Fungal pollution of indoor environments and its management

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Abstract Indoor environments play important roles in human health. The health hazards posed by polluted indoor environments include allergy, infections and toxicity. Life style changes have resulted in a shift from open air environments to air tight, energy efficient, environments, in which people spend a substantial portion of their time. Most indoor air pollution comes from the hazardous non biological agents and biological agents. Fungi are ubiquitous in distribution and are a serious threat to public health in indoor environments. In this communication, we have reviewed the current status on biotic indoor air pollution, role of fungi as biological contaminants and their impact on human health.

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1. Introduction

All around the world, life style changes have resulted in a shift from open air environments to air tight, energy efficient environments at home and work places, where people spend a substantial portion of their time (Chao et al., 2003; Molhave, 2011). In these environments, improper maintenance, poor building design or occupant activities often result in a condition called as “Sick Building Syndrome” (SBS), where occupants experience adverse health effects that appear to link with the time spent in a building (Ebbehoj et al., 2002; Zeliger, 2003). The complaints may be localized to a particular room or widespread throughout a building and relief usually occurs soon after leaving the building (Bholah and Subratty, 2002; Bakke et al., 2008). Headaches, pressure on the head and throbbing, and feelings of tiredness are the most common signs of SBS.

Various abiotic agents like dust, particulate matter, wall coverings, synthetic paints, glue, polishes, and Volatile Organic Compounds (VOCs) may contribute to indoor pollution and cause SBS (Chao et al., 2002; Horner, 2003). Most of the air pollution comes from sources inside the building itself like, hair spray, perfume, room deodorizer, paints, thinners, home appliances, photo copiers, printers, computers, and air purifiers (Rosnagel, 2000; Wilson and Straus, 2002; Rylander, 2004). Use of disinfectants (linear alkyl benzene sulfonates) and fatty acid salts (soap) in cleaning agents (rub shampoo) can cause enhanced eye and airway irritation (Herbarth et al., 2003; Guo, 2011).

The release of gases from solvents used indoors, such as chlorine (used for drinking water disinfection), α-pinene, β-pinene (ingredients of synthetic paints and disinfectants) and formaldehyde (from building materials) can cause health problems (Hagmolen et al., 2007). Fiber glass or rock wool, floor coatings of linoleum, polyvinyl chloride, and use of glulamdehyde can cause throat and facial dermal symptoms (McDonnell and Burke, 2011). Odors associated with bioaerosols may lead to anhedonic responses (Hiipakka and Buffington, 2000; Hintikka, 2004). Cigarette smoke is another source of not only VOCs, but also of toxic chemicals, carcinogens, and dust particles that may affect the lungs (Roussel et al., 2008). VOCs may become a problem in buildings that are being renovated or constructed.

The increase in temperature and humidity also affects the release of VOCs (Reijula, 2004; Hatfield and Hartz, 2011). The outdoor air that enters a building can be a source of indoor air pollution. For example, outdoor air may contain pollutants from motor vehicle exhausts (Reynolds et al., 2001). Physical and design characteristics of buildings such as lighting, ergonomics and noise may lead to chronic health effects. The nocturnal artificial lighting suppresses melatonin and may predispose people to breast and colon cancer (Li and Yang, 2004).

Bacteria, fungi, pollen, viruses, rat droppings, mites, insect body parts or bird droppings can be sources of biological contamination (Nevalainen and Seuri, 2005; Khan and Karuppayil, 2010). The indoor bacterium – Legionella causes both Legionnaire’s Disease and Pontiac Fever (Nakayama and Morimoto, 2007). Common contributors to biological pollutants are water damage to homes during flooding or storm damage, leaks in plumbing, roofs or air conditioners, humidifiers, and bathrooms; and ice damming on building roofs allows water to seep through the roof sheathing (Cunningham et al., 2004; McNern et al., 2008). Humidifiers in the ventilation circuit provide place for proliferation of microorganisms (Farley and Franklin, 1992; Yamashita et al., 2005; Li et al., 2010).

An assessment of dust samples in schools and day care centers shows that dog, cat and mite allergens could cause severe health problems in children (Aydogdu and Asan, 2008). Allergens detected in mattresses, floor dust and curtains are found to increase the risk for asthma (Adgate et al., 2008). The design and operation of Heating, Ventilation and Air Conditioning systems (HVAC’s) have an impact on the distribution of airborne infectious organisms (Mc Grath et al., 1999).

