Concentration and determinants of molds and allergens in indoor air and house dust of French dwellings

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ABSTRACT

Molds and allergens are common indoor biocontaminants. The aims of this study were to assess the concentrations of common molds in indoor air and floor dust and the concentrations of house dust mite, cat and dog allergens in mattress dust in French dwellings, and to assess predictors of these concentrations. A sample of 150 houses in Brittany (western France) was investigated. Airborne *Cladosporium* and *Penicillium* were detected in more than 90% of the dwellings, *Aspergillus* in 46% and *Alternaria* in only 6% of the housings. Regarding floor dust samples, *Cladosporium* and *Penicillium* were detected in 92 and 80% of the housings respectively, *Aspergillus* in 49% and *Alternaria* in 14%. House dust mite allergens Der p1 and Der f1 were detected in 90% and 77% of the mattress dust samples respectively and Can f1 and Fel d1 in 37% and 89% of the homes. Airborne and dustborne mold concentrations, although not statistically correlated (except for *Aspergillus*) shared most of their predictors. Multivariate linear models for mold levels, explaining up to 62% of the variability, showed an influence of the season, of the age of the dwelling, of aeration habits, presence of pets, smoking, signals of dampness, temperature and relative humidity. Allergens in the dust of the mattress were strongly related to the presence of pets and cleaning practices of bedsheets, these factors accounting for 60% of the variability. This study highlights ubiquitous contamination by molds and underlines complex interaction between outdoor and indoor sources and factors.

1. Introduction

Molds are a ubiquitous contaminant, found both in outdoor and indoor environments. In closed environments, presence of moisture allows them to grow on a large amount of surfaces and materials. This moisture can have different sources, linked to the structure of the building (such as poor insulation), to human activities (bathing, showering, cooking…) or events that release water in the building materials and structure (Bornehag et al., 2004). There is a global endeavor regarding building design and maintenance policies striving to reduce energy consumption, with often low-quality insulation and inadequate air ventilation. Associated to the decrease in temperature due to reduced heating (notably for economic reasons), this results in higher humidity in dwellings, thus promoting development of molds (Reboux et al., 2009). This is of major concern in large parts of industrialized countries (USA, Canada and Europe), since (i) between 4 and 40% of dwellings in these countries appear to be affected by dampness, (ii) people spend between 80 and 90% of their time indoor and (iii) many studies already demonstrated adverse effects of dampness and mold exposure on respiratory health particularly in children (Bornehag et al., 2001; Deguen et al., 2012; Fisk et al., 2010; Heinrich, 2011; Kanchongkittiphon et al., 2015; Sharpe et al., 2015; World Health Organization, 2009).

Potentially hazardous exposures and sensitization can occur through inhalation of airborne spores or through dermal contact with settled dust, especially for children (Jovanovic et al., 2004).

Assessing the related health risk implies to be able of accurately estimating exposure. Several studies have been carried out using various methods to assess the fungi spectrum and levels in dwellings but, to date, the exposure assessment methods are not standardized and fully validated (Méheust et al., 2014, 2012; Tischer and Heinrich, 2013). Most of the published papers

investigated either indoor air or dust compartments, but studies measuring simultaneously both environments are still few (Jovanovic et al., 2004; Reponen et al., 2010).

The literature also remains controversial regarding the main determinants of indoor air and dust mold concentrations. Being under the influence of outdoor sources, indoor concentrations exhibit a wide geographical and temporal variability. Therefore, it is very difficult to draw a general picture of the contamination of dwellings across countries, as shown by the heterogeneous results of the 13 studies from Europe, USA and Asia summarized by (Nevalainen, 2014). In France, a recent study in 3193 dwellings evidenced 6 profiles of dwellings characterized by their composition in microorganisms (including molds) and showed differences in their geographical distribution (Rocchi et al., 2015). Moreover, the association of molds measurements and objective characteristics of the dwellings, particularly signs of dampness, remains unclear. Several authors found dampness signs or visible mold to be good predictors for fungal biomarkers (Sordillo et al., 2011) or airborne or dustborne mold concentrations (Haas et al., 2007; Roussel et al., 2008), while other found associations only with objectively measured ambient parameters like temperature and relative humidity (Ren et al., 2001) or no association with visible signs of dampness or moldy odor in the dwelling (Holme et al., 2010). Outdoor levels also have been reported as the main determinant for total fungal and *Cladosporium* indoor concentrations (Dassonville et al., 2008).

House dust also can be a vector of exposure for other indoor biological contaminants, particularly allergens. Also associated to allergies and asthma (Gaffin and Phipatanakul, 2009; Kanchongkittiphon et al., 2015), allergens most commonly found in dwellings include house dust mite (HDM) Der f1 and Der p1, cat allergen Fel d1 and dog allergen Can f1. Several studies reported dust allergen levels in USA (Perry et al., 2006; Peterson and Ownby, 2003; Rosenfeld et al., 2011), but studies in Europe are, to our knowledge, limited to the United-Kingdom

(Luczynska et al., 1998), Poland (Wardzyńska et al., 2012) and the Netherlands (Rijssenbeek-Nouwens et al., 2002). In France, very few data is available; one study within the national survey carried out by the French Observatory for Indoor Air Quality (OQAI) reported air levels of Can f1 and Fel d1, and dust levels of Der f1 and Der p1 (Kirchner et al., 2007). Regarding their determinants, several authors investigated the influence of building characteristics and inhabitants' behavior but there is still a high variability in the established relationships (Cho et al., 2006; Gross et al., 2000; Luczynska et al., 1998; Perry et al., 2006; Peterson and Ownby, 2003; Visitsunthorn and Chirdjirapong, 2010; Wardzyńska et al., 2012).

