CONSERVING OUR CULTURAL HERITAGE: THE ROLE OF FUNGI IN BIODETERIORATION

Hanna Szczepanowska and A. Ralph Cavaliere

ABSTRACT

The objects of cultural heritage are composed of varied materials which can be affected by diverse microbial communities. The study of these complex and heterogeneous assemblies of materials and microorganisms require an inter- and multi-disciplinary approach. Development of a strategy towards prevention, mitigation of biodeterioration and removal of microorganisms, especially fungi begins with the understanding of the materials' fabric, assessment of causes behind the biodeterioration, and the context in which it occurs.

Three aspects of biodeterioration of cultural heritage are discussed: 1) the multitude of bio-agents' on cultural heritage materials, 2) fungal interaction with substrates, and 3) prevention and conservation of biodeteriorated artworks. The challenges of conservators' work in dealing with bio-degraded museum collections are discussed based on the case studies of biodeteriorated art on paper, exemplifying two types of fungal interaction with the substrate: 1) surface deposits of pigmented spores/conidia, and 2) pigmented fruiting structures embedded in the matrix of the substrate.

The microbial metabolites deteriorate the substrates on which they grow resulting in chemical and physical changes of the material bulk and surface, at times leading to structural weakening. We focused our studies on black stains which are prevalent on art rendered on paper, a subject that has received very little attention. Our techniques of analysis included three-dimensional topographic imaging and visualization, structural characterization and optical microscopy, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM).

INTRODUCTION

Conservation of art aims to prevent deterioration of objects which are in good condition and conserving those which have already been damaged. Biodeterioration which is one of many forms of artworks' deterioration is particularly complex because it involves living microorganisms interacting with a variety of highly complex heterogeneous materials. Biodeterioration in the context of cultural heritage can be defined as decay of culturally, historically or artistically significant objects induced by microorganisms, such as bacteria, fungi or lichens. It differs from biodegradation which refers to the breakdown of materials in nature.

Two main phases occur in the initial stage of biodeterioration- physical contact of spores with surface and attachment to surface. Biodeterioration starts as soon as microbial spores land on a surface where molecular water is present. Liquid water that is essential for microbial growth becomes available on the surface when it rea-

ches dew point temperature, and water condenses on the surface (Dew point temperature, private communication with Dr. Marion Mecklenburg, Senior Scientist, Emeritus, MCI- SI). It is a simplified shortcut to a synergistic work of relative humidity, temperature and the type of material involved that leads to the formation of liquid water on the surface. In addition to the need for liquid water, numerous authors list physico-chemical involvements in the first stage of fungal attachment to surfaces. Furthermore, spores secrete adhesions, such as protenaceous and/or polysaccharide materials which aid in the attachment of spores to the surface.

Fungal pigments produced on paper

Paper is a network of plant fibers derived from cotton, flex, or wood combined with particles of mineral fillers such as kaolin or calcium carbonate added to produce a desired, specific surface. Paper is a structurally complex, heterogeneous material susceptible to biodeterioration caused by fungi, one of which is staining caused by fungal pigments. Many species of fungi produce pigments which serve different functions - varying from a protective action against lethal photo-oxidations (carotenoids- in bacteria) to protection against environmental stress (melanins- in fungi) and acting as cofactors in enzyme catalysis (flavins). In addition, bio-pigments often protect the organism against extremes of heat and cold and against natural antimicrobial compounds produced by other invading microbes (allelotic metabolites) (Liu and Nizet, 2009, Paolo et al., 2006, Medentsev and Akimenko, 1998). Phylogenetic diversity of pigmented species, and the chemical diversity of the pigments themselves, precludes a single unifying hypothesis for their evolution and persistence. In general, pigmentation produced by microorganisms results from complex bio-chemical processes occurring in their cell and are referred to as secondary metabolites.

It has been well established that dark pigments associated with melanins are produced in some fungi as well as in organisms across many kingdoms. Melanins are amorphous substances with a remarkable ability to absorb-infrared, visible and ultraviolet radiation which makes their study using optical, light-based instruments, particularity challenging (Buskirt et al., 2011). The biosynthetic pathways of pigment formation are known only to some extent and the molecular structure of the pigment and its organization still remains unclear (Bochenek and Gudowska-Nowak , 2003). Fungal, animal and synthetic melanins share a number of similarities in their chemical makeup. Many of the fungi producing melanin are filamentous forms which belong to the class Dothideomycetes, members of the large, well characterized Phylum (or Division) Ascomycetes (Sterflinger et al., 1999, Sterflinger, 2006).

Black pigmented fungi, which grow on art works, may also be pathogenic. Pigments in fungi are used in presumptive clinical diagnosis (Liu and Nizet, 2009) therefore have been researched extensively in the medical field (Jacobson 2000, Icenhour et al., 2003). Fungal pathogenesis in plant and animal hosts has been of

main concern to agricultural studies. Some of these same pathogens of the Dothideomycetes have been identified on paper.

The extent of staining depends on where within their structure fungi produce pigments. It can occur in spores/conidia and mycelium as powdery deposits on surfaces, in their fruiting bodies, as inclusions in substrate, or as pigmented products of metabolism. Each scenario has impact on the conservation strategy (Fig.1).

Figure 1: 1920 etching, covered with black fungi induced stains along the top margin. Adhesive applied along the top to attach the artwork to a window mat provided nutrient for fungi.

Two phenomena occur with respect to the interface of fungi and the matrix of paper. One is the attachment of pigmented spores and mycelium to paper fibers facilitated by secretion of adhesives. The other is intertwining of paper fibers with pigmented fungal elements, such as perithecial hairs and hyphae. We studied both types of fungus-paper interfaces on the original works of art on paper.

MATERIALS AND METHODS

Considering the limitations of examining the original artworks, and often strict rules applied to sampling the material, the examination protocol relied on the choices that are permitted in the real life situation. In addition to optical microscopy, a confocal laser scanning microscopy was utilized which is a novel application in the examination of fungal stains on paper.

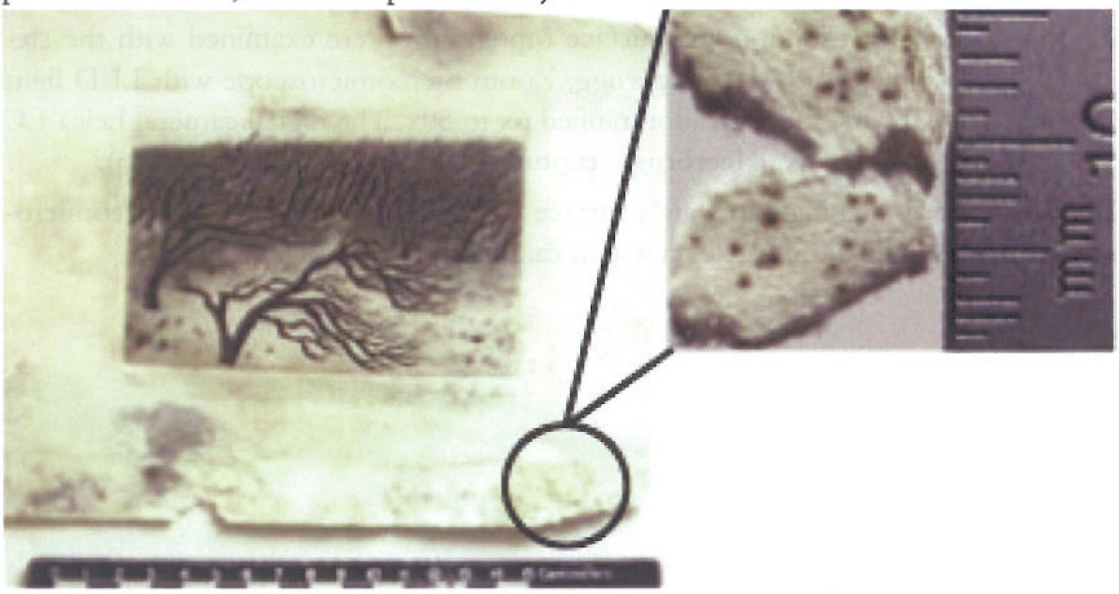
Two, twentieth century, original artworks (1920 and 1958), and one ca 17th century study-paper, affected by fungi-induced black stains were selected for study. The papers' characteristics indicated compact and calendared paper (etching 1920), unsized, printing-paper (1958) and soft, blotter-like paper (17th c). Stains on these papers were characterized as follows:

