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MICRO-ORGANISMS (BACTERIA AND FUNGI) IN BUILDINGS

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Building Service Engineering

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DESCRIPTION

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Abstract The main aim of this thesis is to get familiar with basic knowledge about fungi and bacteria, also to provide information about them, its sources and causes, its health effect, methods of its measurement and prevention and also about its critical mass concentration in the indoor air. Methods that were used are literature studies and measurements at Mikkeli University of Applied Sciences premises. In the result there are analyses of the condition that can affect on micro-organisms growing in buildings.		
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1. INTRODUCTION

Every animal on the Earth, including humans, needs oxygen. When people are breathing, oxygen enters the lungs and then spreads to the body through blood. Oxygen is saturating cells and allows body to produce energy that body needs. Every minute people are breathing for about 15 times.

Some people say that “we are what we eat”, but also we are what we breathe. For example, when you go to the mountains, or sea, or even village you can feel something like vertigo. It is because of the high quality of air there. But while you are in the city, no matter where: in office, or maybe in your own flat, or in summer café your feelings is absolutely the opposite: you feel suffocation, headache or even suffer from allergic reaction. That is why it is so important to breathe only pure air.

This thesis will look into the sources, which can pollute and spoil the air. To be more specific, this work look into the microorganisms that could not only mar air, but even be dangerous for our health.

Nowadays, problems connected to indoor air quality is very significant. In all world there are a lot of scientists, who are making researches about this theme. But it also important to provide information about these kind of problems for all people. It is so because these problems can accure in every house and can seriously harm health of its occupants. That is why this thesis will be very usefull not only for people who involved in the building process, but also for everyone.

The main aim of this work is to provide information about reasons that can affect on micro-organisms growing, information about harm that they can cause and methods of its prevention.

Also will be discussed how the measurements for microorganisms must be done. Because the knowledge about micro-organisms concentrations in buildings can help us to predict indoor problems and prevent it. In this thesis there are measurement results from the Mikkeli University of Applied Sciences. They are oriented to explain the dependence between amount of micro-organisms (bacteria and fungi) and factors like occupancy, purpose of room and time period.

2. IMPORTANCE OF THE CLEAN AIR

The Earth's atmosphere composition has not changed for thousands of years. Some natural processes, like volcanic eruptions, have spoiled the air, but these events have occurred infrequently and have never reached scale of a global catastrophe. Our ancestors breathed the air, whose composition is shown in the Table 1:

Table 1. The composition of the pure atmospheric air /1/.

Substance	Concentration in the dry clean air
Nitrogen (N ₂)	78.084%
Oxygen (O ₂)	20.9476%
Argon (Ar)	0.934%
Carbon Dioxide (CO ₂)	0.034%
Other gases	0.0004%

The other gases in the Earth's atmosphere are: neon, krypton, methane, helium, xenon, nitrogen oxides, carbon monoxide, hydrogen, ammonia, sulfur oxides, hydrogen sulfide, hydrocarbons. The share of these substances is only about 0.0004%. Nevertheless, when the maximum allowable concentration of these substances exceed the norm, they are essentially poison the man's life.

The last decades of rapid scientific and technological progress gives us not only conveniences and amenity, they also gives us a numberless amount of substances, which poison our air. So that is why in our time cleaning of indoor air has such a significant role.

The results of new research prove that a good indoor air quality has a significant positive effect on productivity of office employees . In the materials of 5th International Congress Cold Climate HVAC is said that Danish and Swedish scientists carried out the studies, which showed dependence between productivity of typical office workers and indoor air quality. It is very interesting, that in one of the cases carpet was the source of pollution, in the others – several extra computers with conventional displays (flat displays less reduce air quality). In this

experiment, different air quality was achieved by changing the intensity of the ventilation rate and by using old and new filters in the ventilation systems. Researches found for example that deviations of air temperatures from “ideal” (about 21°C), no matter upwards or downwards, has a negative impact on productivity. So I think that additional costs on installation and operation of the climate equipment will worth itself when productivity will increase and number of errors during the process will be reduced. /1/

3. POLLUTERS

When speaking about air quality, there are some factors, whose influence is much bigger, than just provoking uncomfortable working or living conditions, they can even harm our health. These harmful compounds are divided to three groups:

3.1 Volatile organic compounds (VOCs)

To the VOCs can be related: unpleasant odors, toxic gases, fumes of vehicles, tobacco smoke, urban smog. The most common VOCs air polluters are specified in Table 2:

Table 2. Major air pollutants, its Threshold Limit Values (TLV) and source /1/.

Major air pollutants	Threshold limit value (mg/m ³)	Sources of pollution
Carbon monoxide (CO)	1.0	Cars, tobacco smokers
Oxides of nitrogen (NO _x)	0.04	Cars, gas cookers
Sulphur oxides (SO _x)	0.05	Combined heat and power plant
Phenol	0.03	Furniture, building insulation
Formaldehyde	0.003	Furniture, building insulation
Styrene	0.002	Building insulation
Benzopyrene	0.000001	Cars

Ozone (O ₃)	0.03	Office equipment, photochemical reactions
Lead	0.03	Diesel
Aromatic hydrocarbons	0.012	Lacquers, paints, wallpaper, waste

This list of substances is a small fraction of pollutants, which can be found on the street. Even in very small amounts these pollutants can be a reason for very serious human poisoning. In addition, VOCs can easily react with oxygen and other oxidants, which lead to the formation of more serious toxic pollutants.

For example, according to the data, which Moskompriroda gives, in the residential areas near the highway levels of contamination by carbon monoxide and nitrogen oxides exceeds TLV in 10-15 times /1/. It means that you can find at your house exactly the same concentration. You can not hide from the VOCs by airtight double-glazed window – fresh air simply nowhere to take. And also the concentration of the VOCs in the room is usually higher, than outside, because of lack of wind pollutants “accumulate” in the building.

Also, even the furniture can act like a pollution source. In that furniture, that is cheap and made of inexpensive modern materials like plywood or chipboard, the phenol-formaldehyde resin is used as a binder. This polymeric compound has set of advantages: it is convenient to work, very inexpensive to produce, almost never burns. But there is a health risk for human health, because it gradually decomposes to phenol and formaldehyde and both of these substances are toxic and carcinogenic /1/.

Tobacco smoke is also very harmful. It contains about 5000 organic compounds and many of them are carcinogenic and mutagenic. The scientist proved that harmful substances from smoking cigarettes can be found in the room even after a month.

The analysis of the indoor air in city apartment can expose about 40 000 of VOCs. They penetrate into the blood through the alveolus and then are spread throughout the body causing harmful health effects. A systematic or periodic flow of relatively small amounts of toxic

substances can be a cause of chronic poisoning. The signs of chronic poisoning are disruption of normal habits and neuropsychical deviation /2/:

- rapid fatigue or a feeling of constant fatigue
- severe mood swings
- drowsiness or insomnia
- weakening of attention
- apathy
- forgetfulness
- distraction

If poisoning of the same substance is chronic it can cause damage of:

- kidneys
- blood-forming organs
- nervous system
- liver

3.2 Dust and Aerosols

To this group can be referred: house dust, dust mites, allergens and house dust mites.

Almost always dust and aerosols appearing because of human activities. Even a dust in an apartment or on the village road is unpleasant and harmful, but it can not be compared to the dust near the gate of chemical plant or paint shop. Particles of dust with layer of water on them are able to absorb quantities of any substances and transfer them for a long distance.

For example, activated charcoal or coal dust is used as a filter in many modern air cleaners. The same coal dust from the exhaust of diesel vehicles contain products of incomplete combustion, such as benzopyrene, aldehydes, and sulfur compounds. It is a very dangerous poison (a carcinogen and mutagen), even at very low concentrations, it can lead to respiratory disorders and during long-term to cancer /1/.

The atmospheric dust can have any combination from pure quartz to a mixture of organic compounds. The size of dust particles varies from 10 microns to 0.01 microns. Particles over 10 microns (sand, pollen) quickly settle, dust particle size from 0.2 microns to 5 microns - are

floating in the air for several days, aerosols of less than 0.1 microns behave like gases. Thus, not all of the dust can be removed by simple vacuum and a wet rag.

Every day at the mucosal airways can get up to 6 billion of dust particles. If its size is less than 5 microns, these particles settle in the alveolus and disrupt the oxygen rich of blood, penetrating into the bloodstream and carried by blood stream transfer to organs and tissues of the body.

