

Original papers

Quantitative assessment of mycological air pollution in selected rooms of residential and dormitory housing facilities

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ABSTRACT. The qualitative and quantitative mycological composition of indoor areas of three private residencies and an academic dormitory in Wroclaw, Poland was investigated. Seasonal fungal samples were obtained using a MAS-100 air sampler. The samples were cultured on three different media: Sabouraud Agar (SAB), Dichloran Glycerol Selective Medium (DG18) and Malt Extract Agar (MEA). The number of colony forming unit (CFU) values ranged from 10 CFU/m^3 to 490 CFU/m^3 depending on the culture medium, season, and sampling site. The identification of the cultured fungi was performed using macro- and microscopic observations and diagnostic keys. Eleven fungal genera were identified. The most common fungi were members of genera *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium*; the least common fungi were members of genera *Geotrichum* and *Paecilomyces*. Seasonal variations in the concentration of fungi were observed with the highest concentration of fungi in the spring and the lowest concentration of fungi in the winter. There were no statistically significant correlations between fungal concentrations and the temperature or the relative humidity of the sample sites.

Key words: fungi, air pollution, housing facilities

Introduction

Indoor air quality is one of the most significant factors affecting the health and well being of individuals who spend between 80–95% of their lives indoors [1]. Atmospheric air contains fixed amounts of natural gas compounds, and various abiotic and biotic contaminants. Biotic contaminants include viruses, protozoa, bacteria, cells and tissue fragments of plants and animals, fragments of fungal mycelia, and fungal spores. Abiotic contaminants include organic dust, various materials stored in the buildings and from the air inflowing from ventilation and air conditioning systems [2,3].

According to the World Health Organization (WHO) roughly 3 billion people around the world suffer from diseases caused by indoor air pollution. Most health problems associated with indoor air quality relate to fungi. Mycological air analyses conducted in selected buildings in the USA and Brazil show that fungi represent circa 70% of all microbial pollution of indoor air [4]. Exposure to some fungal genera can cause primarily irritations, infections, allergies, and serious toxic effects. In addition, a large number of fungi produce mycotoxins, secondary metabolites, and volatile organic compounds that can affect human health. In susceptible or highly exposed individuals these can lead to invasive mycosis [5].

The objective of this study was to evaluate the extent of mycological air pollution in private residential houses and an academic dormitory by a determination of their respective fungal composition and number.

Materials and Methods

Mycological comparative analyses were carried out in three private homes and an academic

