



## Biological pollutants in indoor air

## Biološki zagađivači u zatvorenom prostoru

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### Introduction

Interest in indoor air quality emerged in the USA in the 1970, when “sick building syndrome” was first described<sup>1</sup>. During those years, due to energy crisis, building environments substantially changed – namely, ventilation, air-conditioning, and other energy-demanding maintenance processes were sparingly used, particularly in public office buildings. Many office workers reported headache, mucous membrane irritation, and difficulty in concentrating during working hours. All symptoms disappeared at home. Understanding of risk factors underlying this epidemic led to improvement of legislation regarding ventilation rates and maintenance, so the number of complaints decreased in the 1990s.

Indoor air pollution is present in virtually each and every indoor space, with the exception of strictly controlled and sterile spaces in pharmaceutical, medical and research facilities. Biological pollutants may originate from human activity, building materials and carpets; they may also penetrate from outdoor environments by forced ventilation, diffusion or infiltration. Humans are inevitably exposed to such pollutants, considering the amount of time spent indoors, but the influence of the pollution on human health may vary, depends on age, health condition, and individual predisposition. Historically, moulds were the very first recognized and recorded contaminants in indoor environment. In Bible, there are several passages where the moulds are mentioned, together with possible health aspect of exposure to them<sup>2</sup>.

Interest in indoor air monitoring is growing worldwide every year, considering the indoor pollution ranged among the first five leading causes of illness in the world<sup>1</sup>. The World Health Organization (WHO) Regional Office for

Europe prepared in 2006 the Guidelines for indoor air quality<sup>3</sup>, according to previously formulated postulate “The right to healthy indoor air”. In these Guidelines, the WHO presents public health risks due to dampness, associated microbial growth and contamination of indoor air. This organization recognized problems of indoor air quality as important risk factors for human health all over the world, regardless the annual income of given country. The importance of this problem is emphasized by the fact that people, particularly vulnerable populations such are children, pregnant women, elderly, ill and disabled, spend a substantial amount of time indoors.

There is a wide range of possible biological contaminants in indoor air, with different origin and patterns of spreading. For example, pollen and spores of plants are predominantly emitted from outside the building, transferring through doors and windows, or by personal contacts. Various species of bacteria, fungi, algae, and protozoa can originate both from outside space and from materials inside the buildings. According to conclusions of a WHO working group<sup>3</sup>, there are no specific microorganisms that can be specifically associated with indoor air pollution; rather they represent common allergens and other pathogens. However, some agents such as house dust mites and pet hairs are predominantly present in indoor air. Considering the variety of microorganisms and their characteristics, it is virtually impossible to quantify their concentrations in a form of tolerable levels of exposure.

Many studies have found that health risks are increased by exposure to microorganisms, but there is growing evidence that exposure in early life to endotoxins and/or fungal agents protects against atopy and allergic disease. A prospective birth cohort study suggested an inverse relation

between the levels of these pollutants and wheezing problems in 4-year-old children<sup>4</sup>. These results are in agreement with findings obtained from several studies of reduced incidence of hay fever, eczema and asthma in children who grew up on farms compared to urban children, and thus supported the “hygiene hypothesis” which suggests the protective role of microbial exposure.

### Effects of dampness on indoor exposure to biological pollutants

Besides the WHO Guidelines mentioned above, another major reviews published in 2004 by the Institute of Medicine (IOM) report on a wide range of health effects of which there were sufficient evidence for associating the presence of pathogens in damp buildings with following diseases and symptoms: nasal and throat symptoms, cough, wheezing, asthma exacerbation, and hypersensitivity pneumonitis<sup>5</sup>. The IOM committee concluded that limited or suggestive evidence existed for associating the same exposure with shortness of breath, asthma development, and lower respiratory disease. Building dampness and mould are present even in high-income countries. Estimations of dampness and mould presence vary from 20% buildings in Scandinavia<sup>6</sup> to 50% buildings in United States<sup>7</sup>. Fewer studies were conducted in low-income countries; nevertheless, they suggest that the problem of indoor dampness is even greater<sup>8</sup>. The dampness and mould are traditionally related to overcrowded accommodations without adequate heating, ventilation and insulation, hence, the lower income is – these problems are more evident. Climatic changes such as global warming with more frequent occurrence of storms and heavy rains lead to gradual increase in sea level. Together with more frequent floods, it results in the increase in the percentage of buildings affected by dampness and mould, particularly in the areas near the rivers. Increased indoor dampness provides optimal conditions for increased growth of dust mites, fungi and bacteria. Furthermore, chemical contamination is promoted too, because dampness accelerates the degradation of building materials releasing their particles into the air. Finally, excess moisture in indoor spaces creates optimal conditions for insects and rodents. These animals release their own allergens into indoor environments, but can also be the reservoir of contagious diseases agents.

