparticles. Starting from the most resistant: molds from the genera *Aspergillus*, *Rhizopus*, *Penicillium* (*A. niger*, *A. versicolor*, *R. nigricans*, *Penicillium digitatum*, *Penicillium radicola*, *P. chrysogenum*, *P. carneum*, *Pennicillium crustosum*), Gram-positive endospore-forming rods from the genus *Bacillus* (*B. subtilis*, *Bacillus megaterium B. licheniformis*), Gram-positive cocci (*Micrococcus* sp., *Micrococcus flavus*, *S. aureus*, *S. lentus*, *S. xylosus*), molds of the genera *Cladosporium*, *Alternaria* and *Mucor* (*Cladosporium cladosporoides*, *Cladosporium macrocarpum*, *A. alternata*, *Mucor racemosus*), yeast-like fungi from the genera *Rhodotorula* and *Candida (Rhodotorula mucilaginosa*, *Candida spherica*, *C. albicans*), mesophilic actinomycetes from the genus *Nocardia* and Gram-negative rods (*Sphingomonas paucimobilis*, *E. coli*).

There are differences in the nanosilver sensitivity of the genus *Bacillus* between the strains *B. megaterium*, *Bacillus pumilus*, *Bacillus licheniformis* and *B. subtilis.* A concentration of 45 ppm was appropriate for disinfection of each *Bacillus* strain, except for *B. subtilis*. The same difference was observed for strains from the genera *Staphylococcus*, *Micrococcus*, *Aspergillus* and *Penicillium*. In the genus *Penicillium*, for example, *P. digitatum* has lower MIC (22.5 ppm) than other strains *P. radicola*, *P. chrysogenum*, *P. carneum*, *P. crustosum* (MIC 45 ppm). However, in the case of *Cladosporium* (*C. herbarum*, *C. cladosporoides*, *C. macrocarpum*) and *A. versicolor*, the values of MIC and MBC are the same for all species and isolates.

Comparison of isolates of the same species, but of different origin, showed that there are differences in the sensitivity of isolates to the silver nanoparticles. The MIC for silver on *B. subtilis* is the same for the collection strain and the isolate from the National Archives (22.5 ppm), but was different for the strain isolated from the Central Museum of Textiles (45 ppm). The same was observed for *S. aureus* and *A. niger* isolates. The studies showed that there are differences in MIC and MBC between environmental specimens and those from the Pure Culture Collection. Considering the comparison between these, it can be seen that studies need to be carried out on microorganisms isolated from the contaminated environment, for example *A. niger*, *B. subtilis* and *S. aureus*, of which the environmental strain is resistant (MBC 45.00 ppm) and the collection strain is very sensitive to a silver nanoparticle preparation (MBC 11.25 ppm). Sensitivity to silver nanoparticles should be studied before application; the microbial contamination needs to be analyzed carefully due to differences in sensitivity.

Following the studies, the recommended concentration of solution containing nanosilver compound is 45 ppm, which kills 94% of the analyzed microorganisms isolated from Museums and Archives.

4. Discussion

In the museum, archive and library areas, microbiological contamination of the air and surfaces of objects was at an average level: 1.5×10^2–7.0×10^3 cfu m^{-3} in the air and 1.4×10^2–1.7×10^4 cfu 100 cm^{-2} on the surfaces, and was comparable to the

Table 3
Quantitative analysis of microbial contamination of the analyzed archives and museums.

