SUMMARY OF PROFESSIONAL ACHIEVEMENTS

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1. Name and surname

Joanna Karbowska-Berent

2. Awarded diplomas and academic/artistic degrees - with the indication of the name, place and year of their attainment as well as the title of the Ph.D. thesis

Ph.D. degree in biological sciences:
18.04.2003, Nicolaus Copernicus University in Toruń, Faculty of Biology and Earth Sciences, Ph.D. thesis "The Role of Actinomycetes of Genus *Streptomyces* in the Biodeterioration of Historic Parchments." Thesis promoter: Prof. Alicja B. Strzelczyk, Ph.D.

Master of Science in Biology: 29.05.1989, Nicolaus Copernicus University in Toruń, Faculty of Biology and Earth Sciences, master's thesis „Location of Ca^{2+} in the Stigma of *Pharbitis nil*". Thesis promoter: Assistant Professor Alicja Górska-Brylass, Ph.D.

3. Information on previous employment in scientific/arts institutions.

since 01.08.2012: Nicolaus Copernicus University in Toruń, Faculty of Fine Arts, Institute for the Study and Conservation of Cultural Monuments, Department of Paper and Leather Conservation, position: senior lecturer.

01.02.2000 – 31.07.2012: Nicolaus Copernicus University in Toruń, Faculty of Fine Arts, Institute for the Study and Conservation of Cultural Monuments, Department of Paper and Leather Conservation, position: assistant professor.

01.05.1994 – 31.01.2000: Nicolaus Copernicus University in Toruń, Faculty of Fine Arts, Institute for the Study and Conservation of Cultural Monuments, Department of Paper and Leather Conservation, position: assistant.

01.09.1989 – 30.04.1994: Nicolaus Copernicus University in Toruń, Faculty of Fine Arts, Institute for the Study and Conservation of Cultural Monuments, Department of Paper and Leather Conservation, position: member of engineering and technical staff.

4. Identification of an achievement pursuant to Art. 16, sec. 2 of the Act of 14 March 2003 on Academic Degrees and Academic Title as well as on Degrees and Title in Art (Journal of Laws from 2017, item 1789)

a) title of the scientific achievement

Chemical disinfection of paper-based cultural heritage items - effectiveness and threats

b) As part of this topic, I published the following monograph:

Joanna Karbowska-Berent, Chemical disinfection of paper-based historic items - effectiveness and threats, 2014, Nicolaus Copernicus University Press, Toruń, pp. 217, ISBN 978-83-231-3088-8
Publishing reviewers:
Marzena Ciechańska, Ph.D., Professor of Academy of Fine Arts in Warsaw
Beata Gutarowska, Ph.D., Professor of Lodz University of Technology
IF - not available
Scoring of the Ministry of Science and Higher Education - 20.

c) Analysis of the scientific purpose of the above listed works and the results achieved, together with an analysis of their possible use

Introduction

Biodeterioration of paper

Paper can become the environment for the development of microorganisms at every stage of its existence - both at the production stage (Zyska and Żakowska, 2005, Gutarowska and Cichocka, 2009), as well as during the storage of finished paper-based products, including cultural heritage objects, such as old books in libraries and museums, archival hand-written or printed documents, paintings (watercolors, pastels, graphics, drawings, sketches), as well as newspapers and magazines, posters, postings, old photographs taken in old techniques, postcards from previous years, maps, architectural projects, banknotes, leaflets or advertising materials (Kowalik, 1980, Nyuksha, 1984). It was found that paper-based objects became subject to the attack of microorganisms if the paper's moisture content amounts to 8-10% of water or more (Nyuksha, 1979). Exceeding this limit of water content in paper may result from storing objects in rooms that are damp, poorly ventilated or poorly heated in the autumn and winter seasons, where relative humidity

amounts to over 60-70%, as well as from breakdowns of water-sewage or air-conditioning installations, or from natural disasters, especially floods.

Over 300 species of filamentous fungi, including *Chaetomium globosum*, *Trichoderma viride*, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Aspergillus niger*, *Stachybotrys atra*, *Trichoderma koningii* and *Chaetomium elatum*, as well as Basidiomycetes and bacteria (Zyska, 1997, Strzelczyk and Leźnicka, 1981), were detected through culturing methods on papers infected with microorganisms. Microorganisms cause the biodeterioration of materials that make up cultural heritage objects, and can also have a detrimental effect on human health. They degrade paper components, such as protein adhesives, starch, cellulose, binders or organic impurities by means of enzymes secreted outside the cells and such components become a source of carbon, nitrogen, other elements and energy. Furthermore, microorganisms secrete organic acids, pigments and other compounds into the paper substrate and their hyphae grow through the cards. The effects of paper biodeterioration are visible in the form of its weakening, brittleness or thinning, up to and including the occurrence of significant losses. In addition to structural changes, the characteristic symptoms of the growth of mould fungi in paper are stains caused by the release of pigments by fungal hyphae or the production of countless amounts of colourful spores - green, black, brown, etc. In the past, when paper-based objects that were moistened and infected by microorganisms dried up naturally, fungal activity gradually decreased, colonies were slowly dying, but the produced spores preserved their vitality for many months or even years (Sussman, 1966).

Paper disinfection

Since the 1950s, owing to the progress of chemistry, chemical compounds of biocidal properties, i.e. biocides[1], have been used in order to eliminate this threat. Thanks to them, the biodeterioration of cultural heritage items can be interrupted within a few minutes or hours. In the post-war years it was a fast and effective solution to many problems of biodeterioration of library, archival and museum collections, damaged as a result of World War II, inappropriate transport conditions or storing in damp rooms (Husarska, 1954). In general, a several dozen chemical compounds were used or tried to disinfect historic paper items (Sequeira, 2012). However, the following years brought disappointment with the excessive use of chemical compounds, because they were noticed to be harmful to human health and the environment, and the attention was also paid to the possible detrimental effects of biocides on paper-based cultural heritage items, as well as insufficient effectiveness of certain disinfection treatments (Jędrzejewska, 1969). The use of a number of

[1] A biocidal product, i.e. a biocide (Greek *bios* - life, Latin *caedo* - I kill), is an active substance or preparation which contains at least one active substance and is intended for the destruction, deterrence, disposal, prevention or control, in any other form, of harmful organisms by means of chemical or biological action (Brycki, 2006)

toxic biocides gradually started to phase out and, at the same time, the efforts of librarians and archivists aimed at improving the storage conditions of collections began to bear fruit and disinfection needs decreased.

At present, a lot of researchers from Europe and North America believe that when it comes to paper-based objects, even in the case of active growth of fungi, it is enough to mechanically clean up mould deposits, dust and dirt, as well as maintain appropriate microclimatic conditions (16-18^{O}C and 50-60% RH) and cleanliness in storerooms. According to those researchers, disinfection is not needed, because fungi will not grow on paper stored in the correct microclimatic conditions and will die after some time, while in the case of moisture or flooding of collections, a new attack of fungi may occur anyway irrespective of the prior disinfection of the collections, because fungal spores are always present in the environment (Fuchs, 1998). Some authors dealing with this subject allow, in extraordinary cases, the use of biocides, most preferably 70% aqueous ethanol solution and, on a rarer basis, gamma radiation or ethylene oxide (Brokerhof et al., 2007, Florian, 2002, Sequeira et al., 2014).

This approach is consistent with the new trends in conservation, which recommend as little intervention in the historic substance as possible, but at the same time it is also controversial because it involves ignoring the biodeterioration processes of cultural heritage items and disregarding health risks from filamentous fungi to conservators and users of the collections. Little is known about the intensity of biodeterioration after drying an object and the time that fungi retain the ability to germinate, but there are a lot of indications that in the paper-based collections these processes can last for many months or even years. Furthermore, mechanical cleaning allows removing microorganisms only from the surface of a cultural heritage item, while they still remain and continue to perform the biodeterioration processes inside the materials.

Disinfection of paper-based cultural heritage items is a complex problem and requires an interdisciplinary approach, i.e. the cooperation between biologists, chemists and conservators. Prior to disinfection, which involves killing live microorganisms that cause damage, it is necessary to evaluate the viability of microorganisms causing the biodeterioration of an item, because only those objects that are currently subject to biodeterioration processes should undergo disinfection. In order to evaluate the degree of contamination of a cultural heritage item with living microorganisms, culturing or biochemical methods can be used, e.g. determination of ATP level on the surface, and the history and storage conditions of an item must be traced at least in relation to recent years.

Another problem associated with the disinfection of cultural heritage items is the selection of the appropriate biocide, its concentration and the methods of its application. To facilitate the selection of appropriate biocides, general requirements for biocidal products

used in the protection of cultural heritage items were laid down, namely it was concluded that on one hand such products must be effective against the microorganisms that are to be combated, while on the other hand they must be safe for the materials to be protected. In addition, they should be durable, easy to use, cheap and have minimal toxicity for humans and the environment. Existing publications related to disinfection are often limited only to research on the effectiveness of the suggested biocides against microorganisms (Fabbri et al., 1997, Dersarkissian and Goodberry, 1980, Nitterus, 2000, Kistenich, 2002), while there are not many publications which examine the influence of biocides on the substrate of a cultural heritage item or on the media, and they usually only concern a narrow group of materials (Strzelczyk and Rożański, 1986, Suzuki and Koestler, 2003, Weiβ, 2006). On the basis of this existing data, conservators in Poland have so far been using several methods for the disinfection of paper-based cultural heritage items, mainly ethylene oxide fumigation (ETO), vapours of 4-chloro-3-methylphenol and, less frequently, baths in aqueous solution of dimethyl leuryl benzyl ammonium bromide. However, the methods used so far do not meet the above-mentioned criteria to a satisfactory degree; therefore, conservators are constantly indicating the need for research in this field. Sequeira et al. (2014) conducted a survey among paper and skin conservators in the world, in which the respondents said that research in this field of biology should primarily concern safe biocides (67% awarded the maximum number of points to this topic), methods of removing fungal stains (40%) and studies of the effects of the release of metabolites to paper (16%).

Regardless of the development of new trends in the conservation of cultural heritage items, there is a wide market offer and new solutions for microbiological problems, presented as effective, convenient and safe, are suggested in libraries, archives and museums. Unfortunately, these advantages are not always confirmed by multilateral research taking into account the effectiveness against microorganisms and interactions with historic matter.

Research objectives

The overriding objective of the said monograph was the multilateral analysis of problems related to the disinfection of paper-based cultural heritage items with the use of chemical biocides. I conducted interdisciplinary research on biocides, combining the elements of microbiology, chemistry, and the conservation and technology of paper. This methodology is presented in the chapter entitled "Materials and methods of research" of the mentioned monograph.

Specific objectives:

1. Selection of biocides so that they are representative of the market offer and at the same time could prove useful in achieving the objectives of the work,

2. Development of a quick and precise method for testing the effectiveness of biocides against filamentous fungi growing on paper,
3. Evaluation of the effectiveness of the selected biocides against filamentous fungi representative of paper biodeterioration,
4. Evaluation of the influence of biocides on the properties of selected types of paper and media on paper,
5. Determining criteria related to the suitability of biocides for the disinfection of paper-based cultural heritage items,
6. Indication of biocides which are the most suitable for the disinfection of historic paper, while taking into account the above determined criteria.

Assumptions and implementation of individual goals are presented in the following subchapters.

Verification of research objectives and hypotheses

Selection of biocides in such a way that they are representative of the market offer and at the same time could prove useful in achieving the objectives of the work

I assumed that the group of biocides selected by me will be varied and will allow to present the diverse influence of biocides on fungi and on the properties of paper and media on paper.