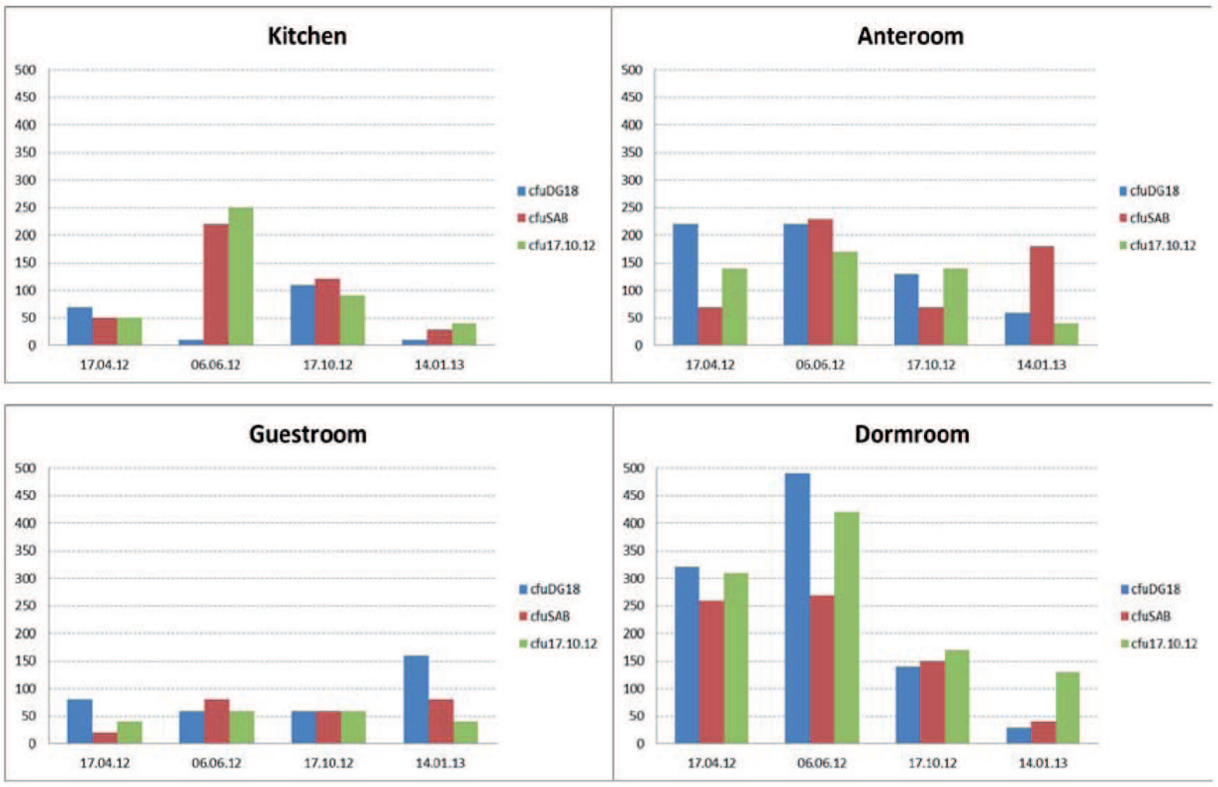

Fig. 1. Fungal CFU/m^3 values of seasonal air samples collected from four separate indoor residential sites and cultured on three different culture media (DG18, SAB and MEA)

dormitory located in Wroclaw, Poland. Indoor air samples were obtained using a MAS-100 air sampler (Merck KgaA, Darmstadt, Germany). Air flow through the sampler was +/– 11 m/s, air volumes were 5–500 liters (depending on expected contamination level) and the sampling rate was 100 l/min. A single sample was collected from each sampling site in each season: spring (17.04.2012), summer (06.06.2012), autumn (17.10.2012) and winter (14.01.2013). At the time of air sampling the relative humidity and temperature were measured with a termohygrometer. All windows and doors were closed at each sampling site.

One room in each of four sites was sampled: site 1, the kitchen of a studio apartment located in the center of the city with moderate traffic and inhabited by two people; site 2, the guestroom of a four room apartment located at the edge of the city with low traffic and occupied by four people; site 3, the anteroom of a family house located at the edge of the city with low traffic and occupied by three people; and site 4, a double room in an academic dormitory located in the centre of the city with the high traffic and occupied by two people. Prior to air sampling the walls of each of the rooms were inspected for the presence of mold and small visible filamentous fungi. Mold and filamentous fungi were detected in the guestroom and anteroom sites.

Three different culture media were used to analyze the air samples: Sabouraud Agar (SAB), Dichloran Glycerol Selective Medium (DG18) and Malt Extract Agar (MEA). Samples were incubated in media-containing Petri dishes for 2–7 days at 26°C. Following incubation the number of CFU/m^3 (Colony Forming Unit/1 m^3 of air) was calculated. After the fungal growth the most dominant species of molds were passed to fresh media.

The specific identification of the fungi was performed using macro- and microscopic observations and commonly accepted methods [6]. Fungal colonies were identified on the basis of color, texture, topography of the surface of the culture, smell of the colony, colour of the reverse of the colony, and the presence of diffuse color pigment. Microscopic features of the fungal colonies (i.e. the presence of macroconidia and microconidia, and their shape and appearance) were then examined and specific fungi identified using diagnostic keys [7].

The statistical significance of the differences in the number of CFU/m^3 observed on the three culture media was determined by the Wilcoxon

test.The Pearson test was used to determine the extent of correlation between the temperature or relative humidity and the number of CFU/m^3 observed.

