



## Spectrum and Concentration of Culturable Fungi in House Dust from Flats in Warsaw, Poland

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### ABSTRACT

Samples of house dust from 18 flats in Warsaw (Poland) were collected from five environments: floor, shelves with books, kitchen, bathroom, and bed. *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* were the most common genera identified in house dust samples. The highest culturable fungal concentrations were recorded in dust from shelves (median value  $8.2 \times 10^4$  CFU/g of dust), and the least one from beds (median value  $8.0 \times 10^3$  CFU/g of dust). Differences in CFUs between environments were statistically significant for *Penicillium*, *Aspergillus* and *Alternaria*. Concentrations of *Penicillium* and *Aspergillus* were significantly higher in autumn than in spring, whereas that of *Cladosporium* was significantly lower in autumn than in spring. The efficiency of ventilation in the bathroom had a significant effect on *Cladosporium* counts. Certain relationships between the concentrations of culturable fungi and some customs of the occupants are discussed.

**Keywords:** *Penicillium*; *Aspergillus*; *Cladosporium*; *Alternaria*; House dust.

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### INTRODUCTION

House dust is a complex of chemical and biological components originating from different internal and external sources. An important biological element of dust is viable mycelia fragments and fungal spores (Butte and Heinzow, 2002; Tatur *et al.*, 2006) and usually their concentration and composition in house dust reflects the fungal flora present in outdoor air (Calvo *et al.*, 1982; Pastuszka *et al.*, 2000; Medrela-Kuder, 2003; Dassonville *et al.*, 2008). Some species of fungi occurring in dust can affect humans, causing a variety of diseases. Among them there are the species of *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus*, which are considered as the most allergenic agents. Substances produced by growing mycelium and spores of these fungi can evoke not only allergic reaction (rhinitis, sneezing, hoarseness, itchy eyes or skin) but also serious diseases such as asthma or lung aspergillosis (Kurup *et al.*, 2000; Cabral, 2010). Moreover, some moulds produce mycotoxins, which increase the risk factor of liver cancer (Bennet and Klich, 2003). In the case when the symptoms and diseases described above encompass many inhabitants of a house we talk about a “Sick Building Syndrome”

(Wang *et al.*, 2008). Some researchers have demonstrated that the “Sick Building Syndrome” may be caused by high concentration of indoor fungi (Cooley *et al.*, 1980; Herbath *et al.*, 2003; Wang *et al.*, 2008). Therefore, an analysis of concentration and composition of fungal species in a house dust seems to be important, because inhalation and direct contact with house dust can be primary routes of exposure to fungal allergens (Garrett *et al.*, 1998; Rogers, 2003).

There are several factors determining the composition and abundance of fungi in the air and dust. Among them, important are climatic conditions (Hjelmroos, 1993; Fernández *et al.*, 1998; Gniadek *et al.*, 2005). Other factors that probably could affect the abundance of fungi in the house dust are related to the behaviour of the inhabitants, e.g., it seems that multiple entrance and exit to an apartment can result in higher concentrations of fungi. Some influence on the composition and abundance of fungi may have a frequency of ventilation. Through the open windows outdoor fungal spores may fall into a flat. Frequency of cooking should also affect abundance of fungi because during cooking increases air humidity and concentration of PAHs (polycyclic aromatic hydrocarbons) - products of gas-combustion and kitchen practices. Increased humidity can promote the growth of fungi, while impact of PAHs is not entirely clear (Bamforth and Singleton, 2005; Leitão, 2009). Another place in a flat where air humidity periodically increases is a bathroom (e.g., during the bath). Therefore, it could be expected that the number of fungi in the bathroom may be higher than in other parts of the dwelling. It also seems that

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the presence of pets and indoor plants can also influence the composition and abundance of fungi in a house.

Additional factors that seem to influence the composition and concentration of fungi in house dust are associated with the localization of house e.g., neighborhood of municipal green or intensity of traffic (Giorgio *et al.*, 1996; Chen *et al.*, 2012). It could be expected that mowed grass and falling leaves can be also a source of fungal spores.

All these are suppositions because our knowledge about the impact of various factors on fungi is insufficient. So far, only a few studies have been devoted to an effect of customs and behaviour of occupants on concentration of fungi in house dust (Su *et al.*, 1992; Verhoeff *et al.*, 1994; Emberlin *et al.*, 1995; Cho *et al.*, 2006) and to the presence of pets (Chew *et al.*, 2003; Salo *et al.*, 2005; Cho *et al.*, 2006; Fujimura *et al.*, 2010).

