WOOD DUST AS A SOURCE OF MICROBIAL CONTAMINATION AT JOINERY

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Abstract

The dust emission during woodworking results in the air pollution with both particulate and microbial aerosols. The aim of this study was to assess a degree of contamination of joinery workplaces with dust, bacteria, fungi, endotoxins, and (1→3)-β-D-glucans released into the air during wood processing and check whether these conditions provoke any changes in cytological picture of nasal lavage fluid in workers after their exposure to studied contaminants. The maximal concentrations of studied pollutants were 3.5mg/m^3, 11473cfu/m^3, 3348cfu/m^3, 3.5ng/m^3 and 394.6ng/m^3, respectively. Exposure to high concentrations of wood dust and (1→3)-β-D-glucans may trigger pathogenic reactions in exposed individuals. Stationary measurements of bioaerosols are poor proxy for workers exposure, hence hygienic evaluation of microbiological hazards in this working environment should be based on personal sampling. The analysis of nasal lavage fluid confirms its usefulness as reliable analytical tool for assessing the health status of joinery workers exposed to harmful biological agents.

Key words: *joinery, wood dust, microorganisms, endotoxins, glucans, nasal lavage, PCR*

1. INTRODUCTION

Wood is a naturally obtained raw material, which is used in industry in both solid and processed forms. Annually, at least 1,700 million m^3 are collected for industrial use (Gioffrè et al. 2012). Occupational exposure to wood dust occurs during woodworking and processing of wood and wood-containing materials and includes logging and sawmill working, pulp and paper industry, cabinetmaking and furniture industry, joinery and carpentry, woodworking machine operating, wood manufacturing and forestry (Binazzi et al. 2015). The health hazards caused by wood dust may be very serious. Wood dust is known to be a human carcinogen. The International Agency for Research on Cancer concluded to assign wood dust to the group 1 of carcinogens to humans (IARC 2012). Exposure to dust derived solely from deciduous trees (hardwood, mainly oak and beech) or from their mixture with coniferous wood (softwood) is correlated with nasopharyngeal adenocarcinoma, whereas non-malignant respiratory effects, except asthma, are not correlated with the specific type of wood (SCOEL 2003). Increased risk of lung cancer was also recently reported in wood dust exposed worker groups (Valličres et al. 2015; Wiggans et al. 2016). Both hardwood and softwood dust can impair the airways patency, causing chronic lung disease. Wood dust in both acute and chronic exposures may act on human body as sensitizer and irritant. These activities mainly concern the respiratory system and skin. Depending on the size of wood dust particles, major adverse health outcomes among woodworkers may refer to both upper and lower respiratory tract. Chronic bronchitis, rhinitis and conjunctivitis as well as skin irritation, have been reported among the occupationally exposed population. Functional changes in the respiratory system and occurrence of occupational asthma were noted among woodworking (mainly furniture) industry workers even at wood dust concentrations below 1mg/m^3 (SCOEL 2003; NTP 2000).

Dust generated from wood processing is a heterogeneous mixture of organic and inorganic particles. It is believed that the adverse health effects of wood dust are caused by the same mechanisms as those provoked by other organic dusts. The critical factors of wood dust exposure, apart from the particles themselves, are chemical compounds naturally occurring in wood. A serious health hazard for workers is also the microbial fraction of wood dust, which includes microorganisms and substances derived from them. In working environment contaminated with wood dust, immunologically reactive bacterial

endotoxins and fungal glucans appear to be particularly important from a health hazard point of view (Mandryk et al. 1999; Gioffrè et al. 2012; Oppliger et al. 2005).

Bioaerosols can consist of: viable microbial particles which are able to produce colonies on proper medium, viable particles unable to grow and produce such structures as well as non-viable microorganisms, fragments or substances derived from the microbial cells. Bioaerosols can be responsible for many adverse health effects from allergic reactions, through infections, to toxic reactions and other non-specific symptoms referred to e.g. as 'sick building syndrome' (SBS) or 'mucous membrane syndrome' (MMS, commonly results in a dry cough, irritation of the eyes, nose and throat and may represent an irritant effect not involving immune responses or mediators). In most situations the observed outcomes are a result of mixed exposure to toxins and allergens (Douwes et al. 2003; Macher 1999).

Endotoxin, known as lipopolysaccharide (LPS), is an integral component of the outer membrane of Gram-negative bacteria. Endotoxins can get into the environment in the course of bacterial cell lysis, during active growth of the cell, or as a result of sudden changes in the hydration state of cell wall when Gram-negative rods are in the aerosol phase. After release, they can occur as components of the whole cell, as cell wall fragments, or as macromolecular aggregates (called "free endotoxins"). According to literature data, an occupational exposure to endotoxins has been linked to various adverse health outcomes, from headache, cough, shortness of breath, through chest tightness and flu-like illnesses to chronic bronchitis, allergic rhinitis, asthma, or an induction of organic dust toxic syndrome (ODTS). Some researchers have reported that LPS, in synergy with other substances present in organic dust, plays an essential role in airway inflammation and bronchoconstriction (Burrell & Ye 1990; Demanche et al. 2009; Liebers et al. 2008; Ławniczek-Wałczyk & Górny 2010; Milanowski 1997; Rylander 2006).

β-glucans are water-insoluble structural cell-wall components of most of the fungi and yeasts, some bacteria, most higher and many lower plants. They are also minor constituents of fungal cytosol. Glucans may account for up to 60% of dry weight of the fungal cell wall, where they are linked to proteins, lipids and carbohydrates. (1→3)-β-D-glucans are non-allergenic agents but have immunomodulating properties and may affect respiratory health. It has been suggested that (1→3)-β-D-glucans have the potential to induce Th_1 as well as Th_2 driven immune responses (Rylander 2010). Initial studies suggest a (1→3)-β-D-glucans role in the development of the adverse symptoms defined as SBS which appeared in the late 80s (Douwes 2005). Since that time, numerous studies have been shown that (1→3)-β-D-glucans modulate various immunological effects by activation of macrophages to cytokine production, neutrophils, eosinophils, T-lymphocyte (Th) and natural killer cells (NK) (Ławniczek-Wałczyk & Górny 2010). Numerous studies have underlined the role of (1→3)-β-D-glucans in adverse health outcomes, especially in bioaerosol-induced inflammatory reactions and diseases including SBS, hypersensitivity pneumonitis and ODTS (Ashitani et al. 2008; Douwes 2005; Fogelmark et al. 1992, 1994; Milanowski 1997; Rylander 2010; Thorn et al. 2001).

In many studies carried out in different occupational environments contaminated with wood dust, inhalation exposure is often assessed on the basis of a questionnaire survey (e.g. Fransman et al. 2003; Jacobsen et al. 2013; Mandryk et al. 1999, 2000; Schlünssen et al. 2004). Typically, the highly personalized feelings about a worker's health condition gathered through a questionnaire survey should be objectified by their verification e.g. using cytological examination of the biological material. In inhalation exposure, nasal lavage (NAL) fluid can be of great analytical importance as a material that is easy to obtain applying a practically non-invasive method. NAL fluid provides the possibility of obtaining a cytological picture of processes (e.g. inflammation) occurring in the nasal mucous membrane of persons exposed to harmful microbial agents (Roponen et al. 2003; Stark 2007).