For the research conducted as part of the presented scientific achievement, I selected a group of 32 biocides while trying to make the group diverse in terms of chemical composition, mechanisms of biocidal activity and the methods of application (solution baths, fumigation, vapour disinfection). The selected biocides usually contained one active substance, sometimes two or more, as well as solvents and possibly modifiers (e.g. pH stabilizers). All active compounds of the selected biocidal products were authorized for use in the European Union and the biocidal products were authorized for use in Poland. Most of them were semi-finished products intended for the production of ready-to-use biocides, some were ready-to-use disinfectants in medicine, food industry or construction, and some were prototype preparations prepared in the laboratory for research purposes. Among the biocidal products that I selected were biocides that had not hitherto been used in the disinfection of paper-based cultural heritage items and, for comparison, biocides that had been used for this purpose for years. The selected biocides contained the following active substances:

a) surfactants, including quaternary ammonium salts (QAC): alkyl dimethyl benzyl ammonium chloride, didecyl dimethyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, didecyl methyl polyoxyethyl ammonium propionate, didecyl

dimethyl ammonium carbonate and N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine,

b) oxidizing agents: sodium dichloroisocyanurate, magnesium monoperoxyphthalate, sodium perborate and, in gaseous form, vaporized hydrogen peroxide and ozone,
c) alcohols and phenolic derivatives - ethanol, sodium 2-phenylphenolate, triclosan, 4-chloro-3-methylphenol,
d) volatile oils from 7 different plant species: grapefruit, eucalyptus, geranium, clove, fir, tea tree, sandalwood,
e) other - ethylene oxide.

Some of the multi-component preparations intended for use in other fields also contained additions of other biocides - guanidine, propiconazole and glutaraldehyde.

Development of a quick and precise method for testing the effectiveness of biocides against filamentous fungi growing on paper

I assumed that the method I developed to test the effectiveness of biocides would be analogous to the conditions of disinfection of cultural heritage items in a conservation workshop, simple to carry out in a microbiological laboratory and the obtained results will be numerical and precise.

For testing the effectiveness of the selected biocides, I chose the following eight cellulolytic strains of filamentous fungi from the collection of the Department of Paper and Leather Conservation: *Trichoderma pseudokoningii* Rifai 1969, *Chaetomidium subfimeti* Seth 1967, *Cladosporium cladosporioides* (Fres.) de Vries 1952*, Penicillium spinulosum* Thom 1910[2]*, Cylindrocarpon hederae* C. Booth 1966*, Paecilomyces variotii* Bain. 1907*, Aspergillus ochraceus* Wilhelm 1877 i *Geomyces pannorum* (Link) Sigler & J. W. Carmich. 1976.

In order to accomplish the specific objective of the work, I carried out an evaluation of the effectiveness of the selected biocides soluble in water or ethanol against the selected fungi using four different methods:

a) diffusion method of paper discs on wort-agar medium: I soaked filter paper discs with a diameter of 20 mm in 35 µl of a biocide solution (the active compound concentration in disinfectant solutions amounted to 1%, 3% and 6%[3]) and put in the centre of the

[2] Presently these strains are included to the collection Kolekcja Czystych Kultur Drobnoustrojów Przemysłowych ŁOCK 105 (*Trichoderma pseudokoningii* ŁOCK 1120, *Chaetomidium subfimeti* ŁOCK 1122, *Cladosporium cladosporioides* ŁOCK 1121*, Penicillium spinulosum* ŁOCK 1123).

[3] If the preparation contained more than one active compound, the concentration was related to the concentration of one compound, mostly from the group of quaternary ammonium salts. I have used this principle throughout my work.

medium; then I inoculated the plate surface with 0.5 ml of the suspension of individual fungal strains with the use of spread plate method, and after 2, 3 and 5 days of incubation I measured the widths of their growth inhibition zones. I used a modified version of this method in order to evaluate the effectiveness of volatile oils. I inoculated the media as described above, turned them upside down and added drops of the selected volatile oils in the amounts of 30 µl, 50 µl, 70 µl or 100 µl, 250 µl and 500 µl; after 7 and 14 days of incubation at room temperature, I visually determined the sensitivity of fungi to oils, based on the intensity of fungi growth according to the 4-point scale which I adopted.

b) the method of bathing media discs with fungal growth in aqueous solutions of biocides; discs with 10 mm diameter and 5 mm height were cut out from the 7-day fungal culture on the malt extract agar and immersed for 1 or 3 hours in the solutions of biocides with active compound concentrations amounting to 0.5%, 1.5%, 3% and 6%; after disinfection discs were shaken intensively in 20 ml of sterile water, and the suspension was used to inoculation with one drop on the hoop new sterile media discs, which were next incubated in humid chambers at 28^{O}C during 2 and 5 days; the control were discs which were not subject to disinfection. I visually evaluated the results of the intensity of growth on the discs according to the 7-point scale which I adopted. On the basis of a comparison of the obtained evaluations of the intensity of fungal growth on discs which were subject to disinfection and on control discs, I calculated the percentage of fungal growth reduction.

c) the method of bathing samples of handmade paper (whole pages measuring 11.5 cm by 19.0 cm) from a doublet of a 19^{th}-century book; I inoculated the samples sterilized in the autoclave by immersing them in a mixture of spore suspensions and the hyphae of the eight above-mentioned fungal strains, and then I placed them in plastic sleeves for 1-2 months. I disinfected the samples using the method of static baths in aqueous solutions of biocides. After the disinfection, I collected the material from the samples with a sterile swab from the surface of 3 cm by 3 cm and shook it in 10 ml of sterile water; I determined the number of fungi in the obtained suspensions by the dilution method. The control groups were infected and undisinfected samples of paper. I showed the results on a logarithmic scale as a reduction in the number of fungi after treatment (R); I assumed that the sample was successfully disinfected if $R \geq 4{,}00$[4].

[4] According to the Polish Standard PN-EN 1650+A1:2013-08E "Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1)"

d) the method of bathing paper samples (5 cm x 5 cm) individually inoculated with the following fungal strains: *Trichoderma pseudokoningii, Penicillium spinulosum*, *Chaetomidium subfimeti, Cladosporium cladosporioides.* I placed the inoculated samples in Petri-dish moist chambers, at the bottom of which there was a layer of constantly moist lignin. After about 3 weeks of incubation at 28^{O}C, when the symptoms of fungal growth became clearly visible on the paper, I disinfected the samples by means of static bathing in aqueous or ethanol solutions, misting, disinfection in the vapours of 4-chloro-3-methylphenol, tea tree volatile oil, vaporized hydrogen peroxide, ozone and, for comparison, by means of fumigation in ethylene oxide. Following the disinfection, the whole samples were shaken intensively in 100 ml of sterile water, and then I determined the number of fungi in the suspensions by the dilution method. The control groups were infected and undisinfected samples of paper. I showed the results on a logarithmic scale as a reduction in the number of fungi after treatment (R); I assumed that the sample was successfully disinfected if R≥4,00.

After carrying out the experiments and comparing the results, I concluded that the most suitable method for determining the effectiveness of biocides was a three-week method of culturing a single fungus strain on a small sample of paper, followed by its disinfection and determining the number of fungi that had survived the treatment. This method enabled to create the conditions of disinfection in a laboratory similar to the disinfection of cultural heritage items in a conservation workshop, and at the same time, owing to this method, I obtained precise and comparable numerical results proving the sensitivity of individual fungi to the applied biocidal products and methods of disinfection. The said method also turned out to be universal, because it was suitable both for the disinfection by means of bathing and also by means of misting, vapourising or gassing. Its biggest disadvantage was the relatively long time of conducting the experiments.

In further research, I gave up on applying a similar version of this method, which consisted in the inoculation of relatively large paper samples with a mixture of spores of eight fungi. It is true, however, that inoculating paper with a suspension of various fungi seems to be a promising and attractive method aimed at recreating the natural mechanisms of the infection of historic paper. In practice, however, after 1-2 months of incubation, almost exclusively the growth of *Chaetomidium subfimeti* could be observed on the paper samples. Such a strong domination of one fungus meant that the test of the effectiveness of biocides actually concerned only the above-mentioned type of fungus and hence the acquired results were unsatisfactory.

In turn, the method of disinfection of media discs with the fungus growth failed due to significant differences in the intensity of fungus growth and the thickness of the mycelium layer, which was much larger on the media discs than on the historic items. This could lead to a false and undervalued evaluation of the efficiency of a biocidal product that failed to kill all cells in such a thick sporing layer of the mycelium, but the same biocidal product would prove sufficiently effective against a much thinner layer of mycelium, which is what we most often deal with in case of historic paper.

The last of the methods used, i.e. the widely known and applied diffusion method of paper discs, was found to be useful in the presented research, but only for a preliminary comparison of the effectiveness of biocidal products in different concentrations against individual fungal strains. Its limitation was also the fact that, above all, it allowed to evaluate the extent to which biocides inhibit germination and the growth of fungal spores, and to a lesser extent, to what degree biocides are capable of killing live hyphae and spores.

Evaluation of the effectiveness of the selected biocides against filamentous fungi representative of paper biodeterioration

I assumed that at least a part of the selected biocides would prove sufficiently effective against the filamentous fungi used. I assumed in the work that a biocide is sufficiently effective if it reduces the number of microorganisms in the logarithmic scale by at least 4 orders of magnitude ($R \geq 4$).

Research on the effectiveness of biocides carried out with the use of the mentioned methods proved that the majority of 32 selected disinfectants was sufficiently effective in combating *Cladosporium cladosporioides*, *Penicillium spinulosum* and *Trichoderma pseudokoningii* fungi, but their effectiveness against *Chaetomidium subfimeti* was much poorer. The following turned out to be the most effective: ethylene oxide (220 g/m^3, for 24 hours), vaporized hydrogen peroxide (250 ppm for 90 mins or 400 ppm for 30 mins), ozone (1.5 g/m^3, 5 hours) and 45% aqueous ethanol solution (in a 45-minute bath), and they were also capable of eliminating *Chaetomidium subfimeti*. The most remarkable is the hitherto underestimated application of water solution of ethanol to disinfection of paper. The reason for this was probably ethanol's ability to dissolve some media on paper, as well as the fast evaporation rate of this compound and the resulting too short contact with fungal spores. Hence, the method of spraying 70% ethanol in water, which was used in the research of Nittérus (2000) and Meier (2006), lacked effectiveness in combating filamentous fungi. Much greater effectiveness was achieved by using baths in a 70% ethanol aqueous solution (Meier, 2006) or vapours of this compound (Bacílková, 2006).

However, the following preparations are those which I found to be ineffective: sodium perborate, magnesium monoperoxyphthalate, commercial preparations used in other fields, and volatile oils except tea tree and geranium oils.

The difficulties in combating *Chaetomidium subphimetic* probably stemmed from the fact that bag spores of this fungus are protected by the peridium and hence they are less accessible for chemical agents. On the other hand, the degree of inhibition of the sprouting of *Chaetomidium subphimetic* ascospores was comparable to other fungi, or even higher (e.g. under the influence of QAC, phenolic derivatives, oxidizing compounds, volatile oils), as demonstrated by the results of the paper disc diffusion method.

During the research, I noticed that some less effective biocides or those used at too low concentrations resulted in more intensive sprouting of spores that survived the disinfection than in control samples. These compounds probably triggered off the activation of sprouting, i.e. the interruption of the spore resting state and the preparation for sprouting (Florian, 1997).