In this context, the present study aimed to document the indoor levels of molds both in indoor air and floor dust, and of allergens in mattress dust of French dwellings in view to assess the exposure of children living in these dwellings. It also aimed to compare the exposure levels with those found in similar studies worldwide. The study area was Brittany, Western France. Another objective of this work was to investigate determinants of these levels in a predictive modeling perspective. Although biological indoor contaminants also include bacterial species or components like endotoxins that have been shown to be associated with human sources (Dannemiller et al., 2015; Hospodsky et al., 2012; Meadow et al., 2014; Qian et al., 2012), this study only focuses on molds and allergens.

2. Materials and methods

2.1. Selection of the dwellings and study design

Environmental measurements of airborne and floor dust molds and mattress dust allergens were carried out in 150 houses from the PELAGIE cohort, a mother-child cohort study conducted in Brittany (Western France) since 2002 described by Petit et al. (2012). These dwellings being part of a larger study where chemical contaminants were measured too, the selection of the dwellings was based on trihalomethanes concentrations in the water supply network, and was described elsewhere (Dallongeville et al., 2015). The dwellings were investigated between September 2012 and October 2013 with 2 visits each performed by trained inspectors. Air samples were collected both in the child's bedroom and in the living room on the first visit, and dust samples from the bedroom's floor (for mold enumeration) and the child's mattress (for allergen quantitation) were collected 5 days after on the second visit (Figure S1). Since the vacuum cleaner used for dust sampling may resuspend settled dust (Veillette et al., 2013), this avoided contaminating air samples with dustborne molds.

2.2. Air and dust sampling for molds

The Sampl'air bioimpactor (AES laboratory, France) was used at a flow rate of 100 L/min to collect two air samples (50 and 100L), both in the child's bedroom and in the living room of the home. Microorganisms were impacted on 3% malt-extract agar (MEA) plates (MEA, Merck, Germany). The sampler was placed at a height of 0.3 to 1 m, in the middle of the room. Previous opening of the windows on the sampling day was recorded for each sample.

Dust samples were collected by vacuuming the floor in the child's bedroom with a Dustream Collector (Indoor biotechnologies, United Kingdom) sampler-fitted vacuum cleaner (40 micron mesh nylon filter, domestic vacuum cleaner LG, 2000W). Sampling was preferentially carried

out on the carpets (when available) or on hard surface floors until filling at least 2/3 of the filter-sampler, in order to ensure collection of at least 10 mg of fine dust. Collected dust was sieved at 300µm, and 10mg of fine dust were resuspended in 1mL of PBS-Tween (0.05%) buffer and shaked at 800 rpm for 1 hour. After dilutions at 1/10 and 1/100, 100µL of these suspensions were spread on MEA and DG-18 (18% Dichloran Glycerol, Agar) agar plates.

2.3. Dust sampling for allergens

Another Dustream Collector filter was used to vacuum the mattress of the child. The upper side of the mattress was entirely vacuumed after removing, when present, drawsheet and bedsheet. The second side was vacuumed too if first side did not allow collection of enough dust (half of the filter). Collected dust was sieved at 425 µm, and 100 mg of fine dust were resuspended in 2mL of PBS-Tween (0.05%) buffer and shaked at 250 rpm for 2h. After centrifugation, the supernatant was analysed by "sandwich" ELISA (enzyme-linked immunosorbent assay).

2.4. Culture and analysis of air and dust samples for molds

Agar plates were incubated at 25°C for 7 days. Fungal colony forming units (CFU) per plate were enumerated on days 3, 5 and 7. For air samples, a positive hole conversion table supplied by the manufacturer was used to account for the probability of several spores going through the same orifice. Calculations were carried-out in accordance to the NF ISO 16000-18 standard, using the formula C=n_ufc/V (with C being the concentration of molds in air, n_ufc the total number of counted unit forming colonies and V the total sampled volume), and results were expressed in CFU/m^3 (colonies forming units per cubic meter of air). For dust samples, results were expressed in CFU per gram of dust. As specified in NF ISO 16000-18 standard, only plates

whith less than 100 colonies can be enumerated. Molds genera were identified on macroscopic and microscopic criteria (after lactophenol-blue coloration on fixed smears) as described in reference mycology manuals (Reboux et al., 2006). *Aspergillus, Alternaria, Cladosporium* and *Penicillium* genera could be identified and counted. Unidentified species were taken into account to determine the total concentration of molds.

2.5. Analysis for allergens

Antigens and antibodies from commercial kits were used for allergen quantitation (Indoor Biotechnologies, kits for *Dermatophagoides pteronyssinus* (Der p1), *Dermatophagoides farinae* (Der f1), dog allergens (Can f1) and cat allergens (Fel d1)). For each plate, a standard calibration curve of 10 points was analyzed with the samples. Curves with a linear determination coefficient over 0.98 and at least 5 points in the linear part of the curve were considered valid and used to assess concentration from optical density. For each sample, if the variation coefficient between two duplicates was above 20%, the sample was analyzed again.