1. 1920 etching: dark cells attached to the surface of paper fibers (*Taeniolella* (Torula) *sp.*)
2. 1958 etching: pigmented fruiting structures embedded into substrate (*Chaetomium sp.*)
3. 17th century paper: pigmented spores/conidia clustered among paper fibers (*Cladosporium sp.*) (*Cladosporium sp.* and *Taeniolella sp.* were identified by De-Wei Li, Ph.D. Research Mycologist, Connecticut Agricultural Experiment Station Valley Laboratory, Windsor, CT.)

The context in which biodeterioration occurred in each case was different and can be deduced from the analysis of the bio-stains location on the artwork, understanding the construction of the artwork and history of its handling.

1) The black stains on the 1920 etching were not coincidentally located along the top edge where adhesive is applied to attach the artwork to a window-mat. Once water became available, it dissolved the adhesive providing rich nutrients to the germinating spores (Fig. 1).
2) Fungal deterioration of the 1958 etching occurred as a result of hurricane Katrina (2005). Dark brown, nearly black pigmented fruiting bodies (perithecia) were entangled into the matrix of the paper. It is unknown how long the paper remained wet, however, long enough to permit formation of the fruiting bodies of the fungus *Chaetomium* (Szczepanowska and Cavaliere, 2000, 2003; von Arx et al., 1986). Consequently the invaded portion of the artwork on which *Chaetomium*, a common cellulolytic fungus grew, was severely structurally deteriorated (Fig. 2).

Figure 2: 1958 etching by Shigeru Kimura, damaged during hurricane Katrina 2005. Individual dark brown spots see inset, are perithecia of *Chaetomium sp.*(Artwork in private collection, used with permission.)



3) On the 17th century paper, the black stains are scattered throughout a fibrous matrix. Although its provenance is unknown, based on the calligraphy of a fragmentary inscription and remnants of pigment (minium- red lead pigment), it was deduced that the paper was manufactured between the 16th and 18th century (Fig. 3).

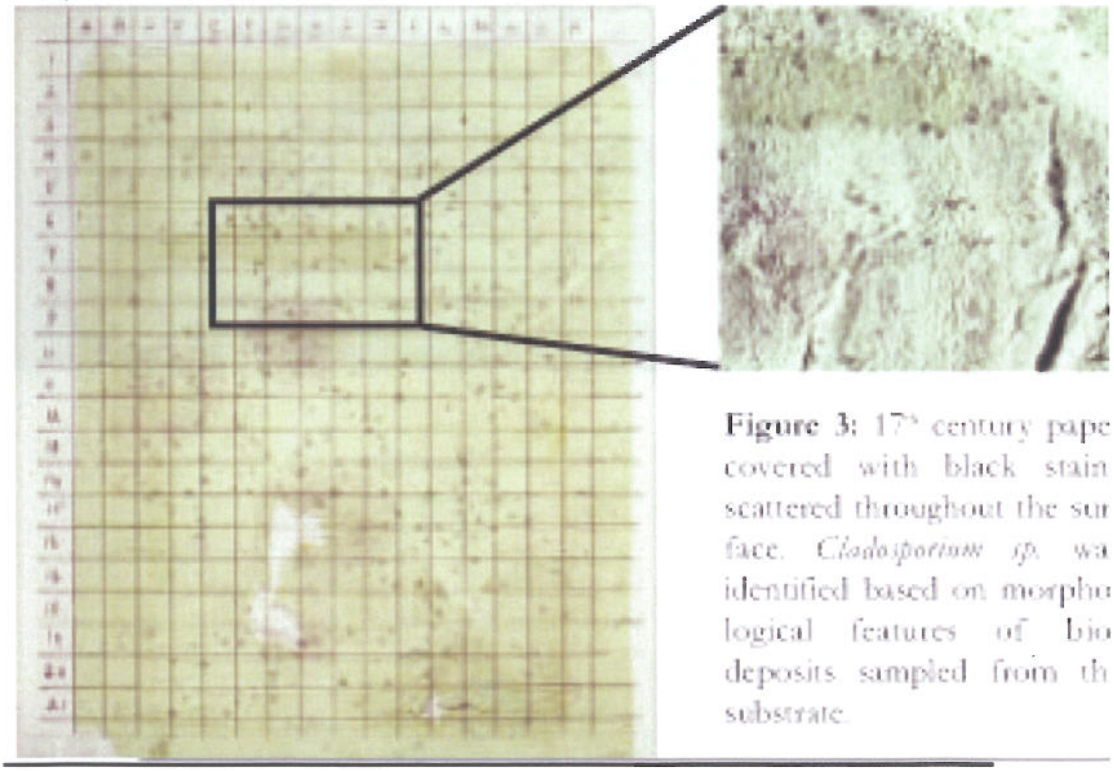


Figure 3: 17th century pape covered with black stain scattered throughout the sur face. *Cladosporium sp.* wa identified based on morpho logical features of bio deposits sampled from th substrate.

Surface characteristics of the bio-mass on the artworks were examined in respect to the fungal particles interaction with the paper fibers matrix. The evaluation regiment was as follows:

1. Bio-deposits distribution and surface topography were examined with the stereomicroscope, Wild M8 Heerbrugg, Zoom Stereomicroscope with LED light ring, Volpi, Swiss. Magnification ranged 6x to 50x. The digital camera, Leica EC 3, Microsoft Systems , Heerbrugg, captured the images (Figs. 4a, 5a, 6a).

Figure 4a: 1920 etching; bio-stain's surface topography examined with streomicroscope,50x magnification; black stain area ca. 05mm x 2mm.

