



## **Transport of Microbial Components in Coarse and Fine Particle Fractions in Office Buildings**

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### **1. Introduction**

Recent years continue to confirm the dynamic development of modern office space in Poland. In 2018, total office space reached the level of almost 10 million m<sup>2</sup>, which makes it the largest office market in Central and Eastern Europe (Savills, 2019). In old and modern interiors, particular attention is focused on the air quality as one of major determinants of the well-being of occupants. Poor room ventilation and dampness can promote the growth of harmful microorganisms in buildings (Douwes et al., 2003; Hardin et al., 2003). Numerous studies tried to characterize airborne microbiota in indoor environments, e.g. schools, offices, shopping centers, libraries etc. (Gołofit-Szymczak & Górny, 2010; Harkawy et al., 2011; Mentese et al., 2012; Frączek et al., 2019). In the air, biological particles can be present as single vegetative bacterial cells or spores, fungal conidia as well as aggregates formed by bacterial/fungal cells and dust particles (Macher, 1999). If inhaled, they may be responsible for various adverse health outcomes in the exposed individuals. Numerous studies indicated that exposure to high concentration of (1-3)- $\beta$ -D-glucans (a component of the fungal cell wall) and endotoxins (a part of the outer membrane of Gram-negative bacteria) may have important impact on human health. Both these compounds can be associated with the development of asthma, airway inflammation and occurrence of non-specific symptoms (headache, dry cough, nasal and eye irritation) (Rylander, 1999; Ross et al., 2000; Bouillard et al., 2005; Ławniczek-Wałczyk et al. 2010; Akpınar-Elci et al., 2013; Park et al., 2018). In the scientific literature, there are very few studies on the airborne transport of such microbial particles in both occupational and non-occupational environments. These available data show that such immunologically reactive components are usually carried on small

dust particles (Górny & Dutkiewicz, 1999; Madsen & Nielsen, 2010; Balasubramanian et al., 2012). The PM<sub>2.5</sub> (particulate matter with a diameter of less than 2.5 µm) is of primary concern as it can reach lower parts of the human respiratory system (Li et al., 2017). The noted adverse health effects of PM<sub>2.5</sub> exposure include respiratory (respiratory irritation, asthma, chronic bronchitis, decreased lung functions, exacerbating existing asthma or chronic obstructive pulmonary disease – COPD, promotion of lung cancer) and cardiovascular (stroke, heart disease, hypertension, atherosclerosis) diseases (Butler et al., 2016). Knowing the sizes of aerosol particles is important as they determine their place of deposition in the human respiratory system and the associated adverse health outcomes. It should be noted, that learning about the ways of transport of microbial particles and factors affecting their spread in the indoor environment can be crucial for the introduction of appropriate measures solutions to minimize their spread and prevent infections and others disorders. In this context, the concentration of biological factors in the indoor air cannot be investigated separately but considerate also evaluation of non-biological particles carrying them. Hence, from the exposure assessment perspective, a detailed characteristic of airborne microbial components carried on dust particles could be of high importance. The aim of this study was to determine the concentrations of endotoxins, (1-3)-β-glucans and culturable microorganisms in coarse, fine and aerosol fractions collected in two office buildings.

## **2. Materials and methods**

### **2.1. Sampling strategies**

The measurements were carried out in summer season in two air-conditioned office buildings in Warsaw, Poland. Two bioaerosol as well as two particulate aerosol samples were taken at each of 12 studied office premises. The studied offices were divided into two groups: open space (for at least 10 workers) and double rooms. The area of examined rooms ranged from 15 m<sup>2</sup> (the smallest) to 64 m<sup>2</sup> (the largest). Additionally, the so-called background samples were taken outside the buildings to determine the outdoor level and to control a possible migration of microbiological contaminants into the indoor environment.

### **2.2. The culturable microorganisms**

A sampling of viable microorganisms was carried out using a six-stage Andersen impactor (model 10-710, Andersen Instruments, Atlanta, GA, USA) at a flow rate of 28.3 l/min for 5 min. The samples were collected on nutrient media (BTL, Łódź, Poland): blood trypticase soy agar for mesophilic bacteria, EMB was applied for Gram-negative rods and malt extract agar for fungi. After

incubation (for bacteria: 1 day at 37°C, followed by 3 days at 22°C and 3 days at 4°C; for fungi: 4 days at 30°C followed by 4 days at 22°C), the bacterial and fungal concentrations were calculated as colony forming units per m<sup>3</sup> (CFU/m<sup>3</sup>). To obtain the bioaerosol concentration in coarse and fine fractions, concentrations from stages I-IV (> 7-2.1 μm) and IV-VI (2.1 -< 0.65 μm) were summed, respectively. The isolated bacterial colonies were identified to the genus and/or species level based on their morphology, microscopic structure and biochemical reactivity (using API tests; bioMérieux, Marcy-l'Etoile, France). The isolated fungal colonies were directly identified under stereo (SteREO Discovery V.12, Carl Zeiss, Göttingen, Germany) and light microscopes (Eclipse E200, Nikon, Tokyo, Japan) based on their macro- and micro-morphological characteristics. The analysis of yeasts was additionally supplemented by biochemical API tests (bioMérieux).

