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INDOOR AIR MOLDS & BACTERIA IN BUILDINGS

A Case Study

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DESCRIPTION

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Indoor Air Molds and Bacteria in Buildings, A Case Study.

Abstract

Unlike air pollution outside, most publicized, the indoor air remained relatively unknown until early 2000. Yet, we spend on average in temperate climate, 90% of our time in closed environments, and a majority of that time in the home: home, work premises or to receive the public, means of transport, in which we can be exposed to many pollutants. The nature of these pollutants depends on particular characteristics of the structure, activities and behaviors (tobacco, paint, etc.) and these pollutants can affect the health and well-being. The quality of indoor air is therefore subject of concern for many years and has emerged as a major public health issue.

Mold development and production of mycotoxins associated in some collective and individual buildings is likely to cause diseases, including allergic (rhinitis, asthma, skin and mucosal allergies). These dangers are of concern to susceptible populations (children, patients whose immunity is depressed, workers, etc…).

This study is based on the small town of Mikkeli in the region of Southern Savonia, specifically the area surrounding the campus of the Mikkeli University of Applied Sciences. The quality of indoor air through the technique of Andersen sampler to collect and identify the different fungi and bacteria that may have a pathogenic effect on humans. According to European Union mold exposure standards, three sampling areas (P_2, P_3 and Y_2) have a seem-like yeast contamination level above 50 CFU/m^3 (First level of contamination: < 50 CFU/m^3 – Very Low). Moreover, two sampling areas that exceeded the first level of contamination for *Actynomycetes* were observed (P_3 and Mt_2). Finally, the first level of contamination was also exceeded for molds on four different sampling areas (P_3, Y_1, Y_2 and Y_3).

Subject headings, (keywords)

Molds, Indoor air sampling, Andersen 6-stage Impactor.

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CONTENTS

1 INTRODUCTION

In recent years, the indoor air quality became a growing concern and a major public health issue. In industrialized countries, people spend on average 80% of their time in indoor spaces enclosed or semi-enclosed (housing, workplaces, schools, recreational areas, sports facilities, shops, transport, ...). The identification and removal of biological contaminants thus become a priority concerning the quality of the indoor environment. Indeed, many compounds as microbial volatile organic compounds (MVOC), allergenic proteins or mycotoxins can be produced in our buildings and thus decrease the indoor air quality. Moreover, the indoor environment is also affected by rising energy costs, since the oil crisis of the 70s, which generated a building insulation improvement demand and a decrease of the aeration to limit heat loss. These changes result in a deterioration in the quality of indoor air and proliferation of bacteria and molds.

Currently, only a small percentage of these microbial contaminants have been implicated in adverse health effects. However, they are majority responsible of the sick building syndrome (SBS), that is to say the set of non-specific symptoms, isolated or associated symptoms, occurring in persons occupying a same building. Furthermore, they could be responsible of inflammation of eyes and respiratory system, fatigue, headache, and in some cases, severe pulmonary problems for people suffering of immunodeficiency. In case of Finland, according to the "Moisture and Mould Programme", approximately 600,000 to 800,000 Finnish people out of 5,5 million are exposed daily to indoor contaminants and impurities caused by microorganisms. Among the people affected, the main buildings responsible for this problem are private homes but also schools [1] and daycare centers. So it is important to perform analyzes to identify the presence of these elements and prevent risks on the populations.

This study is based in the town of Mikkeli, a 58 000 inhabitants city in the region of Southern Savonia, close to the area surrounding the campus of the Mikkeli University of Applied Sciences (MAMK). Theoretical part of this study presents the different techniques of analysis that can be done concerning pollution of indoor air by microorganisms, but also the various analytical results obtained. Concerning health impacts, several hypotheses could be formulated. Indeed, having only the analysis for the Anderson sampler, the evaluation of the possible health problems thanks to this technique can not be

sufficient to obtain a rigorous conclusion on the health impacts. Finally, we can comment theoritical and analytical part concerning the different air sampling (different population, concentration…)

2 THEORY

Before introducing the subject, it is necessary to make a presentation of the concept of mold and mycotoxins, but also different parameters for microbial growth. In addition, an explanation of the sick building syndrome is required to understand the issues related to the quality of indoor air and a presentation of different standard limits for molds and bacteria.

2.1 Presentation of molds and mycotoxines

Among Eukaryotes, the Fungi are neither plants (since they don't perform photosynthesis) or animals but form a full reign. Formerly classified with algae, they now form an autonomous kingdom [1, 12]. Through the metabolism of photosynthesis, green plants can directly fix carbon dioxide from the air: they are said autotrophic. This isn't the case of fungi which are heterotrophic: they must find the carbon needed for their life in their immediate environment, in the form of organic matter. They feed organic matter dissolved by osmotrophy the following nutrition modes:

- ✓ *Saprophytism*: fungi can feed on dead organic matter or decaying (dead leaves, plant or animal debris, feces). They contribute to the recycling of organic matter.
- ✓ *Mutualism*: fungi can live in harmony with other living beings autotrophes to the point that one can not live without the other.
- ✓ *Commensalism*: A fungus is called "commensal" if he takes advantage of his host without harming it (he uses eg as a carrier).
- ✓ *Parasitism*: fungi can also take advantage of the living organic matter. They are parasites and live at the expense of a living on their own account. Often pathogenic, they cause disease and sometimes cause death to their hosts.
- ✓ *Carnivory*: Some mushroom species are carnivorous, they deploy traps to capture their food.

Fungi play a central role in many ecosystems, such as symbionts of trees, but mostly as decomposers completing the carbon cycle and many elements. With bacteria, they are

decomposers most involved in the degradation of organic matter and humus production in terrestrial ecosystems and play a crucial role in biogeochemical cycles and food chains. Some fungi are active in wetland and aquatic environments. Under certain conditions, a cell, called the spore will germinate by emitting a filament. During the development of the mold, while a network of filaments grows concentrically around the spore. The mold then forms a colony called mycelium colonizing the support in the degrading. All fungi are saprophytic, meaning they grow on and at the expense of very various inert materials such as paper, wood or food [3].

The presence of indoor mold has become over the years a concern for both health professionals and the population in general. Indeed, in recent years more and more studies conducted in North America and Europe have highlighted a possible link between the presence of indoor mold, the mycotoxines produced and various health damage. Mycotoxins are toxins produced by various species of mold can be toxic to humans. They are low molecular weight molecules (< 1000 Daltons) that are difficult to degrade.

The effects of mold on the health of occupants depending on the mode and extent of exposure, the nature of the agent involved and susceptibility of exposed individuals (health, age, etc.). The main symptoms due to the presence of indoor air bacteria and molds [4]:

TABLE 1. Symptoms and impacts on health of indoor air molds and bacteria

Symptoms od the respiratory system	Cough, sputum, irritation of the nose and throat, runny nose, sneezing, noisy breathing (wheezing), difficulty breathing, chest pain.
Respiratory allergies	Rhinitis, alveolitis, bronchitis, asthma, pneumonitis hypersensitivity.
Non-respiratory symptoms	Eye irritation, lesions and tissue infections.
Skin allergies	Skin irritation, dermatitis.
General toxic effects	Fever, chills, headache, nausea, vomiting, diarrhea, immune system deficiencies, fatigue, hair loss.

2.2 Temperature and humidity : Impacts on microbial growth

The management of temperature, humidity and ventilation is an important element in the interraction between humans and microorganisms. Indeed, these factors are primarily responsible for microbial growth as well as their distribution in the indoor environm-

-ent. As regards the temperature and humidity it is seen that the optimum temperature for microbial growth is between 40°F (≈ 4.4°C) and 100°F (≈ 37.8°C), relative humidity, for its part, must be higher than 70% (FIGURE 1).

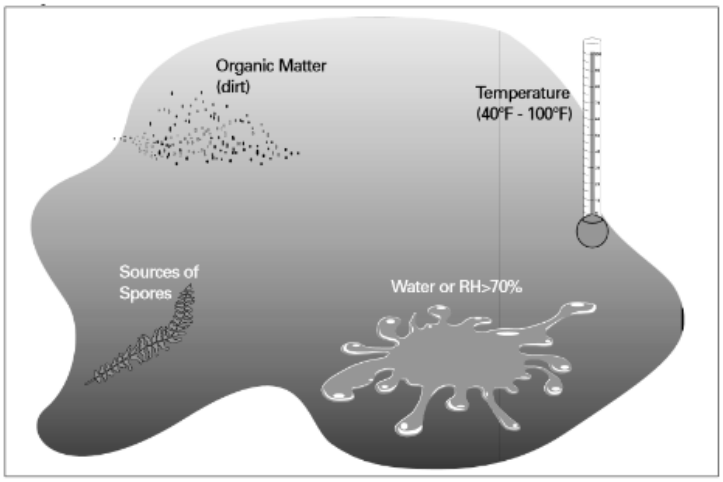


FIGURE 1. Main elements for microbial growth

(Source : Trane, 2002, "A guide to Understanding ASHRAE Standard 62-2001")

2.3 Dampness of buildings

In the indoor air environment, many viable or non-viable micro-organisms are present, thus forming a complex of biological contaminants. These contaminants can take different forms such as bacteria, pollen, fungi, for example, and can be highly allergenic for people living in the medium. The presence of these elements is induced by dampness problems, that is to say when the different materials in a building become sufficiently damp to generate material damage or visible mold growth. This moisture thus generates the growth of these biological contaminants which may result symptoms on human health such as asthma or respiratory symptoms. Dampness can also be an indicator of poor ventilation who can cause an increase of other potentially harmful indoor pollutants. Three types of moisture which are generally found in a building [11]:

1. *Condensation*: Most common cause of dampness in buildings. This moisture have an impact on old or new buildings and is directly associated with mold growth. It's cause warm and moist air meets a cooler surface.
2. *Rising Dampness*: Principally caused by the absorption of the humidity of the ground by the walls.
3. *Penetrating Dampness*: This phenomenon is caused when water enters through the walls.

2.4 Techniques for measuring biological contaminent and Legislation

The detection, sampling and identification of microorganisms, especially of fungi, are important to mitigate the indoor air quality problems. Concerning the sampling methods, there are two types, culturable methods and non-culturable methods [6].

Culturable methods are traditional microbial detection methods based on culturing of microosganisms on culture mediums. The advantages of this method are the possibility to identify the microbial genera or species in samples and the huge amount of reference materials concerning the microorganisms. However, this methods includes binding limitations. Indeed, only 10% of microbes occurring in indoor air (or materials) are culturable. Moreover, problems with the culture mediums is observed, the culture mediums and the incubation conditions can affect the results. There is no single medium that permits the growth of all microbes or there is some species that grow poorly on most media. One of the biggest problems of culturable methods is the interactions between the microbial colonies developing on the agar medium which may influence the results. The culturing is time consuming and labor intensive, but also require a significant number of materials such as growth media and therefore, a costly technique [28].