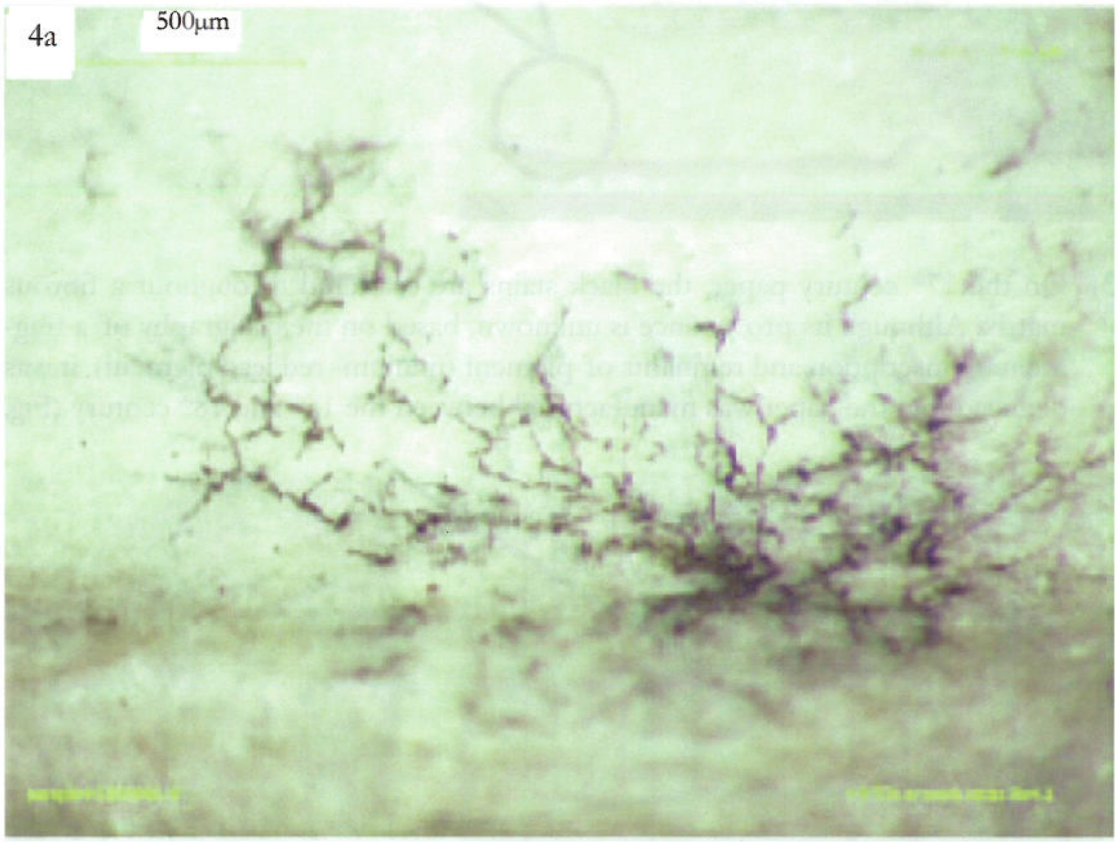


Figure 5: 1958 etching: pigmented fruiting structures embedded into substrate (*Chaetomium sp.*) **5a** Dark-brown perithecia visible as defined spots; stereo-micrograph, 50x. Size of perithecia: 0.3mm to-0.6mm.

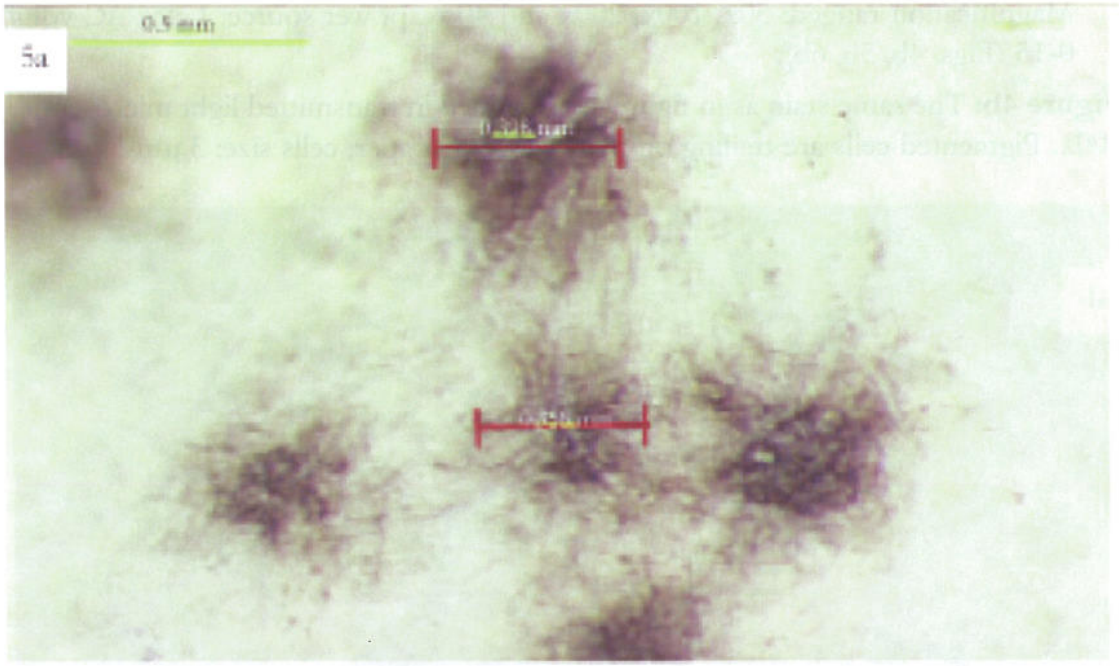
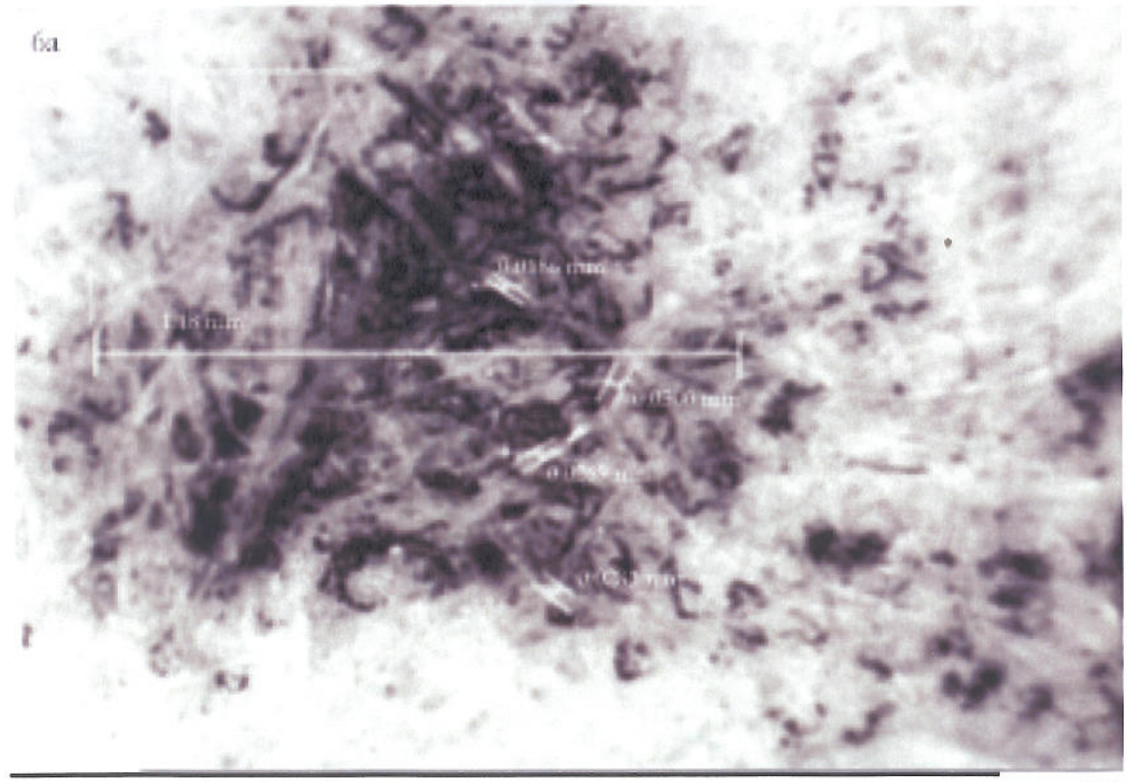


Figure 6: A 17th century paper: pigmented spores/conidia clustered among paper fibers (*Cladosporium sp.*). **6a** Stereo-micrograph, 50x of the black stains; stains size varied: 05mm x 2mm.



