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VISIBLE FUNGI GROWTH AND DAMPNESS ASSESSED USING A QUESTIONNAIRE VERSUS AIRBORNE FUNGI, (1→3)-β-D-GLUCAN AND FUNGAL SPORE CONCENTRATIONS IN FLATS

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Abstract
Objectives: The study aimed at determination of the usefulness of the subjective assessment of selected signs of fungi growth in flats and microclimate parameters to indicate the actual air contamination with culturable fungi, (1→3)-β-D-glucans and fungal spores. **Material and Methods:** This analysis covered 22 flats, the inhabitants of which declared in a questionnaire interview the presence of the developed mycelium on solid surfaces in the flat. Air samples for determination of the culturable fungi, (1→3)-β-D-glucans and (viable and non-viable) fungal spores concentrations indoor and outdoor the flats during the heating period were collected. During bioaerosol sampling microclimate parameters were measured. Predictive models for concentrations of the tested biological agents with regard to various ways to assess fungal contamination of air in a flat (on the basis of a questionnaire or a questionnaire and microclimate measurements) were built. **Results:** The arithmetic means of temperature, relative humidity, CO_2 concentration and air flow velocity in the flats were respectively: 20.5°C, 53%, 1431.6 ppm and 0 m/s. The geometric mean concentrations of airborne fungi, (1→3)-β-D-glucans and fungal spores in these premises amounted to 2.9×10^2 cfu/m^3, 1.6 ng/m^3 and 5.7×10^3 spores/m^3, respectively. The subjective assessment of fungi growth signs and microclimate characteristics were moderately useful for evaluation of the actual airborne fungi and (1→3)-β-D-glucan concentrations (maximum percent of explained variance (VE) = 61% and 67%, respectively), and less useful in evaluation of the actual fungal spore concentrations (VE < 29%). In the case of fungi, higher usefulness was indicated of the questionnaire evaluation supported by microclimate measurements (VE = 61.2%), as compared to the evaluation only by means of a questionnaire (VE = 46.9%). **Conclusions:** Subjective evaluation of fungi growth signs in flats, separately or combined with microclimate measurements, appeared to be moderately useful for quantitative evaluation of the actual air contamination with fungi and their derivatives, but more extensive studies are needed to strengthen those findings.

Key words:
Glucans, Fungal spores, Airborne fungi, Questionnaire survey, Dwelling contamination, Residential exposure

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INTRODUCTION

The studies carried out in recent years in European countries, Canada and the United States indicate that on average 20% of residential premises exhibit some signs of dampness [1]. In Poland the dampness problem may refer to as many as 43% of flats [2]. Dampness of usable areas in closed rooms contributes to the growth and dissemination of fungi and their derivatives, especially fungal spores and (1→3)-β-D-glucans – components of the cellular wall of fungi released as a result of fungi degradation [3,4]. Consequently, indoor air of the flats exhibiting signs of dampness and fungi growth may become contaminated with fungal spores [5] and fragments of fungi [6]. Because of their usually very small sizes, they may be deposited in the inhabitants' airways and reach even alveoli, causing infectious or allergic effects [3,4,7]. On the other hand, the effects of (1→3)-β-D-glucans in the indoor air on human body are still a matter of dispute [8]. However, (1→3)-β-D-glucans are commonly used in the studies as non-specific fungal marker. The use of this marker allows to determine (in contrast to the culture method) viable and not-viable fungal cells [9,10].

Many epidemiological studies point to a correlation between inhabiting or long staying in premises showing signs of dampness and fungi growth and an increased risk of the occurrence of various ailments and diseases of the respiratory tract (e.g., allergy including initiation of asthma as well as exacerbation of existing asthma), both in adults and in children [11–14]. In most studies focused on health effects of exposure to fungi in residential flats, the assessment of fungal contamination in the flats is based only on the information obtained from the inhabitants or from inspectors' observations during their short visits. Because of possible subjective bias of the data obtained from the inhabitants and random character of the observations conducted by inspectors, such assessment may often differ from the true state. On the other hand, assessment of contamination of flats with airborne fungi and their derivatives based on measurements of these agents is expensive and time-consuming.

The analysis presented in this article is aimed at determination of the usefulness of the subjective assessment of selected signs of fungal growth in flats and microclimate parameters to assess the actual extent of air contamination with fungi and their derivatives. The results of the conducted analysis could be used for a preliminary assessment of home exposure to fungi.

MATERIAL AND METHODS

Flat selection

The studies were carried out in the municipal agglomeration in central Poland during 2011–2012 period. This publication presents the results obtained for 22 flats chosen in a purposeful way from 754 flats as a simple random sample selected from the official register of territorial division of the country (TERYT) conducted by the Central Statistical Office of Poland (Główny Urząd Statystyczny – GUS), the inhabitants of which gave their informed consent to participate in the study. The criterion of purposeful selection was the presence of the developed mycelium on solid surfaces in the flat, as declared by the inhabitants in the questionnaire interview. The interview was conducted by trained pollsters during their visits to each of the randomly selected flats. The interview was based on a questionnaire specifically prepared for the purpose of this study. It included questions on, e.g., intensity of the occurrence of various signs of dampness in the premises, intensity of the occurrence of various signs of fungal growth, as well as the way the flats are used, as this may also contribute to the dampness and fungal growth.

Air sampling and measuring microclimate parameters

In each of the investigated flats, the air in the living room and additionally in other rooms was analyzed if it exhibited signs of fungi growth, and then the results were averaged for the whole flat. Air samples for determining

the concentrations of culturable fungi, (1→3)-β-D-glucans and fungal spores in each of the investigated rooms were collected at the same time. The samples were collected in a stationary way at the height of approx. 1.0–1.2 m from the floor level during the heating period in normal conditions of heating, airing and using the rooms. At the same time, to evaluate the 'background' concentrations for the tested agents near the flats covered by the study, atmospheric air samples were collected in a similar way.

Air samples for the analyses of culturable fungi and (1→3)-β-D-glucans were collected using separate measuring sets consisting of a GilAir 5 pump (Sensidyne, USA) and the open-faced aerosol sampler (Two-Met, Poland), with a 37-mm diameter glass-fiber filter (Whatman International Ltd, UK). The open-faced sampler was disinfected with 70% ethanol solution before each loading with a filter, then loaded with a filter inside of a Class II Biosafety cabinet (Esco Micro Pte Ltd, Singapore), and transferred in a dust-free bag to the sampling sites. The sets were operated at a flow rate of 2 l/min. The measuring sets were calibrated before each sampling procedure using a Gillibrator 2 calibrator (Sensidyne, USA). The sampling took approximately 24 h. All the samples were collected in 2 repetitions. After the sampling, the filters with collected biological material were transported to the laboratory in a tightly closed bags.

