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Introduction

The investigations described herein were a first step in a scientific project entitled "Investigations of biodeterioration of historical objects based on the analysis of volatile organic compounds emitted by moulds". The project is financially supported entirely by the National Science Centre in Poland.

The primary aim of our investigations was to establish the usual concentration and composition of microorganisms in the museum's indoor air. This was the key step in the project because the species of mould collected and identified in the museum facilities, which are described in the literature as deteriorating natural fibres, were then used for inoculation on model samples chosen to represent historical objects (including cellulose, wool, silk, parchment). An analysis of Microbial Volatile Organic Compounds (MVOCs) emitted by moulds growing in these conditions was also carried out. The analytical technique chosen to perform the analysis was Solid Phase Microextraction (SPME) – Gas Chromatography (GC) – Mass Spectrometry (MS). The results obtained with MVOC analysis will be used for the fast identification of mould-induced infestation in historical objects. This can be a useful tool for the rapid detection of biodeterioration processes running in situ, in real objects of art and cultural heritage.

The Department of Microbiology at the Faculty of Commodity Science, Cracow University of Economics, is currently taking part in a few scientific projects concerned with investigating the biodeterioration of historical, art and sacral objects. Classical microbiological methods are applied in investigations beside the MVOC analysis mentioned above. Further, molecular biology methods are used to identify microorganisms that infest objects of cultural heritage. The Department of Microbiology has for many years been collaborating with various museums, churches and cultural institutions not only in the area of biodeterioration of objects but also in microbial contamination of indoor air and surfaces, and it continues to be open to further opportunities.

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1. Microbial Quality of Indoor Air in Museums

The microflora present in the air can originate from water, soil, the surfaces of various objects, as well as animals and plants. The air, however, does not make the living environment for microorganisms, but is rather a medium in which they float and move. Bioaerosol occurring in the air may contain plant pollen, small algae, protozoa, viruses, bacteria, fungal spores, fragments of mycelium and yeasts (Florian 2002). It can also contain dead microorganisms, fragments of cell structures and metabolites of living microorganisms that cannot be tested with cultivating methods, but only with analytical techniques (Florian 2002, Flannigan 2001). Four processes can be used to determine the change in concentration of bioaerosol present in the air (Rüden *et al.* 1978):

– active increase of concentration, resulting from the relocation of people and animals that emit microorganisms,

– passive increase, resulting from the rising and desorption of microorganisms from the surface of water, soil and various objects,

– active decreasing, due to environmental factors (rainfall) or the death of microorganisms,

– passive decreasing, due to sedimentation or absorption on the surface of water, soil or other surfaces.

Microorganism viability in the air depends on numerous factors, the most important of which is humidity. The group of microorganisms that are quite resistant to dehydration includes spores and other spore forms of fungi and bacterial endospores. Vegetative forms of microorganisms, on the other hand, die in the air relatively quickly. The speed of microorganism translocation in the air and time of suspension in the form of aerosol depends on their shape, size, surface, mass and the presence of electrical charge. On the other hand, quantity and species composition of microorganisms present in the air are associated with climatic conditions, or microclimate for indoor spaces. In Poland's climatic zone, the greatest quantity of microorganisms in the air is observed in the spring and autumn months. With December and January come low temperatures, low air humidity and frozen ground, limiting the quantity of microorganisms.

Evaluating the composition of microorganisms present in aerosol in open spaces reveals that fungi such as *Cladosporium* predominate. In some instances

their spores account for as much as half of the spores detected in the air (Florian 2002, Flannigan 2001, Mullins 2001). Other quite frequently detected species come from the genera *Aspergillus, Penicillium,* and *Alternaria*. However, species composition and the quantity of microorganisms in the air are subject to constant changes due to humidity, temperature, precipitation, wind speed, time of day, time of year, height above mean sea level (AMLS), and the degree of urbanisation. For indoor spaces, the list of these factors has to be modified as appropriate and completed with additional consideration given to the number of windows and doors, ventilation, air-conditioning and heating systems, wall and floor finishing and, above all, the presence of people and the type of activities they engage in. It is possible that the presence of plants and animals should also be taken into consideration (Szostak-Kot, Syguła-Cholewińska & Błyskal 2007).

Paper, wood, fabrics, parchment, leather, wax, plastics, metals and alloys are among the materials commonly found in the historic objects stored in various museums. The objects are at risk of degradation that may be due to both the effects of external factors, such as temperature, relative humidity, light, environmental pollution, microorganisms (Zou, Uesaka & Gurnagul 1996, Barański *et al.* 2001, Haillant, Fromageot & Lemaire 2005, Havermans *et al.* 1994, Szostak-Kot & Syguła-Cholewińska 2012), and internal ones including composition, acid content and moisture level. Most of these factors determine the physicochemical degradation of historic objects, while microorganisms are responsible for a process known as biodeterioration.

Museums must protect their collections against destruction due to physicochemical phenomena, biodeterioration or both of these factors together. Preventive measures are required during storage, maintenance, conservation treatment, transport, exhibition and repeated storage of an object – in fact, through the entire "cycle of its life". To combat physical factors such as temperature, humidity and light, objects can be protected by providing the appropriate microclimatic conditions adapted to specific requirements for objects made of a given material (including the selection of proper light intensity) (Florian 2002, Camuffo *et al.* 2001, Thomson 1994, ASHRAE 2007). The American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) guidelines are a useful tool for developing the appropriate microclimatic conditions that should be maintained in museum facilities (ASHRAE 2007). The guidelines cover five major microclimatic classes – AA, A, B, C, D – and three additional classes, including two for objects at risk of chemical degradation (low-temperature storage) and one intended for objects made of metals (dry storage). The most rigorous conditions assumed for microclimate stability are provided in class AA. Here, the acceptable short-term temperature fluctuations are ±2K, and humidity ±5%, while

seasonal temperature change has to be within the range of ±5K and any change in humidity is unacceptable.

The effects of gaseous environmental contaminants, i.e. NO_x, SO_2, O_3 and volatile organic acids can be prevented with the aid of appropriate filters in ventilation systems in museums, the development of sorbent-based systems or airtight museum display cases with a controlled, modified internal atmosphere. Internal factors that contribute to the degradation of historic objects, such as acidity, can be eliminated during conservation treatments. A significant challenge for museums, however, is to protect objects against biodeterioration caused by bacteria and fungi.

Microorganisms present in the indoor air of museum facilities can originate from various sources. They get into the air from infected historic objects, from materials used for the construction of display cases, and from contaminated buildings. They are further introduced into museum interiors by way of out-of--order, uncleaned or inappropriately constructed ventilation and air-conditioning systems, through windows and doors, as well as via employees and visitors. To illustrate the scale of microbial contamination in indoor air, the quantity of fungal spores in 1 g of dust in the air of closed spaces can be between $6 \cdot 10^3$ and $3.2 \cdot 10^6$ CFU (colony forming unit) (Korpi *et al.* 1997). At the same time, the quantity of mould spores in 1 m^3 of indoor air in a museum can be between 1 CFU and $4.5 \cdot 10^6$ CFU (Yi-Ping, Ying & Jun-Gang 2010). In this context, a broken down heating, air-conditioning or ventilation system can lead to rapid changes in air temperature and humidity values, posing a serious risk of microorganism growth on the surface of historic objects (Camuffo *et al.* 2001, Martens 2012). An equally serious hazard can occur when there are long-lasting seasonal changes in microclimate parameters inside museum facilities; these facts highlight the importance of guidelines developed by ASHRAE.

Microorganisms present in museum facilities can develop and grow only under conditions that are optimal for them. Thus the method to protect collections against biodeterioration is to maintain the desired microclimate parameters inside the buildings where they are housed (Martens 2012). Most of the institutions like libraries and museums or archives usually have climate-controlled rooms and keep a low relative humidity (RH), but there are still places where indoor environment monitoring does not exist. This can pose the real risk that episodes of high RH will occur, thus allowing moulds to develop. Similar episodes can also take place in storerooms with controlled microclimates when air conditioning, ventilation or central heating systems fail and as well when water pipes or a roof leak. A high RH value can also occur inside rooms when seasonal weather fluctuates quickly and the microclimate controlling system cannot keep up to balance the changes. In all of these cases, the real danger of microflora activation on

historical objects exists. Thus development of a tool for the early detection of deterioration in objects is of the utmost importance to a global programme aimed at protecting collections.

Within the last twenty years, attitudes to microclimatic parameter values in museum facilities that favour microorganism growth on historic objects have varied. It was initially thought that RH below 60% protects museum objects against the development of fungi, while when RH climbs above 75% they develop intensively (Michalski 1993, Scott 1994). However, further studies demonstrated that for the development of microorganisms on historical objects, the RH value near the surface of an object is far more important than the global RH value in the room (Adan 1994). This seems reasonable given that RH near the surface of an object is modified by its temperature. Consequently, it was recommended that the RH value measured near the surface of the object be used to assess the risk of the object developing microorganisms. The score is called Time-of-Wetness (TOW), i.e. the proportion of time when RH at the surface of an object is above 80% to total, periodic measurement time, which is assumed to be 24 hours. For TOW below 0.5 it is believed that there is no risk of fungi developing (Adan 1994).

Clarke *et al.* (1996) presented equations together with so-called curves of limit for mould growth that made it possible to determine for what combinations of minimum temperature and RH values the mould growth on an object occurs. Unfortunately, their elaboration looked at only six mould species and did not take into consideration the most important factor – the availability of nutrients. A solution to this problem was suggested in another, and currently the most advanced, model that makes it possible to evaluate the risk of fungal growth on historic objects by involving a combination of the following parameters: temperature, RH, spore activation time, mould growth rate and the availability of nutrients (Sedlbauer 2001).

According to this model, the specific, defined values of temperature, RH and nutrient availability have to be met simultaneously for the established period of time required for spore activation. If the time of the optimal parameter combination exceeds that needed to activate the spores, mould develops on a historical object, and the rate of their daily growth (mm/day) again depends on the concomitant occurrence of specific values of temperature, RH and nutrient availability. The higher the temperature and humidity values are, the shorter the spore activation time and the greater the mould growth rate will be. Obviously for this model there is a certain combination of temperature and RH values under which spore activation does not occur. The model was developed for two types of surfaces of historic objects: organic materials that can be bio-deteriorated (I), and inorganic and porous materials (II), where organic matter trapped in the pores is a source of nutrients.

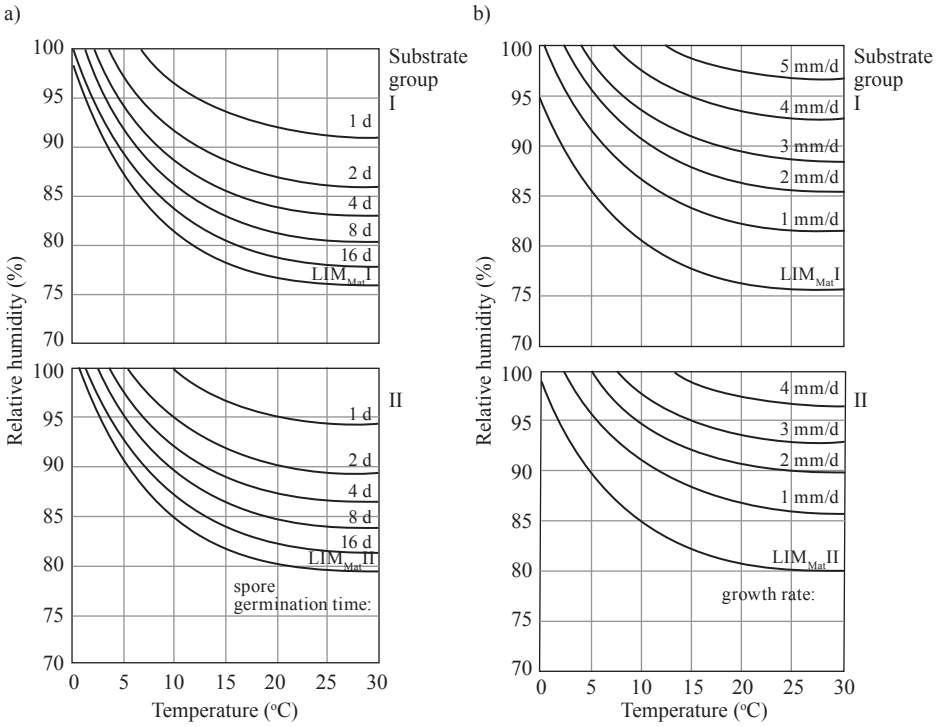

Fig. 1. Spore Activation Rate (a) and Mycelium Growth Rate (b) versus Air Temperature and Relative Humidity at the Surface of Objects that Belong to the Ist or IInd Group

Source: Sedlbauer (2001).