It has been reported earlier that fungal spores may enter flats from outside (Medrela-Kuder 2003; Ponce-Caballero *et al.*, 2010) but the most important sources are usually within the flats (Reponen *et al.*, 1992). Therefore, there is a need for searching sources of indoor fungi and need for information about an impact of various factors on the composition and abundance of fungi in house dust. Such an information could be useful for searching ways to reduce of fungal abundance in house dust and minimizing the effects of exposure to fungi dangerous to the health of people. So far, the interest of researchers was focused mainly on examining the fungal spores in house dust from carpets or smooth floor (Gravesen *et al.*, 1986; Dybendal *et al.*, 1989; Beguin and Nolard, 1996), mattresses (Beguin, 1995), or upholstered furniture (Schober, 1991). In our study we analyze the fungi in the dust collected not only from the mattress, floor or carpet, but also from furniture and equipments in a kitchen, in a bathroom, and from bookshelves in living room. In each of these places in a house conditions for the development of fungi are slightly different, therefore, it can be expected that the composition and abundance of fungi in house dust in these environments will be also different. Additionally, the spectrum of fungi and their concentration were analyzed in relation to flat location and characteristics and to some customs and daily activities of the inhabitants.

## MATERIALS AND METHODS

### *Sampling and Culture Method of Fungi*

Samples of dust were collected from 18 flats located in the largest multi-family housing estates in different districts of Warsaw. Flats in some respects were similar; all of them were situated in high-rise blocks (from 5 to 10 floors) aged 20–30 years, and were heated by central heating system supplied from a municipal power plant, therefore the temperature and relative humidity of air in the flats were similar (a survey was conducted during the heating period, which in Poland lasts from mid October to mid April). The temperature ranged from 20 to 23°C in the autumn and from 22 to 25°C in the spring. Relative humidity ranged from 60 to 65%. In kitchens during cooking and in bathrooms during the bath humidity periodically increased to 90%. In

addition, all bathrooms had no windows. However, flats differed in some aspects resulting from the location (traffic intensity on the nearest streets, neighbourhood of municipal green), facilities and equipment, habits and day-to-day activities of residents (frequency of cleaning, cooking, ventilating, number of exits and entries into flat per day) and also the presence or absence of pets and indoor plants (Table 1).

Sampling took place once in autumn 2003 (8 flats) and once in spring 2004 (10 flats). The occupants did not clean the flat at least one week before the dust sampling. In each flat dust samples were collected from five environments: floor (carpeted floor) in the living room, shelves (book shelves or shelves with various decorations in the living room), bathroom (furniture, water or gas pipes and vent – depending on the equipment), kitchen (furniture, water or gas pipes, vent, kitchen hoods refrigerator – depending on the equipment) and mattress in a bedroom. For each environment 3 samples were taken, each from a square of the area of 100 cm<sup>2</sup>.

Dust was collected with a sterile swab placed in a sterile 10-mL test tube (Eurotubo, I.A.S.A., Spain). Before and after sampling swab was weighed with accuracy of  $\pm 0.0001$  g. Mass of the dust samples ranged from 2 mg to 13 mg. Fungi from dust samples were isolated by the dilution plate method according to Pepper *et al.* (1995), with slight modification. To each test tube 5 mL aqueous solution of Triton-X (0.05%) was poured. Then the tubes content was vortexed at maximum speed (2500 rpm) for 5 min and 0.5 mL planted onto Rose Bengal Agar (with chloramphenicol). The samples were cultured at 23–25°C for 5–7 days. For each sample of dust 5 replications were prepared. Pure swabs (without dust) were treated as a control and subjected to the same procedure as swabs with collected dust. From pure swabs any fungal colony was isolated. After incubation, the colonies of fungi were counted and identified to genus level based on their morphological characteristics (Gilman, 1957; Barnett and Hunter, 1972; Domsch *et al.*, 1980). The number of culturable fungi was expressed in colony forming units per gram of dust (CFU/g of dust) according to a formula given by Pepper *et al.* (1995).

### *Questionnaire and Statistical Analysis*

The inhabitants filled out a questionnaire concerning the location of flat, equipment and facilities, and the habits and activities of the inhabitants (Table 1). Based on this information, the following factors, which could affect the concentration and spectrum of fungi were selected: intensity of traffic, neighbourhood of municipal green, efficiency of ventilation in the bathroom, presence of pets, number of indoor plants, number of entries and exits, frequency of ventilation, cooking and cleaning habits.

It was assumed that not all of the analyzed factors have a legitimate impact on the concentration of fungi in surveyed environments (e.g., ventilation efficiency in a bathroom does not affect the concentration of fungi in the bed). Therefore in particular statistical test not all environments are included (Table 1).

