E. Sources & exposure
E.1 Source control

INDEX OF INDOOR AIRBORNE FUNGAL SPORES POLLUTION IN BRUSSELS HABITAT

Chasseur C.[1] , Bladt S.[2], Wanlin M.[3]

[1]Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium
[2]Brussels Institute for the Management of the Environment, Brussels, Belgium
[3]Fonds des Affections Respiratoires ASBL, Brussels, Belgium

[*]Corresponding email: Camille.chasseur@wiv-isp.be

Keywords : Habitat, Indoor Airborne Moulds, Green Ambulance

SUMMARY

The abnormal presence of any mould in any location within a building, presents a health risk, especially for young children and asthmatic people. In Brussels, the Regional Unit for Indoor Pollution Intervention assists the general practitioner in his diagnosis when there is a suspected link between his patients health and his home. For moulds, the most evident and easiest evaluation tool consists in a visual inspection. But hidden contaminations are often present and require air sampling methods. An index of indoor pollution with airborne fungal spores has been developed on the basis of over 1600 surveys in dwellings. This index is based on several airborne concentrations percentiles obtained for some specific moulds. In Brussels, the best represented airborne moulds in the visited dwellings were *Penicilium spp., Aspergillus versicolor* and *Cladosporium sphaerospermum*. These 3 taxa are the basis of the index. But when other specific toxigenic moulds are present, they are taken into account.
In conclusion, this index helps to decide when a measured airborne fungal concentration may be considered as abnormal. The index provides additional information in order to improve the environmental analyses, and is particularly useful when the presence of moulds is suspected but when they remain invisible.

INTRODUCTION

Concerning biological contaminants in damp dwellings, moulds represent in terms of frequency and growth, a factor that cannot be underestimated. It has been demonstrated that moulds are potent sources of a large variety of allergens (*Mazur, 2006; Simon-Nobbe, 2007; Crameri, 2014*). Non immune effects are also noted such as irritant or toxic symptoms (*Mazur, 2006*). Epidemiological studies (*Zureik, 2002 ; Fisk, 2010 , Tischer, 2011, Mendell, 2011; Sharpe, 2015*) support the significant link between mould exposure and certain pathologies, generally respiratory such as an increase of asthma, dyspnea, wheezing, cough, or respiratory infections like rhinitis and bronchitis. Among affected populations, children are particularly at risk : wheeze and persistent cough in the first year of life (*Belanger, 2003)* and asthma development (*Rosembaum, 2010; Chen, 2011)* are mainly mentioned.
In our areas, moisture excess in buildings is frequent and is responsible for mould

developments. More than one hundred species of moulds were listed on water damaged building materials. The most frequent species identified in damp dwellings are *Cladosporium sphaerospermum*, *Aspergillus versicolor*, several species of *Penicillium*, followed by *Alternaria spp. Chaetomium spp.* and *Stachybotrys chartarum* (*Reboux, 2009*).
But outdoor air is also an important source of natural airborne fungal spores, and it should be taken into account during a fungal survey. Some of them present an important seasonal character and may be collected in high concentrations. It is mainly the case for three allergenic moulds, *Alternaria alternata* (June-September), *Cladosporium herbarum* and *C. cladosporioides* (May-October).
In addition to the natural airborne moulds, specific contaminations may be produced by human activities. It is the case during the demolition of an old building or due to some nearby activities such as garbage dump, public waste sorting, industrial composting centre, and so on. The proximity of these activities could influence the outdoor and indoor quality in the near surroundings (*Heer, 2003; Albrecht, 2008*).
In Brussels, the Regional Unit for Indoor Pollution Intervention (French acronym CRIPI) provides assistance to medical diagnosis when a physician suspects a link between a health problem and the patient's habitat. A set of chemical and biological samples is collected from the patient's home on the basis of a standard protocol (*Bladt, 2010; Chasseur, 2014*). These samples are then analysed, identified and quantified, as well as their potential sources. This environmental diagnosis is completed by recommendations and advices to residents in order to reduce or even eliminate the nuisances (*Bladt, 2008*). But for air, results are not easily interpretable because there are no health based standards on the acceptable number of micro-organisms in the indoor environment and there are no international regulations for airborne concentrations of moulds or mould spores. But in practice, threshold values may help, especially in cases with weak detected contaminations.
Concerning surveys in Brussels, an index of indoor airborne fungal spores was developed on the basis of percentiles calculated for the most relevant mould species. This index permits to compare the results obtained during a survey with those obtained from a large panel of similar environments. It is a reference to detect abnormal specific air composition compared with a panel of 1600 dwellings. Of course, these anomalies are not automatically linked to health, and require further medical investigations. In the case of CRIPI, they are given to the physician as new clues for the medical diagnosis.