Non-Culturable methods are based on finding compounds from microorganisms like MVOCs, mycotoxins could be linked to the dusts, the use of markers to search for structural or constituent components of microorganisms, etc … These methods can be effective to assess pollution of indoor air. However, they do not identify precisely the microorganisms found in the contaminated area.

Concerning the identification methods, we can distinguish the phenotypic identification including “traditional” method based on the macroscopic and microscopic characteristics, the methods based on physiological characteristics and finally, the molecular methods based on the characteristics of sequences nucleic acids (DNA).

2.4.1 Enzymatic & PCR methods in analysis

Thanks to advances in the field of molecular biology, some precise and rapid techniques have been developed. They are based on different structures necessary for the proper functioning of cells and their interractions. These two different structures are:

Enzymes: These are proteins consisting of amino acid chains allowing the activation or acceleration (catalyst) of chemical reactions.
DNA: Deoxyribonucleic acid is a macromolecule composed of a succession of nucleotides (adenosine, cytidine, guanosine and thymidine), carrier of genetic information [3].

2.4.1.1 Mycometer

The mycometer is a test method for onsite quantification of fungal particles in air building. The methodology measures the level of fungal particles in terms of viable and most nonviable spores and hyphal fragments. It quantifies fungal particles in the air by measuring the level of the enzyme, β-N-acetylhexosaminidase (NAHA) from airborne fungal particles captured on a 0.8 μm MCE (Mixed Cellulose Ester) filter in sampling cassette.

FIGURE 2. Mycometer® Air

(Source : MOULDNMORE, 2015, "On site mould testing")

The enzyme present in the fungal cells hydrolyzes the enzyme substrate, so a fluorescence can be observed when the mixture is excited with ultraviolet light (365 nm). For sampling, it is necessary to collect 300 L to 600 L at a flowrate of 20 L/min. In addition, it should be performed at approximately 1,45 meters from the ground in order to obtain representative samples from the medium [7].

To analyse the cassette filters, 1 mL of the enzyme substrate will be added to the filter and incubate for around 30 minutes (the exact time being set by the room temperature). Thereafter 2 mL of an alkaline buffer is added to the filter and the liquid in the filter holder is sucked out through the filter and collected in a cuvette. The fluorescence of this fluid can be read on fluorometer and the fluorescence value for a control filter is

deducted from the sample value. To obtain a more robust measure and avoid non-informative figures, the values read in the fluorometer are divided by ten and given as a round figure to express the N-acetylhexosaminidase (NAHA) enzyme activity in units (EU per m^3). So, the amount of fluorescence correlates to the amount of fungi present in the sample [7].

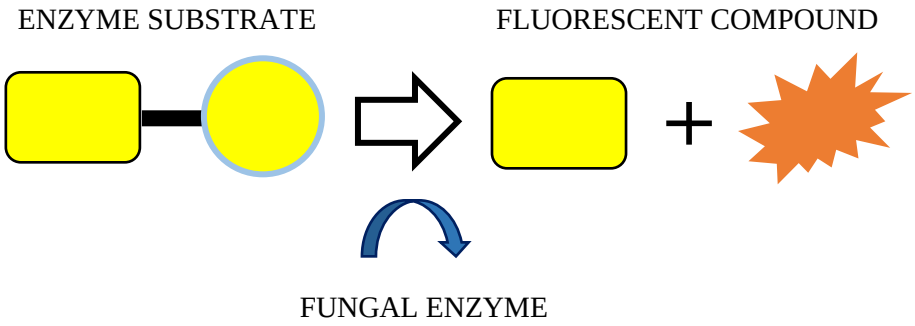


FIGURE 3. Fluorometric Detection of Fungal Enzyme Activity

(Source : CETEC, 2015, "Mycometer® Air")

After fluorescence analysis, there are three levels represented by three different colours where yellow and red colour mean a mold grow. Concerning the green colour, it means that the level of mold is not above normal background level (2.4.2 Legislation ; < 200 CFU/m^3). For the yellow color, the level of mold is above normal background level (> 200 CFU/m^3). It is typically due to high concentrations of spores, hyphal fragments in dust deposits but may in some cases indicate the presence of mold damage (mold growth). And finally, for the red colour, the level of mold is high above normal background levels due to mold growth (>1000 CFU/m^3).

2.4.1.2 PCR and other molecular methods for identification

There are several molecular biology techniques for identifying different species of mold. These techniques have a high identification rate, because they use the DNA as a reference and using PCR to amplify specific DNA sequences for identification. Among these molecular biology techniques, two of them will be detailed. First, the quantitative Polymerase Chain Reaction (or qPCR), and the second, the use of specif oligonucleotide (short segments of nucleic acids, as RNA or DNA, of a few tens of nucleotides) arrays In order to introduce these techniques, it is necessary to make a presentation of the PCR and its principle, to understand their identifications systems.

The Polymerase Chain Reaction (PCR) is a melcular method of genic amplification *in vitro* allowing duplication of an DNA or RNA sequence known from a low amount of nucleic acid (specific DNA sequence) and specific primers consisting of synthetic oligonucleotides of 20 to 25 nucleotides (FIGURE 4**)** [6].

The three steps constituting one cycle of PCR are performed at different temperatures to control the enzymatic activity :

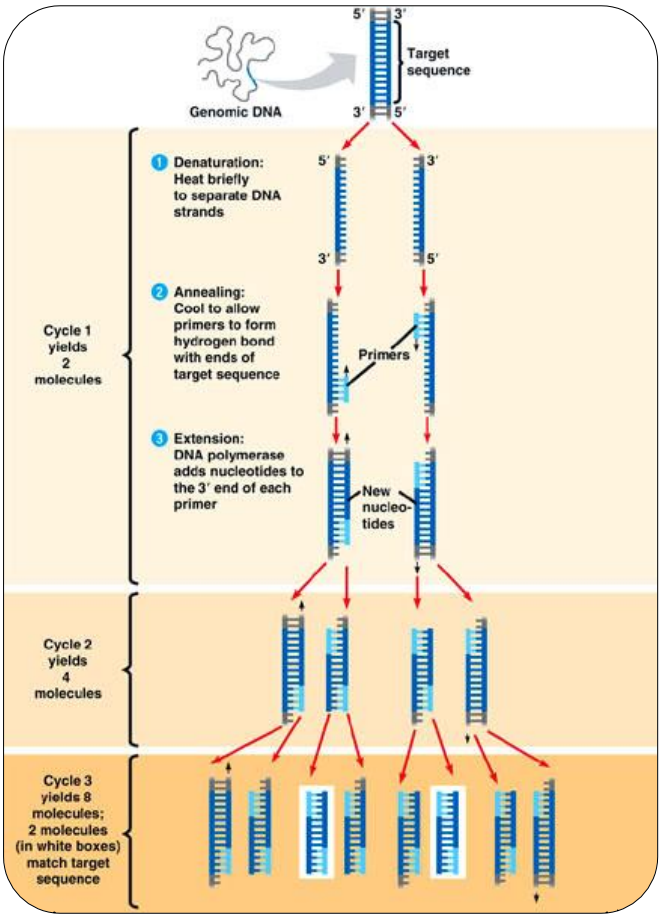


FIGURE 4. Principe of PCR

(John Pavlus, 2007, Scienceblogs)

1. Denaturation of DNA by heating (10 to 15 minutes at 95°C). The hydrogen bonds are broken and the two strands of DNA separate. DNA passes as single stranded in the medium.
2. Hybridization of the primers to the extremities of the desired sequence (2 to 60 seconds at 50 to 60°C). Generally, the primers are in large excess.
3. Elongation thanks to the action of a DNA polymerase which extends the primers by incorporating complementary deoxyribonucleic sequence (4 to 120 seconds at 72°C).

This cycle is repeated many times to obtain an exponential increase of the target DNA sequence. In addition, in the development of the PCR reaction, the choice of primers is crucial. They will have a dual role: By hybridizing to the DNA template, they delimit the DNA region to be amplified (step 2 of the cycle) and, with their extremity 3' OH free, serving as a primer for DNA polymerase (step 3 of cycle). The oligonucleotide primers hybridize to the ends of the sequence that will be amplified, it is necessary to know the nucleotide sequences of the extremities of the amplified DNA region. This is at the extremities that the oligonucleotide primers will hybridize [6].

Quantitative PCR (qPCR) is based on the ability to track over time the PCR process using fluorescence (FIGURE 5). Fluorescent probes are fixed either on double-stranded DNA (SYBR technology) or on a specific DNA sequence (Taqman and Beacon technology). The fluorescence of the probes appear once they are attached to DNA.

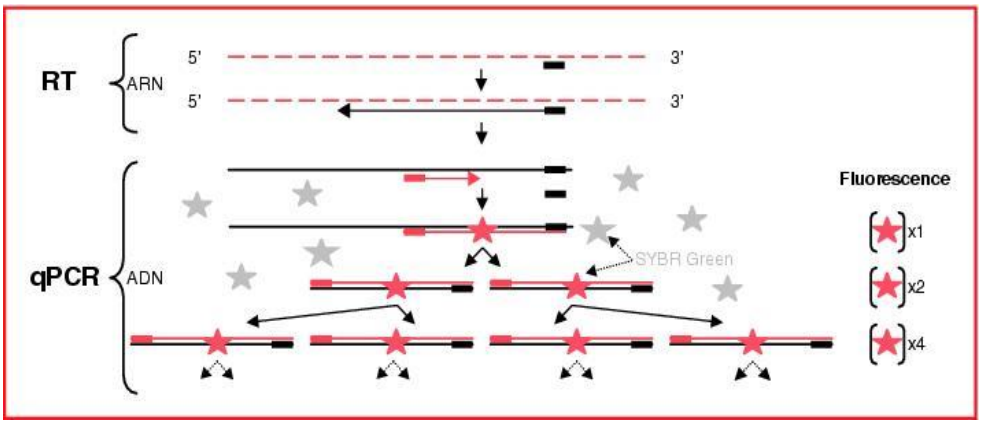


FIGURE 5. Principe of qPCR with SYBR Green

(Plumet S and Gerlier D, Virol Methods, 2005)

All the DNA present in the sample is extracted, then identified and quantified using fluorescently-labeled probes that are specific to the mold species of interest. Air samples will be taken on a polycarbonate filter with an air volumes of 7000 L or more, permitting an overloading (common for qPCR samples, greatly increasing the sensitivity of air sampling). This technique allows us to identify the different kinds of mold harvested and to be able to quantify them with precision [8].

Through sequence databases and a matrix table, it is possible, after PCR, to recognize the different molds thanks to various probes of oligonucleotides specific to each species. Oligonucleotide probes are designed from the Internal Transcribed Spacer (ITS) regions.

