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Culturable and Total Fungi in Dust Accumulated in Air Ducts in Single-Family Houses

ANNA-LIISA PASANEN[1]*, LIISA KUJANPÄÄ[1], PERTTI PASANEN[1], PENTTI KALLIOKOSKI[1] AND GÖRAN BLOMQUIST[2]

Abstract Fungal spore content in dust accumulated in air ducts was investigated in 24 mechanically ventilated single-family houses of which 15 had also a central air heating system. Dust was collected from the ducts simultaneously with cleaning of the ventilation systems. Besides spore concentrations and flora of culturable fungi, total fungal spore concentrations were determined in dust samples by the aqueous two-phase technique and spore counting with epifluorescence microscopy. Culturable spore concentrations in the dust varied from 10^4 to 10^7 CFU/g and total spore concentrations from 10^7 to 10^8 spores/g. Total spore concentrations in the duct dust were significantly higher in the air heated houses than in the other mechanically ventilated houses. The difference resulted mainly from a higher proportion of recirculation air and a higher age of the air heated houses. *Cladosporium*, *Penicillium*, *Aspergillus* and yeasts consisted of >90% of fungal flora in the dust. Although total spore concentrations were at the same level both in the exhaust and in the supply ducts in both types of house, culturable fungal spore concentrations were slightly higher in the exhaust ducts than in the supply ducts. The proportion of culturable spores was<5% of total spores in dust accumulated in the ducts.

Key words Accumulated dust; Central air heating system; Duct work; Dwelling; Mechanical ventilation; Micro-fungi

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Introduction

It is well known that ventilation systems, even the systems without water-containing components, may act as significant microbial sources in indoor air, if moisture and dust accumulate in supply air ducts and other components of ventilation systems. Biocontamination in supply air filters and ducts has been investigated in Denmark, Sweden and Finland. In air filters, fungal spore concentrations have been reported to vary widely (up to 10^4–10^5 CFU(colony-forming units)/g of filter material or 10^3–10^4 CFU/m^2 of filter) depending on spore concentrations in outdoor air, flow rate through the filter, filtering efficiency, moisture conditions and loading time of the filter (Valbjørn et al., 1990; Sverdrup & Nyman, 1990; Martikainen et al., 1990; Pasanen et al., 1991; Pasanen et al., 1995).

Dust and microorganisms accumulated in supply air ducts may originate from contamination during construction, from outdoor air due to leakages between the filter cassette and the assembly frame, or from insufficient filtering efficiency of the filter (Pasanen, 1994). Surface densities and fungal spore concentrations of accumulated dust in air ducts of office buildings, schools and dwellings reported in the literature are presented in Table 1. The average annual accumulation rate of dust in supply air ducts has been estimated to range from 0.7 to 1.8 g/m^2 · year in office buildings and to be about 0.1 g/m^2 · year in dwellings (Valbjørn et al., 1990; Pasanen, 1994; Kalliokoski et al., 1995). However, there are only a few studies dealing with dust accumulation in ventilation systems of single-family houses. Fungal spore concentrations in dust accumulated in air ducts seem to vary widely, although the results are not necessarily comparable between different studies, because of variations in sampling and cultivating methods (Table 1). Culturable fungal spore concentration does not, however, reflect completely fungal contamination in accumulated dust, because ventilation systems provide unfavorable conditions for survival of fungal spores. It has been estimated that theoretical survival time of microorganisms on air filters is about three days (Martiny et al., 1994). Thus, the proportion of culturable spores in accumu-

[1]University of Kuopio, Department of Environmental Sciences, P.O.Box 1627, FIN-70211 Kuopio, Finland; [2]National Institute for Working Life, Analytical Chemistry Division, P.O.Box 7654, S-90713 Umeå, Sweden. *Corresponding author: Tel. 358-17-163 157. Fax. 358-17-163 230.
Email: anna.pasanen@uku.fi

Table 1 Surface density and culturable fungal spore concentrations in dust accumulated on air ducts in non-industrial buildings. Air ducts of the buildings studied had not been cleaned. Ventilation systems of the buildings do not include water-containing components or samples, the results of which, as presented here, had been taken from other parts of the system

