

Contamination with microscopic fungi measured by the concentration of ergosterol in dusts of various types of wood with different granulation

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Abstract: *Contamination with microscopic fungi measured by the concentration of ergosterol in dusts of various types of wood with different granulation.* Dusts are a mixture of compounds generated both in the anthropogenic and natural environment and they are a by-product of combustion and abrasion or crushing of solid materials. Dust generated in wood industry plants as a result of mechanical wood processing is particularly dangerous. Wood dusts - both dispersed in the air inside a production plant and deposited - also contain the work environment's so-called biological agents. This term refers to microorganisms and the structures and substances produced by these organisms, which have an adverse effect on the human organism and may cause numerous diseases. The aim of this study was to determine the level of contamination with microscopic fungi of dusts from various types of wood depending on the granulation. The method of analysis of the chemical ergosterol marker was used here, the concentration of which is correlated with the number of microscopic fungi. On the basis of the study, it was found that dusts with finer granulation from deciduous wood are characterized by a higher concentration of ERG, i.e. a higher level of contamination with microscopic fungi. It is important information from the point of view of health and safety at work.

Keywords: wood dust, microscopic fungi, ergosterol, work safety

INTRODUCTION

Dusts are a mixture of compounds generated both in anthropogenic and natural environment. They are a by-product of combustion and abrasion or crushing of solid materials. Dust generated in wood industry plants as a result of mechanical wood processing is particularly dangerous. The dust generated during sawing, machining, grinding or milling settles on walls, floors and machines, creating a hazard at the workplace. Moreover, dust particles with sizes $\leq 80 \mu\text{m}$ settle on surfaces very slowly, and even finer ($<10 \mu\text{m}$) remain suspended in the air (Dolny and Hlásková, 2015; Očkajová et al., 2020; Reg. MPiPS, 2014). Dusts with particles larger than $5 \mu\text{m}$ affect the upper respiratory tract, posing a serious threat to health (Asgedom et al., 2019; Tureková et al., 2019). The toxic effect of dusts, both deciduous and coniferous species, affects the mucosa of the nose, eyes, throat and may cause skin irritation and allergies, and may even lead to a reduction in lung capacity and chronic diseases (Douwes et al., 2001; Mračková et al., 2015; Schlünssen et al., 2018).

During wood conversion and woodworking, to a lesser or greater extent large amounts of waste composed of fine wood dust particles are produced, which may be dispersed in the air causing contamination of the work place. Wood dusts - both dispersed in the air inside a production plant and deposited - contain also the so-called biological agents of the work environment. This term refers to micro- and macroorganisms, as well as the structures and substances produced by these organisms, which have an adverse effect on the human organism and may cause numerous diseases. Common hazardous biological agents include e.g. microscopic fungi (Directive 2000/54/EC). This health hazard is mainly the result of the

size of spores produced by microscopic fungi, ranging from around a dozen to several dozen micrometers (Kozakiewicz and Laskowska, 2016). Due to their dimensions they may easily penetrate human airways and skin pores. To date, there are only few reports on sterol contents in wood dusts. The greatest exposure of workers to wood dust contaminated with mycobiota and their toxic metabolites is recorded in the manufacture of wood furniture and in joinery workshops, mainly during manual and machine sanding. Due to the high risk of occupational diseases resulting from the exposure of employees to wood dust numerous safety measures have been implemented as part of good manufacturing practices. However, this is frequently insufficient to eliminate the hazards connected with the presence of molds in the work environment. For this reason, in this study it was decided to analyze the contamination with mycobiota in wood dust at various work stations in a plant producing wooden furniture elements and other small wood products. The species origin of the analyzed wood dust is closely related to the type of raw material used in that plant. Microscopic fungi in dust can be analyzed using the following methods: chemical for testing the concentrations of ergosterol (ERG) and adenosine 5'-triphosphate (ATP), microbiological for determining e.g. colony-forming units (CFU), and genetic, such as ELISA tests. Among the above-mentioned methods, the analysis of ERG concentration is becoming increasingly the method of choice for testing various types of materials, not only biological. ERG is a good indicator of living and dead fungal contents in plant material.

The aim of this study was to determine the level of contamination with microscopic fungi of dusts from various types of wood depending on the granulation. The method of analysis of the chemical ergosterol marker was used here, the concentration of which is correlated with the number of microscopic fungi.

MATERIALS AND METHODS

Wood was selected for the tests: beech, oak, hornbeam, ash, alder, walnut, larch, pine, spruce. Sanding was performed using a prototype narrow belt sanding machine designed and made in the laboratory of Department of Furniture Design (Faculty of Wood Technology, Poznań University of Life Sciences PULS). The sanding paper type EKA 1000 F (Ekamant, Poland) in the form of belts with dimensions 1000 × 80 mm was used. The grit sizes of the paper were P60 and P180. Cutting speed of 14,5 m/s and sanding pressure of 0,65 N/cm² were applied.

