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CONTENTS OF MICROSCOPIC FUNGI IN DUSTS COMING FROM CEREAL ANALYSIS LABORATORIES

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Abstract: Microscopic fungi – components of bioaerosol found in the workplace environment of individuals employed in the agricultural sector – constitute a considerable hazard for their health. This study includes quantitative and qualitative analyses of mycobionta contained in 20 samples of dusts collected from laboratories conducting analyses of cereals. A total of 27 species of viable microscopic fungi were isolated. The most frequently isolated genera *Penicillium* and *Aspergillus*, accounting for 27% and 26% of analyzed isolates. The content of fungal biomass was determined quantitatively using a fungal marker, ergosterol (ERG). Concentrations of this metabolite for all samples ranged from 0.48 mg/kg–212.36 mg/kg. Based on the analyses, it may be stated that the concentration of microfungi in settled dust from laboratories conducting analyses of cereals was varied, and in several cases markedly exceeded admissible concentration levels.

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INTRODUCTION

Indoor air in the workplace environment, depending on occupational activities and contaminants emitted while they are performed, differs in its composition both in terms of inorganic and organic matter [27]. Both settled and suspended dusts contain the so-called biological agents of the workplace environment. This term refers to micro- and macroorganisms, as well as structures and substances produced by these organisms, which have a harmful effect on the human organism and may cause occupational diseases.

Commonly found biological factors, which are harmful for human health, include among other composition also microscopic fungi. Occupational groups particularly at risk of exposure to different fungi are farmers, gardeners, workers in grain silos as well as individuals employed in laboratories conducting cereal analyses. In the EU member countries, issues of protection of health of employees against risk connected with exposure to biological factors in the workplace are regulated by Directive No. 2000/54/EC. The Ordinance of the Minister of Health of 22 April 2005 on biological factors harmful for human health in the workplace environment and the protection of health of employees exposed to these factors in connection with their occupation (official Gazette Dziennik Ustaw No. 81, item 716) [40] gives, e.g. the classification and a list of harmful biological factors, as well as a list of activities exposing workers to the action of biological factors, including mould fungi, particularly their spores and hyphal fragments, which are a considerable hazard. This is mainly due to the fact that spores range in size from around a dozen to several dozen micrometers, and due to their dimensions may easily penetrate the airways and be deposited in skin pores. Microscopic fungi cause numerous occupational diseases of farmers, such as inhalant and skin allergies, allergic pulmonary alveolitis and mycoses (pityriasis versicolor, ringworm of hairless skin and the scalp with deep reaction) [5, 30]. Apart from human tissue infection with fungal

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mycelium, pathogenic processes may also occur, caused by secondary metabolites produced by most fungi, i.e. mycotoxins (*dermato-mycotoxicosis professionalis*). There are studies indicating a significant relationship between the occurrence of fungi with the presence of mycotoxins in the plant material [26, 42, 45]. Moulds are important allergens for many occupational groups. Allergies to fungi acquirepd at work are frequently sources of considerable problems in diagnostics and medical expert opinions concerning the incidence of the disease, i.e. the potential diagnosis of an occupational disease. To date, several cases have been described of chronic diseases of workers of the agricultural sector, caused by their contact with both moulds and their metabolites. One of such groups of workers in this sector comprises laboratory workers employed in laboratories performing analyses of cereals. They have contact with many samples of cereals coming from different farms [22]. Observation of basic safety precautions when working on this type of material is crucial, mainly for the health of these workers [24].

The aim of this study was to determine the level of air contamination with microscopic fungi in laboratories conducting analyses of cereals in western Poland in terms of quantity and quality. In quantitative determinations of total fungal biomass the chemical method of analysis of a specific fungal marker, such as ergosterol (ERG) was used. This is a component of the cell wall in fungi and is used as an indicator of mycobionta content in analyzed material [31, 32, 33]. Moreover, isolated viable fungal strains were identified based on colony morphology and microscopic specimens. After their identification, the species composition of mycobionta, in analyzed dusts was determined.

