AIRBORNE TRANSPORT OF BACTERIAL SPORES AND FUNGAL CONIDIA ON NATURAL AND MAN-MADE FIBERS

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Abstract

Bacteria and fungi together with the structures and substances they produce may exert a harmful influence upon exposed individuals leading to numerous adverse health outcomes. As far as a transport of microbial agents on dust particles is relatively widely studied, a role of fibers as their carrier is not well recognized. Natural and man-made fibers' structure and arrangement supporting such transport were described based on samples derived from both occupational and non-occupational environments. The ability of fibers to convey microbial propagules loaded with different number of elementary charges in both dry and humid air was studied in newly built aerosolization chamber. The results showed that from 1g of fibers in natural state is possible to isolate up to 9×10^5 culturable microbes, including pathogens. Tested fibers (especially natural ones) were able to transport in the air up to 100 bacterial spores or fungal conidia per 1 fiber.

Key words: *fibers, microorganisms, electrical charge, airborne transport, air humidity*

1. INTRODUCTION

Fibers are ubiquitous in the environment. They may arise from different sources including those of natural and man-made origin. Inorganic fibers can naturally derive from the soil and rocks. They have widespread uses in construction, power, automotive, shipbuilding, and aerospace industries. Fibers can be also abundant in plastics, textile and other numerous industry branches being available as organic and inorganic ones from both natural and man-made sources. As such, the exposure to fibers may happen in both occupational and non-occupational environments (Kulkarni et al. 2011; Lippmann 1990; Mauderly et al. 2000; Moeller 2005; Schneider et al. 1983; Wang 2005).

The term "fiber" is usually applied to a broad variety of non-spherical particles having an elongated shape. From the physical point of view, the behavior of fibers suspended in the air is a function of their dimensions, especially length and diameter. Fibers are considered to be elongated structures with a length/diameter (aspect) ratio of greater than 3:1 (Kulkarni et al. 2011). As inhalation is the most important route of exposure to fibers, the respirable ones are usually in the center of attention. The World Health Organization has defined a respirable fiber as a particulate with length greater than 5µm, diameter smaller than 3µm and aspect ratio of 3:1, at least (Foster & Costa 2011; Ruzer & Harley 2013). Fiber dimensions are known to impact its pathogenicity. Inhaled particles may be deposited within respiratory tract by five mechanisms: sedimentation, impaction, diffusion, interception, and electrostatic precipitation (Mauderly et al. 2000). Interception is likely to be the most effective deposition mechanism for fibers. For such shapes, deposition can occur when particles contact an airway wall although its center of mass remains on a fluid streamline. Such elongated particle orientation is a critical factor describing interception. The probability of interception increases as an airway diameter decreases, but it can also be fairly significant in both the upper respiratory tract (e.g. larynx) and upper tracheobronchial tree. While interception probability grows with increasing fiber length, the aerodynamic behavior of a fiber and the probability of impaction or sedimentation are influenced by fiber dimension (Sbirlea-Apiou et al. 2010, Schlesinger 2000). The respirability of fibers is also largely depended on density and cross-sectional area. Fibers with aerodynamic equivalent diameters between 5µm and 10µm can deposit in the larger airways. Those less than 5µm can reach the terminal bronchioles and alveoli. This latter aerodynamic diameter is equivalent to a fiber diameter of approximately 3µm (Mauderly et al. 2000). So far, experimental fiber deposition data in humans are

not available. Studies in animals supported by mathematical and physical models revealed that long fibers (>10μm) tend to show enhanced deposition in the tracheobronchial region and reduced deposition in the pulmonary region, compared to shorter fibers. However, fibers which are very long (>50μm) and thin (<0.5μm) can reach distal conducting airways being deposited in the alveolar region. The fiber shape is also important in terms of their deposition as straight fibers penetrate more distally than curly ones (Foster & Costa 2011; Schlesinger 2000).

The fiber pathogenicity depends on three critical features, i.e. dose, dimension, and durability. In case of studies relating the incidence of disease, the establishment of dose is usually based on quantitative estimates of external (to the body) exposure using air monitoring. This generally only gives a proxy measure of the internal dose (supported usually by mathematical models), which is what would lead to a pathological effect. The issue of dimension relates to both the ability of a fiber to penetrate the distal lung and deposit in the alveolar region as well as the ease with which alveolar macrophages can clear material from this region of the lung. Long fibers may be removed rapidly from larger airways of the lung by the mucociliary system, which propels fibers and mucus out of the respiratory system. Small fibers (like other small particles), if deposited in the proximal alveolar region, are phagocytosed with ease by alveolar macrophages, either persisting within the macrophage if durable or moved up onto the mucociliary regions and ejected from the lung. Fibers deposited in non-ciliated regions must be dealt with by alveolar macrophages. When a fiber is longer than maximal length that a macrophage can phagocytose (~15μm to 20μm), the whole process may not be successful. Such so-called "frustrated phagocytosis" leads to the release of various toxic components, which can damage surrounding cells and cause inflammation. Inflammation may have both positive (if they activate inflammatory cells to heal the body) and negative results (if such process is uncontrolled and occurring repeatedly). If the deposited fibers cannot be cleared by macrophages, they may persist in the alveoli or may translocate out of the lung, where they may retain and drive a pathogenic response. Finally, fiber durability is defined as ability to resist damage or modification that would substantially alter its structure in a biological environment. Exposure to a biopersistent fiber, i.e. fiber that will not break, split or dissolve in the lungs, may lead to severe pathological effects (Foster & Costa 2011; Ruzer & Harley 2013).

