

Key words

Moulds • Spores • Asthma • Rhinitis • COPD • Allergens • mVOC

Indoor exposition to molds and health outcome

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Summary

Molds are ubiquitous organisms that are organized to form a body called the mycelium, which spread spores. Mold spores contain a variety of chemical substances (allergens, mycotoxins, volatile organic components (VOCm)...) which main health effects include allergic reactions, irritation phenomena, toxic phenomena, infections (such as fungal skin infections, pulmonary infections ...). Epidemiological studies have indicated that in Europe and in the rest of the world up to 50% of homes are contaminated by molds and have confirmed that exposure to molds is associated with a significantly increased prevalence of rhinitis, asthma, COPD, and lung function reduction. The eviction of the mold and hence the prevention of mold-related health effects is possible. Climate change will increase moisture and mold. Appropriate mitigation and adaptation to limit exposure to molds of human beings should be developed.

Introduction

Molds and fungi are natural elements of the environment that play an essential role in the decomposition of leaves, trees and debris. These microorganisms can enter the indoor environments directly or in the form of spores suspended in the air and contaminating them. Excessive mold constitutes a major health risk, which has only recently begun to be quantified.

Characteristics and types of molds in indoor environment

Molds are ubiquitous microorganisms in any indoor or outdoor air, soil, water, and on any surface. Molds are organized to form a body, named mycelium, that can produce spores, elements of spherical shape and very small size very similar to pollen, which are dispersed in the air. It a naturalist from Florence, Pier Antonio Micheli (1679-1737), a botanist at the court of Grand Duke Cosimo II de Medici, who first discovered that fungi reproduce by spores, thus repudiating the theory of "spontaneous generation of fungi" widespread in scientific circles of his time.

A mold mycelium develops on any material containing water in the amount that meets its needs. The spores of mold enclose a variety of chemical substances, mainly including allergens, mycotoxins and volatile components. The list of fungal allergens is very long

(<http://www.allergome.org>) and is not exhaustive. The fungal allergens generally have a high molecular weight. Mycotoxins are secondary metabolites of molds low molecular weight (between 200 and 10,000 daltons) that are secreted only by certain molds. Many molds release, as a byproduct of the metabolism, the microbial compounds called volatile organic compounds (mVOC). It is the latter that is responsible for the odor that characterizes the mold. The Center for Disease Control and Prevention (CDC) of the United States of America indicates that all molds are potentially dangerous to health. Data in the literature indicate that the molds most commonly found in the indoor environment and therefore potentially more dangerous to the health of individuals who live there are: *Aspergillus sp.*, *Penicillium sp.*, *Fusarium sp.*, *Trichoderma sp.*, *Memnoniella sp.*, *Cladosporium sp.*, *Alternaria sp.*, and *Stachybotrys chartarum* (also called *Stachybotrys atra*) a potentially very toxic mold.

Identification of the presence of molds and determination of the spores

The identification of the presence of molds can be subjective or objective.

The presence of mold in the environment is perceived by the human eye when they are organized to form the mycelium, which

looks like a patina. For this reason, in population studies molds indoor environments were initially identified by the response to a standardized questionnaire on the presence of moisture and/or brown spots, mold, water leaks ... on the walls of the house in general. It is in this case a subjective identification of the mold that has been shown to be inappropriate when the molds are not visible (because hidden behind a cupboard for example). The objective identification of spores allows the quantification and speciation of the fungal species and can be done in several ways. In some rare epidemiological study, the mycelium was collected on tape and placed in culture for species identification. The concentration of fungal spores dispersed in air was carried out with the same methodology used for the pollen. The spores are captured by a dedicated sampler, transferred onto a glass slide, recognized and counted under a microscope. The concentration of fungal spores was also recovered on the ground by means of suction of the powder in which the spores are recognized and counted under a microscope. This method also allows to extract the fungal DNA. More recently, fungal contamination was assessed using the electrostatic wipes stored for extended periods (10 weeks on average) in the contaminated areas, a collection that allows to quantify, for Q-PCR, up to 40 fungal species. The quantification of molds in closed rooms was also conducted through the dosage of metabolites of molds such as ergosterol, the β -glycans and the extra-cellular polysaccharides. These metabolites do not allow us to characterize the fungal species and have been used only as a "proxy" (surrogate) of fungal contamination. More recently, the measurement of microbial VOC (mVOC) specific mold has allowed to identify the presence of mold not visible¹. Even this method allows us to characterize the fungal species.