The mass of airborne dust (in mg) collected on the filters for the analysis of (1→3)-β-D-glucans was evaluated by the gravimetric method. The filters were weighed before and after sampling with accuracy equal to 0.01 mg using CP 225D scales (Sartorius AG, Germany). Then the filters were stored at –20°C and determinations were performed after all the samples had been collected. The filters for assessment of culturable fungi were analyzed immediately after the measurement.

Air samples for analysis of fungal (viable and dead) spores were collected using a Personal Volumetric Air Sampler for glass slides (Burkard Manufacturing Co Ltd, England) at a flow rate of 10 l/min. The samples were collected in 2 repetitions. Before each sampling, the sampler was disinfected with 70% ethanol solution and fitted with a standard glass microscope slide on which, in the central part (2×2 cm), the optically clear adhesive vaseline (Laboratorium Galenowe Olsztyn Sp. z o.o., Poland) was mounted. The sampling took 30 min. After the sampling, the slides were removed from the sampler, transported to the laboratory in special boxes and analyzed.

During indoor and outdoor bioaerosol sampling at the same point, the basic parameters, such as: temperature, relative humidity, CO_2 concentration and air flow velocity were measured. The measurements were carried out using the microclimate multifunction meter Testo 435–2 (Testo AG, Germany) at the height of 1–1.2 m over the floor level during 10 min. The values of individual parameters were noted every minute, then the result was averaged for a given measurement point.

Sample analysis

The filters for assessment of culturable fungi were put into sterile containers, covered with 10 ml of Phosphate Buffer Solution (BTL, Poland) and the biological material on the filters was eluted by shaking on a platform shaker (Johanna Otto GmbH, Germany) (shaking time: 60 min, shaking rate: 420 revolutions per minute). A series of 10-fold dilutions was made from the obtained eluates. Plates with Malt Extract Agar (soy peptone – 3 g/l, agar – 15 g/l, malt extract – 30 g/l (BTL Sp. z o.o., Poland)) supplemented with streptomycin sulphate (130 mg/l, Sigma-Aldrich Chemie GmbH, Germany) and chloramphenicol (50 mg/l, PPH Galfarm Sp. z o.o., Poland) were inoculated with given volumes of eluates and their dilutions by a superficial method. In order to determine the total number of fungi, agar plates were incubated at 25±1°C for 7 days. The colonies which grew on the plates were counted, and taking into account the degree of the sample dilution and the volume of aspired air, the obtained fungi concentration

was expressed in terms of the number of colony forming units per 1 m^3 of the examined air (cfu/m^3). The detection limit was 3.5 cfu/m^3.

The analysis of (1→3)-β-D-glucan concentrations in the air samples collected on the filters was carried out using Glucatell test in kinetic version (Associates of Cape Cod Inc., USA) according to the procedure described by Cyprowski et al. [15]. For all the samples, the analysis was carried out separately for water-soluble (WS) and alkali-soluble (AS) fractions of (1→3)-β-D-glucans. Final concentration of (1→3)-β-D-glucans was the sum of the 2 determined fractions. Taking into account volumes of the air collected on the filters, the concentration values were expressed in ng/m^3. The detection limit was 0.008 ng/m^3.

The slides for assessment of fungal spores were stained with basic fuchsin and analyzed microscopically. The fungal spores were counted under the 1000 × total magnification of a light microscope (BX41, Olympus Corporation, Japan) using immersion oil. Before the start of analysis, the size of a single field of view obtained at this magnification (0.038 mm^2) and the size of trace on the adhesive substance (14 mm^2) (which was created during air sampling), were determined using an objective micrometer scale with an accuracy of 0.01 mm. At least 100 randomly selected microscopic fields per sample trace on slide were examined. Fungal spores were distinguished from other airborne particles based on their morphological characteristics with the aid of the illustrated identification key available in the literature [16].

The total spores present in the sample trace on each slide were estimated from total spores counted in all the analyzed microscopic fields and the fraction of total trace area represented by the analyzed fields. The fungal spore concentrations in the air samples were estimated taking into account the total spores per sample trace and the volume of collected air. Results were expressed as $spores/m^3$. The detection limit was 12 $spores/m^3$.

Statistical analysis

The concentrations of airborne culturable fungi, (1→3)-β-D-glucans and fungal spores inside and outside the flats with fungi problems were characterized using geometric mean (GM), geometric standard deviation (GSD) and the range of the observed values (min.–max). The values of indoor and outdoor microclimate parameters were characterized by the use of arithmetic mean (AM), standard deviation (SD) and the range of the observed values. Spearman's rank correlation coefficients were determined to test the association of environmental variables and the biological contamination of air in the flats under the study. The analysis did not include the correlation of the investigated agents with the air flow velocity, because the value of this parameter in all the investigated flats was equal to 0 m/s.

Linear regression was used to compare airborne culturable fungi, (1→3)-β-D-glucan and fungal spore concentrations with respect to subjectively assessed intensity of dampness and signs of fungi growth in flats, the way the flat is used (that could potentially affect the occurrence of fungi) and microclimate measurements. For this analysis, the measured values of each microclimate parameter (with the exception of air flow velocity) were categorized into 2 groups with the ranges of values favorable or unfavorable for the occurrence of fungal growth. The group with the range of unfavorable values for the occurrence of fungi growth in a flat was the reference category.

In the case of subjective assessments of fungi growth, the reference category was the group of questionnaire answers pointing to the occurrence of the developed mycelium having the smallest reported size ($< 0.25\ m^2$) and color of mycelium other than black and/or brown. In relation to other signs of dampness and fungal growth, the answers indicating the lack of a given sign were the reference category. For the subjective assessments of the way the flat was used, the reference category was a group of questionnaire answers showing that the flat was used in a way which

does not predispose to the occurrence of fungal growth. The dependent variables were log transformed, with the exception of microclimate parameter values. P-value of 0.05 was used as the level of significance.

Predictive models for concentrations of the tested biological agents with regard to various ways to assess the degree of fungal contamination of the air in a flat (on the basis of a questionnaire or a questionnaire and microclimate measurements) were built. Partial least squares (PLS) regression was used to build the predictive models. Number of components in the PLS model was selected using crossvalidation (CV). We used root mean square error (RMSE) and percent of explained variance (% VE) as measures of predictive performance.

R Statistical Package (Version 3.0.1) was used to conduct all the calculations [17].

RESULTS

Analysis of the questionnaire data indicates that of the 22 tested flats in which the mycelium, developed to a different degree, was observed on stable surfaces, in 15 flats the inhabitants, additionally reported the presence of some signs of dampness on the walls or ceiling, and in 6 – dampness was accompanied by the peeling off paint. In 19 of the 22 flats the water vapor precipitated on windows or other surfaces during the heating period, but only in half of the flats the presence of perceptible moisture in flats was reported. In 18 flats a developed mycelium was observed on the surface of at least 0.25 m^2, and in the other 4 – on a smaller area. In over half of the flats (59%) mycelium was black and/or brown, whereas in the other cases there were other colors of the mycelium. The presence of perceptible mustiness was reported only in 8 of the 22 flats. Inhabitants of 9 of the 22 investigated flats admitted that during the heating season a lower temperature prevailed in the premises – no more than 20°C, while in the other flats the temperature above that value was maintained. Besides, in 9 cases, the rooms were aired several times a week, whereas the other flats were aired every day during the heating season.