2. Bio-mass interaction with substrate and morphological features of bio-mass were examined with the transmitted light microscope, Leica DM LM, using phase contrast illumination, dark field and bright light illumination. Magnification ranged: 50x, 100x, 200x and 40ox, power source: Leica AC volts 0-15 (Figs. 4b, 5b, 6b).

Figure 4b: The same stain as in figure 4a examined in transmitted light microscope, 200x. Pigmented cells are trailing on the surface of paper; cells size: 3 μm-5 μm.

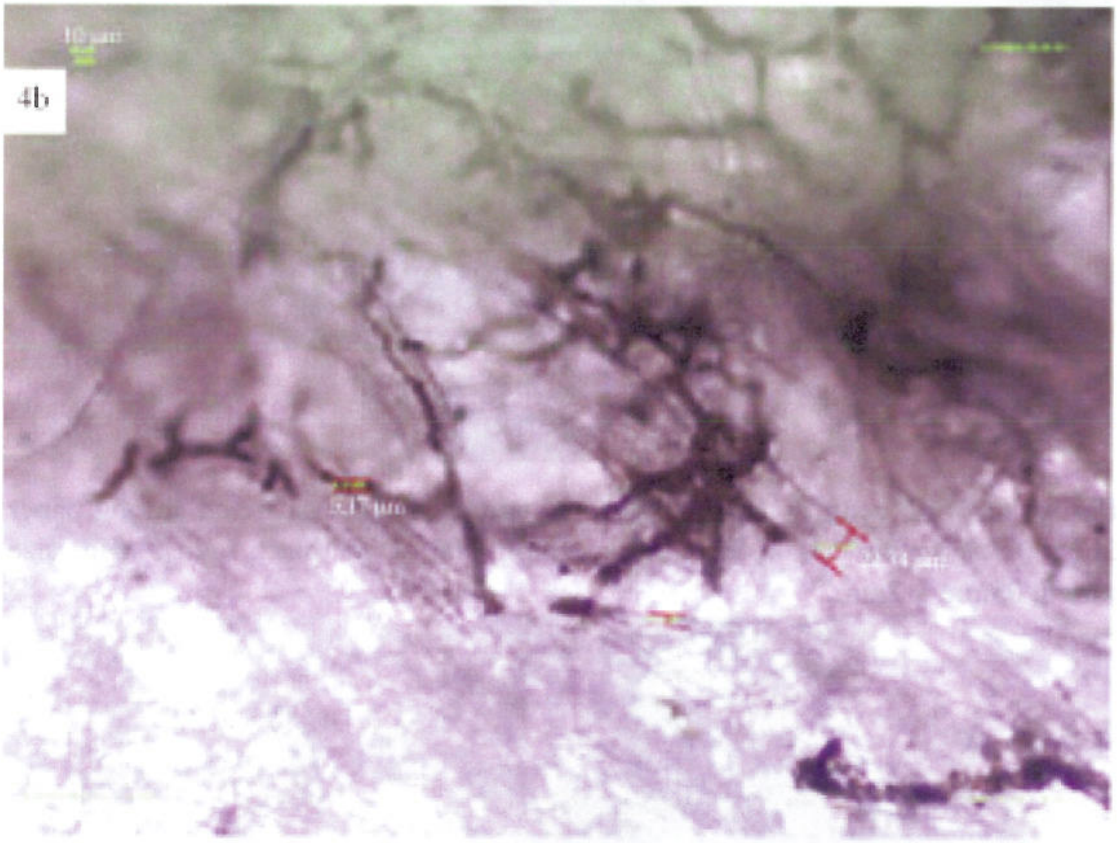


Figure 5b: Same specimen as in figure 5a transmitted light micrograph, 200x, an individual fruiting structure. Melanized, dark perithecial hairs are entangled with paper fibers, here visible as transparent light blue filaments.

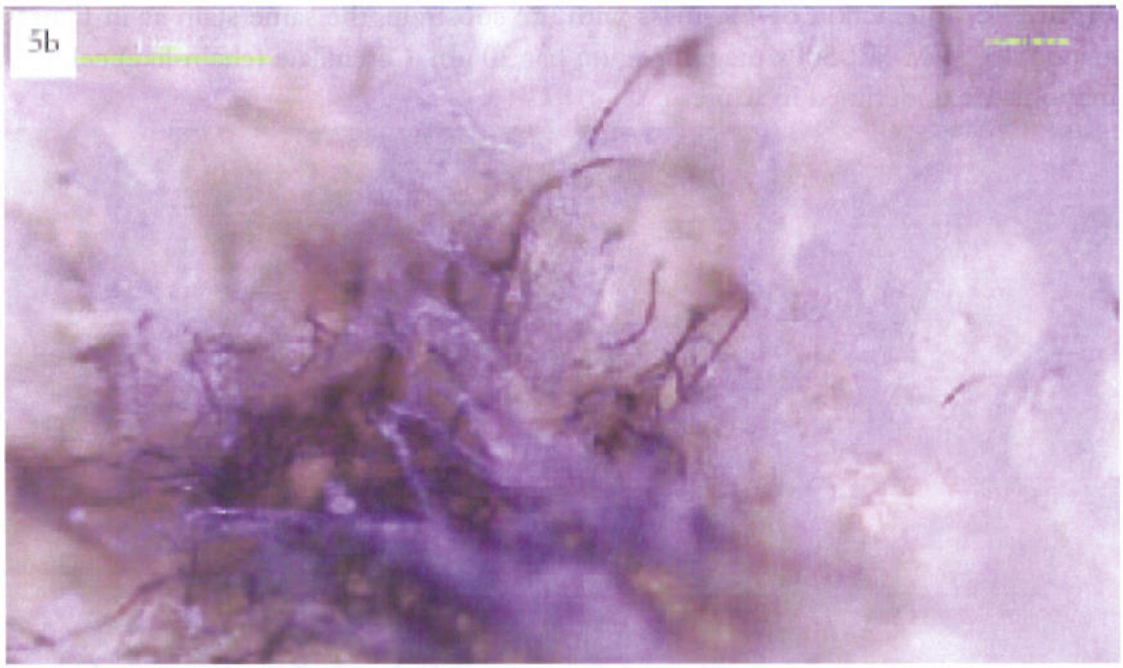
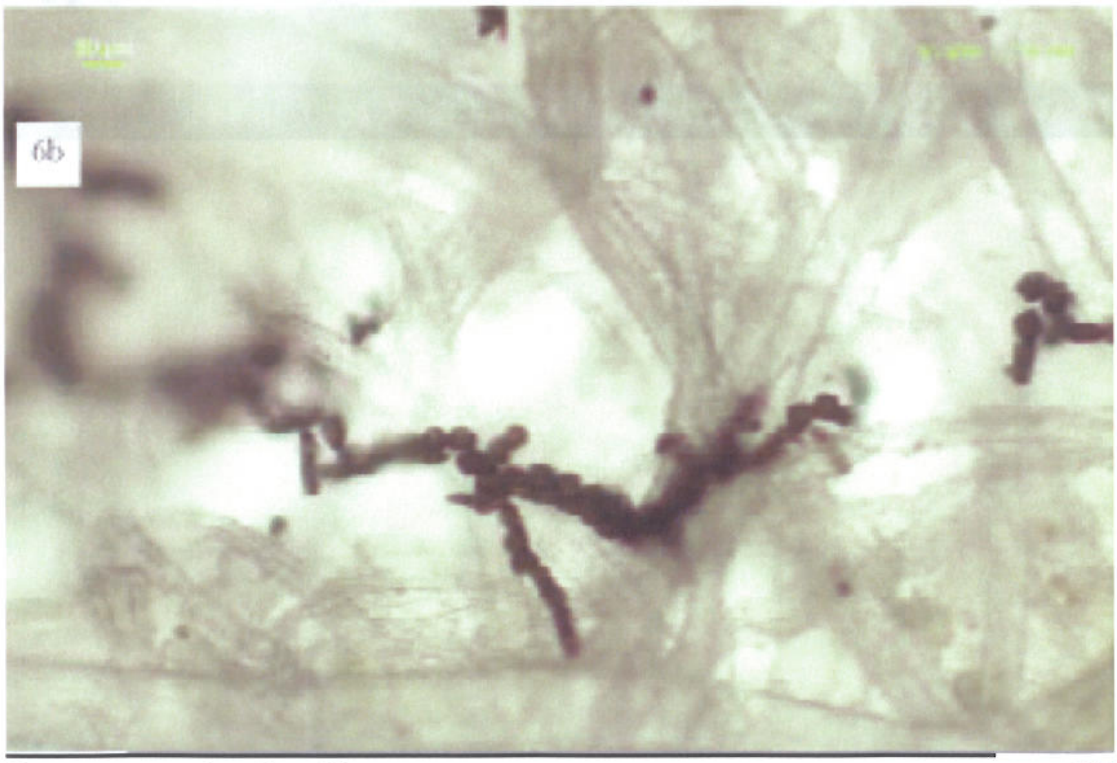