The minimum RH value that is assumed in the model to be required for the development of fungi on historical objects is very close to the values specified in the ASHRAE guidelines (2007), though these guidelines apply only to RH (without any consideration given to temperature) and are provided for materials which bio-deteriorate easily, such as cotton, leather, and parchment, all of which fall under category I in the model.

The optimum conditions (temperature and RH) specified in the literature for the exhibition and storage of various historical objects are correlated with the nature of material of which the objects were made (wood, paper, wool, silk, metal), in order to ensure the risk of mechanical or chemical degradation of museum objects is reduced to the greatest possible extent. Simultaneously, these assumed temperature and RH values usually also ensure that the risk of microorganism development on objects is minimised (Thomson 1994, ASHRAE 2007).

Rapid changes of weather conditions, a significant increase in the number of visitors, the breakdown of microclimate control system in a museum or uncontrolled, long-term, seasonal changes of microclimate in museum facilities can all cause the temperature and relative humidity values to obtain the combination which can be optimum for microorganism development. If these microclimate parameters last for a long enough period of time (see Fig. 1), the activation and growth of spore forms of microorganisms present on objects' surfaces occur, thus commencing the bio-deterioration process.

Bio-deterioration is mostly the result of the metabolic activity of moulds. By means of enzymes that they form, they can directly deteriorate historical objects they have infested, and even lead to the complete degradation of artefacts in a longer or shorter period of time. Objects that are especially susceptible to microbiological decomposition are those containing in their composition organic compounds such as: cellulose – books, documents, fabrics, furniture, paintings and wooden sculptures; proteins – parchment, leather, silk, and wool. Moreover, moulds produce a quite significant amount of various metabolites such as organic and inorganic acids, vitamins, amino acids, purines, sulphur containing organic compounds, antibiotics, toxins, chelates and dyes (Ciferri, Tiano & Mastromei 2000, Mandrioli, Caneva & Sabbioni 2003, Valentin 2003). These compounds are frequently associated with the so-called microbiological corrosion of objects, causing their structures to be damaged by chemical reactions between metabolites and the surface, and not by direct decomposition, as in the case of enzymatic activity. Microbiological corrosion may be observed on historical objects made, for example, of metal or ceramics, when they are infected by moulds that metabolise organic matter present in dust that has been deposited by the air on the surface of an object.

The surface of historical objects can be considered "a new type of ecosystem", where some limited number of mould species predominate depending on the relative humidity of air and nutrient availability (Grant *et al.* 1989, Nielsen 2002):

– the first species that can appear on historical objects are those that are able to grow at a water activity (a_w) of $a_w < 0.8$. This group includes: *Penicillium chrysogenum*, *Aspergillus versicolor* and *Aspergillus fumigatus*, *Aspergillus niger*, *Eurotium* representatives, *Penicillium brevicompactum*, *Penicillium commune*, *Paecilomyces variotii*;

– the next group of colonising species includes moulds that require water activity a_w of 0.8–0.9 to grow. This includes the following species: *Alternaria* sp., *Cladosporium* sp., *Ulocladium* sp. These species are able to grow even under intensive humidity changes within a single day;

– the third group consists of moulds that appear on objects being flooded or when extremely high air humidity occurs at their surface. They require water

activity of $a_w > 0.9$, and include numerous toxin-producing species. This group includes *Chaetomium globosum*, *Stachybotrys chartarum* and representatives of *Trichoderma* genus.

The rate of biodeterioration of historical objects and the extent of the damage they wreak depend on how long the optimum conditions for mould growth occur and on how quickly preventive measures are taken. The most frequent damages of historical objects that are caused by microorganisms are spots of various colours and damage to the material structure, including an object's wholesale deterioration.

Some of the metabolites produced by moulds can be hazardous not only to historical objects, but also to the health of visitors and museum staff. This group of compounds includes toxic organic compounds called mycotoxins, as well as volatile organic compounds emitted by fungi into the environment, or MVOCs, and substances present in their cells, e.g. β-D-glucans, which can be found in cell walls. Mycotoxins are organic compounds of small molecular weight and varied chemical structure. Of particular importance are aflatoxins and trichotecens, mainly when workplace air toxicity is studied. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* while trichotecens are produced by *Stachybotrys chartarum* (Midgeley, Hay & Clayton 1997, Miller 1994, Pasanen *et al.* 1992). The harmful effects of β-D-glucans, found not only in the fungal cell wall but also in conidia, are allergenic and attack the respiratory system.

The last group of compounds are microbial volatile organic compounds, which are emitted by fungi and contain a mixture of various organic compounds, including short-chain alcohols and aldehydes, ketones and organic acids, which are thought to cause a disease called chronic fatigue syndrome (Flannigan 2001, Wiszniewska *et al.* 2004, Nielsen 1999). The emission of MVOCs by moulds can be used to detect this group of compounds (Bingley *et al.* 2012).

Today's commonly used mould detection methods include visual examination and traditional microbial tests. The former can be used to detect mould only when it grows on exposed surfaces and when the infestation is advanced enough to see the overgrowing mycelium. Microbial tests require sampling at the surface of the object with a swab or using a contact method (pressing the broth directly on the object) or even employing a destructive method such as cutting off and scratching off pieces of the object. In the last two mentioned cases, the sampled spores and hyphae from the swab or the gathered pieces of objects have to be transferred onto microbial broths (Kraková *et al.* 2012). The samples prepared in any of these three methods need to be kept in incubators for about one week in defined conditions, during and under which the growth can be assessed both quantitatively and qualitatively. This means microbial tests are time-consuming, rely on a sampling technique which requires contact with the object (or even altering its structure),

and yield results only when the moulds are growing on an accessible surface of the object not in the bulk. Molecular methods, which are used to identify moulds growing on paper or other cellulosic objects, are also common today, but sometimes require intensive sampling from the surface of the object (i.e. a large enough sample of DNA has to be acquired) causing alterations of the surface itself either by scratching it or cutting off fragments (Mesquita *et al.* 2009).

Thus, the best alternative to the described methods will be a contact-free technique, making it possible to detect moulds even at an early stage of growth, and identifying their presence even if they grow inside the structure of the objects. The literature data (Fiedler, Schütz & Geh 2001, Lancker *et al.* 2008, Matysik, Herbarth & Mueller 2008) and our preliminary results (Sawoszczuk 2014) allowed us to confirm the effectiveness of the method which meets these expectations: namely, analysis of Volatile Organic Compounds (VOCs) emitted by moulds or the Microbial Volatile Organic Compounds. Moulds emit these compounds at every stage of mycelium growth (even from the very beginning) and MVOCs are released from the objects by diffusion even if fungi grow inside the object. Moreover, the MVOC sampling technique is contactless. It is based on sorption of volatile compounds just from above the surface of the objects. The two most common methods used for MVOCs sampling are, first, absorption into a sorption tube filled with various adsorbents and, second, solid phase microextraction (SPME). In the latter, VOCs are collected on a small amount of sorbents or their mixture, which is attached to the end of a metal needle; the whole setup is called fibre. In the first case, the absorbed MVOCs are analysed in a thermal desorber (TD) – gas chromatography (GC) – mass spectrometry (MS) system (Betancourt *et al.* 2013). In the second, MVOCs gathered on SPME fibre are analysed using a GC-MS system (Bingley *et al.* 2012).

The main goal of the project, which is financed by the National Centre of Science, is to measure MVOCs emitted by moulds growing on various materials used to represent historical objects (cellulose, wool, silk, parchment). The measurements are done with Solid Phase Microextraction (SPME) – Gas Chromatography (GC) – Mass Spectrometry (MS). Because the presence of microorganisms in museums' indoor air can pose a risk for historical objects and the health of visitors and staff, it is especially important to control the microbial quality of the air there. Of course, the level of air contamination with microorganisms in museum facilities throughout the world varies and can reach a concentration, for example, for mould fungi of from 1 CFU/m^3 of air up to $4.5 \cdot 10^6$ CFU/m^3. The latter figure was obtained during research conducted in the Emperor Qin's Terra-Cotta museum on days when the greatest tourist attendance was noted (Yi-Ping, Ying & Jun-Gang 2010).

2. Studies on Air Microbial Quality in Selected Facilities of the National Museum in Cracow

2.1. The Aim of the Research

The main aim of the investigations presented in this work was to determine the concentration and species composition of microorganisms in indoor air in selected facilities of the National Museum in Cracow. The investigated rooms were located in various National Museum buildings in Cracow. Moreover, the air quality was also evaluated in two of the museum's storerooms located in the Royal Castle at Niepołomice, outside the city. The qualitative study of microflora was carried out only for fungi because they seem to be the most dangerous microorganism for historical objects of all those that can be identified in the indoor air of museums, archives and libraries as they activate at a relatively low RH. Some mould species develop when RH was as low as 75%, whereas bacteria require much higher relative humidity to grow. Fungi activate and develop at relatively lower relative humidity than bacteria. This is why our investigations of biodeterioration of historical objects based on the analysis of volatile organic compounds looks only at moulds. There is a higher probability that after rapid changes to the microclimate parameters, it will be moulds, rather than bacteria or actinomycetes, that biodeteriorate objects. Hence, only fungi were identified during the investigations.

The other aim of the research was to compare:

– the effectiveness of two techniques of air sampling that were used in the analysis of air microbial quality measurements: Koch's sedimentation method, and aspiration method using an aeroscope (air sampler);

– the microbial quality of indoor air in selected museum facilities before and after measures aimed at limiting the microbial contamination in these places.

2.2. Characteristics of the Facilities

The facilities of the National Museum in Cracow that were subjected to examination are located in old buildings from various historical periods. Some of them are located in buildings that have been renovated. Other places still have their natural historical character. The research on the microbial quality of indoor air was carried out in two kinds of places: storerooms and conservation workshops. Exhibition rooms were not included in these studies. In all of the places examined, the microclimatic parameters: temperature (T) and relative humidity (RH) were monitored constantly. The investigations of microbial air quality were carried out in the spring and summer months of 2013.

In all of the locations, the parameters of microclimate were maintained at the level corresponding to detailed specification established for storing historical objects made of specific materials (Thomson 1994). Special attention was given to the storeroom of the National Museum collection located in the Princes Czartoryski Museum in the arsenal building on Pijarska street in Cracow, where water had infiltrated the western wall from the outside, as confirmed by salt efflorescence on the wall. The storerooms and conservation workshops selected for the research differed by location (various buildings in the city and outside the city), position in their respective buildings (levels –1 to 3), dimensions, number of windows and doors, walls, floors and ceiling finishings, ventilation, heating and air-conditioning system. The characteristics of the facilities are presented in Table 1.