Wood dusts are produced as a by-product of the wood and wood-based materials processing mainly in sawmills, woodboard and plywood factories, furniture, carpentry, wooden and matched packaging plants etc. Wood dust exposure studies cover many of these work environments; however, in this context, relatively little attention has been so far devoted to the health hazards caused by wood dust at joineries. Hence, the aim of this study was to assess a degree of contamination with dust, bacteria, fungi, endotoxins, and (1→3)-β-D-glucans released into the air during wood processing at joinery and

to check to what extent the observed combined dust and microbiological contamination of this working environment affects the manifestation of adverse health effects after inhalation exposure of workers based on the cytological image of nasal lavage.

2. MATERIALS AND METHODS

2.1. Joinery short characteristics

This study was carried out in small joinery located in central Poland. The investigated joinery was mainly involved in the furniture production using both solid woods (i.e. pure stand softwood and hardwood) and wood-like boards (e.g. hardboard, chipboard, plywood etc.). During 8-hour shift, three workers were usually present at work. Their major tasks involved: sawing, drilling, fretting, mortising, moulding, grooving, planing, veneering, gluing, twisting etc. The studied joinery was naturally ventilated and workers avoided, despite its availability, the use of respiratory protection equipment.

2.2. Sampling strategy

The measurements were carried out in summer season. For this study, aerosol particles were collected using stationary and personal sampling methods. All particulate and microbial aerosol measurements were performed in duplicate.

Wood dust measurements were carried out as personal sampling at workplaces and stationery sampling outdoors using conical inhalable sampler (CIS) equipped with APEX pumps (Casella Measurements Inc., Bedford, Great Britain) operated at a flow rate of 3.5l/min for 4h. Before each measurement, CIS samplers were sterilized with 1% solution of E-Toxa-Clean (Sigma-Aldrich Sp. z o.o., Poznań, Poland). Particles were collected on 37-mm Teflon filters with 2µm pore size (SKC Ltd., Eighty-four, PA, USA). During indoor measurements, each sampler was clipped onto a worker's collar. During outdoor sampling, each sampler was placed at a height of 1.5m above ground level. In both cases, such localization simulated aspiration of particulate aerosol from the human breathing zone. The wood dust mass were gravimetrically determined by weighting the filters before and after sampling with ultra-microbalance (model XP2U, Mettler Toledo, Zürich, Switzerland), following in both cases a 24h equilibration period at constant air temperature and humidity (22±3°C and 45±5%, respectively). After gravimetric analysis, each filter was transferred into a 50ml pyrogen-free polypropylene tube filled with 10ml of sterile pyrogen-free water (Lonza, Basel, Switzerland) containing 0.05% Tween 20 (Sigma-Aldrich Sp. z o.o.) and vortexed on a platform shaker (model Promax 1020, Heidolph Instruments GmbH & Co., Schwabach, Germany) at room temperature for 1h. After extraction, the resulted suspension was used to determine bacterial, fungal, endotoxin and glucan concentrations. For assessment of microbial concentrations in wood dust samples, the spread plate method was applied, where 0.2ml of the obtained suspension was spread evenly over the same media as used for bioaerosol sampling (see below). The rest of obtained suspension was used for quantitative analysis of endotoxins and (1→3)-β-D-glucans.

Viable (understood in this study as culturable) bioaerosol stationary samples were simultaneously taken in both outdoor and indoor environments using a 6-stage Andersen impactor (model 10-710, Andersen Instruments, Atlanta, GA, USA) at a flow rate of 28.3l/min for 5min. At the beginning of each measurement cycle, bacterial aerosol was collected on blood trypticase soy agar (TSA) with 5% sheep blood (Becton, Dickinson & Co., Sparks, MD, USA) and, after impactor reloading, fungi were aspirated on malt extract agar (MEA, Oxoid Ltd., Basingstoke, UK). The sampling time was 5min for both bacterial and fungal aerosols, and their concentrations (after laboratory elaboration – see below) were expressed as colony forming units in $1m^3$ of sampled air (cfu/m^3). During the tests, Andersen impactor was placed at a height of 1.5m above ground level to simulate aspiration from the human breathing zone.

2.3. Microbiological analyzes of the air samples

The collected bioaerosol samples were incubated at the temperature of: bacteria – 1 day at 37°C, followed by 3 days at 22°C and 3 days at 4°C; fungi – 7 days at 25°C. After incubation, the bacterial

and fungal concentrations were calculated as cfu/m^3. The isolated bacterial colonies were identified to the genus and/or species level based on their morphology (after Gram staining; 111885 Gramcolor stain set; Merck KGaA, Germany) (Fischer & Dott 2003), microscopic structure (Eclipse E200, Nikon, Tokyo, Japan) and biochemical reactivity using analytical profile index (API) tests and APIweb database (bioMérieux, Marcy-l'Etoile, France). The taxonomical origin of isolated pathogens was additionally confirmed using molecular techniques (see below). The isolated fungal colonies were directly identified under stereo (model SteREO Discovery V.12, Carl Zeiss, Gottingen, Germany) and light microscopes (Nikon) based on their macro- and micro-morphological characteristics. Filamentous fungi were taxonomically systematized according to their morphology using several identification keys: Fisher & Cook (1998), Murray et al. (2013), Samson et al. (2004) and St-Germain & Summerbell (2011). The analysis of yeasts included Gram staining (Merck KGaA) and biochemical reactivity evaluation based on API 20C AUX test (bioMérieux).

The isolated microbial pathogens were additionally qualitatively characterized using polymerase chain reaction (PCR) amplification of 16S rRNA gene fragment, followed by its sequence analysis. Genomic DNA from each bacterial isolate was extracted from pure culture grown on TSA agar using Genomic Mini Kit (A&A Biotechnology s.c., Gdynia, Poland) according to the protocol recommended by the manufacturer (including lysozyme, lysostaphin and/or mutanolysin pretreatment). The concentration and purity of DNA isolates were spectrophotometrically assessed (model DS-11, DeNovix Inc., Wilmington, DE, USA).The results of sequencing were compared with the sequences published in the Gene Bank database using the Blast tool (from the National Center for Biotechnology Information).

Endotoxins from wood dust samples were quantitatively analyzed using Kinetic-QCL Limulus Amebocyte Lysate (LAL) assay (Lonza), following the manufacturer instructions. After extraction and vortexing of the samples, the remaining (from bioaerosol analysis) dust suspensions were centrifuged at 1,000×g for 10min (model 5804 R, Eppendorf AG, Hamburg, Germany) and divided into 2 equal parts. The first part of the supernatant was spectrophotometrically (model Sunrise, Tecan Group Ltd., Männedorf, Switzerland) analyzed at wavelength of 405nm in 37°C for endotoxins using LAL assay with a potency of 12EU/ng against *Escherichia coli* 055:B5 standard endotoxin. The concentration of airborne endotoxins was expressed in ng/m^3.

The second part of the supernatant was again vortexed (model BioVortex V1 Plus, Biosan, Riga, Latvia) for 2 more minutes, followed by an additional 10min agitation in an ultrasonic bath (model Sonic 5, Polsonic, Warsaw, Poland). Directly afterwards, 0.6M NaOH was added and the suspension additionally shaken for 1h at room temperature in order to unwind the glucan triple-helix structure and make them water soluble. The concentrations of (1→3)-β-D-glucans were also spectrophotometrically (Tecan Group Ltd.) assayed at wavelengths of 405nm and 490nm in 37°C using the quantitative kinetic Glucatell assay (Associates of Cape Cod, East Falmouth, MA, USA) and expressed in ng/m^3.