### 2.3. Particulate aerosol, endotoxins and (1-3)-β-D-glucans

The concentrations of particulate aerosol were measured using Sioutas impactor (SKC Ltd., Eighty Four, PA, USA) at a flow rate of 9 l/min for 240 min. Particles were separated in five aerodynamic particle diameter ranges: <0.25 (stage E), 0.25-0.5 (stage D), 0.5-1.0 (stage C), 1.0-2.5 (stage B) and 2.5-10 μm (stage A). Particles were collected on 25-mm (stage A-D) and 37-mm PTFE filters (stage E) with 0.5 μm and 2 μm pore sizes, respectively (SKC Ltd.). The mass concentrations of particulate matter in all samples were gravimetrically determined before and after sampling following in both cases a 24 h equilibration period of filters at constant air temperature and humidity. PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub> values from stages A-E, B-E and C-E were summed, respectively. After gravimetric analysis, each filter was transferred into a 50-ml, pyrogen-free tubes (Lonza, Basel, Switzerland) with 10 ml of sterile pyrogen-free water and extracted for endotoxins and (1-3)-β-D-glucans. A detailed description of analytical procedures has already been presented by Ławniczek-Wałczyk et al. (2013). The concentrations of (1-3)-β-D-glucans and endotoxins were quantified using GlucateLL (Associates of Cape Cod, East Falmouth, MA, USA) and Kinetic-QCL LAL (Lonza) assays, respectively. The concentration of airborne endotoxin was expressed in EU/m<sup>3</sup>, whereas the (1-3)-β-D-glucans concentrations in ng/m<sup>3</sup>.

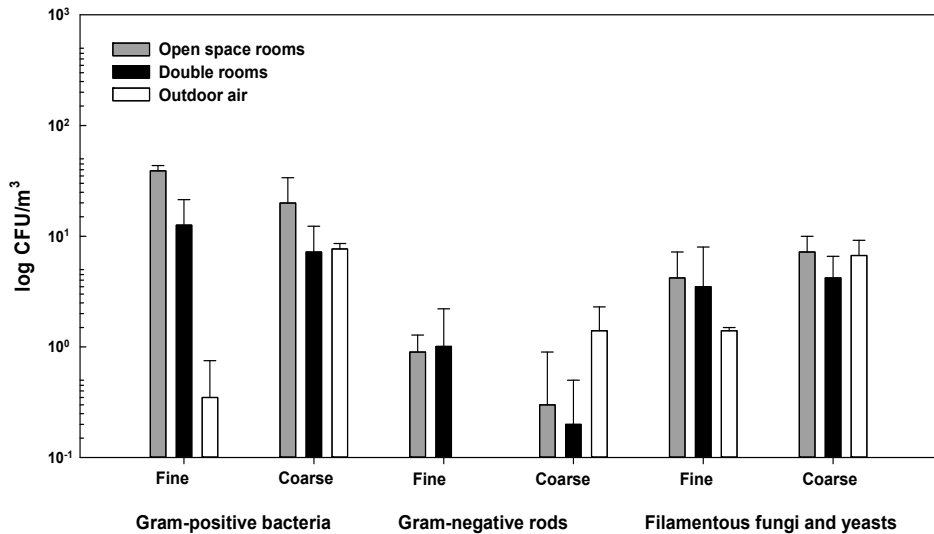
### 2.4. Statistical analysis

The collected measurement data were subjected to the analysis of variance (ANOVA) supplemented with a post-hoc (Scheffe) test and correlation analysis using the STATISTICA software package version 7.1 (StatSoft, Inc., Tulsa, USA), assuming *p* values below 0.05 as statistically significant.

### 3. Results and discussion

#### 3.1. The concentrations of culturable microorganisms

The concentrations (mean, standard deviation) of culturable microorganisms in examined offices are presented in Figure 1.



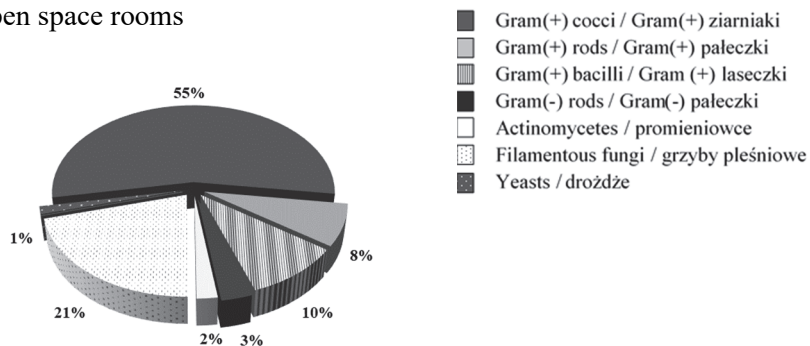
**Fig. 1.** The microbial fine and coarse concentrations (arithmetic mean, standard deviation) in the air of the examined offices and outdoor air

In both types of examined rooms, the higher levels of bacteria were observed in fine bioaerosol fraction (average:  $3.9 \cdot 10^2$  CFU/m<sup>3</sup>) than in coarse ( $p < 0.01$ ). Moreover, the concentrations of Gram-positive bacteria in fine and coarse fractions recorded in open space rooms were significantly higher than in double rooms and outdoor background ( $p < 0.05$ ). The higher levels of fungi in both groups of studied offices were observed in coarse bioaerosol fraction (average: of  $5.6 \cdot 10^1$  CFU/m<sup>3</sup>). For outdoor background, higher bacterial and fungal aerosol concentrations were observed in coarse fractions ( $p < 0.01$ ). The comparison between indoor and outdoor concentrations of Gram-positive bacteria and fungi showed that their indoor concentrations only in open space rooms were higher than in outdoor air ( $p < 0.05$ ). In case of Gram-negative bacteria, the higher concentrations in coarse fractions were noted in outdoor background than in indoor spaces. It is well known that people are the main active source of bacterial aerosol in the indoor environments. These microorganisms are emitted in a great