1.1. Fungal pollution of indoor environments

Fungi are ubiquitous in distribution and are a serious threat to public health in indoor environments (Samet and Spengler, 2003; Khan, 2009). Many fungi that are reported to cause allergy belong to Ascomycota, Basidiomycota or anamorphic fungi. There are many reports on fungi isolated from indoor environments (Table 1) (Portnoy, 2003; Khan et al., 2009). Fungi are able to grow on almost all natural and synthetic materials, especially if they are hygroscopic or wet. Inorganic materials get frequently colonized as they absorb dust and serve as good growth substrates for Aspergillus fumigatus and Aspergillus versicolor (Samet and Spengler, 2003). Wood is highly vulnerable to fungal attack. Cladosporium and Penicillium (Penicillium brevicompactum and Penicillium expansum) are reported to infest wooden building materials. Kiln dried wood surfaces are more susceptible to fungi (Sailer et al., 2010). Acylated wooden furniture, wood polyethylene composites, plywood and modified wood products are susceptible to infestation by Aspergillus, Trichoderma and Penicillium (Thacker, 2004; Doherty et al., 2011).

Inner wall materials used in buildings, such as prefabricated gypsum board, highly favors the growth of Stachybotrys
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<td>Comparative survey of airborne fungal spores</td>
<td>Chakraborty et al. (2000)</td>
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<tr>
<td>Dust from carpet, Erfurt, Hamburg</td>
<td>Germany</td>
<td><em>Alternaria, Aspergillus, Cladosporium, Penicillium</em></td>
<td>To compare exposure to mold spores in two German cities</td>
<td>Koch et al. (2000)</td>
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<tr>
<td>Damp buildings, Lyngby</td>
<td>Denmark</td>
<td><em>Penicillium, Aspergillus, Chaetomion, Ulocladium, Stachybotrys, Cladosporium</em></td>
<td>To elucidate problems with fungal infestation in indoor environments</td>
<td>Gravesen et al. (1999)</td>
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<tr>
<td>Indoor environments, Riyadh</td>
<td>Saudi Arabia</td>
<td><em>Mycosphaerella Yeasts, Fusarium, Penicillium, Aspergillus, Alternaria, Cochliobolus</em></td>
<td>To identify and enumerate the different airborne fungi</td>
<td>Ismail et al. (1999)</td>
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<td>Indoor environments</td>
<td>Kuwait</td>
<td><em>Aspergillus, Penicillium, Bipolaris, Cladosporium, Alternaria</em></td>
<td>Study provides information on the prevalence of allergenic fungi in indoor environments of Kuwait</td>
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<td>Water damaged buildings</td>
<td>Denmark</td>
<td><em>Stachybotrys chartarum, Aspergillus versicolor, Trichoderma spp.</em></td>
<td>To verify the production of mycotoxins from <em>A. versicolor, S. chartarum, T. harzianum, T. longibrachiatum</em> and <em>T. atroviride</em> grown on artificially inoculated building materials</td>
<td>Nielsen et al. (1998)</td>
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<td>Indoor atmosphere, Ismailia</td>
<td>Egypt</td>
<td><em>Aspergillus flavus, Aureobasidium pullulans, Cladosporium cladosporoides</em></td>
<td>Fungal spore population was studied</td>
<td>Wahid et al. (1996)</td>
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<td>Saw mills, Lucknow</td>
<td>India</td>
<td><em>Alternaria, Aspergillus, Curvularia, Drechslera, Epicoccum, Fusarium, Penicillium</em></td>
<td>Fungi in different seasons in saw mill and their allergic potential were studied</td>
<td>Tewary and Mishra (1996)</td>
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<tr>
<td>Household environments, Riyadh</td>
<td>Saudi Arabia</td>
<td><em>Alternaria, Aspergillus, Cercospora, Chaetomion, Cladosporium, Curvularia, Drechslera, Embellisia, Fusarium, Mucor, Penicillium, Rhizopus, Scytalidium, Trichoderma, Tordula, Ulocladium</em></td>
<td>Fungi inhabiting household environments in the West, East and Central localities of Riyadh city were screened</td>
<td>Bokhary and Parvez (1995)</td>
</tr>
<tr>
<td>Hospital New Delhi</td>
<td>India</td>
<td><em>Aspergillus flavus, A. niger, A. versicolor, Cladosporium, Alternaria, Fusarium oxalicum, Penicillium citrinum</em></td>
<td>Fungal airspora of hospital</td>
<td>Singh et al. (1994)</td>
</tr>
</tbody>
</table>
1. Health hazards of indoor fungi

Inhalation or ingestion is a principal route of exposure to fungal propagules. Products of mold growth such as Microbial volatile organic compounds (MVOC) or Microbial volatile break down products may contribute to symptoms of illness or discomfort independently on exposure to fungal biomass (Bezhold et al., 2008). The role of indoor fungi in irritative disorders i.e. primarily non-infective diseases such as allergy and asthma, has long been recognized. Bioaerosols of fungal origin, consisting of spores and hyphal fragments are readily respirable and are potent elicitors of bronchial irritation and allergy (Britton, 2003). At least 600 species of fungi are in contact with humans and less than 50 are frequently identified and described in epidemiologic studies on indoor environments (Phipatanakul, 2003; Khan et al., 2009).