Results and Discussion

The results of this mycological case study suggest that the pattern of fungal air pollution varied between the four residential sites studied (Table 1). Furthermore, the number of fungal colonies depended on the type of culture medium used (Fig. 1).

The fewest numbers of colony forming units (CFU/m^3) on MEA and SAB media (45.0 CFU/m^3 and 60.0 CFU/m^3, respectively) were recorded from air samples obtained from the guestroom of the four room apartment, while the highest number of CFU/m^3 was obtained in the dormitory room (MEA 232.5 CFU/m^3 and SAB 180.0 CFU/m^3).The other two sites (kitchen in the studio apartment and anteroom in the family house) had similar average concentrations of molds (MEA 170.0 CFU/m^3, SAB 105.0 CFU/m^3).

For air samples cultured on DG18 medium the highest average annual CFU/m^3 value (245.0 CFU/m^3) was observed in samples obtained from the dormitory room, while the smallest average annual CFU/m^3 value (47.5 CFU/m^3) was detected in the city center apartment.

The Pearson correlation test ($p>0.05$) indicated that neither the temperature or the relative humidity in the selected rooms had a statistically significant effect on the measured fungi concentrations.

There was a slightly greater fungi number detected on the MEA medium (average 2.70 bred strains), when compared with the fungi number observed on the SAB medium (average 2.25 bred strains). This could be due to the fact that SAB is a universal medium which is applicable for isolation of both outdoor and indoor fungi**,** while MEA and DG18 are media primarily recommended for

Table 1.The fungal CFU/m^3 values of seasonal air samples collected from four separate indoor residential sites and cultured on three culture media (DG18, SAB and MEA)

Examined room	Date	Temperature [°C]	Relative humidity [%]	CFU/m^3			Average annual CFU/m^3		
				DG18	SAB	MEA	DG18	SAB	MEA
kitchen	17.04.12	25.2	45.7	70	50	50	47.5	105.0	170.0
	06.06.12	23.1	47.3	10	220	250			
	17.10.12	22.1	59.6	110	120	90			
	14.01.13	23.6	47.4	10	30	290			
guestroom	17.04.12	20.6	56.8	80	20	40	90.0	60.0	45.0
	06.06.12	21.3	56.1	60	80	60			
	17.10.12	21.0	56.1	60	60	60			
	14.01.13	19.5	46.0	160	80	40			
anteroom	17.04.12	17.7	46.8	220	70	140	157.5	137.5	145.0
	06.06.12	21.4	59.6	220	230	170			
	17.10.12	16.4	71.8	130	70	140			
	14.01.13	15.8	63.0	60	180	130			
dormroom	17.04.12	22.0	42.2	320	260	310	245.0	180.0	232.5
	06.06.12	21.1	44.4	490	270	420			
	17.10.12	22.1	58.1	140	150	170			
	14.01.13	21.0	60.7	30	40	30			

Table 2. The most common seasonal fungal genera isolated from sampling sites located in three residential houses and a dormitory room, Wrocław, Poland (2012–2013)

Season 2012/13	**Fungi genera present in four different indoor locations**			
	Site 1 Kitchen	**Site 2 Guestroom**	**Site 3 Anteroom**	**Site 4 Dormitory**
Spring	*Penicillium* *Cladosporium* *Aspergillus* yeast	*Aspergillus* *Cladosporium* *Penicillium* *Paecilomyces* yeast	*Penicillium* *Cladosporium* *Aspergillus* *Geotrichum*	*Cladosporium* *Penicillium* *Aspergillus* yeast
Summer	*Rhizopus* *Penicillium* *Cladosporium* *Alternaria* *Fusarium*	*Penicillium* *Alternaria* *Cladosporium* *Aspergillus* *Syncephalastrum* yeast	*Cladosporium* *Penicillium* *Trichoderma* *Aspergillus* yeast	*Penicillium* *Aspergillus* *Cladosporium*
Autumn	*Penicillium* *Aspergillus* *Cladosporium* *Fusarium* *Alternaria* yeast	*Cladosporium* *Paecilomyces* *Alternaria, Aspergillus* *Penicillium* *Acremonium* yeast	*Cladosporium* *Penicillium* *Aspergillus* *Geotrichum* yeast	*Aspergillus* *Penicillium*
Winter	*Aspergillus* *Penicillium* yeast	*Cladosporium* *Aspergillus* *Paecilomyces* *Fusarium*	*Penicillium* *Paecilomyces* *Aspergillus* yeast	*Penicillium* *Fusarium* *Aspergillus* *Cladosporium* yeast