METHODOLOGY

The CRIPI enquiries were conducted in dwellings in the 19 administrative districts of the Brussels-Capital Region, both in poor and wealthy neighborhoods. Each investigation included three parts: the completion of a questionnaire by the inhabitant, preferably the patient; chemical and biological sampling of the habitat.
Concerning the fungal parameters, air samplings are made in various rooms and outdoors, with an RCS+ (Biotest®, 80 liters for mesophilic moulds, 160 liters for thermophilic moulds). Strips filled in our laboratory with HS agar media are used and incubated at 25°C during 5 days to isolate and identify mesophilic and hydrophilic moulds, and at 45°C during 2 days to isolate and identify thermophilic moulds (*Beguin, 1994*). An outdoor air sampling is always taken to assess the possible outdoor airborne fungal flora (natural or accidental contaminants) and to distinguish them from possible indoor contaminants.

These analyses are systematically carried out in the main rooms of the dwelling (living, kitchen, bathroom and bedrooms). Other rooms can be taken into account depending on the case, the cellar for instance, often source of moisture, or the attic, often significant source of dust.
Specific percentiles were calculated (XLSTAT) from a total of 5219 indoor and 1342 outdoor air samples. The lower limits of analytical determination (LoD) were 12.50 CFU/m^3 and 6.75 CFU/m^3 for mesophilic fungi and thermophilic fungi respectively. Threshold limits or percentiles were rounded to the nearest integer. Because of technical considerations of the RCS+ and the sampled air volume, the highest values recorded for the mesophilic species were restricted to 204 CFU/strip (2550 CFU/m^3). This limit value was also used to determine the cases with confluence of colonies.

RESULTS AND DISCUSSION

Indoor and outdoor moulds

The percentage of samples with the presence of hydrophilic mesophilic and thermophilic moulds species was calculated from 5219 indoor air samples and from the 1342 outdoor air sampled during each survey to serve as daily reference. On figure 1, we note that four moulds presented a strong outdoor origin: *Cladosporium herbarum* (Pers.) Link with 76.3% outdoors versus only 37.8 % indoors, *Aspergillus fumigatus* Fresen. with respectively 39.3% vs 19.0%, *Alternaria spp.* Nees: Fr., with respectively 14.8% vs 5.0%, and *Botrytis cinerea* Pers. with respectively 10.9% vs 3.3%. Sterile mycelia on agar cultures also present a strong outdoor origin (figure 1). Among abundant outdoors species, some important seasonal variations are well known, *Cladosporium herbarum* and *Alternaria alternata* (Fr.) Keissl for instance (*Belgian aerobiological surveillance network*).

On the other hand, two moulds presented a strong indoor origin : *Aspergillus versicolor* (Vuil.) Tiraboshi, with 39.5% indoors vs only 17.4 % outdoors, and *Cladosporium sphaerospermum* Penz, with 22.4% indoors vs only 12.0% outdoors. Less frequent, the two cellulolytic moulds *Stachybotrys chartarum* (Ehrenb.) S. Hughes, with respectively 3.3% vs 1.7 % and *Chaetomium spp.* Kunz, with respectively 3.0% vs 1.0%, were among species with higher indoor records (figure 1).