Among airborne particles in both occupational and non-occupational environments, their substantial amount is always composed of those of microbiological origin. Among them can be single bacterial cells or spores, single fungal conidia, their microbial aggregates of different shapes and sizes as well as agglomerates built from both biological and non-biological (natural, synthetic or semi-synthetic particulate or fiber) constituents. In case of filamentous fungi and certain bacterial groups such as *Actinomycetes*, the environmental forms of their propagules may physically resemble and behave in the air like fibers (Fig. 1). For example, microscopic observations revealed that *Aspergillus versicolor* and *Penicillium melinii* having thin and long conidiophores, can release into the air long "fiber-like" chains composed of up to 60–80 single spores, whereas other filamentous fungi like *Cladosporium cladosporioides* having different hyphae structure with short and thick conidiophores can produce shorter (up to ~30 elements) spore chains (Górny et al. 2001).

a b

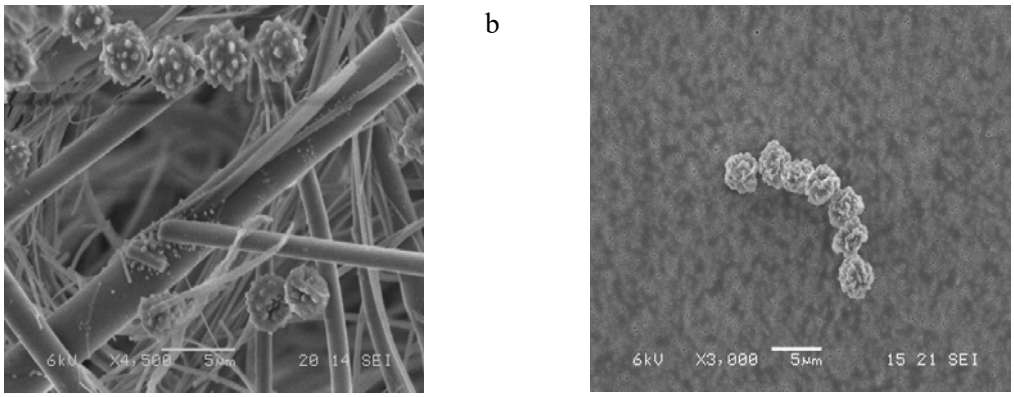


Fig. 1. Scanning electron microscope photographs of fungal spores released into the air from the colonies growing on malt extract agar: "fiber-like" spore chains of *A. versicolor* (a) and *P. melinii* (b).

So far, the coexistence of natural, synthetic or semi-synthetic fibers and microbial particles in aerosol state did not obtain special studies dedicated to this topic. In the scientific literature, there are no results of the research devoted to fibrous aerosols as a means of transport of microbial particulates either in the working or other non-industrial environments. Also, the health effects associated with an exposure to such fibro-microbiological aerosols are not so far fully characterized. Considering the above, the aims of this study were to evaluate the possibility of transport of microbiological particles by natural, synthetic and semi-synthetic fibers as well as to assess the potential of those fibers to carry bacterial and fungal particles when loaded with different number of elementary charges in both dry and humid air.

2. MATERIALS AND METHODS

The sampling was carried out at: three industrial facilities processing natural (cotton, silk, flax, hemp, wool) and synthetic (polyamide, polyester, polyacrylonitrile, polypropylene, viscose) fibrous materials, at four homes where dogs or cats were kept, one stable where horses were bred and one zoo pavilion where lions were kept. At each of these facilities, fibrous aerosol was sampled (in duplicates) using 37-mm open-faced cassette connected with APEX pump (Casella CEL, Bedford, Great Britain) on sterile Teflon filter with pore size of 2µm (PTFE, SKC – Omega Specialty Division, Eighty Four, PA, USA) at 4L/min during "routine" activities, i.e. during final stages of fibrous material manufacturing cycle, horse currying and cat, dog or lion grooming. Each time, the sampler was placed at a height of 1.0–1.5m above the floor level to simulate aspiration from the human breathing zone and $3m^3$ of the air was filtered. All man-made and natural fiber samples were weighed (on analytical balance; ABJ 220-4M, KERN & SOHN GmbH, Balingen, Germany) and laboratory analyzed for their microbiological purity. At the beginning, all fibers were washed out from the filters using 10ml of 0.9% NaCl solution (Baxter Manufacturing Sp. z o.o., Lublin, Poland) with 0.05% additive of Tween 80 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) by 1h vortexing (Promax 1020, Heidolph Instruments GmbH & Co., Schwabach, Germany) in a room temperature. The resulted suspensions were subsequently diluted (from 10^{-1} to 10^{-6}) and 0.1ml of each dilution (in triplicates) was spread evenly over the following microbiological media: a) blood tripticase soy agar, TSA (bioMérieux SA, Marcy-l'Etoile, France) – for aerobic mesophilic bacteria, b) eosin-methylene blue agar, EMB (BTL, Łódź, Poland) – for aerobic Gram-negative bacteria, c) blood Schaedler's agar (bioMérieux SA) – for anaerobic bacteria, d) ISP Medium 2 agar (Becton-Dickinson Microbiology Systems, Sparks, MD, USA) – for actinomycetes, and e) malt extract agar, MEA (BTL) – for fungi. Then, the plates with bacteria were incubated for 1 day at 37°C followed by 3 days at 22°C and another 3 days at 4°C. The plates with fungi were incubated for 4 days at 30°C followed by 4 days at 22°C. After incubation, the qualitative and quantitative analyses of growing microorganisms were performed. The bacterial and fungal concentrations were expressed as colony-forming units per 1g of fibers (cfu/g). Bacterial strains were identified by Gram staining, their morphology, and finally by the biochemical API tests (bioMérieux SA). Fungi were identified according to their morphology using several identification keys: Barnett & Payne (1986), Domsch et al. (1995), Fisher & Cook (1998), Klich (2002), Krzyściak et al. (2011), Pitt (2000), Samson et al. (2004), and St-Germain & Summerbell (2011).

To check the structure and arrangement of fibers that may support transport of microbial particles, a scanning electron microscope (SEM) analysis was performed on each second sample obtained at each sampling point. In this study, three different microscopes were used: "table-top" type SEM (TM-3030; Hitachi, Tokyo, Japan), low-vacuum and low acceleration voltages type SEM (SU3500; Hitachi), and ultra-high resolution type SEM (SU8010; Hitachi). After sampling, if necessary, the material gathered on filter was coated with gold or carbon (SC7620 Sputter Coater, Quorum Technologies Ltd., Lewes, Great Britain).