The arithmetic mean of temperature and relative humidity measured in flats during the study reached 20.5°C (SD = 2.4, range: 14–23°C) and 53.0% (SD = 9.5, range: 38–76.4%), respectively and outside the buildings: 8.8°C (SD = 4.5, range: –2.7–14.0°C) and 48.5% (SD = 17.6, range: 23–73.6%), respectively. At the same time, the arithmetic mean of CO_2 concentration and the air flow velocity in the flats were 1431.6 ppm (SD = 983.1, range: 810–5606 ppm) and 0 m/s, respectively and in the background – 489 ppm (SD = 49.9, range: 432–600 ppm) and 0.4 m/s (SD = 0.2, range: 0.2–1 m/s), respectively.

Table 1 shows the geometric mean concentrations of culturable fungi, (1→3)-β-D-glucans and fungal spores in the indoor air of the investigated residential premises and in the outdoor air. The geometric mean concentration of culturable fungi, (1→3)-β-D-glucans and fungal spores in the indoor air of the flats amounted to 2.9×10^2 cfu/m^3, 1.6 ng/m^3 and 5.7×10^3 spores/m^3, respectively.

The correlation analysis (Table 2) indicated that there was a significant strong positive correlation between the concentration of culturable fungi in the indoor air of the investigated flats and the concentration of fungal spores, the value of relative humidity and the level of CO_2 ($p < 0.05$). In the case of fungal spore concentration, a significantly strong positive correlation was observed between the value of relative humidity and the level of CO_2 in the flats ($p < 0.05$). The value of relative humidity inside the investigated flats was significantly strongly positively correlated with the concentration of CO_2 in the indoor air ($p < 0.05$).

Table 3 presents the results of the analysis of the indoor concentrations of airborne fungi and their derivatives, depending on the intensity – as declared by inhabitants – of the occurrence of various signs of dampness, fungal growth, declared temperature in the flat and intensity of airing of the flat during the heating season, and the measured values of the indoor microclimate parameters. In

Table 1. Concentrations of culturable fungi and their derivatives in the indoor air of the flats with signs of fungi growth and in the outdoor air

Agent	Concentration of agent					
	indoor air of flats (N = 22)			outdoor air (N = 22)		
	GM	GSD	min.–max	GM	GSD	min.–max
Culturable fungi ($\times 10^2$ cfu/m^3)	2.88	0.05	0.22–67.28	0.53	0.02	0.14–1.50
(1→3)-β-D-glucans (ng/m^3)	1.56	1.97	0.28–4.13	2.06	1.52	0.42–2.86
Fungal spores ($\times 10^2$ spores/m^3)	57.51	0.03	10.38–2 331.49	13.64	0.02	0.61–35.64

N – number of samples; GM – geometric mean; GSD – geometric standard deviation; min. – minimal value of the range; max – maximal value of the range.

Table 2. The matrix of correlation coefficients (r) for bioaerosol components and parameters of the microclimate inside the flats under the study (N = 22)

Variable	Correlation coefficient				
	1	2	3	4	5
1. Culturable fungi					
2. (1→3)-β-D-glucans	0.21				
3. Fungal spores	0.64*	–0.09			
4. Temperature	0.19	0.16	0.16		
5. Relative humidity	0.56*	–0.02	0.56*	0.05	
6. Concentration of CO_2	0.66*	–0.17	0.94*	0.14	0.66*

* $p < 0.05$.

the case of culturable fungi, such analysis indicates the occurrence of significantly higher concentrations in the flats, where signs of developed mycelium were found on the surface larger than 1 m^2 ($p < 0.05$). In the case of (1→3)-β-D-glucans, significantly elevated concentrations were found to be associated with the declared damp surface which does not exceed 1 m^2, signs of fungi growth on the surface larger than 1 m^2 and with only black and/or brown mycelium ($p < 0.05$).

Analysis of the concentrations of fungi and their derivatives in the indoor air, depending on measured values of temperature, relative humidity and concentration of CO_2 inside the flat indicated significantly elevated concentrations, both in the case of culturable fungi and fungal spores at relative humidity higher than 60% ($p < 0.05$).

The multivariate analysis showed a moderate usefulness of the investigated variables in evaluation of the actual levels of culturable fungi and (1→3)-β-D-glucans, allowing to explain no more than 61% and 67% of the variability of actual concentrations, respectively. This analysis showed lower usefulness of the investigated variables in evaluation of the actual levels of fungal spores (VE < 29%). In the case of assessment of the actual concentrations of culturable fungi, the questionnaire evaluation supported by measurements of selected parameters of indoor microclimate (VE = 61.2%) was shown to be more useful as compared to the evaluation based exclusively on the questionnaire data (VE = 46.9%) (Table 4).

Figure 1 presents the ranks of subjectively ascertained signs of dampness and fungal growth, declared microclimate

Table 3. Concentrations of culturable fungi and their derivatives in the indoor air of the flats depending on the declared intensity of the occurrence of various signs of dampness and fungi growth and on the way the flat is used, as well as measured values of indoor microclimate parameters (N = 22)

Investigated trait / parameter	Flat with analyzed trait (n)	Culturable fungi ($\times 10^2$ cfu/m^3) [GM (GSD) min.–max]	p	(1→3)-β-D-glucans (ng/m^3) [GM (GSD) min.–max]	p	Fungal spores ($\times 10^2$ spores/m^3) [GM (GSD) min.–max]	p
Size of surface (wall/ceiling) with traces of dampness							
≤ 1 m^2	6	6.34 (0.04) 0.44–23.32	0.12	2.42 (1.64) 1.27–4.13	0.04*	72.18 (0.03) 21.67–467.03	0.38
> 1 m^2	9	2.85 (0.07) 0.22–67.28	0.43	1.53 (1.91) 0.48–2.86	0.33	68.14 (0.05) 10.38–2 331.49	0.38
Condensation of water vapor on the windows and other surfaces in the flat during the heating season							
yes	19	2.79 (0.05) 0.22–67.28	0.82	1.50 (2.03) 0.28–4.13	0.51	60.71 (0.04) 10.38–2 331.49	0.62
Perceptible moisture in flat							
yes	11	2.37 (0.03) 0.37–23.32	0.58	1.74 (1.85) 0.72–4.13	0.47	42.50 (0.02) 10.38–226.95	0.27
Signs of dampness and peeling off paint on the walls and/or ceiling							
yes	6	3.59 (0.05) 0.37–23.32	0.71	1.84 (1.78) 0.72–3.58	0.50	68.36 (0.04) 10.38–467.03	0.70
Size of surface with traces of developed mycelium (wall / ceiling / windowsill / seal windows / furniture)							
0.25–1 m^2	10	2.65 (0.04) 0.22–18.72	0.14	1.56 (1.96) 0.48–4.13	0.09	48.80 (0.03) 10.38–467.03	0.56
> 1 m^2	8	6.60 (0.05) 1.13–67.28	0.02*	2.15 (1.53) 1.02–3.58	0.02*	95.41 (0.05) 12.28–2 331.49	0.16