2.6. Measurement of ambient parameters

Temperature, humidity and carbon dioxide were measured every 10 min in the living room during the 5 days separating the two visits (Q-Trak 7575, TSI Incorporated, Shoreview, USA). The air exchange rate (AER) was calculated from each CO_2 concentration decay sequence, assuming that there was no production during this period. Under that hypothesis, AER was determined using a simple mass balance equation and linear regression on log-transformed CO_2 concentrations (Ramalho et al., 2013). Each decay was taken into account only if it had more than 9 points (1h30 of decay) and if the determination coefficient of the linear regression was

higher than 0.9. For each dwelling, 1 to 21 decays were selected, and the weighted average AER was used to characterize the ventilation.

2.7. Questionnaire

A detailed questionnaire was fulfilled by each family in order to describe house characteristics, habits and daily activities of residents during the sampling period. Items investigated in the questionnaire were collected in the literature and in similar studies carried out previously (Dassonville et al., 2009; Roda et al., 2011). The questionnaire included general information about the building (period of construction and renovation, surface, number of rooms), specific information about the rooms where sampling was carried out (child's bedroom and living-room) and the kitchen, often directly linked to the living-room (floor, wall and ceiling materials, presence of pressed-wood furniture, heating and ventilation systems), daily living habits in general and specifically during the sampling period (heating, aeration, cooking, type and frequency of cleaning, number of occupants and pets, specific activities or use of specific products). Questions about humidity included dampness spots, visible mold, moldy odor, peeling-off wallpaper, blistering paint, condensation, perceived moisture and damp bedding.

2.8. Handling of missing data and statistical analysis

Variables of the questionnaire having more than 40% of missing data, zero-variance variables (variables where all individuals presented the same modality) and near zero-variance variables (variables where only one individual presented a different modality than the rest of the sample) were removed. In order to complete remaining missing data, and since single imputation may underestimate the variability of the estimation, multiple imputation was implemented. Expectation-maximization algorithm was applied to jointly impute the missing values (with

number of imputations = 10, maximum number of iterations = 10 000, number of burn-in iterations = 200).

Classical descriptive statistics were determined for each variable of the questionnaire and for each mold and allergen concentrations. Concentrations below the limit of quantification were replaced by half of this limit of quantification (LOQ/2). For airborne molds, since only 16 bedroom and 20 living-room samples showed a value above the upper limit of quantification recommended by NF ISO 16000-18 standard, the value was replaced by this limit of quantification (2000 ufc/m^3). For univariate and multivariate analyses, concentrations were log10-transformed to approach normality of the distributions. To select the determinants to include in the multivariate model, univariate linear regression models were run for every predictor, and each predictor leading to a linear model p-value below 0.1 was included in the multivariate analysis. For these, the variance inflation factor (VIF) corrected by the number of degrees of freedom (Df) ($VIF^{1/2*Df}$) of each predictor was calculated to investigate potential multicolinearity. The analyses were performed using the R 3.1.0 software.

3. Results

3.1. Characteristics and ambient parameters in the investigated dwellings

Among the investigated dwellings, 81% were located in rural areas. Sampling took place in winter for 30% of the dwellings, the other being equally distributed between spring, summer and fall (23% in each season). Regarding history of the buildings, 20% were built before 1948, 49% between 1949 and 1999 and 31% after 2000. About 66% of the dwellings had undergone renovation work, among which 14% within the year before sampling. According to the questionnaire, windows were opened less than 30min per day in 46% of the living rooms and 56% of the bedrooms. Pets were present in 56% of the dwellings: 21% of the families owned a dog, 38% at least one cat, 8% fishes and 8% one rodent.

Dampness signs were reported in 13% of the living-rooms, 18% of the bedrooms and 14% of the kitchens. Overall, 31% of the dwellings reported a dampness sign in at least one room.

Objective measurements of relative humidity showed 5-days mean values ranging from 27.8 to 71.3%, with a median of 53.9%. Considering the intra-dwellings variability, the standard deviation of relative humidity in each dwelling was below 20% of the 5-days mean value. The 5-days mean CO_2 concentrations varied between 371 and 1332 ppm, with a median value of 621 ppm. The derived mean air exchange rate (AER) ranged from 0.12 to 0.92 h^{-1} (median: 0.30 h^{-1}).

3.2. Air and floor dust mold concentrations

Air sampling could be carried out in the 150 dwellings. Quantification of total mold concentration and of the various genera was possible in more than 90% of the dwellings (Table 1), which plates were not contaminated or invaded by one particular genus. Regarding the levels of contamination, 28% of the dwellings had a total concentration in the living room lower than 250 CFU/m^3, 17% between 250 and 500 CFU/m^3, 21% between 500 and 1000 CFU/m^3 and the

major part (34%) showed concentrations above 1000 CFU/m^3. Results were comparable in the bedrooms, with respectively 17, 25, 20 and 37% of the dwellings in these categories. The total mold concentrations ranged from 13 CFU/m^3 in the bedroom and 40 CFU/m^3 in the living-room to more than 2000 CFU/m^3 in both rooms (in 11% of the bedrooms and 13% of the living-rooms).

Cladosporium and *Penicillium* were the most abundant genera in both sampled rooms, being present in more than 80% of the dwellings, and reaching maximal concentrations above the upper limit of quantification of 2000 CFU/m^3. *Aspergillus* was present in a lesser extent (in about 40% of the dwellings), whereas *Alternaria* was present in only 6 to 7% of the housings.