Based on the results of the "cultivation" phase of the study, the abilities of 4 natural (cotton, hemp, cat, and horse) and 2 man-made (polyester and viscose) fibers to transport bacterial (*Streptomyces albus*) spores and fungal (*Aspergillus versicolor*) conidia loaded with elementary charges in dry (RH<30%) and humid (RH>60%) air were analyzed in laboratory conditions using newly built aerosolization chamber (patent pending). The humidity conditions within the chamber were regulated using

ultrasonic fogger (M1, Happet, Luboń, Poland) and real-time controlled by thermohygrometer (Omniport with RH/T probe V/T, E+E Elektronik GmbH, Engerwitzdorf, Austria). Each of microbial aerosols was loaded with elementary charges (–4500V, –3000V, –1500V, –500V, 0V, +500V, +1500V, +3000V, +4500V; Mainelis et al. 2001) using Topas electrostatic aerosol neutralizer (EAN 581, Topas GmbH, Dresden, Germany) and generated (for 10min) into the chamber from water suspension by Collison nebulizer (MRE CN25, BGI Incorporated, Waltham, MA, USA) or from contaminated agar surface using tube generator (patent pending). Bioaerosol concentrations were measured with optical particle counter (1109, GRIMM Aerosol Technik GmbH & Co. KG, Ainring, Germany). In the chamber, each of the tested strains was subsequently mixed (for 10min at air stream velocity typical for indoor environment, i.e. 0.3m/s as measured with Omniport with V/T probe; E+E Elektronik GmbH) (Górny et al. 2001) with each of the studied fibers (their concentrations were controlled using real-time fiber monitor; 7400AD, MSP Corporation, Shoreview, MN, USA). Such mixture was sampled (in duplicates) for 5min at 5L/min with IOM cassette on polycarbonate filter with a pore size of 0.8µm (SKC Inc., Eighty Four, PA, USA). After sampling, all collected fibers carrying microbial particles were washed by vortexing from the filter using 0.9% NaCl solution with Tween 80. Spread plate method was applied to assess culturable bacterial or fungal concentrations in such obtained suspensions using earlier described methodology. Finally, the number of culturable bacterial spores or fungal conidia loaded with different number of elementary charges and transported on natural (animal and plant), synthetic as well as semi-synthetic fibers in dry and humid air was calculated and expressed as colony-forming units per 1 fiber (cfu/f).

All collected data were statistically elaborated using analysis of variance (ANOVA) followed by Scheffe's test, as well as t-test and correlation analysis using Statistica (data analysis software system) version 10—2011 (StatSoft, Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

3.1. Microbiological contamination of fibers

Both animal and plant fibers were microbiologically polluted (Fig. 2); whereas all five tested synthetic and silk fibers were free from such contamination. Among animal fibers, the highest microbial load (ANOVA: $p<0.01$) had horse hair (up to $9\times10^5/5.3\times10^3$ and 6.3×10^4cfu/g for aerobic/anaerobic bacteria and fungi, respectively), then lion fur (up to $7.1\times10^5/1.2\times10^3$ and 1.5×10^4cfu/g), dog coat (up to $3.3\times10^5/9.1\times10^4$ and 4.3×10^3cfu/g) and cat fur (up to $4.5\times10^3/1\times10^0$ and 2.5×10^3cfu/g). The less contaminated was sheep fleece (up to $3\times10^2/2.3\times10^2$ and 2.3×10^2cfu/g).

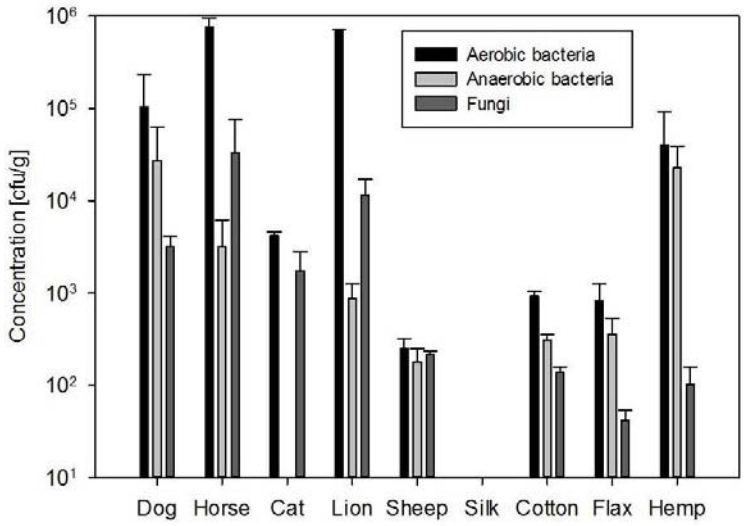


Fig. 2. Microbial contamination of animal (dog hair, horse hair, cat fur, lion fur, sheep fleece, silk fiber) and plant (cotton, flax, hemp) fibers.

From among the plants, the most contaminated were hemp (up to $7.7\times10^4/3.4\times10^4$ and 1.4×10^2cfu/g), flax (up to $1.1\times10^3/4.8\times10^2$ and 5×10^1cfu/g) and cotton fibers (up to $9.2\times10^2/3.1\times10^2$ and 1.4×10^2cfu/g); however, those differences were not statistically significant (ANOVA: $p>0.05$).

Quantitative analysis of fibers' microbiota revealed that majority of isolated microorganisms was aerobic. Among animal fibers, those microbes constitute 98% isolates of lion fur, 95% of horse hair, 77% of dog hair, 71% of cat fur and 39% of sheep fleece. Among plant fibers, the percentages were as follows: 67%, 64% and 57% for flax, cotton and hemp fibers, respectively (Fig. 3). The most frequently present on tested fibers were aerobic endospore-forming (hemp, cotton, wool) and non-sporing Gram-positive rods (wool, dog, horse), Gram-positive cocci (flax, horse) and Gram-negative rods (flax, cotton, wool, lion, cat, sheep); however, from 0.8% (horse) to 43.3% (hemp) and from 0.1% (hemp) to 10.3% (cotton) of microbiota were constituted by anaerobic bacteria and fungi (mainly molds), respectively.