Evaluation of the influence of biocides on the properties of selected types of paper and media on paper

I assumed that:

a) at least a part of the selected biocides will not alter, in a statistically significant manner, the basic properties of paper, i.e. its pH and optical and strength properties,
b) at least a part of the selected biocides will be safe for media on paper,
c) after the application, it will be possible to remove biocides from the paper, because they are alien compounds in the historic material and leaving them in the paper may, long-term wise, result in difficult-to-predict changes.

In order to verify the hypothesis a), I analyzed the influence of the selected biocides on two types of test paper: paper A - white, containing only cellulose, to a certain degree equivalent to handmade paper, linens, free of adhesives due to the activity of microorganisms, and paper B - slightly yellowish, containing 80% wood pulp, 20% kaolin, rosin-sized, the equivalent of machine-made paper, produced since the mid-19th century[5]. I focused on the comparison before and after the disinfection, as well as after the artificial aging of the following paper properties: pH, total color difference ΔE, parameter ΔR_z and tensile strength. Research after artificial aging was aimed at demonstrating the changes that take place in papers over a long period of time. A long time period was simulated by placing

[5] Although, in practice, papers with such a high content of wood pulp are rarely encountered, nevertheless in research they were used in order to highlight possible harmful phenomena that could remain undetected in case of other papers.

the samples for 3 weeks in a climatic chamber at the temperature of 80^{O}C and RH of 65%[6] without light.

As a result of the research and comparisons, I noticed and characterised the two most significant negative side effects for the paper substrate, related to the disinfection with chemical biocides, i.e. optical changes and decreasing of tensile strength.

I assumed that the total colour difference ΔE caused by disinfection should not exceed the value of 2,00, i.e. it should be unnoticeable or noticeable only by an experienced observer (Drzewińska, 2002). The optical changes observed as a result of disinfection concerned mainly paper B and consisted in its yellowing, which was caused by a very chemically reactive lignin present in the wood pulp. Due to this harmful influence, I negatively evaluated the suitability for the disinfection of paper-based cultural heritage items of the following biocides: N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine, didecyl dimethyl ammonium carbonate, most oxidizers and tea tree volatile oil. Although the tea tree oil is of natural origin, its active substances are chemical compounds, as is the case with active substances present in biocidal products. Moreover, they are present in the oil together with at least several dozen other compounds, whose types and content may vary depending on the growing season, and their influence on paper is difficult to estimate.

Ethanol, ethylene oxide and 4-chloro-3-methylphenol did not cause any optical changes of both papers, while some acceptable changes were caused by didecyl methyl polyoxyethyl ammonium propionate, lauryl dimethyl benzyl ammonium bromide and sodium phenylphenolate. The vaporized hydrogen peroxide exerted an uneven influence on the test papers, because it caused a clear and undesirable brightening of paper B, but as a result of artificial aging its colour differed slightly from the colour of the control samples.

The other undesirable side effect, i.e. the deterioration of tensile strength, mainly concerned paper A after the disinfection in water baths, except for the aqueous solutions of ethanol and sodium 2-phenylphenolate, and was associated with the lack of sizing. To a large extent, this undesirable side effect can be attributed to the activity of water molecules during the disinfection and during water rinsing after the disinfection, as well as to the activity of quaternary ammonium salts as surfactants. In conservation practice, however, this effect can be eliminated by reinforcing paper sheets by means of brushing, e.g. with a methyl cellulose solution, followed by pressing in the press, during which bonds among cellulose molecules (hydrogen, covalent, van der Waals and friction forces) responsible for its mechanical strength are restored.

Among the other biocides, the highest deterioration in tensile strength was caused by ozone dosed at 1,5 g/m^3 for 5 hours, as well as tea tree oil in relation to paper A. Ethylene

[6] Modification of the Polish Standard PN-93/P-501174/03 "Paper and board. Accelerated aging. Moist heat treatment at 80^{O}C and 65% relative humidity"

oxide did not affect the paper tensile strength to a statistically significant degree, while 4-chloro-3-methylphenol, as well as baths in a 45% ethanol solution influenced the tensile strength to a small extent. Weiß (2006) in her research related to the disinfection of the samples of two types of cellulose paper and two types of paper with wood pulp with the use of a 70% ethanol solution did not observe any differences in strength properties; however, the disinfection time in those tests amounted to 2,5 minutes only.

Most biocides did not cause any statistically significant changes in pH. Sodium dichloroisocyanurate and ozone (1,5 g/m^3, 5 hours) were the exceptions that caused a statistically significant reduction in the pH of both test papers, whereas sodium phenylphenolate brought about a statistically significant increase in the pH of both test papers by about 1-1,5, even though the sodium ions were completely removed from both papers as a result of a 1,5-hour rinse in running water. These biocides cannot therefore be recommended for the disinfection of historic paper.

In order to verify the hypothesis b) I determined by visual method the influence of biocides on the media, i.e. writing and image carriers, on paper. The diversity of media on paper used in the past and now is huge (e.g. black print, coloured overprints, pencils, iron gall ink, pens, markers, dyes and binders, images in photographs), but little attention in professional literature was given to their changes in relation to disinfection (Fuchs, 1998, Suzuki and Koestler, 2003, Yamamoto, 2004). Despite the fact that in the presented monograph the results of research on the influence of disinfection on media on paper were obtained by means of visual method and for a relatively small number of media, they showed a large variety of influences and harmful changes, e.g. blurring of dark-red overprints, disappearing, blurring or changing the colour of notes taken with a red or black fineliner, brightening or blurring of lines made with a blue pen and carbon paper lines, brightening of handwriting made with iron gall ink, brightening or changing of the shades of black-and-white and colour photography (from the years 1998-2005, i.e. without digital photographs).

Most changes were caused by those biocides, which were also surfactants, i.e. quaternary ammonium salts, oxidizing agents and tea tree oil. The application of biocides at higher concentrations generally resulted in the worsening of negative changes as compared to biocides at lower concentrations. Oxidizing agents caused the brightening of notes taken with iron gall ink as a result of the oxidation of Fe(III) ions, as well as the browning of black-and-white photographs taken in the DOP technique, while they did not significantly affect the black-and-white photographs taken in POP technique (e.g. those from the years of World War I) or colour photographs. Tea tree oil caused brightening and slight pinking of black-and-white photographs taken in the DOP technique. 4-chloro-3-methylphenol turned out to be neutral for most media on paper, and the only observed changes were related to colour

photographs, especially the green colour, and notes taken with a pen. Among quaternary ammonium salts, the least changes were caused by didecyl methyl polyoxyethyl ammonium propionate.

The most stable media in relation to the majority of biocidal products used were black print, pencil lines and, to a lesser extent, notes made with iron gall ink, while dark-red overprints and lines made with fineliners and a blue pen turned out to be very sensitive. Therefore, before performing the disinfection of objects in which coloured media are used, conservators are obliged to carry out tests of their resistance to a given biocide.

To verify the hypothesis c), I examined the capacity of the above-mentioned papers to retain the residues of biocides applied by means of the water bath method.

As a result of an hour-and-a-half rinsing in running water, I was able to almost completely remove the residues of quaternary ammonium salts and sodium phenylphenolate from paper A, which contained pure cellulose. On the other hand, I did not remove the most part of the residues from paper B, which contained 80% wood pulp, rosin and other components. This poses a threat that the residues of biocides in the paper will continue to affect its components and may accelerate its aging. This threat was to even greater degree related to the disinfection by means of misting (2-3 misting cycles using a 0.5% solution of didecyl methyl polyoxyethyl ammonium propionate). Hence, I stated that misting as a method of disinfection of paper cards should be rejected. Misting with a 0.5% solution of didecyl methyl polyoxyethyl ammonium propionate may possibly be allowed in case of the *in situ* disinfection of a large collection in which mould growth is superficial and applies only to bindings, although the outcomes of the presence of biocides in binding materials (leather, parchment, canvas, cardboard, plastics) are currently unknown.

More favourable in this respect were such biocides as vaporized hydrogen peroxide, ethanol and ozone, which evaporated quickly or were unstable and spontaneously degraded after the disinfection. The disinfection in the vapours of 4-chloro-3-methylphenol left particular odour in the samples, which means that this compound did not completely evaporate from the paper.

Determining criteria related to the suitability of biocides for the disinfection of paper-based cultural heritage items

I assumed that the experience gained during the research would allow to formulate more precise requirements for biocides suitable for the disinfection of paper-based cultural heritage items.

Having analysed all results of the research, I formulated the following detailed criteria related to the suitability of biocides for the disinfection of paper-based cultural heritage items:

1. a biocide should reduce the number of filamentous fungi by more than 4 rows on a logarithmic scale (R≥4 log), and the disinfection in the laboratory should be carried out in conditions analogous to those of a conservation workshop,
2. a biocide must not cause any significant changes in the pH of the paper (±0.5 pH unit at most), especially its acidification; it is advantageous to slightly raise the pH value (but not higher than pH 8.0),
3. a biocide must not cause changes in the colour of the paper (ΔE<2); the increase in the whiteness of the paper resulting from the washing out of contaminations during bathing is beneficial,
4. a biocide should not cause a significant deterioration of the mechanical properties of the paper,
5. no residues of biocides may remain in the paper - after the disinfection the residues should be completely rinsable in water, ventilable or they should spontaneously degrade or evaporate,
6. a biocide must not dissolve print, inks or other media on paper, cause any changes in their colour or cause any other changes whatsoever.

Indication of biocides which are the most suitable for the disinfection of historic paper, while taking into account the criterion of effectiveness and the lack of negative impact on paper and media on paper.

In order to evaluate the suitability of a large group of biocides for the disinfection of historic paper, I applied relatively simple and easily available methods, such as examining the number of microorganisms that had survived disinfection or examining the changes in optical properties, pH or tensile strength of the paper. I assumed that among the selected biocides categorised into different groups in terms of chemical structure and type of action, it will be possible to indicate the product(s) that will meet the requirements to a greater degree than the previously used biocides.

The majority of the selected biocidal products (27 out of 32) were evaluated as unsuitable for this purpose due to their low effectiveness or harmfulness for paper or media on paper. None of the tested preparations met all the listed criteria. However, I found the following five biocidal products to be usable: ethylene oxide, 4-chloro-3-methylphenol and lauryl dimethyl ammonium bromide, all of which had long been used for this purpose, as well as didecyl methyl polyoxyethyl ammonium propionate and ethanol - two products that had not been used so far. I also evaluated one product (vaporized hydrogen peroxide) as

controversial and requiring further research. Unfortunately, it turned out that even the best biocides can be used only after taking into account the associated limitations, e.g. weakness against *Chaetomidium subfimeti,* yellowing of papers containing large amounts of lignin or causing changes in some coloured media. I also stated that new biocides for paper disinfection should not be sought among preparations intended for other purposes, but a formula for a separate preparation should rather be prepared for this purpose.

Significance of the obtained research results

As a result of the performed experiments and their analysis, I undoubtedly extended my knowledge on the possibilities and limitations of using biocides to disinfect paper-based cultural heritage items infested with filamentous fungi. The conclusions drawn at the end of the work are particularly important for art conservators and biologists dealing with biodeterioration and disinfection of cultural heritage items, as well as for all carers of paper-based collections.

In my work I showed that chemical disinfection may involve threats to paper-based cultural heritage items, but on the other hand, leaving live microorganisms in an item carries the risk of the progression of biodeterioration. I believe that the type of biocide and disinfection parameters should be chosen in such a way so as to minimize the risks to virtually zero. Therefore, at this initial stage of conservation, it is already necessary to possess a thorough knowledge about an item undergoing conservation, in particular related to the manufacturing technology and materials used, e.g. information about the content of wood pulp and lignin in paper or about the sensitivity of coloured media.