The significance of the effect of a frequency of cleaning,

**Table 1.** Factors and environments subjected to statistical analysis and sample size.

Factor (flat characteristics and habits/activities of occupants)	Factor range	Environment included in the statistical analysis*	Sample size**
Traffic intensity (car/hour)	≤ 10 11–50 ≥ 51	S, F, K, B	72
Neighbourhood of municipal green (in meters)	≤ 50 51–200 ≥ 200	S, F, K, B	72
Ventilation in a bathroom	efficient or inefficient	Bth	18
Exits/entries into flat (number/day)	≤ 10 ≥ 10	F, S	36
Cooking (frequency/week)	approx. 1 2–7 ≥ 8	K	18
Ventilation (hours/day)	≤ 1 2–20 ≥ 24	S, F, K	54
Cleaning (frequency/month)	≤ 1 2–14 ≥ 15	S, F, K, B, Bth	90
Number of indoor plants	≤ 3 4–10 ≥ 11	S, F, K, B	72
Pets (cat or/and dog)	Yes or no	F, B	36

\* F - floor, S - shelves, K - kitchen, B - bed, Bth - bathroom.

\*\* number of environments multiplied by the number of flats.

cooking, ventilation, number of indoor plants, number of exits and entries, traffic intensity and neighbourhood of municipal green on the concentration of fungi was estimated by non-parametric Kruskal-Wallis (H) test. Another non-parametric test - Mann-Whitney (U) test was used for evaluation of the effect of season, efficiency of ventilation in the bathroom and the presence of pets on the concentration of fungi. The same test was applied for evaluation of the differences between the concentrations of fungi in five analyzed environments. Differences with  $p \leq 0.05$  were considered as significant.

## RESULTS

### *Composition and Concentration of Culturable Fungi in Different Environments*

The highest total concentration of culturable fungi was found in the dust from the shelves and the least one in the dust from the beds:  $8.2 \times 10^4$  CFU/g and  $8.0 \times 10^3$  CFU/g of dust (medians), respectively (Fig. 1). In the dust from the shelves the total concentration of culturable fungi ranged from  $1.3 \times 10^4$  to  $4.3 \times 10^5$  CFU/g, whereas in the dust from the beds it ranged from 0 to  $3.8 \times 10^4$  CFU/g. In three flats of the eighteen no culturable fungi were detected in dust collected from beds, and in seven flats the culturable fungal counts did not exceed  $10^4$  CFU/g of dust (Fig. 2).

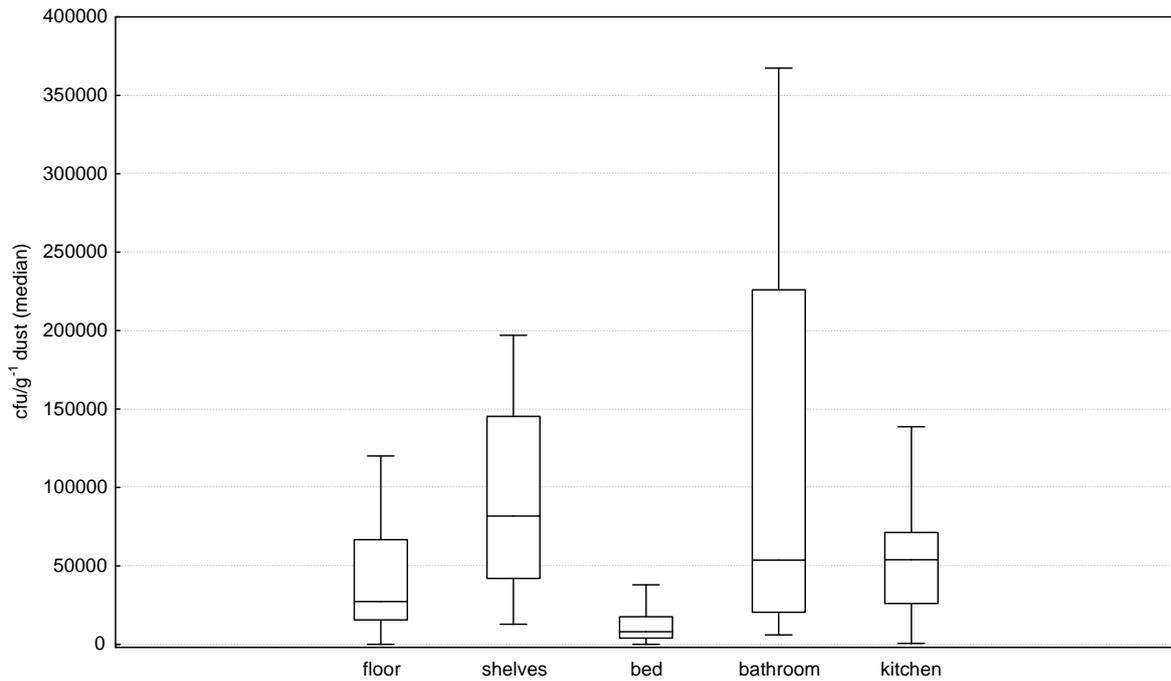
The concentration of culturable fungi presented as a median value in the dust obtained from the bathrooms was similar to that from the kitchens (for both environments it