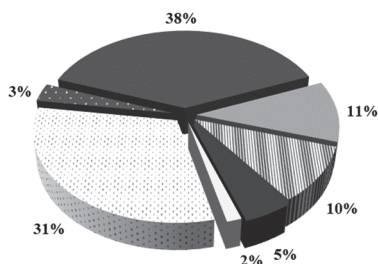
number into the air during talking, coughing, sneezing or peeling of skin scales, whereas fungal aerosol particulates (such as intake spores, mycelium fragments, etc.) can be released into the air from the colonies growing on dead organic matter, plants, soil as well as other organic and inorganic substrates. Hence, an infiltration of outdoor air into the building envelope seems to be the major mechanism responsible for fungal contamination in studied offices. Hospodsky et al. (2012) noted that human activities may increase the total aerosol mass and bacterial concentration in PM<sub>10</sub> and PM<sub>2.5</sub> size fractions in classrooms.

The one of the mechanisms increasing microbial concentration in coarse fraction of air in homes, classrooms, and buildings is resuspension. It was also shown that resuspension from the carpet was significantly higher compared to a smooth floor (Fromme, 2012; Hospodsky et al., 2012). In both types of examined offices, the most common organisms were Gram-positive mesophilic bacteria (37-70% of all identified isolates) followed by fungi (constituting 20-34% of the total microbiota) (Figure 2). Gram-positive bacteria from *Micrococcus*, *Staphylococcus*, *Enterococcus*, *Streptococcus* and *Kocuria* genera dominated in both types of studied offices. They belong to the normal human skin microbiota, but also can be commonly found in the outdoor environment (soil, water, etc.). Such non-pathogenic species present in the air may become an opportunistic pathogen under certain conditions (for example when they are in extremely high concentrations) (Hospodsky et al., 2012). It is well known that e.g. peptidoglycan, the major component of Gram-positive bacterial cell wall, could have a negative effect on human and animal health and if inhaled may contribute to adverse health processes such as infection, endotoxemia and other systemic inflammations (even with organ failure) (Poole et. al., 2012). Gram-positive bacilli and non-sporing rods of the *Microbacterium* genus, which naturally inhabit plants and soil, were also present in the studied premises. Among Gram-negative rods prevailed species from genera: *Sphingomonas*, *Pseudomonas* and *Klebsiella*. In the observed levels, they should not pose a threat to human health. The most frequently isolated fungi belonged to *Penicillium*, *Aspergillus*, *Cladosporium* and *Acremonium* genera. These fungi are naturally present in soil, plants and polluted water, and usually, they do not pose a threat to humans. When analyzing the presence of fungi in enclosed spaces, it should be remembered that their spores can persist for a long time on interior furnishings, offices equipment and elements of heating installations. Due to their allergenic properties, they may pose a potential threat to people staying inside buildings; therefore, the efficient operation of the ventilation system in these interiors is an extremely important issue. In the collected air samples, there was also a small number of yeast of the genera *Candida* and *Rhodotorula*. They inhabit human skin, and in people with dermatological problems may be the reason of mycosis (Flannigan et. al., 2011).

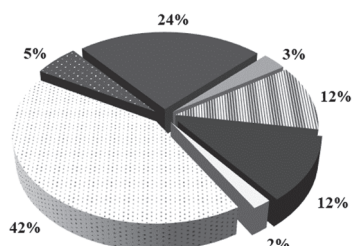
## a) Open space rooms



## b) Double rooms



## c) Outdoor air



**Fig. 2.** The percentage contribution of microbial groups to the total isolated microbiota in indoor and outdoor air

To assess the exposure of office workers to harmful microbiological agents presented in the air, the regulation contained in European Directive 2000/54/EC on the protection of workers from risk related to exposure to biological agents at work were applied. In the studied premises, 8 bacterial (*Actinomyces* spp., *Corynebacterium* spp., *Klebsiella pneumoniae*, *Klebsiella* spp., *Staphylococcus aureus*, *Streptomyces* spp., *Streptococcus* spp., and *Streptococcus pyogenes*) and 1 fungal (*Aspergillus fumigatus*) species were classified to risk group 2 according to the Directive 2000/54/EC and based on that might be recognized as hazard to workers' health. However they, that concentrations were relatively low and did not exceed their levels normally observed in this environment. Nowadays, there is a lack of commonly approved criteria for the assessment of exposure to airborne bacteria and fungi as well as health-based guideline or threshold limit values for airborne microbial contaminants. In Poland, however, such threshold limit values (TLV) were proposed by the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment. For bacteria and fungi in the air of residential and public utility premises as well as in outdoor air in all cases the TLV is equal  $5.0 \cdot 10^3$  CFU/m<sup>3</sup>. The obtained bacterial and fungal concentrations in offices and in outdoor air were below the Polish TLV (Górny et al., 2011).