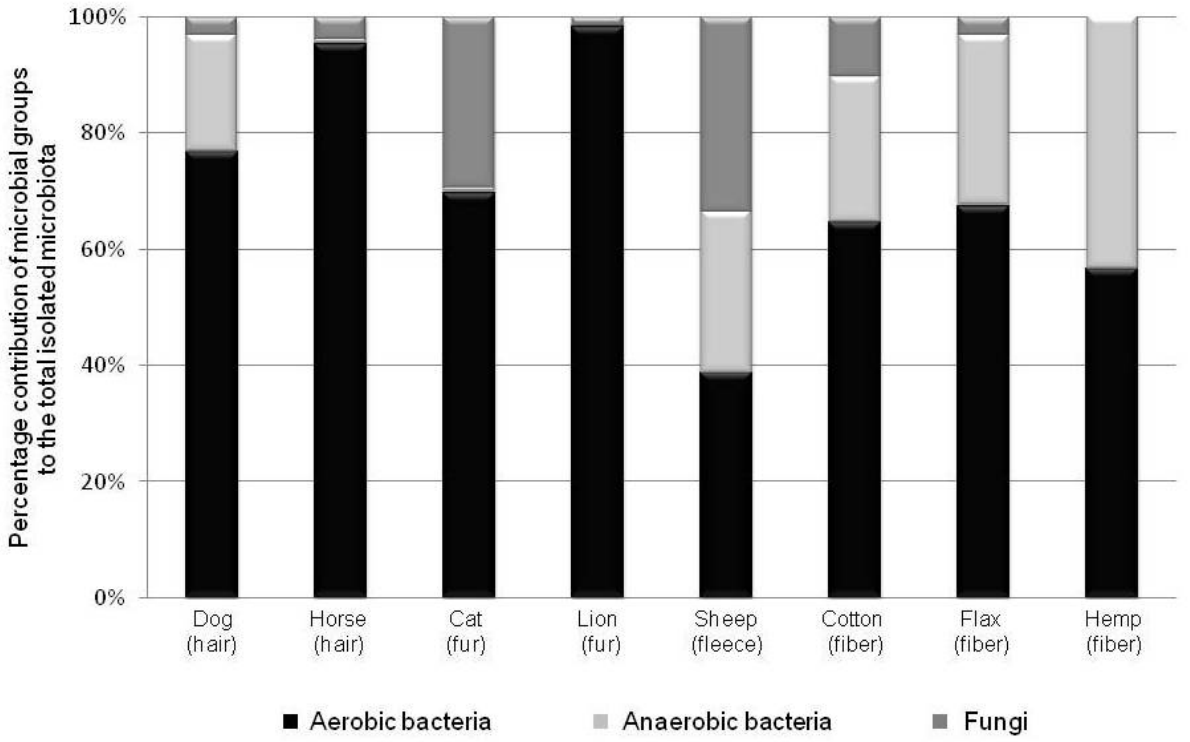


Fig. 3. Percentage contribution of microbial groups to total culturable microbiota isolated from animal (dog hair, horse hair, cat fur, lion fur, sheep fleece) and plant (cotton, flax, hemp) fibers.

As it was shown in this study, the microbiota present on natural fibers comprised wide spectrum of species, including pathogenic strains as classified by the Directive 2000/54/EC and the Ordinance of Polish Minister of Health to hazard group 2 (see Tab. 1). Among different structural components of microbial colonies, spores or conidia are usually the hardest ones and, due to a relatively impermeable chitin-containing wall, represent the best protected parts of the colony structure. These dormant components are extremely resistant to environmental stresses like desiccation, radiation damage (e.g. ultraviolet light from the sun) as well as man-made or naturally produced toxins and are capable of surviving harsh conditions for long periods (even for more than 20 years, like it was proven in case of *Aspergillus* or *Penicillium* conidia) (Gravesen et al. 1994; Haines et al. 1999; Macher 1999; Mandrioli

et al. 2003). Some of them, if present in the environment, can be attached to the fibers and transported in the air as so-called "firm tandems". Taking into account their above described features, bacterial spores of *Streptomyces albus* and fungal conidia of *Aspergillus versicolor* were selected for the subsequent experiments aimed to test the effect of electrical charges on fibers' ability to transport microbial particles in different humidity conditions (see below).

Fiber type	**Pathogenic microorganisms from hazard group 2**
Dog (hair)	*Streptomyces albus*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Aspergillus fumigatus*
Horse (hair)	*Actinomyces naeslundii*, *Aspergillus fumigatus*
Sheep (fleece)	*Actinomyces naeslundii*, *Aspergillus fumigatus*
Lion (fur)	*Actinomyces naeslundii*, *Actinomyces* spp., *Aspergillus fumigatus*
Cotton	*Actinomyces naeslundii*

Table 1. Pathogenic actinomycetes and filamentous fungi isolated from animal (dog hair, horse hair, cat fur, lion fur, sheep fleece) and plant (cotton, flax, hemp) fibers. All pathogens are classified to the hazard group according to the Directive 2000/54/EC and the Ordinance of Polish Minister of Health.