2.4. Nasal lavage analysis

Nasal lavage (NAL) fluid analysis is a noninvasive and reliable method allowing to investigate causal relationships between bioaerosol exposure and adverse health effects (Roponen et al. 2003; Stark 2007). In this study, NAL samples were collected after work shift according to the modified Greiff et al. method (1990). In the beginning, 20ml of physiological salt solution was forced using a sterile syringe with a soft rubber tip into one nostril. During the lavage process, the studied subject turned his chin into the syringe side closely holding the irrigated nostril tightly closed. Then, the resulting fluid was carefully gathered into the sterile Falcon type tube and its volume was measured. The percentage of recovered NAL fluid ranged between 75-85% of the initial volume of saline. In the next step, the NAL fluid was centrifuged at 1200rpm for 10min in 4°C (model 5804 R, Eppendorf AG, Hamburg, Germany) and the remaining washings were resuspended by shaking (Heidolph Instruments GmbH & Co.) in 1ml of PBS at pH of 7.2-7.4 (Dulbecco's buffer, SERVA Electrophoresis GmbH, Heidelberg, Germany) for the cytological analysis. The total number of cells in the nasal washings was determined to produce a cytological image of the nasal mucosa exposed to studied aerosols. From each NAL sample elaborated in that way, the smear on glass slide was gently sprayed with Cytofix (SAMKO, Klembów, Poland) to fix the cells to the slide, and after drying stained with May-Grunwald/Giemsa

method (Sigma-Aldrich Chemie GmbH). Stained preparations were evaluated in a light microscope (Nikon). Total cell counts (epithelial cells, neutrophils, eosinophils, basophiles, lymphocytes and monocytes) were administered as the number of cells in 1ml of recovered washings. Nasal lavage sampling was performed in duplicate. NAL sampling got approval of bioethics committee.

2.5. Measurement of microclimate parameters

The environmental conditions (temperature and relative humidity of the air) in studied cars were checked during every sampling session using hytherograph (model Omniport 20, E+E Elektronik GmbH, Austria). All these measurements were performed in duplicate.

2.6. Statistical analysis

The measurement data were statistically elaborated by a single-factor analysis of variance (ANOVA) with post-hoc analysis (Scheffe's test) as well as t-test and Pearson correlation analysis using the Statistica data analysis software system, version 7.1-2006 (StatSoft Inc., Tulsa, OK, USA). Probability values were treated as statistically significant at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis of particulate and microbial aerosols

The concentrations of wood dust, bacterial and fungal aerosols as well as endotoxins and glucans in wood dust sampled at studied joinery and in outdoor air are presented in Table 1. The average concentrations (and their ranges) for all studied aerosol elements, i.e. for particulate (wood dust) aerosol, airborne bacteria, airborne fungi, endotoxins and glucans in wood dust, measured using personal samplers were as follows: 2.77 (1.6-3.5) mg/m^3, 5858 (1339-11473) cfu/m^3, 1655 (444-3348) cfu/m^3, 2.51 (0.9-3.5) ng/m^3 and 280.97 (176.1-394.6) ng/m^3, respectively. All these concentrations were significantly higher than those obtained using stationary sampling (t-test: $p<0.05$-0.01). For the bioaerosol measurements performed as personal and stationary sampling, analysis of variance revealed statistically significant differences between bacterial and fungal aerosol personal concentrations at workplaces and their stationary concentrations obtained both indoors and outdoors (Scheffe test: in both cases $p<0.05$). The correlation analyzes showed that an increase of bacterial contamination of the air resulted in an augmentation of its endotoxin pollution level ($r=0.84$ at $p<0.05$). Similar trend was observed when airborne fungal concentrations were compared with (1→3)-β-D-glucan concentrations ($r=0.98$ at $p<0.05$). The measured levels of both immunologically active microbial components also significantly correlated with the concentrations of particulate (wood dust) aerosol ($r=1$ at $p<0.05$ and $r=0.9$ at $p<0.05$ for endotoxins and glucans, respectively).

Sampling location	Average concentration (SD)				
	Dust [mg/m^3]	Bacteria [cfu/m^3]	Fungi [cfu/m^3]	Endotoxins [ng/m^3]	(1→3)-β-D-glucans [ng/m^3]
Joinery	2.77 (1.02) *	5858 (4907) * 1018 (70) #	1655 (1385) * 145 (35) #	2.51 (1.41) *	280.97 (109.51) *
Outdoor air	0.02 (0.01) #	300 (45) #	78 (60) #	0.04 (0.01) #	0.02 (0.01) #

*) – personal sampling; #) – stationery sampling; SD – standard deviation

Table 1. Wood dust and microbial aerosol concentrations in indoor (joinery workplaces) and outdoor (atmospheric air) environments.

Occupational exposure to inhalable wood dust has been reported in many studies. The wood dust concentrations at workplaces usually ranged between: 0.1mg/m^3 and 100mg/m^3 in furniture industry

(Douwes et al. 2017; Gioffrè et al. 2012; Glindmeyer et al. 2008; Jacobsen et al. 2010; 2013; Schlünssen et al. 2004; Szewczyńska & Pośniak 2017), 0.1mg/m^3 and 74.05mg/m^3 in sawmills (Douwes et al. 2000; Glindmeyer et al. 2008; Mandryk et al. 1999, 2000; Oppliger et al. 2005; Park et al. 2010), 1.8mg/m^3 and 5.66mg/m^3 in woodchipping mills (Mandryk et al. 1999), 0.21mg/m^3 and 131mg/m^3 at joineries (Douwes et al. 2017; Harper & Andrew 2006; Mandryk et al. 1999; Rongo et al. 2004), 0.4mg/m^3 and 35.6mg/m^3 in fiberboard factories (Dutkiewicz et al. 2001), 1.1mg/m^3 and 29.1mg/m^3 in chipboard factories (Dutkiewicz et al. 2001), 0.09mg/m^3 and 11.28mg/m^3 in carpentry (Gioffrè et al. 2012), 0.9mg/m^3 and 29.5mg/m^3 in plywood facility (Glindmeyer et al. 2008), and 0.07mg/m^3 and 10.24mg/m^3 during chipping operations (Magagnotti et al. 2013). In a few of these studies, the concentrations of bacterial and fungal aerosols as well as endotoxins and glucans were also measured. Bacterial aerosol contamination at workplaces ranged between 106cfu/m^3 and 21173cfu/m^3 in sawmills (Gioffrè et al. 2012; Oppliger et al. 2005; Park et al. 2010) or 131cfu/m^3 and 2000cfu/m^3 in carpentry (Gioffrè et al. 2012). Fungal contaminants in the air noted the following concentrations: from 108cfu/m^3 to 95100cfu/m^3 in sawmills (Gioffrè et al. 2012; Mandryk et al. 1999, 2000; Oppliger et al. 2005; Park et al. 2010), from 4500cfu/m^3 to 12600cfu/m^3 in woodchipping mills (Mandryk et al. 1999), and from 400cfu/m^3 to 36500cfu/m^3 at joineries (Mandryk et al. 1999).

