

Assessment of multi-contaminant exposure in a cancer treatment center: a 2-year monitoring of molds, mycotoxins, endotoxins, and glucans in bioaerosols

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Abstract Indoor air quality in health care facilities is a major public health concern, particularly for immunocompromised patients who may be exposed to microbiological contaminants such as molds, mycotoxins, endotoxins, and (1,3)- β -D-glucans. Over 2 years, bioaerosols were collected on a monthly basis in a cancer treatment center (Centre F. Baclesse, Normandy, France), characterized from areas where there was no any particular air treatment. Results showed the complexity of mycoflora in bioaerosols with more than 100 fungal species identified. A list of major strains in hospital environments could be put forward due to the frequency, the concentration level, and/or the capacity to produce mycotoxins in vitro: *Aspergillus fumigatus*, *Aspergillus melleus*, *Aspergillus niger*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Purpureocillium*

lilacinum, and *Penicillium brevicompactum*. The mean levels of viable airborne fungal particles were less than 30.530 CFU per m³ of air and were correlated to the total number of 0.30 to 20 μ m particles. Seasonal variations were observed with fungal particle peaks during the summer and autumn. Statistical analysis showed that airborne fungal particle levels depended on the relative humidity level which could be a useful indicator of fungal contamination. Finally, the exposure to airborne mycotoxins was very low (only 3 positive samples), and no mutagenic activity was found in bioaerosols. Nevertheless, some fungal strains such as *Aspergillus versicolor* or *Penicillium brevicompactum* showed toxigenic potential in vitro.

Keywords Air monitoring · Bioaerosols · Cancer treatment center · Mold · Mycotoxins

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Introduction

Indoor air quality in hospitals is a major public health concern, particularly for sensitive patients who can be exposed to microbiological contaminants like molds, endotoxins, (1,3)- β -D-glucans, and mycotoxins. These contaminants can cause a wide range of health effects because of their allergenic, infectious, inflammatory, and/or toxigenic effects (Douwes et al. 2003). Previous data has also exposed that inflammation is a critical component of the tumor microenvironment (Coussens and Werb 2002). Environmental monitoring of microbial

contaminants was important in hospitals to limit adverse effects of allergenic and toxigenic molds and to prevent fungal infections in immunodeficient patients.

Microbial contamination in controlled hospital environments with specific air treatment has been widely studied (Caggiano et al. 2014; Falvey and Streifel 2007; Perdelli et al. 2006; Sautour et al. 2009). Although a 2-year-long survey of bioaerosols in operating theaters (air filtration by HEPA filters and positive pressure systems) in Spain showed fungal levels below 1 CFU per m³ (Ortiz et al. 2009), little is known about human exposure to airborne filamentous fungi in non-controlled air environments, particularly in cancer treatment centers. Many people evolving in the different floors of the hospital may be sensitive to biological hazards, particularly patients with immunodeficiency caused by chemotherapy or chronic corticotherapy treatments. Immunocompetent humans could even be concerned by indoor fungal bioaerosols which can cause dysfunctions of the respiratory and immunological systems (Curtis et al. 2004). Indoor airborne mold particles and mycotoxins can be absorbed through the respiratory route and cause health effects through the mechanisms of allergy, toxicity, and infection.

The real frequency of fungal infections is often underestimated because of the difficulty in determining its cause, directly linked to the lack of air quality monitoring. In addition, available studies mainly focused on *Aspergillus fumigatus* and did not include other opportunistic species, or did not provide information on the types of species (Howard 2014).

Several fungal genera such as *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* were commonly associated with the production of allergens causing respiratory and skin symptoms (Twaroch et al. 2015). Another study revealed that high levels of airborne *A. fumigatus* spores were correlated with outbreaks of invasive aspergillosis in intensive care units (Peláez et al. 2012). Environmental exposure to *A. fumigatus* has been associated to nosocomial aspergillosis in cancer patients (Anderson et al. 1996). However, no study provided an extensive characterization of mycoflora and microbial contaminants in areas without specific air treatment.

The incidence of filamentous fungal infections has increased worldwide, and although *A. fumigatus* was most commonly isolated, other opportunistic species belonging to *Aspergillus*, *Fusarium*, *Mucor*, *Paecilomyces*, or *Rhizopus* are capable of causing a wide variety of infections in immunodeficient patients

suffering from AIDS, diabetes, old age, transplantation, or cancer (Enoch et al. 2006; Nucci and Marr 2005). Moreover, emerging molds were associated with resistance to conventional antifungal agents used in clinical practice the past 10 years. For example, azole resistance in *A. fumigatus* isolates has been increasingly reported in several countries (Chowdhary et al. 2013).

In the present study, ergosterol, a sterol in fungal membrane, was used to evaluate its possible utilization as a biomarker for quantifying mold contamination in hospital bioaerosols. A previous study has utilized the fungal biomarker ergosterol to monitor fungal contamination in atmospheric aerosols (Lau et al. 2006).

Other microbial compounds like endotoxins of gram-negative bacteria or β -glucans from bacterial, plant, or fungal cell walls are major markers of microbiological contamination and induce inflammation of the airways (Thorn and Rylander 1998; Ławniczek-Wałczyk and Górny 2010). Although endotoxins seem to have a protective effect against atopic asthma and allergy development in early childhood, their role in cancer risk is still controverted: often reported to reduce lung cancer risk in agricultural cohorts, or more recently considered as a possible lung cancer promotion among women textile workers (Checkoway et al. 2014). Park et al. (2006) showed interactions and effects between exposure to fungi and endotoxin in water-damaged buildings and respiratory health.

The β -glucans are cell wall components of fungi, some bacteria, and plants. These contaminants have immunomodulating properties and can affect respiratory health (Ławniczek-Wałczyk and Górny 2010; Bellanger et al. 2011). During a chronic co-exposure to endotoxins and β -glucans, a persistence of neutrophils in the lung has been observed (Rylander and Holt 1998).

Mycotoxins are secondary metabolites produced by various toxigenic molds (Pitt 2000). These compounds are able to initiate various effects in humans from allergic responses to immunosuppression and cancer. Studies on mycotoxins like aflatoxins, ochratoxin A, or trichothecenes have focused on human or animal diets. Mycotoxins are excreted by fungal cells and could be aerosolized from conidia and fungal fragments (Fischer and Dott 2003; Fischer et al. 2000). The pulmonary exposure to airborne mycotoxins is less considered and needs to be investigated more thoroughly.

