ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2004, 11, 67–73

AIRWAY TOXICITY OF HOUSE DUST AND ITS FUNGAL COMPOSITION

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Piecková E, Wilkins K: Airway toxicity of house dust and its fungal composition. *Ann Agric Environ Med* 2004, **11**, 67–73.

Abstract: House dust is an important source of different toxic metabolites as well as allergens, including those of fungal origin, in the indoor environment. A bio-assay employing 1-day-old chick tracheas was used to characterize airway effects of 2-butanone and dimethylsulphoxide (Me_2SO) extracts of 23 dust samples collected from water damaged (13) and control (10) Danish schools. Direct microscopical analysis of samples, followed by cultivation on dichloran 18% glycerol agar at 25ºC for 10 days to establish their mycoflora, was performed. The *in vitro* ciliostatic potential of the chloroform-extractable endo- and exometabolites of 41 representative fungal isolates was determined. Nine dust extracts in 2-butanone (2 from damp rooms) or 10 (6) in Me_2SO showed some ciliostatic activity in the 3-days' experiment. Fungal composition of dust from buildings with leakage was almost identical with that from undamaged houses, as well as the fungal colony counts from the damp schools and the control samples. *Aspergillus* spp. were prevalent in the samples - 31 or 40% of all fungi, followed by *Penicillium* spp. and *Cladosporium cladosporioides*. *Alternaria* spp., *Chaetomium* spp., *Mucor* spp., Mycelia sterilia, *Paecilomyces variotii*, *Rhizopus* sp., *Ulocladium* sp. and yeasts were each isolated in less than 8% of the fungal content. No *Aspergillus flavus* isolate (8 in total) was aflatoxigenic *in vitro*. *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Penicillium* spp., *C. cladosporioides*, *Chaetomium* spp. and *Ulocladium* sp.; in total, 88% of all fungi tested, produced ciliostatically active metabolites. These toxigenic strains were also present in 4 dust samples from controls and 5 dust samples from water damaged buildings. Extracts of these dust samples were also toxic in bioassay. There were bio-detectable concentrations (10–20 µg of extracts/ml of the organ culture medium) of toxic compounds in house dust. Contribution of fungal metabolites to its toxic effect should be studied further.

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Key words: house dust, fungi, organ cultures, airway ciliostatic activity, toxic extracts.

INTRODUCTION

House dust is a complex mixture of particles, usually up to 1 mm in diameter, in which soil/sand, combustion products, paint chips, hairs, dander, insects, pollen and microorganisms can be present. Thus, it is an important indoor source of exposure to animal allergens (dust mites, pets' hairs etc.), bacteria and associated endotoxin, fungi and their metabolites, but also chemicals such as persistent organic pollutants (e.g. pesticides, polycylic aromatic hydrocarbons, polychlorinated biphenyls), volatile organic compounds (organic acids, aldehydes, alkanes, alcohols, ketones etc.) as well as nonvolatiles, e.g. phtalates, lead, etc. [e.g. 9, 16]. Qualitative and quantitative microbial

Received: 15 November 2003
Accepted: 6 April 2004
Presented at the 1st International Scientific-Training Congress *Organic Dust Induced Pulmonary Diseases*, 10-12 Oct. 2003, Kazimierz Dolny, Poland