Location/n	Age (years)	Supply air duct		Exhaust air duct		Reference
		Surface density of dust (g/m^2)	Fungal spore concentration or density	Surface density of dust (g/m^2)	Fungal spore concentration or density	
offices, day-care centres, dwellings (n=12)	n.m.	n.a.	1–15 CFU/m^{2A}	n.a.	1–1900 CFU/m^{2A}	Sverdrup & Nyman, 1990
schools, offices (n=13)	5–29	1.1–50.9 mean 6.8	0.07–6.2 · 10^3 CFU/g^B	n.m.	n.m.	Valbjørn et al., 1990
offices, school, dwelling (n=6)	5–11	3.6–140.8 mean 18.2	0.02–2.3 · 10^4 CFU/m^{2C}	n.a.	n.a.	Laatikainen et al., 1991
dwellings (n=33)	0–45	<269.9 mean 19.2	0–8.0 · 10^4 CFU/m^{2C}	1.2–1560.1 mean 639	n.a.	Auger, 1994
offices (n=14)	3–34	1.2–158 mean 13.2	0.03–2.0 · 10^4 CFU/g^B	n.a.	n.a.	Pasanen, 1994
office (n=1)	n.m.	n.a.	0.8–6.0 · 10^4 CFU/m^{2A}	n.a.	0.02–1.0 · 10^5 CFU/m^{2A}	Tarvainen et al., 1994
dwellings (n=24)	2–16	0.2–3.9	0.02–27 · 10^6 CFU/g^B	0.2–4.3	0.04–61 · 10^6 CFU/g^B	Kalliokoski et al., 1995*

[A] Swap method
[B] Cultivation of dust derived from air ducts
[C] Contact plate method
* the data of the present study
n.m. not mentioned
n.a. not analyzed

lated dust in ducts can be expected to be quite high. In addition, it should be emphasized that most fungal spores probably retain their allergenic properties even when the spores are no longer culturable (Levetin, 1995).

Direct counting techniques, e.g. for an epifluorescence microscope, have been applied for enumeration of microorganisms in environmental samples (Palmgren et al., 1986), and the CAMNEA method (collection of airborne microorganisms on Nuclepore filters, estimation and analysis) has been used successfully for evaluating total concentrations of fungal spores in air samples (Blomquist et al., 1984a; Karlson & Malmberg, 1989; Hanhela et al., 1995). However, direct counting techniques, including fluorochrome (acridine orange) staining and epifluorescence microscopy, as such, are not useful for analyzing microorganisms in dust samples, because nonspecific binding of fluorochromes to organic dust particles in the sample causes high background fluorescence and makes counting of microorganisms impossible. A Swedish research group applied the aqueous polymer two-phase technique, a well-known separation method for soluble macromolecules (cells, cell particles) (Albertsson, 1986), to partition different fungal genera and species from each other (Blomquist et al., 1984b; Ström, 1986; Ström & Blomquist, 1986; Ström et al., 1989) or to separate microorganisms from other organic particles (Ström et al. 1987). Thus, the aqueous two-phase system can be used as a sample processing method before staining and epifluorescence microscope counting of dust samples.

Dust accumulated in supply air ducts has been found to contain 20% organic matter (Laatikainen et al., 1991; Pasanen, 1994). The confounding effect of organic matter in dust on the analysis is probably a reason why total fungal spore concentrations in accumulated dust have not been reported earlier. The aim of the present work was to estimate fungal content in dust settled on air ducts in single-family houses. The main objective was to determine total fungal spore concentrations (both culturable and total) in the dust samples by using the two-phase technique as a separation method for fungal spores before epifluorescence microscopy.

Materials and Methods

The Single-Family Houses Studied

Dust samples accumulated in air ducts originated from 24 single-family houses situated in or in the vicinity of Kuopio city (80000 inhabitants) in central Finland. All the houses had mechanical supply and exhaust ventilation and, in addition, central air heating was used in 15 houses. In the air heated houses, heating energy was transferred to the rooms by a ventilation system, and the proportion of recirculated air was about 85–95%. The houses with air heating were somewhat older (10–16 years of age) with a smaller floor area (104–161 m^2) than the other houses (2–12 years of age; floor area

107–260 m^2). In 22 houses, the ventilation system was equipped with a coarse air filter (EU3 or 4 with an average dust weight arrest for artificial dust of 80–90%), and two houses had a EU5 filter (an average dust spot efficiency is 40–60%). The houses were selected among customers of a professional cleaning company from which the cleaning of ventilation systems had been ordered. The air exchange rate was low in most of the houses, ranging from 0.05 to 0.66 L/h, and various faults, e.g. unbalanced and too high a proportion of recirculation air and blockage of the ventilation grills, were commonly observed before cleaning. Detailed information on ventilation faults and the effect of cleaning and readjusting of the ventilation system on the air exchange rate is presented elsewhere (Kalliokoski et al., 1995).