Endotoxins and glucans as markers of microbial contamination of workplaces in wood processing sector are also controlled. The hitherto obtained results show that airborne endotoxin levels ranged: from 0.04ng/m^3 to 78.4ng/m^3 in sawmills (Dennekamp et al. 1999; Douwes et al. 2000; Gioffrè et al. 2012; Mandryk et al. 1999, 2000; Oppliger et al. 2005; Sabolić Pipinić et al. 2010; Park et al. 2010), from 2ng/m^3 to 4.87ng/m^3 in woodchipping mills (Mandryk et al. 1999), from 0.1ng/m^3 to 40ng/m^3 at joineries (Harper & Andrew 2006; Mandryk et al. 1999), from 1.62ng/m^3 to 197.4ng/m^3 in fiberboard factories (Dutkiewicz et al. 2001), from 0.32ng/m^3 to 21.74ng/m^3 in chipboard factories (Dutkiewicz et al. 2001), and from 0.14ng/m^3 to 6.93ng/m^3 in carpentry (Gioffrè et al. 2012), whereas glucan concentrations fluctuate between: 0.16ng/m^3 and 226300ng/m^3 in sawmills (Douwes et al. 2000; Mandryk et al. 1999, 2000; Ronald et al. 2003), 0.27ng/m^3 and 10.4ng/m^3 in woodchipping mills (Mandryk et al. 1999) as well as 0.11ng/m^3 and 3.60ng/m^3 at joineries (Mandryk et al. 1999). Against this background, the wood dust, bacterial and fungal aerosol and endotoxin concentrations measured in the studied joinery can be treated as normally observed in this branch of industry, however, some of them should be treated as high and hazardous.

Scientific studies reveal that furniture and cabinet making, joinery, sawmill, plywood and particle-board mill operations are associated with the highest exposures to wood dust (especially during machine sanding and similar operations, frequently resulting in air concentrations of wood dust greater than 5mg/m^3) (Binazzi et al. 2015; IARC 2012). In the present study, wood dust concentrations did not exceed the Polish occupational exposure limit (OEL) of 4mg/m^3 established for inhalable fraction of wood dust except beech and oak dusts but crossed OEL of 2mg/m^3 accepted for beech and oak dusts as well as for mixed wood dusts containing beech and oak dust particles (Ordinance 2016). Scientific literature data indicate an increased incidence of allergic and non-allergic respiratory symptoms, including nasal irritation, throat, eye, cough, nasal obstruction, rhinorrhea, sinusitis, mucosal clearance, wheezing, asthma, decrease in lung function parameters in workers exposed to wood dust (both coniferous and deciduous) at concentrations of ≤1mg/m^3 (SCOEL 2003). Taking this level into consideration, it can be concluded that in studied joinery wood dust concentrations were always higher. It suggests that observed exposure may provoke at least some of the listed above adverse health outcomes.

All the obtained at joinery bioaerosol concentrations were compared with Polish proposals of OEL for bacteria, fungi and endotoxins in working environments contaminated with organic dust (1×10^5cfu/m^3, 5×10^4cfu/m^3, and 2×10^2ng/m^3, respectively) (Górny et al. 2011). As it is seen, all the controlled parameters were below Polish OEL proposals and a degree of workplace contamination seems to be on acceptable level. Regarding endotoxins, their concentrations at examined joinery did not also exceed the OEL proposed by the Dutch Expert Committee for Occupational Standards (i.e. 9ng/m^3=90EU/m^3) as well as the safety level (100ng/m^3) proposed by Clark and Rylander (Ławniczek-Wałczyk & Górny 2010). All measured endotoxin concentrations were also below the level (200ng/m^3) suggested by Rylander as a limit value for toxic pneumonitis (Ławniczek-Wałczyk &

Górny 2010). On the other hand, Mandryk et al. (1999) studying correlations between lung function indices and personal exposures found that airborne wood dust and biohazards associated with wood dust (endotoxins, (1→3)-b-D-glucans, fungi, and Gram-negative bacteria) have negative effects on the pulmonary function of woodworkers. Douwes et al. (2000) stated that exposure to endotoxin concentrations higher than 5ng/m^3 is already sufficient to potentially contribute to the development of respiratory symptoms. Similar conclusions drew Fransman et al. (2003) who studying plywood mill workers observed that average inhalable dust concentrations of 0.7mg/m^3 and endotoxin concentrations of 23ng/m^3 appear to increase a risk of developing work-related respiratory symptoms. In the air of studied joinery, the relatively high concentrations of (1→3)-β-D-glucans (ranging from 176.1ng/m^3 to 394.6ng/m^3) were observed. Glucans, similarly as endotoxins, persist in the environment much longer than viable microbial cells from which they derived. They are able to maintain biological activity even after a death of parental cells and, by that, may serve as indicators of long-term exposure. The evidence that glucans play a role in airway symptoms among woodworkers is scarce. There are few studies concerning an exposure to (1→3)-β-D-glucans in wood industry. Unlike endotoxins, there are neither exposure standards nor OEL proposals for (1→3)-β-D-glucans. Nevertheless, Rylander et al. (1992) demonstrated that exposure to low levels of (1→3)-β-D-glucans (0.1-5.2 ng/m^3) may increase the frequency of various non-specific adverse health outcomes, such as headache, dry cough, throat irritation, nasal irritation, and fatigue. It is also believed that concentrations of (1→3)-β-D-glucans above 12ng/m^3 may cause more frequent appearance of some health complaints such as phlegm, hoarseness, chest tightness or stomach problems (Gladding et al. 2003). Considering this, it could be concluded that (1→3)-β-D-glucans concentrations at the examined joinery were higher than the above mentioned values, which may be related to the adverse health effects among exposed workers.

All bacterial and fungal species, isolated from the examined joinery and outdoor air, are presented in figure 1 as well as in tables 2 and 3. A total of 19 bacterial species belong to 11 genera were identified in the air at joinery workplaces. The most prevalent bacterial group in indoor air was endospore-forming Gram-positive rods constituting up to 37.2% of all recovered bacteria (among them *Bacillus* representatives were predominant), followed by Gram-positive cocci (mostly *Staphylococcus lentus*) that accounted for up to 31.9 %, nonsporing Gram-positive rods (mainly *Microbacterium* spp.) – up to 16.5%, and mesophilic actinomyces (mainly *Rhodococcus* spp.) – up to 10% (Fig. 1). Airborne concentrations of Gram-negative rods (*Flavimonas oryzihabitans*), if occurred, were generally very low (<1%). When compared percentage contribution of bacterial strains to the total microbiota isolated from the air at joinery by personal and stationery sampling methods, it was noted that stationary sampling allows to achieve as rich taxonomical spectrum as personal one (15 v. 17 species) (table 2).