Up to 80% of reserve capacity of immune system is spent on deactivation of dust antigens, which comes to the respiratory tract to the bloodstream. During the time, the reserves of the organism ending and occurs a failure of immune system and development of disease /1/.

3.3 Microbial contaminants

To this group can be referred: viruses, bacteria, fungi and mold. The most vivid example is a flu virus.

It is amazing, but one of the most harmful source of pollution it is man itself, his pets and furniture and clothes. Because of them viruses and allergens, bacteria and dust mites, and also fungal spores (mold) are spreading through our houses.

The United States Environmental Protection Agency gives following information about sources of microbial contaminants:

“ Pollens originate from plants; viruses are transmitted by people and animals; bacteria are carried by people, animals, and soil and plant debris; and household pets are sources of saliva and animal dander. The protein in urine from rats and mice is a potent allergen. When it dries, it can become airborne. Contaminated central air handling systems can become breeding grounds for mold, mildew, and other sources of biological contaminants and can then distribute these contaminants through the home.

Common biological contaminants include mold, dust mites, pet dander (skin flakes), droppings and body parts from cockroaches, rodents and other pests or insects, viruses, and bacteria. Many of these biological contaminants are small enough to be inhaled.” /3/

Following chapters are about most widely-spread bacterial and biological contaminants.

4. LEGIONELLA PNEUMOPHILA

4.1 What is *Legionella*?

The appearance of *Legionella* in the HVAC systems is a very huge problem. This problem became widely known after “The New England Journal of Medicine (NEJM)” made an article about epidemic of pneumonia during the annual convention of the American Legion:

“An explosive, common-source outbreak of pneumonia caused by a previously unrecognized bacterium affected primarily persons attending an American Legion convention in Philadelphia in July, 1976. Twenty-nine of 182 cases were fatal. Spread of the bacterium appeared to be air borne. The source of the bacterium was not found, but epidemiologic analysis suggested that exposure may have occurred in the lobby of the headquarters hotel or in the area immediately surrounding the hotel. Person-to-person spread seemed not to have occurred. Many hotel employees appeared to be immune, suggesting that the agent may have been present in the vicinity, perhaps intermittently, for two or more years. /4, p.1189/

In the World Health Organization made book, named “*LEGIONELLA* and the prevention of legionellosis”/5/ placed researches about this problem. This is what they say about history of Legionnaires’ disease:

“In 1976, an outbreak of severe pneumonia among the participants of the American Legion Convention in Philadelphia led to the description of Legionnaires’ disease by Fraser et al. (1977). The disease was found to be caused by the bacterium *Legionella pneumophila* (*Legionella* after the legionnaires who were infected at the convention; *pneumophila* meaning “lungloving”), belonging to the family *Legionellaceae*. The generic term “legionellosis” is now used to describe these bacterial infections, which can range in severity from a mild, febrile illness (Pontiac fever) to a rapid and potentially fatal pneumonia (Legionnaires’ disease).”/5, p.1/

So here is a first question: what is this *Legionella pneumophila* bacterium?

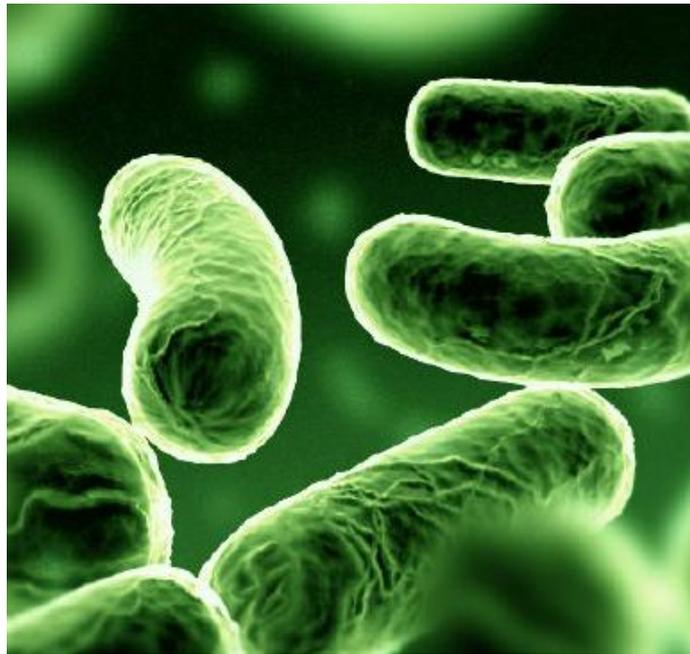


Figure 1. *Legionella pneumophila* bacteria /18/

“The *Legionella* bacterium is a small, aerobic, waterborne, gram-negative, unencapsulated bacillus that is nonmotile, catalase-positive, and weakly oxidase-positive. *Legionella* is a fastidious organism and does not grow anaerobically or on standard media.” /6/

This bacterium is also very viable:

“*Legionella* are ubiquitous in natural and artificial water environments worldwide, and survive in a range of environmental conditions. The bacteria are acid tolerant (they can withstand exposure to pH 2.0 for short periods) and they have been isolated from environmental sources ranging from a pH of 2.7 to 8.3. *Legionella* have been found in sources as diverse as water on plants in rainforests, groundwater. The bacterium also survives in artificial sources of salt water. In certain natural aquatic environments (e.g. in groundwater that is contaminated by soils or subsoil and has a temperature below 20 °C), *Legionella* may present.” /5 p.28-29/

4.2 Sources of *Legionella*.

Legionella can be found worldwide. It can be found not only in water, but in composted material and moist soils. It can exist both in natural and anthropogenic conditions.

“Aside from their natural habitat, *Legionella* bacteria are also able to colonize the man-made water systems such air conditioning systems, cooling towers, hot water systems, whirlpools, vegetable misters and dental-unit water lines. These ecosystems seem to favor the growth of *Legionella* bacteria as they are found in higher concentrations than in their natural environment. With the exception of natural hot springs where temperature ranges from 35 °C to 40 °C, the sources of legionellosis are exclusively man-made water systems. In water, a temperature range between 20 °C to 45°C favors the growth of *Legionella pneumophila*. At lower temperatures, *Legionella* appears to enter into a dormant stage until exposed to more favorable conditions. In these environments, *Legionella* species can be present as planktonic cells but also as monospecies or multispecies biofilms.” /5 p.2/

The main sources of *Legionella* are indicated in Table 3:

Table 3. Sources of *Legionella* /5 p.12/

	Community acquired	Travel associated	Nosocomial
Sources of <i>Legionella</i>	Cooling towers; hot and cold-water systems; spa pools, thermal pools, springs; humidifiers; domestic plumbing; potting mixes and compost	Cooling towers; hot and cold-water systems; spa pools, thermal springs and pools; humidifiers	Cooling towers; hot and cold-water systems; spa pools, natural pools, thermal springs; respiratory therapy equipment; medical treatment

4.3 Health effect of *Legionella*. Legionellosis and Pontiac fever

Legionella bacteria can be acquired by inhalation of infected aerosols or by micro-aspiration of ingested infected water. Legionellosis is not spread from person to person. It's impossible to get from animals.

It is also very important to know, that not all of *Legionella* species can cause Legionnaire's disease:

“There are currently 53 species and 70 serogroups in the genus *Legionella*, only few of which have been associated with legionellosis. While some are recognized as a cause of legionellosis, such as *Legionella pneumophila* and *Legionella longbeachae*, most have only been isolated from the environment such as lakes, rivers or thermal waters” / 7, p.2 /

Legionnaire’s disease has very serious symptoms like fever, chills, cough, chest pain, diarrhea, nausea and abdominal pain. Also this illness has neurological symptoms: headache, lethargy, encephalopathy and mental status change.

This fact about how heavily this bacteria can harm to human health is amazing. For example, in Burke A Cunha, MD article said that:

“The mortality rate may approach 100% in patients with underlying disease. In untreated patients, the mortality rate may be as high as 80%.”/6/

Also in this article there is a very useful information about factors which increase risk of infection. For example elderly people have a bigger risk of infection. Also men have a greater risk. Smoking, chronic heart or lung disease, some immunosuppressive medicines like corticosteroids also can weaken defense from this bacteria.

But *Legionella pneumophila* can cause not only Legionnaire’s disease. It also can be a causative agent of Pontiac fever. Pontiac fever is not as grave illness, as Legionnaire’s disease.