It is important to note that the identification of the presence of molds and the determination of the spores carried in population studies may not be representative of the exposure of individuals to the real mold because performed only *una tantum*. No study has tested the stability and variability of the measures over time. Moreover, the study of the impact of health of the molds should take into account that individuals may be exposed to mold in other environments, indoors as well as outdoors at certain seasons of the year.

Percentage of domestic exposure to molds

Population studies show that mold can contaminate up to 50% of principal residences in Europe and the rest of the world (Fig. 1). These same studies show that the contamination depends on climatic conditions, mold growth being a function of humidity and temperature, and social factors. Recent data from

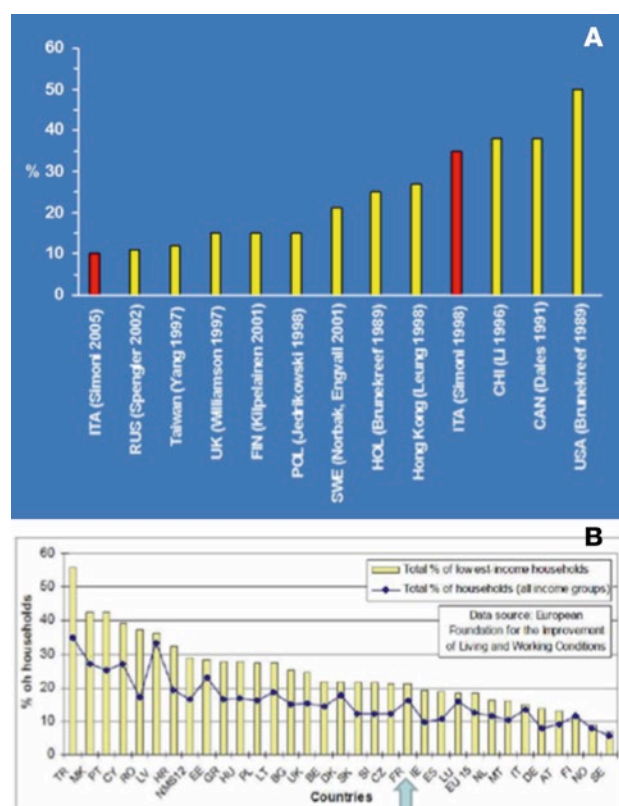


Fig. 1. Domestic exposure to molds in Europe and in the worlds (A) and according to social class (B).

WHO have shown that the European homes of the poorest social classes are more contaminated by molds than those of the rest of the population (http://www.euro.who.int/__data/assets/pdf_file/0017/43325/E92645.pdf).

Effects on health of molds

Molds are environmental pollutants that multiplying and spreading in the air can come into contact with skin, eyes, nose, or mouth. The spores of molds incorporate within them a variety of chemical substances responsible for heterogeneous effects on human health. Among the most documented health effects at the level of the general population are asthma and allergies.

Effects of fungal allergens

Molds are responsible for the aggravation of allergic symptoms but also for their development through the allergen sensitization. In terms of acute effects, the mold spores that are dispersed in the air can cause seasonal or perennial allergies, mostly

inside the houses. The reactions occur because of an allergic-type inflammation (release of histamine and numerous other mediators in response to the allergen). The generally more important mold as a cause of allergy in our regions is *Alternaria*, followed by *Cladosporium*, *Aspergillus*, *Penicillium* and *Epidermophyton*. It is estimated that 8-10% of allergic subjects have positive skin tests and/or IgE antibodies to one or more of these fungi. At the population level, the prevalence of sensitization to *Alternaria* does not exceed 3%. The monosensitization to *Alternaria* is less than 1%². *Alternaria* is present in the air for most of the year, but reaches effective concentrations to cause symptoms especially at the end of the spring, summer and autumn (September-October). *Aspergillus* is a genus that includes species of ubiquitous fungi, some of which may develop even at temperatures above 50°. The highest concentrations are found especially in the fall and winter months. Sensitization and allergy to *Aspergillus* are relatively infrequent. *Cladosporium* is present in very high concentrations in the air, even many thousands of spores per cubic meter in the late spring and summer. However, sensitization to spores of this fungus is rare. At the level of the general population, a recent meta-analysis² showed that the humidity and the presence of a mold are significantly statistically associated (odds-ratio (OR) > 1) with the development, aggravation and the diagnosis of asthma, wheezing, cough, allergic rhinitis and eczema in allergic individuals as well as in non-allergic individuals (Table I). The causal role of molds is strongly suggested in the case of childhood asthma. However

Table I. Impact of moisture and mold on asthma and allergies³.