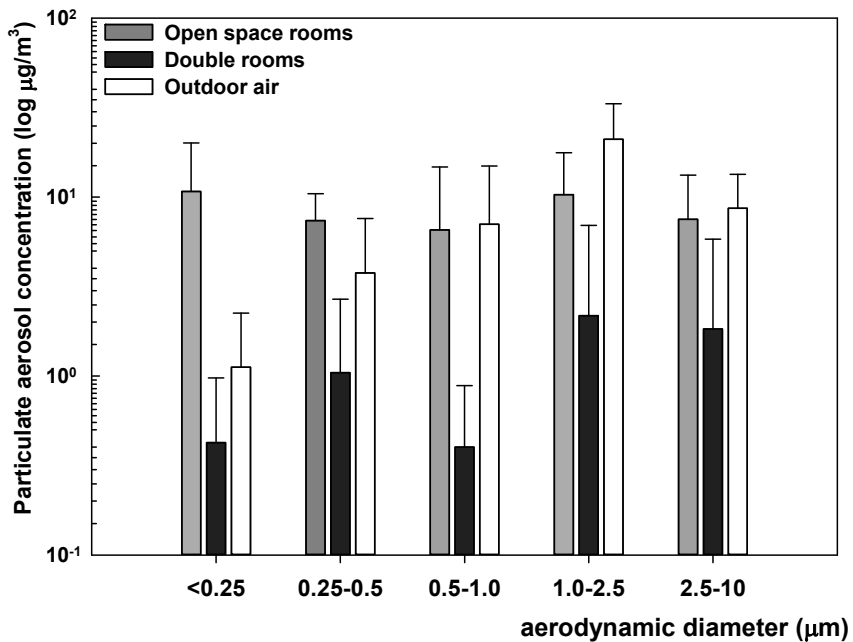
### 3.2. PM Concentrations

The PM levels are often monitored as parameters determining a degree of air pollution in the office buildings. The concentrations of particulate aerosol in the examined offices are presented in Figure 3. It was found that PM concentrations varied between the studied rooms. In open space offices the mean concentrations of PM<sub>1</sub>, PM<sub>2.5</sub> and PM<sub>2.5-10</sub> were at the level of 24, 35 and 7.5 µg/m<sup>3</sup>, respectively, and were significantly higher than in double rooms (1.9, 4 and 1.8 µg/m<sup>3</sup>, respectively) ( $p < 0.01$ ). The obtained results showed that the concentrations of fine particle fraction (PM<sub>2.5</sub>) were higher than coarse ones (PM<sub>2.5-10</sub>). During this study, indoor PM<sub>1</sub> accounted for 67% and 65% of the PM<sub>2.5</sub> fraction in the open space and double rooms, respectively.

The comparison between indoor and outdoor particulate aerosol concentrations showed that outdoor PM concentration in the all measured fractions was significantly higher than those observed in double rooms ( $p < 0.05$ ). The concentration of PM<sub>1</sub>, PM<sub>2.5</sub> in open space offices were higher than in outdoor background, but the differences were not significant. It was noted, that outdoors PM<sub>1</sub> and PM<sub>2.5</sub> concentrations were higher than PM<sub>2.5-10</sub>.

The PM measurements carried out by other researchers show that concentrations of PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub> in public buildings are usually in the range of  $1.1 \cdot 10^1$ - $1.8 \cdot 10^2$  µg/m<sup>3</sup>, and  $0.4 \cdot 10^1$ - $6.7 \cdot 10^1$  µg/m<sup>3</sup>, and  $0.4 \cdot 10^1$ - $3.4 \cdot 10^1$  µg/m<sup>3</sup>,

respectively (Reynolds et al., 2001; Gemenetzi et al., 2006; Menetrez et al., 2009; Balasubramanian et al., 2012; Szigetzi et al., 2014; Morawska et al., 2017). The mean concentrations of studied fractions obtained in double rooms were lower or close to those observed by other researchers, and lower than 24 h WHO recommended values of  $50 \mu\text{g}/\text{m}^3$  and  $25 \mu\text{g}/\text{m}^3$ , respectively for  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  (WHO, 2006). The high values of  $\text{PM}_{2.5}$  observed in open space rooms may be related to the occurrence of internal emission sources (e.g. emission from office devices), increased activity of people, and insufficient ventilation of the rooms (Morawska et al., 2017; Butler et al., 2016).



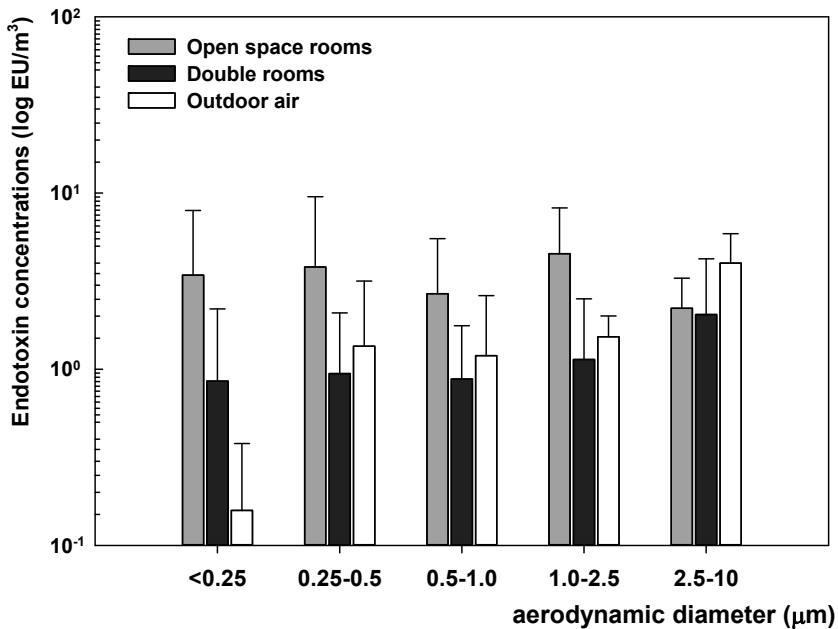
**Fig. 3.** The concentration (arithmetic mean, standard deviation) of particulate aerosol fractions in the examined offices