Perceptible musty odor in flat							
yes	8	5.47 (0.05) 0.37–67.28	0.17	1.81 (1.70) 0.72–3.58	0.46	47.81 (0.03) 10.38–226.95	0.61
Color of the developing mycelium							
only black and/or brown color	13	4.77 (0.05) 0.44–67.28	0.08	2.15 (1.54) 1.13–4.13	0.005*	64.96 (0.03) 21.67–2 331.49	0.60
Temperature maintained in the flat during the heating season (on the basis of questionnaire)							
> 20°C	13	3.00 (0.05) 0.22–57.99	0.90	1.61 (2.14) 0.28–4.13	0.81	79.82 (0.04) 17.81–2 331.49	0.15
Frequency of the flat airing (repeal, open windows / doors) during the heating season							
every day < 1 h	10	5.21 (0.05) 0.44–67.28	0.27	1.81 (1.48) 1.02–3.49	0.62	72.21 (0.05) 12.28–2 331.49	0.64
at most a few times per week	9	1.83 (0.05) 0.22–23.32	0.89	1.36 (1.98) 0.48–3.58	0.91	47.36 (0.03) 10.38–467.03	0.98
Actual temperature in the flat							
> 20°C	16	2.71 (0.06) 0.22–67.28	0.77	1.52 (2.17) 0.28–4.13	0.78	57.24 (0.04) 10.38–2 331.49	0.98

Table 3. Concentrations of culturable fungi and their derivatives in the indoor air of the flats depending on the declared intensity of the occurrence of various signs of dampness and fungi growth and on the way the flat is used, as well as measured values of indoor microclimate parameters (N = 22) – cont.

Investigated trait / parameter	Flat with analyzed trait (n)	Culturable fungi ($\times 10^2$ cfu/m^3) [GM (GSD) min.–max]	p	(1→3)-β-D-glucans (ng/m^3) [GM (GSD) min.–max]	p	Fungal spores ($\times 10^2$ spores/m^3) [GM (GSD) min.–max]	p
Actual relative humidity in the flat							
> 60%	4	17.93 (0.04) 3.76–67.28	0.009*	1.68 (1.48) 1.13–2.77	0.82	235.25 (0.06) 34.63–2 331.49	0.009*
Actual concentration of CO_2 in the flat							
> 1 000 ppm	16	3.55 (0.06) 0.22–67.28	0.34	1.52 (2.15) 0.28–4.13	0.76	71.05 (0.04) 10.38–2 331.49	0.21

Abbreviations as in Table 1.
* $p < 0.05$.

Table 4. Predictive values of multivariate models (root mean square error – RMSE) and the percent of explained variance (% VE) for concentrations of fungi and their derivatives in the indoor air in relation to the 2 ways of assessing fungal contamination of the air in a flat (based only on a questionnaire and based on a questionnaire and microclimate measurements)

Variable	Assessment of fungal contamination of the air in a flat			
	on the basis of a questionnaire		on the basis of a questionnaire and microclimate measurements	
	RMSE	VE (%)	RMSE	VE (%)
Culturable fungi	1.35	46.9	1.65	61.2
(1→3)-β-D-glucans	0.61	66.6	0.54	64.1
Fungal spores	1.36	25.8	1.46	28.9

RMSE – root mean square error; VE – variance explained.

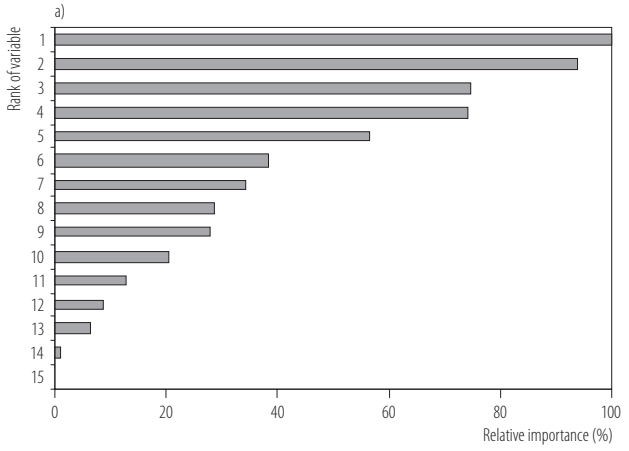
conditions maintained in the flat, and objective measurements of selected microclimate parameters in evaluation of the actual concentrations of fungi and their derivatives in the indoor air. The figure also presents the relative importance of these variables, assuming the value of the most important variable as 100%. In the case of culturable fungi (Figure 1a), the group of the most important traits for appropriate evaluation of the indoor air contamination with this agent includes: subjectively reported presence of dampness on the surface up to 1 m^2, traces of developed mycelium on the surface larger than 1 m^2, only black and/or brown mycelium and perceptible musty odor. In the case of (1→3)-β-D-glucans (Figure 1b) the group of the most important traits includes the same subjective signs, except for the musty odor. As regards fungal spores (Figure 1c), an important role in evaluation of the actual degree of the indoor air contamination with this agent was that of: declared, by the inhabitants, maintenance of temperature above 20°C in the flat during the heating season, the signs of dampness and peeling off paint on the walls and/or ceiling, dampness of the surface up to 1 m^2, signs of mycelium on the surface larger than 1 m^2, and measurement of relative humidity irrespective of the obtained values.

Additionally, to evaluate the reliability of the questionnaire interview used in this study (as the cheapest and simplest method) for assessment of the indoor air quality, the predictive values of the levels of the tested agents in the air of the analyzed flats were calculated. The calculations were based on the predictive model, taking into account only the subjective evaluation of fungal growth in the flats (without measurement of the microclimate). Figures 2a–c list the stipulated (according to the predictive model) and measured values of fungi and their derivatives in the indoor air of the investigated flats. Figures 2a and 2c also highlight the level of the recommended reference value for a given agent or if there is a lack of this value – the level of the limit value at which adverse symptoms of the respiratory tract may occur. The reference to these values was a basis to assess the usefulness of the questionnaire interview used in this study for a qualitative analysis in epidemiological studies.