Particle-size determination and calculation of the content of fine dust particles were carried out according to methods described by (Hlásková et al., 2016; Pędzik et al., 2020; Piernik et al., 2019; Rogoziński et al., 2015). A set of sieves with aperture sizes of 250, 125 and 63 was used due to the high level of wood dust fineness. Based on the results of sieve analysis the particle mean arithmetic diameter was calculated as follows:

$$\bar{x} = \sum_{i=1}^n x_i \cdot q_{3_i}$$

where:

q^3 – particle distribution by mass,

x – mean value of particle size class,

n – number of particle size classes.

The content of particles <10 and μm was calculated. Particles with these sizes can cause the air pollution by airborne particles (Aro et al., 2019).

RESULTS

On the basis of the conducted research, significant differences in the amount of ERG were found between dust granulation. In most cases, the P60 dust contained lower ERG concentrations than the P180 dust (Fig 1). Significantly higher concentration of ERG was found in beech, hornbeam and larch wood. These results indicate a higher contamination of fungi, and microscopic dust of finer graining. Comparing the dust between wood species, it is clearly visible that the wood of deciduous trees has a significantly higher amount of ERG than the softwood (Fig. 2). On the basis of average ERG concentrations, it can also be seen that the P60 dust for all tested samples was characterized by a significantly lower amount of ERG than P180.

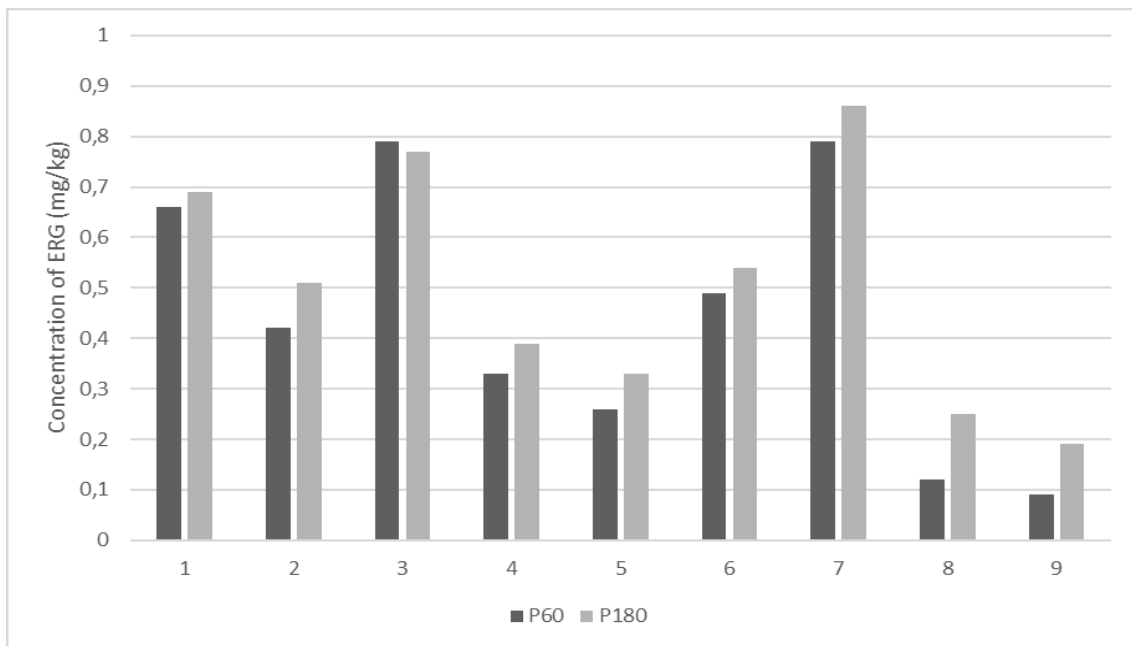


Figure 1. Concentration of ERG (mg/kg) dust: 1- beech, 2-oak, 3-hornbeam, 4-ash, 5-alder, 6-walnut, 7-larch, 8- pine, 9-spruce.