Concentrations in the bedroom and in the living-room were strongly and significantly correlated for each genus and for total mold concentration, with Spearman's rho of 0.49 for *Alternaria*, 0.71 for *Penicillium*, 0.81 for *Aspergillus*, 0.89 for *Cladosporium* and 0.85 for total mold (each $p<0.001$).

Floor dust sampling could be carried out in only 133 out of 150 dwellings for technical reasons. Concentrations of molds in floor dust showed a similar pattern as in air samples regarding the frequencies of detection (Table 2). Total mold concentrations in floor dust ranged between 1,000 and 38.10^5 CFU/g. *Cladosporium* and *Penicillium* were detected in respectively 92% and 80% of the samples, *Aspergillus* in 49% and *Alternaria* in 14%. *Cladosporium* and *Penicillium* were the most contributive genera to the total concentration. Among the four identified genera and total molds, only *Aspergillus* concentrations in air and floor dust were significantly correlated (Spearman's coefficient: 0.20, $p<0.05$).

3.3. Mattress dust allergen concentrations (µg/g of dust)

Sampling and analysis of mattress dust could be carried out in only 134 out of 150 dwellings due to technical reasons. House dust mite (HDM) allergens *Der p1* and *Der f1* were detected in 90% and 77% of the samples respectively and could be quantified in 66% and 61% respectively of the homes (Table 3). Values ranged between lower limit of detection (0.05 µg/g of dust) to 25.7 (respectively 128) µg/g, with a median value of 1.5 (resp. 2.3) µg/g. For the dog Can f1 allergen, 37% of the 137 samples could be quantified, showing values up to 46.4 µg/g but with a relatively low median of 0.19 µg/g. Finally, cat allergen Fel d1 was quantifiable in almost all samples where it was detected (89%), reaching a maximal value of 101 µg/g, relatively high compared to the median of 0.97 µg/g.

3.4. Main determinants

Since airborne concentrations were significantly different in the dwellings sampled in different seasons (Wilcoxon's p-value below 0.05 for all concentrations, except *Aspergillus* in the living-room), analysis of determinants were carried out separately for dwellings sampled in the "warm" season (March to September) and in the "cold" season (October to February).

3.4.1. Determinants of airborne mold concentrations

In the univariate analysis, the main factors influencing mold concentrations were: season, age of the building, aeration, heating during sampling and, over all, ambient parameters, including relative humidity, temperature and CO_2 concentration. Presence of pets (especially cats), plants, signs of dampness and smoking into the dwelling were significant contributors too. Table 4 shows an example of the model for airborne molds in, the living-room during the cold season.

Others models are for airborne concentrations available in supplementary information (Tables S1 to S3).

The performance of the multivariate models varied depending on the season and the genera. None of the models for *Penicillium* concentrations was significant, and models for the warm season in the living-room were significant only for *Cladosporium* and total molds. The explained variance varied, in the bedroom, between 22% (*Aspergillus*, warm season) and 62% (*Cladosporium*, warm season) and between 13% (*Alternaria*, cold season) and 56% (*Cladosporium*, warm season) in the living room.

In both rooms, sampling in the cold season (October to March) was associated with increased *Aspergillus* and *Penicillium* concentration and decreased *Alternaria*, *Cladosporium* and total mold concentrations. Opened windows previous to the sampling did not influence significantly the concentrations, although a trend was present in the univariate analysis.

More recent buildings (built 1990-2000 or 2000-2005 vs. before 1948) showed higher concentrations of *Alternaria* in winter (in both rooms) and lower concentrations of *Cladosporium* in the warm season. Dwellings in rural areas and located near a garden had higher total molds concentrations, but this association was only a non-significant trend in living-rooms sampled in the cold season.

Presence of running mechanical ventilation was associated with decreased *Penicillium* and *Alternaria* concentrations in bedrooms in the cold season. Aerating the rooms longer and more frequently (at least once a week) was associated with increased *Alternaria*, *Cladosporium* and total concentrations, but decreased *Aspergillus* ones. Frequency of aeration only remained significant in the multivariate analysis for *Cladosporium* in bedrooms sampled in hot season.

Dwellings where heating was running were more contaminated by *Aspergillus* and *Penicillium*, but less by *Cladosporium*. Presence of pets, especially cats only, had a minor effect on mold concentrations (increasing *Alternaria* and decreasing *Cladosporium*, the latter as a non-significant trend).

Presence and number of plants in the living-rooms was associated with increased *Cladosporium* concentration in the cold season. Signs of dampness were not associated with total molds, but with increased *Aspergillus* and *Penicillium* contamination in the living-room and *Aspergillus* and *Alternaria* in the bedroom in the cold season. More frequent vacuum-cleaning was slightly associated with reduced *Aspergillus* concentration in the bedroom in summer. Regular or occasional smoking was associated with increased *Alternaria* in summer and *Aspergillus* in winter.

Finally, factors having the most important influence on the concentrations were the measured ambient parameters. In the linear univariate models, their contribution to total variance (i.e. the adjusted r-squared of the model) was up to 10 times higher than for other parameters.

In the warm season, median temperature and median relative humidity were associated with increased *Cladosporium* and total mold concentrations. Air exchange rate had no significant impact, but high median CO_2 concentrations decreased *Cladosporium* and total molds (the latter as a trend).