On the other hand, it is obvious that there is no need to disinfect cultural heritage items on which microorganisms died a long time ago, or objects which are only covered with dust. Thus, when accepting, for example, new files into archival resources, there is no need to disinfect all new items in ethylene oxide, which is a common practice, but the files should be reviewed and there should be an evaluation of the degree of moisture, the state of preservation and microbiological damage. Only those items that demonstrate the symptoms of the growth of microorganisms or insects should be pre-selected for the possible disinfection. However, not all objects bearing visible signs of microbial growth should be disinfected. Those symptoms may have arisen years ago, for example as a result of the last war or even earlier, and during the next several decades of storing in dry conditions, microorganisms could have partially or completely died and the need for disinfection becomes debatable. Therefore, the final decision whether to carry out disinfection should be thoroughly considered and preceded by a microbiological examination of an item and, in particular, by an evaluation of the degree of viability and the activity of microorganisms on the infested surface. As far as I am concerned, only those item that are currently subject to

biodeterioration processes caused by living microorganisms should be disinfected. The cautious approach to disinfection presented above is in line with the principles of remedial conservation of cultural heritage items. Remedial conservation is connected with badly preserved objects and aims to immediately impede the causes of deterioration (Bochenek and Michaś-Bailey, 2014).

After the publication of the monograph, I observed with satisfaction that it became an inspiration for further research related to the application of biocides in the disinfection of paper-based cultural heritage items, in particular for the team from the Faculty of Biotechnology and Food Sciences of the Lodz University of Technology. The team carried out the research on the application of volatile oils (Matusiak et al., 2018) and vaporized hydrogen peroxide (Wawrzyk et al., 2018) in the disinfection of historic paper and fabrics. I also continued to work on this issue, and particularly on disinfection in vapours of 45% aqueous ethanol solution. After 18 hours, disinfection in vapours of 45% aqueous ethanol solution resulted in the complete elimination of the above-mentioned filamentous fungi and caused no significant changes in pH, optical properties or tensile strength of the samples of both types of paper. In addition, I visually observed that ethanol vapours caused far fewer undesirable changes in the media on paper than a 45% aqueous ethanol solution. I presented the results of this research in the form of a poster at the 16th International Biodeterioration and Biodegradation Symposium in Łódź (**Appendix 4, section IIIB, item 1, p. 23**). I intend to continue carrying out research on this method of disinfection so that this new method can be recommended in practice in the future.

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5. Overview of other scientific and research (artistic) achievements

A) Scientific achievements

In 1984, I started my full-time studies at the Faculty of Biology and Earth Sciences at the Nicolaus Copernicus University in Toruń in the field of biology. After the second year of studies I chose the specialization: general biology. From the fourth year, the subject of my interest was cell biology and plant embryology. The first research, conducted as part of the master's thesis at the Department of Plant Cytology and Genetics, was aimed at determining the location of Ca^{2+} ions at various development stages of the stigma of the pistil of *Pharbitis nil* L. In the initial stages, I detected Ca^{2+} ions related to plasmalemma and membranes, while in the mature stigma in vacuoles, mitochondria and the cell wall. The changes in the distribution of Ca^{2+} ions during maturation of the stigma of the pistil indicated the participation of these ions in transporting processes and the exocytosis of secretory vesicles, as well as the incorporation of the secreted materials into the pellicle. Based on the obtained results, I prepared my master's thesis entitled "Location of Ca^{2+} in the stigma of *Pharbitis nil*" under the promotorship of Ms. Alicja Górska-Brylass, Ass. Professor, Ph.D. and the direct supervision of Ms. Elżbieta Bednarska, Ph.D. The results of the master's thesis were included in the joint

publication (**Appendix 4, section IID, item 21, p. 9**). Participation in courses at the Department of Plant Cytology and Genetics taught me how to notice scientific problems, how to solve them in a variety of manners and how to think independently, which is greatly beneficial in my entire professional life at the Faculty of Fine Arts, where on a day-to-day basis I co-operate with representatives of a different professional group, i.e. conservators of works of art.

After graduating, on 1st September 1989, I started working at the Faculty of Fine Arts of the Nicolaus Copernicus University at the Department of Paper and Leather Conservation, where I have been employed since then. Within the Department of Paper and Leather Conservation, at that time managed by a microbiologist - Prof. Alicja B. Strzelczyk, Ph.D - there was a small microbiological laboratory dealing with the research related to the microbiological damage of cultural heritage items, as well as the selection of biocides suitable for their disinfection, and sometimes disinfestation, too. The precursor of this research in Poland was Prof. Romuald Kowalik, Ph.D. from the Institute of Industrial Organic Chemistry in Warsaw, who in the 1960s conducted university courses on microbiology with respect to conservation at the Nicolaus Copernicus University. Prof. Alicja B. Strzelczyk, Ph.D. continued these activities and her long-time scientific-research and teaching activity made conservation microbiology a recognizable branch of microbiology in Poland.

For the first four and a half years I worked at the Department of Paper and Leather Conservation as a technician engineer and already at that time I began my research on actinomycetes of genus *Streptomyces* in the biodeterioration of historic parchment, especially in the degradation of collagen. Since 1994, I have been employed as an academic - initially as an assistant, then as an assistant professor, and since 2012 as a senior lecturer. Our microbiology workshop has the equipment typical for a microbiological laboratory, i.e. high-quality optical microscopes for observation in transmitted and reflected light with the option to photograph images, an autoclave, a sterilizer, scales of different accuracy, a fume hood, a pH meter, a water deionizer, a laboratory refrigerator, as well as a bioluminometer, a small sample homogenizer, two samplers for testing microbiological air quality, a colony counter, a hygrometer for wood and walls, a paper hygrometer and an air thermo hygrometer. In modern science, however, even more specialized equipment and knowledge are necessary, therefore I often establish co-operation with other centres, e.g. with the Institute of Technical Microbiology of Lodz University of Technology (Lodz University of Technology Professor Beata Gutarowska, Ph.D.), the Laboratory of Microbiocide Chemistry (Bogumił Brycki, Ph.D.), CIOP-PIB (Central Institute for Labour Protection - National Research Institute) Professor Rafał L. Górny, Ph.D., Professor Hanna Kwaśna, Ph.D. from Poznan University of Life Sciences, Professor Stanisław Ignatowicz, Ph.D. from Warsaw

University of Life Sciences and Tomasz Sawoszczuk, Ph.D. from the Cracow University of Economics.

My work as a biologist supporting the conservation of cultural heritage items is inherently interdisciplinary, which consists in combining the elements of microbiology, chemistry, materials technology, entomology, as well as conservation and restoration of cultural heritage items. The questions that I am asked by conservators are mostly related to the causes and nature of the observed symptoms of deterioration caused by living organisms, as well as protection methods of cultural heritage items against microorganisms, that is methods of their combating and preservation. Below is a description of my other achievements apart from the accomplishment specified in section 4a of this summary of professional accomplishments.

Short descriptions of major achievements in other publications and conference speeches

Characteristics of participation of actinomycetes in biodeterioration of cultural heritage items

I have been dealing with issues related to the importance of actinomycetes in the biodeterioration of cultural heritage items since the beginning of my work at the Department of Paper and Leather Conservation at the Nicolaus Copernicus University due to the topic of my Ph.D. thesis ("The Role of Actinomycetes of Genus *Streptomyces* in the Biodeterioration of Historic Parchment") and these issues have still remained the topic of my interest. Indeed, bacteria of the order actinomycetes (*Actinomycetales*) play a significant role in the biodeterioration of cultural heritage items due to their being rich in enzymes, especially extracellular hydrolases that are capable of degrading complex organic compounds (cellulose, chitin, collagen, keratin, calcium caseinate and others), as well as their higher resistance to drying than in case of other bacteria. Nevertheless, until the 1980s and 1990s, publications on their role in the biodeterioration of cultural heritage items were scarce.

As part of the specified achievement, I published a review article and, together with Professor Alicja B. Strzelczyk, Ph.D., a monograph in the English language "The Role of Streptomycetes in the Biodeterioration of Historic Parchment" (**Appendix 4, section IID, item 2, p. 5)**, which was based on my Ph.D. thesis.

The growth of actinomycetes of the genus *Streptomyces* on parchment is visible in the form of round stains with the diameter up to 5 mm in the colours of grey, creamy white, pale pink, pink purple, dark purple or brick red, sometimes merging and forming extensive stains. Long-growing colonies are often accompanied by cavities that indicate the diminution

of collagen. While working on the problem of biodeterioration of historic parchments, I characterized and identified 11 strains of streptomycestes isolated from parchment to the cluster level and then, on the samples of new parchment, I reproduced the damage observed on the historic items on this substrate, i.e. the weakening of collagen structure up to the formation of cavities, as well as grey and pink purple stains. All isolated strains were capable of degrading collagen, however to a varied extent. Losses in the mass of parchment samples on which I cultivated the individual strains for two months ranged from 10% (*S. rochei*) to 55% (*S. anulatus*). In subsequent extractions of samples in the waters of increasing temperatures - 20^{o}C, 40^{o}C, 60^{o}C and 80^{o}C - I found products of parchment degradation containing hydroxyproline - an amino acid typical for the main, helical part of collagen's polypeptide chain. After all extractions, only 5% of the initial mass was left in case of a sample inoculated with *S. anulatus*, 18% in case of *S. diastaticus* and approximately 47% in cases of *S. rochei* and *S. griseoruber*. After 1-6 weeks, the image of the electrophoretic separation of products of collagen degradation showed the disappearance of fractions α, β and γ, typical for natural collagen, and simultaneous appearance of numerous "blurred" stripes with masses smaller than the mass of fraction α, which was the evidence of the collagenolytic activity of the mentioned strains. This discovery was important because the ability of microorganisms to degrade collagen is a relatively rare property in nature, the reason being, among others, the complex structure of this protein.

On the samples of parchment inoculated with *S. griseoruber* and *S. rochei* , I also reproduced the grey and pink stains of this substrate. The pink purple pigment *S. griseoruber* changed colour depending on pH (red in acid medium and purple in alkaline medium).

In the review publication (**Appendix 4, section IID, item 10 p. 7**) I presented the role of actinomycetes in biodeterioration of cultural heritage items on the basis of Polish and foreign reference books. Apart from discussing their role in biodeterioration of parchment and historic vegetable-tanned leather, I paid particular attention to the issue, widely discussed in professional literature, of the role they play in biodeterioration of wall paintings in churches. There, one can mainly find representatives of the genera *Streptomyces* and, less frequently, *Arthrobacter, Nocardia* or *Micromonospora.* Moreover, I presented the importance of actinomycetes in underground environments, such as tombs, catacombs, underground churches and caves. The reason for undertaking research related to blends of microorganisms in the underground environment is usually their participation in biodeterioration of cultural heritage objects located there, especially prehistoric rock paintings. Actinomycetes were usually isolated from white or white grey "patinas" which cover images or from dense white deposits. One could also notice purple stains on the walls caused by the growth of actinomycetes. In the walls and ceilings of the Spanish caves of Altamira and Tito Bustillo, the colonies with a diameter of a few millimeters formed by

actinomycetes could be seen with the naked eye. Owing to molecular biology techniques, actinomycetes of other genera than *Streptomyces* were found in these environments, e.g. *Nocardia, Amycolatopsis, Arthrobacter, Brevibacterium, Aureobacterium, Rhodococcus, Corynebacterium*. Strains of the genus *Streptomyces* were identified as, among others, *S. xanthophaeus, S. flavotrichini, S. roseoviridis and S. flavogriseus.*

Determining the relationship between the storage conditions of collections in library and archive storerooms and the state of their preservation and the health of employees

Between 1997 and 2006, I worked with teams dealing with a broadly defined issue of threats related to Polish library and archive collections. Initially (1997-2000) research topics focused on the biodeterioration of collections caused by the flood, which took place in 1997 in Lower Silesia. We showed the extent to which the number of microorganisms decreased after each stage of the initial conservation of flooded collections. We determined that lyophilisation had not sufficiently eliminated microorganisms and we recommended performing the disinfection of collections in ethylene oxide (**Appendix 4, section IID, item 18 p. 8**).