content of house dust samples has already been studied extensively. Regarding fungi in house dust, they reflect the mycobiota found in indoor air - alternariae, aspergilli, cladosporia, fusaria, penicillia, *Rhodotorula* sp., *Trichoderma* sp., *Wallemia* sp. [13], although, some others (*Aureobasidium* sp., ulocladia, yeasts, etc.) were isolated more frequently from dust than indoor air. House dust is composed mostly of organic material (82%), in contrast to outdoor dust that contains only 18% of organic matter, and has a nearly optimal pH for microbial growth. House dust provides sufficient nutrients, though carbon-limited, for initial growth of xerophilic fungi (eurotia, some aspergilli) even at 75–76% air relative humidity (RH) [8]. At 84–98% RH, i.e. 10% of the moisture content in the dust, the fast growth of primary fungal colonizers (aspergilli and penicillia) is supported within 3–11 days and tertiary colonizers (fusaria, yeasts) appear [9]. Fungi growing in house dust can produce a wide spectrum of volatiles - some of them perhaps species-specific, as well as mycotoxins in their metabolism [4, 11]. Fungally contaminated house dust also represents a suitable environment for fungivorous mites that are also dependent on higher humidity levels [3]. Exposure to allergens and chemicals in the home environment is an important risk factor to develop and/or exacerbate respiratory disorders, especially in allergic people and children. It has been stated that the risk to infants from pollutants in dust may be 40-times higher than in adults [16].

The aim of this study was to characterize the potential airway toxic effect of house dust, and of its mycoflora in particular, with a bioassay employing organ cultures of 1 day old chick tracheas.

MATERIALS AND METHODS

House dust. In total, 23 surface dust samples were collected in water damaged (13) and control (10) Danish schools (class rooms, offices, club and day care room, storage room) with a vacuum cleaner fitted with a special attachment (Vacumark mouthpiece, Bach-Petersen aps, Bjerringbro, Denmark). Coarse material was sieved away through sieves DIN 6 (pores 1.25 mm). The obtained fine dust samples (50–100 mg) were stored in new zip-sealed plastic bags at 4ºC prior their extractions and mycological analysis [6].

Dust mycobiota. First, direct microscopical analysis of all samples was performed (magnification 400×; Olympus BX50 by Olympus Optical Co., Ltd., Tokyo, Japan). Next, viable fungal content of dust was analysed by the dilution plate method in sterile saline with 0.5% Tween 80 using dichloran 18% glycerol agar (DG18) (Hi Media Laboratories Pvt. Ltd., Bombay, India) - a low water activity medium with emphasis on enumeration of indoor fungi [18] and cultivation at 25ºC for 8 days. Three agar plates were used for each sample. Every mould isolate was identified according to its macro- and micromorphology, and average total fungal counts were expressed in colony forming units (cfu) per g of dust.

The isolates of *Aspergillus flavus* were tested for ability to produce aflatoxin B1 *in vitro* on the liquid medium with 20% sucrose and 2% yeast extract at 25ºC after 14 days, as described in [1].

Dust extracts. Dust samples (20 mg) were twice extracted by 5 ml of 2-butanone (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) or dimethylsulphoxide (Me_2SO; Polysciences, Inc., Warrington, PA, USA) - both analytical grade, followed by vortexing for 5 min (MS2 Minishaker, IKA Works, Inc., Wilmington, NC, USA). The extracts were removed, pooled and evaporated to dryness under vacuum (Büchi 461 Water Bath, Büchi RE 121 Rotavapor, Flawil, Switzerland). The weighed residues were re-extracted with Me_2SO with a ratio of dust/solvent, 1mg/ml and stored in a medicine flask with a teflon plug at 4ºC.

Fungal metabolites. Forty-one representative fungal isolates were tested for the production of potentially toxic metabolites [14]. Moulds were grown on the liquid medium containing 2% of yeast extract and 10% of sucrose at 25ºC for 10 days.

Two types of chloroform extracts were used in the experiments:

1) Chloroform extracts of cultivation medium (exo-metabolites): After filtration of the biomass, the medium was extracted twice, the pooled extracts dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to dryness (Büchi Water Bath and Rotavapor).

2) Chloroform extracts of biomass (endometabolites): Biomass filter cakes were also extracted twice in a blender (Stomacher Lab-Blender 400, Seward Medical UAC House, London, England) for 10 min. and the dry extracts were obtained, as mentioned above. All residues were reextracted with Me_2SO as above.