MATERIALS AND METHODS

Analyzed material consisted of settled dusts collected in autumn 2008 from 20 locations in 3 replications from laboratories performing cereal analyses in western Poland. Samples of settled dust were collected using the gravimetric method of free settling (Polish Standard PN-Z-04008-7:2002) [36], consisting in the exposure of a known surface onto which dust settles passively, by gravity. Due to the specific character of conducted investigations, the surface of free settling could be neglected, while the weight of settled dust was of greater importance. Exposure lasted for 7 days. The weight of collected dust was approx 2 g.

Methodology of mould determination

The analysis of fungi occurrence in dusts. The probes of dusts from grains were collected in different specialistic laboratories conducting analyses of cereals. The analyses of the composition of viable fungi species occurring in dusts' probes were analyzed. The dilution method was used: 1 g of dust was placed into 10 ml of sterile distilled water and mixed with a magnetic stirrer for 2 min. Next, the 1 ml of suspension was carried on the PDA medium (Potato-Dextrose Agar) in Petri dishes and spread with the aid of a sterile glass stick on the medium surface. The Petri dishes were incubated at 25°C for 7 days. Growing mycelia were isolated on the PDA and SNA (Syntetic Nutrient – Poor Agar) mediums to identify the fungi species. Fungi were determined according to the manuals by Raper and Thom [39], Arx [1] and Domsch *et al.* [9].

Analysis of ergosterol (ERG). A 100 mg sample was placed in a 17 ml culture tube, suspended in 2.5 ml of methanol, treated with 0.7 ml of 2 molar (M) aqueous sodium hydroxide, and tightly sealed [25, 34]. Then the culture tube was placed in a 250 ml plastic bottle, sealed, and placed inside a microwave oven. The sample was irradiated (400 W) for 20 s, and after 3 min for an additional 25 s. After 15 min cooling, the content of the culture tube was neutralized with 0.25 M aqueous hydrochloric acid, 3 ml of methanol were added and extracted with pentane (3 × 4 ml), which was carried out within the culture tubes. Combined pentane extracts were evaporated to dryness under a stream of nitrogen. Prior to the analysis, samples were dissolved in 4 ml of methanol, filtered through a 13 mm syringe filter (Fluoropore Membrane Filter 0.5 µm FH Millipore, Ireland), evaporated to dryness with a nitrogen stream and dissolved in 1 ml of methanol.

A high-performance liquid chromatograph (Waters Corporation, Milford, MA, USA) with a UV detector (Waters 486 Tunable Absorbance Detector) was used, equipped with a Solvent Delivery System Waters 501 HPLC pump, a Waters U6K syringe loading injector by Waters Corporation, 25 µl (Hamilton, USA) or 100 µl (Kloehn Waters, USA) syringes, a 150 mm × 3.9 mm I.D. stable still analytical column packed with Nova-Pak C_{18} – d_p 4 µm (Waters Corporation, Milford, MA, USA) and coupled with a Waters Guard-Pak™ Insert Nova-Pak® C_{18} (4 µm particle size) precolumn. The mobile phase consisted of the methanol portion (90%) and acetonitril (10%, v/v) and was pumped at a flow rate of 0.6 ml/min. The ergosterol peak was monitored at 282 nm. The total run time was 15.0 min with ergosterol eluting at 12 min.

Ergosterol methanolic standard stock solutions and subsequent dilutions were prepared in appropriate volumetric flasks. The calibration curve for ergosterol was constructed within the range of 0.1÷200 µg/ml. Ergosterol content was estimated by comparing peak areas with those of the external standard. The presence of ergosterol was confirmed by a comparison of retention times with those of the external standard and/or by co-injection with a standard solution. LOD was 0.01 mg/kg.

RESULTS AND DISCUSSION

Diseases caused by the harmful effect of microscopic fungi found in the workplace atmosphere have been described extensively in literature [13, 16, 17, 19, 21, 23, 35,

Table 1. Percentage proportions of individual fungal species isolated from dusts coming from cereal analysis laboratories.