“Pontiac fever is a non-fatal, non-pneumonic, febrile, influenza-like illness with a manifestation rate of 90% and an incubation time of 1–2 days. Transmission of Pontiac fever only takes place by inhalation of aerosols. In contrast to Legionnaires’ disease, *Legionellas* do not multiply in the human respiratory system and are therefore not culturable from respiratory tract secretions; however, seroconversion usually occurs. *Legionella pneumophila*, *Legionella micdadei*, *Legionella anisa* and *Legionella feeleeii* have been implicated as agents of Pontiac fever-like illnesses” /8, p.61/

5. FUNGI

5.1 General information about fungi

Fungi are widely spread and play role in human everyday life. For example it is used in food industry: cheese with mould, also mould strain could *Botrytis cinerea* take a part in the process of vine maturing.



Figure 2. Cheese with mould. The example of using of mould in food industry /19/.

Fungi are used in medicine. It is widely known that in 1928 Sir Alexander Fleming discovered antibiotic *Penicillin* from mould. After his discovery, many other antibiotics were derived from mould. Also from mould can be derived drug that called *Cyclosporine*. It is used to suppress the rejection of transplanted organs.

But overall, in a question of indoor air quality fungi are another big problem.

“Fungi are ubiquitous organisms that make up approximately 25% of earth's biomass.

They can be subdivided somewhat artificially by gross morphology into yeasts, mushrooms and molds - the fungi of most importance for indoor air.” /9/

Mould is all species of microscopic fungi. It is growing in the form of multicellular filaments, called hyphae. In contrast, microscopic fungi that grow as single cells are called yeasts, a

connected network of tubular branching hyphae has multiple, genetically identical nuclei and is considered a single organism, referred to as a colony. /10 p.2/

Mold has good adaptive ability. It can colonize dead and decaying organic matter (e.g. textiles, leather, wood, paper) and even damp, inorganic material (e.g. glass, painted surfaces, bare concrete) if organic nutrients such as dust or soil particles are available. /9/

According to the EPA's Mold Guide /11, p16/ all fungus can be divided to the groups, three most common will be Zygomycetes, Ascomycetes and Basidiomycetes. Each of these groups can contaminate buildings, but most common fungi that colonize building materials belong to Ascomycetes. Also fungi can be divided to Saprophytic, Parasitic and Symbiotic.

“Most fungi are saprophytes, and saprophytic fungi thrive by first exuding enzymes and acids that act on surrounding dead and decaying materials and then by absorbing nutrition from the breakdown, fulfilling a critical ecological role by degrading waste material.” /11, p.16/

5.2 Sources of fungi and factors that influence on their growing.

As the example of most common fungi, we can name *Acremonium* (including some species formerly classified under *Cephalosporium*) its source are water-damaged indoor materials including drywall, wood, and paper products. It is moisture-loving fungus. Species that may be found indoors are *A. kiliense*, *A. butyri*, *A. furcatum*, and *A. murorum* (synonym *Gliomastix murorum*).

Also *Aspergillus fumigatus*. It could be found at filters of air-conditioning systems and air ducts, decaying plant materials, compost, wood chips, hay and crops, stored grains and stored sweet potatoes. It is also very important that thermotolerant-thermophilic species - can grow in a temperature range of 12°C to 57°C, with an optimal range of 37°C to 43°C.

At Appendix A, placed the Table A1 with most common fungi and its sources according to EPA Mould Guide Appendix A /11/.

All mould reproduces by tiny spores, which are invisible for human eyes. These spores float through indoor and outdoor air. Mould can start to grow indoors only when mould spores land on a wet surface. None of all mould species can grow without moisture.

“Main requirements for fungal growth in buildings are a source of inoculums (spores, etc), suitable temperature, nutrients, oxygen and moisture” /12, p.519/

There are a lot of factors, which influence mould growth in buildings. Several of them are presented at Figure3.

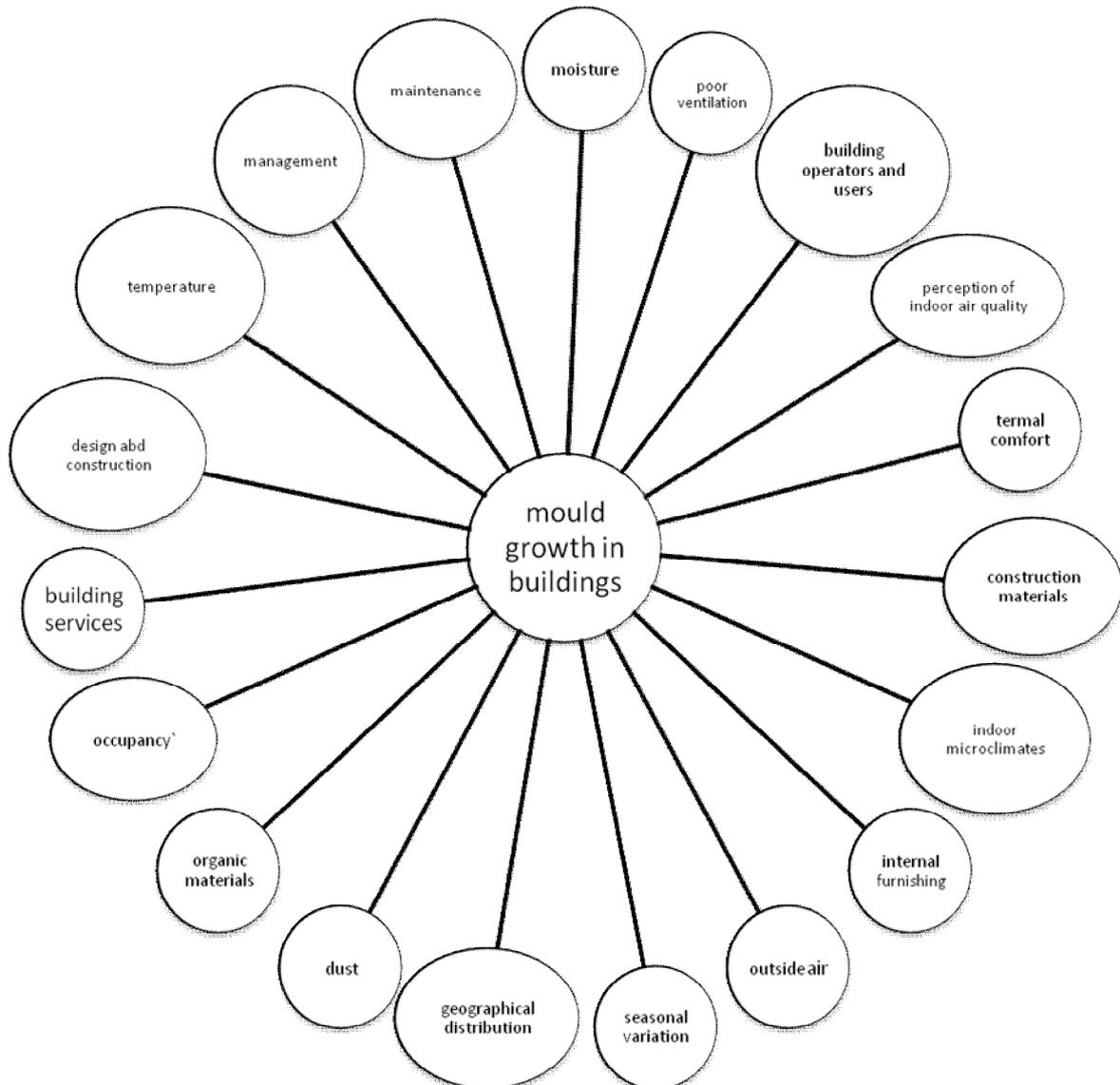


Figure 3. The various factors influencing mould growth in buildings /12, p.519/.