Indoor air contains numerous microorganisms of very different types. For example, house dust mites are small arachnids. Among numerous various species, few are of major importance for indoor air contamination, and their growth is directly related to relative humidity. Moreover, in house dust mites living in mild and temperate climatic conditions, moisture represents a major factor of their increased growth. For survival, development and multiplication, they require a relative humidity in excess of 45–50%, but their activity, including feeding and maturation is more rapid at higher rates of relative humidity which was confirmed in field studies<sup>9,10</sup>. The common foods for house dust mites are skin scales, but they are adapted to use other food sources, as well. House dust mite allergens are commonly produced by

*Dermatophagoides pteronyssinus* (proteolytic allergens Der p I and Der p II), and *Dermatophagoides farina* (Der f I). The faecal particles containing these allergens are predominantly found in house dust, mattresses and pillows<sup>11</sup>.

Other common indoor air pollutants are fungi. Their presence in indoor air is a result of transportation from outside environment *via* building materials, carpets, furniture, wallpapers, etc. Ventilation and air-conditioning systems are another common ways of penetrating of fungi into the buildings. The rate of further growth, spreading and multiplication depends exclusively on moisture content in indoor air, regardless the type of surface. Even the primary colonizers, or xerophilic fungi, which may grow on less moisture surfaces, require relative humidity in excess of 50%. Secondary colonizers require more humidity in their substrates, while tertiary colonizers, or hydrophilic, need sheer water content in liquid phase for their germination and mycelia growth<sup>12</sup>; hence, they are present only in buildings with severe condensation problems. Natural food source for fungi vary from plant, animal and human particles in house dust, to fragments of construction materials such as floor and wall textile coverings, furniture, residue of cooking traces, food storage, paper materials. Since these materials are in ample in every building, and considering that optimal temperature for fungi growth ranges from 10–35 °C, the only limiting factor for development of fungi and mould contamination is dampness. Fungi may be extremely harmful for human health, but may also destruct the building itself, particularly wooden parts, such as roofs, timbers, and other materials.

Some fungi species produce strong allergens, which initiate immune reaction type I (IgE mediated). For example, the indoor contamination with *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium* spp., is related to asthma and other allergic respiratory diseases. Some of these species, such as *Penicillium* and *Aspergillus* can also induce type III allergy (IgG mediated), while at high concentrations, may also initiate combined type III and IV reaction manifested as hypersensitivity pneumonitis. Major fungal allergens are isolated and identified (such as Cla h I from *Cladosporium herbarum*, Alt a I and Alt a II from *Alternaria alternata* and Asp f I and Asp f III from *Aspergillus fumigatus*). Most of them are glycopeptide enzymes, produced during germination and released through spores and hyphae, i.e. live particles<sup>13</sup>. Nevertheless, even dead particles carry substantial health risk, because they may contain possibly harmful (1→3)-β-D-glucans with the potential to impair respiratory functioning<sup>14</sup>, and mycotoxins. The harmful effect of mycotoxins is manifested by interference with RNA synthesis leading to DNA damage. Sometimes this toxicity is beneficial – e.g. penicillin, a strong bactericidic antibiotic, is a mycotoxin produced by fungi *Penicillium*. But, in general, fungi mycotoxins have strong genotoxic, cancerogenic, and immunotoxic potential. The cancerogenic effects of aflatoxin (mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*) are well known. The most important mycotoxins related to indoor air contamination are trichothecenes, generated by fungi *Stachybotrys chartarum* (macrocytic trichothecenes, trichodermin, sterigmatocystin and satratoxin G)<sup>15</sup>.