1.3. Respiratory symptoms

Sinusitis similar to the common cold due to inflammation of para nasal sinuses is reported in homes with visible mold or water damage. Damp concrete floors increased the risk of irritated stuffy or running nose, and itching, burning or irritated eyes. A study showed association between nasal polyps and skin reactivity to Candida albicans in patients exposed to indoor pollution (Burge and Rogers, 2000). Exposure to air borne fungal spores is associated with persistent cough in infants whose mothers had asthma (Bush, 2008). Mucous membrane irritation syndrome is characterized by symptoms such as rhinorrhea (running nose), nasal congestion and sore throat, and irritation of nose and eyes. This syndrome is common not only in agricultural environments, but also found in people exposed to damp buildings (Lanier et al., 2010).

An allergen exposure increased the chances of allergic sensitization and was a risk factor for an early asthma onset as well as enhanced disease severity (Jaakkola et al., 2002; Dukiewicz et al., 2002). In a study of the patients with a history of respiratory arrest, 91% had positive skin prick test for Alternaria alternata whereas that proportion was only 31% for the 99 matched control subjects with asthma and no history of respiratory arrest (Downs et al., 2001). Thus, sensitization to molds especially to Alternaria alternata may be involved in severity of asthma in children and young adults.

1.4. Hypersensitivity syndromes

Various environmental antigens in the air have been found as elicitors of hypersensitivity, including fungi. Majority of the fungi that mediate hypersensitivity are due to occupational exposures. In non-industrial, non-agricultural settings, some case reports suggested that high airborne levels of fungal particles had caused hypersensitivity where patients exhibited pneumonia-like symptoms (Fung and Hughson, 2003). Hypersensitivity pneumonitis or extrinsic allergic alveolitis are a granulomatous lung disease due to exposure and sensitization to antigens inhaled. This disease can be acute or chronic. Exposure to buildings contaminated with fungi and mycotoxin (trichothece) may develop hypersensitivity pneumonitis (Franks and Galvin, 2010).

Inhalation fevers or humidifier fever are a heterogeneous group of stimuli which result in influenza like syndrome. This is a potential problem of damp indoor environment (Cleri et al., 2007). Humidifier fever is an illness accompanied by respiratory tract symptoms and fatigue is common in industrial settings where workers are exposed to microorganisms growing in humidification systems (Gaffin and Phipatanakul, 2009). Organic dust toxic syndrome (ODTS) is a noninfectious illness after inhalation of heavy organic dust (mixture of fungi and bacteria) This occurs within few hours after exposure to dust and symptoms are similar to hypersensitivity pneumonitis but are not due to immune response. This problem is common in workers handling material contaminated with fungi (Jacobs and Andrews, 2003).

1.5. Respiratory infections

Exposure to a variety of fungi such as Aspergillus spp. and Fusarium spp. may result in serious respiratory infections in immunocompromised persons (Boyacioglu et al., 2007; Varani et al., 2009; Jain et al., 2010; Hedayati et al., 2010; Uztan et al., 2010). People with impaired immune system who spend most of their time in indoor environments contaminated by fungi may develop serious fungal infections (Marcoux et al., 2009; Wang et al., 2010a,b). Chronic obstructive pulmonary disease, asthma, cystic fibrosis are disorders among persons potentially infected with Aspergillus (Baxter et al., 2011). In cystic fibrosis or asthma patients, Aspergillus spp. can develop allergic broncho pulmonary aspergillosis, invasive or semi-invasive pulmonary aspergillosis and pulmonary aspergilloma (Kawel et al., 2011).