2.3. Materials and Methods

The microbial qualities of the indoor air in selected places of the National Museum in Cracow (see Table 1) were examined by sampling the air with chosen techniques. Air temperature and relative humidity were measured during each sampling, and each place also underwent visual inspection.

At the first stage of measurements, in the Paper and Leather Conservation Workshop, samples were acquired by using two methods: Koch's sedimentation method and aspiration method (or aspirating sampling method, using Merck's MAS 100 Eco air sampler) in order to compare the effectiveness of these two methods. However, in other locations, only aspiration was used (see Section 3.2.6). The reason for this decision is explained below.

Table 1. Characteristics of the National Museum Facilities Examined for Indoor Air Microbial Quality

Facility Examined	Location of Facility	Area	Number of Investigation Points	Characteristics of the Stored Objects
The Paper and Leather Conservation workshop (before UV disinfection)	National Museum in Cracow, the Łoziński Townhouse, 1st floor, Piłsudskiego street	38 m^2, height 3 m, three window openings with blinds, one door opening, in the room there are two tables for the conservation of objects, a cupboard for chemical reagents, fume cupboard, three vents, tiled floor, walls painted with common paint	4 points – sedimentation sampling 4 points – aspiration sampling	Objects made on paper, including pastels and books, before and after conservation, before and after disinfection
The Paper and Leather Conservation workshop (after ventilation system repaired and UV disinfection)	National Museum in Cracow, the Łoziński Townhouse, 1st floor, Piłsudskiego street	As above	As above	As above
Pastel Paintings Storeroom I	National Museum in Cracow, Main Building, 1st floor, Aleja Mickiewicza	132 m^2, height 4 m, no window openings, two door openings, several cupboards with objects in the centre of the storeroom and lattice constructions by the walls on which objects are hung. Six ventilation inlets and six outlets – forced ventilation. Parquet floor, walls painted with paint (white colour)	8 points – aspiration sampling	Objects made on paper, mostly pastels, some of them with visible growth of moulds – framed objects
Pastel Paintings Storeroom II	National Museum in Cracow, Main Building, 1st floor, Aleja Mickiewicza	152 m^2, height 4 m, two door openings, no window openings, inside there is an extra room (72 m^2) which is an integral part of the main one but is separated only with bars. Inside this room pastels hang on mobile frameworks. In the centre of the room there are two lines of cupboards with objects. Six ventilation inlets and outlets – forced ventilation. Parquet floor, walls painted with paint (white colour)	11 points – aspiration sampling	Objects made on paper, mostly pastels and documents, small amount of textiles, no objects with visible microbial contamination

Table 1 cnt'd

Facility Examined	Location of Facility	Area	Number of Investigation Points	Characteristics of the Stored Objects
Branch Storeroom I	Royal Castle at Niepołomice. Storeroom, 2nd floor	40 m^2, height varying from 2 to 3 m, no windows. Part of the floor made of metal framework is shared, as a ceiling, with the Storeroom II below. Rooms are separated with OSB boards (Oriented Strand Board) put on the framework. No window openings. Walls painted with common paint	2 points – aspiration sampling	Objects made on paper and oil paintings on canvas, mostly packed into various boxes. Not possible to unpack the objects to evaluate microbial contamination
Branch Storeroom II	Royal Castle at Niepołomice. Storeroom, 1st floor	25 m^2, height 3.5 m. A part of the ceiling is shared with the floor of Storeroom I above it. Two windows, permanently shaded. A room with frameworks, on which objects are hung. Parquet floor. Walls painted white	2 points – aspiration sampling	Objects in the storeroom include mostly oil paintings and objects made on paper hung on the framework
National Museum storeroom at the Princes Czartoryski Museum	Armoury, level –1, Pijarska street	400 m^2, height 4 m. Window openings shaded, one door opening. Storeroom separated into two parts with a lattice that does not disturb air circulation. Parquet floor. Walls: bricks not covered with plaster	3 points – aspiration sampling	Various types of objects, made of various materials: metal, ceramics, wood, paper, glass, textiles
The Curiosity Storeroom	The Princes Czartoryski Museum, 2nd floor, Western and Eastern Room, Sławkowska street	Western Room 17 m^2, Eastern Room 16 m^2, height 3 m. One window and one door frame in each of the rooms. Glazed cupboards of room height along the walls	1 point in each of the rooms – aspiration sampling	Objects made of metal as well as textiles and paper, including books, stored in the cupboards

Source: the authors' own studies.

Sedimentation involves directly exposing microbiological broths prepared in Petri dishes (ϕ = 10 cm) at the sampling site, after removing a lid, so that microorganisms present in the air can freely sediment onto the medium surface for a specific time (in this case 10 min). During sampling, disturbances of natural air circulation were avoided to prevent sampling method error that could affect the results of the measurements. When the sampling was finished, the plates were closed and put into an incubator.

For aspiration, Petri dishes containing microbiological medium (ϕ = 9 cm) were put into an air sampler immediately after removing the lid. The cover was then attached to the sampler and air was aspirated at the rate of 100 litres/minute, for one minute, in order to take 100 litres. The Petri dish was then removed from the sampler, closed and put into an incubator. The volume of air to be taken was determined based on initial tests.

The number of points at which the sampling was carried out was chosen, for each of the locations, based on their volume (Table 1). When aspiration was applied, the sampler was located on the floor, and the sampler head was set at a 60° angle in various directions to analyse all of the areas around the examined point. In exceptional cases, especially when various spatial barriers occurred to prevent proper air sampling (without measurement error), samples were taken at the height of 1–1.5 m.

Sedimentation-based sampling was done in exactly the same locations and at the same heights as the aspiration. After sampling, Petri dishes were put into incubators at $30^{o}C \pm 2^{o}C$ and relative humidity of 60–70% for two weeks. The growth of colonies was examined with the naked eye and under a stereoscopic magnifying glass after 3, 5, 7, 9 and 14 days from the beginning of incubation. Fungal and bacterial colonies grown on the broths were counted, and the fungi were identified.

At each of the examined points the samples were taken on four types of microbiological broths:

– TSA – Tryptic Soy Agar (BTL) – to determine aerobic mesophilic bacteria (due to incubation temperature),

– MEA – Malt Extract Agar (BTL) – to determine total number of fast-growing fungi,

– DG18 with chloramphenicol (BTL) – to determine xerophilic fungi,

– Czapek-Dox (BTL) with sterile Whatman filter paper disc – for the growth of cellulolytic fungi.

Microbiological media used in the measurements varied in composition, especially as they have various sources of carbon, nitrogen and other elements that were available for microorganisms. Detailed characteristics of individual media are presented in Table 2. In the case of Czapek-Dox (Cz-D) medium, the only

source of carbon was cellulose, applied as Whatman filter paper disc, in order to detect cellulolytic fungi in the indoor air of the rooms tested, as mentioned above.

Table 2. Composition of Media Used in the Tests

Medium Type	Composition
TSA	casein peptone 15 g, soy flour peptone 5 g, sodium chloride 5 g, agar 15 g
MEA	maltose extract 20 g, peptone 1 g, dextrose 20 g, agar 15 g
DG18	glucose 10 g, peptone 5 g, KH_2PO_4 1 g, $MgSO_4$ 0,5 g, chloramphenicol 0,05 g, agar 15 g
Czapek-Dox	$NaNO_3$ 3 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, agar 15 g, sterile Whatman filter paper disc

Source: the authors' own studies.

This research assumption seems fully justifiable given that the objects stored in the places tested (see Table 1) included ones containing cellulose such as pastels, books, posters, paintings as well as furniture, frames, and sculptures. Chloramphenicol applied in DG18 medium limited the growth of bacteria and species in the genera *Aspergillus*, *Penicillium*, and *Mucor.*

3. Results

3.1. General Remarks

The number of sampling points for each location was adapted not only to room size, but also to the order of the arrangement of, for example, window and door openings, vents, objects and furniture found there. The whole sampling procedure was described above. After sampling the indoor air onto the microbiological broths, the incubation was started by placing them in laboratory incubators, then the colonies of microorganisms were counted and macroscopic assessment was carried out according to this schedule:

– after 2 days from beginning cultivation on TSA medium,
– after 5 days from beginning cultivation on MEA medium,
– after 7 days from beginning cultivation on DG18 medium,
– after 9 days from beginning cultivation on Czapek-Dox medium with filter paper.

Bacteria began to grow on the TSA media within 24 hours of incubation, but macroscopic inspection of their growth and colony counting was carried out only after 48 hours from the beginning of the incubation. The results were verified by reassessing after 72 hours. Fungal growth was evaluated on the third day of cultivation, mostly on MEA media. At this stage growth was visible as non-sporulating, white mycelia. After 5 days of incubation most of the fungi occurred as sporulating colonies that spread through the medium surface, while their quantity remained unchanged, which was confirmed by evaluation after 9 and 14 days from initiating incubation. In a few cases the number of colonies was observed to increase, mostly due to the dissemination of fungal spores that had already grown on the medium. The growth of xerophilic fungi was observed on DG18 broth after 5 days from the beginning of the cultivation. The fungal colonies growing on these media were counted after 7 days from the initiation of the incubation, since keeping the broths in incubators longer did not further affect the results (see growth on MEA). In the case of Czapek-Dox media with filter paper, the cellulolytic fungi colonies were counted 7, 9 and 14 days after incubation was begun. The results obtained after 9 and 14 days did not differ from each other.

To obtain pure fungal cultures, the individual species were isolated from the colonies growing on the media, usually between the 7th and 9th days of the culture.

3.2. Paper and Leather Conservation Workshop

3.2.1. Aims and Objectives

In order to compare the effectiveness of methods, aspiration and sedimentation were used in the Paper and Leather Conservation Workshop. The tests were carried out in two time limits: before repairing the ventilation system and UV disinfection of the room and after. The results of these investigations were supposed to provide information on changes in microbial quality of the indoor air in the room, which was sterilised with UV radiation. Results of measurements are presented in Table 3, separately for each tested point due to the very specific arrangement of objects in the room and complexity of the research task. The obtained values are expressed as colony-forming units (CFU) per 1 m^3 of air. They were calculated based on the number of colonies growing on the broths, but:

– for samples collected with sedimentation, the counts were converted by Omeliański's equation, modified by Gogoberidze (PN-Z-04111-02:1989 Ochrona…, PN-Z-04111-03:1989 Oznaczanie…, PN-EN 14583:2008 Powietrze…, PN-EN 13098:2007 Powietrze…), so the obtained results were expressed in CFU/m^3 of air;

– for samples collected with aspiration, the counts were recounted in accordance with conversion table, which is a step in the proper interpretation of results obtained with an MAS 100 Eco sampler. After they were recounted, they were multiplied by a factor of 10, so the final results were shown in CFU/m^3 of air.

Results for the presence of cellulolytic fungi test, which was carried out on Czapek-Dox medium with Whatman filter paper, were expressed as the number of mould colonies growing on the filter paper.

The microclimate parameters present when the measurements were taken were:

– for investigations before disinfection: temperature 21.6 °C, relative humidity 36.7%,

– for investigations after disinfection: temperature 22.1 °C, relative humidity 36.7%.

3.2.2. Comparison of Results Obtained from Two Methods of Sampling

As mentioned above, one of the research tasks accomplished at the analysis of microbial quality of indoor air in the Paper and Leather Conservation Workshop was to compare two methods of sampling: Koch's sedimentation method and aspirating sampling method, with MAS 100 Eco air sampler by Merck. The results obtained for the samples taken when using both methods – Koch's sedimentation method and aspirating sampling method, with MAS 100 Eco air sampler by Merck – in four various examined points, before and after repairing and UV radiating, are presented in Table 3, respectively. Following an initial data analysis, it may be established that the results obtained for sedimentation method, regardless of the type of the medium used, i.e. TSA, MEA, DG18 or Cz-D with filter paper, are non-homogenous and characterised by large variability in values. Moreover, the room topography and how the windows and doorways and vents were arranged had a noticeable impact on the results obtained for this method. This is confirmed by interpretation of the values obtained for air samples taken under the fume cupboard, where microbiologically contaminated objects were stored.