Several fungi also produce volatile organic compounds as the result of their metabolic processes, but their effects on human health are yet to be investigated. The assessment of fungi contamination in indoor air is very difficult. In a study conducted by Pietarinen et al.<sup>16</sup> culture methods identified only few of species that were recognized and quantified by quantitative polymerase chain reaction (qPCR). *Penicillium*, *Aspergillus* and *Streptomyces* were predominantly identified by both methods. But, culture method successfully identified *Aspergillus fumigates* only in samples containing the amount of total viable fungi more than  $10^6$  cfu/g. Likewise, culture method was able to detect *Stachybotrys chartarum* only in samples with a very high level of fungi contamination, contrary to qPCR method. These results are in agreement with another Finnish study which confirmed the highest prevalence of *Penicillium/Aspergillus* species in house dust, with more precise results obtained by qPCR method<sup>17</sup>. The same authors indicated that concentrations of fungi differ significantly between the seasons with the highest concentrations of *Aspergillus* in winter (more than 10,000 cells/mg of dust).

Numerous species of bacteria are also common contaminants of indoor air. Contrary to relatively harmless saprophytic species originated from people, the species that actively grow in indoor substrates may be potentially harmful. Although the health aspects on moulds and fungi in indoor air are extensively studied, similar investigations of bacteria influence have been of little interest so far. The common feature for both types of microorganisms are requirements for water and temperature ranges for optimal growth and development. Hence, we can fairly assume that bacteria grow in the same sites as fungi, preferably on damp substrates. This suggestion is confirmed by evidences that species such as *Streptomyces*, which are not normally present in indoor environments, easily grow on wet surfaces, so their presence is used in screening for moisture problems in buildings<sup>18</sup>. Very few studies were conducted so far regarding this problem, apart from several investigation conducted by Finnish authors who identified *Streptomyces* and *Mycobacteria* in indoor surfaces<sup>16, 19-21</sup>. The latter bacteria have particularly strong immunogenic potential originated from cell wall components. The majority of culturable bacteria in indoor dust and air are Gram-positive *Micrococcus*, *Staphylococcus* and *Bacillus* strains. Similarly to fungi, there is a certain doubt regarding the method for determination of bacterial load in house dust. Culture method is relatively simple, but only 1% of airborne bacteria in indoor air are culturable. Culturable bacterial concentrations range from  $7.3 \times 10^4$  to  $1.85 \times 10^7$  cfu/g (public buildings) and  $1.1 \times 10^4$  to  $2.1 \times 10^7$  cfu/g in samples of house dust<sup>15</sup>. Chemical markers analysis, i.e. detection of chemical compounds that build the bacterial cell wall (3-hydroxy fatty acids for Gram-negative bacteria

and muramic acid for Gram-positive bacteria), has limited value, since these compounds are non-specific, and the gas chromatography-mass spectrometry method requires complex sample preparation. Simultaneous usage of all the three methods, as reported by Karkkainen et al.<sup>21</sup>, reveals only a moderate correlation between them. Another study conducted in Finland also indicated that culture method failed to detect *Aspergillus fumigates*, while qPCR in the same samples detected the average of  $2.21 \times 10^3$  cells/g. The average concentrations of *Penicillium spp.* and *Aspergillus spp.* were significantly lower when detected by culture method than qPCR ( $9.01 \times 10^3$  cfu/g vs  $1.96 \times 10^5$  cells/g and  $1.35 \times 10^4$  cfu/g vs  $5.44 \times 10^6$  cells/g, respectively)<sup>19</sup>.

Finally, protozoa may also be present in indoor air in damp buildings. Yli-Pirila et al.<sup>22</sup> detected amoebae in 22% of 124 samples of various materials collected from buildings with evident moisture damage; among them there were 11 samples (collected from the most severely damaged surfaces) contained ciliates and flagellates. Field studies on the presence and concentrations of protozoa in indoor air, as well as health aspects of these microorganisms in given conditions are still lacking, with the exception of one *in vitro* study conducted by the same authors, who suggested that amoebae act synergistically with certain bacteria, enhancing their cytotoxic and proinflammatory potential<sup>23</sup>.

## Conclusion

Epidemiological, clinical and toxicological evidences suggest that microbiological contamination of indoor air may be related to numerous diseases and health conditions. Damp and humid environments are obligatory factors for growth, development and multiplication of microbes, hence, the main public health goal should be targeting these problems. Considering the variety of microorganisms, possible synergistic effects, the fact that the most endangered populations are children, women, elderly (who spent relatively substantial time indoors), disadvantages of determination techniques and lack of evidence-based risk assessment, it should be concluded that further investigations are needed.

In Serbia, the very first study on the presence and concentrations of biological pollutants in indoor air is ongoing, financially supported by the Ministry of Education, Science and Technological Development. The first results expected to be available within 2014.

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