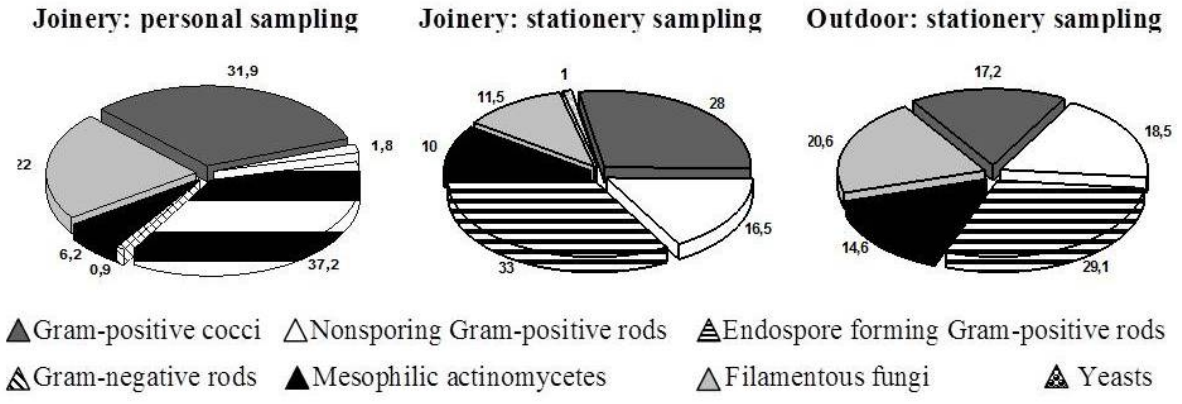


Fig. 1. Percentage contribution of particular microbial groups to the total isolated microbiota.

A total of 16 fungal species belong to 10 genera were identified in the air at joinery workplaces (table 3). On the average, filamentous fungi accounted for 22% and 11.5% of the total isolated microbiota when sampled using personal and stationary method, respectively (Fig. 1). Among all fungal isolates, species from *Cladosporium* genera (especially *C. herbarum*) were predominant. Interestingly, the stationary sampling, compared to personal one, significantly limited the taxonomical spectrum of isolated airborne mycobiota (13 v. 9 species). On the other hand, yeasts were recovered from the air samples only when stationary sampler was used. Such tendency was probably connected with aerodynamic diameter of these particles and sampling technique. Yeasts as big particles have short half-lives in the air and fast sedimentation process dominates their airborne behavior. Also, personal sampler, operating at relatively low flow rate compared to Andersen impactor, was not able to aspirate coarse particulates having such a high inertia.

Bacteria	Joinery		Outdoor air
	Personal sampling	Stationery sampling	Stationery sampling
Gram-positive cocci			
Micrococcus spp.	+	+	–
Staphylococcus capitis	+	–	–
Staphylococcus epidermidis	+	+	–
Staphylococcus haemolyticus	+	+	–
Staphylococcus lentus	+	+	+
Endospore-forming Gram-positive rods			
Bacillus cereus	+	+	+
Bacillus licheniformis	+	+	+
Bacillus megaterium	+	+	+
Bacillus pumilus	+	+	+
Bacillus spp.	+	+	+
Bacillus subtilis [a]	+	+	+
Paenibacillus spp.	+	–	–
Nonsporing Gram-positive rods			
Brevibacterium spp.	+	+	–
Cellulomonas spp.	–	+	+
Microbacterium spp.	–	+	–
Gram-negative rods			
Flavimonas oryzihabitans	+	–	–
Mesophilic actinomycetes			
Actinomyces spp. [a]	+	+	+
Rhodococcus spp.	–	+	–
Streptomyces albus [a]	+	–	+

Table 2. Bacteria identified in the air of the studied environments. The symbols represent: "+" - presence and "–" - absence of the species or genus; "a" - pathogenic species or genus according to the level of risk of infection (after: Directive 2000/54/EC and Ordinance 2005)

Taxonomical speciation of airborne microbiota at studied joinery was characteristic for the environments contaminated with wood dust. Similar qualitative picture of air pollution was described by e.g. Gioffrè et al. (2012) in Italian carpentries and sawmills, however, the quantity and presence of different species of Gram-negative bacteria was there much more abundant. Airborne microbiota of studied joinery workplaces (19/11 and 16/10 bacterial and fungal species/genera, respectively) was also much more numerous than that of outdoor environment (10/5 and 6/6 bacterial and fungal species/genera, respectively); however, all isolated outdoor species were also identified in the air at joinery. Such picture shows that in the absence of mechanical ventilation in this factory, an infiltration of atmospheric air enriches freely indoor microbiota. On the other hand, however, the processing of wood and wood-based materials, naturally contaminated with certain amount of fungi, is still a major source of mycological pollution at joinery (see figure 1 and table 3).

Fungi	Joinery		Outdoor air
	Personal sampling	Stationery sampling	Stationery sampling
Filamentous fungi			
Acremonium spp.	+	+	+
Acremonium strictum	+	+	–
Alternaria alternata	+	+	+
Alternaria spp.	+	+	–
Aspergillus clavatus	+	–	–
Aspergillus ochraceus	+	–	+
Cladosporium cladosporioides	+	+	–
Cladosporium herbarum	+	+	+
Cladosporium spp.	–	+	–
Fusarium spp.	–	+	+
Paecilomyces variotii	+	–	–
Penicillium spp.	+	–	–
Penicillium verrucosum	+	–	–
Rhizopus stolonifer	+	+	–
Ulocladium chartarum	+	–	+
Yeasts			
Rhodotorula glutinis	–	+	–

Table 3. Fungi identified in the air of the studied environments. The symbols represent: "+" - presence and "–" - absence of the species or genus; "a" - pathogenic species or genus according to the level of risk of infection (after: Directive 2000/54/EC and Ordinance 2005)

Among all isolated microbiota, three bacterial human pathogens were identified: *Bacillus subtilis*, *Streptomyces albus* and *Actinomyces* spp. *Bacillus subtilis* is endospore-forming Gram-positive rod most commonly found in soil environments and on plant undergrowth. Due to ability to form stress-resistant endospores and uptake of external DNA, which allow the bacteria to adapt by recombination, this bacterium is relatively abundant in both indoor and outdoor air (Murray et al. 2013; Yates et al. 2016). Inhalation of *B. subtilis* spores may result in allergic alveolitis type of reaction; hence this species was classified in risk group 2 according to their level of risk of infection (Ordinance 2005).

Actinomyces spp. are Gram-positive bacteria that, compared to other natural groups of bacteria, have evolved an unusual diversity of form and function. They are uniquely adapted to life on solid surfaces

and they occurrence on rocks, plants, animals, clothing, food, and other exposed surfaces can be explained by an ability of their spores to withstand desiccation and to survive at low moisture content of the substrate. They can occur in both outdoor and indoor air, but their numbers and types differ widely, depending on the environment and the source. If moisture is not widely present, they are rare in the environment and therefore their presence indoors may be considered as indicator of indoor pollution source. *Actinomyces* constitute also part of the normal human biota of oral cavity and respiratory tract, but infection of organs and bloodstream may occur upon disruption of tissue barriers. *S. albus* is an actinomycete common in soil, dust, straw, fodder, and compost and on plant surfaces. Outdoor air is seldom free of its spores, although their numbers in nonfarming environments are often small. *Streptomyces* appears to be one of the most important indicators for indoor moisture problems and is often found in buildings with odor problems. Due to the immunological reactivity of their fine (i.e. submicrometric in diameters) spores, both *Actinomyces* and *Streptomyces*, if inhaled, may provoke a strong allergic effect (Górny et al. 2003; Murray et al. 2013). These properties caused that all representatives of the genus *Actinomyces* and *Streptomyces albus* species were classified in risk group 2 according to their level of risk of infection (Ordinance 2005).