### 3.3. The concentrations of endotoxins and (1-3)- $\beta$ -glucans

The PM concentrations of endotoxins in the studied offices are presented in Figure 4. In open space offices, the mean concentrations of endotoxins in  $\text{PM}_1$ ,  $\text{PM}_{2.5}$  and  $\text{PM}_{2.5-10}$  were at the level of 9.9, 14.4 and 2.2  $\text{EU}/\text{m}^3$ , respectively, and were higher than in double rooms (2.6, 3.8 and 2.1  $\text{EU}/\text{m}^3$ , respectively) ( $p < 0.01$ ). It was found that the concentrations of endotoxins in fine fraction were significantly higher than in coarse ones ( $p < 0.01$ ). The concentrations of endotoxins in outdoor samples of  $\text{PM}_1$ ,  $\text{PM}_{2.5}$   $\text{PM}_{2.5-10}$  (2.7, 4.2 and 4  $\text{EU}/\text{m}^3$ ) were



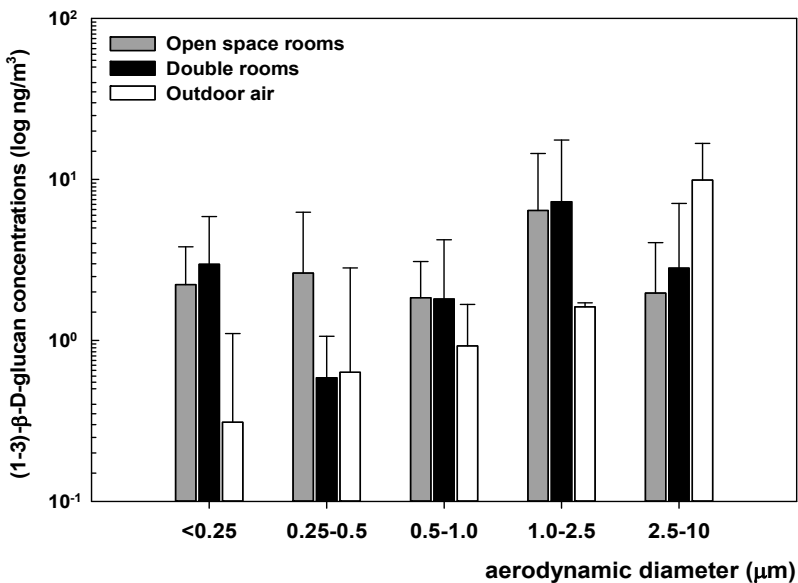
lower than in open space rooms ( $p < 0.05$ ), but higher than in double rooms. However, this last-mentioned difference was not significant. A few studies conducted in public buildings have reported that concentrations of endotoxins in total dust and  $PM_{2.5}$ , may be in the ranges of 0.1-9,2 and 0.05-90  $EU/m^3$ , respectively (Menetrez, et al., 2009; Balasubramanian et al., 2012; Bródka et al., 2012). The measurements carried out in homes have shown that endotoxin concentrations in the  $PM_{2.5-10}$ ,  $PM_{2.5}$ , and  $PM_1$  dust fractions can be at the level of 0.01-0.08; 0.01-39, 0.1-2.5  $EU/m^3$ , respectively (Górny et al., 1999; Chen & Hildemann, 2009; Singh et al., 2011b; Balasubramanian et al., 2012; Yoda et al., 2017). The endotoxin concentrations obtained in this study were similar (double rooms) or higher (open space offices) than those hitherto reported in the scientific literature. Based on these observations, it can be assumed that the air-conditioning system did not properly reduce endotoxins and PM levels in studied open space offices.



**Fig. 4.** The concentrations (arithmetic mean, standard deviation) of endotoxins of particulate aerosol fractions in the examined offices

The concentration of (1-3)- $\beta$ -D-glucans in the examined offices are presented in Figure 5. There were no significant differences in (1-3)- $\beta$ -D-glucans levels between examined offices. Their mean concentrations in  $PM_1$ ,  $PM_{2.5}$ , and  $PM_{2.5-10}$  were at the level of 6.7, 13.1 and 2  $ng/m^3$  in open space rooms, and 5.4,