In winter, median temperature was associated with increased only *Cladosporium* concentration in the bedroom. Median relative humidity increased *Cladosporium* and total concentration, as in summer. Air exchange rate and median CO_2 concentrations did not have any significant effect.

3.4.2. Determinants of floor dust mold concentrations

Except for *Alternaria*, there was no significant difference between floor dust concentrations measured in the hot or cold seasons for each genus (Wilcoxon rank sum test). As a consequence, the analyses were carried out on the whole sample (Table 5).

The multivariate models were significant for every single genera, but not for total molds (p-value of 0.079). However, the explained variance was low compared to models for airborne concentrations (10% for *Cladosporium*, 9% for *Penicillium*, 14% for *Alternaria* and 19% for *Aspergillus*).

Having pets, especially cats, was associated with increased *Cladosporium* concentrations in floor dust. As for air concentrations, aeration of the room had a significant impact. Long aeration (more than 30min per day) increased *Cladosporium* concentrations. Frequent aeration increased *Cladosporium* and decreased *Penicillium* (for 1 to 4 times per week) and *Aspergillus* (for every modality) concentrations. Influence of heating period lost significance in the multivariate analysis, whereas it led to more *Aspergillus* and *Penicillium* and less *Alternaria* contamination in univariate analyses.

Reported dampness signs were associated with higher *Penicillium* concentrations. Presence of a carpet and the nature of the flooring material showed no impact on floor dust mold concentrations. As for airborne molds, regular smoking in the dwelling had a significant positive effect on *Aspergillus* concentrations. Regarding ambient parameters, median relative humidity decreased significantly *Aspergillus* and *Penicillium*, while the effect of median temperature (positive on *Alternaria*, negative on *Penicillium*) lost significance in multivariate analysis.

3.4.3. Determinants of mattress dust allergens

The season had an effect only on Der p1 concentrations which were higher during the cold season (September to March, Wilcoxon's test p-value of 0.001).

Multivariate models were all significant, and their explanatory performance was relatively high (32% of the explained variance for Der p1, 46% for Fel d1, 60% for Can f1), except for Der f1 (5%) (Table 6).

Age of the building influenced positively Der p1, Fel d1 and Can f1. Having dogs and cats in the house was strongly associated with the concentrations of the corresponding allergens and was quite predictive in univariate analyses (58% of the explained variance for dogs and 44% for cats). Presence of drawsheet on the bed reduced Can f1 levels, and lower frequency of cleaning bedsheets (less than once per week) increased Der f1 concentrations. Der p1 concentrations decreased with higher aeration frequency and temperature, and increased with median CO_2 concentrations.

4. Discussion

4.1. Airborne and floor dust mold levels and mattress dust allergen levels

Our study assessed both cultivable airborne and floor dust fungi and mattress dust allergens in 150 homes in Brittany, western France. This study sheds light on the very few data that are available on mold and allergen contamination in French dwellings. This dataset gives a realistic overview of the levels found in Brittany, characterized with an oceanic humid climate.

Several molecular methods are now available for fast and accurate assessment and identification of fungal species and derivation of a "moldiness index" (Méheust et al., 2013; Vesper, 2011). However, despite various drawbacks like the limitation to cultivable microorganisms, culture of molds is still very common, mainly due to its simplicity and low cost for environmental studies

with a sample overcoming a few dozens of dwellings. Moreover, allowing identification at the genus level, this technique is still useful in exposure assessment.

Our data indicate that cultivable airborne and floor dust fungi were found in each dwelling. *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* were, in this order, the most detected genera both in air and dust. This result is consistent with a previous study carried out in eastern France (Reboux et al., 2009), where *Penicillium* and *Cladosporium* were detected in the air of respectively 88% and 82% of investigated dwellings. This last study examined fungal contamination in unhealthy dwellings (n = 32) (homes with visible mold contamination and adverse health outcomes reported by the occupants), dwellings occupied by allergic patients (with medical diagnostic and positive prick-tests for molds) (n = 27) and matched control dwellings (n = 59). Our values for *Cladosporium* and for total molds were higher than those from the control dwellings of this study. One possible explanation may be that, contrary to this study, the respiratory health status of the children living in the study homes did not interfere in our selection of dwellings. Another explanation may be the strong influence of outdoor sources and parameters like climatic conditions (Kinney, 2008), that varies strongly across France. (Rocchi et al., 2015) had already shown that microbial contamination of French dwellings was highly heterogeneous. In our dwellings, *Cladosporium* and *Penicillium* are the most prevalent genera, whereas other French authors, who used surface and dust sampling to collect 338 samples from 61 dwellings across 10 years, found *Aspergillus* to be more frequent than *Penicillium* (Santucci et al., 2007). Consequently, this variability also appears strongly when comparing our levels to foreign studies. If the rank order of genera were the same in studies carried out in England (Hunter et al., 1988), Spain (de Ana et al., 2006), USA (Shelton et al., 2002) and Australia (Dharmage et al., 1999), occurrence of *Aspergillus* also was found to be more frequent in other dwellings in USA (Ren et al., 1999), Southern Taiwan (Su et al., 2001) and Taiwan (Li and Kuo,

1994). This trend also holds true for mold contamination of non-dwelling buildings as schools, for example in Canada (Bartlett et al., 2004). Regarding the quantified levels, they are also highly heterogeneous due to different sampling methods and culture conditions, and are therefore not completely comparable.