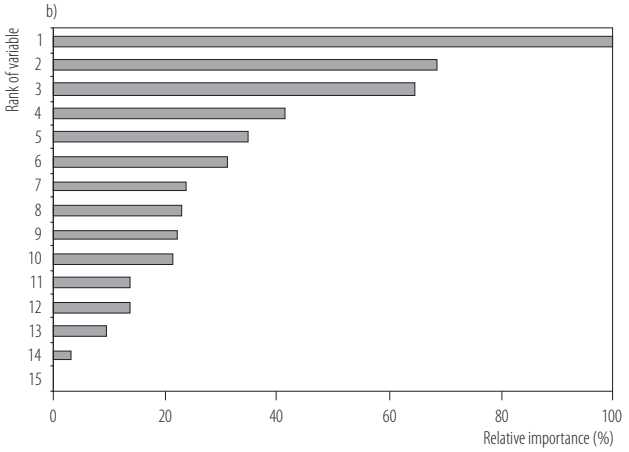
In the case of culturable fungi (Figure 2a) it has been demonstrated that at predicted values of their concentrations below 5×10^2 cfu/m^3, the upper limit of 95% predictive intervals for these values seldom exceeded the reference value recommended in Poland (5×10^3 cfu/m^3) [18]. In such cases, assessment of the indoor air contamination in the investigated flats could be based only on the results of the questionnaire, because the ranges of fungi concentration values stipulated according to these results usually did not exceed the reference value. With the predicted values of fungi concentrations above 5×10^2 cfu/m^3, the upper limits of predictive intervals for these values considerably exceeded the reference value recommended in Poland. This wound point to the demand for verification of questionnaire information in these cases, by the measurement of fungi concentrations aimed at an appropriate assessment of the indoor air contamination with this agent.

In the case of (1→3)-β-D-glucans (Figure 2b), no reference or limit value for the concentration of this agent in the indoor air is accessible in the relevant literature. For this reason, evaluation of the usefulness of the questionnaire applied in this study for the qualitative analysis of air contamination with this agent is difficult.

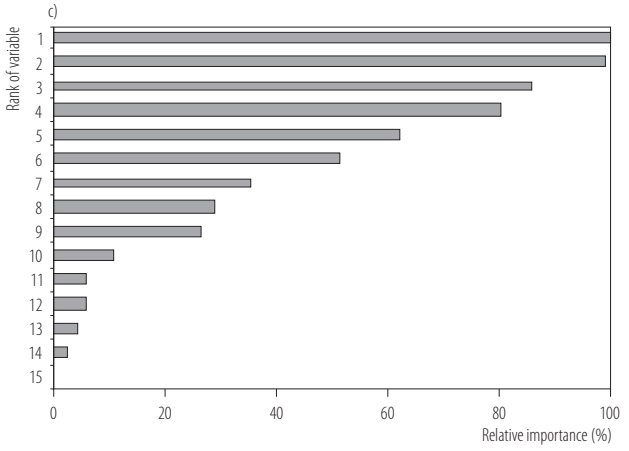
As regards fungal spores, when predicted values of spore concentrations ranging below 1×10^4 spores/m^3 are obtained, the upper limit of predictive intervals for these values did not exceed the value equal to 10^5 spores/m^3 (Figure 2c). This value is defined as the lowest observed effect level (LOEL) in relation to non-allergic people [19]. In such case, these results suggest a possibility to base assessment of the investigated flats indoor air contamination with spores only on the questionnaire data. When spore concentrations predicted values above 1×10^4 spores/m^3 were obtained, the upper limits of predictive intervals for these values considerably exceeded the LOEL. In such



1 – dampness on the surface (wall/ceiling) up to 1 m^2; 2– perceptible musty odor in the flat; 3 – black or brown mycelium on the surface; 4 – fungi growth on the surface (wall / ceiling / windowsill / seal windows / furniture) bigger than > 1 m^2; 5 – the flat airing during the heating season every day < 1 h; 6 – relative humidity measurement in the flat; 7 – the flat airing several times per week during the heating season; 8 – dampness on the surface (wall/ceiling) bigger than 1 m^2; 9 – damp stains and peeling off paint on the walls/ceiling; 10 – temperature measurement in the flat; 11 – condensation of water vapor on the windows and other surfaces in the flat during the heating season; 12 – temperature in the flat during the heating season > 20°C (based on the questionnaire); 13 – perceptible moisture in the flat; 14 – CO_2 concentration measurement in the flat; 15 – fungi growth on the surface (wall / ceiling / windowsill / seal windows / furniture) of 0.25–1 m^2.



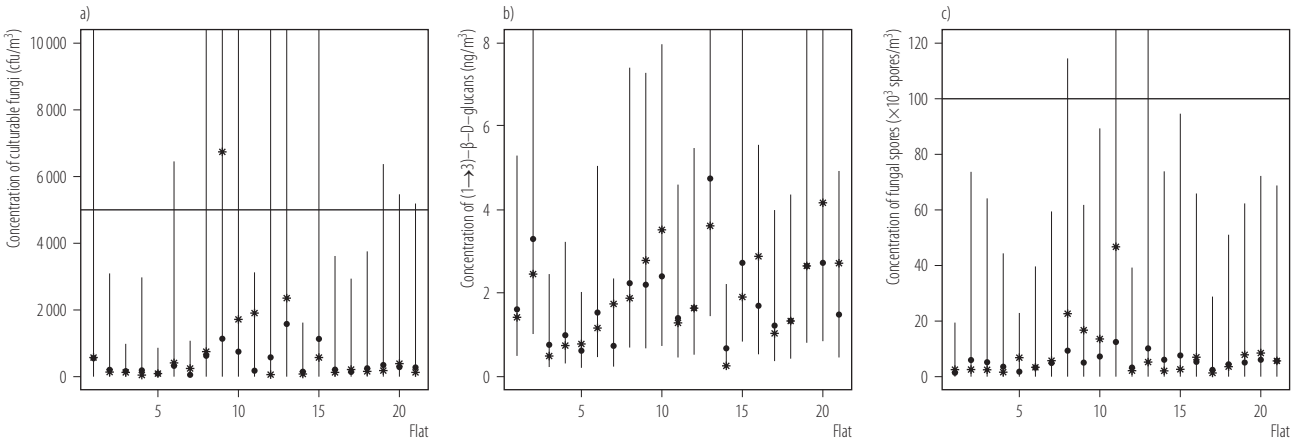
1 – black or brown mycelium on the surface; 2 – fungi growth on the surface (wall / ceiling / windowsill / seal windows / furniture) bigger than > 1 m^2; 3 – dampness on the surface (wall/ceiling) up to 1 m^2; 4 – CO_2 concentration measurement in the flat; 5 – the flat airing during the heating season every day < 1 h; 6 – the flat airing several times per week during the heating season; 7 – perceptible moisture in the flat; 8 – condensation of water vapor on the windows and other surfaces in the flat during the heating season; 9 – damp stains and peeling off paint on the walls/ceiling; 10 – perceptible musty odor in the flat; 11 – temperature in the flat during the heating season > 20°C (based on the questionnaire); 12 – temperature measurement in the flat; 13 – relative humidity measurement in the flat; 14 – fungi growth on the surface (wall / ceiling / windowsill / seal windows / furniture) of 0.25–1 m^2; 15 – dampness on the surface (wall/ceiling) bigger than 1 m^2.