Fungi are ubiquitous eukaryotic organisms, comprising an abundance of species. They may appear at workplaces being transported in the air or with raw materials. Fungi are therefore found in the dust and on surfaces almost everywhere. Fungi can affect human health in a variety of ways. Possible reactions generally manifest as: allergic reactions (sensitization and immune responses, i.e., asthma, allergic rhinitis or hypersensitivity pneumonitis), infections (e.g., aspergillosis), and toxic responses (connected mainly with mycotoxins or glucans) (WHO 2009). All identified in the studied joinery fungal species are ubiquitous in the environment and their presence was strongly connected with organic matter of plant origin. Although, in observed concentrations, they should not be harmful for the exposed workers, their conidia may survive on different surfaces for even more than two decades preserving biological activity (Mandrioli et al. 2003).

The use of a 6-stage Andersen impactor allowed obtaining data on particle size distribution of airborne microbiota in both joinery and outdoor air (figure 2). Taking into account the aerodynamic sizes of bacteria and fungi from the groups predominant in the air, such analysis allowed also defining the forms of studied bioaerosols and determining possible depth of their penetration into the human respiratory tract. In the case of bacterial aerosol, significantly higher concentrations of particles were found at workplaces compared to concentrations in ambient air (t-test: $p<0.05$). This predominance was particularly evident for particles with an aerodynamic diameters >1.1µm. This demonstrates an additional emission of this type of pollution generated from the raw material processed during the routine operations performed by joinery workers. Two distinct concentration peaks (1131cfu/m^3 and 1466cfu/m^3) were observed in particle size ranges of 2.1-3.3µm and >7µm. This indicates an airborne presence of microbial particles in the form of both fine and large bacterial and/or bacterial-dust aggregates. In case of fungal aerosol, the analysis of size distribution curves for joinery and atmospheric air showed that at workplaces fungi were present both as single conidia (0.65-2.1µm) as well as small and large fungal or fungal-dust aggregates (>3.3µm). The fungal aerosol concentrations in the whole range of aerodynamic particle diameters were higher than those measured in atmospheric air. It indicates that for this type of bioaerosol, its primary source is also the raw material and technical processes performed during workers' activities are responsible for its emission into the air.

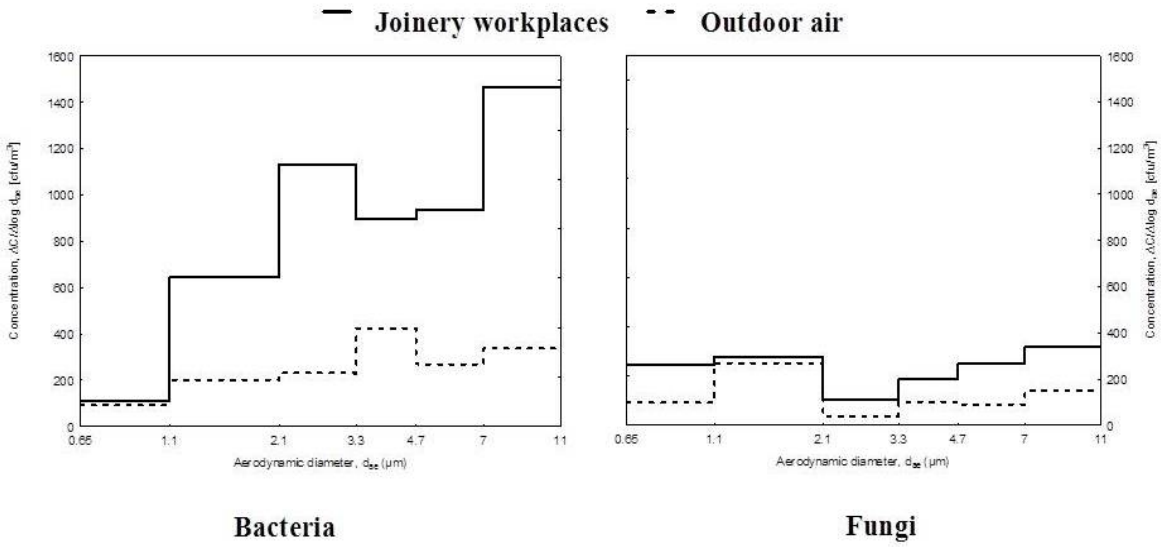


Fig. 2. Size distribution of microbial aerosols in indoor (joinery workplaces) and outdoor (atmospheric air) environments.

Based on size distribution data, it can be concluded that the highest "load" of workplace bioaerosol may reach in the human respiratory tract: in case of bacteria – nasal and oral cavities as well as secondary bronchi, in case of fungi – also nasal and oral cavities as well as terminal bronchi, bronchioles and alveoli. Interaction between aerosol particles and respiratory system cells largely depends on the place of their deposition. As most of the bioaerosol particles were within the aerodynamic range of: bacteria – 2.1-3.3µm and >7µm, and fungi – 0.65-2.1µm and >3.3µm, the potential effects of their influence may address all stages of the respiratory tract being responsible in exposed individuals for numerous adverse health effects from the nose and eyes' irritations, through asthmatic reactions, to allergic alveolitis type outcomes.

Most of the inhaled air during normal breathing enters via the nose and, therefore, the nasal mucosa serves as the primary barrier against inhaled pollutants. NAL is a simple, non-invasive and well tolerated method to assess inflammatory changes in the upper airways (Stark 2007; Steerenberg et al. 1996). Roponen et al. (2003), analyzing NAL fluids from persons exposed to bioaerosol particles, found individual variations as well as seasonal and gender differences in the concentrations of different proinflammatory mediators. In their study, the individual variation in the basal levels of measured inflammatory markers was low, whereas differences between individuals were considerable. Ławniczek-Wałczyk et al. (2013) studying occupational exposure to bioaerosols in poultry houses found significant positive correlations between endotoxin and Gram-negative rod concentrations as well as between (1→3)-β-D-glucan and fungal aerosol concentrations. Professional duties performed by poultry farms employees in endotoxin-contaminated environment resulted in a statistically significant increase in the total number of cells in nasal lavage compared to the control group of office workers. There were also statistically significant correlations between concentration (1→3)-β-D-glucans and the total number of cells in nasal lavage during the handling of biomass (including wooden chips) at power plant (Ławniczek-Wałczyk 2013). In the current study, NAL fluid was analyzed in the cytological context only and immunological reactivity as an answer to environmental microbiological triggers was assessed based on the specific cell content and its correlation with concentrations of dust and microbial pollutants. Here also, statistically significant correlations between the total number of cells as well as bacterial, fungal and glucan concentrations in the air were observed ($p<0.05$ for $r=0.96$, $r=0.98$ and $r=0.93$, respectively). Hence, all of the above described relationships indicate the possibility of respiratory inflammation occurrence in exposed individuals and confirm that nasal lavage can be a reliable analytical tool for assessing the health status of workers exposed to harmful biological agents.