Isolates belonging to *Aspergillus* genus and collected from patients at a tertiary-care cancer center produced gliotoxin, a mycotoxin that contributes to the

pathobiology of invasive aspergillosis (Lewis et al. 2005). Several species of *Aspergillus* isolated from hospital rooms have exhibited cytotoxicity (Gniadek et al. 2011). Recently, a study demonstrated that a co-exposure of mycotoxins and β -glucans exhibited synergistic pro-inflammatory interactions (Korkalainen et al. 2016).

All this data suggests that a comprehensive approach is necessary to assess exposure and air quality in hospital environment, particularly in a sensitive area like a cancer treatment center.

The aim of this study was to propose a multi-contaminant approach that investigates the molds and other microbial contaminant levels in air non-controlled environments located in a cancer treatment center. This study constitutes the first global assessment of indoor air quality from bioaerosols collected in a cancer treatment center.

Material and methods

Bioaerosol collection

The study was performed in a multi-storey cancer center located in Normandy (France) (49°11.151'N, 0°21.5472'W). The Centre François Baclesse is one of the French cancer treatment centers (Unicancer) that receive 55,000 patients per year and provides cancer diagnosis and fully integrative treatment.

Environmental sampling was performed in patient care environment in three non-controlled areas, i.e., without specific air filtration system. These three floors were monitored on a monthly basis for a 2-year period:

- The fourth floor that provides supportive care in cancer,
- The fifth floor that provides medical care for cancer patients (all types of cancers) admitted to hospital from Monday to Friday,
- The sixth floor that provides medical care for cancer patients (digestive, aerodigestive, and thoracic cancers) admitted to hospital for an unlimited period.

The aerosols were taken in a fixed location, in the middle of the corridor on the 4th, 5th, and 6th floor, at a measured height of 1.20 m. The samples were collected in sterile liquid using a cyclonic air sampler Coriolis μ @ (Bertin Technologies, France)

calibrated to draw air at 300 L min⁻¹ for 10 min. Four liquid samples were made for each site.

During the sampling process, particles between 0.3 and 20 μ m, relative humidity (%), and temperature (°C) were monitored every 6 s by a portable aerosol spectrometer (Model 1.108, GrimFunm Technologies, Inc., Douglasville, GA, USA).

Identification and quantification of airborne molds

The liquid sample collected from Coriolis μ @ was directly used and diluted at 10⁻¹, 10⁻², and 10⁻³. One-milliliter aliquots of each solution were deposited into triplicate plates, and MEA (malt extract (1.5%)/agar (1.5%) supplemented with chloramphenicol (0.02%, w/v) was poured. The plates were incubated at 25 and 30 °C for 7 days and checked daily. The resulting colonies were counted and reported as colony-forming units per cubic meter of air (CFU per m³).

Each fungal colony was isolated and purified on MEA medium. Before identification of *Aspergillus* and *Penicillium* strains, cultures at 25 and 37 °C were performed on Czapek Yeast Autolysate agar medium and 25% glycerol nitrate agar medium as recommended by Klich (2002) and Pitt (1979). *Fusarium* species were cultured both on MEA and potato dextrose agar media.

The identification of all fungal isolates was conducted using macroscopic and microscopic features following Booth (1966), Domsch et al. (1980), Klich (2002), Pitt (1979), Samson et al. (2002), Samson and Pitt (2000), Samson and Frisvad (2004), and Von Arx (1981). Moreover, the phenotypes of *Aspergillus* and *Penicillium* species were compared with reference strains preserved in the fungal collection at our laboratory. When necessary, PCR reactions were also made using the primer set ITS1 and ITS4 (Visagie et al. 2014).

Quantification of endotoxins and (1,3)- β -D-glucans

The assessment of exposure of airborne endotoxins was conducted using the LAL method, as recommended by the standard EN 14031 (European Committee for Standardization 2003). The endotoxins and (1,3)- β -D-glucans were quantified using the Endosafe®-PTS™ system (Charles River, USA). The disposable FDA-licensed endotoxin cartridges utilized LAL reagents for a real-time endotoxin quantification, with a sensitivity range of 0.005 to 0.5 UE mL⁻¹. The disposable glucan cartridges have a sensitivity range of 10–1000 pg mL⁻¹.

To perform each test, 25 μL of sample was deposited into each of the four sample cartridge reservoirs.

Quantification of ergosterol

The ergosterol was quantified using gas chromatography coupled to mass spectrometry (GC-MS). A 2-mL aliquot of liquid sample was first hydrolyzed in 3 mL of 10% methanolic KOH at 90 °C for 1 h, and 1 μg of cholesterol (Sigma, USA) was added to the samples as an internal standard. After 3 successive extractions using 1 mL of cyclohexane followed by evaporation, 200 μL of N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Sigma, USA) was added, and then incubated for 30 min at 90 °C.

The derivatized solution was then cooled and injected into GC-MS. The analyses were realized with an Agilent gas chromatograph 7890 equipped with an apolar Agilent HP 5 MS fused silica capillary column (30 m $\times$ 0.25 mm internal diameter $\times$ 0.25 μm film thickness). The carrier gas was helium (24 mL min^{-1}). The GC apparatus was directly interfaced with an Agilent mass spectrometer 5975. The transfer line was maintained at a temperature of 280 °C. The ionization method chosen was electronic impact. The GC operated in the splitless mode. The m/z value for the ergosterol quantification was of 363.3, and a quantification limit of 0.01 μg per m^3 was determined. The retention times of ergosterol and cholesterol were, respectively, of 19.4 and 17.7 min.

Multi-mycotoxin quantification

An ultra-high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) was used to quantify 24 and 25 mycotoxins from fungal cultures and bioaerosols, respectively (Table 1).