1 – temperature in the flat during the heating season > 20°C (based on the questionnaire); 2 – dampness on the surface (wall/ceiling) up to 1 m^2; 3 – damp stains and peeling off paint on the walls/ceiling; 4 – relative humidity measurement in the flat; 5 – fungi growth on the surface (wall / ceiling / windowsill / seal windows / furniture) bigger than > 1 m^2; 6 – perceptible moisture in the flat; 7 – CO_2 concentration measurement in the flat; 8 – dampness on the surface (wall/ceiling) bigger than 1 m^2; 9 – condensation of water vapor on the windows and other surfaces in the flat during the heating season; 10 – temperature measurement in the flat; 11 – the flat airing during the heating season every day < 1 h; 12 – the flat airing several times per week during the heating season; 13 – fungi growth on the surface (wall / ceiling / windowsill / seal windows / furniture) of 0.25–1 m^2; 14 – perceptible musty odor in the flat; 15 – black or brown mycelium on the surface.

Relative importance of these variables are presented, assuming the value of the most important variable as 100%.

Fig. 1. The ranks of subjectively found signs of dampness and fungal growth, declared microclimatic conditions maintained in the flats and objective measurements of selected microclimatic parameters for assessment of actual airborne concentrations of: a) culturable fungi, b) (1→3)-β-D-glucans, c) fungal spores in the flats



* The measured value of concentration of a given agent in the indoor air of tested flat.

• The predicted value of concentration of a given agent in the indoor air of tested flat, calculated on the basis of the predictive model, taking into account the questionnaire responses (without the microclimate measurement).

Vertical lines in the figure represent 95% predictive intervals for individual predicted values of the concentration of given agent; horizontal line in the figure represents in the case of culturable fungi a) – the level of the recommended in Poland reference value of this agent concentration in the indoor air of flats [18] and in the case of fungal spores c) – airborne concentration defined as the lowest observed effect level (LOEL) in relation to non-allergic people [19].

Fig. 2. The predictive values based on the predictive model (taking into account only the questionnaire evaluation of the flat's fungal contamination) and measured values of the indoor airborne concentrations of: a) culturable fungi, b) (1→3)-β-D-glucans, c) fungal spores in the flats under the study

cases this would point to the need to verify the questionnaire information through the measurement of fungal spore concentrations for an appropriate assessment of the indoor air contamination with them.

DISCUSSION

The subjective evaluation, by the users, of fungal contamination of flats with developed mycelium on stable surfaces in these houses also showed many other signs of fungi growth and presence of dampness and often also creation, by the inhabitants during the heating season, of microclimatic conditions which support the growth of fungi (insufficiently heated flats and too low a frequency of airing the premises). However, the measurement of microclimate parameters in the investigated flats showed a moderate average temperature and relative humidity, which may result from a brief measurement of these parameters or may reflect a discrepancy between actual microclimate conditions in the flat and their assessment by the respondents. In turn, an elevated average concentration of CO_2 indoors and lack of air flow noted in the investigated flats may suggest a limited ventilation of the flats and confirm the questionnaire data related to their rare airing.

Despite the declared – in the questionnaire – occurrence of the signs of indoor fungi growth, only in few cases (2 flats) the indoor airborne concentration of fungi exceeded the reference value of 5×10^3 cfu/m^3 recommended by Polish experts for domestic environment [18]. The concentrations of fungi were comparable to the results obtained by various measuring methods for winter season in a similar Polish research carried out by Pastuszka et al. [20], but lower than those obtained by Gutarowska et al. [21] (that author did not specify the season in which the study was carried out). Besides, those levels were lower than the results obtained in winter in Austrian houses with visible traces of fungal growth [22], but slightly higher than those

noted in Finnish houses [23]. At the same time, the obtained airborne fungi concentration in the investigated flats was even by one order of magnitude higher than that found in the winter in houses without any traces of flooding or growth of fungi [23–25], but only slightly higher than in similar studies carried out by Haas et al. [22]. These results may point to rather moderate contamination of the investigated flats' indoor air with fungi, although indirectly they may confirm the signs of fungi growth in the premises, as declared in the questionnaire.

Evaluation of the concentrations – found in our study – of fungal derivatives, i.e., (1→3)-β-D-glucans and fungal spores in the indoor air, is difficult because of a lack of reference values of these agents in Polish conditions. Similarly, comparing them with the results obtained by other researchers in the studies carried out in flats with fungal growth and those free from fungal signs is difficult because different analytical methods were used, and the results are presented for total number of flats or seasons. According to the accessible literature data, the levels of (1→3)-β-D-glucans noted in our study were in the lower part of a wide range of concentrations of this agent (0.2–106 ng/m^3) found in houses with different extent of fungal contamination [9,10]. They were simultaneously corresponding with the level of glucans detected by Lee et al. [26] in American houses without a clear growth and odor of fungi, which may probably result from different climates. However, this comparison may suggest a rather low degree of fungal contamination of air in the investigated flats, especially because (1→3)-β-D-glucans are contained not only in fungi but also in pollen, plants and some bacteria [27], which may constitute a source of this agent in the indoor air.

The airborne concentration of fungal spores (viable and non-viable) noted in the investigated flats was much lower than in the houses contaminated with fungi after flooding [28], but by one order of magnitude higher than in the houses free from fungi [25,26]. This fact may confirm the inhabitants' questionnaire declarations related to the occurrence of fungal contamination signs in their flats. In one of the investigated flats, the airborne concentration of that agent exceeded the value of 10^5 spores/m^3, determined as the lowest observed effect level (LOEL) in relation to non-allergic people [19].

An additional premise of the existence of an internal source of fungi in the investigated flats is the fact of reaching higher maximum concentrations of fungi and their derivatives in the indoor air, as compared to those in the outdoor air, during the analyzed heating season, when the inflow of these agents from the outside is usually limited due to a lower frequency of airing the premises.

The statistical analysis indicated a significant positive correlation of culturable fungi concentrations in the indoor air with spores, similarly as in the research conducted in houses with fungal contamination by Rao et al. [28] and in "pure" houses – by Lee et al. [25], but there is no significant correlation of the levels of each of the mentioned agents with the amount of (1→3)-β-D-glucans, contrary to the results obtained by these researchers [26,28] and by Reponen et al. [29]. A strong correlation between the concentration of culturable fungi and airborne fungal spores seems to be natural, because it results from the fact that the number of fungal spores presented in the studies and determined by the microscope counting method constitutes the total of viable and non-viable spores. The lack of correlation between the levels of fungi and spores and the levels of glucans contained in fungal cellular wall may be associated with the differentiated content of these compounds in various species of fungi [30] or with the existence of other sources of glucans in flats, e.g., pollen, plants and certain bacteria [27], which may disturb this correlation. The concentration of culturable fungi and fungal spores correlated significantly positively also with the values of relative humidity and levels of CO_2 in flats, which may confirm that dampness and poor ventilation of premises enhance the growth of fungi and their density in the indoor air.