1.6. Rheumatologic and other immune diseases

Rheumatic diseases are due to inflammation and stiffness in muscles, joints or fibrous tissue. These diseases are exacerbated by environmental conditions, which include dampness, fungi, and their products indoors (Breda et al., 2010). Systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Sjogren’s syndrome and psoriatic arthritis are found in persons who work in water damaged buildings with microbial growth which include molds (Muise et al., 2010). The effect of inflammatory marker in blood of non smoking persons in homes with high (>4 ng/m²) airborne concentrations of (1–3)-β-D-glucan indicate mold exposure (Pedro-Botet et al., 2007; Kalyoncu, 2010).
1.7. Allergy

The major allergic diseases caused by fungi are allergic asthma, allergic rhinitis, allergic sinusitis, broncho pulmonary mycoses, and hypersensitivity pneumonia (Pieckova and Wilkins, 2004). Concern regarding human exposure to fungi in indoor environments is mainly related to direct mucosal irritation and elicitation of an IgE-mediated hypersensitivity response that precipitates rhinitis and upper airways irritation, eye irritation, and sinusitis that characterize allergic syndrome (Jaakkola et al., 2002; Yike, 2011). The symptoms of allergy are not manifested until sensitization, in which an individual is repeatedly exposed to an allergen. During this process, antigen specific IgE is produced that attaches to receptors on mast cells which are concentrated on gastric and respiratory mucosa. The principal fungal allergens are either cell wall components (1–3)-β-D-glucan or water soluble glycoproteins. These allergens become airborne when these materials are aerosolized (Katz et al., 1999).

A link between respiratory exposure to fungal material and seasonal allergy was first proposed in 1873 by Blackley who listed 106 fungi genera including members who elicited allergy (Blackley, 1873). Allergenic enzymes are produced upon germination of certain fungal spores and exposure to these compounds resulted from inhalation of germinable propagules, followed by germination on upper respiratory tract mucosa. Allergenic cross-reactivity is a consequence of correlated exposures because mites may occur together with fungi on water damaged indoor materials (Lander et al., 2001). In addition, mite fecal pellets often contain large numbers of intact and partially degraded fungal spores because these materials are a preferred food for many dust borne mite taxa (Portnoy, 2003; Santilli and Rockwell, 2003; Khan et al., 2009). After exposure to fungal spores or mycelial particles, susceptible individuals may develop nasal allergy commonly called as – hay fever or allergic rhinitis (Husman, 1996). The symptoms of fungi induced allergic rhinitis are usually indistinguishable from those caused by inhalation of pollen, dust, animal danders, and insect allergens (Savilahti et al., 2010).

1.8. Neuro psychiatric problems

People who inhabit moldy buildings were reported with cognitive defects and difficulties in concentration (Yates et al., 1986; Drappatz et al., 2007). S. chartarum and Aspergillus spp. were identified in air samples when occupants of buildings were checked for neuro psychological tests (such as Grooved pegboard test and Verbal learning test) (Otto et al., 1990).

2. Fungal constituents of indoors

2.1. Volatile fungal metabolites (VFM)

During exponential growth, many fungi release VFMs as products of secondary metabolism. These compounds comprise a great diversity of chemical structures including, ketones, aldehydes and alcohols (Wilkins et al., 2003). Cultural studies of some common household fungi suggest that the composition of VFM’s remain stable over a range of growth media and conditions (Nilsson et al., 2004; Moularat et al., 2011). Determination of VFMs has been suggested as a measure of fungal contamination monitoring in grain storage facilities (Weir, 2000). Limited evidence suggests whether exposure to low concentrations of VFMs may cause respiratory irritation independent of exposure to allergenic particulates (Weinhold, 2007).

Volatile organic compounds may also cause indirect metabolic effects. A well-known example of this is the fungal degradation of urea formaldehyde foam insulation (Shinoj et al., 2011). Fungal colonization of this material results in the cleavage of urea from the polymer releasing formaldehyde, contributing to a decline in indoor air quality (Kreja and Seidel, 2002; Asan et al., 2010). VOCs may have strong and unpleasant odors and exposure to these VOCs has been linked to symptoms such as headache, nasal irritation, dizziness, fatigue and nausea (Burton et al., 2008). The gas chromatography – mass spectrometry (GC–MS) is used for chemical analysis of air samples to assess volatile organic compounds produced by fungi as suitable markers which correlate with fungal growth (Bornehag et al., 2005).