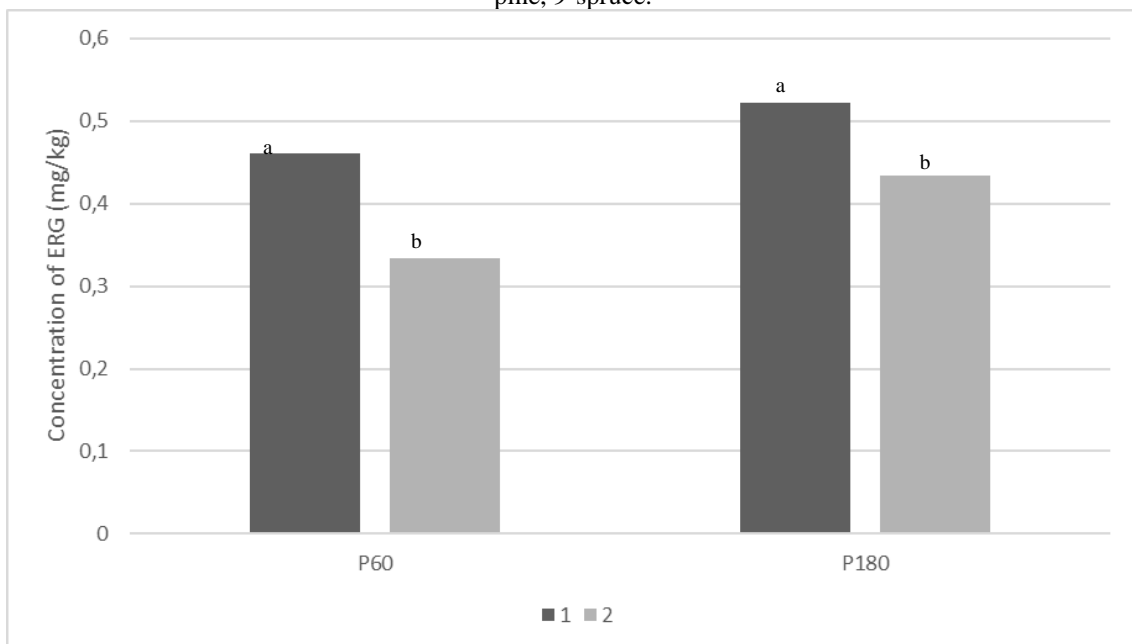


Figure 2. Concentration of ERG (mg/kg) in 1- hardwood, 2 – softwood.

a,b- the same letters indicate no significant differences in the confidence level $p=0,05$

In this study, one of the methods for determining the level of mycobionta contamination was used, namely the analysis of ergosterol concentration (ERG). Ergosterol is

the main sterol of the cell wall of microscopic fungi. This method has been widely used in various branches of the agri-food industry, agriculture, as well as to assess the level of mycobiotic contamination in livestock buildings, residential buildings, etc. both dead and alive microscopic fungi. Little information has been found in the literature on the ERG content of wood dust. Gutarowska and Cichocka 2002 determined the ERG content in paper raw materials from various stages of paper production from a paper mill in Poland. Among other things, the ERG content in the bark (a mixture of pine and birch) was determined to be 1 mg/kg. The mentioned authors also compared the chemical method for the determination of fungal biomass with the method of cultivation on plates with MEA medium. The content of fungi in the bark samples, determined as the number of colony forming units, was 5.4×10^5 (cfu/g), and the types of fungi isolated were: *Cladosporium sp.*, *Penicillium sp.*, *P. chrysogenum*, *Trichoderma viride*, *Rhizopus nigricans*, yeast. The determination of ergosterol, however, has a significant advantage over the cultivation methods because the time of the analysis is short (2-3 hours, in the cultivation method 5 days) and it assesses the total amount of fungi present in the analyzed sample, both active and inactive. This should be borne in mind when interpreting results and comparing methods. The ERG analysis method used in this work is a recognized analytical method. Based on the determined significant correlations between the number of microscopic fungal colony-forming units (CFU) and the concentration of ERG, the maximum concentration of this metabolite in the tested material is determined, indicating a safe level of fungal contamination. The limit of ergosterol concentration in construction materials (e.g. wood), on the basis of which the mold condition is estimated, is 4 mg/kg of material. On the other hand, the value of ERG concentration of 3 mg/kg (Stuper-Szablewska and Perkowski, 2017) was adopted as the safe content of fungal mycophore in healthy cereal grain. Pasanen et al. 1999 reported that in pure mushroom culture (ERG concentrations vary slightly depending on the type and species of fungi), the average concentration of ERG is about 1850 mg/kg. In the light of these data, it can be concluded that the concentration of ERG in dust samples was determined in this study it is low. Stuper-Szablewska et al., 2017 and Szwajkowska-Michałek et al., 2020 publications stated that the amount of mycobiotic measured by the ERG concentration depends on the tree species.

CONCLUSION

On the basis of the study, it was found that dusts with finer granulation from deciduous wood are characterized by a higher concentration of ERG, i.e. a higher level of contamination with microscopic fungi (Fig 2). It is important information from the point of view of health and safety at work. Dusts with a lower granulation of hardwood wood pose a greater threat to workers affected by wood processing than those of higher granulation and originating from softwood.

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Streszczenie:

Zanieczyszczenie mikroskopijnymi grzybami mierzone stężeniem ergosterolu w pyłach różnych gatunków drewna o różnej granulacji.

Pyły drzewne - zarówno rozproszone w powietrzu wewnątrz zakładu produkcyjnego, jak i osadzone - zawierają również tak zwane czynniki biologiczne środowiska pracy. Termin ten odnosi się do mikroorganizmów oraz struktur i substancji wytwarzanych przez te organizmy, które mają niekorzystny wpływ na organizm ludzki i mogą powodować liczne choroby. Celem pracy było określenie stopnia zanieczyszczenia pyłami z różnych gatunków drewna mikroskopijnymi grzybami w zależności od uziarnienia. Zastosowano metodę analizy chemicznego markera ergosterolu, którego stężenie jest skorelowane z liczbą grzybów mikroskopowych. Na podstawie przeprowadzonych badań stwierdzono, że pyły z drewna liściastego o drobniejszej granulacji charakteryzują się wyższym stężeniem ERG, czyli wyższym poziomem zanieczyszczenia mikroskopijnymi grzybami. To ważna informacja z punktu widzenia bezpieczeństwa i higieny pracy.

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