The comparison of airborne concentrations of fungi and their derivatives, depending on the declared intensity of the occurrence of various signs of dampness and fungi growth and on the way the flat is used showed significant differences in the case of several reported signs of flat contamination. Significantly higher levels of fungi occurred, similarly as in the studies conducted by Haas et al. [22], when the declared surface covered by the growth of fungi was > 1 m^2 (as compared to small or point traces covering < 0.25 m^2). The same correlation was observed for fungal derivatives – (1→3)-β-D-glucans. This may imply that contamination of smaller areas only insignificantly contributes to air contamination. Such correlations were not observed for spores. Probably this results from short sampling which because of a high variability of airborne spores distribution within 24 h [23] may not wholly present its actual contamination [31,32].

The study also showed a significantly elevated concentration of (1→3)-β-D-glucans with reported dampness of the ≤ 1 m^2 surface, as compared to its lack, which is hard to explain especially because no such correlation was found with the > 1 m^2 dampness surface. This correlation is not confirmed by other studies in houses, which may suggest that it should be treated with a certain caution. On the other hand, the observed black and/or brown color of mycelium, as opposed to other colors of traces of the fungi growth, was significantly associated with the elevated airborne concentrations of (1→3)-β-D-glucans. This may probably be explained by the fact that the dark color of the colonies of some fungi which are found in the houses contaminated with fungi (e.g., *Cladosporium*, *Alternaria*, *Stachybotrys*) [3,21,33,34] may often indicate the maturity of fungal culture and intense sporulation, which presumably supports the air contamination with fungi and their derivatives.

A comparison of airborne levels of fungi and their derivatives in the investigated flats, depending on measured values of the indoor microclimate parameters favourable or unfavourable for the occurrence of fungi growth in premises, showed significantly elevated concentrations of culturable fungi and fungal spores only at the air relative humidity above 60%. This complies with earlier findings according to which, exceeding of such value in flats may support the growth of fungi on different surfaces [35], and consequently contaminate the air with spores.

The multivariate analysis indicated moderate usefulness of subjective evaluation of the analyzed signs of dampness and fungal growth in flats and characteristics of microclimate for evaluation of the actual concentrations of culturable fungi and (1→3)-β-D-glucans in the indoor air and limited usefulness for evaluation of fungal spore concentrations. In the case of evaluation of the actual concentrations of culturable fungi, higher usefulness of questionnaire evaluation was demonstrated, supported by measurements of selected parameters of the indoor microclimate, as compared to the evaluation based exclusively on the questionnaire data. A limited correlation between the reported visual and sensory signs of dampness and fungi growth, and found out airborne levels of fungi and their derivatives, may result from the "randomness" of inspectors' observation, which may affect the quality of their evaluation. This premise may point to the possibility that the data collected in the questionnaire are encumbered with certain error. Another explanation of the lack of the correlation between subjective evaluations and objective measurements may be the lack of the air flow in the investigated flats. This fact may inhibit suspending of fragments of fungi and fungal spores in the air, even with their high levels released from the infected surfaces.

The study also showed a discrepancy between fungi and glucans, and fungal spores, in arrangement of the importance of subjectively found signs of dampness and fungal growth, declared microclimatic conditions maintained in the flat and objective measurements of selected microclimatic parameters for assessment of the actual concentrations of the investigated contaminants. Presumably this may point to different factors affecting the airborne levels of fungal spores,

as compared to fungi and glucans. Of the analyzed traits, the declared presence of mycelium on the > 1 m^2 surface was characterized by high importance for correct assessment of the concentrations of all the tested agents. This would suggest that it is necessary to include the question related to this trait in the structure of questionnaires assessing the indoor air contamination with fungi and their derivatives.

Despite the limited precision of the quantitative assessment of actual contamination of the indoor air with the investigated agents carried out with the use of a set of questions, such evaluation could be useful in epidemiological studies for qualitative evaluation of the indoor air. As revealed in the predictive models analysis, the subjective evaluation of fungal contamination signs in flats, based on the applied questionnaire, could allow for prediction of possible occurrence of concentrations of a given agent in the indoor air which exceed the reference or limit value. This analysis also demonstrates that the questionnaire used in this study in some cases could confine the need to conduct additional measurements of biological agents and their derivatives without any adverse effects on reliability of evaluation of the quality of air in the flats.

Because of the small sample size (N = 22) the presented analysis has significant limitations and the results are not very conclusive. Therefore, further studies with a larger number of flats are needed.

CONCLUSIONS

The actual mycological contamination of the indoor air was significantly correlated with the size of the surface – as declared by the respondents – covered by the growth of fungi, surface with traces of dampness, observed color of the developing mycelium and the actual, supported by measurements, relative humidity level within the premises. Subjective evaluation of specific signs of fungal contamination of flats, separately or combined with measurement of microclimate parameters, appeared to be moderately useful for quantitative evaluation of the actual degree of air contamination with fungi and their derivatives and could be recommended for use in epidemiological studies if identification of home exposure to fungi is important. More extensive studies are needed to strengthen the results of this analysis.

REFERENCES

1. Institute of Medicine. Damp indoor spaces and health. Washington DC: National Academies Press; 2004.
2. Central Statistical Office of Poland, Social Statistics Division. [Polish population's living conditions during 2004–2005 – Results of EU-SILC study]. Warszawa: Główny Urząd Statystyczny; 2007. Polish.
3. Flannigan B, Samson RA, Miller JD, editors. Microorganisms in home and indoor work environments. Diversity, health impacts, investigation and control. New York: CRC Press LLC; 2001.
4. Grajewski J, editor. [Mycotoxins and mould – threats for humans and animals]. Bydgoszcz: Publisher of the Kazimierz Wielki University in Bydgoszcz; 2006. Polish.
5. Eduard W. The Nordic Expert Group for criteria documentation of health risk from chemicals. 139. Fungal Spores. Stockholm: National Institute for Working Life; 2007.
6. Górny RL, Reponen T, Willeke K, Schmechel D, Robine E, Boissier M, et al. Fungal fragments as indoor air biocontaminants. Appl Environ Microbiol. 2002;68(7):3522–31, http://dx.doi.org/10.1128/AEM.68.7.3522-3531.2002.
7. Green BJ, Tovey ER, Sercombe JK, Blachere FM, Beezhold DH, Schmechel D. Airborne fungal fragments and allergenicity. Med Mycol. 2006;44 Suppl 1:245–55, http://dx.doi.org/10.1080/13693780600776308.
8. Douwes J. (1→3)-β-D-glucans and respiratory health: A review of scientific evidence. Indoor Air. 2005;15(3):160–9, http://dx.doi.org/10.1111/j.1600-0668.2005.00333.x.
9. Beijer L, Thorn J, Rylander R. Mould exposure at homes relates to inflammatory markers in blood. Eur Respir J. 2003;21(2):317–22, http://dx.doi.org/10.1183/09031936.03.00283603.