isolation of indoor fungi. Also MEA medium is recommended for isolation from rooms with high humidity levels, while DG18 medium is recommend for isolation from rooms with low relative humidity levels. However, the differences observed in this study are not statisticaly significant when analyzed by the Wilcoxon test (p=0.47).

A quantitative interpretation of results describing indoor air quality is difficult due to the lack of widely accepted normative and reference values.

Universally applicable standards defining an acceptable level of indoor air contamination with fungal organisms have not yet been established. In this study the evaluation of the air quality in the designated sampling sites was based on the sanitary standards for non-industrial areas formulated by Górny and Dutkiewicz in the World Health Organization (WHO) Expert Meeting, Berlin, 2002 [8]. According to these standards residential fungal bioaerosol concentrations should not exceed 5000 CFU/m^3. The highest concentration found in our study (490 CFU/ m^3) was almost ten times less than the maximum acceptable level proposed by Górny and Dutkiewicz. The presence of the pathogenic fungus *Aspergillus niger* and other fungi such as *Penicillium* and *Trichoderma* could potentially result in the production secondary metabolites which could compromise the health and well-being of humans [9]. The majority of these fungi are known to be potential respiratory allergens. Mold can live practically anywhere and have particularly favourable growth conditions inside residential houses and dormitory rooms. Out of 11 detected fungal genera (Table 2) the most frequent species in the spring and summer were *Penicillium* (5.9% and 100%, respectively) and *Cladosporium* (7.7% and 60.0%, respectively). *Aspergillus* predominanted in autumn and winter (4.55% and 93.3%, respectively). Representatives of the genera *Paecilomyces*, *Geotrichum*, *Rhizopus*, *Alternaria*, *Syncephalastrum*, *Trichoderma*, *Fusarium*, and *Acremonium* were sporadic and sparse. Similar results was found by Cetinkaya et al. in the air of residential areas in the city of Afyon, Turkey [10].The authors identified the following types: *Cladosporium* (representing 31.9% of the total fungi microbiota), *Aspergillus* (18.6%), *Penicillium* (15.5%) and *Alternaria* (13.0%). According to Sen

and Asan *Penicillium* (28.61%), *Cladosporium* (16.08%) and *Alternaria* (15.98%) were also the dominant genera in different residential houses in Tekirdag City, Turkey [11].

The majority of the fungal genera identified in this study are potential respiratory allergens and are associated with allergic respiratory diseases, especially in people with impaired immune systems. For example, *Aspergillus* and *Fusarium* are important producers of mycotoxins, secondary metabolites, and volatile organic compounds; and the spores of *Cladosporium*, *Penicillium* and *Alternaria* may carry allergens, antigens, and polysaccharides such as the β(1→3)-glucans which can cause allergic respiratory disease in susceptible individuals. The presence of these and other fungi in all of the sampling sites could potentially compromise the health and well-being of the humans occupants of the sites [9].

Conclusions

The extent of mycological air pollution in four different indoor sampling sites was evaluated. Air samples were collected with a MAS-100 air sampler. Samples were then cultured on MEA, SAB, and DG18 media which allowed for qualitative and quantitative mycological analyses. In this case study, a total of eleven fungal genera were identified with *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *and Fusarium* the most common genera identified. Most of the genera identified are associated with allergic respiratory diseases. However, their potential risk for respiratory disease at the sample sites, estimated by CFU/m^3 (Colony Forming Unit/1 m^3) number, appears small. The procedures developed in this study may aid in future studies concerned with the health and well-being of individuals exposed to mycological air pollution.

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