Regarding dustborne mold concentrations, our results are of the same order of magnitude than those from (Lignell et al., 2008) for total fungi and *Cladosporium*, but 10 times higher for *Penicillium* and *Aspergillus*. Moreover, these authors also report a 1:100 ratio between culturable and nonculturable fungal material in house dust, thus suggesting that people in the investigated dwellings might be exposed to higher levels. Similarly, our results are slightly higher than those from (Choi et al., 2014), but show the same order of magnitude than those from (Jovanovic et al., 2004). These discrepancies may result from differences in sampling and analysis methods, as well as from the high geographical and temporal variability of mold concentrations.

Concerning allergens, our levels of Der p1 and Der f1 are very similar to those found during a national survey (median values of 1.6 and 2.2 µg/g, respectively) carried out 2002-2005 (Kirchner et al., 2007). They are also comparable to those found by (Luczynska et al., 1998) in the United-Kingdom (geometric mean of 2.0 µg/g for Der p1), and slightly higher than those reported by (Wardzyńska et al., 2012) in Poland (median of the sum of Der p1 and Der f1 of 1.29 µg/g in rural dwellings). For cat allergens, our median levels are higher than those found in the latter study (0.43 and 0.61 µg/g, respectively) and in the USA (Rosenfeld et al., 2011) with a median level of Fel d1 of 0.48 µg/g. For Can f1 however, our levels are below those reported in Poland (median of 0.35 and 2.44 µg/g in rural and urban dwellings, respectively). Comparison with studies carried out in USA by Perry et al. (2006) and Peterson and Ownby (2003) is difficult, since these authors sampled floor dust and not mattress dust.

4.2. Determinants of airborne mold concentrations

Our results should be interpreted with caution since most of the determinants were collected through questionnaires completed by the families. Some studies already highlighted that while this mode of collecting information can be reliable for technical elements of the building, it could be questioned regarding dampness and mold problems (Engman et al., 2007).

Among the identified molds, the four genera may have both indoor and outdoor sources. However, *Aspergillu*s and *Penicillium* are usually more considered as indoor geni, whereas *Cladosporium* and *Alternaria* contamination are more linked to outdoor sources (Meklin et al., 2007). This may contribute to explain the differences of concentrations related to the sampling season, heating period and aeration of the rooms. During the cold season, concentrations of indoor genus were higher, and longer or more frequent aeration increased a frequent outdoor genus, *Cladosporium*. This is confirmed by the significant negative association between *Cladosporium* and the median CO_2 concentrations, directly linked to the confinement of the atmosphere. This supports the importance of outdoor sources, and the strong effect of factors linked with the season, that may involve exchanges with the outdoor environment (aeration) or not (heating) (Dassonville et al., 2008). Moreover, it could explain why some associations (like the presence of plants) are significant in winter and disappear in summer, their contribution being negligible compared to outdoor sources. However, our study was limited to one single visit in each dwelling, which impaired investigating more precisely the influence of seasonal effect.

The relationship between objective mold measurements and signs of dampness remains controversial. While some authors found musty odor or visible molds to be predictive of mold contamination (M.H. Garrett et al., 1998; Krop et al., 2014) , others did not report an influence of these factors (Reponen et al., 2013) or an association depending on which signs of dampness

were considered (Reponen et al., 2010). In our dwellings, visible signs of dampness or musty odor were associated with *Aspergillus*, *Penicillium* and *Alternaria* concentrations. However, comparisons with other studies have to be made carefully, considering the type of media that was sampled (indoor air, indoor dust) and the analyzed contaminant (spore concentration, β-glucans, mold DNA…). Moreover, there might exist a bias when people report dampness signs in their dwellings. Beyond these methodological considerations, these differences might also be related to the variations in water needs across genera and species. While various species of *Aspergillus* can grow on surfaces with water activity coefficients (Aw) lower than 0.8, *Cladosporium* is considered as mesophilic and require an Aw between 0.8 and 0.9 (Li and Yang, 2004).

Regarding objective measurements of relative humidity in air, associations found with *Cladosporium* and total molds are consistent with the literature (Dassonville et al., 2008; M H Garrett et al., 1998; Haas et al., 2007), although not all of the associations reported by these authors are significant. However, this result has also to be considered with caution, since higher relative humidity has already been suggested for other mold genus to reduce aerosolization of fungal spores (Madsen, 2012). We did not find any information about such an effect on the genera identified in the present study in the existing literature.

Age of the building, whose influence appears for several airborne genera, may be interpreted in terms of building design related to reduction of energy consumption. Increasing insulaton lead to increase the dampness and so far the development of mold. The fact that its effect varies across seasons may be due to insulation quality, a parameter interfering more in the cold than in the warm season when air exchanges with the outdoor environment are more important due to opening of windows.

Pets already have been described as a factor influencing mold contamination in the dwellings, with a positive or negative effect across studies (Chew et al., 2003; Dassonville et al., 2008;

Gehring et al., 2001; Ren et al., 2001; Roussel et al., 2008). In our study, having cats is associated with reduced *Cladosporium* in the warm season, an association already described elsewhere (Dassonville et al., 2008). This may be interpreted in terms of time spent outside by cats, possibly greater in the warm season, the animal thus carrying less spores inside the home. However, the information collected through the questionnaire did not allow investigating this hypothesis.