Figure 6b: The same stain as in figure 6a examined in transmitted light microscope, 400x; melanized dark brown cells are attached to paper fibers. Large pores in paper permit air supply necessary for the cells growth.



3. Bio-mass interaction with the substrate on a nano-scale was examined with the scanning electron microscopy in variable pressure. SEM-VP Hitachi S 3700N was used, magnification range: 500x to 3000x (Figs. 4c, 5c, 6c).

Figure 4c: Interaction of bio-mass with the substrate, the same stain as in figures 4a and 4b, SEM-VP, 500x magnification; bar 20 μm. Catenulate (chain-like) cell formations are underlined in white.

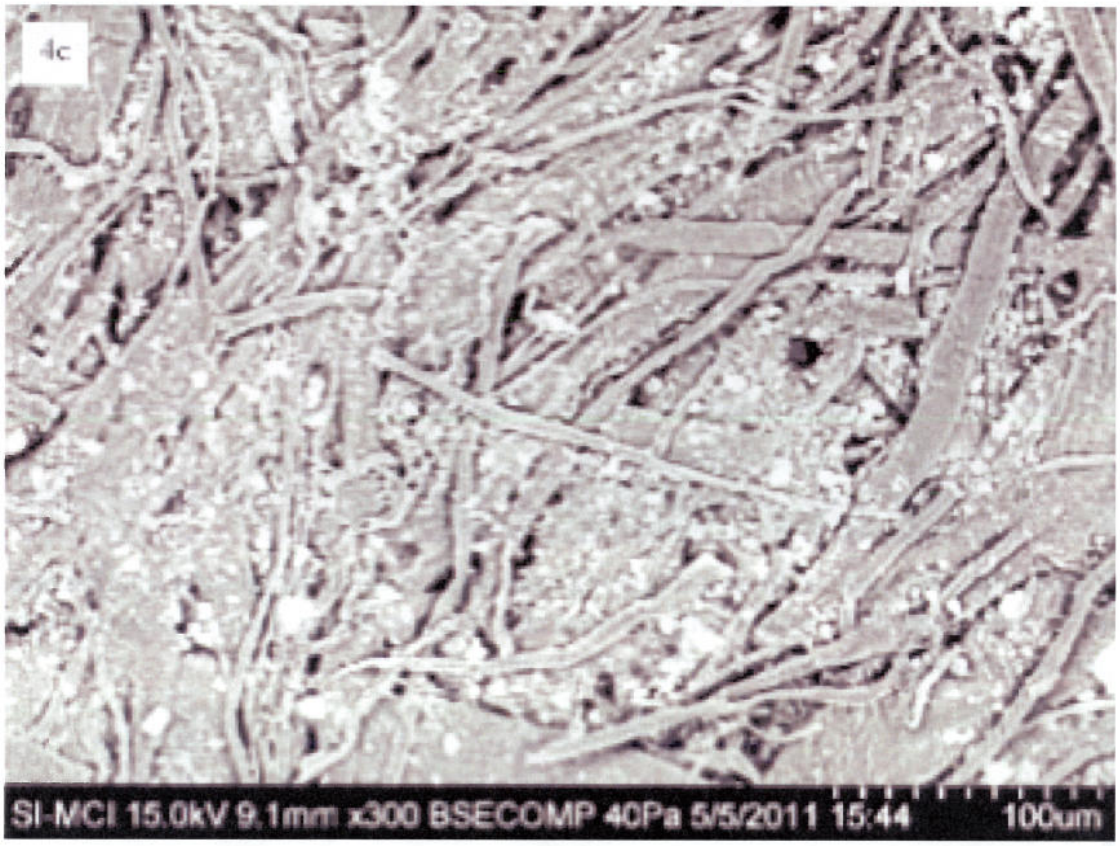


Figure 5c: The same specimen as in figures 5a and 5b SEM-VP 500x reveals characteristics of perithecial hairs, smooth walled, tubular, ca. 3μm in diameter.