For the samples collected with sedimentation method, the determined bacterial concentration (growth on TSA medium, Table 3) was only 64 CFU/m^3 of air, while measurements carried out with the air sampler provided almost 20 times higher results, i.e. 1260 CFU/m^3, which is more probable if the presence of contaminated objects under the fume cupboard (see also Table 3) is taken into consideration. Similarly, a noticeable difference was observed for concentrations of fungi in the air under the fume cupboard (cultures on MEA and DG18 media, Table 3).

For the samples taken with aspiration, the number of fungal colonies on broth was uncountable due to the high intensity growth, while for sedimentation method fungal concentration determined in air under the fume cupboard was 446 CFU/m^3 (MEA) or 2420 CFU/m^3 (DG18). It should be noted that the results of sedimentation are markedly underestimated, while there is a five-fold difference in the results obtained for both media (see also Table 3). At the same time, there is almost no difference in the results obtained for the growth on MEA and DG18 media, when aspiration method was used, e.g. for samples taken near the window. The results obtained for sedimentation in the fume cupboard were also underestimated, and unforeseeably variable, due perhaps to the fact that even when the ventilators were off, a current of air remained present under the fume cupboard: room → vent that clearly, though it randomly affected the bioaerosol sedimentation onto the broths. Likewise, all vertical air movement in the room

Table 3. Microbial Quality of Indoor Air in the Paper and Leather Conservation Workshop before and after Preventive Actions Carried out in the Workshop

Sampling Place	Sampling Method	Medium Type							
		TSA		MEA		DG18		Cz-D	
		B	A	B	A	B	A	B	A
Near the door	aspiration	920	440	340	180	430	130	3***	–**
	sedimentation	382	318	127	–**	1082	64	–**	2***
Under the fume cupboard (contaminated objects present)	aspiration	1260	340	homogenous growth*	280	homogenous growth*	340	1***	–
	sedimentation	64	127	446	64	2420	64	–**	–
Centre	aspiration	650	480	450	160	400	260	1***	3***
	sedimentation	318	318	127	191	318	–**	–**	–
Near the window	aspiration	880	430	350	230	440	270	1***	–
	sedimentation	191	255	309	64	64	64	–**	–

B – before preventive actions carried out in workshop; A – after preventive actions carried out in workshop. The concentrations of microorganisms in indoor air presented in the table are expressed in CFU/m^3, except for Cz-D medium results.

* homogenous growth – means that following 24 hours of incubation there were at least 453 colonies growing on the broth – too many to be counted with the unaided eye.

** no growth of microorganism colonies was detected based on observation with the unaided eye.

*** number of cellulolytic fungi colonies that grew on Whatman filter paper.

Source: the authors' own studies.

resulting from temperature differences, or horizontal movements resulting from people moving or opening windows or doors, can have a significant impact on the results of an air microbial quality investigation done with sedimentation. This is confirmed by the results obtained for the concentration of airborne microorganism measurements from both laboratory measurement methods (sedimentation and aspiration), both before and after corrective measures were taken at the sampling point: near a window (see Table 3), where heaters were used, which forced the intense vertical air movements up. For both the bacteria concentration (TSA medium) and the fungi concentration (MEA and DG18) tests carried out at this test point (before and after disinfection), the evidently underestimated

number of microorganisms in air was measured for sedimentation sampling method, probably due to the vertical movement of the air in this location, which makes it impossible to catch all of the spores using sedimentation (see photos in Chapter 5). This means that the sedimentation technique of air sampling used for the microbial quality examination of air can be imprecise, affecting the results of both quantitative and qualitative analysis.

These observations concerning sedimentation are in line with the data from the literature, where it is emphasised that results obtained for this method are poorly correlated with results obtained with more accurate quantitative methods (e.g. using a sampler) (Radmore & Luck 1984). Moreover, some researchers regard sedimentation to be totally non-quantitative (Buttner, Willeke & Grinspun 1997), since results are related to the size and shape of bioparticles and air movements (Nevelainen *et al.* 1993), and therefore the air volume from which microorganisms sediment is never known.

These arguments, and the measurements we carried out, allowed us to establish that the results obtained for sedimentation are rather estimative in terms of quantity, and consequently quality, and, unlike aspiration, will not enable reliable results to be obtained (Sayer, MacKnight & Wilson 1972, Sayer, Shean & Ghosseiri 1969, Solomon 1975).

The above comparison of sedimentation and aspiration leads to the conclusion that the latter yields more reliable analytical results in terms of both quality and quantity. This is because the values obtained in the tests with aspiration are not determined by various factors including horizontal and vertical air movements, and the shape, size or mass of bioaerosol particles. In addition, the test volume of air is always known. Based on these arguments it was decided that the microbial quality of indoor air in the Paper and Leather Conservation Workshop of the National Museum in Cracow would be done only based on the results obtained from the sampling carried out with aspiration method. The results of the sedimentation testing were not interpreted. Studies of microbiological quality of air in other, selected locations of the National Museum in Cracow were also done only using aspiration.

3.2.3. Quantitative Analysis of the Airborne Microflora prior to Preventive Actions

The greatest amount of bacteria from the four points examined at the Paper and Leather Conservation Workshop before disinfection was found to be under the fume cupboard – up to 1260 CFU/m^3 of air. This result is more than once higher than the number of bacteria in the air, determined for the other three

points measured: 650 CFU/m^3 in the centre, 880 CFU/m^3 at the window and 920 CFU/m^3 next to the door.

Distribution of obtained bacteria concentrations inside the room could be understood when the topography and specifics of this room are taken into account:

– under the fume cupboard there were some microbiologically contaminated objects that were a significant source of microorganism emission into the air;

– over the door there were vents, and the door was opened before measurements: these two factors contributed to the induction of forced air movements that raised microorganisms up from the surfaces and floor, which led to fairly high concentrations being obtained;

– near the window there were radiators in use that heated the air and induced it to move vertically, thus leading to high bacteria concentration being measured, as they were raised up from the radiator surfaces;

– there were no heating elements, vents or contaminated objects in the centre of the room, so the lowest bacteria concentrations from among all examined points was measured here (two times lower than under the fume cupboard; sampling with aspiration method).

The calculated mean value of bacteria concentration in the Paper and Leather Conservation Workshop before disinfection, after excluding the results obtained for the fume cupboard, was 817 CFU/m^3. That is well below the acceptable bacteria concentration limit of $5 \cdot 10^3$ CFU/m^3 in the air of living quarters and offices in the forthcoming standard (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002). The amount of bacteria in the workshop air was also lower than the threshold values for libraries, museums and galleries ($2{,}5 \cdot 10^3$ CFU/m^3) provided by the Chinese standard (GB 9669-1996 Hygienic…).

The analysis of the amount of airborne fungi in the air of the workshop under examination, calculated based on the number of colonies that grew on MEA and DG18 media, allows several conclusions to be drawn. The quantity of colonies that grew on both types of broths provided for the cultivation of fungi is comparable, and the fungi concentration in the air that was calculated based on the colony counts is within the range of 340–450 CFU/m^3, except for air samples that were collected in the fume cupboard. As in the studies of bacterial concentration, here too there was a very high concentration of fungal bioaerosol in the air. It was so high that the growth of fungi on both media, as quickly as two days after incubation, was such that the colonies could not be counted. The large number of fungi in the air under the fume hood was attributable to microbiologically contaminated objects, which, again, have been a major source of microorganism emission into the environment.

Distribution of fungi concentrations determined for individual measuring points in the workshop air did not demonstrate, contrary to the results obtained with bacteria, any dependence on room topography or specifics. This is because the morphology (shape, size and weight) of the particles included in fungal bioaerosol is distinct from bacteria, hence the mechanics of their motion in air differs from that of bacteria and the presence of ventilation flow or upright air movements due to heating does not significantly affect the variability of their quantity in the room. The number of fungal colonies that grew on DG18 medium, in the case of samples taken near the door and windows (where vents are present or radiators are used, factors that reduce humidity) was slightly higher than the number of colonies that grew on the MEA medium. This confirms that the selection of the microbial media for the investigations was accurate, since, according to the specification, DG18 medium is intended for use with xerophilic fungi.

The average value of the fungi concentration in the workshop air examined (with the exception of the fume cupboard) was 402 CFU/m^3 of air. According to recommendations found in the literature (Miller 1994):

– the air in the room should not contain any pathogenic or toxic fungi, such as *Aspergillus fumigatus* and *Stachybotrys chartarum*; and

– the presence of more than 50 CFU of one fungal species per 1 m^3 of air indicates worsening air quality and suggests the need for further aerobiological tests to be carried out in the given room;

– it is acceptable that in 1 m^3 of air up to 150 CFU is present if there is a mixture of several species of fungi;

– it is acceptable that in 1 m^3 of air up to 500 CFU is present if fungi occurring in the air belong to *Cladosporium*, *Alternaria* or other species typical of atmospheric airmicroflora.

According to the guidelines developed by the Italian Ministry of Heritage and Cultural Activity, the acceptable limit for fungi concentration in museum air may not exceed 150 CFU/m^3 (*Atto di indirizzo…* 2000). Unfortunately, the value obtained – 402 CFU/m^3 – does not meet the assumed requirements. However, if one considers the value of $5 \cdot 10^3$ CFU/m^3, which is to be the acceptable limit for fungi concentrations in air of living spaces and offices proposed in the standard being developed in Poland (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002), then fungi concentrations in the workshop air we examined was much lower than the acceptable limit (note, however, that this standard is intended to protect the health of staff, not historical objects).

In terms of the growth of fungi on Cz-D media with Whatman filter paper, three cellulolytic fungal colonies were observed for the air sample that was taken near the door, while for the samples from three other measuring points one colony in each sample was obtained. The level of air contamination with cellulolytic

fungal species determined in the workshop is not high and in fact corresponds to the level present in the outdoor air. This may function as a warning signal to staff, since mostly cellulose-containing objects are stored in this particular workshop.

3.2.4. Quantitative Analysis of the Airborne Microflora Following Preventive Measures

The highest concentrations of bacteria taken in four measurement points in the Paper and Leather Conservation Workshop after the ventilation system underwent maintenance and room disinfection with UV radiation was measured in the centre of the room, at 480 CFU/m^3. This was 2.6 times lower than the highest bacteria concentration found in the air in this workshop before disinfection, for the samples taken under the fume cupboard (1260 CFU/m^3). These results show that the maintenance performed and the air disinfection procedure were effective, as the bacteria concentration in the air under the fume cupboard was reduced considerably, down to 340 CFU/m^3. A similar trend was observed for the other measuring points (see Table 3). Average bacteria concentration in the workshop air after disinfection, excluding the results obtained for the fume cupboard, is up to 450 CFU/m^3 – almost two times lower than the average value of bacteria concentration in the room before disinfection, calculated with the same method.

It should also be noted that repairing of the ventilation system and radiating the workshop with UV not only reduced the amount of bacteria in the air, but also equalised their concentration at the individual examined points (standard deviation SD = 26 CFU/m^3). Therefore the determined concentrations do not show such strong variability between the individual examined points, as observed for the results obtained before disinfection (when there was a strong connection between the topography and characteristics of the room, and consequently the standard deviation was as much as SD = 147 CFU/m^3).