TABLE 2. Example of sequences of molds

(Wen-Tsung Hung, Shu-Li Su, Lin-Yi Shiu and Tsung C Chang, Rapid identification of allergenic and pathogenic molds in environmental air by an oligonucleotide array, 2011)

Microorganism	Probe					
	Code[a]	Sequence (5'-3')[b]	Length (nt)	T_m (°C)	Location[c]	GenBank accession no.
Acremonium strictum[d]	Acstr2-5	CTGCGTAGTAGCACAACCTCGCAttttttttttt	23	59.1	431-453 (2)	AJ621771
Alternaria alternata[e]	Alalt3	CGCACTCTCTATCAGCAAAGGTCTAGCATC	30	63.5	461-490 (2)	AY625056
Aspergillus flavus[e]	Asfla4	CGAACGCAAATCAATCTTTTTCCAGGT	27	63.1	512-538 (2)	AY373848
Aspergillus fumigatus[e]	Asfum2-1	GCCAGCCGACACCCAACTTTATTTTTCTAAttttttttttt	30	65.4	213-242 (2)	AY230140
Aspergillus niger[e]	Asnig2	ACGTTTTCCAACCATTCTTTCCAGGT	26	60.9	517-542 (2)	AY373852

This probes have been developed to identify a wide variety of pathogenic molds. ITS is a non-coding region and highly polymorphic of ribosomal DNA. It is a non-coding region and highly polymorphic (**FIGURE 6. ITS regions**). For this reason, it is very frequently used in cell biology to demonstrate a genetic difference between two species, particularly in fungi.

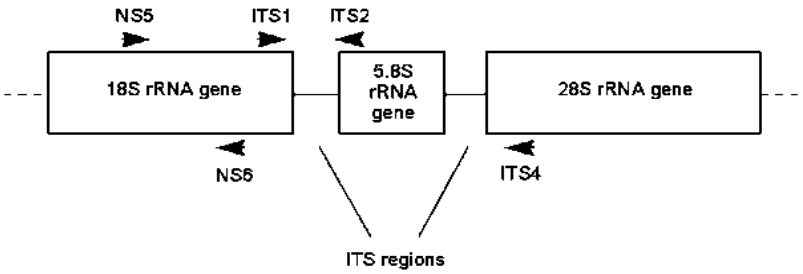


FIGURE 6. ITS regions

(Hart, Development of diagnostic systems for the study of the molecular epidemiology and taxonomy of the *Aphanomyces* fungus associated with EUS of tropical and freshwater fishes, 1997)

2.4.2 Legislation

One of the major problems with the quality of indoor air is the absence of specific limitations on mold and bacteria. Many factors affect on the establishment of specific limitations like human sensitivity, particle toxicity or spore size or mass.

Every human have a different sensitivity for allergic reaction against molds. Indeed, they are a wide variation in individual human reaction (better resistance to allergies, faster lymphatic reactions ...), allergenicity, pathogenicity, and toxicity for molds.

Moreover, cause of the different substrates upon which molds is growing, molds can have different impact on the human health [20].

Particle toxicity: The different toxins produce by microorganisms varies between molds species. The toxicity can ranging from none to probably highly toxic. Moreover, the toxicity of a same molds can varies with specific conditions (substratum, moisture...).

Spore size or mass : A large spore contains potentially more harmful material than a small spore with the same toxicity.

However, some references are used by the different organisms like European Union mold exposure standards (TABLE 3) [11] :

TABLE 3. Indoor air molds counting levels

(Baubiologie MAES, Building Biology Evaluation Guidelines SBM-2008, 2008)

Indoor air molds counted (CFU/m³)	Level of contamination
< 50	Very low
< 200	Low
< 1000	Medium
< 10000	High
> 10000	Very high

Concerning WHO (World Health Organisation), the pathogenic and toxinogenic fungi aren't acceptable in indoor air. Moreover, from 50 CFU/m³ of air of a single fungal species, the source of contamination needs to be identified [11].

3 MATERIALS AND METHODS

During this study, several elements were used to allow the growth of the samples, their observations as well as their identifications. Thus, it is necessary to understand the use of this material and methods to carry out this case study.

But, before every sampling, a wide range of parameters were measured (temperature, humidity, ...) in order to define parameters necessary for a good understanding of the results.

3.1 Indoor air sampling and cultivating

In this study, all samplings were carried out with the Andersen sampler technique, a culturable method. It is one of techniques called “routine procedure” in laboratories.
The use of an Andersen sampler (or Andersen impactor) is one of the most used cultural method in order to carry out indoor air samples. Indeed, it allows, through the use of a pump, filters and appropriate growth media, to cultivate molds and bacteria present in the air [7].

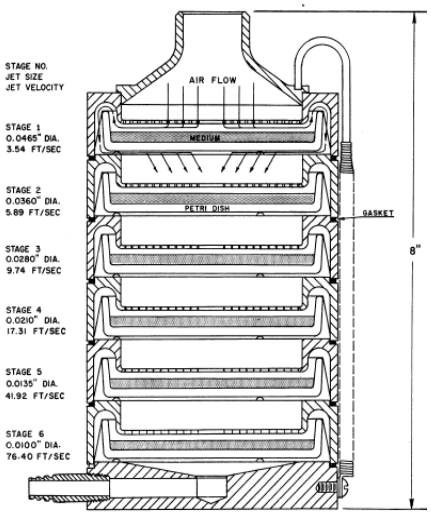


FIGURE 7. Different stages of Andersen Sampler

TABLE 4. Stage size

Stage	Size (µm)	Analogy
n°1	> 7,0	Nasal cavity
n°2	4,35 – 7,0	Pharynx
n°3	3,3 – 4,65	Trachea and primary bronchi
n°4	2,1 – 3,3	Secondary bronchi
n°5	1,1 – 2,1	Terminal Bronchi
n°6	0,6 – 1,1	Alveoli

To proceed with sampling, it is necessary to previously clean the Andersen sampler with alcohol at 70° to avoid possible external contaminations, due to its storage and/or transport. This is composed of six floors (**FIGURE 7 & TABLE 4**) with supports for depositing the growth medium as well as different pore diameters.

Indoor air passes through the various filters of the Andersen sampler with a flow rate of 28.3 L/min during 10 to 15 minutes (depending on hypothesis). This has the consequence the retention of spores and bacteria, allowing inoculation of the agar. The culture media are incubated for one to two weeks at ambient temperatures, allowing the development of different microorganisms.

A growth medium (petri dishes) containing molds and bacteria on the sixth floor can induce a higher risk for the resident of the building. In this study, Malt Agar 2%, DG-18 agar and THG agar were used.

Malt Agar 2%: The agar malt extract is used for the enumeration of yeasts and molds. It contains malt extract, bacteriological agar-agar as well as chloramphenicol, an antibiotic growth promoting molds and yeasts and inhibiting the growth of bacteria.
DG-18 agar: Dichloran Glycerol agar is recommended for the enumeration of yeasts and molds. It reduces the size of the mold of fronds and yeast colonies, to facilitate counting. It contains tryptone, glucose, potassium dihydrogen phosphate, magnesium sulfate, dichloran, chloramphenicol, glycerol and bacteriological agar- agar.
THG agar: The trypton-yeast extract-glucose agar is used for the enumeration of bacteria. It contains tryptone, the autolytic yeast extracts, glucose and bacteriological agar-agar. Natamycin is added to the agar to prevent mold growth.

Molds and bacteria are then identified by macroscopic and microscopic observation to assess the damage and potential toxins generated by them.

3.2 Identification

In this study, all of analysis were carried out with the macroscopic and microscopic observation. This technique doesn't require much equipment or reagent, then it is very easy to implement. Indeed, the microscope and binocular loupe are the only instruments used during the observations.

3.2.1 Macroscopic observation

Macroscopic criteria are based on the observation of colonies and their front and back color, size, relief, appearance (filamentous, sticking), transparency (opaque, translucent), the shape of the contours and pigmentation [7]. The smell of mold can be a very important element of identification, but given the potential risk of inhalation of spores and others, it is not advisable to proceed with an olfactory examination of cultures.

3.2.2 Microscopic observation

The microscopic criteria are based on the morphology of the different structures of mold: the type of thallus (septate or not), the hyphae (color, presence or absence of partitions, approximate diameter, and special structures), the structure and arrangement of spores (color, shape, partitions, ornamentation, and size), the origin of the spores

(endogenous or exogenous) and the reproductive organs (location, color, shape ...).

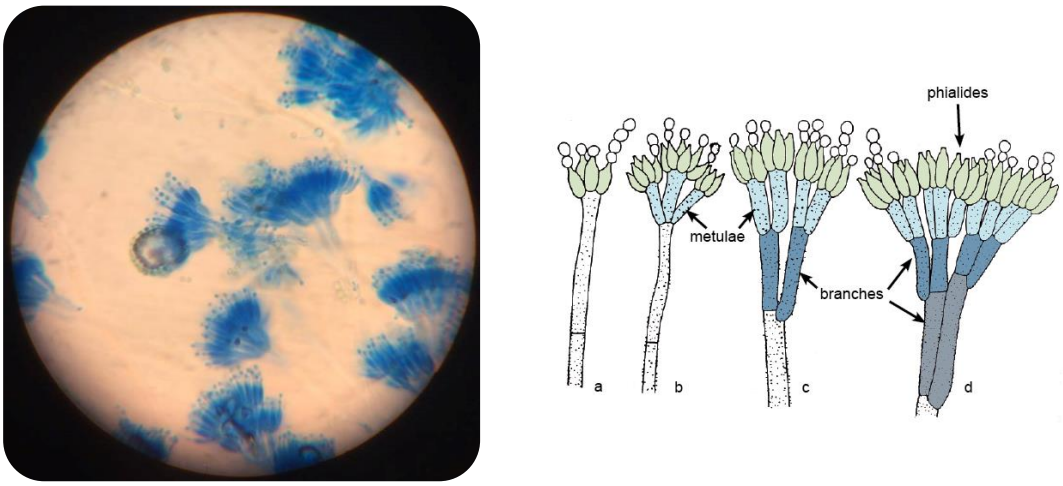


FIGURE 8. Microscopic observation of *Penicillium* with lactophenol cotton blue

(Source : LEBRAT Julien, 2016)

All observations were made with a binocular optical microscope with ×10, ×40 and ×100 objectives. In addition, a camera for microscopy was used to make some shots. To distinguish the different structures, lactophenol cotton blue staining is performed on the mold. Lactophenol Cotton blue (or methyl blue) is an acid dye. This is the dye the more suitable for general mycology because it is specific for callose, which is a major constituent of the cell wall of fungal hyphae. This coloration allows a good conservations of structures avoiding contraction and swelling of the cells.