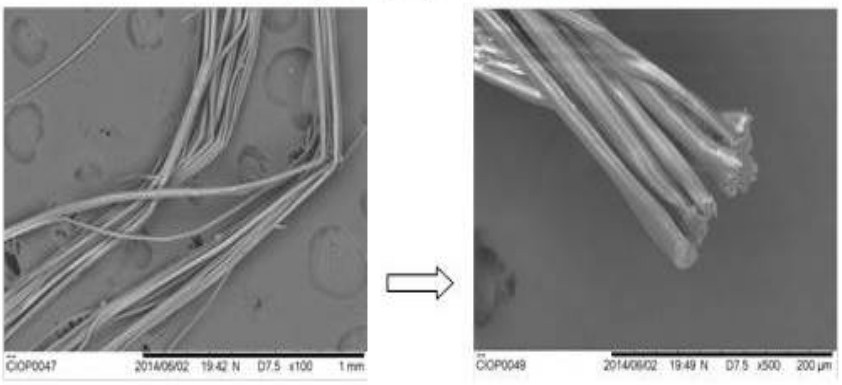
3.2. Structure and arrangement of fibers supporting microbial transport

The arrangement and structure of 14 natural (animal and plant), synthetic and semi-synthetic fibers was studied using different SEMs. It was revealed that all synthetic and silk fibers "naturally" occurring in occupational environment have loose packing and smooth surfaces what in practice substantially reduces or even eliminates the possibility of deposition of microorganisms. In case of animal fibers, although their natural arrangement is also typically loose, due to rough, lamellated surface structure they are able to transport significant amounts of material including those of microbial origin. On the other hand, among the plant fibers, both the arrangement (sheaf packing as in the case of flax and hemp or twisted ribbon as in the case of cotton) and the surface structure (septated as in the case of flax or corrugated as in the case of cotton) play a key role in transport of microbiological particles. As it was shown, natural fibers with loose packing and rough surface (i.e. wool, horse hair, lion fur), sheaf packing and septated surface (e.g. flax, hemp) or present as twisted ribbons with corrugated surface (cotton) were able to carry up to $9\times10^5/3.4\times10^4$ and 6.3×10^4 cfu/g of aerobic/anaerobic bacteria and fungi, respectively. Despite having longitudinally indented surface, semi-synthetic (viscose) fibers have not been able to carry microbial propagules. A few examples of structure and arrangement of natural (animal and plant), synthetic and semi-synthetic fibers are given in Figure 4.

Taking into consideration a degree of microbial contamination of studied fibers as well as a heterogeneity of microbiota present on them, for the subsequent experiments aimed to test the effect of electrical charges on ability to transport microbial particles in different humidity conditions the following fibers were selected: a) from animal ones – dog and horse hair; b) from plant ones – hemp and cotton fibers, and c) from synthetic and semi-synthetic – polyester and viscose fibers.