Species	Dusts																				% total
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	
Alternaria alternata (Fr.) Keissl.	74.9	13.3	38.3			16.7	12.7								17.6	35			15	16.9	15.30
Arthrinium phaeospermum (Corda) M.B. Ellis		1.7	12.8				9.1			2.3			23.5	4.6	58.9						11.60
Aspergillus nigricans Cooke (1885)		21.7		52.5		21.9	14.5	4.3	95.5	9.1				61.2	17.6		9.1		3.7	5.6	15.70
Aspergillus ochraceus G. Wilh.			6.4	2.5		16.7		4.3													1.30
Aspergillus versicolor (Vuill.) Tirab.	4.2	5	2.1	15	5.6	11.2			4.5	9.1			41.2	18.2			18.2		30.3	22.5	7.48
Aureobasidium pullulans (de Bary) G. Arnaud																5					0.30
Chaetomium cochlioides Palliser														2.3		5					0.30
Chaetomium globosum Kunze						5.6		4.3		2.3											0.30
Cladosporium macrocarpum Preuss																	9.1				0.17
Humicola grisea Traaen			2.1																		0.16
Paecilomyces farinosus (Holmsk.) A.H.S. Br. & G. Sm.					27.6	16.7															1.30
Paecilomyces marquandii (Massee) S. Hughes								34.8													1.30
Penicillium brevicompactum Dierckx		21.6								4.6											2.40
Penicillium chrysogenum Thom		16.7		5	16.8			4.3		25	100	38.9	5.9	2.3		2.5			24	12.4	10.50
Penicillium citrinum Thom							9.1														0.80
Penicillium expansum Link				2.5																	0.16
Penicillium lanosum Westling			14.9																		1.77
Penicillium spinulosum Thom				2.5						2.3							9.1				0.30
Penicillium verrucosum Dierckx			21.3	2.5	50		34.6	39.4		2.3				11.4					5	10	10.9
Phoma eupyrena Sacc.						11.2				40.7			29.4			5					4.50
Phoma exiqua Sacc.	16.7			17.5			20	8.6								32.5	9.1				6.30
Trichoderma atroviride P. Karst.												44.4									1.30
Trichoderma koningii Oudem.	4.2	20								2.3		16.7					9.1				3
Trichoderma viride Pers.															5.9			100	22	32.6	1.80
Non-sporulating 1																12.5					0.75
Non-sporulating 2																2.5					0.15
Non-sporulating 3			2.1																		0.16
Number of species: 27	**4**	**7**	**8**	**8**	**3**	**7**	**6**	**7**	**2**	**9**	**1**	**2**	**3**	**5**	**4**	**7**	**6**	**1**	**6**	**6**	

44]. However, the allergenic mechanism of moulds has not been thoroughly clarified yet [3, 7]. This depends on several factors, mainly on individual preferences and co-existence of different fungal species generally described as synergistic. In the case of the presence of several fungal species and mycotoxins in cereal dust, their action may accumulate and cause disease symptoms in the form of acute pulmonary episodes, and other types of symptoms [8]. A potential health hazard for laboratory workers is posed by numerous mould species found in grain and cereal dust, known as etiological factors of allergies and immunotoxic diseases of the respiratory system [10, 11].

Dutkiewicz reported in his monograph on organic dust [11], potentially constituting a health hazard for humans, described contents of individual groups of microorganisms in dusts coming from different environments. He stated