A first observation of structures is carried out with the ×40 objective to discern the general structure of the mold. To accurately observe spores and key structures allowing better identification, immersion observation is made with the ×100 objective and with the immersion oil to increase the resolving power of objectives.

Many paper documents (book, publication, ebook…) [22] and several identification sites were needed to make identifications. In addition, with the help of the expert in the field, my tutor Maritta Jokela, many molds have been accurately identified. For example, the use of the database of the University of Adelaide [23] allows to find a Gender of mold very precisely thanks to the macroscopic and microscopic aspects. The use of Lucid 3.5 software allows us, through a series of choices concerning structural information (color colonies, forms spores ...), to make a selection among the known mold and identify it with a good accuracy.

However, this identification method is difficult, it requires specialized training and years of experience. In addition to requiring up to two weeks for identification, this method can be very inaccurate because of the variety and the resemblance of some species. It is often difficult to get an accurate identification of mold, which can stop to the Genus. In conclusion, it is necessary to be critical with these results and realize them several times.

4 RESULTS

All my analyzes were performed around the university campus. However, considering the various aministrative procedures performed with the Mikkeli University of Applied Sciences, I'm not allowed to disclose the location of some buildings that needs an access authorization. Of the six buildings analyzed, five are part of the university, and among these, two buildings have required access authorization. Following a request from an organization other than the University of Mikkeli, we were able to perform analysis in a building that had some problems due to water damage. Thanks this request of a this person responsible for the building, we were able to perform indoor air bacteria and mold analysis.

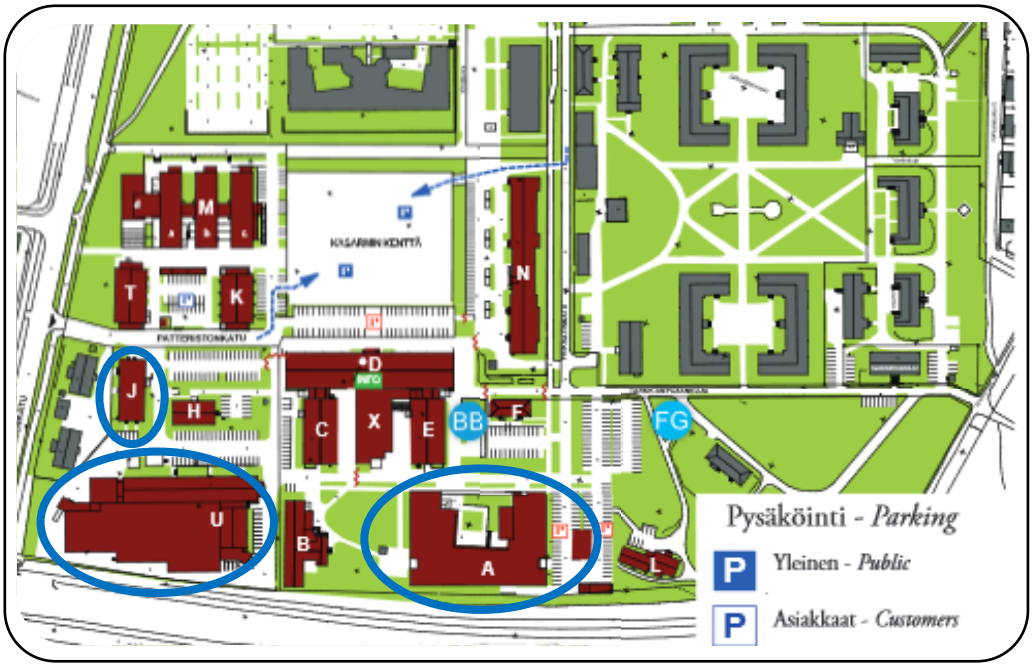


FIGURE 9. Campus of Mikkeli University of Applied Sciences

(Source : MAMK, 2016)

A total of thirteen different rooms were analyzed in different six buildings, so 36 sampling of 10 and one of 15 minutes were performed to obtain optimum results on various

types of agars. Around 222 agar were inoculated and analyzed to identify different microorganisms present in buildings that may have an impact on human health.

The first analysis was made in the second floor of the building J, just above the microbiology and chemistry lab. This place is generally use by teachers or by some students who make their intership. The results of the various parameters to be realized before analysis:

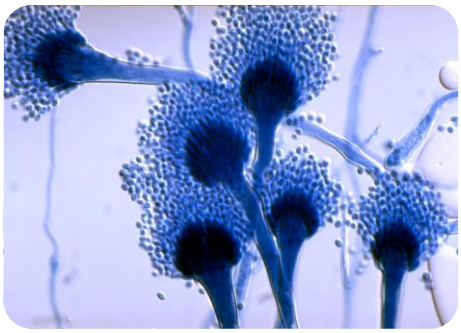
TABLE 5. Parameters of the 2nd floor of the Laboratory

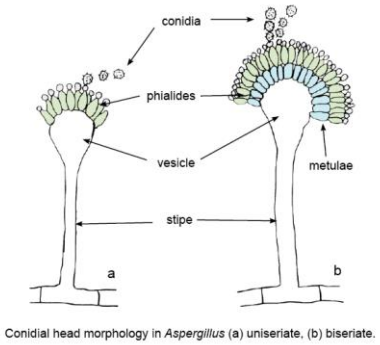
Room	Laboratory 2nd floor	**Relative humidity**	48.6%
Breeding ground	Malt 2%, DG-18, THG	**Ventilation - Activities - Closed**	Ventilation OK – Door open
Sampling time	10 min	**Window open - Captive**	1 - Closed
The sample taken at	15/01/16 - 13h20	**Number of persons during sampling**	5
The sampling height	0,95 m	**Pets**	No
Inside temperature	21,9°C	**Activities during the measurement**	No activity
Outside temperature	-15,6°C	**The number and location of visible mold**	No

Concerning the various molds obtained, we were able to analyze some of them. In fact we have noticed the presence of a particular colonies of molds on the stage 4 and 5 in Malt 2% agar. After staining with lactophenol, the structure of these molds are reminiscent of *Penicillium* (**FIGURE 8**). Indeed, the colonies are in shades of green and made of a dense felt of conidiophores.

In addition, the hyphae are branched or unbranched conidiophores and metulae are more or less cylindrical with smooth walls, with three to six phialides in the form of bottle. The analysis show the presence of long chains of small spores, round to oval (2.5-5 microns). All these elements enable us to hypothesize the presence of *Penicillium*.

We have detected the presence of another type of easily identifiable mold. This mold is found on DG-18 agar, more precisely on the stage 4. So, the observation show the presence of conidiophores erected and terminated by a vesicle carrying a single row of phialides, or a row of phialides and a row of metulae. These various parameters allows us to identify the presence of the mold *Aspergillus*. In addition, the fluffiness of the mold is also an indication concerning its identification.





Conidial head morphology in *Aspergillus* (a) uniseriate, (b) biseriate.

FIGURE 10. Microscopic Observation of Aspergillus with lactophenol cotton blue

On the stage 2 of THG agar, we can observe one colony different from the others. Indeed, an overhead structure as well as a mushroom appearance seems to be present. We observe the presence of Gram^{+} bacteria which gives rise to colonies composed of hyphae. Moreover, we can smell a strong bad smell from the agar. All of these elements indicate the presence of *Actinomycete*.

FIGURE 11. Macroscopic observation, example of *Actinomycete sp*.

(Source : LEBRAT Julien, 2016)

Finally, regarding the rest of the colonies on different stages of agars, we were able to detect the presence of yeast. The presence of no specific structure was observed (only presence of unicellular cell), and the overall macroscopic appearance of the colonies confirms the hypothesis of the presence of yeast.

TABLE 6. Results for the Second floor of J building, Molds

	Localisation:	**Second floor laboratory**	
	Stage	***Number & type of colony***	***Identification***
Type of agar: Malt 2%	n°4	1 colony → Small molds - White colonies - Stringy	YEAST
		1 colony → Big molds - Middle : Light green - Intermediate : White - Border : Yellow - Bellow : Yellow/Green	PENICILLIUM
	n°5	1 colony → Big molds - Middle : Light green - Intermediate : White - Border : Yellow - Bellow : Yellow/Green.	PENICILLIUM
		5 colonies → White colonies - Stringy	YEAST

Type of agar: DG-18	n°4	4 colonies → Big molds - Middle : Dark green - Intermediate : White - Border : Yellow - Bellow : Yellow/Green.	ASPERGILLUS
	n°5	2 colonies → White colonies - Stringy	YEAST

At the end of the identification phase, so we can count the number of microorganisms (CFU) per m^3 of air pumped for each agar. To do this, simply perform the following calculation:

$$\frac{Conversion\ factor\ (L\ to\ m^3)}{(Pump\ flow \times Sampling\ time\ with\ pump)} \times \frac{Number\ of\ colonies\ on\ agar\ (Molds\ \boldsymbol{or}\ Yeasts)}{(Pump\ flow \times Sampling\ time\ with\ pump)} = Colonies\ forming\ units\ per\ m^3 of\ air$$

EQUATION 1. Parameters to obtain the number of colonies per m^3 of air

And in this table for the bacteria and *Actinomycetes*:

TABLE 7. Results for the Second floor of J building, Bacteria & Actinomycetes

Type of agar: THG	n°1	1 colony → Small colony - Yellow - Sticky.	BACTERIA GRAM $^+$
		1 colony → Small colony - White - Sticky.	SEEM LIKE YEAST
	n°2	1 colony → Small colony - Middle : Dark Yellow - Border : Yellow- Sticky.	ACTINOMYCETE
	n°3	1 colony → Small colony - White - Sticky.	SEEM LIKE YEAST
	n°6	1 colony → Small colony - Yellow - Sticky.	SEEM LIKE YEAST

For the Malt 2% agar, with a total of 8 colonies developed on it, we obtain an average of 4,7 CFU/m^3 for *Penicillium* and 14,1 CFU/m^3 for yeast. *Penicillium* represents 25% of microorganisms present on this agar against 75% for yeasts. Concerning DG-18 agar, with a total of 6 colonies, we obtain an average of 9,4 CFU/m^3 for *Aspergillus* and 4,7 CFU/m^3 for yeast. *Aspergillus* represents 66.7% of microorganisms present on this agar against 33.3% for yeasts. Finally, on THG agar with a total of 5 colonies, we obtain an average of 2,4 CFU/m^3 for *Actynomyces*, 7,1 CFU/m^3 for seem-like yeast and 2.4 CFU/m^3 for bacteria. So, *Actynomyces*, represents 20% of microorganisms present on this agar against 60% for seen-like yeasts and 20% for bacteria.

