Isolation and identification of filamentous fungi from HVAC system

M. Butala[a], M. Črnigoj[a], P. Zalar[a], V. Butala[b,*]

[a]*University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Ljubljana, Slovenia;*
[b]*University of Ljubljana, Faculty of Mechanical Engineering, Aškerčeva 6, Ljubljana, Slovenia*

ABSTRACT
During the research, air samples were taken by exposure of agar plates and taking smear samples from the AC equipment. Sampling took place during the autumn because the concentration of spores at that time reaches its peak. Different enumeration media for filamentous fungi were used to assure the germination of reproductive structures and inhibition of growth of fast growing saprophytes: dichloran rose-bengal chloramphenicol medium (DRBC), dichloran glycerol medium (DG18) and malt extract medium (MEA). Additional culture media were used for the identification of the fungi-colonizing environment mentioned above. In particular, the species of *Cladosporium* and *Alternaria*, which are commonly found in our everyday environment, proved that they have the ability to accumulate and grow in almost every compartment of air-handling equipment. In consideration of the results, we arrived at conclusions on how the air-quality in the working environment is affected by HVAC filtering systems. Research confirmed that the presence of fungi in the HVAC systems influences the quality of indoor air by disseminating the spores into the working environment.

INDEX TERMS
Fungi; Air-conditioning equipment; Sampling method; Identification

INTRODUCTION

Heating, ventilation and air-conditioning (HVAC) systems are used in buildings in order to maintain and to regulate the indoor environment. Under normal circumstances, a filtration system is expected to significantly reduce the level of particulates coming from outdoors, whilst the interior of the building should prevent the growth of microorganisms. HVAC systems offer a number of locations where populations of microorganisms can flourish, and can therefore be the source of their growth and dissemination. Certain opportunistic fungi can cause allergies and other health problems to individuals without a normal immune response. For this reason, such organisms should be reduced to minimum in our working and living environment.

HVAC systems are installed in buildings in order to maintain and regulate internal environment parameters, such as temperature and relative humidity, air quality. The air-conditioning system operates fully when the premises are occupied, and, in order to conserve energy, stops operating or operates at reduced power when they are empty. However, they operate sufficiently to remove pollutants from the premises. Research has shown that fungi can thrive in HVAC systems and that their dissemination through the ventilation system cannot be excluded (Neumeister-Kemp *et al.*, 1999; Pejtersen, 1999). That is why microorganisms can be transported from the locus of contamination to the occupied space and affect the indoor air-quality (Health Canada, 1995; Halonen *et al.*, 1999).

The presence of moisture and dust in indoor environments can, under the certain conditions, result in microbial contamination (Nathanson, 1996). Microorganisms, which

* Corresponding author.

include bacteria and fungi, are ubiquitous in both outdoor and indoor environments. Fungal spores, especially *Cladosporium* and *Alternaria*, are common in outdoor air during the growing season, and the most common fungi that grow on leaves constitute 60–70% of the spores in the air. Species of fungi that have the ability to grow and accumulate indoors, or in air-handling equipment, can be different from common plant and leaf fungi. Certain fungi can cause allergies and other health problems in sensitive individuals (certain toxic metabolites may participate) (http://sis.agr.gc.ca/brd/fusarium/key.html).

The variety and concentration of spores in the air is subject to continuous diurnal and seasonal variation. Contributory factors include availability of substrates which have a direct effect on the release of spores into the air. In the context of microbial growth, a problem building almost certainly means that chronic leaks or damp conditions exit. Thus, filamentous fungi may grow on biodegradable water, damaged or damp finishing and construction materials. In addition to fungi, mites may reproduce on damp interior surfaces where there is an abundance of human skin scales. Fungi such as *Fusarium* and yeasts, as well as Gram-negative bacteria, may grow in water or on wet surfaces in heating, ventilation and air-conditioning systems (Flannigan *et al.*, 2001).

We analysed fungi in air-conditioning systems installed in a building located in the city centre. The building has an air-conditioning system installed on the roof on the eleventh floor with a capacity of 45 000 m^3/h of outdoor air. The structure of the air conditioner (Figure 1) enables the preparation of primary air for the four built-in duct system induction convectors that are installed in the premises. The AC has built-in class F5 filters which are replaced, on average, every 30 days. Humidification is adiabatic with plastic water drop eliminator. Regular mechanical maintenance of the AC equipment is performed once a month with a high-pressure (vacuum) cleaner, during which the water in the humidification chamber is replaced. Occasionally, depending on the amount of water, the Bascal 100 antibacterial agent may be put into the humidification chamber. The primary preparation of airflow to individual premises through the induction convector takes place in the air-conditioning unit, where the air finally undergoes thermodynamic treatment, depending on the requirements of individual premises. Air leaves the room through an opening in the ceiling.