Institution	Number of fungi in the air (cfu m^{-3})	Number of bacteria in the air (cfu m^{-3})	Total number of microbes in the air (cfu m^{-3})	Percentage contribution in the air (%)		Number of fungi on surfaces (cfu 100 cm^{-2})	Number of bacteria on surfaces (cfu 100 cm^{-2})	Total number of microbes on surfaces (cfu 100 cm^{-2})	Percentage contribution on the surfaces (%)	
				Fungi	Bacteria				Fungi	Bacteria
The National Archives in Lodz	Av: 3.8×10^1 Min: 2.0×10^1 Max: 1.6×10^2 SD: 1.2×10^2	Av: 1.1×10^2 Min: 2.0×10^1 Max: 8.5×10^2 SD: 5.2×10^2	Av: 1.5×10^2 Min: 2.0×10^1 Max: 8.5×10^2 SD: 1.0×10^2	25.68	74.32	Av: 4.0×10^2 Min: 5.0×10^0 Max: 2.6×10^3 SD: 1.2×10^3	Av: 5.0×10^2 Min: 5.0×10^0 Max: 1.2×10^4 SD: 3.9×10^3	Av: 9.0×10^2 Min: 5.0×10^0 Max: 1.2×10^4 SD: 6.3×10^2	44.44	55.56
Library in Lodz	Av: 5.0×10^1 Min: 2.0×10^1 Max: 4.0×10^2 SD: 1.3×10^2	Av: 2.7×10^2 Min: 2.0×10^1 Max: 3.3×10^3 SD: 1.3×10^3	Av: 3.20×10^2 Min: 2.0×10^1 Max: 3.3×10^3 SD: 3.7×10^1	15.63	84.38	Av: 1.0×10^3 Min: 5.0×10^0 Max: 6.4×10^3 SD: 3.9×10^3	Av: 1.0×10^1 Min: 5.0×10^0 Max: 1.5×10^1 SD: 7.3×10^0	Av: 1.1×10^3 Min: 5.0×10^0 Max: 6.4×10^3 SD: 3.9×10^2	90.91	9.09
The Central Museum of Textiles in Lodz	Av: 7.7×10^1 Min: 0.0 Max: 2.4×10^2 SD: 5.8×10^1	Av: 4.2×10^2 Min: 0.0 Max: 1.3×10^3 SD: 3.0×10^2	Av: 2.5×10^2 Min: 0.0 Max: 8.2×10^2 SD: 2.8×10^2	15.44	84.56	Av: 1.0×10^2 Min: 0.0 Max: 8.0×10^2 SD: 2.2×10^2	Av: 2.0×10^2 Min: 1.3×10^1 Max: 1.2×10^3 SD: 3.4×10^2	Av: 1.5×10^2 Min: 0.0 Max: 9.0×10^2 SD: 2.9×10^2	30.73	69.27
The Museum of Independence Traditions in Lodz	Av: 1.4×10^4 Min: 1.0×10^1 Max: 5.3×10^4 SD: 2.1×10^4	Av: 3.4×10^2 Min: 1.8×10^2 Max: 5.8×10^2 SD: 1.5×10^2	Av: 7.0×10^3 Min: 1.0×10^1 Max: 5.3×10^4 OS: 1.6×10^4	97.64	2.36	Av: 3.4×10^4 Min: 0.0 Max: 2.7×10^5 SD: 9.5×10^4	Av: 2.6×10^1 Min: 0.0 Max: 7.2×10^1 SD: 2.9×10^1	Av: 1.7×10^4 Min: 0.0 Max: 2.7×10^5 OS: 6.7×10^4	99.92	0.08
The Museum of Archeology and Ethnography in Lodz	Av: 4.2×10^2 Min: 1.0×10^1 Max: 1.3×10^3 SD: 4.5×10^2	Av: 5.6×10^2 Min: 1.5×10^2 Max: 2.4×10^3 SD: 5.6×10^2	Av: 4.9×10^2 Min: 1.0×10^1 Max: 2.4×10^3 SD: 5.06×10^2	43.24	56.76	Av: 8.9×10^2 Min: 0.0 Max: 5.9×10^3 SD: 1.8×10^3	Av: 1.2×10^2 Min: 0.0 Max: 3.1×10^2 SD: 1.0×10^2	Av: 4.6×10^2 Min: 0.0 Max: 5.9×10^3 SD: 1.2×10^3	85.93	14.07
The National Museum in Warsaw	Av: 7.5×10^1 Min: 0.0 Max: 4.6×10^2 SD: 1.2×10^2	Av: 3.4×10^2 Min: 7.0×10^1 Max: 8.6×10^2 SD: 2.0×10^2	Av: 2.1×10^2 Min: 0.0 Max: 8.6×10^2 SD: 2.1×10^2	18.26	81.74	Av: 6.3×10^1 Min: 8.0×10^0 Max: 2.0×10^2 SD: 5.5×10^1	Av: 2.1×10^2 Min: 1.3×10^1 Max: 6.5×10^2 SD: 2.0×10^2	Av: 1.4×10^2 Min: 8.0×10^0 Max: 6.5×10^2 SD: 1.6×10^2	23.17	76.83

Av – arithmetic mean.
Min/Max – minimum/maximum value.
SD – standard deviation.

Table 4
Species composition of microorganisms and frequency of their isolation in the air and on the surfaces in analyzed museums, libraries and archives.