The obtained average number of bacteria in the air of the workshop, after maintenance procedures, was below $5 \cdot 10^3$ CFU/m^3, which is to be the acceptable level of bacterial concentration in the air of living spaces and offices, as per for the forthcoming standard (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002). In addition, it is also lower than that called for in the guidelines developed by the Italian Ministry of Heritage and Cultural Activity, according to which the acceptable limit for bacterial concentration in museum air may not exceed 750 CFU/m^3 (*Atto di indirizzo*… 2000).

The concentrations of fungi in the air measured after the maintenance to the ventilation system and room disinfection were carried out, at each of the individual evaluating points (determined based on the count of colonies grown on

MEA and DG18 media) fell considerably. The highest concentration of fungi was measured in the air under the fume cupboard (280 CFU/m^3 for MEA media and 340 CFU/m^3 for DG18). However, these values are still considerably lower than the results obtained before disinfection, when the amount of fungi colonies on broths was uncountable due to the extent of their growth.

Analysis of fungi amounts in the air that were measured for other evaluation points also allows us to conclude that preventive measures taken in the workshop resulted in a considerable reduction of fungi concentrations, from about 340–450 CFU/m^3 before disinfection to 130–270 CFU/m^3 of air following it, with the average value of fungi concentration in the workshop air (excluding the fume cupboard) being 205 ± 57 CFU/m^3 of air. This figure is nearly twice lower than the one obtained for air in the same workshop before disinfection, i.e. 402 ± 47 CFU/m^3.

Distribution of the fungi concentration in the air after disinfection, with the highest value determined under the fume cupboard, differs from the results obtained for bacteria, for which the lowest count was observed under the fume cupboard, and the highest in the centre of the room. A considerably reduced, but still quite high quantity of fungi in the air under the fume cupboard results from the permanent storage of microbiologically contaminated objects there; they may constantly emit microorganisms into the environment.

Although the determined average value of fungi concentration in the workshop air after preventive actions was lower than the data obtained before, it still failed to meet the requirements for indoor air quality specified in the literature (Miller 1994). It is also higher than the values presented in the guidelines developed by the Italian Ministry of Heritage and Cultural Activity, according to which the acceptable limit for fungi concentration in museum air should not exceed 150 CFU/m^3 (*Atto di indirizzo…* 2000). On the other hand, considering the value of $5 \cdot 10^3$ CFU/m^3, which will be the acceptable limit of fungi count in air of living spaces and offices in the standard being under development for a long time in Poland (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002), it can be agreed that a fungi concentration in air of the Paper and Leather Conservation Workshop is much lower than the acceptable level.

It should be noted, however, that the value proposed in Poland's standard (which has not yet come into force) seems to be overestimated compared to the standards and guidelines that are applied throughout the world (CEC 1993). Therefore monitoring of fungi concentration in air in the workshop should be continued and further corrective actions should be taken to ensure the fungi quantities in the air are reduced.

Observations of fungal colony growth on Cz-D medium with Whatman filter paper, for which air samples were taken after disinfection of the workshop,

showed that the amount of fungi fell. Three colonies of cellulolytic fungi were detected for one evaluation point only – the centre. This is a much lower number than was present before disinfection, where three cellulolytic fungi colonies were found in the air samples taken near the door, while one colony was found for samples taken in another three points as well.

3.2.5. Qualitative Study of Fungi Microflora

Establishing the fungi composition in the museum's indoor air was an especially important step of the project because the species of mould identified in the museum facilities were subsequently used for inoculation on samples modelled after historical objects (including cellulose, wool, silk, parchment), and an analysis of MVOCs emitted by moulds growing during these experiments was carried out.

The qualitative analysis of fungi growing on broths, after their sampling from indoor air in the Paper and Leather Conservation Workshop, was carried out when the pure fungal cultures were obtained by isolating species from the colonies that were growing on MEA, DG18 and Cz-D with Whatman filter paper, between the 7th and 9th days of incubation (Flannigan 2001, Campbell, Johnson & Warnock 2013, Silva *et al.* 2011). Among the species detected, representatives of the following genres predominate: *Aspergillus* (4 species), including: *Aspergillus niger* (see Photos 1–3, 5, 6 in Chapter 5), *Aspergillus fumigatus* (see Photos 1, 3, 5 in Chapter 5), *Aspergillus flavus*, *Aspergillus versicolor*, and *Penicillium: Penicillium brevicompactum*, *Penicillium funiculosum*, *Penicilium chrysogenum*. *Paecilomyces variotii* and *Alternaria alternata* were also identified. On Cz-D media with Whatman filter paper, these cellulolytic species were detected: *Chaetomium globosum* (see Photo 7 in Chapter 5) and *Trichoderma viride* (see Photo 12 in Chapter 5) fungi.

3.2.6. Evaluating the Effectiveness of Preventive Measures Taken in the Workshop

Comparison of the results of microbial indoor air quality analysis carried out in the Paper and Leather Conservation Workshop before and after preventive actions (see Table 3), together with the details discussed in the above sections, indicate that maintenance of the ventilation system and disinfection with UV radiation resulted in:

– a nearly two-fold reduction in the fungi concentration in the workshop air (from 402 to 205 CFU/m^3 of air);

– a considerable reduction in the fungi concentration in air under the fume cupboard (where infected objects are usually stored). The fall went from a level at which fungal colonies that were developing on media were uncountable due to vigorous growth down to 340 CFU/m^3 of air;

– a nearly two-fold reduction in the bacterial concentration in the workshop air (from 817 CFU/m^3 to 450 CFU/m^3 of air);

– a significant reduction in bacterial concentration in the air under the fume cupboard (from 260 CFU/m^3 to 340 CFU/m^3 of air);

– a reduction in the amount of cellulolytic fungi incubated on Cz-D media with Whatman filter paper (from 6 colonies from all evaluation points before disinfection, to three colonies after disinfection);

– a reduction in the variability among the results obtained for all evaluation points, this phenomenon was observed for data collected before preventive actions (there was a strong correlation between measured values and topography and the characteristics of the workshop – standard deviation was as much as 147 CFU/m^3).

The obtained results demonstrate that the preventive actions taken in the workshop significantly improved the microbial indoor air quality. The concentration of bacteria in the workshop air, both before and after the measures met the requirements defined as an acceptable concentration level in various standards. The only exceptions are the results obtained for measurements done with aspiration prior to the preventive measures. These results are higher (for some evaluation points) than values recommended in the Italian guidelines (750 CFU/m^3). Note that the bacteria are not considered to be very hazardous for historic objects, since the relative humidity must be very high (close to 100%) for them to develop. A much more serious hazard for objects kept in the workshop was the quite high concentration of fungi in the air, which the preventive steps, nearly halved. Unfortunately, this still failed to achieve the requirements laid out in the literature (Miller 1994) or in the guidelines developed by the Italian Ministry of Heritage and Cultural Activity, according to which the acceptable limit of fungi concentration in museum indoor air may not exceed 150 CFU/m^3 (*Atto di indirizzo*… 2000).

3.3. The Pastel Paintings Storeroom at the Main Building of the National Museum in Cracow

3.3.1. Quantitative Analysis of Airborne Microflora

The microbial quality of the indoor air in the two Pastels Storerooms (I and II, see Table 1), located in the Main Building of the National Museum in Cracow, was

measured with aspirating method only. The results of measurements are presented in Table 4, separately for each of the investigated rooms, mostly because their interior volumes were different and therefore a different quantity of evaluation points had to be selected in each of them. The obtained values are arithmetic averages of microbe concentrations in air and they are expressed as CFU per 1 m^3 of air. These values were calculated in a similar way as in Section 3.2.

With the examination of the indoor air for the presence of cellulolytic fungi, which was carried out on Czapek-Dox medium with Whatman filter paper, the results were expressed as the number of mould colonies that grew on the filter paper.

The microclimate parameters during measurements were:

– Storeroom I investigation: temperature 21.9°C, relative humidity 46.9%,
– Storeroom II investigation: temperature 22.2°C, relative humidity 48.3%.

Table 4. Microbial Quality of Air in Pastels Storerooms I and II

Sampling Place	Sampling Method	Medium Type			
		TSA	MEA	DG18	Cz-D
Storeroom I	aspiration	440 ± 150	30 ± 8	30 ± 14	1***
Storeroom II	aspiration	270 ± 78	45 ± 7	40 ± 14	2***

The concentration of microorganisms in indoor air presented in the table is expressed in CFU/m^3, except for Cz-D medium results.
*** number of cellulolytic fungi colonies that grew on Whatman filter paper.

Source: the authors' own studies.

The analysis of the data summarised in Table 4 demonstrates that the concentrations of bacteria in the air of both repositories is low, reaching 440 CFU/m^3 and 270 CFU/m^3, respectively. These values were determined based on the number of bacteria colonies growing on the TSA broths. They were calculated as the average value of the results obtained for 8 (Storeroom I) and 11 (Storeroom II) evaluation points. The measured values are much lower than the concentrations determined in indoor air of the Paper and Leather Conservation Workshop (see Table 3). Note that the concentration of bacteria determined in the air of both repositories is also considerably lower than the acceptable levels of concentrations presented in the Polish, Chinese and Italian guidelines (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002, GB 9669-1996 Hygienic…, *Atto di indirizzo*… 2000).

The concentration of fungi in the indoor air of both storerooms presented in Table 4 was determined based on observations of mould growth on MEA and DG18 broths, after sampling of the air in the same evaluation points for bacteria. As with the bacteria, the concentrations are the mean values calculated for results

acquired from several measuring points. The average concentration of fungi in the air of a given storeroom as determined based on their growth separately for both different broths (MEA and DG18) was identical in repository I, or differed only slightly, in repository II. Comparison of the results demonstrates that there is a slightly higher concentration of fungi in the air of Storeroom II. This may be because in this storeroom, unlike in Storeroom I, a larger number of objects is kept and they are a potential source of microorganism emission. The designated concentrations of airborne fungi in both repositories are far below the limit values given in the literature, i.e. 150 CFU/m^3 of air (Miller 1994, *Atto di indirizzo…* 2000). They are also almost five times lower than the concentrations of fungi measured in the air of the Paper and Leather Conservation Workshop after disinfection.

Based on observations of growth of fungi colonies on Cz-D medium with Whatman filter paper, for air samples that were taken in repository I only one cellulolytic fungus colony was found, while for the samples from repository II two colonies were cultivated. The level of indoor air contamination by cellulolytic species of fungi was not high, corresponding to the level in the outdoor air, but the presence of these fungi in the storeroom air should be monitored, as it is mostly cellulose-containing objects that are kept there.

3.3.2. Qualitative Study of Fungi Microflora

To qualitatively analyse fungi that grew on the broths, after the air was sampled on their surface with aspiration in Storerooms I and II, the pure fungal cultures were isolated between the 7th and 9th days of incubation, from numerous colonies that developed on MEA, DG18 and Cz-D media with Whatman filter paper. Representatives of the following genuses predominated: *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus versicolor*, and *Penicillium brevicompactum*, *Penicillium funiculosum*. Representatives of *Alternaria alternata* were also present. *Aspergillus fumigtus*, a cellulolytic species, were detected on Cz-D media with Whatman filter paper as well. The composition of airborne fungi found in Pastel Storerooms I and II was much more limited when compared to the composition identified in the Paper and Leather Conservation Workshop.

3.4. The External Storerooms of the National Museum in Cracow in the Royal Castle at Niepołomice

3.4.1. Quantitative Analysis of the Airborne Microflora

The investigations of microbial quality of indoor air in two external Storerooms (I and II, see Table 1) in the Royal Castle at Niepołomice were done with aspirating method, as explained in Section 3.2.2. The results of the analysis are presented in Table 5, separately for each of the examined storerooms, due to their different volumes and differences in their characteristics (e.g. only in Storeroom II are there windows), different characteristics of the objects that are stored in each and the diverse storage methods used: in Storeroom I, objects were packed in boxes, whereas and in Storeroom II they were hung on frames. Note that between Storerooms I and II there is a metal framework built into the Storeroom I floor (1/3 of the floor area) and the entire ceiling of Storeroom II. It is covered with OSB boards (Oriented Strand Board) that only partly cover the framework surface and therefore do not limit the air exchange between the storerooms.