Using microbiological techniques, we took samples of various parts of the AC equipment and attempted to determine whether fungi settle in AC equipment and whether the air filtration system affects the quality of the air inside the premises. Different researchers estimate that there are approximately 1.6 million species of fungi, of which humans are susceptible to 200 on a daily basis. Fungi may be found in the air both in nature and in enclosed spaces. Contact with the spores or parts of the mycelium can cause health problems in people with a weakened immune system (Committee on the Assessment of Asthma and Indoor Air, 2000).

MOULD SAMPLING

Materials and Methods

We used two sampling methods for the air-conditioning units and the ventilation system. The first method was to expose the agar plates to the air. The method comprises a 15-min exposure of the agar plates to air in an enclosed, functioning AC unit, under conditions of normal ventilation inside enclosed premises. In the second method, we took smear samples from different parts of the AC unit while it was turned off. The samples were taken during the autumn because the concentration of spores at that time reaches its peak. The exact date was 23 November 2001.

Petri dishes with three different types of culture mediums were exposed to the air: DRBC (inhibits the growth of fast-growing fungi, which prevented them from encroaching on slow-growing fungi on the initial agar plates), DG18 (contains glycerol which reduces osmotic

activity, thus creating sufficiently dry conditions such as may be found in AC equipment) and MEA (malt extract sugar—more nutritious than DG18, used as a classical culture medium for the fungi). In all culture media an antibiotic, chloramphenicol, which inhibited the growth of all bacteria susceptible to antibiotics present in the AC equipment, was added.

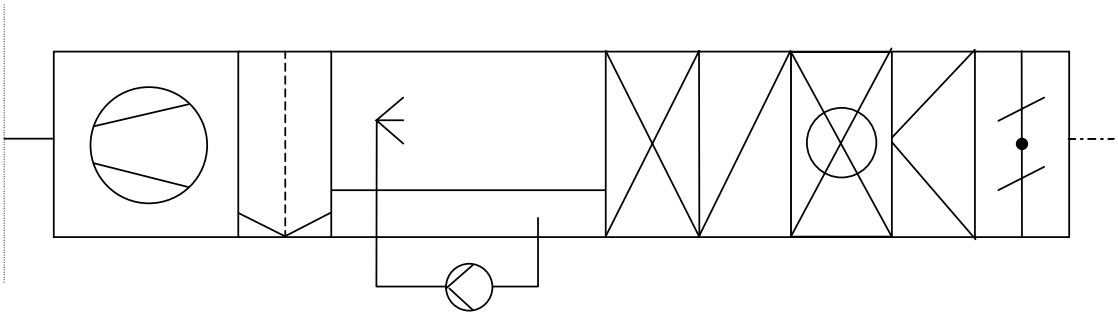
The smear samples were also transferred to the laboratory and aseptically plated into the above-mentioned culture media.

We also used other culture media for the correct identification of all species of fungus. We thus facilitated the development of species-specific reproductive structures on Oat Agar (OA), Potato Dextrose Agar (PDA), Malt Extract Sugar (MEA) and defined minimum media, Synthetic Nutrient Agar and Czapek agar (SNA and CZ).

Dichotomous keys were used to identify the fungi (Synoptic keys (api 20C AUX© test, a test for clinically significant yeast) were used to identify yeasts (Samson *et al.*, 2000). The computer key—Fuskey—was used for identifying the order of Fusarium (de Hoog *et al.*, 2000).

RESULTS

The fungi were isolated from the surface of the air-conditioning unit, which consists of: louvers to conduct air (I), class F7 air filter (II), recuperator (III), pre-heater (IV), refrigerator (V), adiabatic nozzles humidifier (VI), water droplet eliminator (VII), ventilating fan with motor (VIII). The Roman numeral IX indicates where the air leaves the air-conditioning unit to zone heaters placed in each floor.



IX. VIII. VII. VI. V. IV. III II. I.

Figure 1 Scheme of the air-conditioning unit.

The above-mentioned methods identified the following types of fungi on the surfaces of the air-conditioning unit.

I. Entry of the air into the air-conditioning unit—louvers in contact with the external environment:

- *Alternaria alternata*
- *Aureobasidium pullulans*
- *Basidiomycota*
- *Cladosporium cladosporioides*
- *Epicoccum purpurascens*
- *Fusarium sambucinum*
- *Penicillium* sp.