Microorganisms	Frequency of isolation from all samples in each institution (%) Air/Surfaces					
	NA	L	CMT	MIT	MAE	NM
Gram-negative rods						
Sphingomonas paucimobilis	0/0	0/0	0/0	75.0/0	0/0	0/0
Pseudomonas alcaligenes	0/0	0/0	0/0	0/0	0/0	0/50.0[4a,b,c,d;6a,e,j,l,m;8;9d]
Gram-positive cocci						
Micrococcus sp.	0/0	0/0	95.9/83.3[1a,g;5a;6a,bc,n;9b,c;6○]	100.0/0	86.1/16.7[6g,p]	95.9/56.3[4a,b,c,d;6a,e,j,l,m;9d]
Micrococcus flavus	92.9/0	100.0/0	0/0	0/0	0/0	0/0
Kocuria varians/rosea	0/0	0/0	75.0/0	0/0	0/0	0/0
Staphylococcus aureus	0/0	0/0	0/0	0/0	100.0/0	0/0
Staphylococcus hominis	0/0	0/0	91.7/0	0/0	0/0	0/0
Staphylococcus lentus	50.0/15.8[7]	50.0/0	0/0	100.0/0	0/0	0/0
Staphylococcus xylosus	0/1.0[7]	33.3/0	75.0/0	0/0	0/0	0/0
Gram-positive endospore-forming rods						
Aneurinibacillus aneurinilyticus	0/0	0/33.3[7]	0/0	0/0	0/0	0/0
Bacillus sp.	0/0	0/0	0/66.7[1g;6n,o;9b]	0/0	0/0	0/0
Bacillus firmus	0/0	0/0	75.0/0	0/0	0/0	0/0
Bacillus licheniformis	21.4/0	8.3/33.3[7]	0/0	0/0	0/0	0/0
Bacillus megaterium	0/0	0/0	0/0	0/0	0/58.3[5b;6c,d,g,p;8]	0/0
Bacillus pumilus	21.4/26.3[7]	8.3/0	0/55.0[1a;5a;6a,b,c,d,o;9a]	0/0	0/75.0[5b;6c,d,g,i,p;8]	0/62.5[3c;4a,b,c;6e,j,l,m;8]
Bacillus subtilis	17.9/10.5[8]	33.3/33.3[7]	0/44.4[1a,g;6n,o;9a,b]	0/0	0/66.7[5b;6c,d,h,i,p;8]	0/0
Brevibacillus laterosporus	0/0	0/0	0/50.0[1a;6○;9b]	0/0	0/0	0/0
Lactobacillus delbrueckii	0/0	0/0	0/50.0[5a;6a,b,c;9c]	0/50.0[2a;6c,e]	0/0	0/0
Paenibacillus polymyxa	0/0	0/0	0/0	0/0	91.7[5b;6c,d,g,h,i,p;8]	0/0
Mesophilic actinomycetes						
Nocardia sp.	21.4/0	41.7/0	0/0	0/0	0/0	0/0
Micromonospora sp.	0/26.3[7]	0/0	0/0	0/0	0/0	0/0
Molds						
Alternaria sp.	14.3/0	0/0	0/0	0/0	0/0	0/0
Alternaria alternata	7.1/0	0/0	0/14.3[5a;6b]	0/0	0/0	0/43.8[4a,b,d;5c;6k,j;8]
Alternaria consortiale	0/0	16.7/0	0/14.3[9a,c]	0/0	0/0	0/0
Aspergillus flavus	0/0	12.5[8]	0/0	0/0	0/0	0/12.5[4d;6m]
Aspergillus fumigatus	7.1/0	0/0	0/0	0/0	0/0	0/0
Aspergillus niger	7.1/0	8.3/0	20.83/28.6[1c;6a,d;9c]	0/12.5[6c]	0/0	4.2/0
Aspergillus ochraceus	0/0	0/0	0/0	0/0	0/0	8.3/0
Aspergillus parasiticus	0/0	0/0	0/0	0/0	0/27.3[6g;8]	0/0
Aspergillus terreus	0/0	0/0	0/0	0/0	0/0	12.5/0
Aspergillus versicolor	14.3/11.1[7]	58.3/0	25.0/0	0/50.0[2a;3a;6e,f]	61.1/0	4.2/0
Aspergillus wentii	0/0	0/0	0/0	0/0	0/0	8.3/0
Aureobasidium pullulans	0/0	0/0	0/0	0/0	0/0	4.2/0
Botrytis sp.	7.1/0	8.3/0	4.2/0	0/0	0/0	0/0
Chrysonilia sitophila	21.4/0	0/0	0/0	0/0	0/0	0/0
Cladosporium sp.	0/0	0/0	4.17/0	0/0	0/0	0/0
Cladosporium cladosporoides	0/0	0/0	12.5/7.1[9c]	0/0	0/0	0/0
Cladosporium herbarum	14.3/33.3[7]	58.3/0	0/0	0/12.5[6e,f]	38.9/0	0/12.5[4b;8]
Cladosporium macrocarpum	7.1/0	58.3/0	0/0	0/0	50.0/0	16.7/18.8[4a;6a;8]
Mucor sp.	0/0	8.3/0	0/0	0/0	0/0	0/0
Mucor circinelloides	0/0	0/12.5[7]	0/0	0/0	0/0	0/6.3[6m]
Mucor globosus	0/0	8.3/0	0/0	0/0	0/0	0/0
Mucor hiemalis	0/0	0/0	8.3/0	0/0	0/0	0/0
Mucor plumbeus	0/0	0/0	0/0	0/0	0/18.9[6i;9]	0/0
Mucor racemosus	0/0	0/0	0/14.3[9a,b]	0/0	0/18.9[5b;8]	0/0
Paecilomyces variotii	0/44.4[7]	0/0	0/0	0/0	0/0	0/31.3[6k,l;8;9d]
Penicillium carneum	28.6/11.1[7]	33.3/50.0[6j]	45.8/57.1[1b,c;5a;6a,b;9c]	0/12.5[6e]	5.6/36.4[8]	0/0
Penicillium commune	0/0	0/0	0/0	0/0	0/0	33.3/0
Penicillium crustosum	21.4/33.3[7]	50.0/25.0[7]	0/0	0/0	0/0	0/0
Penicillium digitatum	14.3/11.1[7]	0/0	0/0	0/0	0/0	4.2/62.5[4a,b,d;5c;6e,j,k;8;9d]
Penicillium gladioli	0/0	0/0	0/0	0/0	0/0	20.8/0
Penicillium italicum	14.3/0	58.3/0	0/0	0/0	0/0	4.2/37.5[4a,b;6e,j,m]
Penicillium paneum	0/0	8.3/0	0/0	0/0	0/0	25.0/18.8[6e;8;9d]
Penicillium polonicum	0/0	0/0	0/7.14[5b]	0/25.0[1f]	0/27.3[6h;8]	8.3/18.8[6l;9d]
Penicillium radicola	0/0	0/0	45.8/0	0/0	0/0	0/0
Penicillium sclerotigenum	0/0	0/0	0/0	0/0	0/0	8.3/0
Rhizopus nigricans	14.3/0	0/12.5[6j]	37.50/14.3[6c,n]	100/0	27.8/0	0/0
Trichoderma viride	0/0	0/0	0/0	0/0	5.6/0	0/0
Yeast-like fungi						
Candida sp.	0/0	0/0	0/0	0/0	0/0	0/31.3[4a,b;6a,j]
Candida sphaerica	0/0	0/12.5[7]	0/35.7[5a;6a,b,d;9c]	0/0	0/0	0/0

(*continued on next page*)