In order to search for mycotoxins from the bioaerosols, 5 mL of the collected liquid was acidified with 1% acetic acid and extracted using a solid phase extraction (SPE) vacuum manifold using Oasis HLB (6 mL, 200 mg) cartridges (Waters). The conditioning of the cartridges was performed sequentially applying 5 mL of methanol and 5 mL of Milli-Q water at a flow rate of 1 mL min^{-1} . The sample was then percolated through the cartridge at a flow rate of 1 mL min^{-1} . After rinsing with 10 mL of Milli-Q water, each column was eluted with 10 mL of methanol at a flow rate of 1 mL min^{-1} . The extract was evaporated to dryness with

Table 1 Mycotoxins and quantification limits in bioaerosols and culture medium (MEA)

Mycotoxin	Quantification limit bioaerosol ($\mu\text{g L}^{-1}$)	Quantification limit culture medium ($\mu\text{g L}^{-1}$)
3acetyldeoxynivalenol	1.0	nd ^a
Aflatoxin B ₁	0.1	0.5
Aflatoxin B ₂	0.1	0.5
Aflatoxin G ₁	0.2	0.5
Aflatoxin G ₂	0.2	0.5
Alternariol	1.0	10.0
Citrinin	0.1	1.5
Cyclopiazonic acid	0.2	5.0
Deepoxydeoxynivalenol	0.1	2.5
Deoxynivalenol	0.1	2.5
Diacetoxyscirpenol	0.1	1.5
Fumagillin	0.1	5.0
Fumonisin B ₁	0.1	2.5
Fumonisin B ₂	0.1	2.5
Fusarenon X	1.0	50.0
Gliotoxin	1.0	10.0
HT2 toxin	2.0	nd ^a
Mycophenolic acid	0.1	2.5
Neosolaniol	0.1	1.5
Ochratoxin A	0.1	2.5
Paxillin	1.0	10.0
Sterigmatocystin	0.1	1.5
T2-Toxin	0.1	1.5
Verrucarol	nd ^a	5.0
Verruculogen	0.1	1.5
Zearalenone	0.1	2.5

^a Not determined

a Büchi evaporator and then under nitrogen. The final residue was dissolved in 50 μL of acetonitrile and then 450 μL water before injection into LC-MS/MS.

A liquid chromatography was performed using the Agilent Technologies series 1200 (Palo Alto, CA, USA) quaternary pump coupled with an autosampler and an Agilent 6460 tandem mass spectrometer. The analytes were separated at 40 °C on a column Acquity BEH C18 2.1 $\times$ 150 mm 1.7 μm (Waters, Milford, MA, USA). The mycotoxins were separated using an elution gradient with acetonitrile (solvent A) and water acidified with 0.1% formic acid (solvent B). The gradient program was as follows: at time 0, 10% solvent A and linear gradient to

100% solvent A within 10 min. The flow rate was 400 $\mu\text{L min}^{-1}$. The injection volume was 5 μL .

An LC-MS/MS was performed with an electron spray ionization (ESI) source in positive and negative modes. The parameters used for the mass spectrometer in all experiments were as follows: capillary voltage, 4 (mode +) and 3.5 kV (mode -); gas flow, 10 L min^{-1} ; gas temperature, 300 °C; pressure of nebulization, 45 psi. The MS/MS was used in the multiple reaction monitoring mode (MRM). For each mycotoxin, at least two specific parent and daughter ion transitions (quantifier and qualifier) were selected. For the quantification, one of the four internal standards, aflatoxin B1–13, zearalenone-13C, fumonisin-13C, deoxynivalenol-13C, was attributed to each mycotoxin.

The ability of fungal isolates to produce mycotoxins in vitro was also evaluated by LC-MS/MS. A total of 183 fungal strains collected from bioaerosols were grown in triplicate on MEA medium during 2 weeks at 25 °C before their multimycotoxin analysis. For each dish, three agar plugs measuring 8 mm in diameter were removed from the central area of the colony (including conidia and mycelium), pooled, weighted, and transferred to 5-mL glass vials. All mycotoxins were extracted with 2 mL of ethyl acetate acidified with 1% acetic acid. After 15 min of centrifugation at 1500 rpm, each extract was evaporated to dryness under a stream of nitrogen. The final residue was dissolved in 50 μL of acetonitrile and then 450 μL and filtered through Millex HV 0.45 μm before the injection into the LC-MS/MS.

Mutagenicity of bioaerosols

Seven liquid samples (aliquot of 2.5 mL) that represent the different types and levels of contaminants were evaporated in a parallel evaporator (Syncore polyvap, Büchi Labortechnik G, Flawil, Switzerland) and then dried under a stream of nitrogen. The final residue was dissolved in 200 μL DMSO and then filtered through Millex HV 0.45 μm .

The preincubation method of the *Salmonella typhimurium* assay was used with strains TA98, TA100, and TA102, without and with Aroclor-induced S9mix preparation, as described by Pottier et al. (2014). Each sample was tested in quadruplicate.

Statistical procedures

Fungal diversity was determined by the Shannon and Weaver index (Shannon and Weaver 1949).

Descriptive statistics (frequency, mean and standard deviation, median, minimum, and maximum) were used to characterize mold contamination, endotoxin, glucans and ergosterol production, mycotoxins, and mutagenicity. Exploratory analyses were conducted to examine their relationship by using the Fisher exact test, the Wilcoxon test, the Kruskal-Wallis test, and the Pearson correlation coefficient as appropriate.

We used multiple linear regression analysis to explore the association between physical and microbial measurements. In the model, the dependent variables were the number of particles, endotoxins, glucans, and total CFU, and the independent covariates were as follows: the floor, the ergosterol, the outdoor temperature, indoor temperature, the relative humidity, the number of people passing in the service, and the time since the last cleaning operation. The multiple linear regression model was used with the stepwise method.

The $P < 0.05$ was considered statistically significant. All analyses were conducted using the SAS version 9.4.

Results and discussion

Mold contamination

A total of 1678 fungal isolates have been identified during the 2 years of monitoring. This study provides the first detailed description of mycoflora in bioaerosols collected in a cancer treatment center. A number of 110 different fungal species has been identified. The Shannon Weaver indexes were not significantly different between the 3 floors ($P = 0.3599$) which indicates no difference in fungal biodiversity between the 3 floors.

Figure 1 shows the repartition of major fungal groups (*Acremonium*, *Cladosporium*, *Aspergillus*, *Penicillium*, and other species) reported on the 4th, 5th, and 6th floors. The mycoflora was dominated by *Aspergillus* and *Penicillium* species. Table 2 summarizes the most recurrent strains that were detected at least 3 months per year.