Penicillium Link is a genus present indoors (on wet building materials, moist fabrics or leather, moulded fruits, cheese, …) as well as outdoors (anthropogenic sources close to the dwelling like demolition site for instance); this requires a certain reserve about its airborne origin (figure 1). Particularly for this taxon, outside air sampling during the survey is useful.

Concerning the most frequent indoor fungal contaminants (*A. versicolor*, *C. sphaerospermum* and *Penicillium spp*.), significant seasonal outdoor variations are not observed, and consequently the same trends are noted indoors (figure 2). Moreover, for these taxa, the measured airborne background is higher inside than outside, and a larger number of high contaminations is noted indoors, whatever the season.

So, for airborne moulds, result interpretations inside a damp dwelling show that the total indoor airborne fungal load does not necessarily reflect an indoor contamination due to damp problems. For this type of control conducted by the “Green ambulances” such as CRIPI, results need to be focused on the specificity.

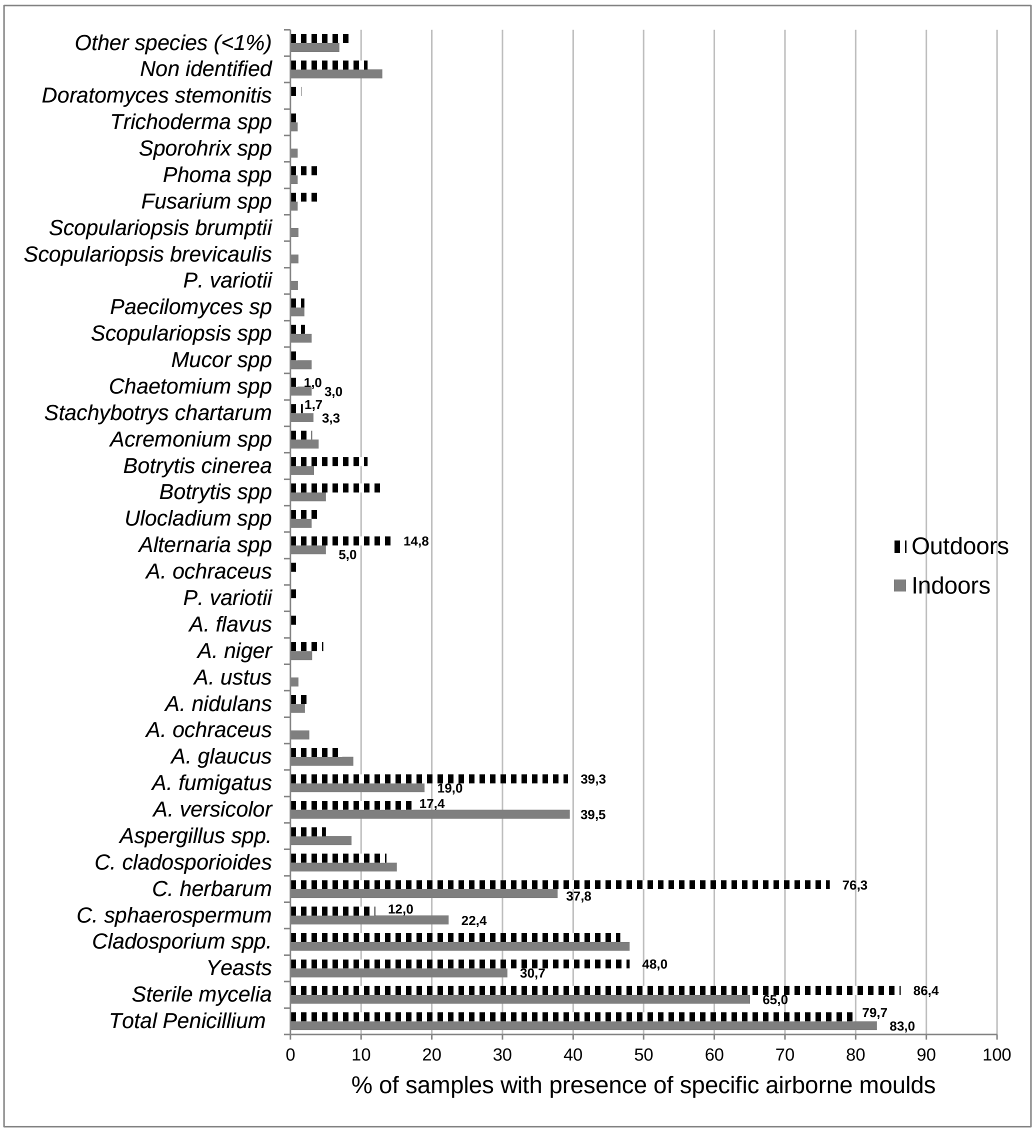

Figure 1: the most frequent airborne moulds sampled indoors during surveys in dwellings in Brussels, on the basis of 5219 samples from indoors, and 1342 samples from outdoors.

Our results show how relevant it is to consider in priority the 3 following taxa: *Aspergillus versicolor, Cladosporium sphaerospermum,* and *Penicillium* spp. In addition, two strong cellulolytic taxa, *Stachybotrys chartarum, and Chaetomium* spp.,. are less frequent in Brussels dwellings but their indoor impact is important.

Quantitative aspect of specific indoor moulds airborne contamination

Looking at percentiles scales of the 5 main taxa able to amplify in damp indoor environments, the most adapted percentiles corresponding to a level of contamination were selected. The main outdoor species were also examined and the other species were treated as a whole (table 1).