equals  $5.4 \times 10^4$  CFU/g). However, the range of total number of culturable fungi observed in the bathrooms was larger than that one noticed for the kitchens, in dust from bathrooms concentration ranged from  $6.0 \times 10^3$  to  $5.0 \times 10^7$  CFU/g, whereas in the kitchens from  $6.7 \times 10^2$  to  $3.5 \times 10^5$  CFU/g of dust (Fig. 1). In two bathrooms the concentrations of fungi were respectively ten and hundred times higher than in the other bathrooms (Fig. 2). In both bathrooms fungal growth was well visible as scattered spots on the walls and ceiling during sampling.

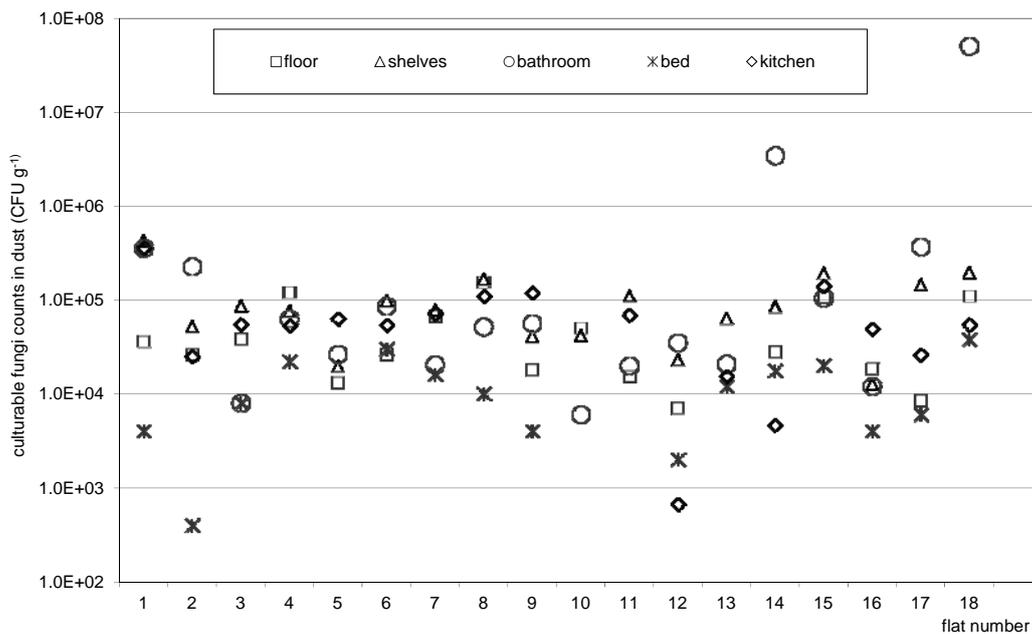
The concentration of culturable fungi in the dust from the floors was higher than in the dust from the beds, but lower than in the dust from the other four environments – median  $2.7 \times 10^4$  CFU/g and ranged from 0 to  $1.5 \times 10^5$  CFU/g of dust.

Statistical analysis indicated that differences in the total concentration of culturable fungi in surveyed environments were significant between the bed and the kitchen ( $p < 0.001$ ), bathroom ( $p < 0.001$ ), shelves ( $p < 0.001$ ) and floor ( $p < 0.01$ ), and between the shelves and the floor ( $p < 0.05$ ).

*Penicillium* spores dominated in the dust samples from the floor (71.5%), shelves (63.2%), kitchen (57.4%) and bed (47.5%). In contrast to this in bathrooms *Aspergillus* (32.8%) dominated, followed by *Alternaria* (28.3%) and *Penicillium* (25.5%) (Fig. 3). Two bathrooms with mould problems, extremely high concentration of *Cladosporium* was found ( $2.2$  and  $2.6 \times 10^6$  CFU/g of dust), a thousand times more in comparison with other tested bathrooms. These two bathrooms were excluded from the analysis