Toxicity *in vitro*. The ciliostatic potential of dust extracts and of fungal chloroform-extractable exo- and endometabolites was determined by bioassay employing chicken tracheal organ cultures [7, 10]. Their toxic effects were compared with the effect of a mycotoxin sterigmatocystin (Sigma Chemical Co., St. Louis, MO, USA). 20 µg extract or mycotoxin in Me_2SO per 1 ml were added to 1-day-old chicken trachea slices (0.1 mm) in culture medium (minimal essential medium according to Eagle with Earl's salts - E-MEM - Institute for Sera and Vaccines, Prague, Czech Republic - with 1% of 3% glutamine, 2.5% of 7.5% $NaHCO_3$ with phenol red - Institute for Sera and Vaccines, 10% of bovine fetal serum, 100 µg streptomycin and 100 U penicillin). Reference media were prepared with 1% Me_2SO instead of extracts and blank with pure rich medium. The organ models were incubated at 37ºC and 5% CO_2. Ciliar movement was checked on 5–7 tracheal rings microscopically (magnification 250×; Olympus BX50) after 0, 1, 2 and 3 days. The assays were carried out in triplicate.

Table 1. Mycological analysis of house dust.

Sample origin		Direct microscopy	Viable fungal counts (cfu/g)	Fungal composition
1 - class room	P	melanized hyphal fragments, alternaria- and cladosporium-type macroconidia	296	Mycelia sterilia, *Penicillium* sp., yeasts
4 - office	P	melanized hyphal fragments, alternaria-type macroconidium	387	*Aspergillus ustus*, *Botrytis cinerea*, *Mucor* sp., *Penicillium* sp., yeasts
5 - club	P	melanized hyphal fragments and microconidium, alternaria- and cladosporium-type macroconidia	387	*A. niger* gr., *A. ustus*, *Chaetomium globosum*, *Penicillium* sp.
6 - day care	P	melanized hyphal fragments, alternaria- and cladosporium-type macroconidia	608	*A. ustus*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, *Penicillium* sp., *Rhizopus* sp.
7 - class room	P	melanized hyphal fragment, cladosporium-type macroconidium	762	*A. glaucus* gr., *Ch. globosum*, *C. cladosporioides*, *P. variotii*, *Penicillium* sp.
8 - class room	P	no fungal particles	279	*Chaetomium* sp., *Ch. globosum*, *C. cladosporioides*, *Penicillium* sp.
9 - class room	P	no fungal particles	904	*A. flavus*, *A. niger* gr., *A. ochraceus*, *C. cladosporioides*, *Mucor* sp., *Penicillium* sp., yeasts
10 - class room	P	melanized hyphal fragments, alternaria-type macroconidia	904	*Alternaria* sp., *A. flavus*, *A. niger* gr., *A. ochraceus*, *C. cladosporioides*, *Mucor* sp., *M. spinosus*, *Penicillium* sp., yeasts
11 - class room	P	alternaria- and cladosporium-type macroconidia	608	*Alternaria* sp., *A. flavus*, *A. niger* gr., *A. ochraceus*, *A. versicolor*, *Penicillium* sp., *Rhizopus* sp.
12 - class room	P	melanized hyphal fragments, alternaria- and cladosporium-type macroconidia	637	*A. niger* gr., *A. ochraceus*, *A. ustus*, *C. cladosporioides*, *Penicillium* sp., *Rhizopus* sp.
13 - class room	P	cladosporium-type macroconidia, aspergillus-type fruit body	835	*A. glaucus* gr., *A. niger* gr., *A. ochraceus*, *A. ustus* (2×), *C. cladosporioides*, mycelia sterilia, *Penicillium* sp.
14 - class room	P	alternaria- and drechslera-type macroconidia	671	*Alternaria* sp., *A. niger* gr., *A. ochraceus*, *Mucor* sp., *Penicillium* sp.
15 - storage	P	no fungal particles	722	*A. glaucus* gr., *A. niger* gr., mycelia sterilia, *Penicillium* sp. (2×), *Rhizopus* sp., *Ulocladium* sp. (2×)
2 - library	C	melanized hyphal fragment, alternaria-type macroconidia	455	*C. cladosporioides*, mycelia sterilia, *Penicillium* sp., *Rhizopus* sp., yeasts
3 - office	C	ulocladium-type macroconidia	1040	*A. flavus*, *A. fumigatus*, *A. glaucus* gr., *A. nidulans* gr., *A. niger* gr., *B. cinerea*, *C. cladosporioides* (2×), *Chaetomium globosum*., mycelia sterilia, *P. variotii*, *Penicillium* sp., yeasts
16 - class room	C	melanized hyphal fragment, drechslera-type macroconidium	671	*A. glaucus* gr., *A. niger* gr., *A. ustus*, *Mucor* sp., *Penicillium* sp.
17 - class room	C	no fungal particles	546	*Alternaria* sp., *A. niger* gr., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp.
18 - class room	C	no fungal particles	637	*A. niger* gr., *C. cladosporioides*, *Ch. globosum*, *P. variotii*, *Penicillium* sp., *Trichoderma* sp., *Ulocladium* sp.
19 - class room	C	no fungal particles	432	*A. niger* gr., *P. variotii*, *Penicillium* sp.
20 - class room	C	melanized hyphal fragments, alternaria-type macroconidia	171	*A. flavus*, *A. niger* gr., *Rhizopus* sp.
21 - class room	C	alternaria-, cladosporium- and drechslera-type macroconidia	421	*A. niger* gr., mycelia sterilia, *Neurospora crassa*, *P. variotii*, *Penicillium* sp., yeasts
22 - class room	C	melanized hyphal fragment, alternaria- and cladosporium-type macroconidia	529	*A. niger* gr., *C. cladosporioides*, *Mucor* sp., mycelia sterilia, *P. variotii*, *Penicillium* sp., yeasts
23 - class room	C	melanized hyphal fragment, alternaria-type macroconidia	637	*A. niger* gr., *C. cladosporioides*, mycelia sterilia, *P. variotii*, *Penicillium* sp.