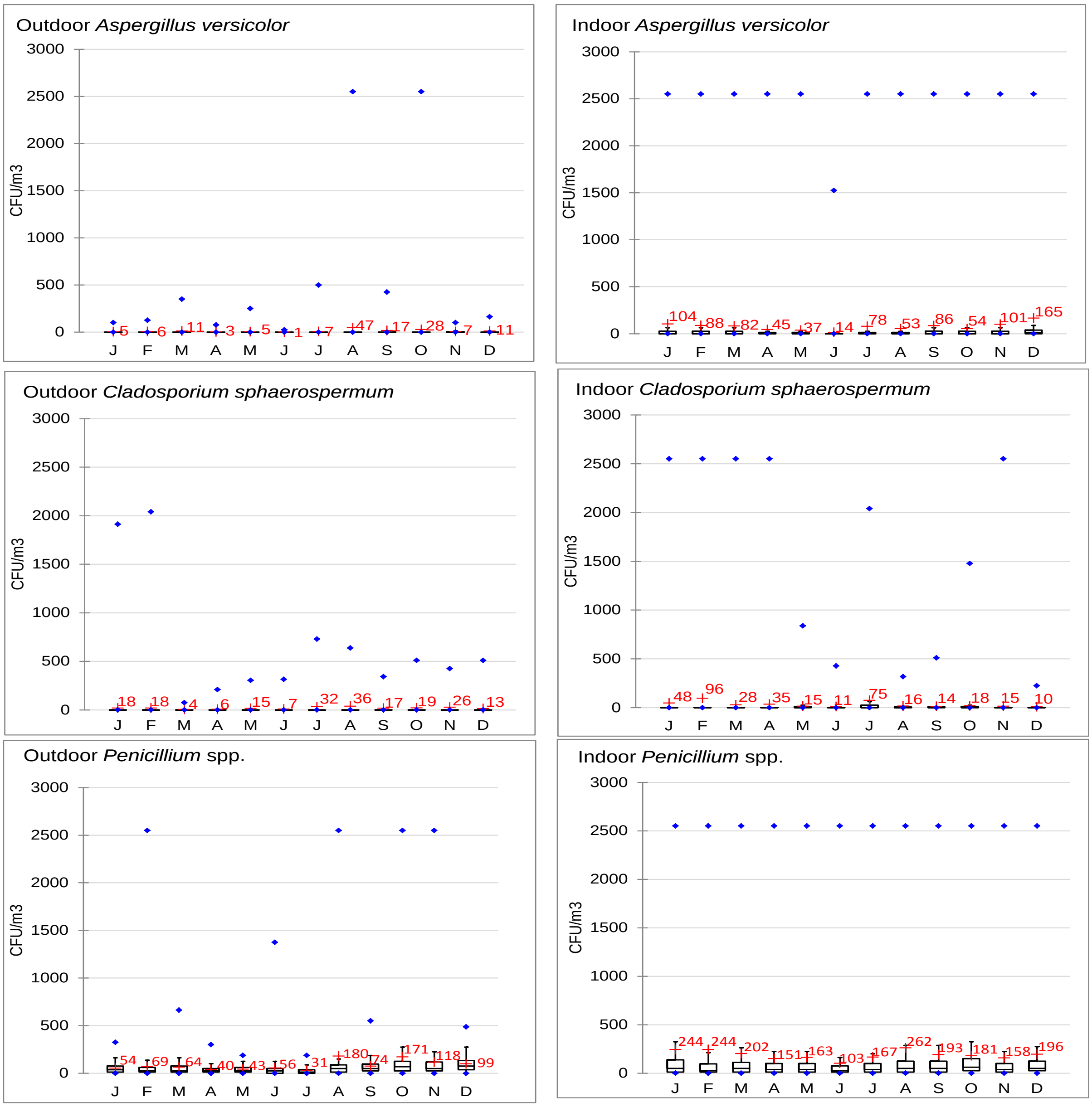

Figure 2 : outdoor and indoor monthly airborne concentrations for A. versicolor, C. sphaerospermum and Penicillium spp.. (+: mean; ◆: maximum measured)

For the 3 most frequent taxa of damp origin, the concentration may be considered as satisfactory until percentiles 75. Between C80-85 the situation changes, and may be considered as average. From percentiles 90, a rapid increase of concentrations is observed, and becomes an alert. For 10% of the surveys, the situation was very abnormal and should be considered as unacceptable. We also noted that concentrations corresponding to these percentiles varied according to the taxa. *A. versicolor* and *C. sphaerospermum*, had rather similar concentrations, optimal at 0-25 CFU/m^3 and very abnormal above 88-200 CFU/m^3. By contrast and with similar reasoning, for *Penicillium*, the contamination level is higher, optimal until 113CFU/m^3 and very abnormal only above 838 CFU/m^3.

For less frequent species but with a high potential to develop on indoor damp papers, the situation was optimal for 95% of visited dwellings (with 0 CFU/m^3) and should be considered as abnormal above 13-25 CFU/m^3 .

	A. versicolor	*C. sphaerospermum*	*Penicillium spp.*	*Chaettomium spp.*	*S. chartarum*	*C. herbarum*	*Alternaria spp.*	*Sterile mycelia*	*A. fumigatus*	*Yeasts*	*Others*
% Outdoors	17	12	80	1	2	76	15	86	39	48	/