The values of temperature and relative humidity of the air ranged from 23.1°C to 23.7°C and from 41.9% to 43.3% as well as from 28.2°C to 28.6°C and from 37.9% to 39.1% for studied workplaces as well as outdoor environment, respectively. None of the microclimatic parameters had statistically significant effects on the concentration of studied bioaerosols.

4. CONCLUSIONS

Joinery workers are exposed to relatively high concentrations of wood dust and airborne glucans that may contribute to the development of adverse health outcomes. Stationary measurements of microbial aerosols are poor proxy for workers exposure to bioaerosols at joinery, hence hygienic evaluation of microbiological hazards in this working environment should be based on personal sampling of these contaminants. The analysis of nasal lavage fluid confirms its usefulness as a reliable analytical tool for assessing the health status of joinery workers exposed to harmful biological agents.

As the number of microbial exposure studied performed at joineries is still very limited, the abilities to draw firm conclusion require to significantly extending in the future both the pool of controlled wood-processing facilities and the range of investigated parameters, especially those of microbial origin.

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REFERENCES

Ashitani, J, Kyoraku, Y, Yanagi, S, Matsumoto, N &, Nakazato, M 2008, ‘Elevated levels of β-D-glucan in bronchoalveolar lavage fluid in patients with farmer’s lung in Miyazaki, Japan’, *Respiration*, vol. 75, 182-188.

Binazzi, A, Ferrante, P & Marinaccio A 2015, “occupational exposure and sinonasal cancer: a systematic review and meta-analysis’, *BMC Cancer*, vol. 15, no. 49, pp. 1-17.

Burrell, R & Ye, SH 1990, ‘Toxic risks from inhalation of bacterial endotoxin’, *British Journal of Industrial Medicine*, vol. 47, pp. 688-691.

Demanche, A, Bønløkke, J, Beaulieu, MJ, Assayag, E & Cormier, Y, 2009, ‘Swine confinement buildings: effects of airborne particles and settled dust on airway smooth muscles’, *Annals of Agricultural and Environmental Medicine*, vol. 16, 233-238.

Dennekamp, M, Demers, PA, Bartlett, K, Davies, H & Teschke, K 1999, ‘Endotoxin exposure among softwood lumber mill workers in the Canadian province of British Columbia’, *Annals of Agricultural and Environmental Medicine*, vol. 6, no. 2, pp. 141-146.

‘Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work’, *Official Journal of European Communities*, vol. L 262/21, pp. 21-45.

Douwes, J 2005, ‘(1→3)-β-D-glucans and respiratory health: a review of the scientific evidence’, *Indoor Air*, vol. 15, 160-169.

Douwes J, Cheung, K, Prezant, B, Sharp, M, Corbin, M, McLean D, ‘t Mannetje, A, Schlünssen, V, Sigsgaard, T, Kromhout, H, LaMontagne, AD, Pearce, N & McGlothlin JD 2017, ‘Wood dust in joineries and furniture manufacturing: an exposure determinant and inventory study’, *Annals of Work Exposures and Health*, vol. 61, no. 4, pp. 416-428.

Douwes, J, McLean, D, van Maarl, E, Heederik, D & Pearce, N 2000, 'Workers exposures to airborne dust, endotoxin, and β(1,3)-glucan in two New Zealand sawmills', *American Journal of Industrial Medicine*, vol. 38, pp. 426-430.

Douwes, J, Thorne, P, Pearce, N & Heederik, D 2003, 'Bioaerosol health effects and exposure assessment: progress and prospects', *Annals of Occupational Hygiene*, vol. 47, no. 3, pp. 187-200.

Dutkiewicz, J, Olenchock, S, Krysińska-Traczyk, E, Skórska, C, Sitkowska, J & Prażmo, Z 2001, 'Exposure to airborne microorganisms in fiberboard and chipboard factories', *Annals of Agricultural and Environmental Medicine*, vol. 8, no. 2, pp. 191-199.

Fischer, G & Dott, W 2003, 'Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene', *Archives of Microbiology,* vol. 179, pp. 750-782.

Fisher, F & Cook, NB 1998, *Fundamentals of diagnostic mycology*, Saunders Company, Philadelphia.

Fogelmark, B, Goto, H, Yuasa, K, Marchat, B & Rylander, R 1992, 'Acute pulmonary toxicity of inhaled β-1,3-glucan and endotoxin', *Agents and Actions*, vol. 35, 50-56.

Fogelmark, B, Sjöstrand, M & Rylander, R 1994, 'Pulmonary inflammation induced by repeated inhalations of beta-(1,3)-D-glucan and endotoxin', *International Journal of Experimental Pathology*, vol. 75, 85-90.

Fransman, W, McLean, D, Douwes, J, Demers, PA, Leung, V & Pearce, N 2003, 'Respiratory symptoms and occupational exposures in New Zealand plywood mill workers', *Annals of Occupational Hygiene*, vol. 47, pp. 287-295.

Gioffrè, A, Marramao, A & Iannò, A 2012, 'Airborne microorganisms, endotoxin, and dust concentration in wood factories in Italy', *Annals of Occupational Hygiene*, vol. 56, no. 2, pp. 161-169.

Gladding, T, Thorn, J & Stott, D 2003, 'Organic dust exposure and work-related effects among recycling workers', *American Journal of Industrial Medicine*, vol. 43, pp. 584-591.

Glindmeyer, HW, Rando, RJ, Lefante, JJ, Freyder, L, Brisolara, JA & Jones, RN 2008, 'Longitudinal respiratory health study of the wood processing industry', *American Journal of Industrial Medicine*, vol. 51, pp. 595-609.

Górny, RL, Cyprowski, M, Ławniczek-Wałczyk, A, Gołofit-Szymczak, M & Zapór, L 2011, 'Biohazards in the indoor environment - a role for threshold limit values in exposure assessment' in MR Dudzińska (ed.), *Management of indoor air quality*, CRC Press - Taylor and Francis Group, pp. 1-20.

Górny, RL, Mainelis, G, Grinshpun, SA, Willeke, K, Dutkiewicz, J & Reponen, T 2003, 'Release of *Streptomyces albus* propagules from contaminated surfaces', *Environmental Research*, vol. 91, pp. 45-53

Greiff, L, Pipkorn, U, Alkner, U & Persson, CG 1990, 'The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions', *Clinical and Experimental Allergy*, vol. 20, no. 3, pp. 253-259.

Harper, M & Andrew, ME 2006, 'Airborne endotoxin in woodworking (joinery) shops', *Journal of Environmental Monitoring*, vol. 8, no. 1, pp. 73-78.

IARC 2012, *IARC monographs on the evaluation of carcinogenic risks to humans – Arsenic, metals, fibers, and dusts – volume 100 C*, International Agency for Research on Cancer, Lyon.

Jacobsen, G, Schaumburg, I, Sigsgaard, T & Schlünssen, V 2010, 'Non-malignant respiratory diseases and occupational exposure to wood dust. Part II. Dry wood industry', *Annals of Agricultural and Environmental Medicine*, vol. 17, pp. 29-44.

Jacobsen, G, Schlünssen, V, Schaumburg, I & Sigsgaard, T 2013, 'Cross-shift and longitudinal changes in FEV1 among wood dust exposed workers', *Occupational and Environmental Medicine*, vol. 70, pp. 22-28.

Liebers, V, Raulf-Heimsoth, M & Brüning, T 2008, 'Health effects due to endotoxin inhalation (review)', *Archives of Toxicology*, vol. 82, 203-210.