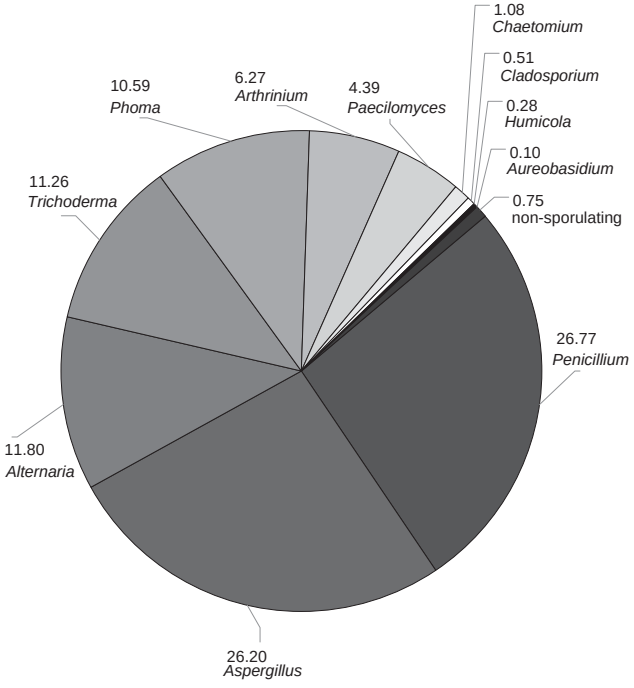

Figure 1. Percentage proportions of fungi isolated from 20 samples of laboratory dusts.

that all dusts contain Gram-negative and Gram-positive bacteria, *Actinomycetes* and microscopic fungi. In the latter group of microorganisms the most frequently isolated fungi are those from the genera *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. candidus*, *A. terreus*, *A. clavatus*, *A. niger*), *Penicillium*, *Eurotium*, *Trichoderma*, *Absidia*, *Mucor*, *Rhizopus* as well as yeasts of *Candida* spp., *Rhodotorula* spp. and *Endomycopsis* spp.

Among samples of dusts analyzed in the course of this study, a total of 27 different cultivar fungal species were isolated (Tab. 1). The highest frequency was recorded for fungi from the genera *Penicillium* (26.77%) and *Aspergillus* (26.20%), while the lowest – for fungi from genus *Aureobasidium* (0.1%). The genus *Penicillium* was represented mainly by *Penicillium chrysogenum* and *Penicillium verrucosum* (Fig. 1). The most frequently observed species among fungi from the genus *Aspergillus* was *Aspergillus nigricans*, accounting for 15.7% fungal microbionta. It was isolated from over 59% analyzed dust samples (Tab. 1). Krysińska-Traczyk *et al.* [19] in analyzed samples of grain dusts collected during threshing of cereals by a combine harvester also identified fungi from genus *Aspergillus* (*A. fumigatus*, *A. niger*, *A. candidus*, *A. ochraceus*) to be dominant. Góra *et al.* [14] identified *A. niger* and *Aspergillus* spp. in the air during grain processing (sacking wheat brans, pouring cleaned wheat grain, production of semolina). Lugauskas *et al.* [21] isolated species from the genera of *Penicillium* Link, *Aspergillus* P. Micheli., *Mortierella* Coem., and *Mucor* P. Micheli from the air at the grain mill, which made up the vast majority of the identified isolates. It needs to be stressed that in samples of analyzed laboratory dusts *Alternaria alternata* constituted over 15% of all isolated fungi. This species belongs to fungi commonly found in cereal and cereal dust [16, 21, 23], and could be a cause of allergic diseases [10, 15]. In literature sources on the subject, there are several reports on the occurrence of this fungus and other species of genus *Alternaria* isolated from dust [2, 15, 37]. Contents recorded in harvest dust of 5 cereals for different species of microscopic fungi, as well as mycotoxins they produce have been described in detail by Krysińska-Traczyk *et al.* [16, 18, 20]. In rye dust she found mainly 3 mould species, i.e. *Monosporium silvaticum* (65.9%), *Alternaria alternata* (21.0%) and *Oidiodendron flavum* (13.1%). Fungus *Alternaria alternata* accounted for as much as 78.5% of all mould species isolated from samples of barley dust. Species isolated from oat dust were *Rhodotorula* spp. prevailed (92%) as well as *Monilia sitophila*, *Cladosporium elegantulum*, *Alternaria alternata* and *Penicillum* spp. The dominant species in samples of buckwheat dust was *Rhodotorula rubra* (62.8%), while in case of maize dust it was *Alternaria alternata* (25.6%). In corn dust, the dominant fungi were *Penicillum* spp. (59.0%), and common species was *Alternaria alternata* (25.6%), *Aspergillus ochraceus*, *Geotrichum candidum*, *Rhizopus stolonifer* (Ehrenb.) Vuill. and *Aspergillus niger* constituted the remaining 15.4% of the grain dust mycobiota [18].