Next, as part of the government ordered grant PBZ MIN 002/H01/2002 "Acidic paper - mass rescue of endangered Polish library and archival collections", group A.2: "The analysis of microbiological and conservation aspects of mass protection of acidified Polish library and archival collections from the 19th and 20th centuries" (2003-2006), I participated in research on the biodeterioration of acidified library and archival collections from the years 1800-1914, stored in 5 selected storerooms located in 4 institutions, i.e. in the Kórnik Library of the Polish Academy of Sciences, Nicolaus Copernicus University Library in Toruń, the Public Provincial Library - the Copernicus Library in Toruń and the 2nd Department of the State Archives in Toruń. My task was to determine, based on the results obtained by a team of researchers, the relationship between the storage conditions of collections in library and archive storerooms and the state of their preservation and the health of employees, as well as to determine the extent to which the storage conditions of collections in the selected libraries and archives meet the recommended requirements. The research team consisted of microbiologists, art conservators, doctors and chemists. In order to accomplish the task, I prepared three publications for publication related to the biodeterioration of the 19th- and 20th-century collections, quantitative and qualitative analysis of bioaerosols in library and archive storerooms, as well as health risks from microorganisms for librarians and archivists in the work environment.

As is known, libraries and archives have been gathering, storing and making available to interested persons thousands of books and various types of archives for hundreds of years. The turbulent history of Poland caused that these collections were often separated, transported, changed owners, were subject to fires and floods, and had often been stored for years in places where they were exposed to moisture, dampening, molding and degradation by insects and rodents. In modern library/archive storerooms, efforts are made in order to ensure that the storage conditions of collections are correct and meet the recommended requirements to the greatest extent. Storage conditions of collections in storerooms are largely influenced by the microclimate in the room, i.e. temperature and air humidity, the amount and type of light reaching a collection, the amount and type of chemical air pollution, as well as the hygiene of collections, i.e. their microbiological purity and air quality in storerooms in terms of the amount and type of microorganisms present in the air. I pointed out in publications that the above-listed parameters depend to a large extent on the technical condition of the building, i.e. the type of soil on which it is built (wet, dry), the condition of damp proof courses, the tightness of roof coverings, the flow capacity of the gutter system and discharge pipes, the thickness and humidity of the walls, as well as the efficiency of heating, ventilation and possibly air conditioning.

In all examined magazines, the microclimate differed from the values recommended for library and archive storerooms (temperature 16-18^{O}C, relative humidity 50-60%), i.e. most often it was too warm (over 20^{O}C) and too dry (below 50%). Moreover, the microclimate parameters were variable and depended on the outside conditions (temperature amplitudes reached several degrees and relative humidity amplitudes varied between 20% and 40%).

In all storerooms, about ¾ of the collections on average were objects with respect to which no deterioration caused by microorganisms or insects was found. However, there was a concern about the fact that the remaining part of the collections - about ¼ on average - was more or less attacked by microorganisms, less often by insects. Objects strongly damaged by microorganisms or insects accounted for 0.2-6.3% of the collections depending on the storeroom. The most frequently observed type of damage was foxing, i.e. rusty brown stains occurring on papers, especially those of inferior quality, from which the following materials were made: book ends, protective cards, paper dividers, as well as stiffening cardboard and papers glued to the insides of files. Less frequent changes were those caused by the growth of mould fungi, i.e. granular, fluffy or powdery deposits in the colours of white or rusty red, as well as various sizes of stains in the colours of white, cream, pink, red, purple or grey, in most cases permeating through several sheets of paper. Changes in colour were accompanied by the deterioration of paper, the visible symptoms of which were its thinning or loosening, weakening and even losses. Microbiological tests (microscopic observations and culturing of samples) proved that these changes were caused by fungi of the genera

Aspergillus, Penicillium, Cladosporium, less frequently by *Chaetomium, Rhizopus, Alternaria, Trichothecium, Sporothrix, Ulocladium, Acremonium Sepedonium, Botrytis, Stachybotrys* and *Botryotrichum sp*. accompanied by gram-positive cocci and actinomycetes. Slightly over ¼ of the cultured microorganisms were capable of degrading cellulose. However, most microorganisms were inactive and non-culturable. The symptoms of their growth were still visible on the collections, but after many years of storage under better conditions, a large portion of microorganisms that caused the symptoms died (**Appendix 4, section IID, item 14, p. 7/8)**.

In cooperation with the team of Professor Rafał Górny, Ph.D., I compiled the results of the research related to settled dust and bioaerosols in the mentioned storerooms. It was detected that settled dust contained 11 species of fungi belonging to 8 genera (*Penicillium* spp., *Alternaria tenuis, Acremonium strictum, Oidiodendron rhodogenum, Oidiodenron flavum, Aspergillus repens, Aspergillus penicilloides, Trichothecium laxicephalum, Aspergillus glaucus, Paecilomyces variotii* and *Cladosporium* sp.), as well as 10 species of bacteria belonging to 4 genera, including representatives of sporulating bacilli of the genus *Bacillus* known for proteolytic properties, non-sporulating bacilli of the genus *Cellulomonas*, known for cellulolytic properties, and *Staphylococcus epidermis* and *S. sciuri*, i.e. bacteria living on human skin and settled on books as a result of contact with readers.

Dust settled on the surface of books/archival materials came from the air in storerooms, therefore its qualitative composition to a large extent reflected the bioaerosol composition in these rooms, although the species diversity of microorganisms in the air was higher (39 species of bacteria belonging to 17 genera, and 18 species of fungi belonging to 12 genera). The largest part of bioaerosol in the air of the majority of storerooms were bacteria (84,2%), and among them mainly gram-positive cocci, while the second most numerous group were mould fungi (15,8%), most often of the genera *Penicillium*, *Alternaria tenuis*, *Trichothecium laxicephalum, Aspergillus regens, Oidiodenron flavum* and *Oidiodenron rhodogenum*.

The concentration of bioaerosol in storerooms proved to be an essential parameter for evaluating storage conditions of the collections. The concentrations of fungal aerosol averaged at 156 cfu/m^3 (in the range of 51 - 490 cfu/m^3), while bacterial at 400 cfu/m^3 (in the range 247 - 712 cfu/m^3). These values were much lower than the limits for bacterial and fungal aerosol concentrations for public facilities, which amount to 5.000 cfu/m^3 in case of bacterial aerosol and 5.000 cfu/m^3 for fungal aerosol[7]. These limits, however, are set to protect human health against microorganisms, which is why I pointed out the need to set

[7] proposals recommended by the Team of Experts on Biological Factors of the Inter-ministerial Committee for Maximum Permissible Concentration and Intensity of Agents Harmful to Health in the Work Environment (Górny, 2009).

limits for library, archive and museum storerooms, which would take into account the threats to the collections. Reference books suggest various values of these limits; in this work I assumed the level of 200 cfu/m^3 as the upper limit for the concentration of fungal aerosol. This threshold value was exceeded only in one of the storerooms, where it reached 712 cfu/m^3, which was caused by frequent rainwater flooding of the underground corridor in the immediate vicinity of the storeroom (**Appendix 4 section IIA item 5, p. 3**)

On the basis of the presented results of joint research on the state of preservation of collections and their current storage conditions in the majority of storerooms, I found it difficult to demonstrate unambiguous relationships between microclimate and microbiological quality of air in a storeroom on the one hand, and the degree of biodeterioration of collections by microorganisms on the other. The main reason for the difficulties was probably the fact that the majority of the examined storerooms had only been used for about 10 years, so too short a time for the conditions in storerooms to significantly affect their condition. The current state of preservation of the collections was indeed influenced by the current storage conditions, but also, to even greater extent, the past of individual books and archival materials - their individual stories consisting of years of storage in close-to-normal conditions, but also wars, floodings, years of storage in damp basements or attics, as well as numerous changes of storage place. Another source of these difficulties was probably unequal susceptibility of different library materials to attack by microorganisms, including various types of paper used in the collections in the examined storerooms, because papers of inferior quality were more often infested by microorganisms.

On the basis of the results presented here, I recreated the sequence of events which influenced the current state of preservation of the collections in the examined storerooms with respect to their biological damage. Good condition of the building and storage rooms is crucial in this case. Defects and damages to the library or archive building, i.e. the lack or the poor condition of damp proof courses, roofing leaks, rainwater flooding of walls due to the obstruction of the gutter system and discharge pipes, the lack of heating in winter, inefficient ventilation or leaks in water installations lead to the dampness of wall barriers in the first place and, in some cases, the collections are directly flooded with water. Water evaporates slowly from the flooded wall barriers and from the collections, which are then infested by filamentous fungi. The relative air humidity in the storage rooms increases, which entails an increase in bioaerosol concentration and in the number of microorganisms in the settled dust. Eventually, there is an increase in water content in the remaining unflooded collections, in which colonies of microorganisms begin to grow, which results in deterioration visible to the naked eye - deposits, stains, weakening of the structure. The pace of these processes varies - in case of floods, microorganisms begin to grow in the collections, to a disastrous extent, within a few or several days, whereas in case of periodic flooding of wall barriers due to leaks

in roofing and fluctuations of microclimate parameters combined with insufficient ventilation, deterioration may occur after a few weeks or months.

The continuation of my interest in bioaerosol-related research was the co-operation with Professor Rafał Górny, Ph.D. regarding the microbiological air quality in conservation workshops (**Appendix 4, section IIA, item 3, p. 3**). In this work, microorganisms were collected from the air using air samplers by impact method directly onto media (using a six-stage Andersen impactor) and by filtration method onto filters with pore diameter of 3 μm. The microorganisms collected on the filters were subsequently rinsed and cultured on microbiological substrates or treated with formaldehyde and stained with acridine orange and then filtered through a black polycarbonate filter (pore size of 0.8 μm), observed and counted under an epifluorescence microscope at a wavelength of λ = 490 nm (the CAMNEA method). The other method allowed to consider all particles of biological origin in the air, not only colony-forming units on the media. The comparison of the results of both methods indicated that the microorganisms cultured on media constituted only 0.1-5.5% of the total number of biological particles detected by the CAMNEA method, which did not require culturing. These results converge with the results of similar comparative research on microorganisms from the soil, which found that culturing methods can detect only 5% of fungi and 12% of bacteria of the total number of microorganisms which live in the soil and are detectable by means of molecular research techniques not requiring culturing (Frąc and Jezierska-Tys, 2010).