Among *Aspergillus* genus, some species were identified during the 2-year monitoring. Figure 2 presents the diversity of *Aspergillus* strains where *A. fumigatus*, *A. melleus*, *A. niger*, *A. sydowii*, *A. ustus*, and *A. versicolor* were the most frequently identified.

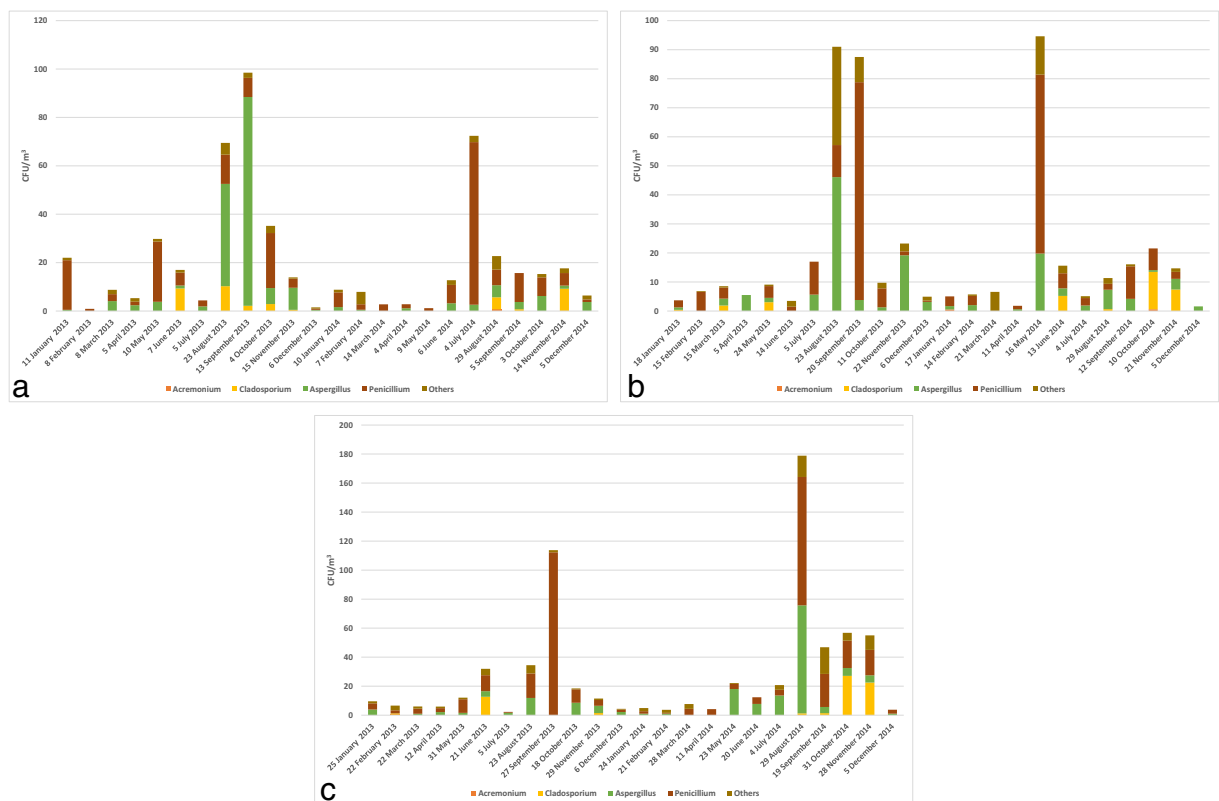


Fig. 1 Representation of major fungal groups (*Acremonium*, *Cladosporium*, *Aspergillus*, *Penicillium*, others) in bioaerosols during a 2-year monitoring period on 4th (a), 5th (b), and 6th (c) floors

The mycoflora was also constituted by various species belonging to *Penicillium* genus (Fig. 3). Among them, *Penicillium brevicompactum* and *P. chrysogenum* were the most recurrent.

Concerning the other filamentous fungi identified, the results indicated that several genera such as *Fusarium*, *Mucor*, and *Purpureocillium*, currently considered as emerging fungi in medical mycology, were recorded in literature. For example, the emerging pathogenic fungus *Purpureocillium lilacinum* (Antas et al. 2012) was identified in our study during 3 months of the first year on the 6th floor.

The concentrations of airborne molds (total CFU per m^3) are presented in Fig. 4. The mean concentrations of culturable airborne fungi did not significantly differ between the floors ($P = 0.4397$). The mean levels of total culturable molds varied from 16.141 to 30.530 CFU per m^3 , according to the floor. In comparison, Tormo-Molina et al. (2012) showed a mean fungal concentration of 24 CFU per m^3 during 2 years in a hospital in Spain. The study of Hao et al. (2011) in 2 tertiary hospitals in China showed median air fungal

load of 91.94 CFU per m^3 in the southwest China hospital and 71.02 CFU per m^3 in the northern China hospital, respectively.

However, seasonal variations were observed in our study, highlighting contamination peaks during autumnal and summer periods, when mold concentrations amounted to 98.5, 94.6, and 178.9 on the 4th, 5th, and 6th floors, respectively. Seasonal fluctuations in the levels of fungi have been reported in previous studies (Tormo-Molina et al. 2012; Hao et al. 2011). Variations in airborne fungi have been also observed in wards of the pneumology department of a Polish hospital with monthly mean concentrations ranging from 9.9 to 96.1 CFU per m^3 (Augustowska and Dutkiewicz 2006). Fungal contamination was at its highest during the summer in Korean hospital lobbies (Park et al. 2013). These findings suggest a need for additional air sampling and patient monitoring during these transient peaks.

In a general way, recurrent molds were also predominant from a quantitative point of view. Table 3 presents quantitative data for 2 recurrent strains, *A. fumigatus* and *P. brevicompactum*.