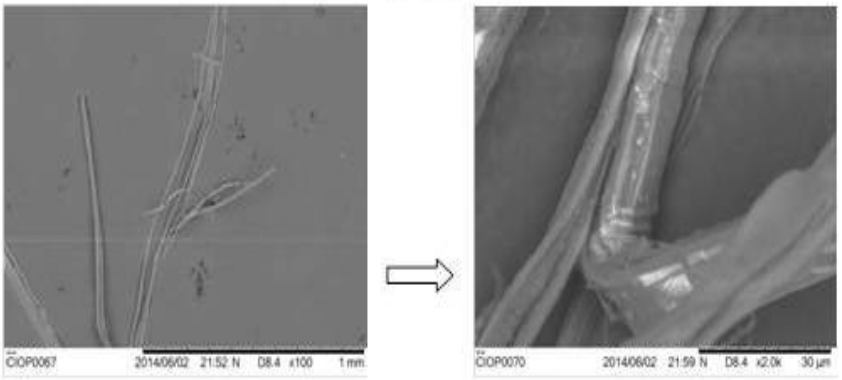
Flax

Sheaf packing/septated structure



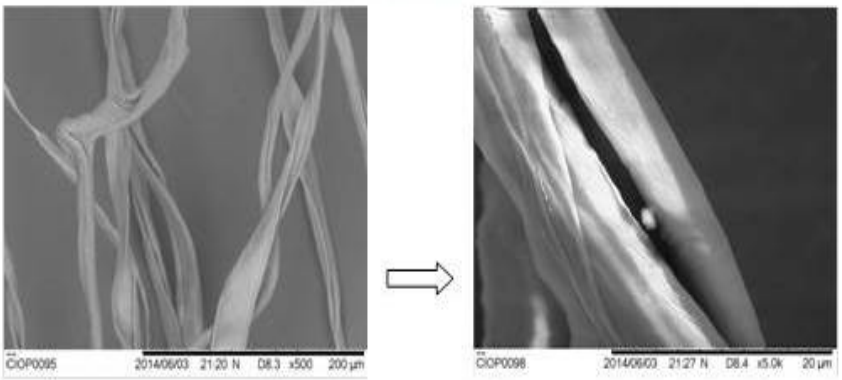
Hemp

Sheaf packing/septated structure



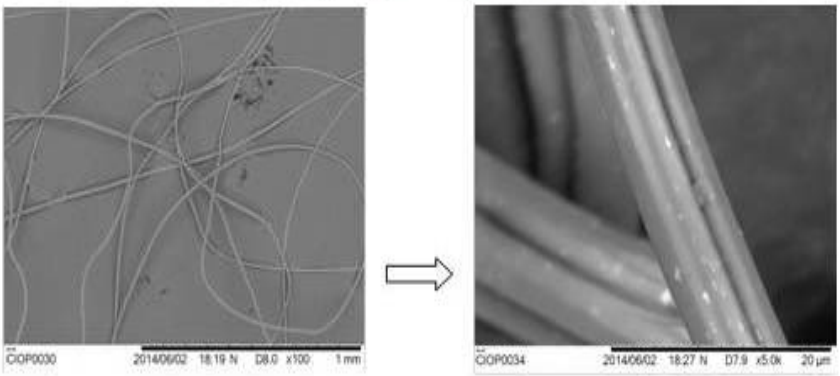
Cotton

Twisted ribbon/corrugated surface



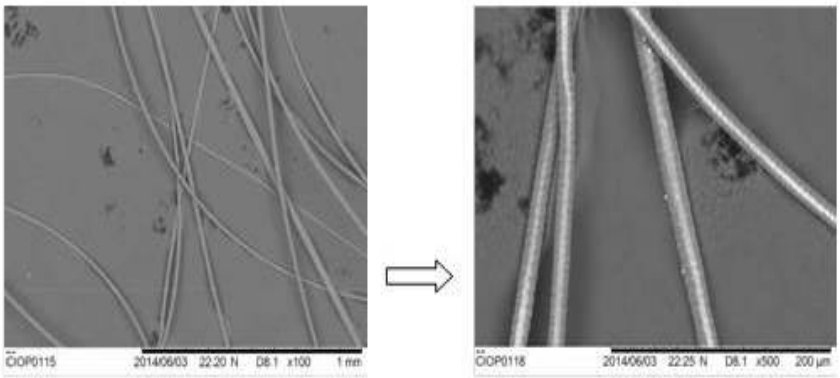
Loose packing/longitudinally indented surface