The advantages of methods that do not require culturing, as well as the experience I acquired during the implementation of the "Acid paper" project prompted me to look for another method, which also does not require culturing, to determine the level of viability of microorganisms on the surfaces of cultural heritage items. The disadvantages of culturing methods used for this purpose include a long result waiting time and insufficient credibility of the result. This is because on culture media one can often notice the growth of numerous microorganisms which ended up on the surface of a cultural heritage object by accident in the form of spores, but are capable of growing rapidly on media, instead of those microorganisms which are actually responsible for deterioration, often weakened or dead. Therefore, for the research on the viability of microorganisms on the surfaces of cultural heritage items, I adapted a bioluminescence method that does not require culturing and had so far been used in the food industry. The bioluminescence method allowed for a quick approximate determination of the amount of adenosine-5'-triphosphate (ATP) - a compound found only in living cells, which is used to store energy in the cells. The bioluminometer presented the results in the Relative Light Units (RLU), which amount is proportional to the amount of ATP in a sample. The level of ATP reflected the degree of contamination of the examined surface by living microorganisms, with the correlation coefficient amounting to 0.63 ($p \leq 0.05$), i.e. the positive average correlation. The correlation coefficient was not only

influenced by the dissimilarity of the methods, but also by the uneven nature of the growth of microorganisms on the used substrates. Nevertheless, I use it as a supplementary method to study the infestation of cultural heritage items by living microorganisms, which is of crucial importance when it comes to deciding on the necessity of disinfecting an item (**Appendix 4, section IID, item 17, p. 8**).

The method of bioluminescent determination of ATP on the surface of objects, as well as the research on microbiological air quality were used by me in another work devoted to the examination of library storerooms (**Appendix 4, section IID, item 12, p. 7**). In this work, I stated that concentrations of fungal aerosols quite accurately reflect storage conditions of collections, i.e. microclimate, possible wall dampness, the quality of ventilation and the degree to which collections were cleaned from contamination, including mould infestation. For this reason, I recommended these methods as necessary to fully determine the conditions for storing collections in a storeroom and the state of their preservation.

During the implementation of the project "Acid paper - mass rescue of endangered Polish library and archival collections" I also participated in preparing a publication on the threats from microorganisms in libraries/archives to the health of employees of these institutions directly dealing with collections (**Appendix 4, section IID, item 15, p. 8**). This work was carried out together with a team from the Institute of Occupational Medicine and Environmental Health in Sosnowiec under the supervision of Dorota Jarosińska, M.D., Ph.D.. In general terms, filamentous fungi can cause three types of diseases in people: allergic diseases (e.g. allergic rhinitis and sinusitis, atopic asthma with leading allergy to fungi and extrinsic allergic alveolitis), fungal infections (e.g. acute bronchitis and acute pneumonia, chronic bronchitis, fungal pneumonia) and fungal poisoning (mycotoxicoses, i.e. diseases caused by the entry of mycotoxins into the human body through the alimentary or respiratory tract). Mycotoxins are present in the human environment usually in low concentrations and generally cause chronic intoxication, which initially manifests itself through non-specific symptoms, such as fatigue, headaches, diarrhoea, muscle aches, runny nose, frequent illnesses similar to influenza. After years, however, they can lead to the damage of liver, kidneys and even to cancer (Piontek, 2004). Apart from mycotoxins, mould fungi release volatile organic compounds into the air (*MVOCs - Microbial Volatile Compounds*), responsible for, among others, a specific "fungal" smell; MVOCs may also contribute to chronic fatigue syndrome.

The aim of the task was to present to what extent the exposure to filamentous fungi causes the above-listed symptoms or diseases in people who work professionally with library/archival collections. In the first stage, two types of questionnaires were prepared, i.e. building status surveys addressed to the administration of buildings and individual employee surveys containing questions about the nature and length of working time with a book

collection, conditions in a workplace, home environment and health condition. The analysis of "building" surveys indicated that nearly a quarter of the surveyed libraries/archives had been flooded in the past. The damage also concerned collections, although only a small part of them (up to 5%) was usually flooded. The current occurrence of moisture stains and/or mould marks on the walls of rooms was confirmed by nearly half of the facilities, while the occurrence of moisture stains and/or mould marks on collections was confirmed by one-third of people who filled in the "building" surveys and as many as half of those who worked directly with the collections.

Almost all surveyed employees confirmed the fact of exposure to harmful agents in the current workplace, mainly to dust and biological aerosol, and mentioned the occurrence of a number of disease symptoms mainly from the respiratory system (itchy nose and/or sneezing, rhinorrhea or a feeling of nasal congestion, dyspnoea, cough, wheezing) or skin (rash and/or redness on the surface of hands and forearms); headaches and the feeling of chronic fatigue were also among the recurring symptoms. In the case of all of the analysed symptoms, most respondents reported that the symptoms intensified in the workplace.

In the second stage, a detailed allergy diagnosis was carried out in a selected group of 46 employees of the Wroclaw University Library, which suffered during the flood in 1997. As a result of the diagnostic tests that included: general examination, a spirometric and rhinomanometry tests, as well as measuring the level of immunoglobulins E in blood (total IgE and specific IgE: the following mould fungi antigens were selected *Alternaria tenuis, Aspergillus fumigatus, Cephalosporium acremonium, Trichoderma viride*), no deviations from the accepted norms were found in the examined librarians. Such results should not prompt us to discontinue the search in this respect, because the results of Wiszniewska et al. (2010), carried out on a group of 200 art conservators and museum workers suggest the role of filamentous fungi as occupational allergens in this professional group (Wiszniewska, 2010).

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Detection of filamentous fungi causing foxing on the drawing of Leon Wyczółkowski "Rynek w Gniewie"

The term "foxing" is used to describe small, red or yellow-brown, irregular stains, which are numerous on paper in books, documents, graphics and other objects, especially from the 19th century. The explanation of the mechanism of foxing formation has been within the scope of interest of many authors all around the world for many years. They indicate that the stains are of microbiological or chemical origin, but it has hitherto not been possible to clearly determine the reasons for their formation. Rarely is it possible to grow microorganisms that are capable of forming foxing and reproduce it in laboratory conditions (Nol et al., 1983, Arai, 2000).

A drawing "Rynek w Gniewie" by Leon Wyczółkowski was once handed over to the Department of Paper and Leather Conservation at the Nicolaus Copernicus University. The drawing was exceptional in that apart from red-brown foxing stains, its surface was covered in whitish or sand-yellow deposits, composed respectively of fungal hyphae or sand-yellow cleistothecia, filled with spherical sacks with ascospores (**Appendix 4, section IIA, item 4, p. 3**). The result from the bioluminometer indicating the ATP level in the area covered with a sandy yellow deposit was 620 RLU, while in a deposit-free area - 160 RLU. The incubation of samples of sand yellow deposits on microbiological media allowed to grow and isolate 5 fungal strains, of which *Eurotium rubrum* W. Bremer, *E. repens* de Bary and *Aspergillus versicolor* (Vuill.) Tirab. secreted large amounts of yellow brown dyes onto the culture media. These strains were successfully used in an attempt to reproduce, on the test papers, the damage visible on the paper of Wyczółkowski's drawing. Red brown foxings appeared on the samples of all four types of test papers placed on one-week-old colonies of *Eurotium rubrum* growing on the medium. Such stains were also caused by *E. repens* and *Aspergillus versicolor*, although the effects varied strongly depending on the species of fungus, the type of medium and the type of paper.

This is a worthy achievement, because it is extremely rare to grow fungi that cause foxing and reproduce it in laboratory conditions. Unfortunately, it does not explain the origin of this damage in every case, and the results of research indicate the multiplicity and diversity of its causes (Soyeon, 2007).

The significant contribution of microorganisms in the formation of foxing on paper samples from three different sources is also shown by the results presented in the publication compiled in collaboration with the teams from the Lodz University of Technology and Rzeszów University of Technology (**Appendix 4, section IIA, item 1, p. 2**). My contribution to the creation of this work consisted in inspiring the topic of research, gathering

research samples and compiling their characteristics. In this work, modern research tools, i.e. metabolomics and metagenomics, were used for the first time in the foxing-related research. Owing to the analyses of the metabolome, it was found that foxing stains contained products of sugar metabolism, which may be cellulose degradation products, yellow and fluorescent compounds, i.e. β-, γ- and δ-tocopherols, 2-methyl-3-phytyl, a precursor in tocopherol biosynthesis, as well as 3-hydroxy-L-kynurenine, belonging to the metabolic pathway of tryptophan. This is the first work in which the compounds responsible for the colour of foxings were identified. In addition, the work also highlighted the role of bacteria, among others *Ralstonia sp*. and *Delftia sp*., in the formation of foxing, whereas most publications had hitherto identified filamentous fungi as responsible for foxing.

In 2017, I published a review paper in the Polish language summarizing the results of long-time research on foxing (**Appendix 4, section IID, item 1, p. 5**).

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Characteristics of blends of microorganisms destroying wall paintings

In the years 1997-2003, together with Professor Alicja B. Strzelczyk, Ph.D., I participated in the work of a team dealing with the issues of biodeterioration and conservation of wall and vault paintings in the church of the Bernardine Fathers in Skępe. The reason for undertaking microbiological research was the occurrence of extensive white patina on paintings and the weakening of the painting layer on paintings on the vault over the monastery choir and in St. Anne's chapel. Owing to SEM observations, I proved that white patina on the paintings in St. Anne's chapel was formed by a biofilm consisting of long filamentous cells with a diameter of 0.3-3 μm. I isolated 13 strains of bacteria, mainly of the genera *Bacillus* (5 species) and *Streptomyces* (3 species), as well as 7 strains of filamentous fungi, and I also characterised their properties that are essential with respect to the state of preservation of the painting. Isolated microorganisms, primarily bacteria from the genera *Streptomyces* and *Bacillus*, showed capabilities which were dangerous to the painting and consisted in decomposing binders used in the painting, i.e. casein and calcium caseinate,

growing in conditions of low water availability and low temperature ($4^{O}C$) and acidifying of a substrate (**Appendix 4, section IID, item 16, p. 8**).

The result of my interest is also a review publication on the biodeterioration of wall paintings in Poland and in the world (**Appendix 4, section IIE, item 12, p. 12**). I also gave a speech on this issue at the symposium in New York (**Appendix 4, section IIK, item 23, p. 22**). Following 1960s, this subject was the topic of numerous research works. The examined paintings were mainly located in Italy, Russia, Romania and Germany. On the basis of the results of these works, I presented in the review publication the sources of the dampness of paintings, which is the main cause of the growth of microorganisms, and discussed the importance of bacteria, fungi, algae (chlorophyta) and lichens in their deterioration. I also showed the mechanism of microorganisms succession in this environment and also mechanisms that lead to biodeterioration (enzymatic degradation of painting adhesives, including modern synthetic binders, release of organic acids, oxidation and reduction reactions, and biofilm formation).

Summary

My scientific achievements consists in the authorship or co-authorship of **51** publications, including **3** book publications (including habilitation thesis), **7** original papers published in journals indexed by Journal Citation Reports, **5** original papers published in journals indexed by ERIH. The total number of MNiSW points for these works, according to the year of publication amounts to **417** (including habilitation thesis), while the total number of the Impact Factor is **10,955** (5-year IF – 11,522). According to the Scopus database, the total number of citations amounts to **67**, without self-citations: **60** (27 March 2019) (**Appendix 4, sections IIF and IIG, pp.15/16**).

During my professional activity I delivered **27** speeches at national and international conferences (**Appendix 4, section IIK, pp. 19-23**) and reviewed five articles sent for publication in international scientific journals (in 4 different ones) and three articles in the national journal (**Appendix 3a, section IIIP, p. 36**).