Table 4 (*continued*)

Microorganisms	Frequency of isolation from all samples in each institution (%) Air/Surfaces					
	NA	L	CMT	MIT	MAE	NM
Rhodotorula sp.	0/11.1[7]	0/0	0/0	0/0	0/9.1[6d]	0/0
Rhodotorula minuta	0/0	0/0	0/0	0/0	0/0	0/6.3[4a]
Rhodotorula mucilaginosa	0/0	0/0	33.33/14.29[1a;9a]	0/0	0/0	0/0

NA – The National Archives in Lodz; L – Library in Lodz; CMT –The Central Museum of Textiles in Lodz; MAE – The Museum of Archeology and Ethnography in Lodz; MIT – The Museum of Independence Tradition in Lodz; NM – The National Museum in Warsaw.
[1] Fabric: a) textile prepared to wash; b) loom (textile); c) Turkmen bound carpet (19th/20th century); d) jacquard fabric ("Eagles" – L. Kintopf, ca 1926, Poland); e) woollen kilim Syon (Eastern Malopolska, 19th century); f) banner attacked by fungi; g) carpet from 16th century prepared for repair. [2] Leather: a) rucksack attacked by fungi (leather part). [3] Painting: a) painting on gantry (from the wall side); b) painting on gantry (from the room side); c) oil painting. [4] Sculpture: a) pedestal of Apollo Lykejos; b) pedestal of Togate Statue; c) collective pedestal in center of gallery; d) pedestal of Jupiter Capitolinus. [5] Steel: a) steel uppers automatic machine; b) steel rack; c) gantry. [6] Wood: a) wooden rack; b) wooden drum-type washing machine; c) on the wooden wardrobe; d) wooden rack; e) wooden desk; f) in the drawer of cupboard; g) wooden sculpture ("Beehive" – S. Mucha); h) wooden chest; i) chair; j) wooden shelf; k) wooden frame; l) restoration bench (fiberboard); m) wooden windowsill; n) table for textile lamination; o) bench for filling gaps; p) wooden washer. [7]Books/Documents. [8]Walls. [9]Other: a) washing basin; b) laboratory bench; c) plastic windowsill; d) plastic table.

Table 5
Sensitivity of the microorganisms isolated from archives and museums and collection strains to silver nanoparticles.

Microorganisms	Origin	MIC (minimal inhibitory concentration) (ppm)	MBC (minimal biocidal concentration) (ppm)
Gram-negative rods			
Escherichia coli	ATCC	11.25	22.50
Sphingomonas paucimobilis	MIT	11.25	11.25
Gram-positive cocci			
Micrococcus sp.	MAE	45.00	45.00
Micrococcus flavus	L	22.50	45.00
Staphylococcus aureus	ATCC	11.25	22.50
Staphylococcus aureus	MAE	22.50	45.00
Staphylococcus lentus	L	22.50	22.50
Staphylococcus xylosus	NA	11.25	>45.00
Gram-positive endospore-forming rods			
Aneurinibacillus aneurinilyticus	L	22.50	22.50
Brevibacillus laterosporus	CMT	11.25	11.25
Bacillus subtilis	ATCC	22.50	>45.00
Bacillus subtilis	NA	22.50	>45.00
Bacillus subtilis	CMT	45.00	>45.00
Bacillus megaterium	MAE	11.25	45.00
Bacillus pumilus	NA	22.50	22.50
Bacillus pumilus	MAE	11.25	11.25
Bacillus licheniformis	L	22.50	45.00
Mesophilic actinomycetes			
Nocardia sp.	L	11.25	22.50
Molds			
Alternaria alternata	NM	22.50	22.50
Aspergillus versicolor	L	45.00	45.00
Aspergillus versicolor	MAE	45.00	45.00
Aspergillus niger	ATCC	22.50	45.00
Aspergillus niger	CMT	45.00	45.00
Cladosporium cladosporoides	CMT	22.50	22.50
Cladosporium herbarum	L	22.50	22.50
Cladosporium macrocarpum	NM	22.50	22.50
Mucor racemosus	MAE	11.25	22.50
Penicillium digitatum	NM	22.50	45.00
Penicillium carneum	L	45.00	45.00
Penicillium crustosum	L	45.00	45.00
Penicillium chrysogenium	ATCC	45.00	45.00
Penicillium radicola	CMT	45.00	45.00
Rhizopus nigricans	NA	45.00	45.00
Rhizopus nigricans	MIT	45.00	45.00
Yeast-like fungi			
Candida albicans	ATCC	11.25	22.50
Candida sphaerica	L	22.50	22.50
Rhodotorula sp.	NA	22.50	22.50
Rhodotorula mucilaginosa	CMT	22.50	22.50

ATTC American Pure Culture Collection; NA – The National Archives in Lodz; L – Library in Lodz; CMT – The Central Museum of Textiles in Lodz; MAE – The Museum of Archeology and Ethnography in Lodz; MIT – The Museum of Independence Tradition in Lodz; NM – The National Museum in Warsaw.