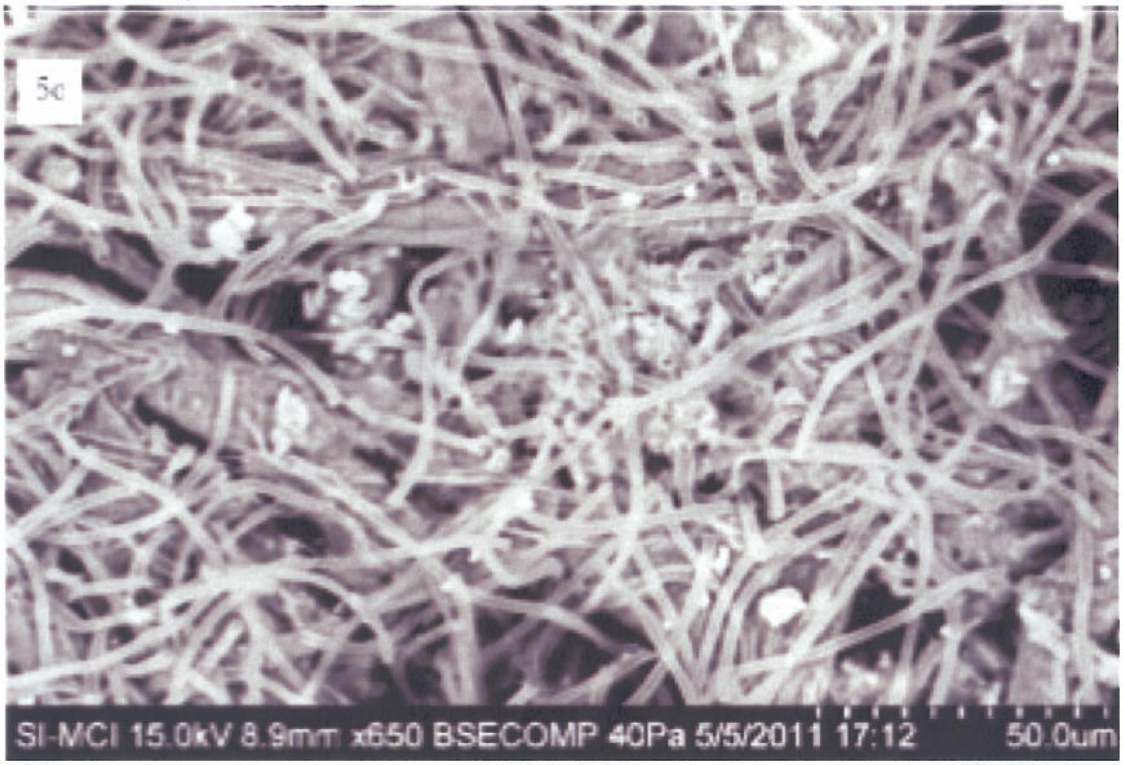
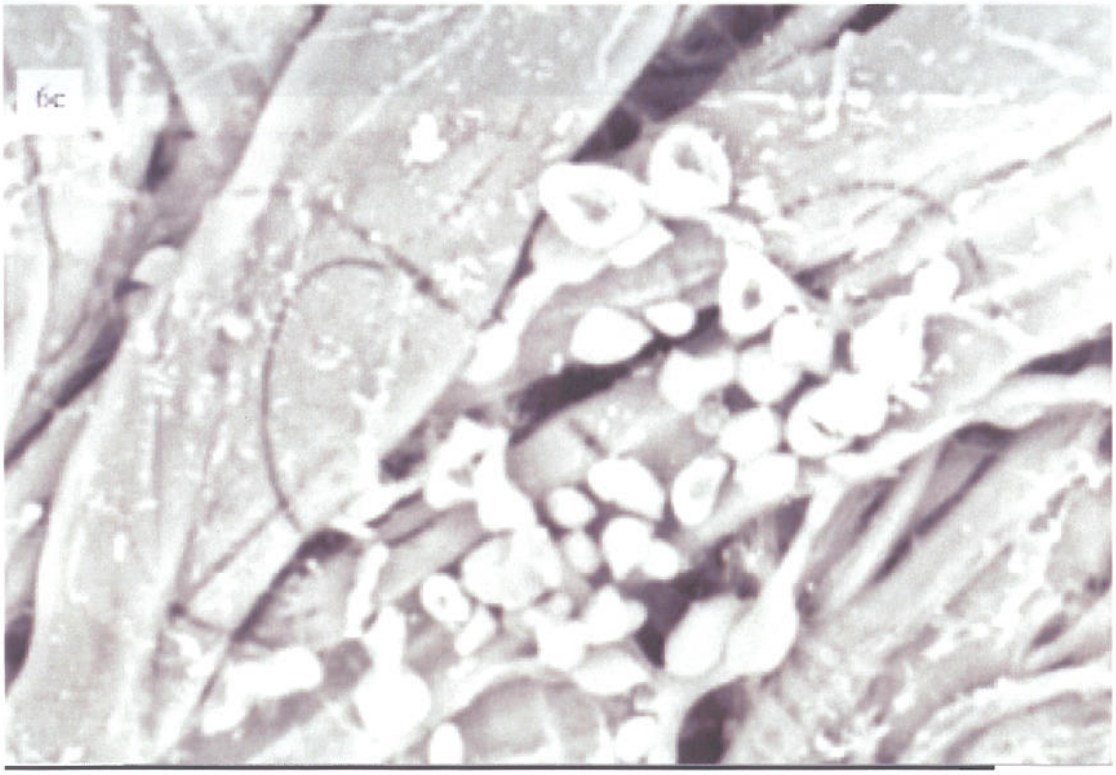


Figure 6c: The same specimen as in figures 6a and 6b SEM-VP, bar 4 μm; cells are clustered and attached to paper fibers; average cell dimensions: 5-9 μm.



4. Surface morphology and topography with and without bio-mass was measured and profiled with confocal laser scanning microscope (CLSM) Keyence VKX at magnification 1000x (Figs.4d, 5d, 6d).

Figure 4d: The same specimen as in figures 4a - c, 3D mapping and surface topography, area measured: x:200 μmy: 100 μm z: 40 μm. Keyence VKX Laser Scanning Microscopy, 1000x. Catenulate cells and cells clusters are marked in black against light grey paper fibers.

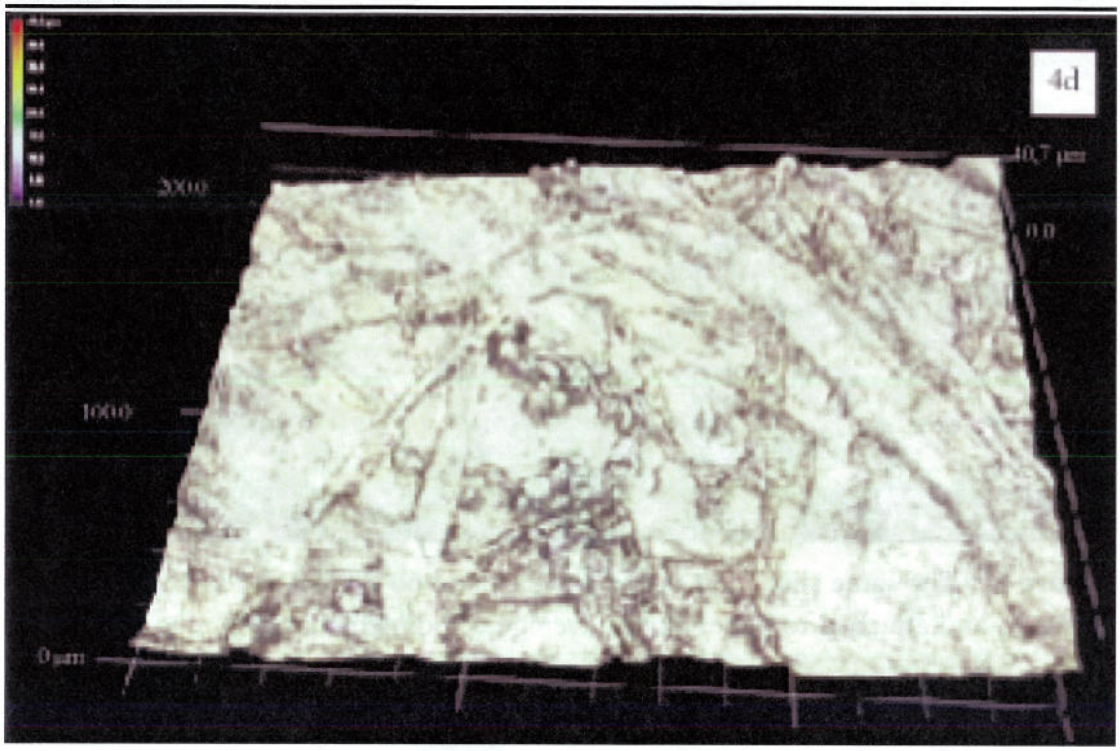


Figure 5d: Same specimen as in figures 5a - c, CLSM, the marked area was measured to produce profile of bio-mass deposit; the highest concentration of the biomass of perithecial hairs is marked with white-circle.