Viscose



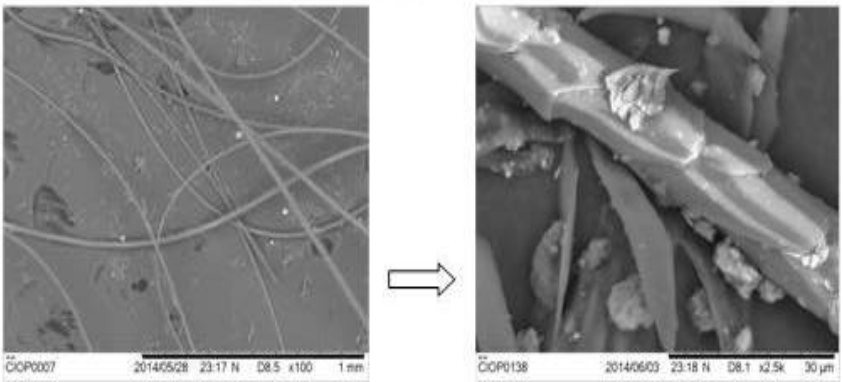
Loose packing/rough surface

Cat

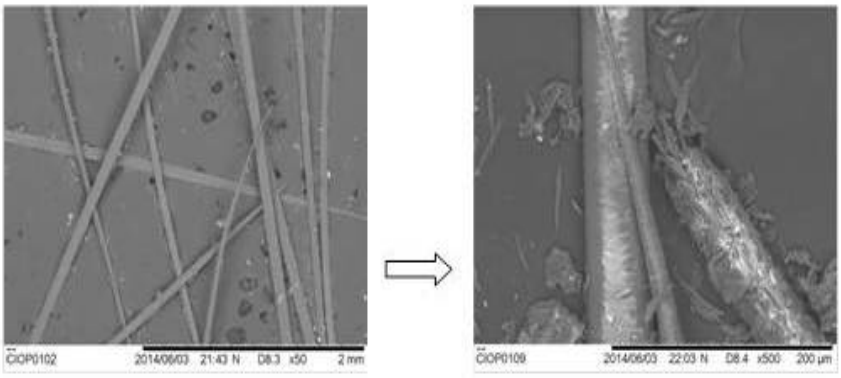


Loose packing/rough surface

Dog



Horse



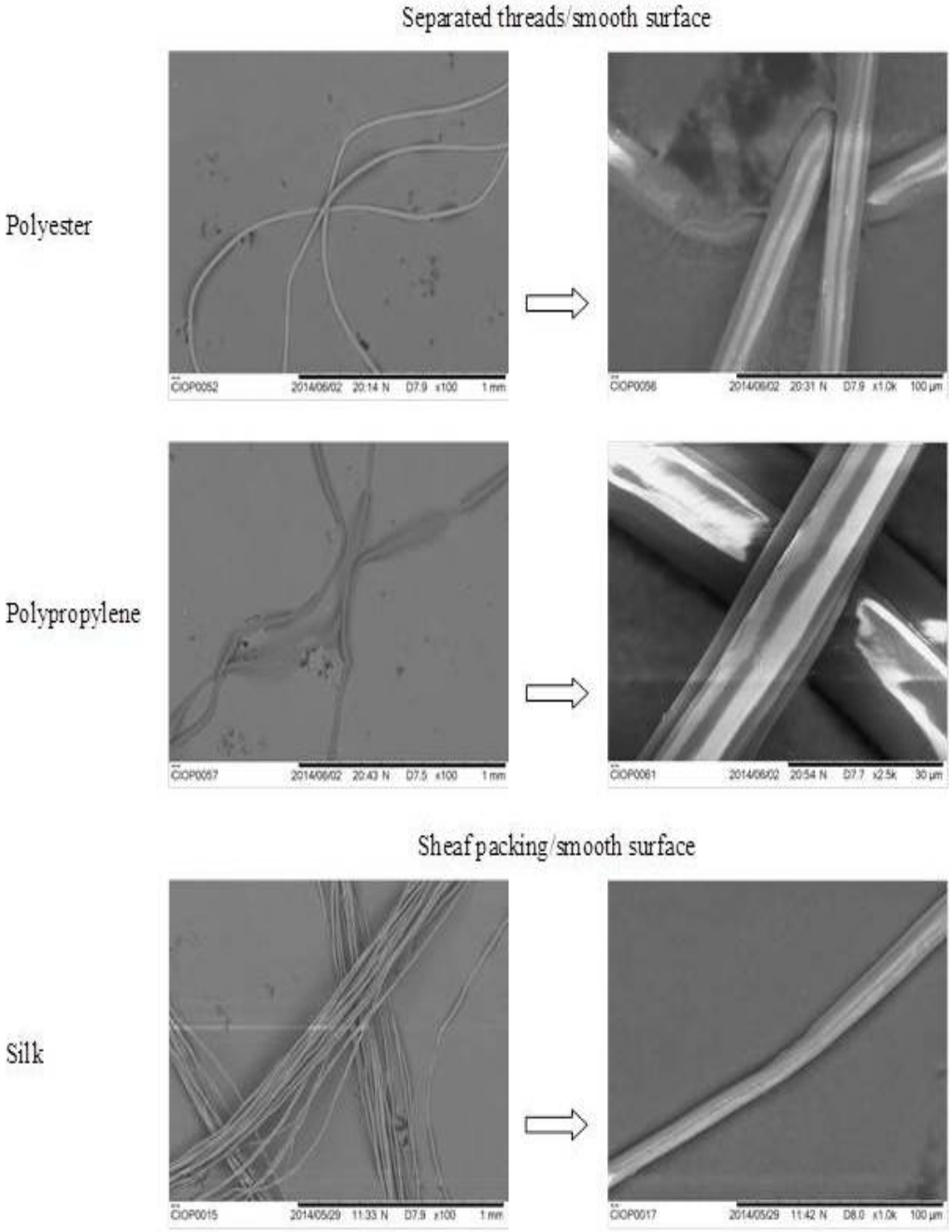


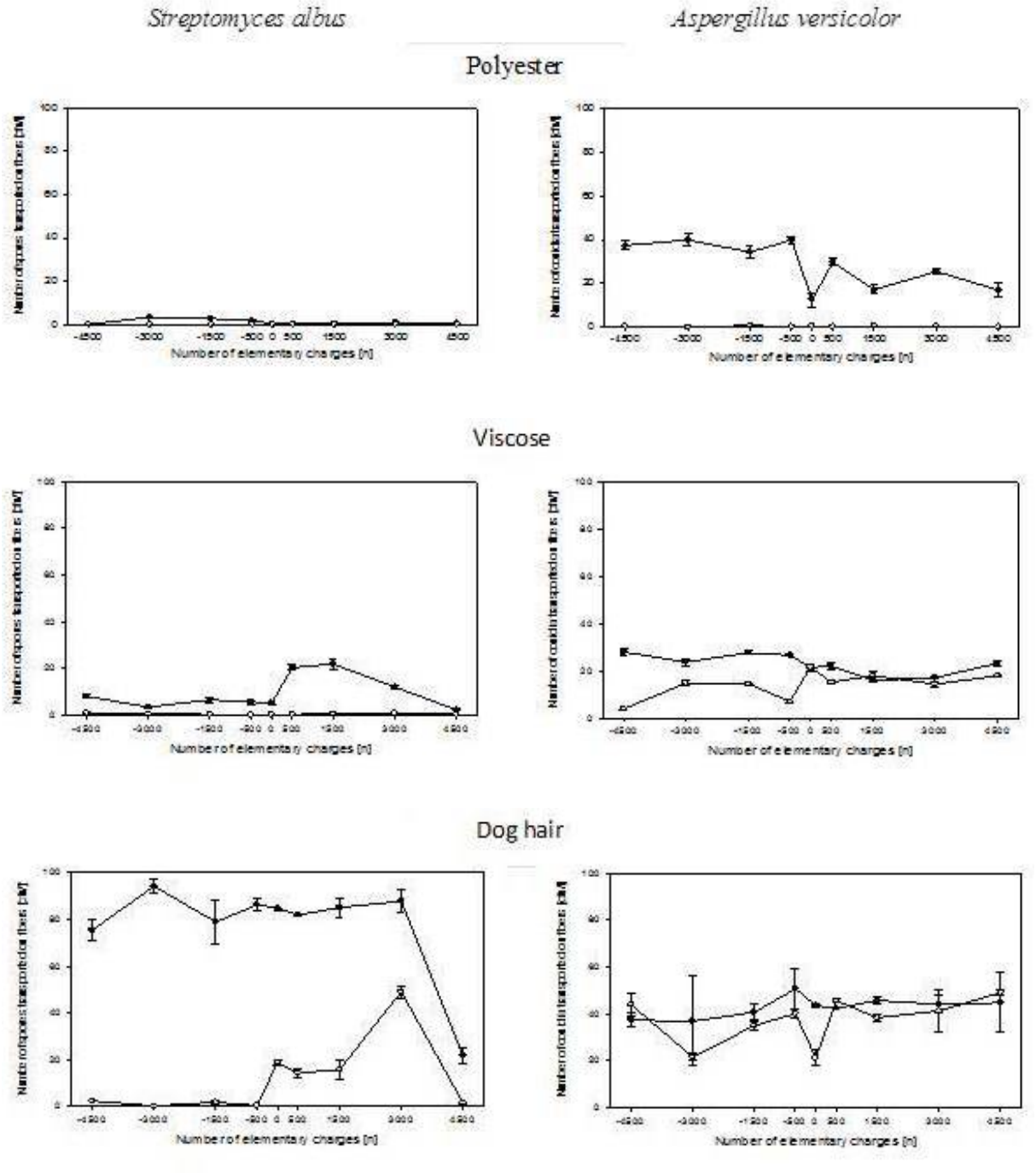
Fig. 4. Characteristic structure and arrangement of natural (animal and plant), synthetic and semi-synthetic fibers.

3.3. Effect of electrical charges on fibers' ability to transport microbial particles in different humidity conditions

In this phase of study, the efficiency with which the selected tested fibers transport microbiological particles was assessed (Fig. 5). The performed experiments showed that fraction of culturable microorganisms generated into the air and subsequently transported on fibers may reach 50% of total bioaerosol particles. In the environment of low air humidity (RH<30%), the percentage of culturable microorganisms was always higher than those for particles aerosolized in high (RH>60%) humidity conditions ($p<0.05$). Moreover, the highest percentage of culturable microorganisms was always noted when the particles (bacterial spores as well as fungal conidia) were free from electrical charge (0V).

Each application of electrical charges (±500V÷±4500V) resulted in decrease in percentage of culturable particles ($p<0.05$). This phenomenon can be explained by a disruptive influence of electrical conduction on viable microorganisms in the environment in which water is relatively easily available.