The values obtained are arithmetic averages of the microbe concentrations in the air and they are expressed as CFU per 1 m^3 of air. Calculations were carried out as described in the previous sections.

Results of air tests for the presence of cellulolytic fungi were expressed as the number of mould colonies that grew on the filter paper on Czapek-Dox medium.

The microclimate parameters during measurements were:

– Storeroom I: temperature 24.0°C, relative humidity 52.5%,
– Storeroom II: temperature 24.0°C, relative humidity 48.8%.

Table 5. Microbial Quality of Air in External Storerooms I and II in the Royal Castle at Niepołomice

Sampling Place	Sampling Method	Medium Type			
		TSA	MEA	DG18	Cz-D
External Storeroom I	aspiration	435 ± 22	60 ± 56	45 ± 49	1***
External Storeroom II	aspiration	550 ± 38	70 ± 28	20 ± 14	–**

The concentration of microorganisms in indoor air presented in the table is expressed in CFU/m^3, except for Cz-D medium results.

** no growth of microorganism colonies was detected based on observation with the unaided eye.

*** number of cellulolytic fungi colonies that grew on Whatman filter paper.

Source: the authors' own studies.

The data presented in Table 5 demonstrate that the concentrations of bacteria (growth on TSA media) in the indoor air of both storerooms are elevated, but both values, i.e. 435 CFU/m^3 (Storeroom I) and 550 CFU/m^3 (Storeroom II), are well below the acceptable limits presented in the literature (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002, GB 9669-1996 Hygienic…, *Atto di indirizzo*… 2000). Moreover, the concentrations of airborne fungi determined for both storerooms did not exceed the acceptable value of 150 CFU/m^3 as specified in various guidelines (Miller 1994, *Atto di indirizzo*… 2000). The values obtained for Storeroom I are fairly scattered – that is, there is a high standard deviation.

This may be because in one part of this storeroom the indoor air is mixed, through the frameworks, with the air of the Storeroom II, whereas in the second part of Storeroom I such an exchange does not occur. Hence the values determined for each specific test point in Storeroom I are characterised by high variability. Comparing the amount of fungi in the air, determined on the basis of their growth on MEA and DG18 broths, leads to the conclusion that in the air of both storerooms there is a reduced amount of xerophilic fungi (growth on the substrate DG18) – a reasonable occurrence given the quite high relative humidity measured in both areas.

Results of air investigations for the presence of cellulolytic fungi that were carried out in both storerooms with the application of Cz-D medium with Whatman filter paper, demonstrate that there is a very low concentration of these fungi in the air examined there. In the samples taken in various investigation points, in both storerooms, only one colony of cellulolytic fungus was detected – *Eurotium*, which is xerophilic. This species can grow on cellulose-containing materials as well as on textiles, leather and furniture surfaces. Its spores can also be found in dust particles, among other places. Although the amount of cellulolytic fungi determined in the air of both rooms was low, monitoring the presence of these fungi in is recommended, since mostly cellulose-containing objects are stored there.

3.4.2. Qualitative Study of Fungi Microflora

As with tests carried out in other places, among the pure fungal cultures isolated from MEA, DG18 media and Cz-D with Whatman filter paper, representatives of the following species predominate: *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium brevicompactum*, *Penicillium funiculosum*, *Penicilium chrysogenum* (see Photo 10 in Chapter 5). The presence of a representative of the following genuses was also observed: *Eurotium chevalleri* (growth on Cz-D broth with Whatman filter paper, see Photo 9 in Chapter 5) and *Aureobasidium pullulans*. The composition of airborne fungal microflora

detected in both storerooms is much more limited compared to the composition determined in the air of Paper and Leather Conservation Workshop or in the Pastel Painting Storerooms.

3.5. Collection Storerooms of the National Museum at the Princes Czartoryski Museum in Cracow

3.5.1. Aims and Objectives

Microbial indoor air quality in the storeroom of the National Museum's collection located in the armoury building of the Princes Czartoryski Museum, and in two other rooms (eastern and western), the Curiosity Storerooms in this museum (see also Table 1), was analysed, in accordance with the previous assumptions, using aspirating method. Table 6 presents the results of the measurements, individually for each of the examined storerooms, each of which had a different interior volume and various specifications. For example, the storeroom for collections is located beneath ground level and there is a lack of active window openings. The Curiosity Storerooms, on the other hand, are located on the first floor and there are windows in use and different characteristics of the objects stored there.

Additionally, in the case of the collections storeroom, which is located in the cellars of the arsenal building, the concentrations of microorganisms in the air were expressed individually for each of the three investigation points that were positioned in a straight line along the axis: inoperative window opening (the western wall) → centre → door area. This was done because, in this storeroom, on the western wall, where there are inoperative window openings, water infiltration from outside was observed, having become evident by the salt efflorescence on the wall. To precisely specify the level of microorganisms in the air at this place and their possible propagation towards the interior of the room, the results obtained for each of the three examined points were presented and interpreted independently (Table 6). We would point out that National Museum staff, fearing the microbial contamination by salt efflorescence of the objects stored near the wall in this storeroom, continuously sterilised the air using a lamp emitting UV radiation during working hours.

The arithmetically obtained average values of airborne microbe concentrations are expressed, as previously, in CFU per 1 m^3 of air. These values were calculated in an identical manner to the measurements discussed above.

In the case of indoor air tests for the presence of cellulolytic fungi, which were carried out on Czapek-Dox broth with Whatman filter paper, the results were expressed, as in other cases, as the number of mould colonies that grew on the filter paper.

Table 6. Microbial Quality of the Air in the National Museum Storeroom Located in the Arsenal Building of the Princes Czartoryski Museum and in the Curiosity Storerooms

Sampling Place	Sampling Method	Medium Type			
		TSA	MEA	DG18	Cz-D
National Museum storeroom armoury building – western wall	aspiration	20 ± 7	10 ± 8	–**	–**
National Museum storeroom armoury building – centre	aspiration	50 ± 6	10 ± 7	–**	–**
National Museum storeroom armoury building – door	aspiration	60 ± 3	10 ± 7	20 ± 4	1***
The Curiosity Storeroom – western room	aspiration	40 ± 7	NA****	40 ± 2	1***
The Curiosity Storeroom – eastern room	aspiration	200 ± 9	150 ± 17	–**	4***

The concentration of microorganisms in indoor air presented in the table is expressed in CFU/m^3, except for Cz-D medium results.
** no growth of microorganism colonies was detected based on observation with the unaided eye.
*** number of cellulolytic fungi colonies that grew on Whatman filter paper.
**** NA – the medium was destroyed, rendering impossible the incubation and counting of colonies.

Source: the authors' own work.

The microclimate parameters during measurements were:

a) in the National Museum storeroom: temperature 19.6 °C, relative humidity 39.6%,

b) in the Curiosity Storerooms:

– western room: temperature 21.0 °C, relative humidity – NA,
– eastern room: temperature 21.0 °C, relative humidity – NA.

3.5.2. The National Museum's Storeroom in the Arsenal Building

Quantitative Analysis of the Airborne Microflora

Based on the results of the quantitative analysis of bacterial growth on TSA broth, which are presented in Table 6, it can be concluded that the amount of bacteria in the indoor air of the National Museum's storeroom located in the arsenal building is very low – the lowest, in fact, from among all the rooms examined. Furthermore, distribution of the bacteria concentrations in this warehouse make it possible to indicate that the lowest amount of bacteria was identified in the air near the western wall, while a slightly higher level occurred in the centre and the highest, but still very low, near the door. The concentration of bacteria measured at this point, reaching 60 CFU/m^3, is 12 times lower than the level laid out in the most stringent guidelines defining acceptable levels of bacteria concentration in museum air, which is 750 CFU/m^3 (*Atto di indirizzo*… 2000).

Analysis of the results of fungi concentration measurements carried out in the arsenal building storeroom (growth on MEA and DG18 broth) leads to conclusions similar to those for bacteria concerning the concentration levels and their distribution in the room. The concentrations of fungi in the air of the room, which were determined based on the analysis of growth of these microorganisms on MEA broth, are low and attain the same value in all of the three examined points in the warehouse, i.e. 10 CFU/m^3. This is the lowest amount of fungi measured in the air among all the rooms surveyed. In contrast, based on analysis of the concentration of airborne fungi in the storeroom, determined on the basis of their growth on DG18 media, it can be concluded that in two investigation points – near the wall and in the centre – no xerophilic fungi capable of growing on this medium were detected. Close to the door, the concentration reached 20 CFU/m^3. The obtained values of airborne fungi concentrations in the examined storeroom are well below the value provided in both the standards (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002) and guidelines (Miller 1994, *Atto di indirizzo*… 2000).

Analysis of the growth of cellulolytic fungi on Cz-D broth with Whatman filter paper shows that these moulds are distributed in the indoor air of the arsenal building storeroom to an extent similar to other microorganisms, i.e. no presence of cellulolytic fungi was observed near the wall or in the centre, while for the sample taken near the door only one colony grew on the medium.

The qualitative analysis of microbial measurements carried out in the this storeroom demonstrate that, from among all of the National Museum rooms examined, the lowest level of microorganisms' concentration – both bacterial

and fungal – occurred here, and are well below the critical limit values specified in standards or guidelines (Miller 1994, Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002, GB 9669-1996 Hygienic…, *Atto di indirizzo*… 2000). This means that preventive actions taken by museum employees to disinfect the air with UV radiation are efficacious.

Indeed, there was concern that the infiltration of water through the wall can increase the relative humidity of the air, thus providing favourable conditions for microorganism growth. However, the systematic monitoring and control of relative humidity in the warehouse as well as the use of UV lamps effectively eliminated the risk. The concentration of microorganisms in the air of the storeroom slightly increased proportionally with the distance of the UV lamp from the area it is meant to correct (western wall → centre → door), further indicating its efficaciousness. Finally, the UV lamp was similarly effective in reducing microorganism concentration in tests done in the Paper and Leather Conservation Workshop (Section 3.2.4).

Qualitative Study of Fungi Microflora

As with the measurements carried out elsewhere, among pure fungal cultures that were isolated from MEA, DG18 broth and Cz-D with Whatman filter paper, representatives of the following species predominated: *Aspergillus niger* (see Photo 11 in Chapter 5), *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium brevicompactum*, *Penicillium funiculosum*, *Penicilium chrysogenum* (see Photos 11 and 13 in Chapter 5). However, *Cladosporium herbarum*, *Myrotecium verrucaria*, *Stachybotrys chatarum* were also identified.

Analysis of Microbial Contamination on Surfaces of Selected Objects Stored in the National Museum Warehouse in the Arsenal Building of the Princes Czartoryski Museum

Analysis of surface microbial contamination was carried out for a few selected objects (paintings) stored in the arsenal building. The surfaces of paintings were sampled with swab and contact methods. With swab method, the investigated surface was gently rubbed with a swab soaked in sterile water. The microorganisms were then extracted from the object onto the cotton swab. The contact method involves placing a ready-made microbial broth on a sampled surface for 10 seconds. In these analyses two types of ready-made medium Rodac ConTact Test (BTL, Łódź) were applied: the first to determine the total number of bacteria and the second type to determine the total number of yeasts and moulds (BTL, Łódź). Both types of medium contained neutralisers, which inhibit the influence of biostatic or biocidal substances (which may occur on the investigated surfaces) on the growth of microorganisms during incubation.