Ławniczek-Wałczyk, A 2013, *Endotoxins and (1-3)-β-D-glucans as markers of microbial contamination in occupational environment*, PhD thesis, central Institute for Labour Protection – National Research Institute, Warsaw.

Ławniczek-Wałczyk, A, Górny, RL, Gołofit-Szymczak, M, Niesler, A & Wlazło, A 2013, 'Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of production cycle', *Annals of Agricultural and Environmental Medicine*, vol. 20, no. 2, pp. 259-268.

Macher, J 1999, *Bioaerosols: Assessment and Control*, ACGIH, Cincinnati.

Magagnotti, N, Nannicini, C, Sciarra, G, Spinelli R & Volpi, D 2013, 'Determining the exposure of chipper operators to inhalable wood dust', *Annals of Occupational Hygiene*, vol. 57, no. 6, pp. 784-792.

Mandrioli, P, Caneva, G & Sabbioni 2003, *Cultural heritage and aerobiology. Methods and measurement techniques for biodeterioration monitoring*, Kluwer Academic Publishers, Dordrecht.

Mandryk, J, Alwis, KU & Hocking, AD 1999, 'Work-related symptoms and dose-response relationships for personal exposures and pulmonary function among woodworkers', *American Journal of Industrial Medicine*, vol. 35, no. 5, pp. 481-490.

Mandryk, J, Alwis, KU & Hocking, AD 2000, 'Effects of personal exposures on pulmonary function and work-related symptoms among sawmill workers', *Annals of Occupational Hygiene*, vol. 44, pp. 281-289.

Milanowski, J 1997, 'Experimental studies on the effects of organic dust-derived agents on respiratory system: comparison between endotoxins and glucans', *Inhalation Toxicology*, vol. 9, 369-388.

Murray, PR, Rosenthal, KS & Pfaller MA 2013, *Medical microbiology. 7th ed.*, Elsevier, Philadelphia.

NTP – National Toxicology Program 2000, *Report on carcinogens – Background document for wood dust*, U.S. Department of Health and Human Services, Durham.

Oppliger, A, Rusca, S, Charrière, N, vu Duc, T & Droz, P-O 2005, 'Assessment of bioaerosols and inhalable dust exposure in Swiss sawmills', *Annals of Occupational Hygiene*, vol. 49, no. 5, pp. 385-391.

'Ordinance of the Minister of Family, Labour and Social Policy of June 27, 2016, amending ordinance on maximum admissible concentrations and intensities for agents harmful to health in the working environment', *Journal of Laws*, pos. 944.

'Ordinance of the Minister of Health of April 22, 2005, on hazardous biological agents in the work environment and the protection of health of workers occupationally exposed to them', *Journal of Laws*, vol. 81, p. 716.

Park, H, Park, H & Lee, I 2010, 'Microbial exposure assessment in sawmill, livestock feed industry, and metal working fluids handling industry', *Safety and Health at Work*, vol. 1, pp. 183-191.

Ronald, LA, Davies, HW, Bartlett, KH, Kennedy, SM, Teschke, K, Spithoven, J, Dennekamp, M & Demers, PA 2003, 'β(1→3)-glucan exposure levels among workers in four British Columbia sawmills', *Annals of Agricultural and Environmental Medicine*, vol. 10, pp. 21-29.

Rongo, LMB, Msamanga, GI, Burstyn, I, Barten, F, Dolmans, WM & Heederik, D 2004, 'Exposure to wood dust and endotoxin in small-scale wood industries in Tanzania', *Journal of Exposure Analysis and Environmental Epidemiology*, vol. 14, no. 7, pp. 544-550.

Roponen, M, Seuri, M, Nevalainen, A, Randell, J & Hirvonen, M-R 2003, 'Nasal lavage method in the monitoring of upper airway inflammation: seasonal and individual variation', *Inhalation Toxicology*, vol. 15, no. 7, pp. 649-661.

Rylander, R 2006, 'Endotoxin and occupational airway disease', *Current Opinion in Allergy and Clinical Immunology*, vol. 6, 62-66.

Rylander, R 2010, 'Organic dust induced pulmonary disease – the role of mould derived beta-glucan', *Annals of Agricultural and Environmental Medicine*, vol. 17, 9-13.

Rylander, R, Persson, K, Goto, H, Yuasa, K &, Tanaka, S 1992, 'Airborne β,1–3 glucans may be related to symptoms in sick buildings', *Indoor Environment*, vol. 1, pp. 263-267.

Sabolić Pipinić, I, Varnai, VM, Beljo Lučić, R, Čavlović, A, Prester, L, Orctt, T & Macan, J 2010, 'Endotoxin exposure assessment in wood-processing industry: airborne versus settled dust levels', *Arhiv za Higijenu Rada i Toksikologiju*, vol. 61, pp. 161-166.

Samson, RA, Hoekstra, ES & Frisvad, JC 2004, *Introduction to food- and airborne fungi. 7th ed.*, Centraalbureau voor Schimmelcultures, Utrecht.

Schlünssen, V, Schaumburg, I, Heederik, D, Taudorf, E & Sigsgaard, T 2004, 'Indices of asthma among atopic and non-atopic woodworkers', *Occupational and Environmental Medicine*, vol. 61, pp. 504-511.

SCOEL 2003, *Recommendation from the Scientific Committee on Occupational Exposure Limits: Risk assessment for wood dust*, SCOEL/SUM/102.

Stark, H 2007, *Inflammatory airway responses caused by Aspergillus fumigatus and PVC challenges*, PhD thesis, University of Kuopio, Kuopio.

Steerenberg, PA, Fisher, PH, Gmelig-Meyling, F, Willighagen, J, Greese, E, van de Vliet, H, Ameling, C, Boink, ABTJ, Dormans, JAMA, van Bree, L, van Loveren, EH 1996, 'Nasal lavage as tool for health effect assessment of photochemical air pollution', Human & Experimental Toxicology, vol. 15, pp. 111-119.

St-Germain, G & Summerbell, R 2011, *Identifying filamentous fungi. 2nd ed.*, Star, Belmont.

Szewczyńska, M & Pośniak, M 2017, 'Assessment of occupational exposure to wood dust in the Polish furniture industry', *Medycyna Pracy*, vol. 68, no. 1, pp. 45-60.

Thorn, J, Beijer, L & Rylander, R 2001, 'Effects after inhalation of (1→3)-β-D-glucan in healthy humans', *Mediators of Inflammation*, vol. 10, 173-178.

Valličres, E, Pintos, J, Parent, ME & Siemiatycki, J 2015, 'Occupational exposure to wood dust and risk of lung cancer in two population-based case-control studies in Montreal, Canada. *Environmental Health*, vol. 14, 1.

WHO 2009, *Guidelines for indoor air quality: dampness and mould*, WHO Regional Office for Europe, Copenhagen.

Wiggans, RE, Evans, G, Fishwic,k D & Barber, CM 2016, 'Asthma in furniture and wood processing workers: a systematic review', *Occupational Medicine*, vol. 66, 193-201.

Yates, MV, Nakatsu, CH, Miller, RV & Pillai, SD 2016, *Manual of environmental microbiology, 4th ed.,* ASM Press, Washington.