contamination investigated by other authors (Kolmodin-Hedman et al., 1986; Rojas et al., 2002; Guiamet et al., 2011; Karbowska-Berent et al., 2011). Microbial contamination at museums was higher, and varied depending on the type of stored objects and the temperature and humidity conditions. Generally, in three museums (excluding the Museum of Independence Tradition in Lodz) the number of microorganisms did not exceed the permissible limit of microbial bioaerosol concentration in the air as recommended in the literature: 2.0×10^2 cfu m^{-3} for fungi and 2.5×10^3 cfu m^{-3} for bacteria (Gorny, 2004) or the occupational exposure threshold 5.0×10^3–1.0×10^4 cfu m^{-3} (Malmros et al., 1992). The highest contamination was found in the Museum of Independence Tradition in Lodz. The average concentration of fungal microorganisms in the air at that museum exceeded the limit value 100-fold for closed rooms. In this museum the relative humidity was at the highest level (41%), warehouses were located in the basement, and museum collections were made from materials such as textiles, wood, leather, which are susceptible to microbial degradation under conditions of high humidity (Strzelczyk, 2004). This was also the case at the Museum of Archeology and Ethnography. The main group of microorganisms in the air and on surfaces in these two museums was fungi, which accounted for 86–98% of all microorganisms. This confirmed the dependency of occurrence of these microorganisms on the conditions of high humidity. Under these conditions, the sorption of cellulosic materials (wood, nonwovens) and of the skin is high, which creates conditions for the fungi growth. However, a much higher level of relative indoor humidity (RH > 70%) at which molds were activated is given in the literature (Flannigan and Miller, 1994).

The fungi which most frequently contaminated the museum objects may pose a threat to museum objects and archives. There were also isolated *Trichoderma viridae* and species from the genus *Aspergillus* (*Aspergillus terreus*, *Aspergillus wentii*), which are known for the production of numerous extracellular enzymes, including cellulolytic, and organic acids (Gutarowska, 2010). These metabolites can damage museum and archival objects (Zyska and Zakowska, 2005). Among the fungi, there were isolated some species known for their allergenic properties, such as *A. alternata*, *Alternaria consortiale*, *A. niger*, *C. herbarum*, *Cladosporium cladosporioides* and numerous molds from the genera *Penicillium* (Kurup et al., 2002; Chapman, 2006). Many isolated species belong to the class of toxic organisms, like *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, especially *A. versicolor* – a sterigmatocistin producer, found at each analyzed site with a frequency ranging from 4.2% to 61.1%, isolated both in the air and on surfaces. The toxicity of these species has been confirmed in numerous scientific studies (Jarvis et al., 1998; Johanning et al., 1999; Nielsen et al., 1999; Tuomi et al., 2000; Flannigan et al., 2001; Bennett and Klich, 2003; Gutarowska et al., 2010).

Bacteria were less frequent. Noteworthy is the presence of pathogenic bacteria *S. aureus* in all air samples in the Museum of Archeology and Ethnography. This fact is disturbing, because *Pseudomonas alcaligenes*, originating from the soil and water, is capable of complex compound decomposition (Sarath Babu et al., 2005) and there is a real risk that the presence of the bacteria negatively affects the stability of the museum collections. Moreover, *P. alcaligenes* can cause infection in people with immune deficiency (Manfredi et al., 2000).

Research into microbial contamination in facilities such as archives, libraries, and museums conducted worldwide focuses on fungus species analysis or the cellulolytic properties of microorganisms isolated in these environments (Chen et al., 2010; Sterflinger, 2010). Cellulolytic bacteria represent a small proportion among the organisms isolated during the study. These observations are confirmed in, for example, the work of Valentin (2007) and Karbowska-Berent et al. (2011), who point to the predominant presence of the bacterial bioaerosol types *Micrococcus*, *Staphylococcus*, *Bacillus*, and also actinomycetes.

The analysis of selected, representative species showed varied sensitivity of strains to a nanosilver preparation. Gram-negative rods and mesophilic actinomycetes proved to be sensitive – for them a nanosilver concentration of 11.5 ppm inhibited the growth, while a concentration of 22.5 ppm had a biocidal effect. Greater resistance to nanosilver was shown by Gram-positive cocci and Gram-positive endospore-forming rods, for which the MIC ranged from 11.2 to 22.5 ppm, and MBC 11.2–45 ppm; for many bacteria from the genus *Bacillus* the biocidal nanosilver concentration was not established (MBC $>$ 45 ppm). There have been no studies so far on fungi's sensitivity to a preparation of nanosilver; this study showed that most molds are resistant to nanosilver, with MIC and MBC in the range from 22.5 to 45 ppm. The most sensitive were *Alternaria*, *Cladosporium* and *Mucor*, while nanosilver-resistant fungi included those from the genera *Aspergillus*, *Penicillium* and *Rhizopus*.

So far, the nanosilver sensitivity studies have been performed mainly on reference strains, like *S. aureus*, *E. coli*, *Salmonella* Tyhpimurium, *B. subtilis* (Silver and Phung, 1996; Xu et al., 1998; Yoon et al., 2007; Ruparelia et al., 2008; Bankar et al., 2010). These studies showed the high sensitivity of *E. coli* and *S. aureus* to nanosilver with a particle size from 1 to 10 nm, in preparations with a concentration of 0.1–10 ppm (Kim et al., 2007; Tien et al., 2009; Chen et al., 2010). It has been found that some microbes are characterized by high resistance to nanosilver, including *Salmonella* Tyhpimurium and *Listeria monocytogenes*, which is associated with the presence of plasmid pMg 101 in their cells (Silver and Phung, 1996; Xu et al., 1998).