II. Surface of the filter:

- *Botrytis cinerea*

- *Chaetomium* sp.
- *Cladosporium cladosporioides*
- *Curvularia geniculata*
- *Epicoccum purpurascens*
- *Fusarium* sp.
- *Mucor* sp.
- *Penicillium claviforme*
- *Penicillium echinulatum*
- *Penicillium spinulosum*

VI. Surface of the nozzle supports and surface of the water from the humidifier:

- *Cladosporium cladosporioides*
- *Cladosporium macrocarpum*
- *Cylindrocarpon* sp.
- *Rhodotorula rubra*

VII. Surface of the water dropt eliminator:

- *Alternaria alternata*
- *Basidiomycota*
- *Cladosporium cladosporioides*
- *Cladosporium herbarum*
- *Epicoccum purpurascens*
- *Geotrichum candidum*
- *Sporobolomyces salmonicolor*

DISCUSSION AND CONCLUSION

Most of the identified fungi are airborne as they create dry, lightweight spores in brittle chains from which they are easily released. One example is *Cladosporium*, which is very common and mostly found in the air—more than 35 000 conidia/m^3 were detected (Committee on the Assessment of Asthma and Indoor Air, 2000).

The air-conditioning unit has proved to be a suitable habitat for the growth of several fungi. They were isolated from all compartments of the air-conditioning unit. Certain fungi are more resistant and better adapted to the environmental conditions, and are therefore more widespread. We thus identified three species isolated from the larger compartments of the air-conditioning unit: *Alternaria alternata*, *Cladosporium cladosporioides* and *Epicoccum purpurascens*. The first of these was isolated at the very entrance of the air into the unit—the louvers, and in the condenser. They are commonly found in humid and warm environments, and their presence in the louvers, where the external air temperature is 5°C, came as a surprise.

Epicoccum purpurascens was more common than the above-mentioned *Alternaria*, as it was located in the filter as well. It can be recognized by its characteristic spores and is commonly found in the air.

Cladosporium cladosporoides is one of the most studied and most commonly found fungi in ‘aero-biology’ and, as expected, was isolated in every compartment we searched. This fungus was not identified as the cause of the sick building syndrome (WHO, 1983: Combination of general symptoms, irritation of eyes, skin symptoms, and manifestations related to the upper and lower airways) (McGrath *et al.*, 1999). It is interesting to observe that its characteristic lemon-shaped spores (range of size within the species: 3-7(-11) × 2-4(5) μm)

grew smaller as the air passed thorough the various compartments, even though we were dealing with a single species.

Fungi were found only in one compartment and did not spread through the entire air-conditioning system. We isolated fungi that were found only on the louvers and pre-filter but were not found in the humidifier.

Two species of fungi were isolated only in the humidifier compartment. A wider variety of fungi was found in the condenser.

REFERENCES

Committee on the Assessment of Asthma and Indoor Air (2000). *Clearing the Air: Asthma and Indoor Air Exposures*. Washington DC: National Academy Press.

Flannigan, B., Samson, R.A. and Miller, J.D. (2001). *Microorganisms in Home and Indoor Environments*. London: Taylor & Francis.

Halonen, R., Reiman, M., Lehtinen, M., Keskikuru, T. and Kokotti, H. (1999). Transport of microbes via supply ventilation ducts. *Proceedings of the 8th International Conference on Indoor Air Quality and Climate—Indoor Air '99*, Vol. 2, pp. 214–219. Edinburgh: Indoor Air '99.

Health Canada, Communication Branch (1995). *Indoor Air Quality in Office Buildings: A Technical Guide*. Ottawa: Tunney's Pasture.

de Hoog, G.S., Guarro, J., Gené and Figueras, M.J. (2000). *Atlas of Clinical Fungi*. Utrecht: Centraalbureau voor Schimmelcultures, and Reus: Facultat de Medicina, Universitat Rovira i J Virgili.

McGrath, J.J., Wong, W.C., Cooley, J.D. and Straus, D.S. (1999). Continually measured fungal profiles in sick building syndrome. *Current Microbiology* **38** (1), 33–36.

Nathanson, T. (1996). Microbials and HVAC systems operation and maintenance in office buildings. *Proceedings of the 8th International Conference on Indoor Air Quality and Climate—Indoor Air* '96, Vol. 2, pp. 1131–1136. Nagoya: Indoor Air '96.

Neumeister-Kemp, H.G., Esposito, W., Kemp, P.C. and Kircheits, U. (1999). Fungi genera throughout the HVAC system in a well maintained New York building: the worth of high cleanliness standards. *Proceedings of the 8th International Conference on Indoor Air Quality and Climate—Indoor Air '99*, Vol. 2, pp. 190–195. Edinburgh: Indoor Air '99.

Pejtersen, J. (1999). Sensory pollution and microbial contamination of ventilation filters. *Indoor Air* **6**, 239–248.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C. and Filtenborg, O. (2000). *Introduction to Food and Airborne Fungi*. Utrecht: Centraalbureau voor Schimmelcultures.