REVIEW ARTICLE

Biomonitoring the indoor environment of agricultural buildings

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Abstract

Introduction. Agricultural hygiene and biomonitoring helps protect people, livestock and crops from pests and disease, including insects, parasites, pathogens and weeds. Optimising the health of animals and crops increases productivity, minimises animal suffering, and ultimately protects human health by ensuring that foodstuffs are safe for consumption. A healthy farm environment also protects the health of the agricultural workers. Ensuring hygiene and health protection is one of the basic construction requirements. Such requirements are examined when commissioning new constructions and examining defects in constructions already in use. One substantial defect is biocorrosion which represents a synergistic process with a complex variety of factors, caused by biochemical manifestations of various micro-organisms (micromycetes). Micromycetes producing mycotoxins therefore play an important role regarding the so-called 'Sick Building Syndrome' (SBS) that has become a global problem nowadays. Therefore, agricultural hygiene and biomonitoring aims to minimise the introduction of additional pathogens and pests, as well as the spread of pathogens and pests in farm environments; this helps protect the safety of foodstuffs further down the supply chain.
Objective. The aim of the presented study is to point out the need to address indoor environment monitoring, summarizing the most commonly used methods for monitoring biological factors, and characterizing the negative effects of biological agents on humans and animals exposed to their negative effects.

Key words

agriculture, biomonitoring, building, environment, health, hygiene, industry, indoor, monitoring methods, micromycetes

INTRODUCTION

A construction, during its economically justifiable lifespan, must satisfy the basic requirements for its use [1], of which the requirements regarding hygiene, health and environment protection are among the most important. According to the applicable regulations [2], a construction must be designed and completed in such a way that the products and materials used in construction do not release harmful substances which may damage human and animal health. There is also a whole host of matters which may result in a situation where a construction, after a certain period of time, becomes unsuitable in terms of hygiene of health risks. This is caused mainly by a combination of different factors, such as underestimating the material or construction solutions applied, or its incorrect use, which, due to excessively high humidity in both the exterior and interior of the construction, causes growth of micro-organisms on the surfaces or inside constructions that may subsequently threaten the internal environment of buildings [3–8]. As a result, microbial pests appear in the internal structures, e.g. moulds (micromycetes), which due to their physicochemical activities, degrade, the materials (masonry, stone, concrete, wood, various types of plaster, paint, etc.), form which the structures are made [9–16]. The effects these degradation processes have on the health of the humans and animals using the premises are even worse. Micromycetes are producers of several organic acids which react with certain elements of building materials and decompose them. Micromycetes producing mycotoxins therefore play their role in the so-called 'Sick Building Syndrome'(SBS) [17–22] presenting a global problem nowadays. The term describes nonspecific difficulties, including upper respiratory tract problems, headaches, fatigue and rashes, which are typically associated with the residents and workers in a particular building. SBS has been increasing since 1970, when older, naturally ventilated buildings have begun to be replaced by more energy-efficient, air-tight buildings.

An increasing number of studies show that an unsuitable quality of the internal environment may cause various health risks. Biological pests are very dangerous for humans or animals [23]. Particularly, dangerous are those types of micromycetes which are potential pathogens. Many micromycetes have allergenic effects and vulnerable persons, such as those with asthma or allergies, can react to small amounts of spores acting as allergens [24]. For the further use of constructions affected in this way, an examination of their corrosion is required.

Methods of monitoring and assessing the indoor environment of buildings. The detection methods of indoor

microbes exposure assessment include a description and quantification of exposing agents. According to Lignell [25], in the case of microbes, this means that microbial communities, including various groups of bacteria and fungi, should be detected properly. Detection methods are based on culturing, microscopy after staining, assessment of total biomass of microbes using chemical markers, as well as on molecular methods. It is self-evident that different microbes can have different effects on occupants and, therefore, identification of the microbes to the genus or species level is needed.