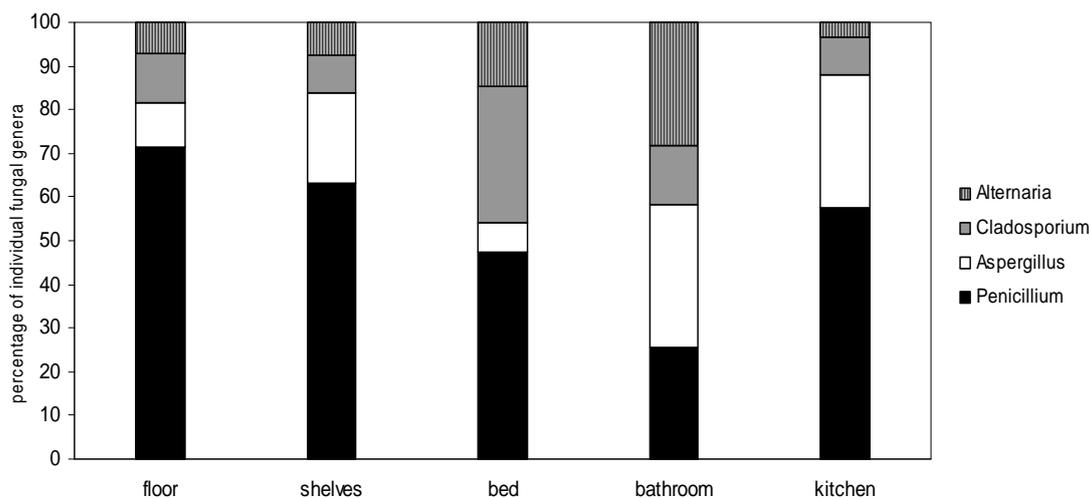
**Fig. 1.** Total concentration of culturable fungi in five environments.



**Fig. 2.** Total concentrations of culturable fungi in different environments in individual flats.

concerning the percentage contribution of fungi in individual environments. Concentrations of *Penicillium*, *Aspergillus* and *Alternaria* differed significantly between environments. For *Penicillium* statistically significant differences in the concentration were found between the bed (median =  $8.0 \times 10^2$  CFU/g of dust) and the kitchen (median =  $2.1 \times 10^4$  CFU;  $p < 0.001$ ), bathroom (median =  $1.1 \times 10^4$  CFU;  $p < 0.001$ ), shelves (median =  $3.7 \times 10^4$  CFU;  $p < 0.001$ ), floor (median =  $1.4 \times 10^4$  CFU;  $p < 0.001$ ) and between the shelves and the bathroom ( $p < 0.05$ ) and floor ( $p < 0.01$ ).

For *Aspergillus* statistically significant differences occurred between the bed (median = 0 CFU/g of dust) and the other four environments; kitchen (median =  $2.0 \times 10^3$  CFU;  $p < 0.001$ ), bathroom (median =  $1.7 \times 10^3$  CFU;  $p < 0.001$ ), shelves (median =  $5.8 \times 10^3$  CFU;  $p < 0.001$ ) and floor (median =  $7.3 \times 10^2$  CFU;  $p < 0.01$ ). For *Alternaria* statistically significant differences were found between the bed (median = 0 CFU/g of dust) and the kitchen (median =  $1.6 \times 10^3$  CFU;  $p < 0.05$ ), shelves (median =  $3.5 \times 10^3$  CFU;  $p < 0.001$ ) and floor (median =  $1.7 \times 10^3$  CFU;  $p < 0.05$ ), and between the bathroom (median =  $7.5 \times 10^2$  CFU/g of dust) and the shelves ( $p < 0.05$ ).



**Fig. 3.** The percentage contribution of dominant genera of fungi in house dust samples in five environments.

**Table 2.** Descriptive statistics of the concentration of culturable fungi from house dust samples collected in autumn and spring.

Season		Fungi concentration $\times 10^3$				
		Total	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Alternaria</i>
Autumn	median	53.0 <sup>n.s</sup>	21.2 <sup>***</sup>	5.2 <sup>*</sup>	0 <sup>***</sup>	1.0 <sup>n.s</sup>
	SD	99.8	60.8	32.9	32.2	7.0
	min	0.4	0	0	0	0
	max	431.0	259.0	169.0	159.3	36.0
Spring	median	26.0	8.7	1.0	2.0	1.0
	SD	7 134.7	19.9	84.4	476.9	85.5
	min	0	0	0	0	0
	max	49 950.0	92.0	590.0	46.0	600.0

Statistical significance: n.s - not significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

The concentration of other fungal genera such as *Mucor*, *Trichoderma*, *Fusarium* and *Scopulariopsis* was much lower (from 10 to 100 times) in comparison with a dominated genera concentration of *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria*.

#### **The Effect of Season on the Concentration of Culturable Fungi**

The total concentration of fungi in dust samples collected in autumn was two times higher than in samples collected in the spring. In autumn concentration of fungi ranged from  $4.0 \times 10^2$  to  $4.3 \times 10^5$  CFU/g of dust (median  $5.3 \times 10^4$  CFU/g of dust), whereas in spring it ranged from 0 to  $1.4 \times 10^5$  CFU/g of dust (median  $2.6 \times 10^4$ /g of dust). Concentration of *Penicillium* and *Aspergillus* was significantly higher in autumn in comparison with spring. In contrast to this the concentration of *Cladosporium* was lower in autumn than in the spring. Concentration of *Alternaria* was similar in autumn and in spring dust samples (Table 2).