Table 2 Most recurrent fungal strains isolated from bioaerosols

Recurrent fungal strain	Frequency (months)		
	4th floor (year 1/year 2)	5th floor (year 1/year 2)	6th floor (year 1/year 2)
<i>Aspergillus fumigatus</i>	6/3	4/3	5/5
<i>Aspergillus melleus</i>	3/3	0/0	3/5
<i>Aspergillus niger</i>	8/8	5/4	6/5
<i>Aspergillus repens</i>	0/3	0/0	0/0
<i>Aspergillus sydowii</i>	0/0	0/0	3/0
<i>Aspergillus ustus</i>	0/0	3/0	0/0
<i>Aspergillus versicolor</i>	4/3	5/6	3/0
<i>Chaetomium globosum</i>	0/0	0/0	4/0
<i>Cladosporium herbarum</i>	4/3	4/4	0/0
<i>Fusarium culmorum</i>	0/0	0/0	0/3
<i>Penicillium brevicompactum</i>	5/5	4/6	5/7
<i>Penicillium chrysogenum</i>	5/5	4/6	7/5
<i>Penicillium citrinum</i>	0/0	3/0	4/4
<i>Penicillium crustosum</i>	0/0	0/0	4/4
<i>Penicillium digitatum</i>	0/0	0/0	0/0
<i>Penicillium expansum</i>	0/3	0/0	0/5
<i>Penicillium glabrum</i>	0/0	0/0	0/3
<i>Penicillium olsonii</i>	0/3	4/0	0/0
<i>Penicillium puberulum</i>	0/0	0/0	3/0
<i>Penicillium spinulosum</i>	0/0	0/0	3/0
<i>Penicillium variable</i>	0/0	3/0	0/0
<i>Penicillium verrucosum</i>	0/0	0/0	0/3
<i>Penicillium waksmanii</i>	0/3	0/0	4/0
<i>Pithomyces chartarum</i>	0/3	0/0	0/0
<i>Purpureocillium lilacinum</i>	0/0	0/0	3/0
<i>Trichoderma viride</i>	0/0	0/0	3/4

Mean concentration values of *Aspergillus fumigatus* did not exceed 1.8 CFU per m³ except on the 4th floor during the first year due to the high levels (up to 59 CFU per m³) observed in August and September. In a previous investigation in oncology wards, *Aspergillus fumigatus* and *A. flavus* have been recovered at a mean 1.83 CFU per m³, but no association was demonstrated between concentrations of *Aspergillus* and an increased incidence of aspergillosis (Hospenthal et al. 1998).

Endotoxins, glucans, and ergosterol

Table 4 presents endotoxins, (1–3)- β -D-glucans, and ergosterol levels in bioaerosols.

Exposure to endotoxins has been shown in various sectors: agriculture, industry, housing, and outside air. To this date, no data is available for bioaerosols collected in hospitals, particularly in cancer treatment centers. A comparison with environmental levels of endotoxins measured in other studies proved to be difficult because of the various aerosol sampling and contaminant quantification methods used in different studies (Duquenne et al. 2013). The maximum levels observed in our study remained lower than those measured in agricultural or industrial environments and were comparable to values measured in homes or outside air (Ławniczek-Wałczyk and Górny 2010; Madsen 2006; Mueller-Anneling et al. 2004).

Few studies were interested in (1–3)- β -glucans exposure although these agents were known to play a role

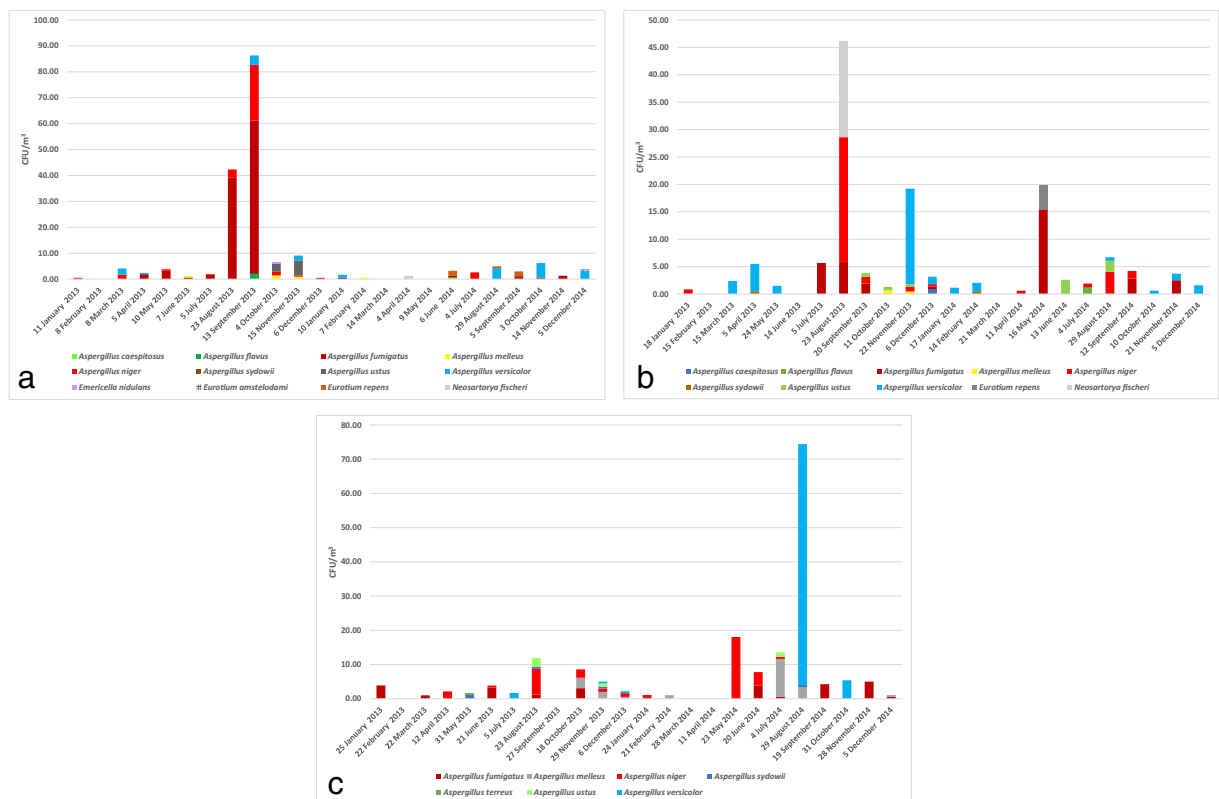


Fig. 2 Representation of *Aspergillus* species in bioaerosols during a 2-year monitoring period on 4th (a), 5th (b), and 6th (c) floors

in inflammatory responses and respiratory symptoms (Thorn and Rylander 1998). In our study, low concentrations of total (1–3)- β -glucans were observed in comparison to levels previously observed in moldy houses (up to 16.0 ng per m^3) or breeding activities (up to 5×10^6 ng per m^3) (Ławniczek-Wałczyk and Górny 2010; Reponen et al. 2007).