Raising qualifications

During my employment at the Department of Paper and Leather Conservation, I have been raising my qualifications. The interdisciplinary nature of my work as a biologist who supports the conservation of cultural heritage items required filling knowledge gaps related to conservation basics, techniques and technologies which helped create the works of art and even the elements of architecture. Hence, I completed postgraduate studies in church art organized by the Institute for the Study and Conservation of Cultural Monuments of the Nicolaus Copernicus University, covering issues related to the history of art, prophylaxis and

fundamentals of church art conservation (258 hours of theoretical classes), as well as a mycological and architectural course entitled: "Protection of Buildings against Biological Corrosion", organised by the Polish Association of Building Mycologists in Wrocław. The other course included 90 hours of lectures and 110 hours of practical classes related to the causes of moisture in architecture, the growth of fungi and insects in building materials and methods of combating them. Moreover, I participated in the international course "Biotechnology and the Preservation of Cultural Artifacts", organised by Fondazione per le Biotechnologie in Turin, Italy, which was devoted to the issues of biodeterioration of cultural heritage items and methods of their protection. The lecturers on this course were internationally prominent scholars in this field, including Prof. Ralph Mitchell from Harvard University, Prof. Claudia Sorlini from the Università degli Studi in Milan, Dr. Giulia Caneva from the Università degli Studi Roma Tre from Rome, Prof. Thomas Wahrscheid from the Institute for Material Science from Bremen and Cesareo Saiz-Jimenez from Instituto de Recursos Naturales y Agrobiologia from Seville.

In the first years of my professional life I participated as a listener in symposia related to biodeterioration and protection of wood "Protection of Wood", organised by the Faculty of Wood Technology of the Warsaw University of Life Sciences (in Rogowo in 1994, 2004 and 2007) and in the symposia of the Polish Association of Construction Mycologists in Wrocław (in Szklarska Poręba, 1995). In order to broaden my knowledge in the field of biocides, I participated as a listener in the symposia entitled "Quaternary ammonium salts and areas of their application in the economy" organised by the Wood Technology Institute in Poznan (in 1996 and 1997).

I also participated in the majority of conferences entitled "Distribution and corrosion of technical materials", organised by the Faculty of Biotechnology and Food Sciences of the Lodz University of Technology, a training seminar on "Microbiological air pollution in industrial plants", organised by the Lodz University of Technology, a scientific and training conference entitled "Health threats related to the work of art conservators and museum employees", organised by Prof. J. Nofer Institute of Occupational Medicine in Lodz and the conference entitled "Integrated protection of museum exhibits against pests", organised at the Warsaw University of Life Sciences. In 1993 I completed a 10-day internship at the Carl von Ossietzky University in Oldenburg, the aim of which was to take photographs of parchment samples damaged by actinomycetes in a high-quality scanning electron microscope, unavailable at the Nicolaus Copernicus University at that time.

In addition, I developed my skills with respect to statistical compilation of results obtained in natural research by taking part in two courses, namely "*Statistica* for medics and biologists", organised by StatSoft Poland in Krakow, and "Fundamentals of data analysis" as part of 18th Analytical Workshops organised by Predictive Solutions in Warsaw (**Appendix 4,**

section IIIL, item 9, p. 30).

B) **Educational and organizational achievements**

Educational activity

I have been teaching at the Department of Paper and Leather Conservation since the beginning of the summer semester in the academic year 1993/1994. My educational activity focuses on widely understood issues related to the protection of cultural heritage items against biological threats - microorganisms, fungi and insects.

At the Faculty of Fine Arts of the Nicolaus Copernicus University, I teach a third-year university course, included in the curriculum of the major: “Conservation and restoration of works of art” (KiR), which is called "Protection of cultural heritage items against biodeterioration" (formerly: "Microbiology with disinfection and disinfestation of cultural heritage items"). The course, which I have been teaching, consists of classes and lectures (lectures I have been teaching since the academic year 2004/2005). This course is compulsory for third-year students of all three specialties of KiR, i.e. conservation of painting and polychrome sculpture, conservation of architectural elements and details, and conservation of paper and leather (**Appendix 4, section III I, pp. 25/26**). Classes include issues related to the microclimate in the places where cultural heritage items are located, the basic characteristics of filamentous fungi, the so-called domestic fungi, bacteria, including cyanobacteria, as well as algae, mosses, lichens and herbaceous plants and insects destroying cultural heritage items; the symptoms of biodeterioration of paper, parchment, canvas, leather, painting layer and repainting, wood, stone, brick, as well as the mechanisms of biological destruction processes are shown and discussed; students are also acquainted with methods and biocides used for disinfecting and disinsecting cultural heritage items, as well as methods of protecting cultural heritage items against biodeterioration. These classes are highly rated in student surveys.

Regardless of these above-mentioned classes, I carry out research related to the destruction of biological origin of cultural heritage items which are subject to conservation by students as part of the cultural heritage conservation workshop (years 3 and 4), as well as student internships or the implementation of diploma theses (years 4 and 5). The outcome of this research involves short reviews prepared by students under my supervision, in which one can find the characteristics of symptoms of damage of a cultural heritage item caused by the activity of living organisms, as well as proposals to protect the item against biodeterioration.

Furthermore, at the **Faculty of History** of the **Nicolaus Copernicus University**, I teach a third-year university course included in the curriculum of the major: "Archival science

and document management", which is called "Prevention and conservation of archives" (**Appendix 4a, section III, I, p. 26**). The topics of this course include materials science of archives (old and modern), damage to archives due to chemical, biological and physical factors, as well as principles of preventive conservation of archives, including storage conditions. At the Faculty of History in the years 1994-2009, I taught a course included in the curriculum for the major "Conservation archeology", which was called "Disinfection and disinsection of cultural heritage items", and in 1998-2008, I taught a course included in the curriculum for the major "Library science and scientific information", which was called "Prevention related to storing library collections".

During my work I was the advisor of 5 master's theses, I independently supervised 17 master's degree students (**Appendix 4a, section IIIJ, pp. 27-29**) and I was the promoter of 1 thesis at the post-graduate studies in the field of museology. I reviewed 9 master's theses. The subject matter of these theses was closely related to my interests and research trend.

Promotional and organizational activity

My significant achievement with respect to teaching and popularizing knowledge is the co-authorship with Prof. Alicja Strzelczyk, Ph.D., of the handbook "Microorganisms and insects deteriorating historic items and their control", intended mainly for conservation students, but also for certified conservators of works of art, employees of museums and libraries, as well as other people involved in the protection of cultural heritage items (**Appendix 4 section IID item 1, p. 4**). In addition, I am the author of the chapter on biological aspects of the destruction of timber and the control of fungi and insects, which can be found in the Polish-Russian collective work "Problems of conservation and research of architectural monuments", published by the European Foundation for Monument Protection in Gdańsk (**Appendix 4, section IIE item 10, p. 12)**.

My greatest achievement in the organizational field was that in 2018 I performed the function of secretary of the 6th Scientific Conference of Paper and Leather Conservators entitled "Cultural Heritage Items - Biology - Conservation. Theory and practice", which took place on 18-19 October at the Nicolaus Copernicus University in Toruń (**Appendix 4, section IIIC item 1, p. 23**). The leading topic of the conference was biodeterioration of collections on paper and methods of their protection against biological factors. I invited prominent specialists in this field to deliver the lectures or prepare posters, and then, based on the proposals I was sent, I put together a conference program. I divided the lectures into 6 thematic sessions:

1. Disinfection - needs and possibilities - at the beginning of the session I gave an introductory lecture entitled: The importance of research on biodeterioration in the field of conservation and restoration of cultural heritage items. Next, the presenters

discussed the biocides which are nowadays used in the material protection, as well as legal issues related to their use.

2. Methods of disinfection of cultural heritage items - this session presented new proposals related to the disinfection of cultural heritage items, such as derivative compounds of 1,2,3-triazole, silver nanoparticles misting, low-temperature plasma, volatile oils and lyophilisation. The presenters emphasized such a choice of disinfection methods so that, on one hand, they would be non-destructive in relation to disinfected objects and, on the other hand, sufficiently effective to eliminate the threatening microbiological factor.
3. Monitoring and controlling insects destroying cultural heritage items - this session was devoted to discussing, on numerous examples, Integrated Pest Management (IPM) methods in libraries, archives and museums.
4. Modern methods of research on the biodeterioration of cultural heritage items - the presenters presented metabolomics and metagenomics, both of which are innovative techniques applied in the identification of microorganisms present in cultural heritage items and the metabolites they emit, owing to which the mechanisms of biodegradation and corrosion of materials can be understood in a better way. Several lectures presented the results of practical applications of these methods in relation to cultural heritage items and their storage environment.
5. Microbiological prevention in storing of archival, library and museum exhibits and
6. Other conservation problems - speeches in the last two sessions were mainly given by paper and leather conservators, who presented their experience related to practical problem solving of biodeterioration in archival, museum and library practice.

The lectures were presented in the form of multimedia presentations, and the conference was accompanied by the exhibition of posters and commercial companies stands dealing with the sale of laboratory equipment, disinfection and disinsection, as well as the exhibition of marble paper with the possibility of making a purchase.

Conference participants emphasized that not only was it an opportunity for them to familiarize with the issues of paper-based cultural heritage protection against biodeterioration, but its most important success was to allow two communities to meet, i.e. the community of practitioners - conservators, museum employees, archivists - and the community of scientists - microbiologists, entomologists and chemists. Lectures and discussions made it possible both to highlight the problems faced by practitioners and to present the current state of scientific knowledge that can be used to solve them. The conference gave the opportunity to build cooperation between practitioners and scientists, to jointly search for solutions to the posed problems, as well as the opportunity to develop new research areas.

Twice I prepared events as part of the Science and Art Festivals organized by the Nicolaus Copernicus University: lectures with a demonstration entitled: "A residential house and a gazebo on the allotment plot - threats from fungi and insects" (1st Festival) and "How much life is in the cubic metre of air? Problems of microbiological air pollution" (5th Festival).

III. Other professional activity

Consulting activity. My knowledge of the issues related to biodetioration of cultural heritage items and their protection against biological threats often results in my being invited to be a part of conservation expert teams, to participate in conservation projects funded by the Ministry of Culture and National Heritage, to carry out analyses of the state of infestation of cultural heritage items by microorganisms or insects, and to develop guidelines related to the methods of protecting cultural heritage items against biodeterioration, including disinfection or disinsection. My opinion was also ordered by conservation and construction companies, museums, foundations, libraries, archives, parishes, doctoral students and private individuals. Initially, I compiled the opinions under the guidance of or together with Prof. Alicja B. Strzelczyk, Ph.D., then independently, and in recent years with Joanna Joanko, M.A., a technical employee. In total, I compiled or participated in the compilation of almost 200 microbiological, mycological and entomological opinions (**Appendix 4, section IIIM, pp. 30-34**).

The most numerous group of objects, to which these opinions were devoted, were paper-based or parchment-based cultural heritage items. Some opinions concerned individual, extremely valuable cultural heritage items, e.g. the 11th-century Codex Aureus Gnesnensis from the Archdiocesan Archive of Gniezno, the 14th-century graduation from the Diocesan Library in Pelplin and the map of Andreas Hindenberg dated 1636 from the State Archives in Katowice. Other opinions concerned the entire historic book collections, e.g. the collection of the Elbląg Library (from the 15th to the 20th century), special collections of the Public Provincial Library - the Copernicus Library in Toruń, the collection of the post-Augustinian library in the Monastery Complex in Żagań, a collection of 19th- and 20th-century books in the Library of the Theological Seminary in Pelplin, or old prints from the Library of the National Maritime Museum in Gdansk. Some opinions concerned archival resources, e.g. from the State Archives in Toruń, or museum collections of paintings on paper, e.g. pastels and watercolors of Leon Wyczółkowski from the collections of the District Museum in Bydgoszcz, collections of graphics, chromolithographic prints, watercolours and drawings from the Museum in Chełmno. There were also opinions regarding contemporary collections, e.g. collections of the Medical Library of Collegium Medicum of the Nicolaus Copernicus University in Bydgoszcz or archival documentation in three storerooms in Ostróda and its surroundings, belonging to a private storage company.