#### **Relationship between Concentration of Culturable Fungi and Flat Characteristics and Habits of Occupants**

Neighbourhood of municipal green did not influence the total concentration of fungi nor the fungi of the genus *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria*,

whereas traffic intensity was significantly associated with *Alternaria* ( $p < 0.01$ ) and *Cladosporium* ( $p < 0.05$ ) concentration. The habits and the day-to-day activities of the residents (frequency of cleaning, cooking, ventilating, number of exits and entries into flat per day) did not influence the total concentration of fungi in the surveyed environments. There were also no significant differences between flats with and without pets and with and without indoor plants.

The effectiveness of the ventilation in the bathroom had a statistically significant importance on the total concentration of fungi ( $p < 0.01$ ), as well as on the concentration of *Cladosporium* ( $p < 0.05$ ). In bathrooms with poor ventilation concentration of *Cladosporium* was 1000 times higher than in the bathrooms with efficient ventilation.

Frequency of ventilation (number of hours when the windows are open) did not influence the concentration of *Cladosporium*, *Penicillium* and *Aspergillus*, but significantly affected the concentration of *Alternaria* ( $p < 0.05$ ). In flats rarely ventilated (less than one hour per day) the concentration of *Alternaria* was  $6.0 \times 10^2$  CFU/g of dust (median). A frequency of ventilation from 2 to 20 hours per day increased the concentration of *Alternaria* two fold ( $1.6 \times 10^3$  CFU/g of dust), whereas ventilation 20–24 hours per day increased spore concentration five fold ( $3.3 \times 10^3$

CFU/g of dust). Number of exits and entries into flat per day was significantly associated with *Aspergillus* concentration, the higher number of entries and exits, the lower concentration of *Aspergillus* in dust from the floor and shelves ( $p < 0.05$ ). Frequency of cooking significantly negatively affected *Penicillium* concentration ( $p < 0.05$ ); the higher frequency of cooking, the lower concentration of *Penicillium*.

## DISCUSSION

### *Composition and Concentration of Culturable Fungi in Different Environments*

This study has shown that genera spectrum and concentrations of culturable fungi in dust in Warsaw flats did not differ from the results obtained in other European countries (Miller *et al.*, 1988; Hyvärinen *et al.*, 1993; Verhoeff *et al.*, 1994; Beguin, 1995; Beguin and Nolard, 1996; Jovanovic *et al.*, 2004). For example, Beguin and Nolard (1996) in carpeted floors in Belgium houses found a concentration of fungi ranging from  $5.0 \times 10^3$  to  $6.6 \times 10^7$  CFU/g of dust. Similar mould spore concentration was detected in German houses by Jovanovic *et al.* (2004) (in dust from the floor median value  $2.8 \times 10^4$  CFU/g, and range from  $1.5 \times 10^3$  to  $1.2 \times 10^6$  CFU/g). In the samples from the beds, Beguin (1995) found that mould concentration ranged from  $1.0 \times 10^3$  to  $7.0 \times 10^7$  CFU/g of dust (median value  $3.0 \times 10^4$  CFU/g of dust). In our surveys, the total concentration of culturable fungi expressed as median values varied from  $8.0 \times 10^3$  CFU/g in the dust from beds to  $8.2 \times 10^4$  CFU/g in the dust from the shelves. In the dust originating from the flats in Warsaw, as well as in the dust from other European houses, the spores of the most allergenic fungi, such as: *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* were found. Therefore, people staying at home (some of them spend there even 90% of their free time) are exposed to agents causing respiratory diseases. Especially exposed to pollutants in dust are infants and children who crawl, and put to mouth their hands and other objects. It is difficult to assess whether the observed density of fungi in the dust is dangerous to human health because so far a threshold concentration of fungi calculated at 1 g of a dust is not a clearly defined (Górny, 2004). It is considered that indoor allergens, including fungi, are the most important risk factor for asthma, in particular for sensitisation in early childhood (Wahn *et al.*, 1997; O'Connor *et al.*, 2004). Various epidemiological studies have shown the associations between the culturable fungi in the air or dust, and increased risk of adverse respiratory symptoms (see Curtis *et al.*, 2004). Matheson *et al.* (2005) found that a doubling of *Cladosporium* exposure was associated with 52% greater odds of asthma attack in the last 12 months, while O'Connor *et al.* (2004) showed that children with asthma had a positive allergy skin test to at least one fungal extract. In homes of those children the most common fungi were *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*. Furthermore, Cho *et al.* (2006) stated that the level of *Alternaria* antigen was associated with some home characteristics and activity of the inhabitants.