<i>Symptom or disease</i>	<i>Study design</i>	<i>No of studies</i>	<i>Odds-Ratio range</i>
Development of asthma	Prospective	4	0.65-7.08
	Retrospective	2	0.63-4.12
	Cross-sectional	3	1.6-2.2
Symptoms in asthmatics (exacerbation)	Intervention	3	-
	Prospective	1	3.8-7.6
	Retrospective	0	1.5-4.9
	Cross-sectional	4	1.0-7.6
Asthma in the life	Prospective	2	1.2-1.3
	Cross-sectional	18	0.6-2.6
Current asthma at the time of the survey	Prospective	1	-
	Cross-sectional	25	0.3-13.0
Dyspnea	Intervention	1	No ORs
	Cross-sectional	11	0.4-9.4
Wheezing	Intervention	1	-
	Prospective	12	0.68-6.17
	Retrospective	1	1.5-2.8
	Cross-sectional	41	0.44-8.67
Bronchitis	Prospective	1	0.7-3.8
	Cross-sectional	11	1.2-2.4
Impaired respiratory function	Intervention	2	-
	Prospective	2	-
	Retrospective	1	-
	Cross-sectional	6	-
Cough	Prospective	2	0.54-2.14
	Retrospective	0	1.18-1.90
	Cross-sectional	26	0.21-5.74
Respiratory infections and otitis media	Prospective	5	0.45-5.1
	Cross-sectional	13	0.48-3.14
Common cold	Prospective	1	0.6-1.8
	Cross-sectional	5	0.98-1.7
Eczema	Prospective	2	1.2-2.9
	Cross-sectional	4	0.3-1.9
Allergy/atopy (except allergic rhinitis and eczema)	Prospective	7	0.6-2.4
	Cross-sectional	15	1.1-1.9
Allergic rhinitis	Prospective	2	1.2-3.2
	Cross-sectional	3	0.7-3.5
	Intervention	1	-
Upper airway symptoms	Prospective	5	1.03-3.2
	Retrospective	1	1.0-1.3
	Cross-sectional	20	0.37-5.92
Other respiratory symptoms	Prospective	5	1.03-1.06
	Cross-sectional	13	0.45-2.4
Total number of studies		103	

Table II. Impact of quantitative measures of molds on asthma and allergies ³.

<i>Quantitative measures</i>	<i>Symptoms or diseases</i>	<i>Studies with positive associations</i>	<i>Studies with negative associations</i>	<i>No. of studies</i>	<i>Odds-ratio Range</i>	<i>References</i>
Ergosterol in the dust, high levels	Current asthma	5 of 6 (83%)		3	0,92-4-fold	Dharmage et al. 2001; Matheson et al. 2005; Park et al. 2008
Endotoxins in the dust, high levels	Wheezing	20 of 25 (80%)		14	0,67-2,8	Iossifova et al. 2007, 2009; Park et al. 2001, 2006; Zhao et al. 2008; Schram-Bijkerk et al. 2005; Bolte et al. 2003; Campo et al. 2006; Douwes et al. 2006; Gehring et al. 2008; Gillespie et al. 2006; Litonjua et al. 2002; Perzanowski et al. 2006
(1→3)-β-D-glucans in the dust, average levels	Wheezing	7 of 8 (88%)		3	0,89-6,05	Douwes et al. 2006; Iossifova et al. 2007, 2009
(1→3)-β-D-glucans in the dust, high levels	Wheezing		10 of 11 (91%)	4	0-1,25	Douwes et al. 2006; Iossifova et al. 2007, 2009; Schram-Bijkerk et al. 2005
Total number of studies				24		