12.7 and 2.8 ng/m<sup>3</sup> in double rooms, respectively. It was also observed, that the concentrations of  $\beta$ -glucans in fine fraction were significantly higher than in coarse ones ( $p < 0.001$ ). It was noted, that outdoors  $\beta$ -D-glucans concentrations in PM<sub>1</sub> (1.8 ng/m<sup>3</sup>) and PM<sub>2.5</sub> (3.4 ng/m<sup>3</sup>) were lower than in studied rooms, while their load in PM<sub>2.5-10</sub> (10 ng/m<sup>3</sup>) were significantly higher in outdoor than indoor samples ( $p < 0.5$ ). Recent investigations conducted in homes in urban areas by Chen & Hildemann (2009) have shown that  $\beta$ -D-glucan concentrations in the PM<sub>10</sub>, PM<sub>2.5</sub> may reach 0.1 · 9 ng/m<sup>3</sup> and 0.1 · 1.4 ng/m<sup>3</sup>, respectively. In Singh et al. studies (2011a,b) carried out in rural homes, (1-3)- $\beta$ -D-glucan levels in PM<sub>1</sub> were in the range between 0.01-29 ng/m<sup>3</sup>. A very wide range of (1-3)- $\beta$ -D-glucan concentrations in the air of office buildings 0.4-52,5 ng/m<sup>3</sup> (TSP) was also obtained by Madsen et al. (2010) and Bródka et al. (2012). Taking into account the above-mentioned values, it can be concluded that the concentrations of (1-3)- $\beta$ -D-glucans in the air of studied office buildings were characteristic for this type of premises.



**Fig. 5.** The concentration (arithmetic mean, standard deviation) of (1-3)- $\beta$ -D-glucans in particulate aerosol fractions in the examined offices

It was also found that endotoxin and (1-3)- $\beta$ -D-glucan levels in PM<sub>1</sub> and PM<sub>2.5</sub> in both types of studies offices were higher than measured in homes (Singh et al., 2011a; Yoda et al., 2017). Moreover in our study, fine fractions (< 2.5 and < 1  $\mu\text{m}$ ) from offices had a significantly higher content ( $p < 0.01$ ) of endotoxin and

(1-3)- $\beta$ -D-glucans compared to outdoor samples. The concentrations of endotoxins showed a strong positive correlation with  $PM_1$  ( $r = 0.61$ ,  $p < 0.05$ ) and  $PM_{2.5}$  levels ( $r = 0.76$ ,  $p < 0.05$ ) as well as with Gram-negative rods in fine bioaerosol fraction ( $r = 0.75$ ,  $p < 0.05$ ). The concentrations of (1-3)- $\beta$ -D-glucans showed positive correlation with  $PM_{2.5}$  ( $r = 0.54$ ,  $p < 0.05$ ) and  $PM_{2.5-10}$  ( $r = 0.28$ ,  $p > 0.05$ ). The concentrations of (1-3)- $\beta$ -D-glucans in  $PM_{2.5}$  showed a positive correlation with fungi in fine bioaerosol fraction ( $r = 0.59$ ,  $p < 0.05$ ). Considering the above-mentioned relations, it can be concluded that the main source of endotoxins in the offices were Gram-negative rods. The sources of (1-3)- $\beta$ -D-glucans were probably both fungal conidia and their fragments of aerodynamic diameters  $< 2.1 \mu\text{m}$ . All endotoxins and (1-3)- $\beta$ -D-glucans levels in each of measured PM fractions showed a weak positive correlation with their outdoor levels. This observation can be explained by the fact that smaller particles may remain suspended in the air of indoor premises for longer period of time. Different human activities (walking, cleaning, etc.) can also cause aerosolization of microorganisms and their fragments into the air of indoors (Fromme et al., 2017; Salimifard et al., 2017).

In this study, the use of Andersen and Sioutas impactors allowed to describe the forms, in which the microbial particles are present in the air of studied office premises as well as their potential depth of penetration in the human respiratory system. The analysis of bioaerosol size distribution confirmed that bacteria occurred in the form of single cells and fungi occurred in the form of small conidia and fungal-dust aggregates. It was also found that the main carriers of endotoxins and (1-3)- $\beta$ -D-glucans in this work environment were fine aerosol fractions with particle diameters  $< 2.5 \mu\text{m}$ . Such particles can penetrate the lower parts of the human respiratory system posing a health risk for exposed people (Ruzer & Harley, 2012). This study is among few investigations evaluating endotoxin and (1-3)- $\beta$ -D-glucan concentrations in fine and coarse fractions. On the world scale, there are currently no widely accepted guidelines regarding interpretation of such measurement data (Ławniczek-Wałczyk & Górny, 2010; Górny et al., 2011). However in Poland, the TLV for occupational exposure to endotoxins in the environments polluted with organic dust ( $2000 \text{ EU}/\text{m}^3$ ) and in non-industrial (public buildings, dwellings) indoor environments ( $50 \text{ EU}/\text{m}^3$ ) were proposed (Górny et al., 2011). It should be emphasized that these values are related to the endotoxin concentration in total dust only. According to these proposals, the levels of endotoxins measured in studied offices were below proposed TLV of  $50 \text{ EU}/\text{m}^3$ . Nowadays, there are only a few studies concerning exposure to (1-3)- $\beta$ -D-glucans in offices. The results of (1-3)- $\beta$ -D-glucans' measurement conducted in the present study are similar to those reported by other authors (Rylander, 1997; Madsen et al., 2010), however, they focused on (1-3)- $\beta$ -D-glucan levels in total dust only. So far, there are no TLV proposals for (1-3)- $\beta$ -D-glucans. Research carried by Rylander (1997) indicated that exposure to  $5 \text{ ng}/\text{m}^3$

may be associated with the occurrence of many non-specific health problems such as headache, irritation of the throat and nose, and general fatigue. In this light, the obtained results suggest that exposure of office workers to (1-3)- $\beta$ -D-glucans may increase the incidence of above mentioned adverse health effects. Hence, the measurements of (1-3)- $\beta$ -D-glucans can be a useful tool supporting the hygienic quality assessment in office as well as in public utility premises.