The incubation of microorganisms sampled directly form the surfaces on the ready-made broths was carried out in incubators at a temperature of 30 ± 2 °C and relative humidity of 60 ± 5%. The microorganisms sampled onto the swabs were inoculated on sterilised microbiological mediums by rubbing their surface with the swabs (surface inoculation). As with microbial air quality investigations, the TSA, MEA and DG18 broths were used for the cultivation (see Section 2.3). The methodology for cultivating, analysing microorganism growth, isolating the pure cultures and identifying the microorganisms was exactly the same as was done to analyse the microbial air quality.

The quantitative analysis of results obtained after incubation of the TSA, MEA and DG18 broths inoculated with swabs demonstrate that there was a small amount of active bacteria and moulds (or their spores) on the investigated surfaces. One exemption was found only for a sample taken directly from a paper sticker attached to the back of a painting with ready-made broth Rodac ConTact Test (for bacteria). In this case, after one day of incubation the broth was completely covered with one species of bacteria. The intense growth of bacteria could mean that it had a very high metabolic activity when it was sampled on Rodac broth. It can be assumed that a bacterium was decomposing organic glue that had been applied to attach paper a sticker to the painting.

The qualitative analysis of fungi growing on broths after direct sampling (Rodac broth) or inoculation with a swab was carried out once the pure fungal cultures were obtained by isolating species from the colonies growing on the MEA and DG18 between the 7th and 9th days of incubation (Flannigan 2001, Campbell, Johnson & Warnock 2013). The representatives of the following species were found: *Penicillum brevicompactum*, *Penicillium expansum*, *Eurotium chevalleri*, *Peacilomyces varotti*, *Scopulariopsis brevicaulis.*

3.5.3. The Curiosity Storerooms (the Western and Eastern Rooms)

Quantitative Analysis of Airborne Microflora

The test results of bacteria concentration measurements in air in the western and eastern rooms of the Curiosity Storerooms, presented in Table 6 (growth on TSA medium) indicate that the concentrations obtained did not exceed the levels determined in any standards or guidelines for indoor air quality in museums (Miller 1994, Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002, GB 9669-1996 Hygienic…, *Atto di indirizzo*… 2000). It should be noted, however, that the amount of bacteria in the air of the eastern room was elevated, i.e. 200 CFU/m^3. That figure is five times higher than the value obtained for the western room, though it still does not exceed the acceptable limits.

The quantitative analysis of results obtained for fungi measurements carried out in both rooms show a similar distribution of concentration levels between rooms, i.e. the concentration of moulds in the western room, at 40 CFU/m^3, is almost four times lower than that measured in the eastern room. The 150 CFU/m^3 measured in this room is at the limit value laid out in the guidelines (exactly 150 CFU/m^3) (Miller 1994, *Atto di indirizzo…* 2000), but is lower than the $5 \cdot 10^3$ CFU/m^3, which is to be the acceptable limit in Poland (Dutkiewicz & Mołocznik 1993, Górny & Dutkiewicz 2002).

Furthermore, the results of investigations of the presence of cellulolytic fungi in the air of both rooms, when Cz-D medium with Whatman filter paper is applied, show a larger quantity of these fungi in the eastern room. The growth of four fungal colonies was observed for the sample taken in the eastern room, while for the sample acquired in the western room only one colony was cultured.

Based on the findings described above, it may be concluded that air in the eastern room is more contaminated than the air in the western room (see Table 6), though the acceptable limits for microorganism concentrations in the air were not exceeded in either.

Qualitative Study of Fungi Microflora

Among pure fungal cultures isolated from MEA and DG18 media, representatives of the following species were found: *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium brevicompactum*, *Penicillium funiculosum*, *Penicilium chrysogenum*, *Penicillium expansum* (see Photo 14 in Chapter 5). On Cz-D medium with Whatman filter paper (air sample taken in the eastern storeroom), colonies of *Trichoderma viride* species, which has cellulolytic properties, were also identified. Although its concentration in the air of the eastern room was low, it is recommended that the indoor air quality there be monitored, as cellulose-containing objects are stored there.

4. Final Conclusions

Examination of the microbial quality of the air in all of the rooms of the National Museum in Cracow that were examined for the presence of bacteria reveals that the highest concentration of these microorganisms was measured in the Paper and Leather Conservation Workshop prior to the renovation of the ventilation system and sterilisation of the room with UV radiation. There was, on average, 817 CFU/m^3 of bacteria in the air in the workshop, exceeding the limit of 750 CFU/m^3 laid down in Italian Ministry of Heritage and Cultural Activity guidelines (*Atto di indirizzo…* 2000). However, the preventive measures taken, including sterilisation with UV light, resulted in a two-fold reduction in the bacteria concentration, so that the room reached the average level reported in other rooms, or approximately 450 CFU/m^3. At 43 CFU/m^3, the lowest amount of airborne bacteria was measured in the storeroom located in the arsenal building. This low concentration was achieved thanks to preventive actions taken by museum employees, including control and regulation of humidity in the room plus air sterilisation with UV radiation. These actions were taken in response to water infiltrating the western wall of the storeroom.

The concentration of bacteria in the storerooms located in the Royal Castle at Niepołomice was elevated but below the acceptable limit. It was expected that the concentration of microflora in the air would be lower than in storerooms in the city. The unexpectedly high amount of bacteria measured in storerooms located outside of the city may be a consequence of the quite high air exchange ratio between the rooms caused by the ventilation and heating systems. This induced the bacteria deposited on the surfaces to rise and bacteria suspended in the air to move intensively.

The highest concentration of airborne fungi concentrations was detected in the Paper and Leather Conservation Workshop, also before corrective steps were taken. The average concentration of fungal bioaerosol was 402 CFU/m^3 of air. However, for air samples taken under the fume hood, fungal growth on broth was so strong that it was impossible to count the colonies. Renovating the ventilation system and sterilising the air in the room resulted in a two-fold reduction in fungal concentration, to 205 CFU/m^3. Nonetheless, this value exceeded the acceptable limit of 150 CFU/m^3 as laid down in the literature (Miller 1994) or in

museum guidelines (*Atto di indirizzo*… 2000). However, in the Leather Conservation Workshop, microbiologically contaminated objects to be disinfected are stored under the fume cupboard.

An elevated concentration of fungi in the air, reaching the acceptable limit (150 CFU/m^3), was also detected in the eastern room of the Curiosity Storerooms. In this case, however, air sterilisation with UV radiation and the increased number of air exchanges in the room should reduce this concentration.

The average concentration of fungi in other rooms in the National Museum in Cracow was maintained at about 43 CFU/m^3 of air – roughly 3.5 times lower than the acceptable limit. As with the bacteria, the lowest amount of airborne fungi, a mere 10 CFU/m^3, was detected in the National Museum storeroom located in the arsenal building. The reasons for such low concentrations measured in this place have been discussed above, as the levels of bacteria concentrations in the air of the National Museum rooms we examined are compared.

The average concentration of fungi microflora in the storerooms in the Royal Castle at Niepołomice was lower than that found in the Paper and Leather Conservation Workshop but slightly higher than established for the storerooms located in the city. While microbial contamination in the storerooms located outside of the city was expected to be lower than in their urban counterparts, the results show otherwise, the reasons for which was described above. The composition of airborne fungal microflora detected in both storerooms in the Royal Castle at Niepołomice was much more limited when compared that for the air of the Paper and Leather Conservation Workshop or in the Pastel Paintings Storerooms.

The levels of microorganisms' concentrations measured in the air of the National Museum's rooms are safe for museum employees and visitors, since they meet the requirements of the guidelines laid out in the literature, which state that the acceptable limit of bacteria concentration in the air of residential buildings is 2000 CFU/m^3, and 300 CFU/m^3 for fungi (Krzysztofik 1992).

As was emphasised at the beginning of this work, one of the main goals of the investigations carried out in various storerooms of the National Museum in Cracow was to establish the fungi composition in the museum's indoor air. This was an especially important step in the ongoing project because the species of mould identified during measurements in the museum facilities were after that bought from the IHEM Culture Collection (Brussels, Belgium) to ensure that in further research the pure isolates of moulds are used. This is the list of moulds: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium brevicompactum*, *Penicillium funiculosum*, *Penicilium chrysogenum*, *Paecilomyces variotii*, *Alternaria alternata*, *Chaetomium globosum*, *Trichoderma viride*, *Eurotium chevalleri*, *Aureobasidium pullulans*, *Cladospo-*

rium herbarum, *Myrotecium verrucaria*, *Stachybotrys chatarum*, *Scopulariopsis brevicaulis*.

To summarise, the results of quantitative analysis of microflora enabled us to determine that, during our study, the microbial contamination in selected facilities of National Museum both in Cracow and in Niepołomice was below the accepted limits defined in the various standards. In the qualitative analysis of fungi microflora, mould strains were identified that:

– exhibit cellulolytic properties, e.g. *Trichoderma viride*, *Stachybortys chartarum*, *Myrothecium verrucaria*, *Chaetomium globosum*, *Cladosporium herbarum* (Flannigan 2001, Eriksson, Blanchette & Ander 1990, Szczepanowska & Cavaliere 2000, Arpan *et al.* 2013, Picart, Diaz & Pastor 2008);

– have the ability to grow on materials containing proteins found in parchment, silk or wool, e.g. *Alternaria alternata*, *Chaetomium globosum*, *Aspergillus niger*, *Penicillium chrysogenum*, *Aspergillus fumigatus* (Borrego *et al.* 2012, Pinzari, Cialei & Piñar 2012, Polacheck 1989, Vivar *et al.* 2013, Kraková *et al.* 2012, Mesquita *et al.* 2009, Gallo, Pasquariell & Valenti 2003, Szostak-Kotowa 2004, Abdel-Kareem & Alfaisal 2010, Muhsin & Salih 2000, Seves *et al.* 1998, Abrusci *et al.* 2005, Abdel-Kareem *et al.* 1997);

– have allergenic properties, e.g. *Alternaria alternata*, *Stachybotrys chartarum*, *Aspergillus fumigatus*, *Cladosporium herbarum* (Florian 2002, Flannigan 2001, Mullins 2001, Midgeley, Hay & Clayton 1997, Miller 1994, Pasanen *et al.* 1992).

This means that species of moulds identified in the air of the storerooms studied are dangerous both for employees and for the collections, and therefore the monitoring of fungi microflora concentration in these places should be carried out periodically, and steps taken to reduce them.

5. Examples of Microorganism Growth on Various Types of Media, after Air Sampling Carried out in Selected Facilities of the National Museum in Cracow

This chapter presents photographs of the microbial broths with the microorganisms' growth. The bacteria and fungi were sampled in the facilities studied at the National Museum in Cracow. The photos were taken by authors.