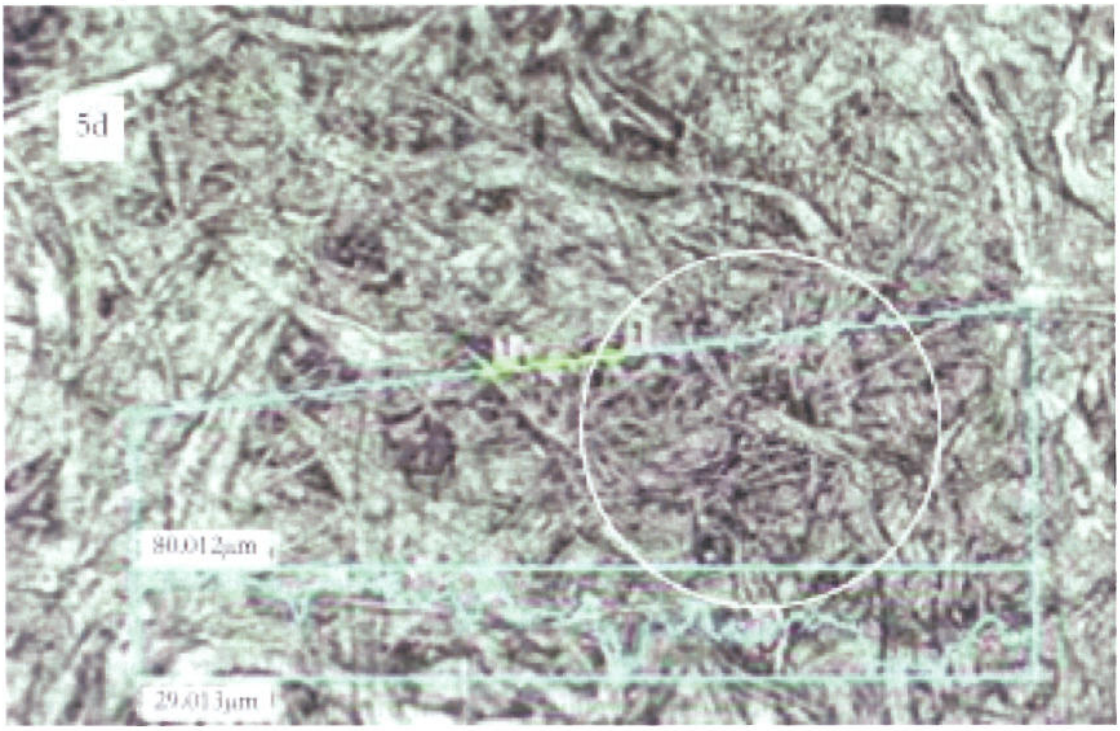
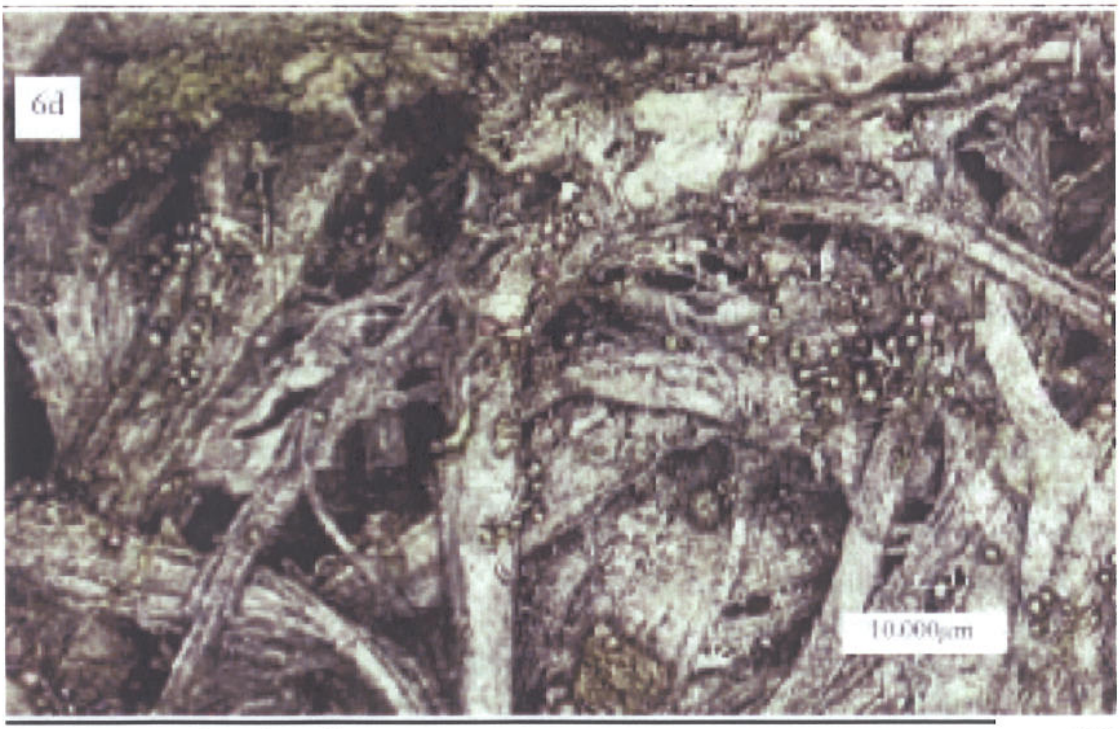


Figure 6d: Images of the stain seen in figures 6a - c, CLSM, 3D mapping of biomass distribution in relation to paper matrix. Cells are of light orange color against dark brown paper fibers. 1000x magnification, bar 10 µm.



Transmitted light and incidental light microscopy has been traditionally employed on biological specimens. Confocal laser scanning microscopy (CLSM) one of the surface metrology tools was developed for applications in high precision industrial environment for the detection of surface imperfections among many others. By scanning the entire surface with a short-wavelength laser, full-focus observation is achieved at resolutions that cannot be attained with an optical microscope. The most important advantage of using CLSM is the reproducibility of the measurements. By measuring the height, width, and cross-section of the fungal elements we obtained a profile capturing quantitatively the differences in surface conditions of both papers with and without fungal deposits.

RESULTS

Each mode of investigation, evaluation of surface topography, morphology, and profiling fungal deposits complemented each other revealing new aspects of fungus stains and their interactions with the substrate. Two species of fungi in the class Dothideomycetes, *Cladosporium sp.* and *Taeniolella sp.* formed thick-walled, pigmented mycelia and conidia, all embedded into the paper matrix. The growth pattern of *Taeniolella* indicated attachment of the hyphae to the fiber surfaces of the paper. However, in 25 of the stained samples there was no evidence of mycelia penetrating into the interior of fibers. The latter disagrees with what was proposed by Nugari (2008) who suggested that mycelium grows in the internal structure of paper fibers.

Distribution of the dark brown spores of *Cladosporium sp.* followed the pattern of pores' location in paper. The spores nested in paper surface indentations which provide natural shielding against their mechanical removal. *Chaetomium,* which produces large perithecial heads, reaching over 0.3-0.4mm in diameter, was embedded in the paper matrix. However, there was no indication that the fruiting structures secreted pigments into the substrate. In all studied cases, the pigmentation was confined to the fungal elements, such as spores, hyphae and elements of the fruiting structures; no pigmented extracellular material was observed as being secreted into the substrate.

Preventive treatment and conservation of artworks

Knowing that moisture is essential for the germination and growth of fungi, all efforts should be made to control the environment and prevent occurrence of water condensation on surfaces. Constant low temperature in the environment in which artworks are exhibited or stored is one of the preventive methods against microbial growth.

Pigmentation induced by microorganisms on cultural material is resilient to removal, or to its reduction of color intensity by chemical means. That is understandable because pigmented bio-polymers are produced in many cases as fungal defen-

se mechanism and cannot be dissolved in water or other solvents. Experiments with bleaching agents used in conservation for the reduction of discoloration and stains produce limited results. A mechanical removal of pigmented fungal structure, either fruiting bodies or spores is recommended only when no disturbance of the artwork occurs (Fig. 7) By mechanical removal it is understood that fungal elements are extracted with a surgical scalpel, tweezers or inoculating needle. Laser removal of pigmented fungal structures using 532 nm wavelength proposed in 1994 (Szczepanowska and Moomaw, 1994) was repeated in 2005 (Scholten, et al., 2005, Ochocinska-Komar et al., 2005; Pilch, et al., 2005) as another successful treatment option.