The air ducts of the houses were cleaned by the rotating brush method, by a professional cleaning company. Duct cleaning had not been carried out earlier in any of the houses. The air heating systems were cleaned just before the heating season and the other ventilation systems were cleaned later in the fall and in the winter. Simultaneously with the duct cleaning, accumulated dust was collected from the ducts on a filter installed in the depressurizing unit and determined gravimetrically. Dust samples were stored in closed packages at room temperature before the analysis.

The range of surface density of accumulated dust in the ducts is presented in Table 1. The mean annual accumulation rate of dust was 0.1 g/m^2 in the supply ducts and 0.2 g/m^2 in the exhaust ducts. The proportion of organic matter in the dust varied from 2% to 74%, being higher in the air heating systems than in the other ventilation systems, and being higher in the exhaust ducts than in the supply ducts (Kalliokoski et al., 1995).

Determination of Culturable Fungi in Dust

On the day after the collection, at the latest, a 0.5 g sub-sample was taken from each dust sample and suspended in dilution water (0.0425 g/L KH_2PO_4, 0.25 g/L $MgSO_4$, 0.008 g/L NaOH, 0.02% Tween80 detergent). Dilution series were prepared and three successive dilutions were plated in duplicate on 2% malt extract agar and on dichloran 18% glycerol agar containing streptomycin and chloramphenicol, respectively, to prevent bacterial growth. The former medium permits the growth of meso-hygrophilic fungi and the latter one xerophilic fungi (Samson et al., 1994). The plates were incubated at 25°C for seven days and fungal colonies were counted and identified on the genus level.

The culturable fungal spore concentrations were presented as colony-forming units (CFU)/g of dust, and the prevalence of fungi as average percentage of fungal genus both in culturable spore concentrations and in dust samples. The significance of differences in culturable spore concentrations and the prevalence of fungal genera in spore concentrations between supply and exhaust duct dust was tested with the Wilcoxon test, and between air heated houses and other mechnically ventilated houses, with the Mann-Whitney U test. McNemar and Fisher's Exact test was used to test the differences in the frequency of the most common fungi in dust samples between supply and exhaust duct dust and between air heated houses and other mechnically ventilated houses, respectively.

Determination of Total Fungal Spore Concentrations in Dust

Preparation of Dust Samples. From each homogenized dust sample, a 0.5 g sub-sample was weighed and suspended in 49.5 mL of 0.05% Tween 80 aqueous solution. The suspension was first placed in a shaker for 30 min and then in an ultrasonic bath for 15 min. This dust suspension was used in the two-phase system.

Preparation of the Two-Phase System. The two-phase system used contained 5% (w/w) sodium dextran sulphate (NaDS; Pharmacia Biotech Norden AB, Sollentuna, Sweden), 4% (w/w) polyethylene glycol^{8000} (PEG; Carbowax 8000, Union Carbide Corp., New York, USA), 0.3 M NaCl, and 10 mM KH_2PO_4 – phosphoric acid buffer (pH 2.5). At first, stock solutions of the phase system components were prepared. Because dextran usually contains 5-10% water, the dextran concentration was first checked by preparing a 22% (w/w) NaDS aqueous solution, determining the concentration with polarimetry, and then adjusting the NaDS stock solution to 20% (w/w). The strength of the other stock solutions were 40% (w/w) PEG, 3 M NaCl, and 0.2 M phosphate buffer (0.2 M KH_2PO_4, pH 2.5 adjusted by 85% phosphoric acid). To prevent microbial contamination, the NaDS stock solution was autoclaved, the other stock solutions were prepared into sterile water, and all the solutions were stored in a refrigerator.

From the stock solutions, 1.0 g NaDS, 0.4 g PEG, 0.4 g NaCl, 0.2 g phosphate buffer, and 1.0 g sterile water were weighed into a test tube. After adding of 1 mL sample suspension, the final weight of the system was 4.0 g. The content of the tube was mixed by inverting it 30 times and the phases were allowed to settle for 20 min.