Traditional microbial detection methods. These are based on culturing. Culturing methods underestimate the total amount of microbes present in the sample. It has been estimated that somewhere between 0.001 – 15 % of bacteria in environmental samples are culturable [26]. For fungi, the proportion of culturability can be higher – a median value of 87 % has been reported [27]. The culture medium and incubation conditions [21, 28] are 2 of the factors that further affect the results. There is no single medium that permits the growth of all microbes. Some genera, such as *Stachybotrys*, grow poorly on most media. In addition, interactions between the microbial colonies developing on the agar medium may influence the results. Rapidly growing fungi, such as *Mucor* spp., may obscure slowly growing ones such as *Wallemia* spp.. In addition, organisms present at high concentrations may inhibit the growth of less abundant species [29–30]. The indoor temperatures are usually in the mesophilic range of microbes and therefore, incubation is usually performed at 25 ± 3 °C for a duration of at least 5 days [31]. The advantages of culture include the possibility to identify the microbial genera or species in samples and to isolate the strains for further characterization. Morphology based identification is usually performed using a microscope, but also direct identification of species by image analysis using accurate digital camera is possible [32]. However, culturing is time-consuming, labour intensive, and therefore a costly technique.

In addition to culturable microbes, non-culturable and non-viable microbes are important as exposing agents because they can also cause adverse health effects by evoking allergic or toxic reactions [33–34]. Methods for detecting these microbes include microscopic techniques, bioassays, immunoassays, chemical methods, and molecular methods. Microscopic analyses, usually by epifluorescence microscopy with acridine orange staining [35], or impaction on coated slides with lactophenol staining, enable total cell counts or spore counts, respectively, but identification of fungal species is not possible [36]. Total cell concentrations of bacterial and fungal bioaerosols can be measured by flow cytometry [37–38].

Microbial communities can also be quantified by chemical methods using markers for the structural or constituent components of microbes. One advantage is that these substances can be used for characterizing and quantifying particular microbial groups. The *Limulus* amoebocyte lysate assay (LAL) is the most commonly used bioassay for endotoxin measurements. Immunoassays for specific antigens and allergens include enzyme-linked immunosorbent assay (ELISA) and the radio allergo-sorbent test (RAST) [39].

Molecular methods. Within molecular the methods in the detection of indoor microbes, the identification of specific bacterial or fungal species has developed greatly as the techniques based on DNA analyses have become available. These methods allow the specific detection of target organisms. With respect to environmental samples, a variety of these techniques has been successfully applied, including the qPCR, restriction fragment length polymorphism (RFLP) analysis, random amplified polymorphic DNA (RAPD) analysis, and multiplex reverse transcription-PCR (RT-PCR) [40–42]. These techniques serve different purposes: qPCR is used for quantitative detection, RFLP, RAPD and RT-PCR are used for, e.g., differentiation of species or strains from each other.

Air sampling methods. According to Lignell [25] and Peccia and Hernandez [43], within air sampling in general, air is an extreme and oligotrophic environment for microbes, e.g., when compared to soil. This means that special features must be taken into the account in the air sampling. Differences in the aerodynamic diameter of particles can affect the collection efficiencies of air samplers. Furthermore, sampling stress can reduce the viability or culturability of many microbes [44].

Air samplers are based on different physical principles and they can be designed for the detection of culturable or nonculturable microbes. Sampling methods for airborne particles can be subdivided into passive samplers using natural aerosol convection, diffusion or gravity, and active samplers using stationary or personal pumps [45]. Stationary sampling is the most widely used method for conducting microbial measurements in indoor environments. In addition, personal sampling has been used especially to characterize exposures during remediation [46], or to analyze total individual exposure to microbes [47]. According to some authors, passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls onto a critical surface, such as in the surgical cut or on the instruments in operating theatres [48–50]. This method is standardly used in practice in our condition. Cyclone personal samplers are also used which collect bioaerosols into microcentrifuge tubes which enable particle size fractioning [51]. Subsequently, these samples can be readily analyzed by, e.g., polymerase chain reaction (PCR) and immunoassays.