The quantitative assessment of culturable microbial particles loaded with elementary charges and transported in such state in the air revealed that fibers were able to carry up to 100 bacterial spores or fungal conidia per 1 fiber. The most effective in this respect were animal (dog and horse) and plant (cotton) fibers (Fig. 5). Both tested man-made fibers were significantly less efficient than natural ones (Scheffe test: $p<0.05$).



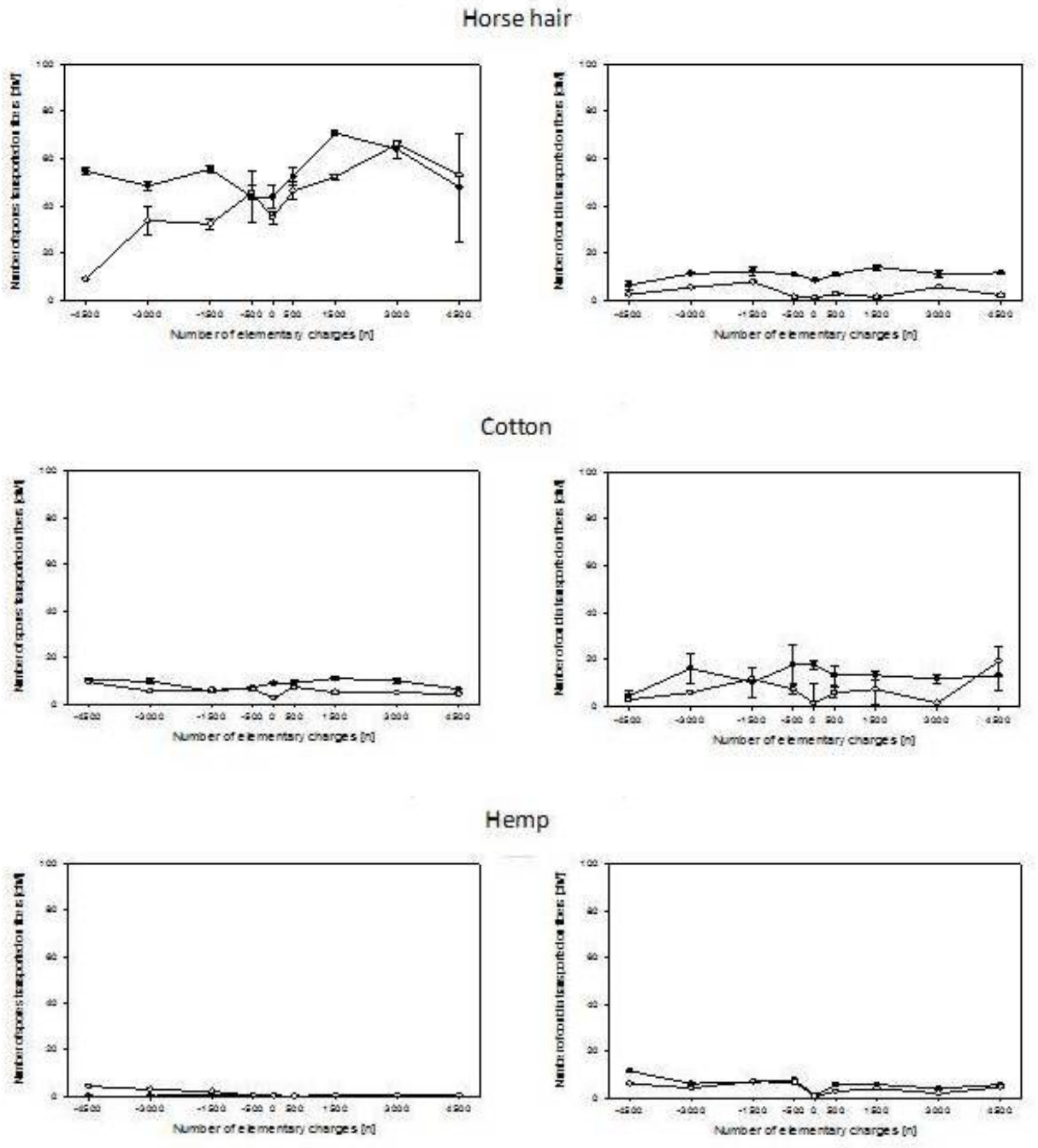


Fig. 5. Number of culturable bacterial spores or fungal conidia loaded with different number of elementary charges and transported on natural animal (dog and horse hair) and plant (cotton and hemp), synthetic (polyester) as well as semi-synthetic (viscose) fibers in dry and humid air. Black dots represent dry spores or conidia; white dots represent wet spores or conidia.

4. CONCLUSIONS

All tested synthetic, semi-synthetic, and silk fibers were free from microbiological contamination. In turn, natural (animal and plant) fibers were able to carry a substantial number of microbial particles, including those of pathogenic properties. The effect on the number of microbial particles transported on fibers had both their natural arrangement and surface structure. As heavily contaminated, animal

and plant fibers should be thoroughly eliminated from the environment and, if possible (e.g. in factories, if manufacturing allows) should be replaced by synthetic, semi-synthetic or silk ones to avoid unwanted dissemination and subsequent inhalation or skin exposure to harmful microbial agents. To restrain such process, the containment measures efficiently limiting their presence and dissemination in both occupational and non-occupational environments should be introduced.

The assessment of the number of culturable microorganisms transported in the air by respirable fibers showed that the fibers loaded with an appropriate number of elementary charges (±500V ÷ ±4500V) were able to carry up to 100 bacterial spores or fungal conidia on 1 fiber. The most versatile in this respect were animal (horse and dog) and plant (cotton) fibers. In this context, the synthetic fibers were significantly less efficient than natural ones. However, it should be pointed out that such feature, i.e. the ease with which natural fibers can transport microbial particulates in the air, can be used to develop health-oriented products. Such for example highly efficient filters or air purifiers made from these fibers may help to maintain a proper hygienic quality both indoors and outdoors.

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