2.2. (1–3)-β-D-glucan

This is a cell wall component of filamentous fungi and yeasts. In moisture damaged building materials, (1–3)-β-D-glucan levels are found in the range of 2.5–210 μg/g (Rylander and Holt, 1998; Rylander et al., 1998; Wan et al., 1999). The mean concentrations of 1.55–2.22 μg/g (1–3)-β-D-glucan in dust are positively related to the culturable fungi isolated from buildings (Wan and Li, 1999). (1–3)-β-D-glucan may cause inflammatory airway reactions and also affect the immune system when inhaled (Rylander and Lin, 2000; Fogelmark et al., 2001). The biological activities of (1–3)-β-D-glucan include host-mediated anti tumor activity, adjuvant effects, activation of neutrophils, eosinophils, macrophages and complement (Walinder et al., 2001). There is the increasing evidence that (1–3)-β-D-glucan causes non-specific inflammatory reactions (Beijer et al., 2002; Rylander et al., 2010; Tercelj et al., 2011). The (1–3)-β-D-glucan is responsible for bioaerosol-induced respiratory symptoms observed in both indoor and occupational environments (Srikanth et al., 2008; Dhouwes, 2003). (1–3)-β-D-glucan levels are readily detected in house dust samples and the presence of textile floor coverings is strongly associated with increased levels of (1–3)-β-D-glucan (Mork, 2002; Reponen et al., 2010; Rylander, 2010; Sykes et al., 2011).

2.3. Ergosterol

This is the most important sterol found in the cell membranes of fungi (Hyvarinen et al., 2006). The presence of ergosterol in the indoor environment indicates fungal contamination. The content of ergosterol in spores differs between different fungal species and cannot be considered as a good marker. <62 μg/g of ergosterol in house dust indicates that the counts of fungi are very high (Park et al., 2008; Heinrich, 2011). Quantitative measurement of ergosterol in fungal biomass in indoor environments by gas chromatography – mass spectrometry (GC–MS) provides a measure of total fungal matter (Cone, 1998).

2.4. Mycotoxins

These are non volatile, secondary metabolites of fungi. Routes of mycotoxin exposure include – inhalation, ingestion or skin
contact. The most well documented mycotoxins in indoor environments are aflatoxins, trichothecenes and ochratoxins (Kilburn, 2004; Zain, 2011). Humans may be exposed to these toxins by airborne or toxin containing spores in agricultural settings or moldy buildings (Vojdani et al., 2003a; Gottschalk et al., 2008). Aflatoxin B1 is the most thoroughly studied mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus and is one of the most potent carcinogens (Thacker, 2004). A. flavus and A. parasiticus are not commonly found on building materials or in indoor environments (Etzel, 2001; Bhetariya et al., 2011).

Much of the information on the human health effects of inhalation exposure to mycotoxins comes from studies done in the work place. Human health effects attributed to inhalation of mycotoxins include: mucous membrane irritation, skin rash, nausea, immune system suppression, acute or chronic liver damage, acute or chronic nervous system damage, endocrine effects and cancer (Olsen et al., 1988; Karunasena et al., 2010). The presence of fungi in a building does not necessarily mean that mycotoxins are present or they are in large quantities. Exposure to mycotoxins can occur by inhaling airborne particulates containing mycotoxins, including dust and fungal components (Vojdani et al., 2003b; Halios and Helmis, 2010).

Toxigenic fungi have been isolated from building materials and air samples in buildings with moisture problems, where the residents have suffered from nonspecific symptoms possibly related to mycotoxin production, such as cough, irritation of eyes, skin, respiratory tract, joint ache, headache and fatigue (Gottschalk et al., 2008; Bonetta et al., 2010). Few studies have established a casual relationship between mycotoxin exposure and building related illnesses (Kuo et al., 2008; Sen and Asan, 2009; Giulio et al., 2010; Asan et al., 2010). Even though some fungi can grow on almost any natural or synthetic construction material, mycotoxin production occurs preferentially on materials that allow these fungi to grow and provide the conditions for mycotoxin production (Soroka et al., 2008). Analysis of mycotoxins from contaminated materials, such as a dry wall, carpet or house dust can be done by GC–MS or liquid chromatography – mass spectrometry (LC–MS) (Chiao et al., 2002).