C - control schools, cfu - colony forming units, gr. - group, P - water damaged buildings; fungal isolates used for toxicity study are underlined.

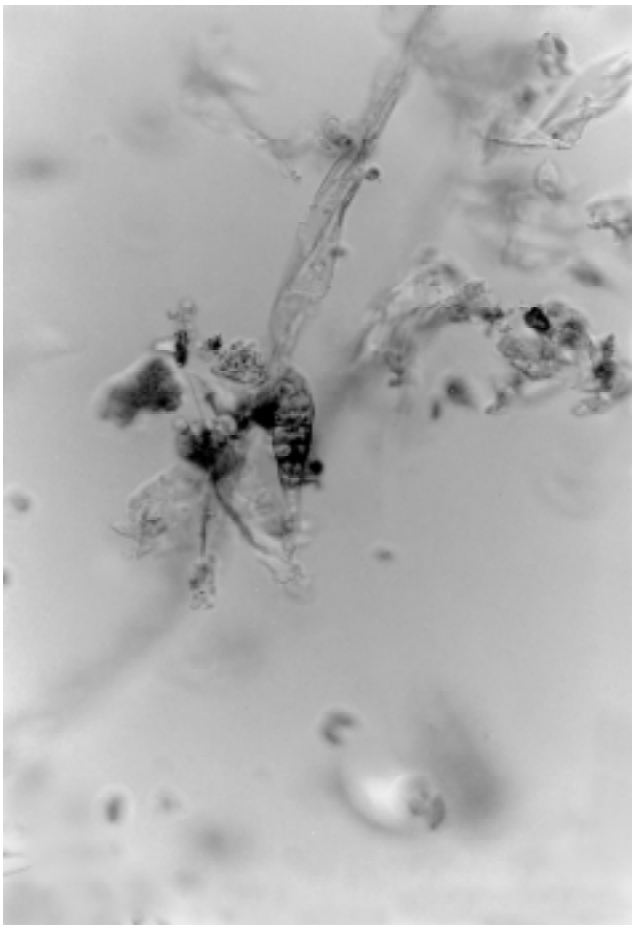

Figure 1. Dust (sample No. 10) with alternaria-type macroconidium, × 400.