But the most significant are moisture and ventilation in buildings. According to EPA Mould Course:

“Common moisture problems includes leaking roofs; leaking or condensing water pipes, especially pipes inside wall cavities or pipe chases; leaking fire-protection sprinkler systems; landscaping gutters, and down spouts that direct water into or under a building;

high humidity (> 60% relative humidity); unvented combustion appliances such as clothes dryers vented into a garage. (Clothes dryers and other combustion appliances should be vented to the outside.)” /13 p.41/

According to EPA Mould Guide:

“Inadequate or poorly maintained ventilation systems that may not provide enough air for dilution or dehumidification or that may themselves harbor sources of mold or disperse mold spores into the occupants’ breathing zone.” /11 p. 3/

5.3 Health effects

Fungi can cause a variety of health problems. According to EPA Mould Guide /11 p.21/, fungi can cause:

- Fungal infections
- Allergic rhinitis
- Asthma
- Hypersensitivity pneumonitis (extrinsic allergic alveolitis)
- Interstitial lung disease
- Bronchopulmonary aspergillosis
- Allergic fungal sinusitis
- Allergic dermatitis
- Irritant symptoms
- Organic dust toxic syndrome
- Pulmonary hemorrhage in infants

All these diseases can be divided into four groups:

- Infections
- Allergic or hypersensitivity reactions
- Irritant reactions
- Toxic reactions

Fungal infections are most common fungal diseases. For example, following fungus can be a source of infection:

“For instance, *Coccidioides immitis* can cause a flu-like syndrome (“Valley Fever”) or sarcoid-like syndrome and pulmonary coin lesions; it typically occurs following inhalation of spores from arid soils in the southwestern United States or Mexico. The origin of this particular fungus is most likely from the outdoors, and it usually would not be considered associated with wet buildings. *Histoplasma capsulatum* can cause interstitial or cavitory pneumonia. It typically occurs in spelunkers and others exposed to bat guano or bird droppings in the Mississippi or Ohio River valleys where Histoplasmosis is endemic. *Cryptococcus* typically causes self-limited infections, although in immuno-compromised individuals it can cause meningoencephalitis or cavitating pneumonia. It has been associated with exposure to pigeon droppings on windowsills or air conditioning units in urban office buildings. Sporotrichosis can be manifest by cutaneous or lymphangitic lesions, or by pulmonary involvement and disseminated disease. It typically occurs in gardeners, often after they have been pricked by thorns. Dermatophytes cause the typical infections of the skin, hair, and nails (e.g., tinea cruris, corporis, and pedis). These skin infections too may have environmental associations. For example, tinea pedis may develop following use of locker rooms at public swimming pools or school gymnasiums.” /11, p.22/

It is widely known, that mould produce allergens:

“Allergic reactions to mold are common and can be immediate or delayed. Repeated or single exposure to mold, mold spores, or mold fragments may cause non-sensitive individuals to become sensitive to mold, and repeated exposure has the potential to increase sensitivity. Allergic responses include hay fever-like symptoms such as headache, sneezing, runny nose, red eyes, and skin rash (dermatitis).” /11, p.19/

Hypersensitivity is a result from immunologic responses to antigens. Multiple components of fungi can serve as antigens.

Mold also can be the cause of irritants reaction of eyes, skin, nose, throat and lungs:

“In sufficient concentrations, fugally derived VOCs may lead to eye irritation, conjunctivitis, skin rashes, rhinitis, laryngitis and hoarseness, cough, and even chest tightness.” /13 p.26/

During the growth, some of the fungi can produce complex secondary metabolites called mycotoxins.

“In recent years, there have been numerous reports in both the medical literature and the popular media that indoor exposure to fungi or fungal toxins has caused significant disease or death in the occupants of water damaged homes or workplaces. These locations had significant (generally visible) fungal growth and odors, typically reported as from the “black mold,” *Stachybotrys chartarum*. (It should be noted here that many molds are “black” in appearance.) *S. chartarum* is a ubiquitous organism, growing on cellulose products exposed to water or high humidity. In moist buildings, *S. chartarum* frequently grows on wallpaper, wallboard, ceiling tiles, carpets (especially those with jute backing), insulation (e.g., urea–formaldehyde foam) in the spaces between inner and outer walls, around leaking window frames or water pipes, and in HVAC air ducts containing lint or other organic debris. Some reports of *Stachybotrys*-related disease have involved celebrities, and these and other incidents have triggered widely publicized litigation against builders and insurance companies. Concerns relating to the health effects of mycotoxins as encountered in indoor environments focus on respiratory, neurological, and dermatologic effects” /13 p.28/

Most common fungi and its effects on the human health are indicated in the Table 4.

Table 4. Most common fungi, possible metabolite and health effects. /9/

Fungal Species	Subtract	Possible Metabolites	Potential Health Effects
<i>Alternaria alternata</i>	moist window-sills, walls	allergens	asthma, allergy
<i>Aspergillus versicolor</i>	damp wood, wallpaper glue	mycotoxins, VOCs	unknown

<i>Aspergillus fumigates</i>	house dust, potting soil	Allergens	asthma, rhinitis, hypersensitivity pneumonitis
		many mycotoxins	toxic pneumonitis, infection
<i>Cladosporium herbarum</i>	moist window-sills, wood	Allergens	asthma, allergy
<i>Penicillium chrysogenum</i>	damp wallpaper, behind paint	mycotoxins	unknown
		VOCs	unknown
<i>Penicillium expansum</i>	damp wallpaper	Mycotoxins	nephrotoxicity
<i>Stachybotrys chartarum (atra)</i>	heavily wetted carpet, gypsum board	mycotoxins	toxic pneumonitis, infection

From all this information it is very obvious, that fungi is dangerous for human health and its appearance should be prevented.

6. PREVENTION

6.1 Prevention of *Legionella* in water systems

Legionella bacteria are living and growing in the water and come to human organism in the form of fine-dispersed bacterial aerosol. So that is why water is the main source. For properly water quality WHO is suggesting to make Water Safety Plan (WSP):

“Developing a WSP is the preferred approach to managing specific health risks of exposure to *Legionella* from water systems (WHO, 2004; Davison et al., 2005). In some jurisdictions, other terms are used; for example, the term “risk management plan” is used by the Department of Human Services, Victoria, Australia. Such plans are similar to a WSP, but are less clearly defined. For the purposes of this document, the term WSP is used. Authorities responsible for water system safety or building safety should develop systemspecific WSPs. Major benefits of developing and implementing such a plan are the

systematic and detailed assessment and prioritization of hazards (biological, chemical or physical agents, or water conditions, with the potential to cause adverse health effects), and the operational monitoring of barriers and control measures.” /5, p. 43/

The key steps of WSP are indicated at Figure 4.