% Indoors	40	22	83	3	3	38	5	65	19	31	/
	Frequent indoor and high amplification potential on damp building materials		Frequent indoor and high amplification potential on various substrate	Less frequent but with high amplification potentiel on cellulose		Important natural outdoor origin, with important seasonal variations (highest in June-September)		Fungus not able to sporulate on synthetic media. Often epiphytic fungus	Important natural or anthropogenic outdoor origin.	Epiphytes for the most part, but also some dermatophytes, intestinal or drug related	Special indoor contamination or anthropogenic one
Main substrate	Plaster, wood, tissue		Plaster, wood, tissue, leather, but also fruits, cheese, ...	Damp paper (wallpaper, books, archives, cardboards box, ...)		Plants		Plants for the most part	Garden soil and potting, compost, natural humus	Plants for the most part, but also diverse origins	/
T°C isolation	25	25	25	25	25	25	25	25	45	25	25
LoD (CFU/m3)	12,50	12,50	12,50	12,50	12,50	12,50	12,50	12,50	6,75	12,50	12,50
P5	0	0	0	0	0	0	0	0	0	0	0
P25	0	0	13	0	0	0	0	0	0	0	0
P50	0	0	38	0	0	0	0	13	0	0	0
P75	25	0	113	0	0	76	0	50	7	13	0
P80	25	13	150	0	0	114	0	63	7	13	0
P85	38	25	225	0	0	114	0	75	7	25	0
P90	75	38	338	0	0	152	0	100	14	38	13
P95	200	88	838	0	0	189	13	138	20	63	13
P99	2550	765	2550	13	25	303	25	288	47	250	75
MAX	2550	2550	2550	263	713	682	100	1963	270	2463	1913