Presence of plants in the living-room increased *Cladosporium* concentration during the cold season but the association disappeared in the hot season, probably meaning that this source is relatively weak when the room is more ventilated.

4.3. Determinants of floor dust mold concentrations

Floor dust concentrations were not correlated with air concentrations, except for *Aspergillus*. This result has again to be interpreted with caution, dust sampling taking place 5 days after air sampling, with some possible micro-environmental changes between these two sampling times. Moreover, even if both measures were made by cultural methods, how microorganisms are cultivated has to be considered. In direct bioimpaction, agglomerates of viable spores will yield only one growing colony whereas shaking of resuspended dust samples allows separating agglomerates, each viable spore thus leading to one fungal colony. This effect may vary across genus (Chew et al., 2003).

Contrary to airborne concentrations, dust concentration did not vary according to the season of sampling. In fact, microbial concentrations in air and dust provide a different information, as already stated by (Chew et al., 2003; Reponen et al., 2010). Floor dust concentrations are less subject to rapid variations and reflect rather long-term cumulative exposure. Further, measures of dustborne concentrations may not necessarily be representative of exposure through inhalation.

In fact, genera and species developing and surviving in dust may be different in air and dust (Chew et al., 2003), and which fraction of dustborne mold can be aerosolized is not well known (Reponen et al., 2010).

Nevertheless, some determinants are shared by airborne and dustborne molds: having a cat (increased *Cladosporium* concentrations), signs of dampness (influencing *Penicillium* in dust, *Alternaria* and *Aspergillus* in air), regular smoking (increased both airborne and dustborne *Aspergillus* concentrations), bedroom aeration frequency and duration (increased both airborne and dustborne *Cladosporium* concentrations, and decreased *Aspergillus*). Unexpectedly, carpeted floor, or at least presence of a carpet in the bedroom, did not show any association with any mold genus, as shown by other authors for fungal biomarkers as ergosterol or β-glucans (Gehring et al., 2001; Giovannangelo et al., 2007; Sordillo et al., 2011). Inverse associations with median relative humidity for *Penicillium* and *Aspergillus* remain difficult to interpret. These parameters being measured in air of the living-room, they may be comparable in the bedroom air, but slightly different in bedroom dust.

4.4. Determinants of mattress dust allergen concentrations

The expected determinants for Can f1 and Fel d1, i.e. hosting a dog or a cat (Nicholas et al., 2010, 2008), were strongly associated with allergens in both the univariate and multivariate models. Concentrations of Can f1 seemed to be lower in buildings built after 1948, but since age of the building and hosting a dog were correlated (p-value = 0.006), this may be an effect of confounding. Washing the bedsheets less than once a week resulted in higher concentrations of Der f1 in our samples, as already shown by (Perry et al., 2006). The frequency of the room aeration showed a significant negative effect only on Der p1 concentrations. Even if the brutal change in temperature and relative humidity in the room by opening the windows, especially in

winter, could reduce viable house dust mite, measured allergens do not reflect only viable population of house dust mites but also dead ones. The type of mattress the samples were extracted from (spring mattress, latex, foam or other) did not show an association with allergen concentrations, although this factor already had been suggested by other authors (Visitsunthorn, 2010).

5. Conclusions

Our study provides an overview of airborne and floor dust mold and mattress dust allergen concentration in 150 dwellings, and investigates several determinants of their occurrence. This study shows an important and ubiquitous contamination, and highlights complex interactions between indoor and outdoor sources and factors. Hence, when assessing indoor mold contamination for epidemiological purposes, outdoor levels should be documented, when possible, in addition to precise description of dwellings characteristics and living habits.

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Table 1. Airborne mold concentrations in the bedroom and the living-room (in CFU/m^3)

	Cladosporium		*Penicillium*		*Aspergillus*		*Alternaria*		Total molds	
	Bedroom	Living room	Bedroom	Living room	Bedroom	Living room	Bedroom	Living room	Bedroom	Living room
Nr of samples	150	150	150	150	150	150	150	150	150	150
Nr of valid samples	143	145	137	138	136	137	136	138	148	150
Nr of samples where genus is present	130	133	123	128	62	65	8	12	148	150
Nr of samples where genus is countable and >0	123	123	120	124	61	65	8	12	114	123
Frequency of detection (% of dwellings)	91	92	90	93	46	47	6	9	100	100
Frequency of quantification (% of dwellings)	86	85	88	90	45	47	6	9	77	82
Minimum	<10	<10	<10	<10	<10	<10	<10	<10	13	40
Median	71	109	61	68	<10	<10	<10	<10	465	510
Mean	295	336	150	168	44	52	<10	<10	684	729
Maximum	>2000	>2000	1186	1165	1112	1418	101	101	>2000	>2000

Table 2. Mold concentrations in the dust of the bedroom's floor (in CFU/g of dust)

	Alternaria	*Aspergillus*	*Cladosporium*	*Penicillium*	Total molds
Number of valid samples	133	133	133	133	133
% of detection	14	49	92	80	100
minimum	0	0	0	0	1000
median	0	0	40.10^3	20.10^3	120.10^3
mean	2 992	43 120	79 459	63 699	235 970
maximum	10.10^4	34.10^5	68.10^4	96.10^4	38.10^5

Table 3. Allergen concentrations in the dust of the child's mattress (in µg/g)

	Der p1	Der f1	Can f1	Fel d1
Number of valid samples	134	134	134	134
% above limit of detection	90	77	54	93
% between LD and LQ	24	16	18	4
% quantified	66	61	37	89
minimum	<0.05	<0.05	<0.05	<0.02
median	1.5	2.3	0.19	0.97
mean	3.8	7.8	2.9	4.3
maximum	25.7	128	46.4	101

Table 4: Linear multivariate analysis for main determinants of airborne mold concentrations in the living-room (cold season). The numbers correspond to the coefficients of each determinant in the multivariate linear model for the airborne concentrations of molds, the symbols indicating the significance of these coefficients (# : p<0,1, *: p<0,05).