Figure 2. Dust (sample No. 16) with drechslera-type macroconidium, × 400.

RESULTS

Fungal composition of dust from buildings with water damage was almost identical with that from undamaged schools. Fragments of melanized hyphae, macroconidia and fruit bodies were found microscopically in both kinds of samples, except for Nos. 8, 9, 15, 17, 18, 19 (e.g. Figs. 1, 2). It is apparent that not all fungal particles present in house dust are still viable, as the genera expected according to direct microscopy were not always actually found after cultivation of samples on DG18 medium.

Absolute viable fungal counts between 279–904 cfu/g of dust or between 171–1040 cfu/g were detected in samples taken from buildings with moisture history or the control buildings, respectively (Tab. 1). There was no statistically significant difference in the counts in damaged and control houses as calculated by 2-sided 2-samples Student's *t*-test ($p = 0.515$).

Aspergillus spp., especially from the *A. niger* group, and *Penicillium* spp. were the most prevalent fungal genera in dust from buildings with leakage (31 and 35 respectively, *A. niger* gr. 14% of all isolates) as well as controls (40 and 19, *A. niger* gr. 30%). *Cladosporium cladosporioides* represented 8 or 7%, while each fungus of *Alternaria* spp., *Chaetomium globosum*, *Mucor* spp., Mycelia sterilia, *Paecilomyces variotii*, *Rhizopus* sp., *Ulocladium* sp. and yeasts less than 8% of the isolates. *Botrytis cinerea*, *Chaetomium* sp., *Mucor spinosus*, *Neurospora crassa* and *Trichoderma* sp. were isolated only once in this study (Tab. 2). No *Aspergillus flavus* isolate (6 from damp and 2 from control schools) was aflatoxigenic *in vitro*.

The extracts of 8 control and 7 dust samples from damaged or control buildings were toxic in the bioassay. The more polar solvent Me_2SO seemed to be more efficient for extraction of compounds with airway toxicity from dust than 2-butanone: 4 control and 6 damp house origin dust samples contained toxic chemicals extractable by Me_2SO, and also 2 extracts (1 control and 1 wet) were effective immediately after 1 day of the exposure (Tab. 3).

Alternaria spp., *Aspergillus* spp., *Botrytis cinerea*, *Penicillium* spp., *C. cladosporioides*, *Chaetomium* spp. and *Ulocladium* sp., in total 88% of all fungi tested, produced ciliostatically active metabolites. These toxic strains were also present in the dust samples, the extracts of which showed certain toxicity in vitro. Endo- and exometabolites of *Aspergillus flavus*, *A. fumigatus*, *A. glaucus* gr., *A. niger* gr., *A. ochraceus*, *Botrytis cinerea*, *Cladosporium cladosporioides* and *Penicillium* spp. were able to stop cilia beating after first 24 h of the action. Other isolates of *A. niger* gr. and *Penicillium* spp., together with *Chaetomium* spp., *A. ustus* and *Ulocladium*

sp. produced strong toxic endometabolites, while anothers of *A. flavus*, *A. ochraceus*, *Chaetomium globosum* and another *Penicillium* spp. as well as *C. cladosporioides* only exometabolites that stopped movement of chick tracheal cilia in a day (Tab. 4).

Extracts of the dust samples Nos. 1, 9, 11 and 15 did not show any ciliostatic activity under experimental conditions, although the fungi present were able to produce toxic exometabolites on the liquid cultivation medium. On the other hand, dust extracts Nos. 6, 13 and 14 were toxic, but exometabolites of their fungi tested were not. Thus, not only fungal metabolites were responsible for potential toxicity of dust samples (Tab. 3, 4).