The second analysis was made in two different rooms of building U, the fitness building (Room U237A & U237B). This place is one of the most frequented spot on campus because they are a lot of students and teachers who use this place everyday for musculation, yoga and others activities…

TABLE 8. Parameters of the Fitness building

Room	U237A & U237B	**Relative humidity**	25.4%
Breeding ground	Malt 2%, DG-18	**Ventilation - Activities - Closed**	Ventilation OK – Door open
Sampling time	10 min	**Window open - Captive**	3 - Closed
The sample taken at	22/01/16 – 9h15	**Number of persons during sampling**	12
The sampling height	1,33 m	**Pets**	No
Inside temperature	24.2°C	**Activities during the measurement**	Sport - Musculation
Outside temperature	-10.3°C	**The number and location of visible mold**	No

The analysis of this building is important because during exercise, respiratory activity is stronger, so the inhalation of contaminated air is more important and can have a more significant impact on health.

FIGURE 12. Room U237A

(Source : LEBRAT Julien, 2016)

However, the building is relatively new and has an efficient ventilation system and the double glazed windows are recent. All the results of this area are in the following table:

TABLE 9. Results of the fitness building

Localisation: U237A	**Type of agar : Malt 2%**	
Stage :	**Number & type of colony**	**Identification**
n°4	1 colony → Big molds - Middle : Light green - Border : Dark green	PENICILLIUM
	1 colony → Small colony - Yellow - Sticky.	SEEM LIKE YEAST

Localisation: U237B	**Type of agar : DG-18**	
Stage :	**Number & type of colony**	**Identification**
n°3	32 colonies → Small colony - Yellow - Sticky.	SEEM LIKE YEAST

The absence of microbial growth on agar DG-18 for the room U237A as well as Malt 2% agar from U237B room was observed. On the stage 4 of the Malt 2% agar of the room U237A , the growth of a ubiquitous mold of indoor air *Penicillium was* observed. It also noted the presence of a yeast at the same stages. On agar DG-18, the exclusive growth and large number of yeasts on the stage 3 of the U237B room was noticed. These results show that, there is a concentration of 3,5 CFU/m^3 of *Penicillium* and yeasts for the room U237A. For the room U237B, we obtain an average of 113,1 CFU/m^3 for seem-like yeast. So, there are 50% of *Penicillium* and 50% of yeasts on Malt 2% agar. It is therefore wise to take more into consideration the U237 room because it contains mold which may have an impact on human despite it low concentration.

Next analyses were taken from building P who requiring an access authorization. The university didn't give me the permission to reveal the position of it, so we will name the area P. Three different pieces were analyzed including a room with a bar, a party room and a meeting room. All these areas are generally used by students during the week, so it is important to take samples.

The first room analyzed will have the name of P_1. At first glance, we see that the building is old of several tens of years, including old window models with double glazing. Moreover, we observe the presence of fabric sofa with a large amount of dust, which could harbor spores or other organic compounds.

FIGURE 13. First sampling area of building P

(Source : LEBRAT Julien, 2016)

The place does not seem to be cleaned very well, but we can observe a ventitation system fairly distributed in the room. In view of figure above, the analysis was carried out on the table is located below the ventilation system, encompassing the entire area.

TABLE 10. Parameters of the P_1

Room	P_1	**Relative humidity**	62.7%
Breeding ground	Malt 2%, DG-18, THG	**Ventilation - Activities - Closed**	Ventilation OK – Door closed
Sampling time	10 min	**Window open - Captive**	5 - Closed
The sample taken at	04/02/16 – 14h30	**Number of persons during sampling**	3
The sampling height	0,9 m	**Pets**	No
Inside temperature	20.8°C	**Activities during the measurement**	No activities
Outside temperature	-5.1°C	**The number and location of visible mold**	No

We can notice that after incubation and observation of different agar the presence of two new kind of mold: *Paecilomyces* and *Cladosporium*. For *Cladosporium*, the colonies are flat and dense, it becomes powdery or velvety (because of the abundance of conidia) and are olive green to olive brown. Moreover, the hyphae are septate and dark. Conidia are cylindrical to elliptical shape and are produced in branched chains. They include well-marked insertion scars. For *Paecilomyces*, phialides are thin, inflated to their bases, elongated at their ends, and are generally grouped into brush. Conidiophores are branched and bear phialides at their ends. As for conidia they are oval and unicellular form.

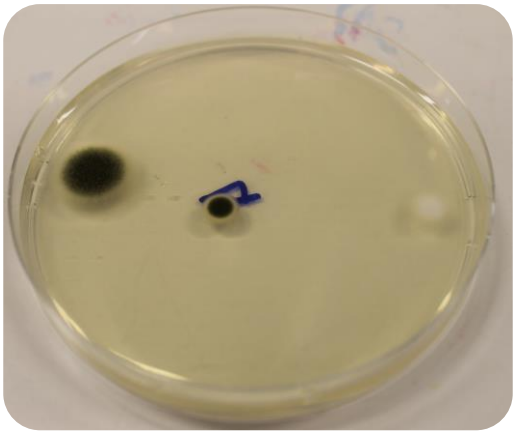


FIGURE 14. Macroscopic observation of *Paecilomyces* (white color) & *Cladosporium* (khaki color)

(Source : LEBRAT Julien, 2016)

They grow as well on Malt 2% agar (stage 4 and 5) and DG-18 agar (stage 4 and 5). Moreover, it was observed the presence of *Actinomycete* on stage 1, 3 and 6 of the THG agar. The rest of different colonies were identified as yeasts. All the results obtained for the zones P_1 are contained in the table:

TABLE 11. Mold of P_1

	Localisation:	**Area P_1**	
	Stage :	**Number & type of colony**	**Identification**
Type of agar: Malt 2%	n°3	1 colony → White color above, Yellow color below	YEAST
	n°4	1 colony → White color above, Yellow color below	YEAST
		1 colony → Dark green color middle, Light green color intermediate, White color side. Same bellow	CLADOSPORIUM
	n°5	2 colonies → Dark green color middle, White color side, Yellow color below	PAECILOMYCES
		1 colony → White color above, Yellow color below	YEAST
Type of agar: DG-18	n°2	1 colony → White color	YEAST
	n°4	1 colony → White color stringy	PAECILOMYCES
		2 colonies → Dark green color middle, Light green color intermediate, White color side. Same bellow	CLADOSPORIDIUM
	n°5	1 colony → Dark green color middle, Light green color intermediate, White color side. Same bellow	CLADOSPORIDIUM

So, on Malt 2% agar, with a total of 6 colonies developed on it, we obtain an average of 7,1 CFU/m^3 for *Paecilomyces*, 3,5 CFU/m^3 for *Cladosporium* and 10,6 CFU/m^3 for yeasts ; So, the concentration total of mold is 10,6 CFU/m^3. *Paecilomyces* represents 33.3% of microorganisms present on this agar, *Cladosporium* 16.7% and yeasts 75%. Concerning DG-18 agar, with a total of 5 colonies, we obtain an average of 10,6 CFU/m^3 for *Cladosporium* (60%), 3,5 CFU/m^3 for *Paecilomyces* (20%) and 3,5 CFU/m^3 for yeast (20%) ; So, the total concentration of mold is 14,5 CFU/m^3.

TABLE 12. Bacteria & Actinomyces of building P_1

	Counted colonies		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum
1	3	0	3
2	0	1	1
3	1	1	2
4	0	3	3
5	0	2	2
6	2	0	2
Total	6	7	13

Finally, on THG agar with a total of 13 colonies, we obtain an average of 21,2 CFU/m^3 for *Actynomyces* (46.2%) and 24,7 CFU/m^3 for seem-like yeast (53.8%).

We moved to the second part of the building, the party room often used by students. A very big mess in this room is present during our arrival. Indeed, many of furniture, carpets and materials littered the floor of the analysis area.

FIGURE 15. Second sampling area of building P

(Source : LEBRAT Julien, 2016)

In addition, the presence of mold encrusted in the parquet and covered with a protective paper is observed (**FIGURE 15**). This room has many old windows and humidity seems important.

TABLE 13. Parameters of the P_2

Room	P_2		**Relative humidity**	62.7%
Breeding ground	Malt 2%, DG-18, THG		**Ventilation - Activities - Closed**	Ventilation OK – Door closed
Sampling time	10 min		**Window open - Captive**	5 - Closed
The sample taken at	04/02/16 – 15h15		**Number of persons during sampling**	3
The sampling height	1,34 m		**Pets**	No
Inside temperature	21.5°C		**Activities during the measurement**	No activities
Outside temperature	-4,9°C		**The number and location of visible mold**	Visible molds on the ground

Above all, the analysis show the presence of mold already observed before: *Penicillium*. Three new molds are emerging during the analyzes. Two of them were identified with greater precision, the first one is *Aureobasidium pullulans*. For it, the spores are produced in great masses along the filaments and occur on short lateral branches. The presence of rough scar, generally produce when the spores are released and *Stachybotrys chartarum* is also observed [18].

For *Stachybotrys chartarum*, it is characterized by clusters of colourless to brown swollen phialides. The dark brown unicellular spores are produced from the tips of the phialides. The last identified mold is *Oidiodendron*.

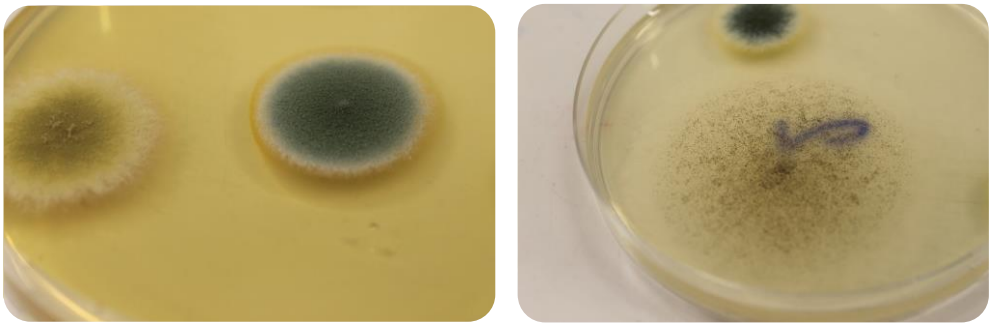


FIGURE 16. Macroscopic observation of Stachybotrys chartarum (on the left) & Aureobasidium pullulans (on the right)

(Source : LEBRAT Julien, 2016)

We find the presence of mold on the stage 3, 4 and 5 on Malt 2% agar and stage 5 on DG-18 agar for *Stachybotrys chartarum*. For *Aureobasidium pullulans*, we can find it on the stage 2 and 4 on DG-18 agar. Finally, the mold *Oidiodendron* is found on stage 5 on DG-18 agar. For this mold, the dark conidiophores are erect, tall and are terminated by a rather irregular system of tree-like branches. The brown to colourless conidia are produced by the fragmentation of the conidiophore branches.