The average concentrations of ergosterol in hospital bioaerosols ranged from 5 to 13 ng per m^3 , whereas a level of 2160 ng per m^3 can be determined in a contaminated environment such as a solid waste plant (Kozajda et al. 2015).

Mycotoxins and mutagenicity

Only one mycotoxin, sterigmatocystin, was found in 3 samples of bioaerosols at levels of 0.31, 0.32, and 1.45 μg per m^3 . This mycotoxin has previously been detected in dust samples (Bloom et al. 2009) and exhibited cytotoxic and mutagenic properties (Despot et al. 2016; Anninou et al. 2014; Krivobok et al. 1987).

For the 7 bioaerosols tested, mutagenicity was negative with strains TA98, TA100, and TA102 which

confirmed the very low exposure to mycotoxins via inhalation in this environment.

However, the present study reports the ability of airborne mold isolates to produce mycotoxins after culture on solid medium. Among the 183 isolates tested in triplicate (which represent 45 different species), we found that 105 isolates mainly distributed in 3 fungal species were capable of producing mycotoxins in vitro (Table 5).

A. fumigatus, *Aspergillus versicolor*, and *P. brevicompactum* were the major groups of molds showing toxigenicity in vitro. Isolates of *A. fumigatus* were found to produce some mycotoxins, in particular gliotoxin, an immunosuppressive mycotoxin that can exacerbate the pathogenesis of aspergillosis (Lewis et al. 2005). Several isolates of *A. versicolor* and *P. brevicompactum* were able to produce high concentrations of sterigmatocystin (up to 2494 $\mu\text{g g}^{-1}$) and mycophenolic acid (up to 593 $\mu\text{g g}^{-1}$), respectively. Figure 5 shows the distribution of toxigenic isolates in *A. versicolor* and *P. brevicompactum* species. Mycophenolic acid is the fungal secondary metabolite displaying a wide array of biological properties, extensively studied

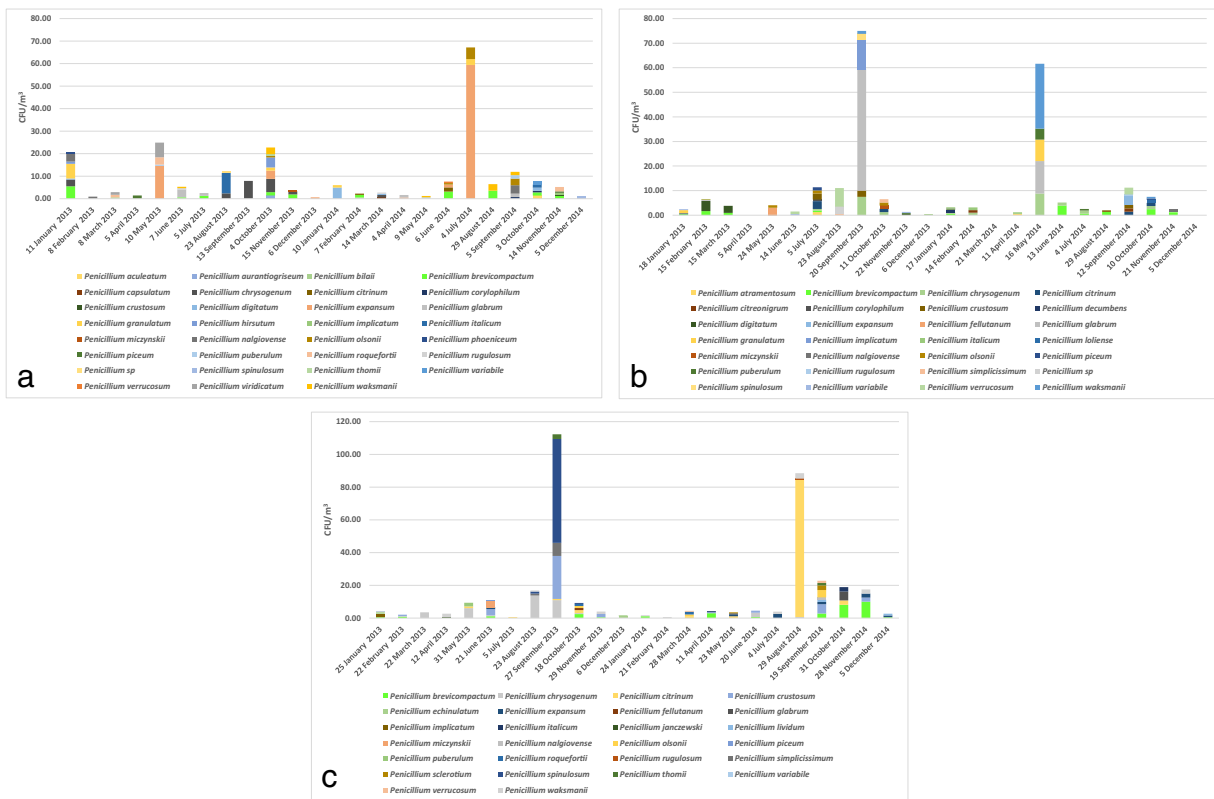


Fig. 3 Representation of *Penicillium* species in bioaerosols during a 2-year monitoring period on 4th (a), 5th (b), and 6th (c) floors