To assess airborne levels of mould spores, Air-O-Cell Cassettes and/or Anderson sampling are commonly used. Air-O-Cell Cassettes are a form of nonculturable sampling that quantifies the number of viable and non-viable mould spores in the air. The samples are taken by pulling a calibrated amount of air through the cassette. In the cassette, air passes over a microscope slide, causing the particulates (mould spores, etc.) to stick to the slide. The slide is then examined in a laboratory to determine the genera of mould spores present [52–54]. However, although there is much published research, procedures have not been firmly established, and there are still debates on the sampling techniques to be used, their frequency of application, and even on the usefulness of such checks and controls [30, 49, 55, 56]. In fact, international standards offer different techniques (active or passive sampling) and different kinds of samplers, thus leaving the choice of system open [55, 57].

Impact of mycotoxins on humans and animals. Mycotoxins by Ostrý [58] are toxic metabolites produced by certain toxigenic microscopic fungi (moulds). Mycotoxins, according to Zain [59], are a structurally diverse group of mostly small molecular weight compounds, produced mainly by the

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secondary metabolism of some filamentous fungi or moulds which, under suitable temperature and humidity conditions, and may develop on various foods and feeds, causing serious risks for human and animal health [60–63]. Mycotoxins are secondary metabolites that have no biochemical significance in fungal growth and development; however, they vary from simple C4 compounds, e.g., moniliformin, to complex substances, such as the phomopsins [64]. Currently, more than 300 mycotoxins are known, scientific attention is focused mainly on those that have proved to be carcinogenic and/or toxic.

Human exposure to mycotoxins may result from consumption of plant-derived foods contaminated with toxins, the carry-over of mycotoxins and their metabolites in animal products, such as meat and eggs [65], or exposure to air and dust containing toxins [66].

According to Zain [59], toxigenic moulds are known to produce one or more of these toxic secondary metabolites. It is well established that not all moulds are toxigenic, and not all secondary metabolites from moulds are toxic. Examples of mycotoxins of the greatest public health and agro-economic significance include *aflatoxins* (AF), *ochratoxins* (OT), *trichothecenes*, *zearalenone* (ZEN), *fumonisins* (F), *tremorgenic toxins*, and *ergot alkaloids*. These toxins account for millions of dollars annually in losses worldwide in human health, animal health, and condemned agricultural products. Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions. Ochratoxin A (OTA) is a secondary metabolite produced by several species of *Aspergillus* and *Penicillium*. The toxin, which is a nephrotoxic and nephrocarcinogenic compound, has mainly been found in cereals as well as in other products like coffee, wine, dried fruits, beer and grape juice. It occurs in the kidney, liver and blood of farm animals by transfer from animal feed. *Fusarium graminearum* and *Fusarium culmorum*, has an osteogenous action and is significantly toxic to the reproductive system of animals [67]. Other serious types of micromycetes occurring in buildings include: *Cladosporium sphaerospermum, Verticillium* sp., *Cladosporium herbarum, Fusarium verticillioides, Rhizopus* sp., *Mucor* sp.

Human food can be contaminated with mycotoxins at various stages in the food chain [68] and the most important genera of mycotoxigenic fungi are *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium* and *Stachybotrys*. The principal classes of mycotoxins include a metabolite of *A. flavus* and *Aspergillus parasiticus*, aflatoxin B1 (AFB1), the most potent hepatocarcinogenic substance known, which has been recently proved also to be genotoxic. In dairy cattle, another problem arises from the transformation of AFB1 and AFB2 into hydroxylated metabolites, aflatoxin M1 and M2 (AFM1 and AFM2), which are found in milk and milk products obtained from livestock that have ingested contaminated feed [69, 70].

CONCLUSIONS

The aim of the presented study was to stress the need to address indoor environment monitoring, summarizing the most commonly used methods for monitoring biological factors, and characterizing the negative effects of biological agents on humans and animals exposed to their negative effects. The key to agricultural hygiene is effective risk management and constant improvement of the hygiene standards at each level of the agricultural process, and as agricultural products move further along into the human food chain. Effective cleaning, disinfecting and pest control regimes are integral. Also diagnosing micromycetes in terms of type and intensity of presence is important for choosing the appropriate measures for their elimination in the internal environment of a construction, and for preventing further corrosion of building constructions.

Acknowledgement

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