Table 1: indoor percentiles for airborne concentrations of main taxa able to amplify in damp dwellings, main outdoor taxa and other species

Concerning *C. herbarum* and *Alternaria spp.*, their presence indoors could influence the results during the season with high airborne spore production in nature (June-September). Mainly of oudoor origin, these 2 taxa should be excluded from the interpretation of indoor data.

Aspergillus fumigatus is also essentially from natural outdoor source. This thermophilic species is involved in the processes of organic matter degradation and humification (litter, soil), processes with high increase of temperature. This species is also produced during industrial human activities, such as compost production. Some authors (*Albrecht, 2008*), noted abnormal airborne concentrations of this mould and of other thermotolerant bacteria, 600-1400m away from the production site, with up to 1–2 orders of magnitude higher compared with natural background levels. So, despite its mainly outdoor origin, airborne concentrations of 14-20 CFU/m^3 should alert us due to possible indoor sources (potting soil) and possible anthropogenic sources in the vicinity of the dwelling. Moreover, thermophilic fungi such as *A. fumigatus* can also be used as indicator organisms.

Yeasts are frequent in nature and their presence indoors may originate from various sources: outdoor epiphyte species, species involved in food spoilage or used in food processing industries (maturing of certain cheeses), and human sources such as skin episaprophytes species, species naturally hosted in the gastrointestinal tracts or used against childhood diarrhea (Enterol), and so on. When total airborne concentrations exceed 38-63 CFU/m^3, identification is recommended up to species level.

Others species are rare and so, their presence above 25 CFU/m^3 should alert us.

The index of indoor airborne fungal spores pollution used by CRIPI in Brussels habitat, is presented in the figure 3.

	0-P75	P75-P85	P85-P90	P90-P95	P95-P99	>P99
A. versicolor	0-25	25-38	38-75	75-200	200-2550	>2550
C. sphaerospermum	0	0-25	25-38	38-88	88-765	>765
Penicillium spp.	0-113	113-225	225-338	338-838	838-2550	>2550
Chaetomium spp.	0	0	0	0	0-13	>13
S. chartarum	0	0	0	0	0-25	>25
C. herbarum, C. cladosporioides, Alternaria	Not to be taken into account					
A. fumigatus	0-7	7	7-14	14-20	20-47	>47
Sterile mycelia	0-50	50-75	75-100	100-138	138-288	>288
Yeasts	0-13	13-25	25-38	38-63	63-250	>250
Other species	0	0	0	13	13-75	>75
	Satisfactory	Average	Bad	Alert	Unacceptable	

Figure 3: index of indoor airborne fungal spores pollution used by CRIPI in Brussels habitat

CONCLUSION

An index of this type can be very useful for surveys of dwellings, however one has to take in mind some important rules when applying it.
Firstly, it is important to respect the sampling protocol.
Secondly, as 'the total mould contamination' is an insufficient criterion, this index is based on the presence of different mould taxa present in the measured spore concentrations.
Thirdly, it is recommended to sample in several rooms of the concerned dwelling. When a concentration gradient of spores is observed, this is of great importance. A concentration gradient may point to the presence of hidden mould sources or to accidental and momentary mould sources as known in the case of *Penicillium spp*. (contaminated fruit, …). In this case, a second survey is requires a few weeks later.
Fourthly, it is also crucial to compare indoor and outdoor spore data to account for any natural or anthropogenic source close to the concerned dwelling.
Finally, during surveys of dwellings, air sampling of mould spores can provide useful information. However, due to the air instability these samplings always need to be accompanied by the analysis of other matrices such as settled dust on furniture and other horizontal surfaces or accumulated dust in carpets, armchairs and mattresses. This index is always used by CRIPI in their final microbial diagnosis that is sent to the general practitioner.