3. Factors influencing fungal colonization

Moisture, nutrients and temperature are the most important factors that influence the growth of fungi on building materials (Rajasekar and Balasubramanian, 2011). Availability of water is expressed in terms of water activity (aw). The requirement for moisture depends on the fungal genus or species. Usually fungal growth is favored at aw of 0.95–0.99, while 0.65–0.90 and 0.88–0.99 are reported to be required for the growth of xerophilic fungi and yeasts (Leong et al., 2011). Nutrients in house dust and water favor fungal growth on building materials. Fiberglass, galvanized steel accumulated with dust or lubricant oil residues, allows the growth of fungi (Kennedy et al., 2004; Rene et al., 2010; Yau and Ng, 2011). The temperature in buildings of about 20–250 °C promotes the growth of mesophilic fungi. However, the temperature below optimum level slows down the growth of fungi. pH range of 5–6.5 in building materials allows the best growth of most of the fungi (Vacher et al., 2010; Hoang et al., 2010). Sufficient light and oxygen are also critical for the growth of fungi in indoor environments (Zadrazil et al., 1991; Airaksinen et al., 2004; Voisey, 2010). Separate collections of organic as well as non-organic house hold waste is a common practice in many countries (Curtis et al., 2004, 2005). This often involves indoor storage of organic waste, including fruits, vegetables and food remain in apartment buildings in densely populated areas until they are disposed off. As a result, decomposition of organic waste may begin inside the waste bin and may act as a source of fungal spores inside the house (Husman, 1996).

4. Quantitation of fungi

In non culture based methods, microscopic counting of spores or cells can determine fungi in the samples (Table 2). Light, epifluorescence and scanning electron microscopy are used for identification of fungi. The choice of microscope type depends on sample preparation. Light microscopy provides the basis for morphological identification (Moularat et al., 2008; Krause et al., 2003). Components or metabolites of fungi can also be used to quantitate fungal population in an environment. Extra cellular polysaccharides can be detected by specific assays for partial identification of fungal genera in indoor environments (Jovanovic et al., 2004). Polyclonal antibody based assays detect a broad range of fungal antigens but cannot detect the spores (Mitchell et al., 2007). Molecular methods for quantitation of fungi include the use of genus/species specific probes, Polymerase chain reaction (PCR) based methods, Restriction endonuclease analysis and Karyotyping (Dean et al., 2005). Mitochondrial DNA (mt DNA) can be used for restriction enzyme analysis and DNA fingerprinting for fungal identification.

5. Sampling methods

For isolation of fungi, surface and air sampling techniques are used (Asadi et al., 2011). Bulk sampling of materials such as settled dust, pieces of wall board, duct linings, carpets etc.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Methods of quantitation of indoor fungi.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Assessment</td>
</tr>
<tr>
<td><strong>Culture based</strong></td>
<td></td>
</tr>
<tr>
<td>Air Sampling Impactor</td>
<td>Organisms are collected on culture medium</td>
</tr>
<tr>
<td>Liquid impinger</td>
<td>Organisms are collected in collection fluid</td>
</tr>
<tr>
<td>Air filtration</td>
<td>Organisms are collected on filter</td>
</tr>
<tr>
<td><strong>Non Culture based</strong></td>
<td></td>
</tr>
<tr>
<td>Air sampling</td>
<td>Microscopy</td>
</tr>
<tr>
<td>Liquid impinger</td>
<td>Simple microscopy</td>
</tr>
<tr>
<td>Air filtration</td>
<td>Electron microscopy, Flow cytometry, Fluorescent in situ hybridization (FISH), Fluorechrome labeled nucleic acid probes, Ergosterol or Fungal extracellular polysaccharides, GC–MS Specific enzyme immunoassays, Volatile fungal metabolites (1–3)-β-glucan mycotoxins</td>
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</tbody>
</table>
are tested to determine the contamination with biological agents (O’Meara and Tovey, 2000; Reponen, 2011). Suction devices are used to collect samples of loose materials like carpet dust. Surface sampling is used to confirm the nature of the suspected microbial growth on environmental surfaces to measure the relative degree of contamination and identify the types of present fungi (Cabral, 2010). In this approach, samples are collected by pressing contact plate or adhesive tape onto a surface, suction device and wet swab. Therefore surface sampling can be of four types that is - contact sampling, agar contact sampling, adhesive tape sampling and surface wash sampling (Yamamoto et al., 2011). Adhesive tape sampling is an important method to examine the fungi in the specimens using a compound microscope. The samples provide the hyphal fragments and the reproductive structures which may help for identification (Ahlen et al., 2003; Aydogdu et al., 2010).

Air sampling for fungi can be done by three standard methods including: impactor, liquid impinger, and air filtration methods (Table 3).

In the impactor method, the air stream is passed through a slit into a culture medium and adhesive microscopic slide or tape strip is used to collect the sample (Zhen et al., 2009). Slit samplers, single stage impactor, multistage impactor, Burkard, rotorod, Andersen, SAS, casella, sierra marple impactor and centrifugal samplers are the common impactor samplers used. The air flow rate is about 2–180 L/min (Engelhart et al., 2007). Liquid impingers collect the samples directly into the fluid and the microorganisms are retained in the liquid until they are cultivated on media or evaluated by techniques like biochemical or immuno assays (Jo, 2011). Shipe sampler, AGT–30, midget, multistage and micro impingers are common impinger devices. The air flow rate is 0.1–55 L/min and the sampling time ranges from minutes to hours. Centrifugal samplers such as RCS, aerojet cyclone are devices with 40–100 L/min air flow rate (Gralton et al., 2011).