the objective measurement of fungal contamination gave contradictory results (Table II). In particular, studies of farmers found that early exposure to mold was inversely associated with the development of allergic diseases (the so-called "Hygiene hypothesis"). Another way to investigate the impact of mold on asthma and allergies consisted in assessing the relationship between allergic sensitization to molds and these conditions. There are data showing that allergic sensitization to molds is a risk factor for the development and severity of asthma. Sensitization is highly dependent on geographical and environmental conditions of exposure to mold allergens. Hereditary factors may also contribute to sensitization, but to date only a few studies have highlighted the role of genetic variants in the sensitization to molds. In the French study on the Genetics and Environment in asthma (EGEA), the combined effect of integrin genes of β-3 (ITGB3) and Toll-like receptor 2 (TLR2) increased the susceptibility to sensitization to the mold *Aspergillus herbarum*, *Cladosporium*, *Alternaria alternata* in 1243 adults with asthma. These results suggest an important role of the integrin/TLR2 complex in the innate immune response to fungal agents.

Effects of other chemical substances contained in molds

INFLAMMATION AND INFECTIONS

The meta-analysis of Mendell has also indicated that moisture and mold are significantly associated with dyspnea, cough, bronchitis,

allergic rhinitis, upper respiratory tract infections and eczema (Table I) ³. The fact that the molds have an impact on respiratory symptoms in non-allergic individuals and that this is not strictly limited to events such as allergic asthma and rhinitis, suggests the existence of an irritant and inflammatory non-allergic role for molds. We were among the first to found in a study carried out in a professional environment that workers whose offices were in excess of molds (according to objective measures carried out locally for a week) suffered significantly more from sick building syndrome (SBS) than workers who were not exposed ⁴. These symptoms included tearing and eye irritation, nasal irritation, cough, skin irritation, headache, fatigue. In other cases cited in the scientific literature the SBS symptoms were attributed to the presence of mold in the air of confined spaces. Within the European project ENVIE intended to establish the chemical and physical hazards of the indoor environment, we have demonstrated through a systematic review of the literature that exposure to molds significantly increased the risk (odds-ratio greater than 1) of symptoms suggesting COPD ⁵.

In schoolchildren included in the Health Effects of School Environment (HESE) study performed in 5 European countries we found that the total amount of spores and fungal DNA was positively associated with increased prevalence of chronic respiratory symptoms and a decrease in FEV1 ⁶. Children exposed to ≥ 300 cfu/m³ spores had more total dry cough at night (OR: 3.10, 95% CI 1.61 to 5.98), more rhinitis in the absence of respiratory infections (OR: 2.86, 95% CI: 1.65 to 4.95), and more persistent cough (OR: 3.79, 95% CI: 2.40 to

5.60) than the other. The DNA of *Aspergillus/Penicillium* was significantly associated with wheezing and the DNA of *Aspergillus versicolor* in wheezing, rhinitis and cough. The DNA of *Aspergillus versicolor* was inversely associated with FEV1. Recently, we observed that the association between COV_m, asthma and chronic bronchitis was strongest among farmers who, as is known, are less frequently affected by allergic diseases (OR = 2.95, 95% CI (1, 10, 7.95) and 3.35, 95% CI (1.33, 8.48), respectively) ⁷.

Toxic phenomena

Toxic phenomena can be induced by molds, whose spores contain mycotoxins. Mycotoxins are associated with mold spores but also with the mycelium and the substrate on which the mold producing toxins had developed. Mycotoxins can exert effects on human health and in particular on the liver, kidney and nervous system as a result of exposure by ingestion, skin contact and inhalation. The most frequently studied mycotoxins are those produced by certain species of *Aspergillus*, *Penicillium*, *Fusarium* and *Stachybotrys* including alfa-toxins produced by *Aspergillus* sp. and the acratossina produced from *Penicillium* sp.

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Climate changes and molds

The WHO has identified the effects that rising temperatures, rising sea levels and the melting of ice, snow and frozen ground will cause on health. Will be more frequent extreme weather events that will affect food productivity, quantity and water quality, air quality and the distribution of plants and animals. With more rains and floods, the houses will become more humid and mold will grow faster. Consequences on allergic diseases and respiratory diseases are expected ^{8,9}.

Prevention of exposure to molds

Limiting the contact with the mold helps to relieve the symptoms and reduce the risk of developing related diseases. This can be achieved through detection and treatment of molds in a home or a building through regular maintenance and repair in order to prevent the accumulation of spores. The effectiveness of the implementation, the use of dehumidifiers and air purifiers to improve the air quality is not documented. In some countries, the doctor may prescribe the intervention of specialized personnel to identify the fungal species and to perform appropriate preventive measures.