	Fungi air concentration				
	Alternaria	*Aspergillus*	*Cladosporium*	*Penicilllium*	Total molds
(Intercept)	0,037	0,625*	-0,961	2,106*	1,554*
Rural dwelling (vs urban)					0,229#
Proximity to a garden (vs no)					0,232
Building date (vs before 1948)					
built 1949-1974	0,007			-0,452*	
built 1975-1989	0,034			-0,272	
built 1990-2000	0,011			-0,446#	
built 2000-2005	0,152*			-0,083	
Vacuum cleaning frequency (vs less than 1 / week)					
1/week	-0,093				
2/week to 1/day	-0,115#				
Visible dampness signs (vs none)					
kitchen		0,409			
dwelling		0,006		0,359*	
living-room		0,876#			
Presence of plants in the living-room (vs no)					
1 to 2			0,536*		
3 to 5			0,756*		
6 or more			0,444		
Presence of cats (vs no cat)	0,108*				
Heating on during sampling (vs no)			-0,627#		-0,260#
Regular smoking in the dwelling (vs. no)		0,942*			
5-days median RH			0,048*		0,018*
5-days mean Air Exchange Rate			-0,297		0,021
adjusted R^2	0,13	0,20	0,45	0,07	0,38
p-value of the whole model	0,017	0,004	0,000	0,069	0,000

Table 5: Linear multivariate analysis for main determinants of dustborne mold concentrations in the bedroom. The numbers correspond to the coefficients of each determinant in the multivariate linear model for the airborne concentrations of molds, the symbols indicating the significance of these coefficients (# : p<0,1, *: p<0,05).

	Fungi dust concentration				
	Alternaria	*Aspergillus*	*Cladosporium*	*Penicillium*	Total molds
(Intercept)	-0,413	9,739 *	2,645 *	7,759 *	4,725 *
Heating on during the sampling period (vs. no)	-0,096	-0,327		-0,145	
Sampling during the cold season (vs. hot season)	-0,954 *				0,293
Bedroom aeration duration in general					
< 30 min/day	-0,033				
> 30 min/day	0,509				
Bedroom aeration frequency in general					
1/day		-0,648	0,482		
1/week to 4/week		-1,644 *	0,911 *		
>1/day		-1,857 *	-0,373		
Bedroom aeration frequency during the sampling period					
< 30 min/day			0,585	0,822 #	
> 30 min/day			1,111 #	0,816	
Bedroom floor wet cleaning	1,041 #		-0,922		-0,529
Dry cleaning of the bedroom furniture			-0,427		
Regular smoking in the dwelling		1,630 *			
Presence of a cat in the dwelling			0,625 *		
Signs of dampness in the bedroom				0,899 *	
5-days mean Air Exchange Rate	-1,297				
5-days median T°	0,042	-0,183 #		-0,106	
5-days median RH		-0,059 *		-0,048 *	
adjusted R^2	0,14	0,19	0,10	0,09	0,02
p-value	0,001	0,000	0,005	0,008	0,079

Table 6: Linear multivariate analysis for main determinants of dustborne allergen concentrations in the mattress. The numbers correspond to the coefficients of each determinant in the multivariate linear model for the airborne concentrations of molds, the symbols indicating the significance of these coefficients (# : p<0,1, *: p<0,05).

	Allergen dust concentration (log-transformed)			
	Can f1	Der f1	Der p1	Fel d1
(Intercept)	-1,314 *	-0,636	-6,416 #	-0,327
Cold season (vs hot)			0,624 *	
Building date (vs before 1948)				
built 1949-1974			-0,024	-0,814 #
built 1975-1989			-0,320	-1,256 *
built 1990-2000			-1,039 *	-0,928 *
built 2000-2005			-0,722 *	-0,942 *
Bedroom aeration duration in general				
< 30 min/day		0,831		
> 30 min/day		0,297		
Bedroom aeration frequency in general				
1/day			-0,365	
1/week to 4/week			-0,241	
>1/day			-1,470 *	
Bedsheet cleaning frequency				
1/month to 1 every 2 months		1,277 #		
1/week to 1/month		0,479		
Heating on during sampling (vs no)			-0,637 #	
Presence of a running mechanical ventilation			-0,498	
Presence of a drawsheet on the bed	-0,687 *			
Presence of a dog in the dwelling	3,477 *			
Presence of a cat in the dwelling		-0,726 #		2,548 *
Dry cleaning of the furniture in the bedroom				-0,708 #
5-days median CO2			4,099 *	
5-days median T°			-0,172 *	
adjusted R^2	0,60	0,05	0,32	0,46
p-value	2,26E-27	4,83E-02	3,07E-08	3,53E-16