Differences in nanosilver sensitivity have been shown between types, species and even strains. There have been no previous studies on strains isolated from the environment; the results presented in this study show large differences in sensitivity between strains of the same species that originated from a strain collection and those which were isolated from the environment. A dose of 45 ppm was established as sufficient to eliminate 94% of all microorganisms. This is a high concentration of silver, which exceeds the effective antimicrobial concentration given in the literature; in the future it is necessary to check the effectiveness of formulations containing nanoparticles of smaller sizes $<$10 nm, as in the literature this is suggested as having a huge impact on the efficiency of the preparation (Zhang et al., 2011). It is also important to demonstrate the difference in nanosilver sensitivity for various physiological cell forms – vegetative cells, bacterial endospores, spores, mycelia. A study conducted for bacteria of the genus *Clostridium* showed a difference in the sensitivity of endospores and vegetative cells to copper (Wheeldon et al., 2008).

4.1. Conclusions

This study gives an indication that sensitivity tests to disinfectants, in this case nanosilver, should be performed on environmental strains. These strains were characterized by increased resistance to nanosilver in comparison with their correspondents stored in the collection. Studies on the optimization of conditions for disinfection using silver nanoparticles on museum objects will be continued in the future.

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References

Abdel-Kareem, O., 2010. Monitoring, controlling and prevention of the fungal deterioration of textile artifacts in the museum of Jordanian heritage. Mediterranean Archaeology and Archaeometry 10, 85–96.

Appendini, P., Hotchkiss, J.H., 2002. Review of antimicrobial food packaging. Innovative Food Science and Emerging Technologies 3, 113–126.

Bankar, A., Joshi, B., Kumar, A., Zinjarde, S., 2010. Banana peel extract mediated novel route for the synthesis of silver nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects 368, 58–63.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clinical Microbiology Review 36, 497–516.

Blanchette, R.A., 2000. A review of microbial deterioration found in archaeological wood from different environments. International Biodeterioration And Biodegradation 46, 189–204.

Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbühler, K., 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalize plastics and textiles. Science of the Total Environment 390, 396–409.

Borrego, S., Guiamet, P., Gomez de Saravia, S., Batistini, P., Garcia, M., Lavin, P., Perdomo, I., 2010. The quality of air at archives and the biodeterioration of photographs. International Biodeterioration and Biodegradation 64, 139–145.

Braydich-Stolle, L., Hussain, S., Schlager, J., Hofmann, M.C., 2005. In vitro cytotoxicity of nanoparticles in mammalian germ line stem cells. Toxicological Science 88, 412–419.

Burd, A., Kwok, C.H., Hung, S.C., Chan, H.S., Gu, H., Lam, W.K., Huang, L., 2007. A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. Wound Repair and Regeneration Journal 15, 94–104.

Chaloupka, K., Malam, Y., Seifalian, M., 2010. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in Biotechnology 28, 580–588.

Chapman, M.D., 2006. Challenges associated with indoor moulds: health effects, immune response and exposure assessment. Medical Mycology 44, 529–532.

Chen, L., Zheng, L., Lv, Y., Liu, H., Wang, G., Ren, N., Liu, D., Wang, J., Boughton, R.I., 2010. Chemical assembly of silver nanoparticles on stainless steel for antimicrobial applications. Surface & Coatings Technology 204, 3871–3875.

Chen, Y.P., Cui, Y., Dong, Y.G., 2010. Variation of airborne bacteria and fungi at Emperor Qin's Terra-Cotta museum, Xi'an, China, during the "Oct. 1" Gold Week period of 2006. Environmental Science and Pollution Research 17, 478–485.

Crispim, C.A., Gaylarde, C.C., 2005. Cyanobacteria and biodeterioration of cultural heritage: a review. Microbial Ecology 49, 1–9.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O., 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. Journal of Biomedical Materials Research 52, 662–668.

Flannigan, B., Miller, J.D., 1994. Health implications of fungi in indoor environments – an overview. In: Samson, R.A., Flannigan, B., Flannigan, M.E., Verhoeff, A.P.,