Partition of Fungal Spores from Organic Dust. The counter current distribution method, with three-step

separation of fungal spores from dust, was used to achieve better partition efficiency. For each dust sample, four test tubes containing the phase system (systems 0 to 3) were prepared, 1 mL of dust suspension was added into tube 0 as described above, and the weight of the other tubes (1–3) was adjusted to 4.0 g with 1 mL of 0.05% Tween solution. After mixing and phase separation, the upper phase was taken away from tube 1, the upper phase of tube 0 (sample tube) was transferred to tube 1, and the fresh upper phase (from tube 1) was added to tube 0. In the second step, the upper phase from tube 2 was taken away, the upper phases from tubes 0 and 1 were transferred to tubes 1 and 2, and the fresh upper phase (from tube 2) was added to tube 0. In the last step, the upper phase from tube 3 was taken away, the upper phases from tubes 0-2 were transferred to tubes 1-3, and the fresh upper phase (from tube 3) was added to tube 0. The volume of the upper phases transferred was 1.5 mL, and the tubes were mixed carefully and allowed to settle between every transfer. The recovery of the three-step separation of fungal spores in the upper phase of the system was estimated to be 93%. After partitioning, 1 mL of the upper phases from tubes 0-3 was withdrawn and combined. This solution was used for an epifluorescence microscope counting.

Counting of Total Spores with Epifluorescence Microscopy. Total fungal spore concentrations were determined with the epifluorescence microscope counting technique as described in detail by Palmgren et al. (1986). Formaldehyde was added to the sample solution (combined upper phase fraction) to achieve the final concentration of 1%. One mL of this solution was stained with 0.01% aqueous acridine orange (Chroma-Gesellschaft, Köngen, Germany) and filtered through a black polycarbonate filter (diam. 25 mm, pore size 0.2 μm; Costar Scientific Corp., Badhoevedorp, the Netherlands). Fungal spores were counted on 40 randomly chosen fields (area of a counting field $6.4 \cdot 10^{-3}$ mm^2) from the filter with an epifluorescence microscope at a magnification of ×1000. The total spore concentration was calculated as spores/g of original dust. The significance of differences in total spore concentrations between supply and exhaust duct dust was tested with the Wilcoxon test and between air heated houses and other mechanically ventilated houses, with the Mann-Whitney U test.

Results

Spore concentrations of meso-hygrophilic and xerophilic fungi, as well as total fungi in dust collected from the supply and exhaust air ducts in both types of system are presented in Table 2. Both culturable and total spore concentrations in dust accumulated in air ducts were approximately 3- to 10-fold higher in the air heating systems than in the other ventilation systems, and the difference in total spore concentrations was statistically significant (P=0.0008 for the supply ducts and 0.0003 for the exhaust ducts). In both types of system, culturable spore concentrations seemed to be about 2 to 7 times higher in dust accumulated in the exhaust ducts than in dust settled on the supply

Table 2 Culturable and total fungal spore concentration in dust collected from air ducts of 24 single-family houses

Fungal spore concentration	Air heated houses (n=15)		Houses with mechanical ventilation (n=9)	
	Supply ducts	Exhaust ducts	Supply ducts	Exhaust ducts
Meso-hygrophilic fungi (CFU/g)				
range	$0.02–27 \cdot 10^6$	$0.04–24 \cdot 10^6$	$0.2.–13.4 \cdot 10^5$	$0.6–20.2 \cdot 10^5$
mean	$2.2 \cdot 10^6$	$4.7 \cdot 10^6$	$4.3 \cdot 10^5$	$5.1 \cdot 10^5$
median	$1.8 \cdot 10^5$	$1.2 \cdot 10^6$	$8.7 \cdot 10^4$	$1.0 \cdot 10^5$
standard deviation	$6.9 \cdot 10^6$	$7.3 \cdot 10^6$	$5.5 \cdot 10^5$	$8.0 \cdot 10^5$
Xerophilic fungi (CFU/g)				
range	$0.04–23 \cdot 10^6$	$0.05–61 \cdot 10^6$	$0.2–13.3 \cdot 10^5$	$0.6–21.8 \cdot 10^5$
mean	$2.0 \cdot 10^6$	$7.1 \cdot 10^6$	$4.5 \cdot 10^5$	$7.1 \cdot 10^5$
median	$2.1 \cdot 10^5$	$1.1 \cdot 10^6$	$9.2 \cdot 10^4$	$1.6 \cdot 10^5$
standard deviation	$5.9 \cdot 10^6$	$1.6 \cdot 10^7$	$5.9 \cdot 10^5$	$9.0 \cdot 10^5$
Total fungi (spore/g)				
range	$0.4–3.3 \cdot 10^{8a}$	$0.5–3.5 \cdot 10^{8b}$	$2.3–6.9 \cdot 10^{7a}$	$2.2–7.9 \cdot 10^{7b}$
mean	$1.6 \cdot 10^8$	$1.6 \cdot 10^8$	$4.8 \cdot 10^7$	$4.2 \cdot 10^7$
median	$1.6 \cdot 10^8$	$1.6 \cdot 10^8$	$4.9 \cdot 10^7$	$3.4 \cdot 10^7$
standard deviation	$9.8 \cdot 10^7$	$9.5 \cdot 10^7$	$1.7 \cdot 10^7$	$1.7 \cdot 10^7$