Among the three standard methods, air filtration is used to collect the samples of indoor air in volume. In this method after sampling, the filters are agitated or sonicated in a solution (Bazaka et al., 2011). The solution is used for the cultivation of microbes or examination with analytical techniques. In air filtration sampling, glass, cellulose ester, polycarbonate and Teflon filters are used. The air flow rate for this sampling is 1–1000 L/min (Muijlenberg, 2003). Tilak air sampler is a modified Panzer’s slide spore collector. The air is sucked through a projecting tube at the rate of 5 L/min and passes onto a transparent cello tape on the slowly rotating drum (Tilak, 1986; Frazer, 1998; Moulaarat and Robine, 2006).

Gravitational settling is a much earlier approach to collect the particles that settle passively on the open Petridish containing the growth medium (Bartlett et al., 2004; Cook et al., 2011). The choice of sampling technique and exposure assessment depends on the purpose of measurement. Air sampling as well as samples of settled dust, surface and contaminated material is used to monitor the environment (Gabrio et al., 2003; Jung et al., 2011).

6. Practices contributing to indoor biotic pollution

The habit of switching off Air Conditioning (A/C) units is a very common practice to save electricity during off business hours (Hsu et al., 2011). This may lead to condensation of water and rise in relative humidity and temperature favoring fungal growth. In rooms where the A/C units are switched off for long periods of time, frequent cleaning of the A/C filters or rooms is necessary (Yau et al., 2011). It is advised to keep the A/C units switched on continuously. To conserve energy, the temperature can be set or programed as per the manufacturer (Hibbett et al., 2011).

A/C filters need to be replaced or cleaned periodically since the filter can be clogged due to dust load and fungal infestation (Ruping et al., 2011). Potted plants kept in A/C rooms may be a risk factor for the residents since soil may act as a reservoir of fungi (Guieysse et al., 2008). Isolation of pathogenic fungi from soils of potted plants kept in A/C rooms is reported (Robbins et al., 1999; Haas et al., 2007). Places where carpets are furnished need periodic shampooing and vacuum cleaning is necessary since carpets can be home to dust borne fungi (Khan and Karuppayil, 2011).

7. Control and precautions

Spore infiltration from outside can be reduced by closing the outlets and using air conditioning for cooling. In one study, the use of window air conditioner with the vent closed showed effective exclusion of spores (Ayoko et al., 2004). The most effective way to manage fungi in a building is to remove the conditions that favor the establishment and growth of fungi (de Blay et al., 2000; Khan and Karuppayil, 2010).

Elimination of growth can be achieved by avoiding available moisture (Barnes et al., 2007). The steps to reduce moisture include, maintenance of indoor relative humidity to less than 50%, sealing the leaks to prevent water intrusion, increasing bathroom and kitchen ventilation, vent cloth dryers to be kept outside, to keep house plants that are watered regularly healthy, keeping the moisture sensitive materials dry, use of dehumidifier in the basement, etc. (Cole and Cook, 1998).

Carpet increases the fungal levels, hence frequent vacuum cleaning may reduce the spore levels (Ferguson et al., 2009). When a carpet is extensively contaminated cleaning may be difficult and it must be replaced with hardwood, tile or firm flooring materials (Ewers et al., 1994; Khan and Karuppayil,

### Table 3 Commonly used air sampling methods for indoor fungi.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sampler</th>
<th>Air flow rate (L/min)</th>
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<tbody>
<tr>
<td>Impactor</td>
<td>Slit</td>
<td>2–180</td>
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<tr>
<td></td>
<td>Single stage</td>
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<tr>
<td></td>
<td>Multi stage</td>
<td></td>
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<tr>
<td></td>
<td>Burkard</td>
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<td></td>
<td>Rotorod</td>
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<tr>
<td></td>
<td>Andersen</td>
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<td></td>
<td>SAS</td>
<td></td>
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<tr>
<td>Liquid impingers</td>
<td>Shipe</td>
<td>0.1–55</td>
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<tr>
<td></td>
<td>AGT–30</td>
<td></td>
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<tr>
<td></td>
<td>Midget</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multi stage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Micro</td>
<td></td>
</tr>
<tr>
<td>Centrifugal</td>
<td>RCS</td>
<td>40–100</td>
</tr>
<tr>
<td></td>
<td>Aerojet cyclone</td>
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</table>
Washable wall papers and paneling can be treated with fungicidal compounds or antimicrobial products. Wall paper or paneling may be removed to reduce the severity of fungal contamination. Contaminated air ducts and filters may be cleaned to reduce fungal prevalence (Burr et al., 2007). Elimination of sources of indoor air borne pollutants can be achieved during the design phase of a new building, but it will be difficult in an existing building (Lange et al., 2004). The selection of building materials, finishes, furnishings and construction techniques are effective approaches to reduce the sources within a building (Menetrez et al., 2008).