Adan, O.C.G., Hoekstra, E.S. (Eds.), Health Implications of Fungi in Indoor Environments. Elsevier, Amsterdam, pp. 3–28.
Flannigan, B., Samson, R.A., Miller, J.D., 2001. Microorganisms in Home and Indoor Work Environments: Diversity, Health Impacts, Investigation and Control. Taylor & Francis, London and New York.
Florian, M.L., 1997. Heritage Eaters: Insects and Fungi in Heritage Collections. James and James (Scientific Publishers) Ltd, London.
Furno, F., Morley, K.S., Wong, B., Sharp, B.L., Arnold, P.L., Howdle, S.M., Bayston, R., Brown, P.D., Winship, P.D., Reid, H.J., 2004. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? Journal of Antimicrobial Chemotherapy 54, 1019–1024.
Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W., Zhang, S., Yang, X., 2007. Preparation and antibacterial activity of Fe_3O_4–Ag nanoparticles. Nanotechnology 18, 604–611.
Gorny, R., 2004. Biohazards: standards, recommendations and proposals for limit values. Principles and Methods of Assessing the Working Environment 3, 17–39 (in Polish).
Guiamet, P., Borrego, S., Lavin, P., Perdomo, I., Gomez de Saravia, S., 2011. Biofouling and biodeterioration in materials stored at the Historical Archive of the Museum of La Plata, Argentine and at the National Archive of the Republic of Cuba. Colloids and Surfaces B: Biointerfaces 85, 229–234.
Gupta, A., Silver, S., 1998. Silver as a biocide: will resistance become a problem? Nature Biotechnology 16, 888.
Gutarowska, B., 2010. Metabolic activity of moulds as a factor of building materials biodegradation. Polish Journal of Microbiology 59 (2), 119–124.
Gutarowska, B., Sulyok, M., Krska, R., 2010. Study of the toxicity of moulds isolated from dwellings. Indoor and Built Environment 19, 668–675.
Gutarowska, B., Kosmowska, M., Wiszniewska, M., Palczynski, C., Walusiak-Skorupa, J., 2011. An investigation of allergenic proteins produced by moulds on building materials. Indoor and Built Environment, Indoor and Built Environment 3. doi:10.1177/1420326X11413911.
Hsina, Y.H., Chena, H.F., Huanga, S., Shihc, T.S., Lai, P.S., Chueha, P.J., 2008. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicology Letters 179, 130–139.
Jarvis, B.B., Sorenson, W.G., Hintikka, E.L., Nikulin, M., Zhou, Y., Jiang, J., Wang, S., Hinkley, S., Etzel, R.A., Dearborn, D.G., 1998. Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated during a study of pulmonary hemosiderosis in infants. Applied and Environmental Microbiology 64, 3620–3625.
Ji, J.H., Jung, J.H., Kim, S.S., Yoon, J.U., Park, J.D., Choi, B.S., 2007. A twenty eight- day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhalation Toxicology 19, 857–871.
Johanning, E., Landsbergis, P., Gereis, M., Yang, C.S., Olmsted, E., 1999. Clinical experience and results of a sentinel health investigation related to indoor fungal exposure. Environmental Health Perspectives 107, 489–494.
Karbowska-Berent, J., Gorny, R.L., Strzelczyk, A.B., Wlazlo, A., 2011. Airborne and dust borne microorganisms in selected Polish libraries and archives. Building and Environment 46, 1872–1879.
Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., Park, Y.H., Hwang, C.Y., Kim, Y.K., Lee, Y.S., Jeong, D.H., Cho, M.H., 2007. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine 3, 95–101.
Kim, Y.S., Song, M.Y., Park, J.D., Song, K.S., Ryu, H.R., Chung, Y.H., Chang, H.K., Lee, J.H., Oh, K.H., Kelman, B.J., Hwang, I.K., Yu, I.J., 2010. Subchronic oral toxicity of silver nanoparticles. Particle and Fibre Toxicology 7, 20,. doi:10.1186/1743-8977-7-20 (open access).
Klasen, H.J., 2000. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns 26, 131–138.
Kolmodin-Hedman, B., Blomquist, G., Sikstrom, E., 1986. Mould exposure in museum personnel. International Archives of Occupational and Environmental Health 57, 321–323.
Kurup, V.P., Shen, H.D., Vijay, H., 2002. Immunobiology of fungal allergens. International Archives Allergy and Immunology 129, 181–188.
Lee, H.Y., Choi, Y.J., Jung, E.J., Yin, H.Q., Kwon, J.T., Kim, J.E., 2009. Genomics-based screening of differentially expressed genes in the brains of mice exposed to silver nanoparticles via inhalation. Journal of Nanoparticle Research 12, 1567–1578.
Liang, Z., Das, A., Hu, Z., 2010. Bacterial response to a shock load of nanosilver in an activated sludge treatment system. Water Research 44, 5432–5438.
Loeschner, K., Hadrup, N., Qvortrup, K., Larsen, A., Gao, X., Vogel, U., Mortensen, A., Lam, H.R., Larsen, E.H., 2011. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Particle and Fibre Toxicology 2011 8 (18). http://www.particleandfibretoxicology.com/content/8/1/18 (open access).
Lopez-Aparicio, S., Smolik, J., Maskova, L., Souckova, M., Grontoft, T., Ondrackova, L., Stankiewicz, J., 2011. Relationship of indoor and outdoor air pollutants in a naturally ventilated historical building envelope. Building and Environment 46, 460–468.
Malmros, P., Sigsgaard, T., Bach, B., 1992. Occupational health problems due to garbage sorting. Waste Management and Research 3, 227–234.
Manfredi, R., Nanetti, A., Ferri, M., Chiodo, M., 2000. *Pseudomonas* spp. complications in patients with HIV disease: an eight-year clinical and microbiological survey. European Journal of Epidemiology 16, 111–118.
Mesquita, N., Portugal, A., Videira, S., Rodriguez-Echeverria, S., 2009. Fungal diversity in ancient documents. A case study on the Archive of the University of Coimbra. International Biodeterioration and Biodegradation 63, 626–629.
Michaelsen, A., Pinzari, F., Ripka, K., Lubitz, W., Pinar, G., 2006. Application of molecular techniques for identification of fungal communities colonising paper material. International Biodeterioration and Biodegradation 58, 133–141.