The state of preservation of the above-mentioned objects and their collections varied. In historic paper-based or parchment-based objects, I often observed stains or deposits caused by the growth of filamentous fungi, mycelium of *Basidiomycetes* or corridors hollowed out by insects, sometimes partially filled with larvae excrements. The said damage was also present in the bindings of historic books, i.e. leather and wooden boards. A significant problem in these works was the identification of biodeteriogenes and the assessment of their viability. This last issue was crucial from the point of view of conservators, because it affected the rendering of the decision whether to carry out or refrain from disinfection or disinsection of an object/collection. The identification of filamentous fungi was difficult because usually they were colonies formed years ago, often at the time of or as a result of wars. Hence, the attempts to grow them on microbiological media were in many cases unsuccessful. The results of the identification on the basis of microscopic observation of the samples from the objects were approximate and in the best case limited to identifying the type of the fungus. Only in cases where the growth of the colony had taken place recently was it possible to grow microorganisms responsible for the damage and correctly identify their species.

It was even more difficult to evaluate the viability of fungi. Initially disinfection was recommended for all cultural heritage items showing symptoms of infection by mould fungi. Over time, however, I became more cautious. Since 2008, I have started using the bioluminometric method to determine the level of adenosine-5'-triphosphate (ATP) on surfaces infested by microorganisms, assuming that ATP occurs only in living cells, whereas in dead cells it degrades rapidly (see pages 12/13 above). Based on many ATP measurements, I assumed for practical purposes that the ATP level not exceeding 200-300 RLU is low and caused by microorganisms from the air and dust, and I took into account the so-called ATP background level, which is the ATP level in a place free from visible signs of damage. I assumed that if the ATP level in the area affected by the microbial attack three times exceeds the background level, then the contamination with living microorganisms in the affected area is significant and the object needs disinfection.

Thanks to the combined use of culture samples on microbiological media, the bioluminometric method for determining ATP levels and the microscopic observation of samples from cultural heritage objects, the evaluation of the viability of the microorganisms responsible for biodeterioration became more objective and it turned out that some cultural heritage items could have been sent for cleaning without disinfection, in other words without exposing them to aggressive biocides.

I also encountered difficulties when identifying insects that were seeking food in library/archive collections. The cases of finding living larvae of beetles at the time of drilling corridors in books were rare. Much more often than imagoes, exuviae or numerous larvae

excrements of shapes characteristic for genus or species could be found in the feeding grounds. I evaluated the viability of feeding grounds in books after their thorough inspection. In recent years, I have started to use and recommend monitoring traps with pheromones or food attractants to monitor the presence of insects in storerooms.

Other opinions concerned very diverse objects, among which were museum collections, as well as wall or vault paintings, burial crypts in churches, archaeological historic objects on different substrates, and even whole buildings, e.g. wooden churches with equipment, tenement houses or manors. The variety of organisms that were responsible for the damage was much greater than in the case of paper- or parchment-based collections. In addition to filamentous fungi and insects, I encountered wood-deteriorating Basidiomycetes, algae, lichens, mosses and seed plants growing on plaster, brick or stone. In order to identify them, I established cooperation with entomologists, lichenologists, algologists, briologists and botanists.

A number of opinions were related to biodeterioration of historic buildings. The subject of the research were entire buildings, such as three Gothic tenement houses in Toruń at Mostowa 6, a manor house "Studzienka" in Gdańsk, a grave chapel of the Scheiblers in the Evangelical Cemetery in Łódź, the Castle of the Masovian Dukes in Ciechanów, a historic "Willa Kapitana" (Captain's Villa) in Sopot, a wooden church in Czarne (Wielgie Commune), a parish church in Ostrowite near Jabłonowo Pomorskie and recently the building of the former "Sokół" Gymnastic Society in Kolomyia in Ukraine. The manor house "Studzienka" in Gdańsk was to a large extent attacked by *Serpula lacrymans*, which is the most dangerous Basidiomycete that causes brown rot of timber. Inside the building one could notice several large fruiting bodies of this fungus, numerous mycelial cords on the walls and brown rot symptoms on the wooden elements of the structure (except for the timber roof truss). In contrast, on the brick facade of the parish church in Ostrowite near Jabłonowo Pomorskie, I came across numerous foliose lichens, which were identified by a lichenologist as *Ramalina pollinaria* (Westr.) Ach., species subject to partial protection. This triggered a discussion related to what should be more protected - endangered species of lichen or a medieval church.

In some historic buildings I dealt only with wooden structural elements, for example a timber roof truss of the castle in Lidzbark Warmiński, a timber roof truss over the dansker in the Teutonic Castle in Toruń or a wooden structure of a wattle and daub wall in the private chapel of the Grand Masters in the Castle Museum in Malbork.

In churches, the subject of microbiological research and opinions were often wall and vault paintings. Among more important examples, it is worth mentioning the vault paintings in the church of the Bernardine Fathers in Skępe, wall paintings in St. Thomas Basilica in Nowe Miasto Lubawskie, wall paintings in St. John the Baptist's Church in Owińska near Poznań,

as well as paintings adorning the walls of cloisters in the Jesuits' pilgrimage complex in Święta Lipka. Vault paintings in the church of the Bernardine Fathers in Skępe were partly covered with white patina, from which, by means of culturing methods, I isolated and identified the presence of several dozen species of bacteria, including actinomycetes and filamentous fungi. Most of them, especially actinomycetes of the genus *Streptomyces*, possessed the capacity to degrade the casein used to preserve these paintings in the 1950s. In turn, persistent recurring fungal growth after subsequent conservations (mainly *Cladosporium cladosporioides* (Fresen.) De Vries) on paintings adorning the walls of cloisters in the Jesuits' pilgrimage complex in Święta Lipka is the result of the sanctuary having been founded on marshy area and unfortunate construction-renovation works. Thus, it has been a challenge for painting conservators for years.

In several historic buildings, e.g. at the castle in Oporów and at the Castle of the Masovian Dukes in Ciechanów, I performed research, in collaboration with microbiologists from the Faculty of Biology and Environmental Protection of the Nicolaus Copernicus University, related to characteristic surface pulverization of Gothic bricks. These symptoms may probably be attributed to the presence of nitrifying bacteria, but this still requires thorough research.

Among particularly interesting museum objects for which I prepared microbiological opinions was a set of six kits of drywall stamping dies for maps for blind and visually impaired children from the Special School and Education Center for Blind Children in Owińska near Poznań. Drywall dies were stored in humid conditions and heavily infested by several species of actively growing filamentous fungi. There were also numerous barkflies from the species of *Liposcelis decolor*, Pearman, 1925 and *Lepinotus reticulatus*, Enderlein, 1905, feeding on mycelium and spores.

Some microbiological or mycological opinions concerned archaeological historic objects. The most interesting object in this group were fragments of archaeological wood from the times of the first Piasts from the excavations in the archaeological reserve at Ostrów Tumski in Poznań. The wood has survived to our times probably due to close-to-anaerobic conditions that have developed over the years deep in the soil. The excavation of wood resulted in the input of oxygen and numerous microorganisms present in the air. Unfortunately, the excavation works were discontinued and when they were resumed after about 3 years, the wood was infested with the white house fungus *Poria vaporaria*, causing brown rot. Disinfection was impeded by the fact that the amount of wood had been estimated at 20 m^3. On my initiative, a group of specialists took part in a meeting at the Archaeological Museum in Poznań, during which a decision was made to disinfect the wood using the misting method. I have also researched fungi in burial crypts several times, e.g. in churches in Świecie, Łabiszyn, Bydgoszcz and the cathedral in Frombork.

Since 2006, a lot of my opinions have taken into account the examination of the environment of cultural heritage items, in particular microclimate and microbiological air quality in museum, library and archive storerooms, i.e. the so-called bioaerosols. I carried out such tests at, among others, the Fr. Dr Władysław Łęga Museum in Grudziądz, the Library of the Theological Seminary in Pelplin, the Elbląg Library (in 2010), Nicolaus Copernicus University Library in Toruń, special collections storeroom of the Gdańsk Library of Polish Academy of Sciences and special collections storeroom of the Public Provincial Library - the Copernicus Library in Toruń. Experience gained during the testing of microbiological air quality in storerooms showed that the concentration of fungal aerosol is a very convenient and helpful tool in evaluating the purity of rooms and collections.

The preparation of opinions gives me an extremely valuable opportunity to directly interact with cultural heritage items and confront my own knowledge with practical conservation problems. During the research, I contacted, above all, conservators, but also archaeologists and architects. Years of work taught me that every object I dealt with represented a new and different challenge and generated new issues. In many cases, I consulted with biologists of other specialties - entomologists, lichenologists, algologists, briologists, botanists and other microbiologists. Working with cultural heritage items is for me an invaluable source of experience which significantly increases my knowledge about biodeterioration of cultural heritage items. The knowledge which I have gained thanks to such work contributed to, among others, enriching lectures and classes for students, and also to preparing several publications (e.g. **Appendix II, section IID, item 8, p. 6, item 9, pp. 6/7 and item 16, p. 8**). I have developed my own opinion template, which I also use during the course "Practical issues in conservation biology" when I discuss the biodeterioration of students' thesis-related objects with fourth-year students of conservation of works of art.

According to the template I have developed, these opinions usually consist of three parts. The first part contains the characteristics of an object with particular focus on the materials it is made of, its history, and especially the description of the recent and past storage conditions, as far as they are possible to be determined, as well as information about any previous disinfection or disinsection activities, prior conservation, added materials or purification methods. The second part lists methods of examining the biodeterioration of an object, i.e. methods of sampling, types of examinations, e.g. microscopic observations, cultures on microbiological media, identification of filamentous fungi or insects according to keys, bioluminometric determination of ATP level on the surface or other. The third part is devoted to the presentation of examination results and to the discussion of recommendations related to disinfection or disinsection and storage conditions of an object in the future.

Summary. The presented overview of my scientific, teaching and counselling activities shows a wide variety of issues which I deal with professionally. This is primarily connected with a large variety of problems which link conservation of cultural heritage items with biology. Many years of cooperation with cultural heritage items' conservators of all specialties have resulted in my gaining a wealth of knowledge and experience and enabled direct contact with biodeteriorated cultural heritage items, which is indispensable in evaluating a problem and diagnosing the causes of deterioration. Owing to the implementation of scientific works and didactic classes and thanks to the experience gained during the preparation of advisory opinions, I am currently one of the few specialists in Poland dealing with the research on the biodeterioration of cultural heritage items and the protection of such items against biological threats. I also continue the activities of my predecessors in this field - Prof. Romuald Kowalik, Ph.D., Prof. Alicja B. Strzelczyk, Ph.D. and Stanisława Leźnicka, Ph.D. At present, other national research centres have also joined the research on biodeterioration of cultural heritage items, such as Lodz University of Technology or Cracow University of Economics. They apply innovative research methods which enable a fuller insight into the processes of deterioration of cultural heritage items by microorganisms. Last year's 6th Scientific Conference of Paper and Leather Conservators "Cultural Heritage Items - Biology - Conservation. Theory and practice", during which I performed the function of a secretary, led to a dialogue between biologists, microbiologists, chemists and the conservators of works of art, and showed the directions for the future development in this field of knowledge. I believe that it is worth continuing the development in this field at the Institute for the Study and Conservation of Cultural Monuments of the Nicolaus Copernicus University, so that this unique achievement in our country can still support the activities of conservators-restorers of works of art for the sake of the protection of cultural heritage items.

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