ACKNOWLEDGMENTS

This project is funded through the Minister of Environment of the Brussels-Capital

Region. Our gratitude is also extended to the members of the team in the field Céline Liebens, François Beaujean and Sylvie Vanderslagmolen, and to the team of the laboratory of Health and Environment of Institute of Public Health in Brussels (IPH), Danny Phillips, Pascal Kanyandekwe and Hakima El Khabbabi.

REFERENCES

Albrecht A et al (2008) Recommendations for study design and sampling strategies for airborne microorganisms, MVOC and odours in the surrounding of composting facilities. Int J Hyg Environ Health, 211, 121-131

Beguin H, Nolard, N. (1994) Mould biodiversity in homes I. Air and surface analysis of 130 dwellings. Aerobiologia, 10, 157-166

Belanger K, Beckett W, Triche E et al (2003) Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. Am J Epidemiol;158, 195–202

Bladt S, Lenelle Y, Bouland C et al. (2010). Index of indoor air chemical pollution in Brussels habitat. Forum geographic, 9, 93-96

Bladt et al. (2008) Interventions and actions against damp and mould, Report on a WHO working group meeting, 28-29 February 2008, Boon, Germany

Chasseur C, Bladt S, Wanlin M (2014) Twenty years of WIV-ISP involvement in indoor environmental health. WIV-ISP Scientific report

Chen YC, Tsai CH, Lee YL (2011) Early-life indoor environmental exposures increase the risk of childhood asthma. Int J Hyg Environ Health, 215, 1, 19-25.

Crameri R, Garbani M, Rhyner C et al (2014) Fungi: the neglected allergenic sources. Allergy, 69, 176-185

Fisk W, Lei-Gomez, Q., Mendell, M.J (2010) Association of residential dampness and mold with respiratory tract infections and bronchitis: a meta-analysis. Environmental Health, 9, 72

Heer CEW (2003) – Effect of bioaerosol polluted outdoor air on airways of residents: a cross sectional study. Occup. Environ Med 2003, 60, 336-342

Mazur LJ, Kim J. (2006) Spectrum of non infectious health effects from molds. Pediatrics, 118, 6, 1909-1926

Mendell MJ, Mirer AG, Cheung K et al (2011) Respiratory and allergic healthy effects of dampness, mold, and dampness-relatied agents : a review of the epidemiologic evidence. Envir Health Perspect, 119(6), 748-56

Rosembaum PF, Crawford JA, Anagnost SE et al. (2010) Indoor airborne fungi and wheeze in the first year of life among a cohort of infants at risk for asthma. Journal of Exposure Science and Environmental Epidemiology, 20, 503-515

Reboux G, Bellanger AP, Roussel S et al (2009) Indoor mold concentration in Eastern France. Indoor Air; 19, 446–453

Sharpe et al (2015) Indoor fungal diversity and asthma: a meta-analysis and systematic review of risk factors. J Allergy Clin Immunol, 135 (1), 110-122

Simon-Nobbe B, Ursula D, Verena P et al (2008) The Spectrum of Fungal Allergy. Int Arch Allergy Immunol, 145, 58–86

Tischer C, Chen, C.M., Heinrich, J. (2011) Association between Domestic Mould and Mould Components, and Asthma and Allergy in Children: A Systematic Review. Eur Respir J., 38, 4, 812-824

Zureik M (2002) Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey, BMJ. 24, 325, 1-7