TABLE 14. Mold results of P_2

	Localisation:	**Area P_2**	
	Stage :	**Number & type of colony**	**Identification**
Type of agar: Malt 2%	n°2	3 colonies → Dark green color middle, White color side, White/Yellow color below	PENICILLIUM
	n°3	2 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		1 colony → Light Khaki color, White color side	STACHYBOTRYS CHARTARUM
	n°4	2 colonies → Dark green color middle, White color side, Yellow color below	PENICILLIUM
		2 colonies → Light Khaki color, White color side	STACHYBOTRYS CHARTARUM
	n°5	1 colony → Dark green color middle, White color side, White color below	PENICILLIUM
		1 colony → Light green color middle, White color side, White color below	STACHYBOTRYS CHARTARUM
		2 colonies → White color, Stringy	OIDIODENDRON
Type of agar: DG-18	n°2	2 colonies → Dark green color middle, White color side, Green/Yellow color below	PENICILLIUM
		1 colony → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS
	n°3	1 colony → Dark green color middle, White color side, Green/Yellow color below	PENICILLIUM
	n°4	2 colonies → Dark green color middle, White color side, Green/Yellow color below	PENICILLIUM
		1 colony → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS
	n°5	2 colonies → Dark green color middle, White color side, Green/Yellow color below	PENICILLIUM
		1 colony → Light green color middle, White color side, White color below	STACHYBOTRYS CHARTARUM

So, on Malt 2% agar, with a total of 14 colonies developed on it, we obtain an average of 28,3 CFU/m^3 for *Penicillium* (57.1%), 14,1 CFU/m^3 for *Stachybotrys chartarum* (28.6%) and 7,1 CFU/m^3 for *Oidiodendron* ; So, the concentration total of mold is 49,5 CFU/m^3. Concerning DG-18 agar, with a total of 10 colonies, we obtain an average of 24,7 CFU/m^3 for *Penicillium* (70%), 7,1 CFU/m^3 for *Aureobasidium pullulans* (20%) and 3,5 CFU/m^3 for *Stachybotrys chartarum* (10%). So, the total concentration of mold is 35,3 CFU/m^3.

TABLE 15. Bacteria & *Actinomycetes* of building P_2

	Counted colonies		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum
1	0	2	2
2	1	3	4
3	1	17	18
4	0	3	3
5	0	0	0
6	0	1	1
Total	2	26	28

On TGH agar with a total of 28 colonies, there was an average of 7,1 CFU/m^3 for *Actynomyces* (7.1%), 88,3 CFU/m^3 for seem-like yeast (89.3%) and 3.5 CFU/m^3 for bacteria (3.6%).

Finally, we study the last part of the building P. During the analysis, several people were present, and that can affect the results. We noticed that the room heat was higher, same for moisture. In addition, we noticed that the ventilation is older in this place, the air is less renewed.

TABLE 16. Parameters of the P_3

Room	P3	**Relative humidity**	66,8%
Breeding ground	Malt 2%, DG-18, THG	**Ventilation - Activities - Closed**	Ventilation OK – Door open
Sampling time	10 min	**Window open - Captive**	1 - Closed
The sample taken at	04/02/16 – 16h00	**Number of persons during sampling**	6
The sampling height	0,95 m	**Pets**	No
Inside temperature	24.1°C	**Activities during the measurement**	Meeting
Outside temperature	-5,5°C	**The number and location of visible mold**	No

However, we can note the presence of contamination on the first stage of the Malt 2% agar. Furthermore, a high concentration of Gram$^+$ bacteria and *Actinomycete* was noted in THG agar. We may suppose that germs were imported by people present during analysis.

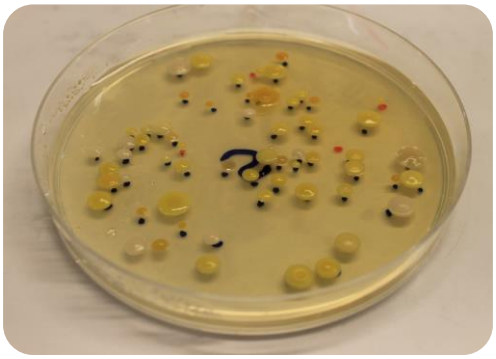


FIGURE 17. Macroscopic observation of Bacteria, Actinomycetes & Seem-likeYeasts on THG agar

(Source : LEBRAT Julien, 2016)

With the results in **TABLE 18**, a high concentration of colonies on all THG agar (approximately 122 colonies) is observed. Cause of this high number, it is necessary to make a correction (APPENDIX 1). For that, we have to sum seem-like yeast and bacteria and find on the table the corrective value. So, we obtain a concentration of 381.6 CFU/m^3 of seem-like yeast and bacteria (85,1%). We also obtain 67.1 CFU/m^3 of *Actinomycetes* (14.9%).

TABLE 17. Mold results of the P_3

	Localisation:	**Area D_3**	
	Stage :	**Number & type of colony**	**Identification**
Type of agar: Malt 2%	n°2	1 colony → Viscous, red/pink color	CONTAMINATION
	n°3	1 colony → Dark green color middle, White color side, Yellow color below	PENICILLIUM
		1 colony → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS
	n°4	3 colonies → Dark green color middle, White color side, Yellow color below	PENICILLIUM
		1 colony → Light green color middle, White color side, White color below	STACHYBOTRYS CHARTARUM
	n°5	4 colonies → White color, stringy	OIDIODENDRON
		1 colony → Dark green color middle, White color side, Yellow color below	PENICILLIUM
Type of agar: DG-18	n°1	1 colony → White color, stringy	OIDIODENDRON
	n°2	6 colonies → Dark green color middle, White color side, Yellow color below	PENICILLIUM
	n°3	5 colonies → Dark green color middle, Light green color intermediate, White color side. Same bellow	CLADOSPORIDIUM
		1 colonies → Dark green color middle, White color side, Yellow color below	PENICILLIUM
	n°4	9 colonies → Dark green color middle, White color side, Stringy	PENICILLIUM
	n°5	5 colony → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS

Concerning the Malt 2% agar, with a total of 12 colonies developed on it, we obtain an average of 17,7 CFU/m^3 for *Penicillium* (41.7%), 14,1 CFU/m^3 for *Oidiodendron* (33,0%), 3,5 CFU/m^3 for *Stachybotrys chartarum* and *Aureobasidium pullulans* (both 8.3%). So, the total concentration of mold is 49,5 CFU/m^3.

For DG-18 agar, with a total of 27 colonies, an average of 56,5 CFU/m^3 for *Penicillium* (59.3%) was obtained, 17,7 CFU/m^3 for *Aureobasidium pullulans* and *Cladosporium* (both 18.5%) and 3,5 CFU/m^3 for *Oidiodendron* (3.7%). So, the total concentration of mold is 95,4 CFU/m^3.

TABLE 18. Bacteria & *Actinomycetes* of building P_3

	Counted colonies			Corrected colonies		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum	*Actynomyces*	Other bacteria	Sum
1	1	2	3	1	2	3
2	5	5	10	5	5	10
3	0	25	25	0	26	26
4	5	26	31	5	27	32
5	6	44	50	6	47	53
6	2	1	3	2	1	3
Total	19	103	122	19	108	127

We change sampling area to another building subjected to access authorization, we will call it the building Y.

FIGURE 18. First sampling place of Y_1

(Source : LEBRAT Julien, 2016)

The latter is even older than the previous building and a strong musty smell is omni-present in all parts. For this sampling, the use of a filter mask is required. The first room is an old kitchen and classroom with a large number of old windows with double glazing. The floor is littered with dust, same applies to different tables. This building has not been used for a long time.

The presence of huge metal ductwork that may be thinking about wood stoves is observed. These give the impression to be hermetically sealed as all the ventilation systems present. All the furnishings are randomly distributed in the room.

TABLE 19. Parameters of the Y_1

Room	Y_1	Relative humidity	72.3%
Breeding ground	Malt 2%, DG-18, TGH	**Ventilation - Activities - Closed**	Ventilation NO OK – Door closed
Sampling time	10 min	**Window open - Captive**	14 - Closed
The sample taken at	12/02/16 – 14h15	**Number of persons during sampling**	7
The sampling height	0,95 m	**Pets**	No
Inside temperature	18,4°C	**Activities during the measurement**	No activitiess
Outside temperature	1,5°C	**The number and location of visible mold**	Yes

After incubation and analysis of the various slants, was observed the presence of 4 new mold:

- ✓ *Geotrichum* with white fluffy appearance, a formation of chains of colourless and slimy spores.
- ✓ *Scopulariopsis*, beige color mold doing streaks on the agar and highly branched and terminating in a brush-like complex bearing flask-shaped annellides.

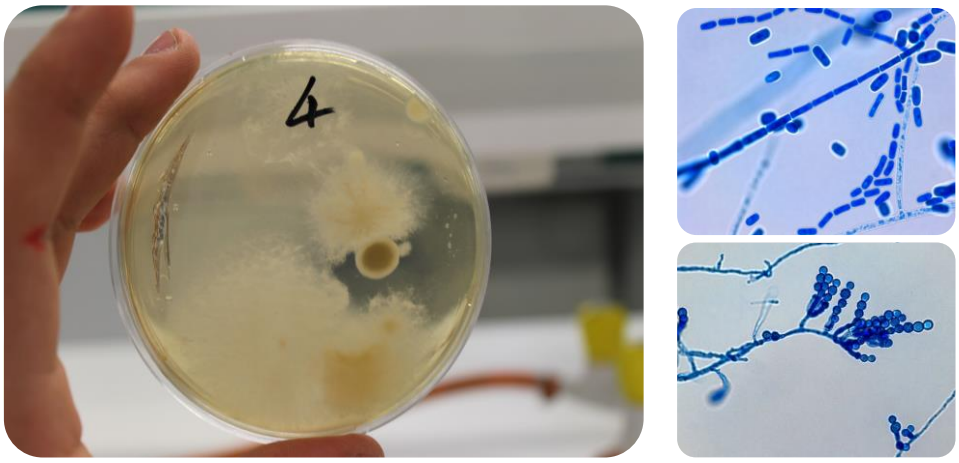


FIGURE 19. Macroscopic & Microscopic observation of *Geotrichum* and *Scopulariopsis*

(Source : LEBRAT Julien, 2016)

- ✓ *Phialophora* an unusual mold blackish color in the center. The phialides are dark, flask-shaped, and have a collar-like or flared apex.
- ✓ *Absidia* having a resemblance Geotrichum but is more stringy.