[a] the difference in spore concentration in the supply ducts was statistically significant between the air heating and the other mechanical ventilation systems (P<0.05)

[b] the difference in spore concentration in the exhaust ducts was statistically significant between the air heating and the other mechanical ventilation systems (P<0.05).

Table 3 Relative proportion of fungal genera in culturable spore counts of dust (average percentages) and among dust samples collected from supply and exhaust ducts of 24 single-family houses equipped with mechanical ventilation. Fifteen houses had also central air heating

Fungal genera	Prevalence of fungal genus (%)							
	Air heated houses (n=15)				Houses with mechanical ventilation (n=9)			
	Supply ducts		Exhaust ducts		Supply ducts		Exhaust ducts	
	in samples	in counts	in samples	in counts	in samples	in counts	in samples	in counts
Cladosporium								
meso-hygrophilic	100	36.1	100	49.0	89	51.7	100	47.1
xerophilic	100	46.9	100	55.6	100	61.9	100	54.5
Penicillium								
meso-hygrophilic	100	35.1	100	32.2	89	17.9[c]	100	33.9[c]
xerophilic	100	32.0	93	33.1	89	17.8[c]	100	30.5[c]
Aspergillus								
meso-hygrophilic	80	10.7[c]	64	5.8[c]	78	2.5	78	3.2
xerophilic	87	10.8[c]	57	3.7[c]	67	3.2	89	4.6
Yeasts	60	7.8	71	4.6	78	14.5	67	5.9
Alternaria, Stemphylium, Ulocladium	60	6.0	43	2.5	22	0.6	22	2.3
Phoma	40	0.3	36	0.6	33	0.2	44	0.6
Aureobasidium	7*	0.1	7*	0.4	22	8.2	22	3.0
Ascomycetes	40	0.4	64	1.9[d]	11*	0.1	22	0.1[d]
Paecilomyces	20	0.3	7*	0.1	11*	0.6	11*	0.5
Rhizopus	13	0.4	7*	0.1	22	0.9	11*	0.1
Trichoderma	20	0.2	14	0.1[d]	44	0.4	56	0.8[d]

* the fungus was observed in the dust samples collected only from one ventilation system.
[c] the difference in the prevalence of fungal genus in dust was statistically significant between the supply and exhaust ducts ($P<0.05$).
[d] the difference in the prevalence of fungal genus in dust accumulated in the exhaust ducts was statistically significant between the air heating systems and the other ventilation systems ($P<0.05$).

ducts although the differences were not statistically significant. The spore concentrations of meso-hygrophilic and xerophilic fungi were approximately at the same level in the dust samples, probably due to the fact that many fungal species were able to grow on both the culture media. However, there was no difference in total spore concentrations between dust collected from the supply ducts and from the exhaust ducts. Thus, the proportion of culturable fungal spores in total spore concentrations was slightly higher in dust accumulated in the exhaust ducts (1.2–4.4%) than in dust settled on the supply ducts (0.9–1.7%), being highest in the air heating systems.