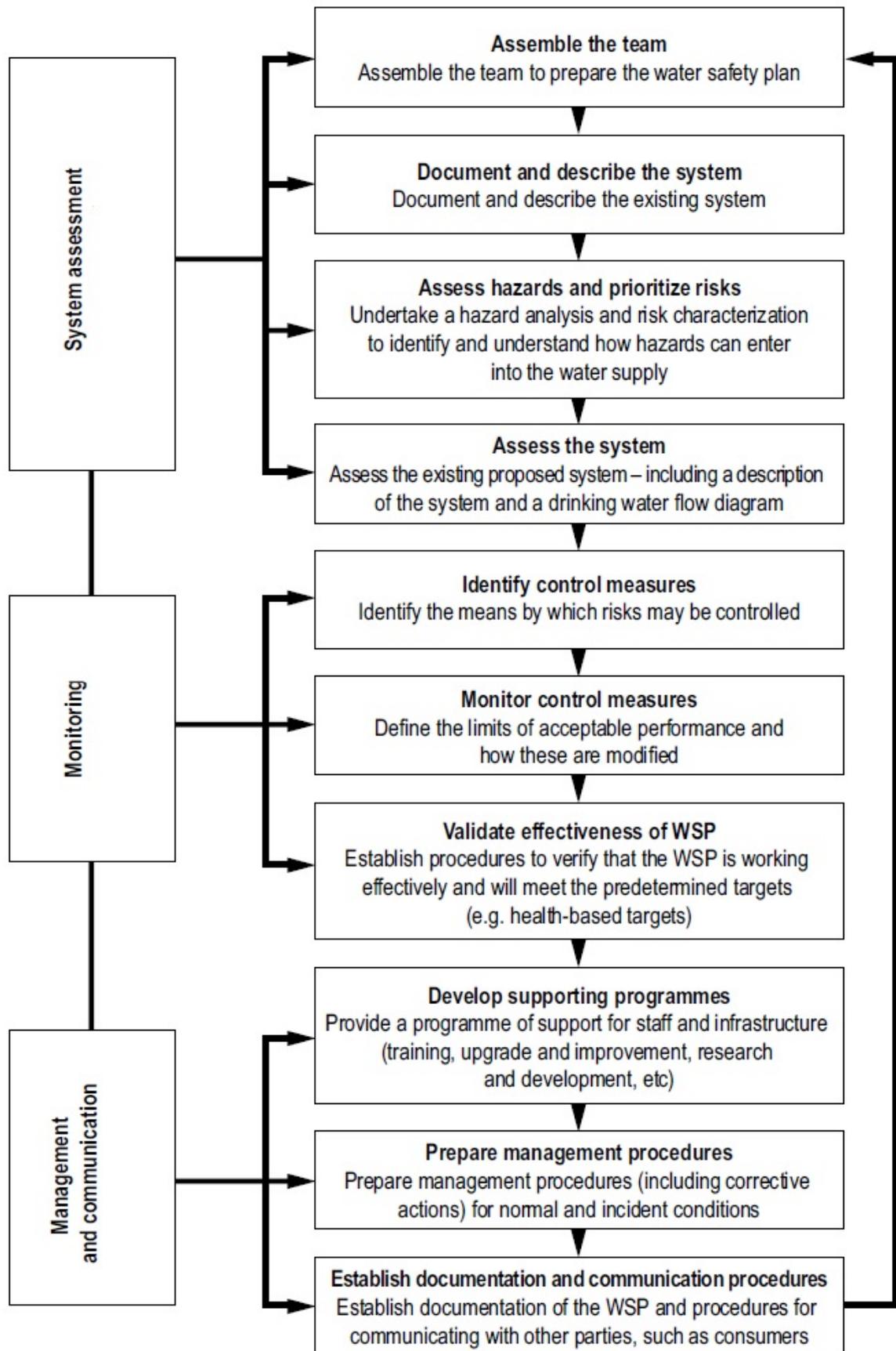


Figure 4. Overview of the key steps in developing a water safety plan /5 p.44/.

All these steps are very important, but one of the most important is monitoring, especially monitoring control measures. These measures include:

- excluding the microorganism
- manipulating the environment to prevent colonization by, and limit growth of, the microorganism (e.g. by controlling nutrient levels, controlling temperature, and preventing low flow and stagnation)
- manipulating the environment to limit growth of the microorganism
- using a disinfectant (e.g. a biocide)

In the different WSP can be used different combination of control methods, or it can base on only one method. Most simple any popular methods are: regulation of temperature, flushing with hot water, dosing with different substances, ionization, UV disinfection. Every method has advantages and disadvantages. They are indicated in Table B1 in Appendix B.

6.2 Prevention of mold problems in buildings

Moisture, especially relative humidity indoors and in structures is the main thing in mould prevention. Some species of mould can grow even if relative humidity indoor will be 62-65%, but after an extensive analysis of published data on laboratory experiments, Rowan et al. (1999) recommended that the relative humidity be maintained below 75% to limit fungal growth in buildings./10, p.38/

In Table 5 indicated critical relative humidity for different materials:

Table 5. Critical relative humidity for various groups of materials. /10, p.38/

Material group	Relative humidity (%)
Wood and wood-based materials	75–80
Paper on plasterboard	80–85
Mineral insulation materials	90–95
Extruded and expanded polystyrene	90–95
Concrete	90–95

Water which comes into the building should be under control. It is a key to mold prevention:

“If water enters a building through a leaking roof or because of a flood or accident, it should be removed immediately and affected areas should be dried out.” /13, p.141/

Also ventilation is very important:

“Ventilation (outdoor airflow into a building) must be adequate to remove and dilute pollutants and humidity generated indoors, although the first alternative for improving indoor air quality should be control of pollutant sources. Ventilation should be energy efficient and arranged so that it does not degrade indoor air quality or climate and does not cause any harm to the occupants or to the building. Ventilation rates should be based on pollution loads, moisture generation and use of the building. To the extent possible, outdoor pollutants should be removed from the air before the air is brought inside the building.” /10, p.41/

In the EPA Mold Course, there are given recommendations for prevention of mould appearance:

- **MOISTURE CONTROL IS KEY**
- Keep the building clean and dry. Dry wet or damp areas within 48 hours.
- Fix leaky plumbing and leaks in the building envelope as soon as possible.
- Watch for condensation and wet spots. Fix the sources of moisture problems as soon as possible.
- Prevent moisture due to condensation by increasing surface temperature or reducing the moisture level in air (humidity). To increase surface temperature, insulate or increase air circulation. To reduce the moisture level in air, repair leaks and increase ventilation (if outside air is cold and dry), or dehumidify (if outdoor air is warm and humid).
- Keep heating, ventilation, and air conditioning (HVAC) drip pans clean, flowing properly, and unobstructed.
- Vent moisture generating appliances, such as dryers, to the outside where possible.
- Maintain low indoor humidity, below 60 percent relative humidity (RH), ideally 30 percent to 50 percent, if possible.
- Perform regular building and HVAC inspections and maintenance as scheduled.

- Do not let foundations stay wet. Provide drainage and slope the ground away from the foundation.
- If you are not experienced with home/building repairs you may want to consult a professional when making repairs, or for assistance with mold-prevention-related changes to your home/building. / 13, p.142/

Also it is very important for bacteria and fungi control to make measurements properly. It will be discussed in the next chapter.

7. AIRBORNE CONCENTRATIONS OF BACTERIA AND FUNGI

7.1 Measurement methods

For measuring the airborne concentrations of bacteria and fungi a lot of different samplers are in use. In the "Healthy Buildings 2000: Microbes, Moisture and Building Physics" book is said, that there are four types of samplers: impactors and sieve samplers, impingers, centrifugal samplers, filter cassette /14/.

7.1.1 Impactors and sieve samplers

These samplers could be: spore traps, slit samplers and cascade impactors.

Their operation principle is based on impaction on agar, sticky surface, glass slide or membranes. Like an example for this device could be: Burkard sampler, Rotorod sampler, Andersen impactor, SAS, Casella impactor, May impactor, Sierra Marple impactor. The air flow through this kind of samplers could be from 2 to 180 l/min. Sampling time could be from minutes to hours, even up to a week. Its possible analyses are cultivation and microscopic analyses.

Illustration for this kind of samplers is at the Figure 5.



Figure 5. Andersen sampler /20/.

7.1.2 Impingers

This sampler is based on principle of impaction which mean, that it use centrifugal force and diffusion into the liquid. The examples of this device are: Shipe sampler, AGI-30 (Figure 6), Midget and micro impinges, Multistage impinger. Air flow rate for this samplers is 0,1 – 55 l/min, sampling time is from minutes to hours. With these samplers cultivation, microscopic analyses, biochemical analyses and immunoassay is made.



Figure 6. SKC Bio Sampler /21/.

7.1.3 Centrifugal samplers

The main principle of these samplers is based on the centrifugal force into liquid, semi-solid or agar medium. As the example, RCS and Aerojet cyclone (Figure 7). Air flow rate of these samplers is 40 – 1000 l/min, sampling time is from minutes to hours. With these samplers cultivation, microscopic analyses, biochemical analyses and immunoassay can be made.

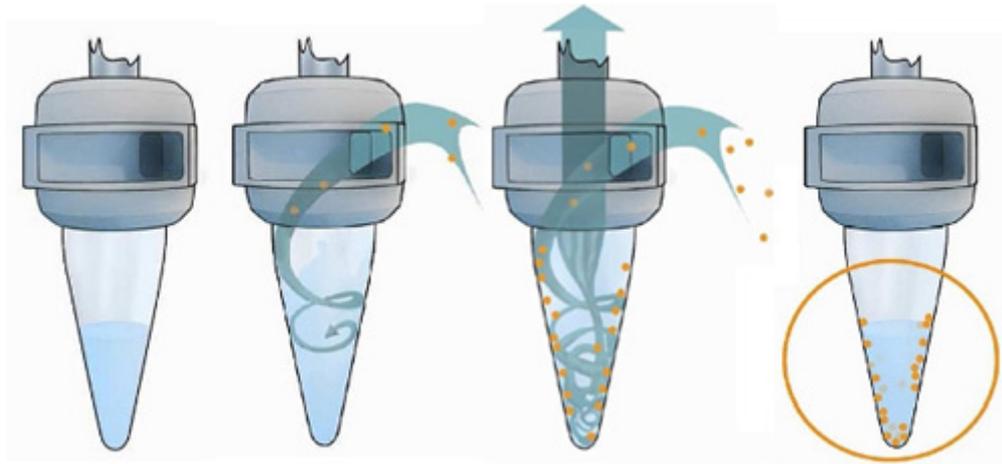


Figure 7. Cyclone sampler /22/.