10. Rylander R, Hsieh V, Courteheuse C. The first case of sick building syndrome in Switzerland. Indoor Built Environ. 1994;3(3):159–62, http://dx.doi.org/10.1177/1420326X9400300311.
11. Bornehag CG, Blomquist G, Gyntelberg F, Järvholm B, Malmberg P, Nordvall L, et al. Dampness in buildings and health. Nordic interdisciplinary review of the scientific evidence on associations between exposure to "dampness" in buildings and health effects (NORDDAMP). Indoor Air. 2001;11(2):72–86, http://dx.doi.org/10.1034/j.1600-0668.2001.110202.x.
12. Bornehag CG, Sundell J, Bonini S, Custovic A, Malmberg P, Skerfving S, et al. Dampness in buildings as a risk factor for health effects, EUROEXPO: A multidisciplinary review of the literature (1998–2000) on dampness and mite exposure in buildings and health effects. Indoor Air. 2004;14(4):243–57, http://dx.doi.org/10.1111/j.1600-0668.2004.00240.x.
13. Bornehag CG, Sundell J, Hagerhed-Engman L, Sigsggard T, Janson S, Aberg N, et al. 'Dampness' at home and its association with airway, nose, and skin symptoms among 10,851 preschool children in Sweden: A cross-sectional study. Indoor Air. 2005;15 Suppl 10:48–55, http://dx.doi.org/10.1111/j.1600-0668.2005.00306.x.
14. Mendell MJ, Mirer AG, Cheung K, Tong M, Douwes J. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: A review of the epidemiologic evidence. Environ Health Persp. 2011;119(6):748–56, http://dx.doi.org/10.1289/ehp.1002410.
15. Cyprowski M, Buczyńska A, Kozajda A, Sowiak M, Bródka K, Szadkowska-Stańczyk I. Exposure to (1→3)-β-D-glucans in swine farms. Aerobiologia. 2012;28(2):161–8, http://dx.doi.org/10.1007/s10453-011-9220-4.
16. Lacey ME, West JS. The air spora. A manual for catching and identifying airborne biological particles. Dordrecht: Springer; 2006.
17. R Core Team [Internet] Vienna: R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2013 [cited 2013 Dec 5]. Available from: http://www.R-project.org.
18. Górny RL. [Biohazards: standards, guidelines, and proposals for threshold limit values]. Podst Metody Oceny Srod Pr. 2004;3(41):17–39. Polish.
19. Eduard W. Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. Crit Rev Toxicol. 2009;39(10):799–864, http://dx.doi.org/10.3109/10408440903307333.
20. Pastuszka JS, Tha Paw UK, Lis DO, Wlazło A, Ulfig K. Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. Atmos Environ. 2000;34(22):3833–42, http://dx.doi.org/10.1016/S1352-2310(99)00527-0.
21. Gutarowska B, Wiszniewska M, Walusiak J, Piotrowska M, Pałczyński C, Żakowska Z. Exposure to moulds in flats and the prevalence of allergic diseases – Preliminary study. Pol J Microbiol. 2005;54 Suppl:13–20.
22. Haas D, Habib J, Galler H, Buzina W, Schlacher R, Marth E, et al. Assessment of indoor air in Austrian apartments with and without visible mold growth. Atmos Environ. 2007;41(25):5192–201, http://dx.doi.org/10.1016/j.atmosenv.2006.07.062.
23. Hyvärinen A, Vahteristo M, Meklin T, Jantunen M, Nevalainen A, Moschandreas D. Temporal and spatial variation of fungal concentrations in indoor air. Aerosol Sci Tech. 2001;35(2):688–95, http://dx.doi.org/10.1080/02786820117763.
24. Horner WE, Worthan AG, Morey PR. Air- and dustborne mycoflora in houses free of water damage and fungal growth. Appl Environ Microbiol. 2004;70(11):6394–400, http://dx.doi.org/10.1128/AEM.70.11.6394-6400.2004.
25. Lee T, Grinshpun SA, Martuzevicius D, Adhikari A, Crawford CM, Reponen T. Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes. Atmos Environ. 2006;40(16):2902–10, http://dx.doi.org/10.1016/j.atmosenv.2006.01.011.
26. Lee T, Grinshpun SA, Kim KY, Iossifova Y, Adhikari A, Reponen T. Relationship between indoor and outdoor airborne fungal spores, pollen, and (1→3)-β-D-glucan in

homes without visible mold growth. Aerobiologia. 2006; 22(3):227–35, http://dx.doi.org/10.1007/s10453-006-9034-y.

27. Stone BA, Clark AE. Chemistry and biology of (1→3)-β-glucans. Victoria Australia: La Trobe University Press; 1992.
28. Rao CY, Riggs MA, Chew GL, Muilenberg ML, Thorne PS, van Sickle D, et al. Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after Hurricanes Katrina and Rita. Appl Environ Microbiol. 2007; 73(5):1630–4, http://dx.doi.org/10.1128/AEM.01973-06.
29. Reponen T, Singh U, Schaffer C, Vesper S, Johansson E, Adhikari A, et al. Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes. Sci Total Environ. 2010;408(22):5565–74, http://dx.doi.org/10.1016/j.scitotenv.2010.07.090.
30. Milton DK, Alwis KU, Fisette L, Muilenberg M. Enzyme-linked immunosorbent assay specific for (1→6) branched, (1→3)-β-D-glucan detection in environmental samples. Appl Environ Microbiol. 2001;67(12):5420–4, http://dx.doi.org/0.1128/AEM.67.12.5420-5424.2001.
31. Pasanen AL. A review: Fungal exposure assessment in indoor environments. Indoor Air. 2001;11(2):87–98, http://dx.doi.org/10.1034/j.1600-0668.2001.110203.x.
32. Martinez K, Rao C, Burton N. Exposure assessment and analysis for biological agents. Grana. 2004;43(4):193–208, http://dx.doi.org/10.1080/00173130410000794.
33. Beguin H, Nolard N. Mould biodiversity in homes I. Air and surface analysis of 130 dwellings. Aerobiologia. 1994;10(2–3):157–66, http://dx.doi.org/10.1007/BF02459231.
34. Zyska B. [Fungi in indoor air in European countries]. Mikol Lek. 2001;8(3–4):127–40. Polish.
35. Zyska B, Żakowska Z, editors. [Microbiology of materials]. Łódź: Publisher of the Technical University of Łódź; 2005. Polish.