The prevalence of the main fungal genera in the culturable spore concentrations and in the dust samples collected from the air ducts of the air heated and the other mechanically ventilated houses is presented in Table 3. *Cladosporium*, *Penicillium*, and *Aspergillus* were the most abundant fungal genera and they comprised, together with yeasts, over 90% of culturable fungal flora in all the dust samples. In the air heating systems, the relative proportion of *Aspergillus* in culturable spore concentrations was significantly higher in the supply duct dust than in the exhaust duct dust ($P=0.006$ for meso-hygrophilic species and 0.02 for xerophilic species). In the other ventilation systems, *Penicillium* species were significantly more frequent in the exhaust duct dust than in the supply duct dust ($P=0.02$ for meso-hygrophilic species and 0.04 for xerophilic species). Some typical outdoor air fungi, such as *Alternaria, Stemphylium, Ulocladium* and *Ascomycetes* were detected more often in the ducts of the air heating systems, and the difference in the relative proportion of *Ascomycetes* in spore concentrations of the exhaust duct dust was statistically significant ($P=0.02$). Correspondingly, yeasts, *Trichoderma* and *Aureobasidium*, were more prevalent in the duct dust of the other ventilation systems. The difference was significant in the prevalence of *Trichoderma* in the exhaust duct dust ($P=0.04$). In addition, some fungi (such as *Absidia, Acremonium, Botrytis, Epicoccum, Fusarium, Monilia* and *Mucor*) were found only occasionally in less than four ventilation systems and their proportion of culturable spore concentrations was less than 0.1%.

Discussion

Significantly lower spore concentrations of culturable fungi have been observed in dust collected from supply ducts in office buildings than those in the present

study obtained in single-family houses (Valbjørn et al., 1990; Pasanen, 199; Table 1). This discrepancy was probably due to the supply air intakes being located higher above ground level and more efficient filtration of supply air in office buildings than in single-family houses. On the other hand, culturable fungal spore concentrations in dust in the air ducts in this study were only slightly higher than those reported in house dust (Miller et al., 1988; Hyvärinen et al., 1993). The same fungal genera were also observed in dust in the present study as reported earlier in dust accumulated in air ducts (Valbjørn et al., 1990; Pasanen, 1994) and in house dust (Miller et al., 1988; Hyvärinen et al., 1993).

In the present study, fungal spore concentrations were distinctly higher in the air heating systems than those in the other ventilation systems, resulting from high recirculation rates and higher age of the houses with air heating (Kalliokoski et al., 1995). However, the season may have had an effect on spore accumulation in ducts; the air heating systems were cleaned and dust was collected in the early fall, when spore concentrations in outdoor air usually vary on the level of $10–10^3$ CFU/m^3 (Pasanen et al., 1990), while the other systems were cleaned later in the fall and winter when outdoor air spore concentrations normally decrease to the level of $1–10^2$ CFU/m^3 (Pasanen et al., 1990). The season may also affect slightly the fungal flora in dust; the prevalence of some outdoor air fungi was higher in the ducts of the air heated houses. However, the main fungal genera were the same in both types of system. Tarvainen et al. (1994) studied the biofilm formation in ventilation ducts during different periods of the year and found that the season had an effect on the quantity and quality of biofilm rather than the rate of the biofilm formation. Fungal spore concentrations in biofilms were higher in the fall than during the winter (Tarvainen et al., 1994).

In the previous studies, culturable fungal spore concentrations have been reported to be higher in dust accumulated in exhaust ducts compared with those in supply ducts (Sverdrup & Nyman, 1990; Valbjørn et al., 1990; Tarvainen et al., 1994; Table 1). This was also observed in the present study although the differences were not statistically significant. However, no difference in total spore concentrations was noticed between the supply and exhaust ducts. The proportion of culturable fungal spores in total spores was only <5%, and it was slightly higher in the exhaust ducts than in the supply ducts. Culturable spores have been estimated to comprise about 1–25% of total spores in the air in an agricultural environment (Karlsson & Malmberg, 1989; Hanhela et al., 1995). The results of this study show that conditions in air ducts, such as variation in temperature and humidity, do not favor viability of fungal spores. The results also support the idea presented by Martiny et al. (1994) that the survival time of microorganisms in air filters is only a few days. A higher proportion of culturable fungal spores in dust in the exhaust ducts is possibly due to more stable moisture conditions for survival of spores and the effect of indoor fungal sources.

Conclusions

This study provided new information on the prevalence of culturable and total micro-fungi in dust accumulated in air ducts in single-family houses. The results revealed that in spite of a lower dust accumulation rate, fungal contamination in air ducts is heavier in single-family houses than e.g. in office buildings. By using a special sample processing technique, it was possible to determine both culturable and total fungal spore concentrations in the dust samples. The results showed that contrary to earlier reports, the accumulation of fungal spores is similar in both supply and exhaust ducts, but only a very small proportion of spores (<2%) is culturable in dust settled on supply ducts. However, the presence of non-culturable spores in accumulated dust and supply air may also be significant because of their possible allergenic properties.

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