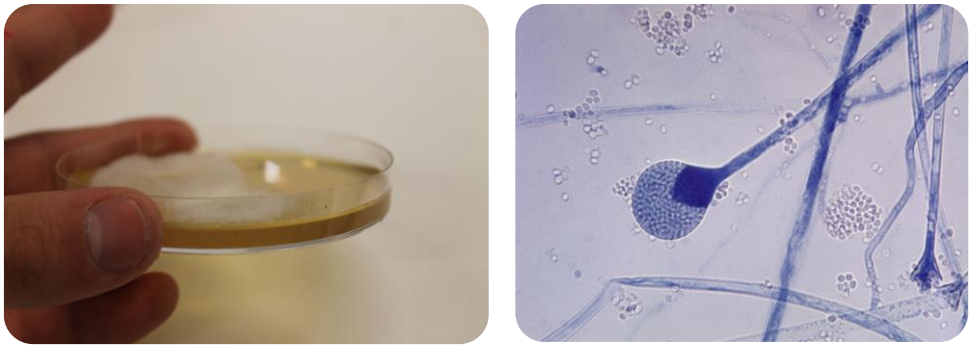


FIGURE 20. Macroscopic & Microscopic observation of *Absidia*

(Source : LEBRAT Julien, 2016)

Thanks to the 27 colonies observed on Malt 2% agar, we obtained a concentration of 60,1 CFU/m^3 for *Geotrichum* (63,0%), 17.7 CFU/m^3 for *Penicillium* (18.5%), 10,6 CFU/m^3 for *Aureobasidium pullulans* (11.1%) and 7,1 CFU/m^3 for *Scopulariopsis* (7.4%). So, the total concentration of mold is 95,5 CFU/m^3.

For DG-18 agar, with a total of 18 colonies, we obtain an average of 24,7 CFU/m^3 for *Absidia* (38.9%), 20,5 CFU/m^3 for *Stachybotrys chartarum* (33.3%), 7,1 CFU/m^3 for *Penicillium* and *Phialophora* (both 11.1%), and fnally 3,5 CFU/m^3 for *Cladosporium* (5.6%).So, the total concentration of mold is 62,9 CFU/m^3.

TABLE 20. Mold results of the Y_1

	Localisation :	**Area Y_1**	
	Stage :	**Number & type of colony**	**Identification**
Type of agar: Malt 2%	n°1	5 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
	n°3	4 colonies → White color, fluffiness, flat, large diameter	GEOTRICHUM
		3 colonies → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS
	n°4	6 colonies → White color, fluffiness, flat, large diameter	GEOTRICHUM
		2 colonies → Cream color, overlay, streaks on the agar.	SCOPULARIOPSIS
	n°5	7 colonies → White color, fluffiness, flat, large diameter	GEOTRICHUM
Type of agar: DG-18	n°1	1 colonies → white color, aerial aspect, stringy.	ABSIDIA
		2 colony → Light green color middle, White color side, White color below	STACHYBOTRYS CHARTARUM
	n°3	4 colony → Light green color middle, White color side, White color below	STACHYBOTRYS CHARTARUM
		1 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		1 colonies → Dark green color middle, Light green color interme-diate, White color side. Same bellow	CLADOSPORIDIUM
	n°4	1 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		6 colonies → white color, aerial aspect, stringy.	ABSIDIA
	n°6	2 colonies → Dark brown color middle, Light Brown color side, inlaid.	PHIALOPHORA RICHARDSIAE

On THG agar with a total of 13 colonies, we obtain an average of 21,2 CFU/m^3 for *Actynomyces* (46.2%) and 24,7 CFU/m^3 for seem-like yeast (53.8%).

TABLE 21. Bacteria & Actinomycetes of building Y_1

	Counted colonies		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum
1	2	2	4
2	0	10	10
3	3	2	5
4	1	7	8
5	0	0	0
6	0	2	2
Total	6	23	29

The second area of analysis is a classroom. As for the first part, a strong musty smell was smell in all thebuilding. In addition, a change was required to mask because many dust had accumulated on the filter.

LOCALISATION 1. Second sampling place of Y_2

(Source : LEBRAT Julien, 2016)

The same stuctures are present in the rooms and we observed the presence of mold in the wood, the main component of the building.

TABLE 22. Parameters of the Y_2

Room	Y_2
Breeding ground	Malt 2%, DG-18, THG
Sampling time	10 min
The sample taken at	12/02/16 – 15h05
The sampling height	0,95 m
Inside temperature	18,1°C
Outside temperature	1,3°C

Relative humidity	72.3%
Ventilation - Activities - Closed	Ventilation NOOK – Door closed
Window open - Captive	8- Closed
Number of persons during sampling	4
Pets	No
Activities during the measurement	No activitiess
The number and location of visible mold	Yes

We note the presence of a new kind of mold, *Ulocladium* in stage 1, 2 and 5 Malt agar 2%. This mold are brown color to olivaceous-black and it is very recognizable microscopically because of the shape of its spores. Conidia are typically obovoid (narrowest at the base) and they are divided into several parts (4, 6 or more). They generally have a brown staining despite lactophenol.

FIGURE 21. Microscopic observation of Ulocladium

(Source : LEBRAT Julien, 2016)

With a total of 23 colonies on Malt 2% agar, we obtained a concentration of 22,2 CFU/m^3 for *Geotrichum* and *Ulocladium* (26.1%), 28,3 CFU/m^3 for *Penicillium* (34.8%), 3,5 CFU/m^3 for *Aureobasidium pullulans* (4.3%) and 7,1 CFU/m^3 for *Acremonium* (8.6%). So, the total concentration of mold is 83,3 CFU/m^3.

For DG-18 agar, with a total of 22 colonies, we obtain an average of 35,3 CFU/m^3 for *Absidia* (45.5%), 3,5 CFU/m^3 for *Stachybotrys chartarum* (4.5%), 31,8 CFU/m^3 for *Penicillium* (41.0%), and fnally 7.1 CFU/m^3 for *Aureobasidium pullulans* (9.0%). So, the total concentration of mold is 77,7 CFU/m^3.

TABLE 23. Mold results of the Y_2

	Localisation :	**Area Y_2**	
	Stage :	**Number & type of colony**	**Identification**
Type of agar: Malt 2%	n°1	2 colonies → Light khaki color middle, White color side, powdery and stringy appearance	ULOCLADIUM
		3 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
	n°3	2 colonies → Light khaki color middle, White color side, powdery and stringy appearance	ULOCLADIUM
		1 colonies → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS
	n°5	5 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		2 colonies → Light khaki color middle, White color side, powdery and stringy appearance	ULOCLADIUM
	n°6	2 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM
		6 colonies → White color, fluffiness, flat, large diameter	GEOTRICHUM

Type of agar: DG-18	n°1	2 colonies → white color, aerial aspect, stringy.	ABSIDIA
		1 colony → Light green color middle, White color side, White color below	STACHYBOTRYS CHARTARUM
	n°2	2 colonies → Black color middle, White color side, Grey color bellow.	AUREOBASIDIUM PULLULANS
	n°3	5 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		1 colonies → white color, aerial aspect, stringy.	ABSIDIA
	n°5	4 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		4 colonies → white color, aerial aspect, stringy.	ABSIDIA
	n°6	3 colonies → white color, aerial aspect, stringy.	ABSIDIA

On THG agar with a total of 25 colonies, we obtain an average of 7,1 CFU/m^3 for *Actynomyces* (8.7%) and 81,3 CFU/m^3 for seem-like yeast (91.3%).

TABLE 24. Bacteria & Actinomycetes of building Y_2

	Counted colonies		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum
1	0	7	7
2	0	0	0
3	0	0	0
4	0	0	0
5	1	12	13
6	1	4	5
Total	2	23	25

Finally, we conducted a final analysis in building Y. This piece is also a classroom that is very similar to previous area. However, a problem occurred during the analysis. Indeed, when retrieving again the laboratory, THG agar was not good, so the results can't be used for this type of agar.

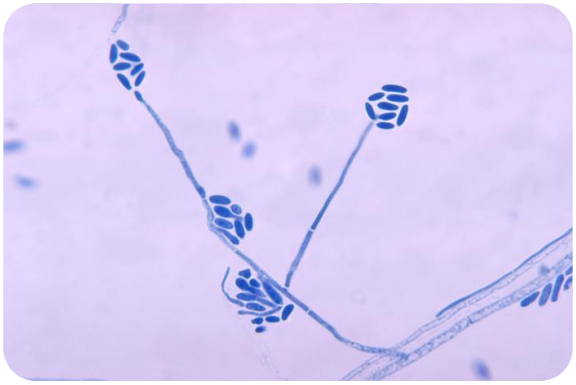


FIGURE 22. Microscopic observation of Acremonium

(Source : LEBRAT Julien, 2016)

However, we can discern the presence of a new kind of mold. *Acremonium* is morphologically very similar to mold *Geotrichum* and *Absidia* but with a different microscopic structure.

Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides with inconspicuous collarettes. Conidia are usually one-celled, hyaline or rarely pigmented and mostly aggregated in slimy heads at the apex of each phialide.

With a total of 22 colonies on Malt 2% agar, we obtained a concentration of 10,6 CFU/m^3 for *Ulocladium* (13.6%), 31,8 CFU/m^3 for *Penicillium* (40.9%), 28,3 CFU/m^3 for *Acremonium* (36.4%) and 7,1 CFU/m^3 for *Scopulariopsis* (9.1%). So, the total concentration of mold is 83,3 CFU/m^3.

For DG-18 agar, with a total of 22 colonies, we obtain an average of 35,3 CFU/m^3 for *Absidia* (45.5%), 3,5 CFU/m^3 for *Stachybotrys chartarum* (4.5%), 31,8 CFU/m^3 for *Penicillium* (41.0%), and fnally 7.1 CFU/m^3 for *Aureobasidium pullulans* (9.0%). So, the total concentration of mold is 77,8 CFU/m^3.

TABLE 25. Results of the Y_3

	Localisation :	**Area Y_3**	
	Stage :	**Number & type of colony**	**Identification**
Type of agar: Malt 2%	n°2	5 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM
		3 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
	n°3	3 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM
		2 colonies → Cream color, overlay, streaks on the agar.	SCOPULARIOPSIS
	n°4	6 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		3 colonies → Light khaki color middle, White color side, powdery and stringy appearance	ULOCLADIUM
Type of agar: DG-18	n°1	1 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM
		2 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
	n°2	2 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM
		4 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
	n°4	6 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM
		1 colonies → white color, aerial aspect, stringy.	ABSIDIA
		1 colonies → Light khaki color middle, White color side, powdery and stringy appearance	ULOCLADIUM
	n°5	2 colonies → Dark green color middle, White color side, Yellow	PENICILLIUM
		1 colonies → White color, inlaid, tough, large diameter, strong smell of anise	ACREMONIUM

During my study, I had the opportunity to perform analysis in another building outside the university campus. Thus request comes from one of the staff members of the organization.

Indeed, last year, the building has suffered water damage, somewhat important, because of the explosion of a pipeline due to very low temperatures during the winter.

One of the main reasons of analysis of this area is the presence of young children in it. Two areas of analysis were selected: One place where children play and the other in the administrative office where the incident took place.