To ensure clean air in the indoors - heating, ventilation and air conditioning system must drive out stale air and replenish it within a building (Gosden et al., 1998). Varying climatic regions demand different thermal performance and conditioning within a building (Buckmaster, 2008). Diffusers and grills should be placed at opposite ends of buildings and be free from any obstructions that may result in the block of air flow into the building (Foarde and Menetrez, 2002). Effective air filtration ensures clean indoor air (Ahmad et al., 2001). Impingement, electronic and adsorption techniques are the three common air filtration technologies designed to get clean air (Escombe et al., 2009). Particulates are removed from the air by impingement and electronic air filters. Adsorptive type of filters eliminate unwanted gases present in the air (Garrison et al., 1993). Dry panel filters, Extended surface (dry) filters, High efficiency particulate air filters (HEPA), Bag filters and Charged media filters are different types of impingement and electronic filters (Hahn et al., 2002).

Regular cleaning prevents the accumulation of debris and particulate matter. Sometimes the cleaning products may also cause indoor air pollution, especially when the products are chemicals or solvent based (Myatt et al., 2008). Hence the use of non toxic cleaning solutions is recommended (Williams, 2004). The cleaning schedule should be during weekends or during periods when the building is not occupied. The maintenance of the HVAC system is very important, as poorly maintained HVAC systems may cause occupant discomfort and illnesses (Manuel, 2004; Gorny et al., 2007). In humidification systems, water drains and drip pans should not become stagnant to avoid air contamination. Portable vacuum systems can be potential sources of airborne particulate matter (Oren et al., 2001). The smaller, more harmful particulates may pass through them and be suspended in the air. Hence central vacuum systems which expel particulate matter to the exterior are the best alternatives (Buemi et al., 2000). A person may get sensitized to fungal spores while cleaning hence well fitted particulate mask with 1 μm particle retention should be used (Gage-White, 1998). Use of mask can avoid fungal allergy during the handling of compost, vacuuming and cleaning (Warsco and Lindsey, 2003; Rengasamy et al., 2004).

There is a renewed interest in the use of germicidal treatment or irradiation to disinfect indoor environments for the control of infectious diseases in hospitals, other health care facilities and the public sector (Cardenas et al., 2008). It has been known for many years that UV light has various effects on fungi (Levetin et al., 2001). Only a few studies have specifically focused on the effects of germicidal UV light. Currently various manufacturers are marketing germicidal UV lamps for controlling contamination, including fungal contamination in indoor environments, as well as Air Handling Units (AHU’s) and ducts (Menzies et al., 1999; Alangaden, 2011).

Control of fungi in the indoor environments has traditionally focused on identifying the source of contamination control, use of filters, cleaning etc. Generally glutaraldehyde, formaldehyde and phenol derivatives such as cresol are used as disinfectants of the floors (Robertson et al., 1942; Weber et al., 1999). Glutaraldehyde shows high toxicity and its vapors irritate eyes, nose and throat (Samimi and Ross, 2003). Formaldehyde stimulates irritation of mucosa and is also reported as a carcinogen. Cresol is less toxic but extensive use may be harmful (Menetrez et al., 2007).

High toxicity and offensive odor of common disinfectants make their use restricted; there is a need for disinfectants which are harmless. There is considerable interest in plant extracts and molecules of natural origin (Khan and Karuppayil, 2010). Biologically active components from plants are reported to eliminate pathogenic microorganisms. A study on antimicrobial activity of vapors of aroma compounds was done to evaluate the practical applications in the indoor environment to reduce microbial count in air. Cinnamaldehyde vapors were reported as strong antimicrobials against airborne microbes (Sato et al., 2006). Essential oils and components of plants are good candidates for the inhibition of growth of environmental isolates. Plant extracts are generally assumed to be more acceptable and less hazardous than the synthetic disinfectants which have similar action (Burt, 2004; Khan and Karuppayil, 2010).

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