In the dust samples analyzed in these studies *Penicillium* and *Aspergillus* dominated. Both genera were also most frequent in the dust originating from London houses (Emberlin *et al.*, 1995) as well as from Belgian ones (Beguin, 1995).

In Warsaw flats, the highest concentration of fungi was detected in dust samples from shelves that could be expected, because the shelves are rarely cleaned. In many cases dust is accumulated on the shelves over the months, or even over the years. In this situation the dust on shelves may become a permanent reservoir of fungal spores. In some papers, one can find a supposition that dust does not favour fungal growth and development. For example, Davies (1960) reported that *Cladosporium* spores lose their ability to germinate in house dust. However, recent studies have shown that organic matter present in dust, rich in volatile compounds, such as aldehydes and organic acids could be a good energy source for airborne fungi and can support their development (Korpi *et al.*, 1997; Mølhav *et al.*, 2000; Tatur *et al.*, 2006).

In our study, in the majority of the surveyed flats, the bed was the environment with the lowest concentration of fungi. In many cases, the concentration of fungi in the beds was negatively correlated with the level of mite allergen (unpublished data in MONIT project). This suggests that fungal spores could also be a good food source for mites, and not only the human dandruff or the shed skin, as it was previously thought (Andersen, 1985).

### *The Effect of Season on the Concentration of Culturable Fungi*

In our study the seasonal differences in *Penicillium*, *Aspergillus* and *Cladosporium* concentrations were observed. In the autumn, the concentration of *Penicillium* and *Aspergillus* increased two- and five fold respectively, in comparison with the concentration in the spring. In Poland, the humidity of the air generally increases in autumn, and the flats are less ventilated due to lower temperatures. Probably the combination of humidity and poor ventilation enhance *Penicillium* and *Aspergillus*. Autumn also offers good conditions for the development of soil born fungi of the genus *Cladosporium*, associated with the falling leaves and debris (Shelton, 2002). On the other hand, the rainfall and the cooler temperature are responsible for the decrease of *Cladosporium* abundance (Mitakakis *et al.*, 1997; Fernández *et al.*, 1998). In our study the samples were collected in November and December, so in the period of low temperatures and abundant precipitation. May be it is a reason why we found a significantly lower concentration of *Cladosporium* in autumn, in comparison with the spring. Seasonal differences in outdoor spore concentrations have been observed by others authors (Larsen and Gravesen, 1991; Mitakakis *et al.*, 1997; Fernández *et al.*, 1998; Medrela-Kuder, 2003).

The seasonal fluctuations in the concentration of fungal spores in air not always reflect the concentration of spores in the dust. The reason for this is that fungi may accumulate in the dust for a long time (Karbowska-Berent *et al.*, 2011). This concerns mainly the dust in places seldom cleaned,

such as e.g., bookshelves. There was no significant difference between their total concentration in autumn and in spring dust samples. However, we found a correlation between frequency of ventilation and *Alternaria* concentration, suggesting that some *Alternaria* spores may come from the outdoor environment via air currents (Giorgio *et al.*, 1996; Chen *et al.*, 2012).

#### **Relationship between Concentration of Culturable Fungi and Flat Characteristics and Habits of Occupants**

It is well known that bad technical condition of the building (poor ventilation, dampness, wet walls) favour the fungal development and the indoors fungal spores concentration (Garrett *et al.*, 1998; Pasanen *et al.*, 2000). In the present study, the most important factor associated with increase of concentration of fungi, especially in the bathrooms, was ventilation. Garrison *et al.* (1993) also showed that in houses with poor ventilation the concentration of fungi increased. Emberlin *et al.* (1995) detected the high concentration of fungi in the houses with high moisture content. We received similar results. In two bathrooms with poor ventilation and with high humidity, the concentration of *Cladosporium* was 1000 times higher than in bathrooms with efficient ventilation. In both bathrooms visible mould growth was evident on walls and ceilings.

Most of the factors related to the characteristics of flats and customs of the inhabitants had no effect on the concentration of fungi in the dust. However, certain relationships for some genera of fungi have been reported. An interesting result was obtained by analyzing the concentration of fungi in the kitchen depending on the frequency of cooking. In dust from the kitchens, where the residents cooked once a week, generally higher concentrations of fungi (although not statistically significant except *Penicillium*) than in the kitchens extensively used by the residents have been recorded. It was expected that the high humidity in the kitchen during cooking could promote fungi whereas one of the factors that could affect the fungi in the kitchen are polycyclic aromatic hydrocarbons (PAHs) - products of combustion of gas and kitchen practices. Tatur and collaborators have shown that in the same flats the level of polycyclic aromatic hydrocarbons (PAHs) was positively correlated with frequency of cooking on gas (Tatur *et al.*, 2009). May be *Penicillium* spores were especially sensitive to PAHs and for this reason concentration of *Penicillium* decreased when the frequency of cooking increased.