Figure 7: Removal of perithecia from the paper's surface. Weak ionic interfacial forces anchoring the bio-mass on the surface allow a mechanical separation of the bio-structures without disturbance of the original matrix.

Preliminary conclusions of work in progress

Detailed studies of fungus distribution in the various types of papers in our studies revealed a broad range of their interactions with the substrate. That diversity is directly related to the morphological characteristics of different fungal species, and the unique topography of each substrate on which they grew. Only partial understanding of the interfacial phenomena of the bio-pigments and substrates was gained using a broad range of analytical instruments. Maintaining the sequence of investigation, from gross (global) evaluation to more detailed (local) analysis was essential, as each technique provided complementary information. Combining optical light microscopy with surface metrological techniques and instruments, such as con-

focal laser scanning microscopy provided information about spatial inter-relations between the fungi and substrate, contributing information towards development of preventive and conservation strategy. However, insight into many unknown behavioral patterns of microorganisms, varied environmental conditions in which they grow and diverse composition of substrates is required to treat each case as a unique, multi-faceted problem. As the research continues more information will be available, elucidating this complex phenomenon of the interaction of fungi and their pigments with substrates.

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BIOAEROSOLS

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TABLE OF CONTENT:

Introduction and Overview 9

Conference Rational, Goals and Learning objectives 11

Climate Change, the Indoor Environment, and Health 17
David A. Butler

PART I:

Health Effects – Epidemiological Research

NIOSH Field Studies on Dampness and Mold and Related Health Effects ... 23
Jean M. Cox-Ganser

How to find, how to manage? Developing guidelines for mold problems through research. 30
Aino Nevalainen, Martin Täubel, Helena Rintala and Anne Hyvärinen

Hypersensitivity pneumonitis 37
E. Neil Schachter

Health Complaints, Lung Function, and Immunologic Effects in German Compost Workers from Long-term Exposures to Bioaerosols 46
Jürgen Bünger, Anja Deckert, Frank Hoffmeyer, Dirk Taeger, Thomas Brüning, Monika Raulf-Heimsoth, Vera van Kampen

Airborne workplace exposure to microbial metabolites in waste recycling plants 52
Stefan Mayer, Vinay Vishwanath, Michael Sulyok

Respiratory Health and Flood Restoration Work in the Post-Katrina Environment 61
Roy J. Rando, John J. Lefante, Laurie M. Freyder, Robert N. Jones

Mold species identified in flooded dwellings 68
Denis Charpin

Attributable fractions of risk factors of respiratory diseases among children in Montreal, Canada 73
Louis Jacques, Céline Plante, Sophie Goudreau, Leylâ Deger, Michel Fournier, Audrey Smargiassi, Stéphane Perron, Robert L. Thivierge

Fungi and chronic rhinosinusitis (CRS): Cause and effect 89
E B Kern, J U Ponikau, D A Sherris and H Kita

Rhinosinusitis and mold as risk factors for asthma symptoms in occupants of a water-damaged building 101
Ju-Hyeong Park

Conclusions on health implications of airborne molds: Analysis of airborne molds in 11 contaminated houses using a new method of evaluation 108
Urban Palmgren, Judith Müller

Observational Epidemiology and Water Damaged Buildings 118
Joseph Q. Jarvis, and Philip R. Morey

Molds and Mycotoxins: Factors That Affect Exposure and Contribute to Adverse Health Effects 125
Karin K. Foarde, Timothy Dean, Doris Betancourt, Jean Kim, Anthony Devine, Grace Byfield, and Marc Menetrez

Does Reversibility of Neurobehavioral Dysfunction by Monosodium Luminol have Diagnostic and Possible Therapeutic Use in Mold/Mycotoxin Exposed Patients? 130
Kaye H. Kilburn

PART II:

Assessment & Remediation

Associations between ventilation and mycological parameters in homes of children with respiratory problems 148
Hans Schleibinger, Daniel Aubin, Doyun Won, Wenping Yang, Denis Gauvin, Pierre Lajoie

The Penetration of Mold Into Fibrous HVAC Insulation Makes Cleaning Impossible 156
Thomas G. Rand and Phil Morey

Past, Presence and Future of Immunoassays for Mycotoxin Testing 163
Erwin Maertlbauer

Health risks of surface disinfection in households with special consideration on quaternary ammonium compounds (QACs) 174
Axel Kramer, Harald Below and Ojan Assadian

Toxicity study of field samples from water damaged houses in flooded areas in Poland 185
Magdalena Twarużek, Jan Grajewski, Manfred Gareis

Mycotoxin screening of indoor environments in sentinel health investigations 195
Eckardt Johanning, Manfred Gareis.

A Comparison of two Sampling Media (MEA and DG18) for Environmental Viable Molds 201
Sirkku Häkkilä

Evaluation strategy in damp buildings and use of infrared thermography.... 207
Yves Frenette

Prevalence of mold observations in European housing stock 215
Ulla Haverinen-Shaughnessy

Indoor Molds and Respiratory Hypersensitivity: A Comparison of Selected Molds and House Dust Mite Induced Responses in a Mouse Model 222
Marsha D W Ward, Yong Joo Chung, Lisa B Copeland, Don Doerfler and Stephen Vesper

What does the development of fungal systematics mean to DNA-based methods for indoor mold investigations? 236
De-Wei Li and Chin S. Yang

Case study: Determination of moisture damages on items of art in exhibitions by the use of microbial analysis 246
Judith Mueller and Urban Palmgren

Investigation of effectiveness of mold disinfectants and chemicals on total cell numbers of mold on building materials 253
Judith Mueller and Urban Palmgren

Microbiological characterization of aerosols isolated from remote lakes in the Chilean Patagonia 259
Escalante G, León C, Campos V, Urrutia R and Mondaca MA.

Use of Culture and PCR Analysis in Mold Assessments 263
Philip R. Morey

Factors promoting the exposure to bioaerosols among swiss crop workers .270
Hélène Niculita-Hirzel and Anne Oppliger

The investigation of mold occurrence inside selected Durban homes 280
Nkala BA, Jafta N and Gqaleni N

Conserving our cultural heritage: the role of fungi in biodeterioration 293
Hanna Szczepanowska and A. Ralph Cavaliere

PART III:

Prevention / Public Health

Risk and Hazard Assessment of Molds Growing Indoors 311
Harriet M. Ammann

Recommendations for detection and remediation of mold growth in indoor environments in Germany.....328
Christiane Baschien, Heinz-Jörn Moriske, Kerstin Becker, Marike Kolossa-Gehring and Regine Szewzyk

Indoor water and mold damage - investigation and decontamination practices in Germany.....336
Wolfgang Lorenz

Update of Canadian and International Mold Guidelines and Standards 347
Donald M. Weekes

Defining "Clean" in terms of the unseen fraction: A Representative Marker in Schools.....359
Richard Shaughnessy, Eugene C. Cole, Ulla Haverinen-Shaughnessy

Effective risk communication concerning environmental change with communities and patients exposed to excessive indoor moisture or mold 363
Paula Schenck and Robert DeBernardo

Critical Issues in Art Conservation After A Water Intrusion Event: Pitfalls of The Emergency Response 372
Karen H. Kahn

INDEX

Index: Fungi and Bacteria .....

Index .....

Authors .....