7.1.4 Filter cassette

The principle of this sampler operation is based on internal impaction, interception, sieving on fibrous, flat or membrane filters. Like an example for this device could be: glass fiber, Teflon, cellulose ester, or polycarbonate filters. The air flow through this kind of samplers could be from 1 to 1000 l/min. Sampling time is hours. Its possible analyses are cultivation, microscopic analyses, biochemical analyses and immunoassay.

Illustration for this kind of samplers is at the Figure 8. /14, p.28/

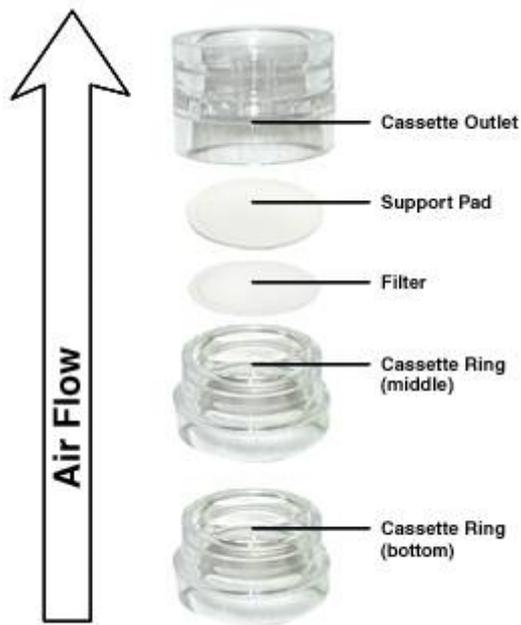


Figure 8. Filter cassette /23/.

7.1.5 Incubation and identification

After sampling, collected materials should be placed into incubator. Temperature and relative humidity in the incubator can vary due to different sampling condition and also it depends on for what kinds of bacteria samples were made. The time of sampling is related to these factors as well.

Also due to factors that described at 7.2 the time period for checking the amount of already grown colonies should be defined. After incubation period is over CFU-value should be calculated (Equation 1):

$$CFU = \frac{\text{summ of all colonies}}{\text{total air flow rate}} \quad (\text{Equation 1}) /15/$$

Total air flow rate should be calculated according to Equation 2:

$$\text{total air flow rate} = \frac{\text{time} \cdot q_v}{1000} \quad (\text{Equation 2})$$

The CFU limit values will be discussed in following sections.

7.2 Concentrations

After sampling procedure is done, measurements results should be compared with standard concentrations. But these number concentrations of airborne microorganisms are closely associated with the sampling and analysis method that were used. These numbers reflect mainly the crowdedness of the room and the efficiency of the ventilation, and thus are a measure of the hygienic quality of the indoor air.

7.2.1 Bacteria

At WHO book “Biological Agents in Indoor Environments Assessment of Health Risks” following concentrations are indicated:

“Concentrations of viable bacteria in indoor environments are usually within the range 10^1 - 10^3 CFU/m³ in homes, and 10^2 CFU/m³ in offices (Pastuszka et al., 2000; Gorny, 1999). A rough estimation might be that a concentration <1000 CFU/m³ can be regarded as “low” and that of >5000 CFU/m³ can be regarded as “high” (Reponen et al., 1992).” /16, p.62/

7.2.2 Fungi

For fungi there are a lot of documents with guidelines. It is indicated in Table 6:

Table 6. Example Quantitative Recommendations for Fungal Concentrations /17 p. 428/

Organization (Document, Year)	Recommendations
American Conference of Industrial Hygienists (Air Sampling Instruments for Evaluation of Atmospheric Contaminants, 1995)	< 100 CFU/m ³ —low 100 – 1000 CFU/m ³ —intermediate, represents general indoor and outdoor concentrations > 1000 CFU/m ³ —high, represents animal handling areas
American Industrial Hygiene Association (The Industrial Hygienist’s Guide to IAQ Investigations, 1993)	Rank order assessment; indoor/outdoor comparison recommended
Commission of European Committees (Report #12: Biological Particles in Indoor Environment, 1993)	Residential structures: $> 10,000$ CFU/m ³ —very high $< 10,000$ CFU/m ³ —high < 1000 CFU/m ³ —intermediate < 200 CFU/m ³ —low < 500 CFU/m ³ —low (on DG18 medium)

	<p>< 50 CFU/m³—very low</p> <p>Commercial, nonindustrial structures:</p> <p>> 2000 CFU/m³—very high</p> <p>< 2000 CFU/m³—high</p> <p>< 500 CFU/m³—intermediate</p> <p>< 100 CFU/m³—low</p> <p>< 25 CFU/m³—very low</p>
Canadian Mortgage and Housing Corporation (Testing of Older Houses for Microbiological Pollutants, 1991)	<p>> 200 CFU/m³ presence of species other than Alternaria and Cladosporium—investigate</p> <p>> 500 CFU/m³ includes Alternaria and Cladosporium—investigate;</p> <p>Indoor/outdoor comparison recommended when \leq 200 CFU/m³</p>
IAQ Association Inc. (IAQ Standard #95-1 Recommended for Florida, 1995)	<p>< 300 CFU/m³ of common fungi—OK</p> <p>< 150 CFU/m³ mixed species, not pathogenic or toxigenic—OK</p>
National Health and Welfare, Canada (disclaimer/IAQ in Office Building: A Technical Guide, 1993)	<p>Toxigenic, pathogenic not acceptable in indoor air</p> <p>\geq 50 CFU/m³ one species—investigate</p> <p>\leq 150 CFU/m³ if mixed species—OK</p> <p>\leq 500 CFU/m³ if common tree/leaf fungi—OK in summer</p>
U.S. Occupational Safety and Health Administration (Technical Manual, 1992)	<p>\geq 1000 CFU/m³—contamination</p> <p>\geq 106 fungi/g dust—contamination</p> <p>\geq 105 fungi/mL stagnant water or slime—contamination</p>
World Health Organization (IAQ: Biological Contaminants, 1988)	<p>Pathogenic/toxigenic unacceptable in indoor air</p> <p>> 50 CFU/m³, one species—investigate</p> <p>\leq 150 CFU/m³, mixed species—OK</p> <p>\leq 500 CFU/m³, if Cladosporium or other common phylloplane—OK</p>

7.3 Measurements of concentrations at Mikkeli University of Applied Sciences

7.3.1 Methods

For this thesis were made some measurements at the Mikkeli University of Applied Sciences. To be more exact first measurements were at the Building A premises: lecturers office №A123, classroom №A232 and cafeteria. Second measurements were at the same places and also at the Mail room in the Building A.

For both measurements 6-stage Andersen sampler was used. It was connected to the fan (Figure 9). Inside the sampler, there were 6 Petri dishes. During all samples flow rate was about 28,3 L/min and time of sampling was 10 minutes.

Petri dishes were marked and temperature, relative humidity and amount of people were measured. These data are indicated in the Table 7:

Table 7. Preparation for measurements.

Place and type of measurement	Used Petri Dishes	Conditions during 1 measurements	Conditions during 2 measurements
Bacteria measurements in the lectures office	BO6, BO5, BO4, BO3, BO2, and BO1.	t= 22, 0°C; RH= 23, 4 %; 4 person in the room	t= 21, 9°C; RH= 15, 5 %; 2 person in the room
Fungi measurements in the lectures offices	FO6, FO5, FO4, FO3, FO2, and FO1	t= 22, 7°C; RH= 23, 4 %; 5 person in the room	t= 22°C; RH= 15, 7 %; 2 person in the room
Bacteria measurements in the classroom	BR6, BR5, BR4, BR3, BR2, and BR1	t= 21, 9°C; RH= 23, 9 %; 1 person in the room	t= 21, 3°C; RH= 18, 3 %; 12 person in the room.
Fungi measurements in the classroom	FR6, FR5, FR4, FR3, FR2, and FR1	t= 21, 9°C; RH= 23, 9 %; 1 person in the room	t= 21, 5°C; RH= 18, 3 %; 12 person in the room
Bacteria measurements in the cafeteria	BC6, BC5, BC4, BC3, BC2, and BC1	t= 21, 1°C; RH= 26, 0 %; 4 person in the room	t= 20, 1°C; RH= 20 %; 7 person in the room
Fungi measurements in the cafeteria	FC6, FC5, FC4, FC3, FC2, and FC1	t= 20, 6°C; RH= 26 %; 3 person in the room	t= 19, 6°C; RH= 17 %; 5 person in the room
Bacteria measurements in the mail room	BM6, BM5, BM4, BM3, BM2, and BM1	-	t= 22, 2°C; RH= 15 %; 6 person in the room
Fungi measurements in the mail room	FM6, FM5, FM4, FM3, FM2, and	-	t= 22, 0°C; RH= 14, 5 %; 4 person in the

	FM1		room
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Figure 9. 6-stage Andersen sampler and fan.