In our study in the flats where the inhabitants had greater activity (number of exits and entries was above 10 per day) concentration of *Aspergillus* was 10 times lower in comparison with the flats where the inhabitants were less active. This may be due to the fact that a small and lightweight fungal spores, such as spores of *Penicillium* and *Aspergillus*, can be raised even by slight air currents caused by the activity of the inhabitants (Górny *et al.*, 2001; Buttner *et al.*, 2002). Therefore, the concentration of these fungi can be reduced in dust samples and higher in air samples.

We expected that the presence of dogs or cats in houses affects the number of fungi, because pets could propagate

spores and other allergens indoors via their feet or fur. Salo *et al.* (2005) and Cho *et al.* (2006) found a significant relationship between *Alternaria* antigen concentration and the presence of dogs in the house, while the present study and that of Emberlin *et al.* (1995) did not confirm any correlation between concentration of culturable fungi and the presence of pets.

#### **CONCLUSIONS AND GUIDELINES**

Present studies have shown that the concentration of fungi, as well as their genera spectrum in dust from Warsaw flats, did not differ from those in other European houses. The results obtained in this study enrich our knowledge about the impact of several factors on the composition and abundance of fungi in house dust. Especially valuable are the results obtained for these factors, which were not analyzed on a broader scale, such as: localization of flats, frequency of ventilation, cooking and frequency of exits and entries into flat per day. We have found that frequency of cooking significantly negatively affected *Penicillium* concentration (the higher frequency of cooking, the lower concentration of *Penicillium*). Moreover, we have found a correlation between the ventilation (number of hours when the windows are open) and *Alternaria* concentration. A ventilation from 20–24 hours per day increased *Alternaria* concentration five fold in comparison to flats ventilated less than one hour per day. The greater number of exits and entries into flat per day decreased the culturable spores of *Aspergillus*. Our study also indicated some differences between the concentration and composition of fungi in different environments in a flat. The highest total concentration of fungi was found in the dust from the shelves and the least one in the dust from the beds. *Penicillium* spores dominated in dust samples from the floor, shelves, kitchen and bed, whereas *Aspergillus* spores dominated in dust samples from the bathroom.

Until now, most of previous studies were focused on an analysis of house dust collected from the floor, carpet or mattress. We included in our study other environments: bookshelves, bathroom and kitchen. The results obtained for these environments provide additional information about the conditions favourable for a survival and development of fungal spores.

It has been reported that the fungal spores accumulated in house dust can maintain their viability for many months (Korpi *et al.*, 1997). It leads to a practical, very simple indication - it is necessary to limit the places where dust could be accumulated e.g. frequent cleaning of the entire house is recommended (Sordillo *et al.*, 2011). Moreover, open shelves should be replaced by closed cabinets. The results obtained in an analysis of dust in the bathroom show an importance of well-functioning ventilation in bathrooms. Most fungi for their development require a relative humidity above 75%. Therefore, actively growing fungi are usually limited to places in the flats such as the bathroom or kitchen, where moisture may increase periodically over 90%. It therefore appears that the key to mould prevention is moisture control, especially in the bathrooms. Numerous studies on indoor air quality suggest an optimum relative

humidity range of 45 to 55%. Increase in relative humidity above this value can promote the growth of mould and also dust mites. On the other hands, too high or too low humidity can cause a variety of health threats and illnesses (Arundel *et al.*, 1986; Tang, 2009).

Our research has several limitations. Firstly, our study involved a small number of flats. It was caused by modest project funds. The second limitation is related to the applied method. To date, no standard methods are available for detecting and enumerating fungi in indoor environments. This considerably limits the possibility of comparing the results from different studies. Using the dilution plate method, applied in this study, only some fungi could be detected, only those which can grow on artificial medium. Thus, the culture-based methods not fully reflect the real abundance of fungi. Nevertheless, these methods allow the identification of many dangerous species of fungi to human health (WHO, 2009). Recent methods such as qPCR, or  $\beta$ -glucan analysis detect both viable as well as non-viable fungal material (Chew *et al.*, 2001; Kaarakainen *et al.*, 2009). These limits cause that our study should be regarded as a preliminary study, which could be helpful in determining the directions for further research.

In conclusion, it appears that it is important to search for sources and factors affecting the concentration of fungi in house dust, because long lasting presence of allergenic fungi in the dust may cause or exacerbate clinical symptoms in the sensitive individuals and extend the period of presence of allergens indoors.

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