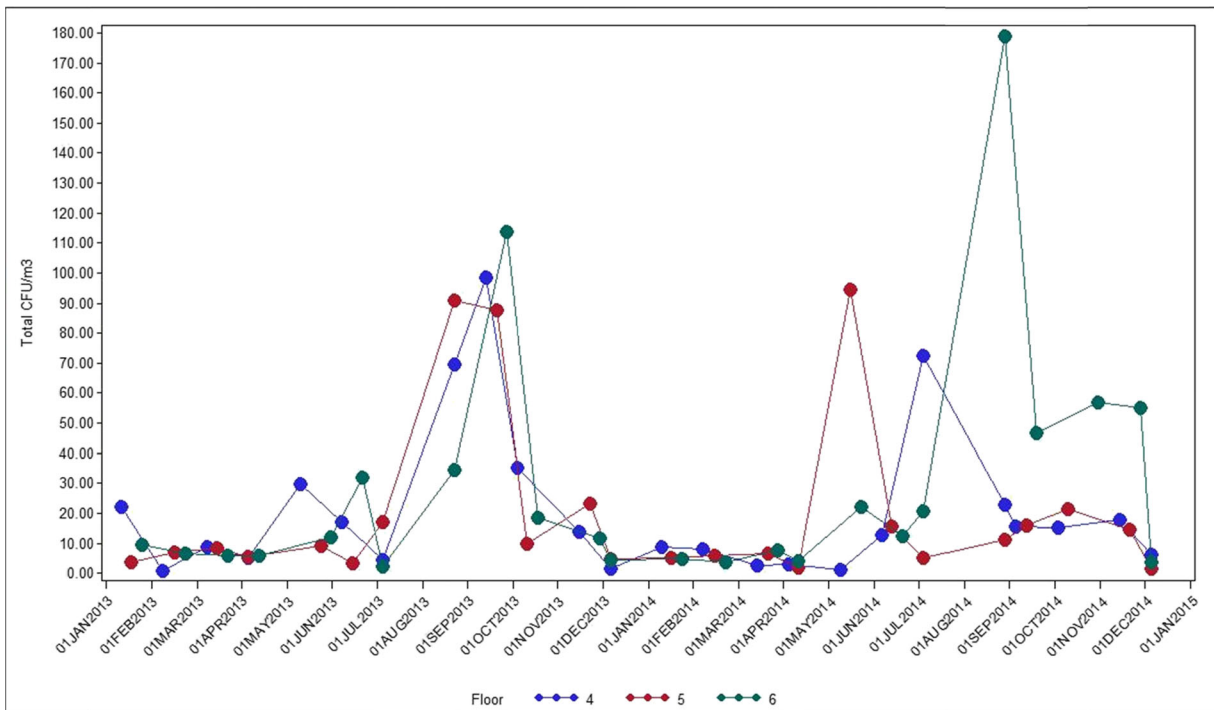


Fig. 4 Airborne mold concentration (total CFU per m³) on the 4th, 5th, and 6th floors during 2-year air monitoring

Table 3 Concentrations of *Aspergillus fumigatus* and *Penicillium brevicompactum* during the 2-year air monitoring

Year	Floor	Fungal strain	Number of months per year	CFU per m ³				
				Median	Minimum	Maximum	Mean	SD
1	4	<i>Aspergillus fumigatus</i>	6	0.300	0.000	59.000	8.829	19.325
	5	<i>Aspergillus fumigatus</i>	4	0.000	0.000	5.850	1.154	2.215
	6	<i>Aspergillus fumigatus</i>	5	0.000	0.000	3.900	1.046	1.512
2	4	<i>Aspergillus fumigatus</i>	3	0.000	0.000	1.300	0.225	0.434
	5	<i>Aspergillus fumigatus</i>	3	0.000	0.000	15.400	1.725	4.427
	6	<i>Aspergillus fumigatus</i>	5	0.000	0.000	4.250	0.871	1.541
1	4	<i>Penicillium brevicompactum</i>	5	0.000	0.000	5.500	0.892	1.605
	5	<i>Penicillium brevicompactum</i>	4	0.000	0.000	1.650	0.304	0.531
	6	<i>Penicillium brevicompactum</i>	5	0.000	0.000	2.450	0.450	0.739
2	4	<i>Penicillium brevicompactum</i>	5	0.000	0.000	3.550	0.879	1.287
	5	<i>Penicillium brevicompactum</i>	6	0.275	0.000	3.900	0.846	1.238
	6	<i>Penicillium brevicompactum</i>	7	0.600	0.000	3.000	0.825	1.058

because of its immunosuppressive properties (Bartman et al. 1981).

Productions of sterigmatocystin by *Aspergillus nidulans* (1 isolate), mycophenolic acid by *Aspergillus repens* (1 isolate), fumonisin B2 by *Aspergillus niger* (1 isolate), and alternariol by *Talaromyces diversus* (1

isolate) were also observed and previously mentioned in literature (Klich 2002; Séguin et al. 2014; Frisvad et al. 2007; Holker et al. 1983).

These results highlighted the potential health hazards posed by toxigenic airborne molds in hospital environments.

Table 4 Endotoxins, (1–3)- β -D-glucans, and ergosterol concentrations

Year	Floor	Contaminant (unit of measure)	Median	Minimum	Maximum	Mean	SD
1	4	Endotoxins (EU per m ³)	2.260	0.180	31.250	4.673	8.702
	5		0.685	0.250	11.490	2.028	3.403
	6		0.490	0.220	55.210	8.924	18.075
2	4	(1–3)- β -D-glucans (pg per m ³)	0.285	0.140	5.070	1.228	1.839
	5		0.375	0.140	3.690	0.893	1.071
	6		0.255	0.110	29.750	3.016	8.458
1	4	(1–3)- β -D-glucans (pg per m ³)	286.640	143.430	1185.000	364.423	293.007
	5		243.068	89.000	589.090	268.864	128.165
	6		278.395	133.200	1188.000	396.240	326.462
2	4	(1–3)- β -D-glucans (pg per m ³)	245.875	162.340	357.180	247.132	66.494
	5		261.535	139.040	842.500	361.460	227.855
	6		294.235	131.500	510.000	279.476	108.060
1	4	Ergosterol (ng per m ³)	9.440	0.000	33.650	11.249	12.384
	5		5.575	0.000	24.350	8.703	9.567
	6		14.585	0.000	39.030	13.936	14.082
2	4	Ergosterol (ng per m ³)	0.000	0.000	35.720	7.211	12.289
	5		0.000	0.000	23.760	5.159	7.691
	6		0.000	0.000	32.330	5.314	12.413

Table 5 Toxicogenic isolates and median levels of mycotoxin production in vitro

Fungal species	Mycotoxins	Number of toxicogenic isolates/total isolates	Median concentration ($\mu\text{g g}^{-1}$ medium)
<i>Aspergillus fumigatus</i>	Fumagillin	29/29	0.478
	Gliotoxin		0.003
	Verruculogen		0.503
<i>Aspergillus versicolor</i>	Sterigmatocystin	16/16	129.326
<i>Penicillium brevicompactum</i>	Mycophenolic acid	60/60	185.757