#### 4. Conclusions

- The concentrations of particulate matter, endotoxins, (1-3)- $\beta$ -D-glucans and microorganisms in the air of open space rooms were higher than those noted in double rooms.
- This study demonstrated that endotoxins and (1-3)- $\beta$ -D-glucans are mainly associated with fine fractions of aerosol particles in office buildings. Such particles can penetrate the lower parts of the human respiratory system posing a health risk for exposed people.
- The main source of endotoxins in the offices were Gram-negative rods. The sources of (1-3)- $\beta$ -D-glucans were probably both fungal conidia and their fragments of aerodynamic diameters  $< 2.1 \mu\text{m}$ .
- The concentrations of airborne endotoxins, bacterial and fungal microorganism in offices were within the range normally observed in this type of facilities and did not exceed the proposed TLV for them.
- The measured airborne (1-3)- $\beta$ -D-glucan concentrations exceeded  $5 \text{ ng/m}^3$ . Such exposure of office workers to airborne fungal components may increase the incidence of adverse health effects.
- Qualitative analysis of bioaerosols enabled to identify bacterial and fungal strains from the risk group 2 (according to the Directive 2000/54/EC), which might pose a health hazard to workers. However, these microorganisms in the observed low concentrations should not pose a threat to the health of employees.
- The constant monitoring of the hygienic condition of studied rooms is suggested, including regular cleaning and replacement of air filters in the air-conditioning system. This should prevent microbiological contamination of the offices in the future.

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## Abstract

In old and modern interiors, particular attention is focused on the air quality as one of major determinants of the well-being of occupants. Exposure to microbiological contaminants in such close indoor space may be associated with the occurrence of various adverse health outcomes in the exposed individuals. Because the size of inhaled particles determines their place of deposition in the human airways and the associated adverse health outcomes, a detailed characteristic of airborne microbial components carried on fine dust particles in office buildings is needed. The aim of this study was to determine the concentrations of endotoxins, (1-3)- $\beta$ -D-glucans and culturable microorganisms in coarse, fine and aerosol fractions collected in two office buildings in Warsaw. The concentrations of particulate aerosol were measured using Sioutas impactors in PM<sub>1</sub>, PM<sub>2.5</sub>, and PM<sub>2.5-10</sub>. Kinetic-QCL LAL and GlucateLL assays were used to detect endotoxin and (1-3)- $\beta$ -D-glucan concentrations, respectively. The bioaerosol samples were taken using six-stage Andersen impactor as coarse (> 7-2.1  $\mu$ m) and fine (< 2.1  $\mu$ m) fractions, as well.

The mean concentrations of particulate aerosol, endotoxins and (1-3)- $\beta$ -D-glucans in all studied offices were: in PM<sub>1</sub> – 6  $\mu$ g/m<sup>3</sup>, 4 EU/m<sup>3</sup> and 5 ng/m<sup>3</sup>; in PM<sub>2.5</sub> – 11  $\mu$ g/m<sup>3</sup>, 6 EU/m<sup>3</sup> and 10 ng/m<sup>3</sup>; and PM<sub>10-2.5</sub> – 3.5  $\mu$ g/m<sup>3</sup>, 2 EU/m<sup>3</sup> and 2.5 ng/m<sup>3</sup>, respectively. The concentrations of endotoxins and (1-3)- $\beta$ -glucans in PM<sub>2.5</sub> were significantly higher than in PM<sub>10-2.5</sub> ( $p < 0.01$  and  $p < 0.001$ , respectively) and accounted for 71% and 84% of their total load in PM<sub>10</sub>. The airborne bacteria occurred mostly in fine fraction (average  $3.9 \cdot 10^2$  CFU/m<sup>3</sup>,  $p < 0.01$ ), while fungi in coarse fraction of aerosol ( $5.6 \cdot 10^1$  CFU/m<sup>3</sup>). The concentrations of endotoxins showed a positive correlation with PM<sub>1</sub> ( $r = 0.61$ ,  $p < 0.05$ ) and PM<sub>2.5</sub> levels ( $r = 0.76$ ,  $p < 0.05$ ) as well as with Gram-negative rods in fine fraction ( $r = 0.75$ ,  $p < 0.05$ ). The concentrations of (1-3)- $\beta$ -D-glucans showed positive correlation with PM<sub>2.5</sub> ( $r = 0.54$ ,  $p < 0.05$ ) and fungi in fine fraction ( $r = 0.59$ ,  $p < 0.05$ ).

This study demonstrated that endotoxins and (1-3)- $\beta$ -D-glucans are associated mostly with fine fraction of aerosol particles. Such particles can penetrate the lower parts of the human respiratory system posing a health risk for exposed people. The main source of endotoxins in the offices were Gram-negative rods. The sources of (1-3)- $\beta$ -D-glucans were probably both fungal conidia and their fragments of aerodynamic diameters <2.1  $\mu$ m. The noted concentrations of endotoxins and microorganism were within the range normally observed in this type of facilities. Nevertheless, constant monitoring of the hygienic condition is suggested, including regular cleaning and replacement of air filters in the air-conditioning system.