The presence of fungi from genera *Alternaria*, *Aspergillus* and *Penicillium* in the wheat harvest and storage cycle was also observed by Beard *et al.* in dusts coming from farms in Colorado (USA) [4] During harvest, genus *Alternaria* predominanted, as it accounted for approx. 69% isolated fungi. Moreover, the presence of *Cladosporium* (17%) was also detected. Dusts collected during wheat storage contained mainly *Aspergillus* (31%), *Alternaria* (12.9%) and *Penicillium* spp. (20%). During winter silo storage of wheat, an increase was found in dust for the total mould count, as well as storage fungi such as *Mucor*, *Fusarium* and *Pencillium*, which accounted for 22.3%, 16.5% and 7.4% of fungi detected in the cereal dust.

Next to the qualitative composition of mycobionta, the total amount of fungal mycobionta in dust is also important in terms of health protection. A high content of mycelia and spores in the airborne dust increases the probability of incidence of infection and allergic symptoms, even in individuals with no allergies [11]. Thus, samples of dusts were also subjected to quantitative analyses. Total mycobionta content was determined based on the determination of ergosterol concentration. It was found that the correlation between ERG and CFU is significant [6, 12, 28, 38, 43]. Contents of this metabolite in samples fell within a very wide range of values. ERG concentration for all samples ranged from 0.48 mg/kg–212.36 mg/kg (Tab. 2). Among analyzed dusts, approx. 41% fell within the range of ERG concentrations from 0.48 mg/kg–7.17 mg/kg, with the same percentage comprised by dusts from the range of concentrations of the analyzed metabolite from 10.13 mg/kg–

Table 2. Ergosterol concentration [mg/kg] in samples of dusts coming from cereal analysis laboratories.

No. of samples	ERG [mg/kg]	No. of samples	ERG [mg/kg]
1	38.10	11	120.62
2	1.86	12	14.98
3	28.16	13	2.14
4	12.30	14	1.13
5	19.51	15	7.16
6	22.31	16	0.48
7	6.13	17	1.04
8	5.31	18	1.84
9	3.89	19	154.28
10	7.17	20	212.36

38.0 mg/kg. In turn, the highest range of ERG concentrations from 120.62 mg/kg–212.36 mg/kg was found in 18% analyzed dust samples.

There is no data in available literature on ERG content in dust coming from laboratories performing cereal analyses. We may only find information concerning concentrations of this metabolite in house dust. Axelsson *et al.* [2] reported ERG concentrations in this type of samples ranging from 6–45 mg/kg. Higher ERG concentrations were observed in similar samples by Saraf *et al.* [41]. He reported ERG concentrations from 20–165 mg/kg. Among data on ERG content in dusts there is no information indicating what concentrations found in dust could pose a hazard for humans exposed to dust. The only information on the safe concentration limit for this metabolite was given by Maupetit *et al.* [25], who adopted the range of ERG concentrations from 1–9 mg/kg as the safe limits for grain for human consumption. In turn, Müller and Schwardorf [29] assumed for the same plant material the limit of 9 mg/kg as safe. On the other hand, Pasanen *et al.* [31] reported that in pure fungal culture (depending on the type and species of fungi values of ERG concentrations varied slightly) mean ERG concentration was approx. 1,850 mg/kg. In view of these data, it is difficult to draw conclusions on the basis of recorded results.

CONCLUSION

The absence of pollutants in rooms in which analyses are performed is crucial, since the level of mycobionta in the air has a significant effect on the health. In analyzed samples, ERG concentrations determined in dusts and numerous identified species of fungi indicate a considerable hazard for the staff. This suggests that it is necessary to observe all safety precautions when working on cereal material and to use high-performance ventilation systems in order to remove dusts found in the laboratory facilities.

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