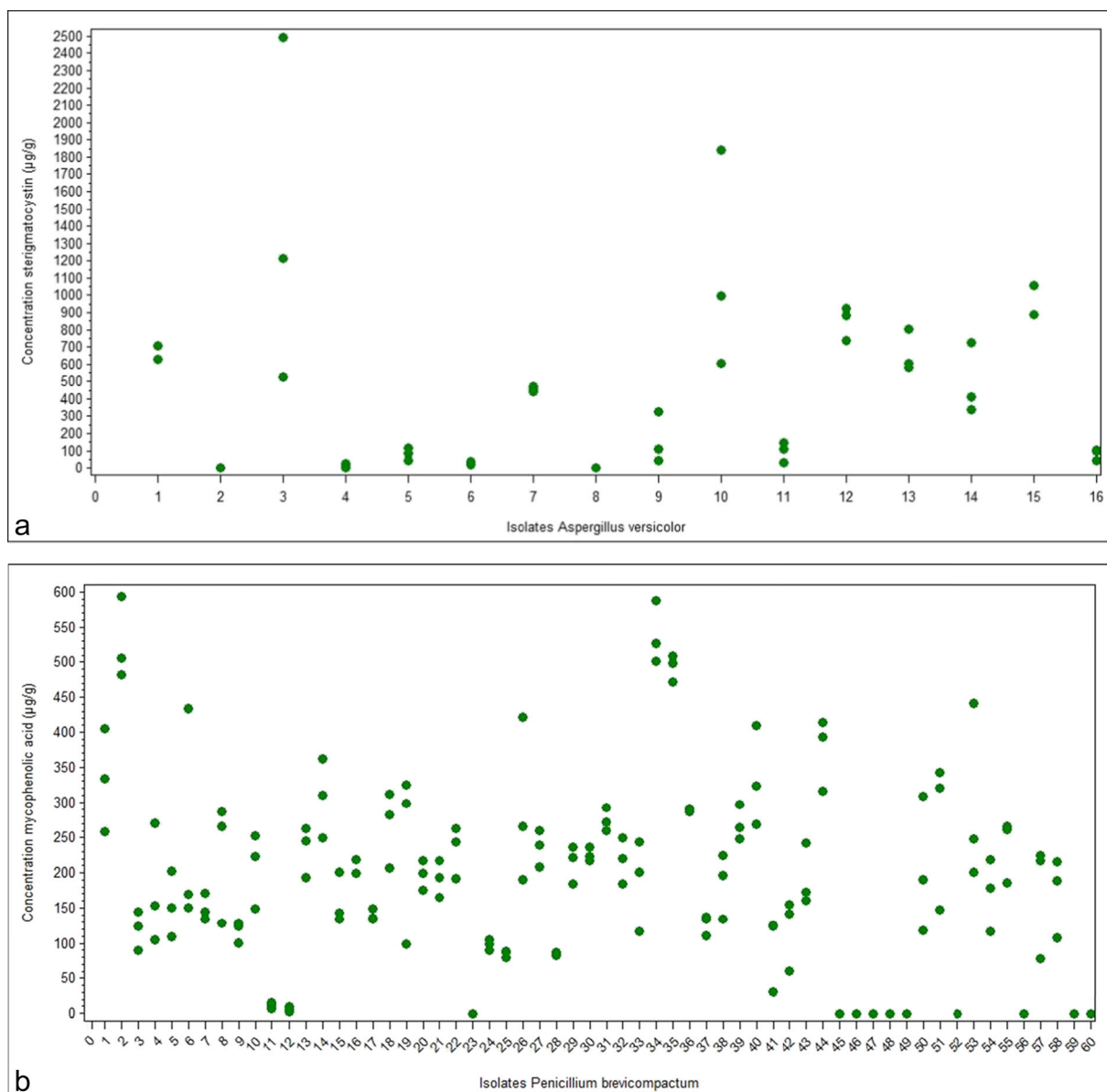


Fig. 5 Distribution of toxicogenic isolates of *Aspergillus versicolor* (a) and *Penicillium brevicompactum* (b)

Interactions between physical and microbial measurements

The multiple regression analysis showed that the relative humidity level can explain the levels of glucans ($P = 0.0082$) and total CFU ($P < 0.0001$). This variable can also explain the concentration (CFU per m^3) of *Aspergillus fumigatus* ($P = 0.0125$) and *Aspergillus niger* ($P = 0.0272$).

Significant associations were demonstrated between several contaminants. Endotoxins were positively associated with glucans ($r = 0.47$; $P < 0.0001$) and ergosterol ($r = 0.32$; $P = 0.0021$). Glucans were also correlated with ergosterol ($r = 0.31$; $P = 0.0032$), and total CFU was significantly associated with total particles ($0.3\text{--}20\text{ }\mu\text{m}$) ($r = 0.31$; $P = 0.0010$). In contrast, ergosterol was not correlated with airborne mold contamination (CFU per m^3) ($P = 0.5168$).

Conclusions

Bioaerosol control is a challenge and a major public health concern in hospital environments, particularly in cancer centers receiving patients with immunological perturbations. This study provides original data on multi-contaminants, particularly molds constituting bioaerosols in this sensitive environment.

The diversity and complexity of mycoflora in bioaerosol were highlighted. This constitutes the first comprehensive monitoring providing a detailed description of airborne fungal species from a French cancer center. Thus, a list of 7 priority fungal strains based on their recurrence and toxigenic potential was established: *Aspergillus fumigatus*, *Aspergillus melleus*, *Aspergillus niger*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Purpureocillium lilacinum*, *Penicillium brevicompactum*.

The present study reported that exposure to molds in non-controlled environments in a cancer center followed fluctuations depending on the seasons. A greater surveillance was advocated when level peaks occurred in the summer and autumn.

The statistical analysis revealed that the relative humidity level could be considered as an environmental determinant of airborne mold (total CFU) and could therefore be used as an indicator of fungal contamination.

Our findings showed low exposure to airborne mycotoxins and no mutagenic activity observed from bioaerosols collected in this environment.

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Compliance with ethical standards

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