Monteiro, D.R., Gorup, L.F., Takamiya, A.S., Ruvollo-Filho, A.C., de Camargo, E.R., Barbosa, D.B., 2009. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. International Journal of Antimicrobial Agents 34, 103–110.
Nielsen, K., 2003. Mycotoxin production by indoor moulds. Fungal Genetics and Biology 39, 103–117.
Nielsen, K.F., Gravesen, S., Nielsen, P.A., Andersen, B., Thrane, U., Frisvad, J.C., 1999. Production of mycotoxins on artificially and naturally infested building materials. Mycopathologia 145, 43–56.
Park, E.J., Bae, E., Yi, J., Kim, Y., Choi, K., Lee, S.H., Yoon, J., Lee, B.Ch., Park, K., 2010. Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environmental Toxicology and Pharmacology 30, 162–168.
Rahman, M.F., Wang, J., Patterson, T.A., Saini, U.T., Robinson, B.L., Murdock, R.C., Schlager, J.J., Hussain, S.M., Ali, S.F., 2009. Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. Toxicology Letters 187, 15–21.
Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles: as a new generation of antimicrobials. Biotechnology Advance 27, 76–83.
Rakotonirainy, M.S., Lavedrine, B., 2005. Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. International Biodeterioration and Biodegradation 55, 141–147.
Rakotonirainy, M.S., Fohrer, F., Flieder, F., 1999. Research on fungicides for aerial disinfection by thermal fogging in libraries and archives. International Biodeterioration and Biodegradation 44, 133–139.
Rojas, T.I., Martinez, E., Gomez, Y., Alvarado, Y., 2002. Airborne spores of *Aspergillus* species in cultural institutions at Havana University. Grana 41, 190–193.
Ruparelia, J.P., Chatterjee, A.K., Duttagupta, S.P., Mukherji, S., 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomaterialia 4, 707–716.
Rutala, W.A., Weber, D.J., 1999. Infection control: the role of disinfection and sterilization. Journal of Hospital Infection 43 (Supplement), 43–55.
Samson, R.A., Hoekstra, E.S., Frisvad, J.C., 1996. Introduction to Foodborne Fungi, fifth ed. Centraalbureau voor Schimmenuturees, Baarn, pp. 6–221.
Sarath Babu, V.R., Patra, S., Thakur, M.S., Karanth, N.G., Varadaraj, M.C., 2005. Degradation of caffeine by *Pseudomonas alcaligenes* CFR 1708. Enzyme and Microbial Technology 37, 617–624.
Sarro, M.I., Garcia, A.M., Rivalta, V.M., Moreno, D.A., Arroyo, I., 2006. Biodeterioration of the Lions Fountain at the Alhambra Palace, Granada (Spain). Building and Environment 41, 1811–1820.
Silver, S., 2003. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiology Reviews 27, 341–353.
Silver, S., Phung, L.T., 1996. Bacterial heavy metal resistance: new surprises. Annual Review of Microbiology 50, 753–789.
Sondi, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science 275, 177–182.
Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents under Dynamic Contact Conditions (ASTM E2149 – 01).
Sterflinger, K., 2010. Fungi: their role in deterioration of cultural heritage. Fungal Biology Reviews 24, 47–55.
Strzelczyk, A.B., 2004. Observations on aesthetic and structural changes induced in Polish historic objects by microorganisms. International Biodeterioration and Biodegradation 53, 151–156.
Tang, J., Xiong, L., Wang, S., Wang, J., Liu, L., Li, J., 2009. Distribution, translocation and accumulation of silver nanoparticles in rats. Journal of Nanoscience and Nanotechnology 9, 4924–4932.
Tian, J., Wong, K.K.Y., Ho, C.M., Lok, C.N., Yu, W.Y., Che, C.M., Chiu, J.F., Tam, P.K.H., 2007. Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem 2, 129–136.
Tien, D.C., Tseng, K.H., Liao, C.Y., Tsung, T.T., 2009. Identification and quantification of ionic silver from colloidal silver prepared by electric spark discharge system and its antimicrobial potency study. Journal of Alloys and Compounds 473, 298–302.
Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E.L., Lindroos, O., Kalso, S., Koukila-Kahkola, P., Mussalo-Rauhamaa, H., Haahtela, T., 2000. Mycotoxins in crude building materials from water-damaged buildings. Applied and Environmental Microbiology 66, 1899–1904.
Vaidyanathan, R., Kalishwaralal, K., Gopalram, S., Gurunathan, S., 2009. Nanosilver – the burgeoning therapeutic molecule and its green synthesis. Biotechnology Advances 27, 924–937.
Valentin, N., 2007. Microbial Contamination in Archives and Museums: Health Hazards and Preventive Strategies Using Air Ventilation Systems Contribution to the experts' roundtable on sustainable climate management strategies, Tenerife, Spain, online at: http://www.getty.edu/conservation/our_projects/science/climate/paper_valentin.pdf.
Wheeldon, L.J., Worthington1, T., Lambert, P.A., Hilton, A.C., Lowden, J.C., Elliott, T.S.J., 2008. Antimicrobial efficacy of copper surfaces against spores and vegetative cells of *Clostridium difficile*: the germination theory. Journal of Antimicrobial Chemotherapy 62, 522–525.

Xu, C., Zhou, T., Kuroda, M., Rosen, B.P., 1998. Metalloid resistance mechanisms in prokaryotes. The Journal of Biochemistry 123, 16–23.

Yamanaka, M., Hara, K., Kudo, J., 2005. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. Applied Environmental Microbiology 71, 7589–7593.

Yoon, K.Y., Byeon, J.H., Park, J.H., Hwang, J., 2007. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. Science of the Total Environment 373, 572–575.

Zhang, X., Niu, H., Yan, J., Cai, Y., 2011. Immobilizing silver nanoparticles onto the surface of magnetic silica composite to prepare magnetic disinfectant with enhanced stability and antibacterial activity. Colloids and Surfaces A: Physicochemical and Engineering Aspects 375, 186–192.

Zotti, M., Ferroni, A., Calvini, P., 2011. Mycological and FTIR analysis of biotic foxing on paper substrates. International Biodeterioration and Biodegradation 65, 569–578.

(in Polish). In: Zyska, B., Zakowska, Z. (Eds.), Microbiology of Materials. Polytechnic of Lodz, Lodz.