DISCUSSION

The health effects of indoor fungi are not limited to allergic diseases, but also may relate to other disorders, mainly of the type respiratory. Primary fungal colonizers (aspergilli and penicillia) belong usually to the most frequent indoor fungi in water damaged buildings [8]. These genera, together with cladosporia, also represent the most common fungal contaminants of the indoor environment in general [16]. All named fungi were also highly dominant in the culturable mycoflora detected in the present study of house dust. Total house dust fungal spectrum can be very wide, e.g. 74 species found in houses in Ryiadh, Saudi Arabia, and 113 in Northeast America, and is affected by outdoor mycoflora, composition of dust, moisture, life style of occupants, etc. Fungal distribution of house dust is supposed to be a less representative part of the indoor funga than the air-borne one [2, 15]. We identified 23 fungal species belonging to 13 genera in dust from the Danish schools. Among our isolates, some potential producers of mycotoxins (*Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. ochraceus*, *A. ustus*, *A. versicolor*, *Chaetomium* spp., *Trichoderma* sp.) and microbial volatile organic compounds (MVOC) (*A. flavus*, *A. versicolor*, *Cladosporium* spp.) were investigated as well.

Table 2. Frequency (%) of fungi in dust samples.

Fungi	Water damaged schools	Control schools
Alternaria sp.	2	2
Aspergillus spp.	31	40
• *A. flavus*	4	2
• *A. fumigatus*	0	1
• *A. glaucus* gr.	2	4
• *A. nidulans* gr.	0	1
• *A. niger* gr.	14	30
• *A. ochraceus*	6	0
• *A. ustus*	4	2
• *A. versicolor*	1	0
Botrytis cinerea	1	1
Chaetomium sp.	1	0
Ch. globosum	4	2
Cladosporium cladosporioides	8	7
Mucor sp.	3	3
M. spinosus	1	0
Mycelia sterilia	3	6
Neurospora crassa	0	1
Paecilomyces variotii	3	8
Penicillium sp.	35	19
Rhizopus sp.	4	3
Trichoderma sp.	0	1
Ulocladium sp.	2	1
Yeasts	2	6
Total	100	100

Note: see Tab. 1

Table 3. Ciliostatic activity of house dust extracts.

Solvent	2-butanone			Me_2SO		
Toxic effect (d) Sample No.	1	2	3	1	2	3
1	–	–	–	–	–	–
4	–	–	–	+	+	+
5	–	–	–	–	+	+
6	–	+	+	–	+	+
7	–	–	–	–	–	–
8	–	–	–	–	–	+
9	–	–	–	–	–	–
10	–	+	+	–	–	–
11	–	–	–	–	–	–
12	–	–	–	–	–	–
13	–	–	–	–	–	+
14	–	–	–	–	–	+
15	–	–	–	–	–	–
2*	–	+	+	–	–	+
3*	–	+	+	–	–	–
16*	–	+	+	–	–	–
17*	–	–	+	–	–	+
18*	–	–	–	–	–	–
19*	–	–	–	–	–	+
20*	–	–	+	+	+	+
21*	–	–	–	–	–	–
22*	–	–	+	–	–	–
23*	–	–	+	–	–	–

– - no toxic effect, + - ciliary movement stopped after day X, Me_2SO - dimethylsulphoxide, * - control buildings.

Table 4. Airway toxic effect of chloroform-extractable fungal metabolites *in vitro*.