In the Petri dishes for fungi was used 2% malt agar. In the Petri dishes for bacteria was used PCA (plate count agar).

Before all samplings outside air temperature and relative humidity should be measured. For these purposes I used Vaisala HUMICAP® Sensor (Figure 10). During the first measurements temperature was $t=+4,6^{\circ}\text{C}$, $\text{RH}=61,5\%$. During to second measurements temperature was $t= - 2,4^{\circ}\text{C}$, $\text{RH}=60,4\%$. According to Finish guidelines, the outside aire fungi measurements are not necessary, while the ground is freeze or there is a snow. At 10.11.2010 and 17.11. 2010 there was snow, so outside measurements were not made.

Before each sampling, air temperature and relative humidity in the room should be measured.



Figure 10. Vaisala HUMICAP® Sensor /24/

Also it is very important to clean the 6-stage Andersen sampler with 70% ethanol after each sampling.

When all measurements are done, all Petri dishes should be placed into the incubator. Temperature in the incubator is $t=+24^{\circ}\text{C}$.

The measurement order:

1st step: measure the temperature and relative humidity room.

2nd step: clean the 6-stage Andersen sampler

3rd step: take six uncovered Petri dishes (all six for bacteria or fungi) into the 6-stage Andersen sampler.

4th step: connect the 6-stage Anderson sampler to the fan.

5th step: take off the protection lid from 6-stage Anderson sampler.

6th step: turn on the fan.

7th step: turn of the fan after 10 minutes.

8th step: disconnect the fan and 6-stage Anderson sampler.

9th step: take out six Petri dishes from 6-stage Anderson sampler, cover it with lids and turn upside-down, to prevent the ingress of moisture to the sampler.

10th step: put all the Petri dishes into incubator.

The samples should stay in the incubator during seven days. Every of these days checking of amount of colonies should be done.

7.3.2 Results

Shorten results of the first measurements included into Table 7. Shorten results of the second measurements included into Table 8. Full results can be found at Appendix C.

Table 8. 10. 11. 2010 measurements results.

Place & type of sampling	Total corrected number of colonies	Concentration cfu/m ³
Bacteria, office	68	240
Fungi, office	6	21
Bacteria, classroom	47	166
Fungi, classroom	1	4
Bacteria, cafeteria	115	406
Fungi, cafeteria	5	18

Table 9. 17.11.2010 measurement results.

Place & type of sampling	Total corrected number of colonies	Concentration cfu/m ³
Bacteria, office	32	113
Fungi, office	1	4
Bacteria, classroom	76	269
Fungi, classroom	2	7
Bacteria, cafeteria	808	2855
Fungi, cafeteria	8	28
Bacteria, mail room	148	523

Fungi, mail room	3	11
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7.3.3 Analysis

From the results a lot of connections between different factors that can influence on the amount of bacteria and fungi can be seen.

First thing that can be noticed in the results, is that concentration of bacteria and fungi in the air depends on amount of people in the room. The best example is results from cafeteria. Result of first measurement in this room was 406 CFU/m³, but second measurements give a result of 2855 CFU/m³. So it is possible to assume that it is due to that during the first measurement there were 4 persons and during the second one there were 7 persons. The bigger amount of people produce the bigger amount of bacteria.

Also in that room the wardrobe is situated, so it is also a source of air pollution, because people carry fungial spores on their outwear. First samples were made at 5 pm, so the main part of student already left the University, but the second samples I made at 2 pm and in wardrobe were a lot of clothes.

The second thing that can be noticed, is that concentration of fungi depends on outdoor weather. Because even if the results of second measurements are a little bit bigger there was much more people and clothes, in another words there was more sources of pollution.

But main purpose of these measurements was to identify, if there are any problems with mold and bacteria in that premises.

So, almost all bacteria concentrations are very low, except concentration of bacteria in cafeteria. But it is 2855 CFU/m³ and according guidelines that are indicated in the chapter 7.2.1, this concentration is much lower than high one (5000 CFU/m³), so it can be assumed, that this concentration is normal.

According to different guidelines that indicated in the chapter 7.2.2, measured concentrations are low or very low.

From all these results can be assumed that there are no bacterial or fungal concentrations are normal in those rooms.

Also bacterial and fungal concentrations in the mail room were measured. It was very interesting to make, because people, that work there, complain to the unpleasant odor. So the task was to determine is this odor connected to the mold problem in the room. But measurement results showed that fungal concentration is normal in that room.

8. CONCLUSION

Nowadays, quality of the air which we inhale indoors and outdoors has great influence to the lungs health. Lungs are very feeble and can be easily damaged by pollutants which are contains in the air. These pollutants increase the risk of appearance of sickness like asthma, allergy, chronic bronchitis, lungs cancer etc.

Biological contaminants like fungi and bacteria can be a cause of bad indoor quality. They could be found worldwide. There are a lot of causes of its appearance; one of them is high moisture indoors, because all micro organisms are in need of water. So that is why moisture control is a key to the clean indoor air.

In present there are a lot of methods that can help in prevention of appearance of microorganisms. Some of them are rather cheap, so almost everyone can afford it. But problem is that information about these methods is not widely known. So, in future, one of the main aims should be the spreading of this kind of information in the community.

GLOSSARY

Most of all definitions are taken from Oxford Dictionary, www.oxforddictionaries.com

Mutagenicity- The capacity to give rise to mutations.

Mycologist - the scientific study of fungi.

Pathogen - a microorganism capable of causing disease.

Xerophilic - (of a plant or animal) adapted to a very dry climate or habitat, or to conditions where moisture is scarce.

Hydrophilic - having a tendency to mix with, dissolve in, or be wetted by water.

Mycotoxin - any toxic substance produced by a fungus.

Taxonomy - the branch of science concerned with classification, especially of organisms; systematics.

Saprophyte - a plant, fungus, or microorganism that lives on dead or decaying organic matter.

Hemolysin - a substance in the blood that destroys red blood cells and liberates hemoglobin.

Chemotype – Chemotype is a strain of a toxigenic fungus that produces a distinct pattern of secondary metabolites.

OR

Strain of a fungal species that produces a distinct pattern of secondary metabolites different from another group(s) of the species.

Inoculum - a substance used for inoculation.

Hyphae - each of the branching filaments that make up the mycelium of a fungus.

Morphology - the branch of biology that deals with the form of living organisms, and with relationships between their structures.

Nosocomial - (of a disease) originating in a hospital.

Anaerobic - relating to or requiring an absence of free oxygen.

Metabolite - a substance formed in or necessary for metabolism.

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APPENDIX A.

Table 1A. Most common indoor fungi /11, Appendix A/

Name of Fungi	Source	Note
<i>Acremonium</i> (including some species formerly classified under <i>Cephalosporium</i>)	water-damaged indoor materials including drywall, wood, and paper products	moistureloving fungus; may be found indoors are <i>A. kiliense</i> , <i>A. butyri</i> , <i>A. furcatum</i> , and <i>A. murorum</i> (synonym <i>Gliomastix murorum</i>)
<i>Alternaria</i>	water-damaged indoor materials; many kinds of plants and other substrates including seeds, soils, foodstuffs, wood and wood pulp, fungicide-treated utility poles, and textiles	more than 20 species; produce mycotoxins
<i>Aspergillus sydowii</i> and <i>Aspergillus versicolor</i>	water-damaged materials; soils, plant parts, paper pulps, photographic optics	<i>A. versicolor</i> is known to produce mycotoxins sterigmatocystin
<i>Aspergillus fumigatus</i>	filters of air-conditioning systems and air ducts; decaying plant materials, compost, wood chips, hay and crops, stored grains and stored sweet potatoes	thermotolerant-thermophilic species - can grow in a temperature range of 12°C to 57°C, with an optimal range of 37°C to 43°C
<i>Chaetomium</i>	water-damaged wood and paper product	<i>C. globosum</i> , <i>C. funicola</i> , <i>C. cochlioides</i> , <i>C. murorum</i> , and <i>C. elatum</i> . <i>C. globosum</i> is the most common and a moisture-loving fungus
<i>Cladosporium</i>	fibrous glass insulation	more than 500 names, the

	materials in heating, ventilation, and air-conditioning (HVAC) systems, window panes, cold storage rooms	most common species are <i>C. herbarum</i> , <i>C. cladosporioides</i> , and <i>C. sphaerospermum</i>
<i>Penicillium</i>	water-damaged environments	250 to 300 species; produce a variety of mycotoxins
<i>Paecilomyces variotii</i>	water-damaged wood products (such as wood subfloor) and dust	good indicator of water damage
<i>Stachybotrys chartarum</i>	cellulose-containing materials, water-damaged paper products	The spores may be dispersed by insects, small animals, water, or through air when disturbed. The fungus has been associated with indoor-air-quality complaints.