## Keywords:

offices, endotoxins, (1-3)- $\beta$ -D-glucans, PM<sub>1</sub>, PM<sub>2.5</sub>, PM<sub>2.5-10</sub>, bioaerosol



## Transport cząstek pochodzenia mikrobiologicznego w drobnej i grubej frakcji aerozolu w budynkach biurowych

### Streszczenie

Zarówno w starych, jak i nowoczesnych wnętrzach budynków biurowych szczególną uwagę zwraca się na jakość powietrza, która jest wyznacznikiem dobrostanu mieszkańców. Narażenie na zanieczyszczenia mikrobiologiczne w takich zamkniętych pomieszczeniach może być związane z pojawianiem się różnych niekorzystnych efektów zdrowotnych u narażonych osób. Ponieważ wielkość wdychanych cząstek determinuje ich miejsce osadzania w drogach oddechowych człowieka i związane z tym problemy zdrowotne, potrzebna jest szczegółowa charakterystyka frakcji cząstek pyłowych transportujących cząstki pochodzenia mikrobiologicznego w budynkach biurowych. Celem niniejszego badania było poznanie zakresów stężeń endotoksyn, (1-3)- $\beta$ -D-glukanów i mikroorganizmów w drobnej i grubej frakcji aerozolu ziarnistego w dwóch budynkach biurowych w Warszawie. Stężenia aerozolu ziarnistego zmierzono przy użyciu impaktorów Sioutas we frakcjach PM<sub>1</sub>, PM<sub>2.5</sub> i PM<sub>2.5-10</sub>. Testy Kinetic-QCL LAL i GlucateLL zastosowano odpowiednio do detekcji endotoksyn i  $\beta$ -D-glukanów. Próbkę bioaerozolu pobrano przy użyciu sześć-stopniowego impaktora Andersena we frakcji gruboziarnistej (> 7-2,1  $\mu$ m) i drobnej (< 2,1  $\mu$ m).

Średnie stężenia aerozolu ziarnistego, endotoksyn i  $\beta$ -D-glukanów w wszystkich badanych biurach wynosiły odpowiednio: w PM<sub>1</sub> – 6  $\mu$ g/m<sup>3</sup>, 4 JE/m<sup>3</sup> i 5 ng/m<sup>3</sup>; w PM<sub>2.5</sub> – 11  $\mu$ g/m<sup>3</sup>, 6 JE/m<sup>3</sup> i 10 ng/m<sup>3</sup> i w PM<sub>10-2.5</sub> – 3.5  $\mu$ g/m<sup>3</sup>, 2 JE/m<sup>3</sup> i 2.5 ng/m<sup>3</sup>. Stężenia endotoksyn i  $\beta$ -D-glukanów w PM<sub>2.5</sub> były znacznie wyższe niż w PM<sub>10-2.5</sub> (odpowiednio  $p < 0.01$  i  $p < 0.001$ ) i stanowiły 71% i 84% frakcji PM<sub>10</sub>. W badanych pomieszczeniach, bakterie występowały głównie w drobnej frakcji aerozolu ( $3.9 \cdot 10^2$  JTK/m<sup>3</sup>,  $p < 0.01$ ), podczas gdy grzyby izolowano najczęściej z frakcji gruboziarnistej aerozolu ( $5.6 \cdot 10^1$  JTK/m<sup>3</sup>). Stwierdzono pozytywną korelację pomiędzy stężeniami endotoksyn a stężeniami pyłu PM<sub>1</sub> ( $r = 0.61$ ,  $p < 0.05$ ) i PM<sub>2.5</sub> ( $r = 0.76$ ,  $p < 0.05$ ), jak również Gram-ujemnymi pałeczkami ( $r = 0.75$ ,  $p < 0.05$ ). Stężenia  $\beta$ -D-glucans wykazały korelację z PM<sub>2.5</sub> ( $r = 0.54$ ,  $p < 0.05$ ) oraz grzybami w drobnej frakcji ( $r = 0.59$ ,  $p < 0.05$ ).

Niniejsze badania wykazały, że głównym nośnikiem endotoksyn i (1-3)- $\beta$ -D-glukanów w pomieszczeniach biurowych były drobne frakcje aerozolu ziarnistego. Cząstki te mogą przenikać do dolnych dróg oddechowych powodując niekorzystne skutki zdrowotne u narażonych osób. Stwierdzono, że głównym źródłem endotoksyn były Gram-ujemne pałeczki. Źródłami (1-3)- $\beta$ -D-glukanów były głównie fragmenty strzępek grzybni (lub spor) o aerodynamicznych średnicach <2,1  $\mu$ m. Odnotowane stężenia endotoksyn i mikroorganizmów w biurach mieściły się w zakresie normalnie obserwowanym w tego typu obiektach. Niemniej jednak sugerowane jest stałe monitorowanie stanu higienicznego tych pomieszczeń, w tym regularne czyszczenie i wymienianie filtrów powietrza w instalacji klimatyzacyjnej.

### Słowa kluczowe:

biura, endotoksyny, (1-3)- $\beta$ -D-glukany, PM<sub>1</sub>, PM<sub>2.5</sub>, PM<sub>2.5-10</sub>, bioaerozol