Sample No., mould	Endometabolites			Exometabolites		
Ciliostatic activity (d)	1	2	3	1	2	3
1, *Penicillium* sp.	+	+	+	+	+	+
3, *Aspergillus fumigatus*, *A. glaucus* gr., *A. niger* gr., *Botrytis cinerea*, *Cladosporium cladosporioides* I	+	+	+	+	+	+
Chaetomium globosum	+	+	+	–	–	–
A. flavus, *A. nidulans* gr., *C. cladosporioides* II	–	–	+	–	–	+
4, *Penicillium* sp.	–	–	+	+	+	+
5, *Ch. globosum*	+	+	+	–	+	+
A. niger gr.	+	+	+	–	–	–
6, *Paecilomyces variotii*	–	–	–	–	–	–
7, *Ch. globosum*	+	+	+	–	–	–
P. variotii	–	–	–	–	–	–
8, *Ch. globosum*	–	+	+	+	+	+
Chaetomium sp.	+	+	+	–	–	+
C. cladosporioides	–	–	–	+	+	+
9, *A. flavus*, *A. ochraceus*	+	+	+	+	+	+
10, *A. flavus*	–	+	+	+	+	+
A. ochraceus	–	–	+	–	–	+
Alternaria sp.	–	–	–	–	–	–
11, *A. ochraceus*, *A. versicolor*	–	–	–	+	+	+
13, *A. ustus* II	+	+	+	–	–	–
A. ustus I	–	–	+	–	–	+
14, *Alternaria* sp.	+	+	+	–	–	–
15, *A. glaucus* gr., *A. niger* gr.	+	+	+	+	+	+
Penicillium sp. I	+	+	+	–	+	+
Ulocladium sp. I	+	+	+	–	–	–
Penicillium sp. II	–	–	+	–	–	–
Ulocladium sp. II	–	–	–	–	–	+
16, *A. glaucus* gr.	+	+	+	–	–	–
A. ustus	–	–	–	–	+	+
17, *Alternaria* sp.	+	+	+	–	–	+
18, *Ulocladium* sp.	+	+	+	–	–	–
P. variotii	–	–	–	–	–	+
Ch. globosum	–	–	–	–	–	–

Note: see Tabs. 1 and 3.

It was shown that house dust contains sufficient nutrients to support fungal growth and metabolism, including a secondary one [9]. The mycotoxin sterigmatocystin, produced by *A. versicolor*, was detected in low concentrations (2–4 ng/g of dust) in carpet dust, and its MVOC (alcohols, ketones and furans) could also have been released from dust growths [4, 11]. The effects of inhalation of mycotoxins and fungal volatiles have not yet been clearly elucidated. However, previously, sterigmatocystin, and chloroform-extractable exo- and endometabolites of *Aspergillus* spp., *Penicillium* spp., *Trichoderma* sp., *Alternaria* spp., *Chaetomium* spp., etc., were found to have very potent ciliostatic effects in the experiments with chicken tracheas [7, 12, 14]. Metabolites of *Penicillium* spp., non-aflatoxigenic *A. flavus*, *A. fumigatus*, *A. glaucus* gr., A. *niger* gr., *A. ochraceus* and *Botrytis cinerea* isolated from house dust tested, showed the highest toxicity *in vitro* in this investigation.

The *in vitro* toxic effect of dust chemical constituents was clear in both kinds of samples - of mouldy or control building origin. Since there were non-toxic samples which contained fungi able to produce ciliostatic metabolites (Nos. 1, 9, 11, 15), or, on the other hand, toxic ones with non-toxic mycoflora (No. 6, 13, 14), the toxic potential of the dust could not be explained solely by the fungal products extracted here. The adverse airway effects of other fungal metabolites: extracellular polysaccharides, ergosterol or its derivatives, β(1→3)-glucans or lipopolysaccharides in house dust, should be also considered when evaluating the toxic potential of this kind of organic dust [3, 5, 17].

In this study, it was found that there are bio-detectable concentrations of toxic compounds in house dust. However, the contribution of fungal metabolites to its toxic effects should be studied in greater detail.

Aknowledgment

Thanks are due to RNDr. L. Wsólová from the Slovak Medical University for the statistical analysis of quantitative experimental data.

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