APPENDIX B

Table B1. Different methods of legionella prevention /5, p.51/

Method	Advantages	Disadvantages
Keeping temperature <20 °C	<ul style="list-style-type: none"> • Simple, effective and easily monitored • Little significant growth of <i>Legionella</i> 	<ul style="list-style-type: none"> • Only really applicable to drinking water systems
Keeping temperature >50 °C	<ul style="list-style-type: none"> • Simple, effective and easily monitored 	<ul style="list-style-type: none"> • Does not eliminate legionellae • Requires circulation temperature to be near 60 °C • Difficult to maintain temperatures in old systems • Requires protection against scalding
Periodic flushing with hot water at 50–60 °C (usually an essential part of control by high temperature, above)	<ul style="list-style-type: none"> • Simple, effective and easy to monitor 	<ul style="list-style-type: none"> • Not applicable in cold-water systems • Requires protection against scalding • Must be maintained and inspected to achieve consistent control • Recolonization occurs within days
Dosing with sodium Hypochlorite	<ul style="list-style-type: none"> • Proven, effective disinfection technique • Simple to use • Relatively cheap 	<ul style="list-style-type: none"> • Formation of trihalomethanes • Needs protection (e.g. carbon filter)

		<p>for dialysis patients</p> <ul style="list-style-type: none"> • Toxic to fish • Affects taste and odour • Not stable, particularly in hot water • Increases corrosion of copper
Dosing with hydrogen peroxide	<ul style="list-style-type: none"> • Simple to use 	<ul style="list-style-type: none"> • Weak disinfectant • Suspected of mutagenicity
Copper and silver ionization	<p>Effective when prescribed concentrations are maintained</p>	<ul style="list-style-type: none"> • Frequent monitoring of copper and silver needed • Pretreatment needed (pH, hardness) • Increased concentrations of copper and silver in water
UV (ultraviolet) Disinfection	<ul style="list-style-type: none"> • Proven disinfection technique • Simple to use 	<ul style="list-style-type: none"> • Effective only at point of application; no control downstream (no residual) • Not suitable for turbid waters • No effect on biofilm formation
Point-of-use filters	<ul style="list-style-type: none"> • Physical barrier • Easy to install (may require some modification of the outlet) • Suitable for hot and cold-water systems • Good for use in systems exposing high-risk patients 	<ul style="list-style-type: none"> • Only suitable at point of use • Must be replaced regularly • Particulates in water may reduce flow and operational life • Expensive

Pasteurization heat with flushing	<ul style="list-style-type: none"> • Disinfection barrier • Useful as short-term remedial measure • Simple to apply in hot-water installation 	<ul style="list-style-type: none"> • Transient effect on Legionella • No limitation of biofilm formation • Scalding risk
Non-oxidizing biocides	<ul style="list-style-type: none"> • Proven technique for cooling systems 	<ul style="list-style-type: none"> • Not suitable for potable water systems • Most not applicable to spa pools • Resistant populations may develop • Need to alternate two different biocides • Often concentrations cannot be readily monitored • Difficult to neutralize for sampling purposes

APPENDIX C

Table C1. 10. 11. 2010 measurements results.

Number of sample	numbers of colonies							Concentration cfu/m ³
	day 1	day 2	day 5	day 6	day 7	total	total corrected	
BO6	0	1	0	0	0	1	1	
BO5	0	3	8	5	0	16	16	
BO4	0	4	5	0	2	11	11	
BO3	0	6	3	4	1	14	14	
BO2	0	8	3	3	4	18	18	

BO1	0	4	3	0	1	8	8	
Total						68	68	240
FO6	0	0	0	0	0	0	0	
FO5	0	0	3	0	0	3	3	
FO4	0	1	1	0	0	2	2	
FO3	0	1	0	0	0	1	1	
FO2	0	0	0	0	0	0	0	
FO1	0	0	0	0	0	0	0	
Total						6	6	21
BR6	0	0	1	0	0	1	1	
BR5	0	4	1	5	1	11	11	
BR4	0	3	10	4	1	18	18	
BR3	0	1	2	1	0	4	4	
BR2	0	3	2	0	0	5	5	
BR1	0	5	2	1	0	8	8	
Total						47	47	166
FR6	0	0	0	0	0	0	0	
FR5	0	0	0	0	0	0	0	
FR4	0	0	0	0	0	0	0	
FR3	0	0	1	0	0	1	1	
FR2	0	0	0	0	0	0	0	
FR1	0	0	0	0	0	0	0	
Total						1	1	4
BC6	0	0	3	1	2	6	6	
BC5	0	4	32	0	0	36	38	
BC4	0	13	12	3	1	29	30	
BC3	0	10	9	2	0	21	22	
BC2	0	2	3	1	0	6	6	
BC1	0	7	4	2	0	13	13	
Total						111	115	406

FC6	0	0	0	0	0	0	0	0
FC5	0	0	1	0	0	1	1	1
FC4	0	0	3	0	0	3	3	3
FC3	0	0	1	0	0	1	1	1
FC2	0	0	0	0	0	0	0	0
FC1	0	0	0	0	0	0	0	0
Total						5	5	18

Table C2. 17.11.2010 measurement results.

Number of sample	numbers of colonies							Concentration cfu/m ³
	day 1	day 2	day 5	day 6	day 7	total	total corrected	
BO6	0	2	2	0	1	5	5	
BO5	0	5	2	0	0	7	7	
BO4	0	4	2	1	0	7	7	
BO3	0	3	2	1	0	6	6	
BO2	0	3	2	0	0	5	5	
BO1	0	1	1	0	0	2	2	
Total						32	32	113
FO6	0	0	0	0	0	0	0	
FO5	0	0	0	1	0	1	1	
FO4	0	0	0	0	0	0	0	
FO3	0	0	0	0	0	0	0	
FO2	0	0	0	0	0	0	0	
FO1	0	0	0	0	0	0	0	
Total						1	1	4
BR6	0	0	1	0	0	1	1	
BR5	0	3	7	2	0	12	12	
BR4	0	6	7	0	0	13	13	

BR3	0	6	12	1	0	19	19	
BR2	0	6	6	0	0	12	12	
BR1	0	9	10	0	0	19	19	
Total						76	76	269
FR6	0	0	0	0	0	0	0	
FR5	0	0	0	0	0	0	0	
FR4	0	0	0	0	0	0	0	
FR3	0	0	1	0	0	1	1	
FR2	0	1	0	0	0	1	1	
FR1	0	0	0	0	0	0	0	
Total						2	2	7
BC6	0	3	32	0	0	35	37	
BC5	0	138	62	2	7	209	296	
BC4	0	122	45	4	0	171	223	
BC3	0	59	47	5	1	112	131	
BC2	0	27	22	1	3	53	57	
BC1	0	22	30	2	5	59	64	
Total						639	808	2855
FC6	0	0	0	0	0	0	0	
FC5	0	1	0	2	0	3	3	
FC4	0	0	1	1	0	2	2	
FC3	0	1	1	0	0	2	2	
FC2	0	0	0	0	0	0	0	
FC1	0	1	0	0	0	1	1	
Total						8	8	28
BM6	0	0	3	0	0	3	3	
BM5	0	12	25	5	0	42	44	
BM4	0	12	20	8	1	41	43	
BM3	0	11	21	3	0	35	37	
BM2	0	3	5	3	0	11	11	

BM1	0	4	5	1	0	10	10	
Total						142	148	523
FM6	0	0	0	0	0	0	0	
FM5	0	1	0	1	0	2	2	
FM4	0	0	0	0	0	0	0	
FM3	0	1	0	0	0	1	1	
FM2	0	0	0	0	0	0	0	
FM1	0	0	0	0	0	0	0	
Total						3	3	11