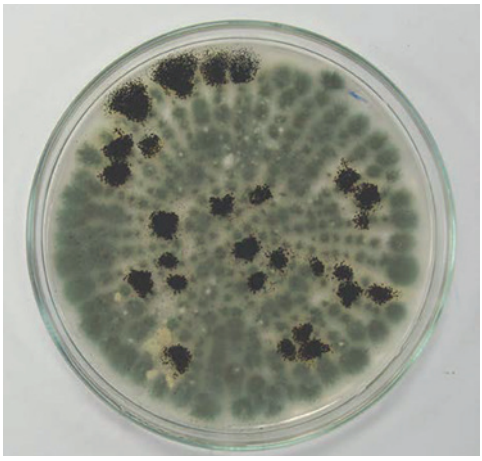

Photo 1. Microorganism growth on DG18 medium (Paper and Leather Conservation Workshop, before disinfection; aspiration method, point: under the fume cupboard)

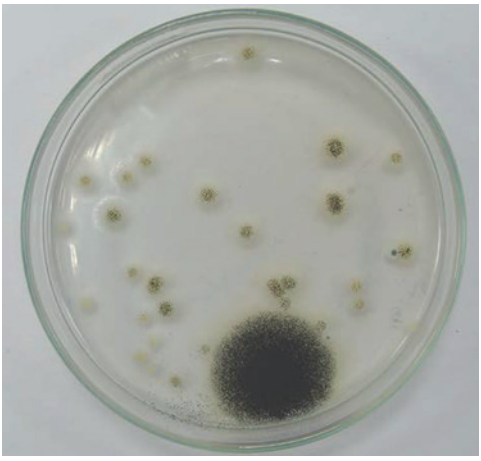

Photo 2. Microorganism growth on DG18 medium (Paper and Leather Conservation Workshop, before disinfection; sedimentation method, point: under the fume cupboard)

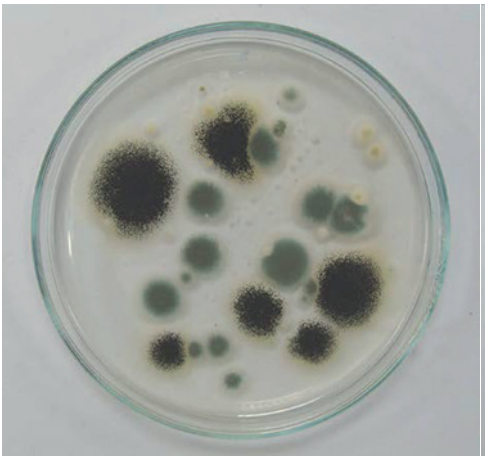

Photo 3. Microorganism growth on DG18 medium (Paper and Leather Conservation Workshop, before disinfection; aspiration method, point: centre)

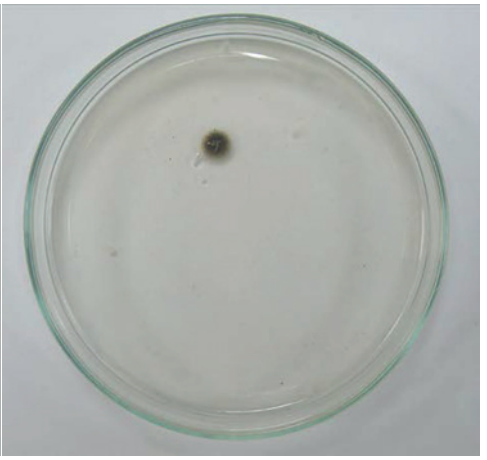

Photo 4. Microorganism growth on DG18 medium (Paper and Leather Conservation Workshop, before disinfection; sedimentation method, point: centre)

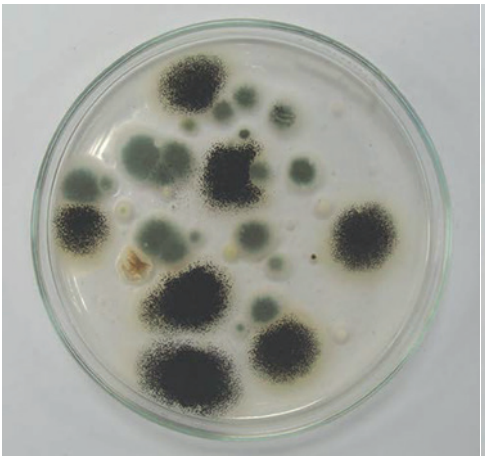

Photo 5. Microorganism growth on DG18 medium (Paper and Leather Conservation Workshop, before disinfection; aspiration method, point: door)

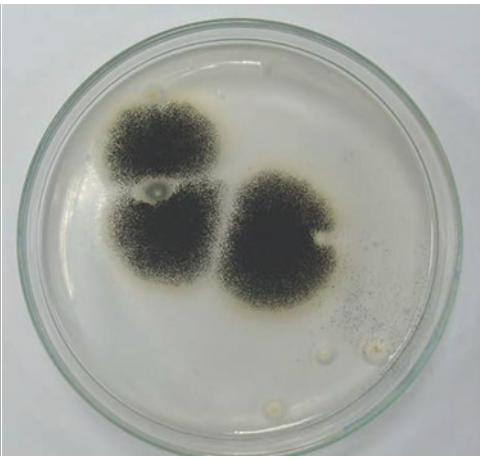

Photo 6. Microorganism growth on DG18 medium (Paper and Leather Conservation Workshop, before disinfection; sedimentation method, point: door)

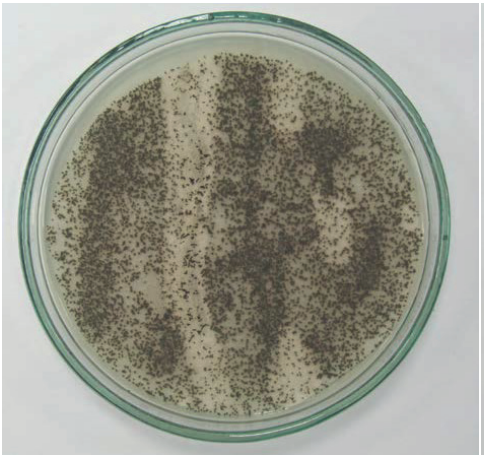

Photo 7. *Chaetomium* sp. growth on Cz-D medium with Whatman filter paper (Paper and Leather Conservation Workshop, before disinfection; sedimentation method, point: centre)

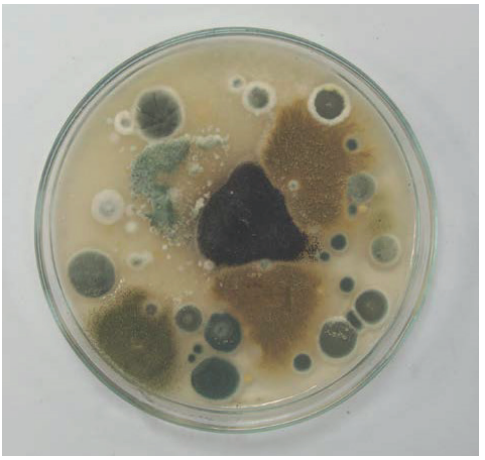

Photo 8. Intensive growth of fungi on MEA medium (Paper and Leather Conservation Workshop, before disinfection; sedimentation method, point: near the door)

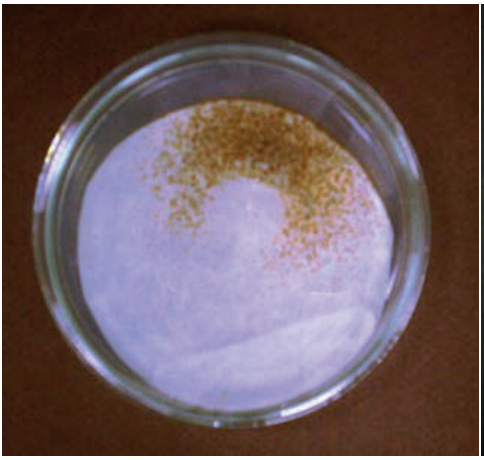

Photo 9. *Eurotium* sp. growth on Cz-D medium with Whatman filter paper (Storeroom I, Royal Castle, Niepołomice)

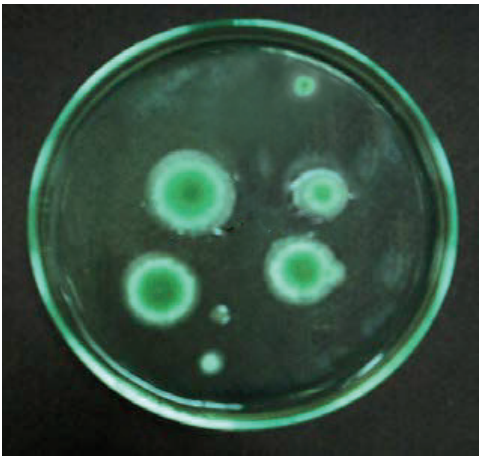

Photo 10. *Penicillium* sp. growth on DG18 medium (Storeroom I, Royal Castle, Niepołomice)

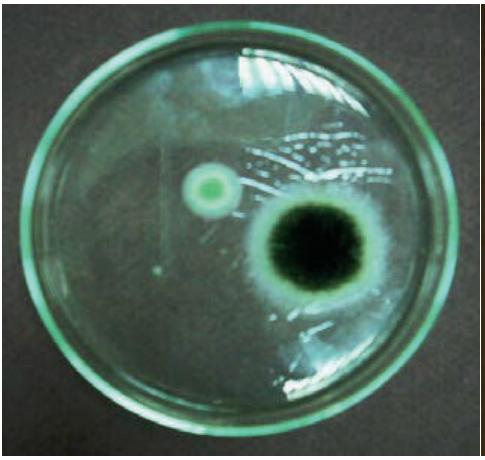

Photo 11. Growth of *Penicillium* sp. and *Aspergillus niger* on DG18 medium (National Museum storeroom in the arsenal building; point: door)

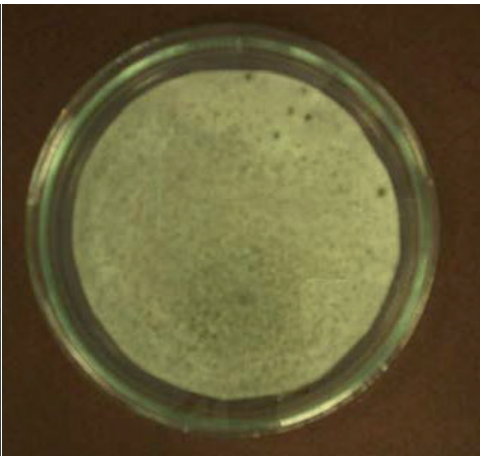

Photo 12. *Trichoderma* sp. growth on Cz-D medium with Whatman filter paper (Paper and Leather Conservation Workshop)

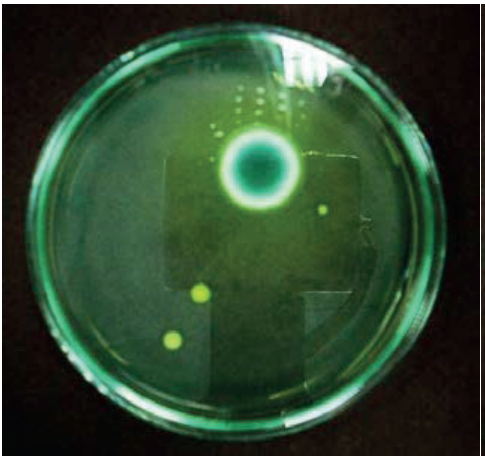

Photo 13. Growth of *Penicillium* sp. on MEA medium (National Museum storeroom in the arsenal building; point: centre)

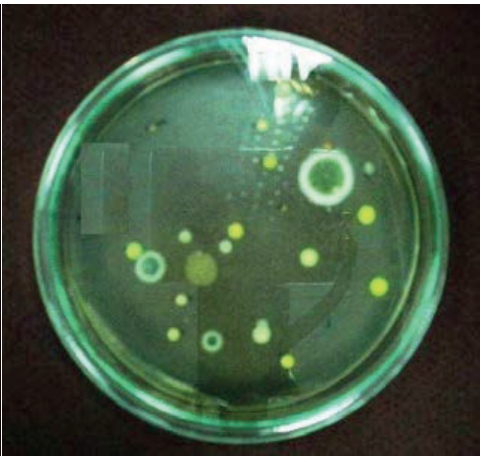

Photo 14. Bacterial and fungal growth on TSA medium (The Curiosity Storeroom, eastern room)

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