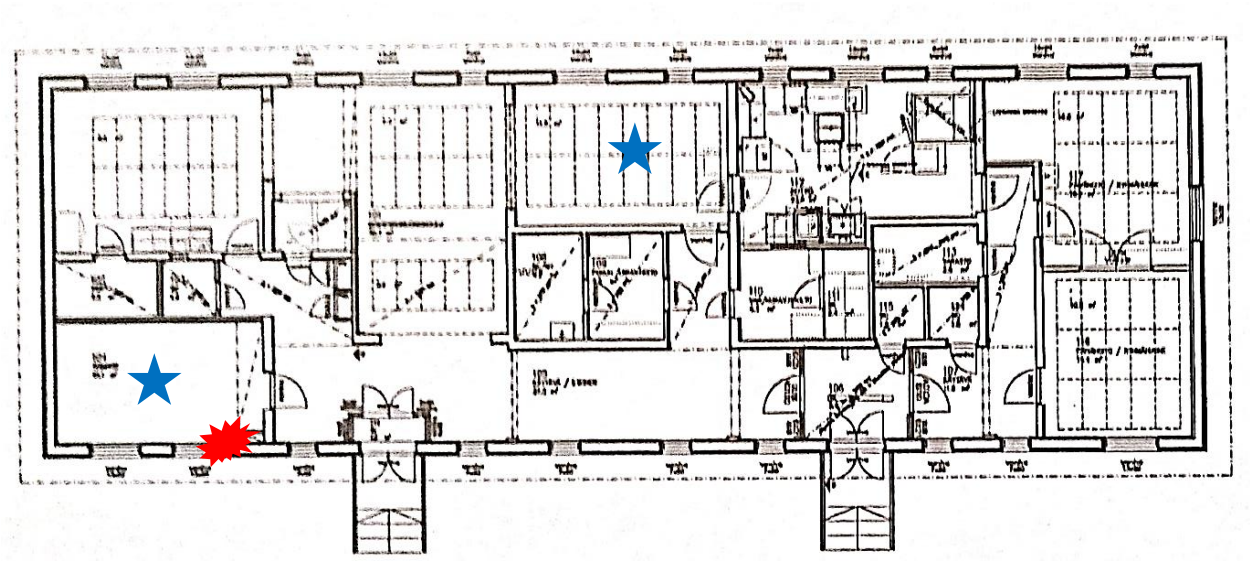


FIGURE 23. Building Mt

We will name this building Mt. In the premises, we noted a high temperature and a musty smell in the administrative room, location of the incident.

TABLE 26. Parameters of the building Mt

Room	Mt
Breeding ground	Malt 2%, DG-18, THG
Sampling time	10 min
The sample taken at	03/03/16 – 7h50
The sampling height	0,92 m
Inside temperature	20,9°C
Outside temperature	-7,8°C

Relative humidity	32.7%
Ventilation - Activities - Closed	Ventilation OK – Door closed
Window open - Captive	2- Closed
Number of persons during sampling	2
Pets	No
Activities during the measurement	No activitiess
The number and location of visible mold	No

However, after the incubations agar, no molds growth was performed on Malt 2% agar and DG-18 was noted. Only *Actinomycetes* have been developed on the THG agar of area Mt_2. On the THG agar of the area Mt_1, we obtained a total of 2 seem-like yeasts, so an average of 7,1 CFU/m^3. For the area Mt_2, with a total of 18 colonies, we obtain an

average of 53.0 CFU/m^3 of *Actynomyces* (83.3%) and 10,6 CFU/m^3 for seem-like yeast (16.7%).

TABLE 27. Results of the building Mt_{1-2}

	Counted colonies in Mt_1			Counted colonies in Mt_2		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum	*Actynomyces*	Other bacteria	Sum
1	0	1	1	9	0	9
2	0	1	1	2	1	3
3	0	0	0	3	1	4
4	0	0	0	1	1	2
5	0	0	0	0	0	0
6	0	0	0	0	0	0
Total	0	2	2	15	**3**	18

Finally, the last two analyzes were carried out in the building A, typically used by Environmental Enginerring and other group classroom. The second last analysis was conducted in a busy area of building A: A183C. This place is different from others because the entire room like a open-plan office.

Despite the ventilation system, the air can migrate from office to office. The final analysis was performed in one of the most used building classrooms A: A240.

TABLE 28. Parameters of the building A

Room	Building A	**Relative humidity**	23,1%
Breeding ground	Malt 2%, DG-18, THG	**Ventilation - Activities - Closed**	Ventilation OK – Door closed
Sampling time	10 min	**Window open - Captive**	6- Closed
The sample taken at	03/03/16 – 79h50	**Number of persons during sampling**	2
The sampling height	0,98 m	**Pets**	No
Inside temperature	25,5°C	**Activities during the measurement**	No activitiess
Outside temperature	-5,9°C	**The number and location of visible mold**	No

After incubation, we can notice that in the staff room, a single mold is developed on stage 4 on Malt 2% agar. No mold growth was observed on the DG-18 agar, only yeasts and *Actinomycetes* are present on the THG agar. No mold growth was observable in the A240 room.

FIGURE 24. Classroom A240

(Source : LEBRAT Julien, 2016)

On the THG agar of staff office room, with a total of 16 colonies, we obtained an average of 46,0 CFU/m^3 of seem-like yeasts (81.3%) and an average 10,6 CFU/m^3 of *Actynomyces* (18.7%). For the room A240 (classroom), with a total of 3 colonies of seem-like yeasts, we obtain an average of 10,6 CFU/m^3.

TABLE 29. Results of the A183C & A240

	Counted colonies in A183C			Counted colonies in A240		
Stage on THG agar	*Actynomyces*	Other bacteria	Sum	*Actynomyces*	Other bacteria	Sum
1	0	6	6	0	1	1
2	0	0	0	0	2	2
3	1	3	4	0	0	0
4	1	2	3	0	0	0
5	1	1	2	0	0	0
6	0	1	1	0	0	0
Total	3	13	16	0	3	3

5 DISCUSSION

With different results, a wide variety of molds according to the physical parameters (temperature, humidity ...) was observed, the growth substrate and the general view of the buildings [23]. *Penicillium* was one of the molds present during the entire study. With these 13 species recognized, as *Penicillium purpurogenum* was observed on some agar, *Penicillium* is part of the most present mold during the year in the indoor and outdoor air. Globally, its concentration is predominant in more than 53% of homes con-

taminated [24]. However, despite its ubiquity in buildings, it can produce organic compounds such as Microbial Volatile Organic Compound (MVOC) and mycotoxins for some species. They are then responsible, if they are in excessive concentration, of irritation and inflammation of the eyes, respiratory system and can cause lethargy and headaches. In addition, for more serious cases may affect immunocompromised people, it may be responsible for hypersensitivity pneumonitis may cause symptoms of fatigue and fever. In the P buildings, there was observed a majority concentration of the genus *Penicillium*, which can have a direct impact on student health. Another mold, *Stachybotrys chartarum*, was found in the majority in this study (>20% in building P_2 and Y_1).

This fungus is the subject of numerous scientific studies because of its potential role in the health problems associated with poor air quality of buildings contaminated with mold or buildings called "unhealthy". These health problems are attributable to the powerful mycotoxins produced by certain strains of *Stachybotrys chartarum*. It has a worldwide distribution and it usually develops in environments rich in cellulose, contaminating foam insulation, textiles, indoor air and building water-damaged materials.

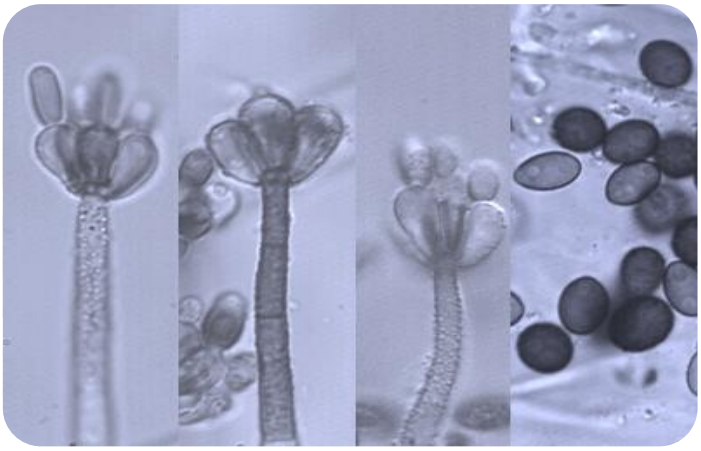


FIGURE 25. Microscopic observation of Stachybotrys

(Source : Phillip Fry, 2010, "Health Dangers and Risks of Stachybotrys Mold")

Despite the problems it causes, it represents only 13% of mold found in contaminated buildings. Among the different mycotoxins produced some cause various harmful effects on humans. The trichothecenes mycotoxins is primarily responsible of respiratory and ocular irritation while satratoxins are known to cause inflammatory reactions.

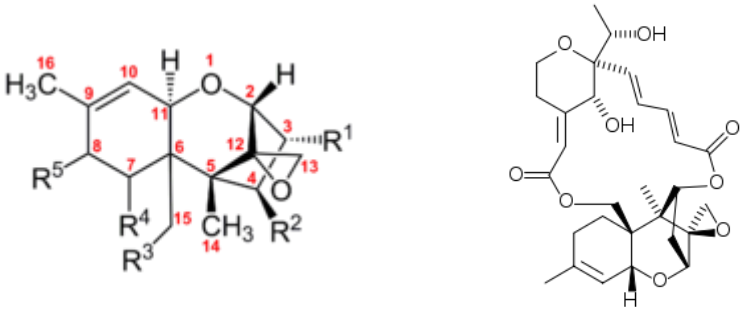


FIGURE 26. Chemical structure of trichothecene and satratoxin

(Source: Wikipedia, 2016, "Trichothecene" & "satratoxin")

Respiratory symptoms may be observed following inhalation such congestion, cough, rhinitis, and, in the most severe cases, pulmonary fibrosis. In addition, chronic exposure to toxins can cause symptoms resembling those of a cold or the flu. This mold was found in both buildings having necessity authorization. Despite the presence of a mask, fatigue and cough effect was felt by me after the analyzes.

In addition, the presence of another type of mold present in these buildings was obseved. The mold *Acremonium* has a worldwide distribution and are usually found in soil and on decaying plant matter. In addition, it produces various hydrocarbons, alcohols, ketones, esters and terpenes, as well in nature as construction materials. Despite the production of mycotoxins allergens associated with allergic rhinitis and asthma, *Acremonium* remains a mold having reduced effects on humans. It is the same for *Ulocladium* that is considered current in the indoor environment and is known to cause low allergic reactions. One can detect its presence in building materials damaged by water and in household dust, especially in domestic air conditioners. *Ulocladium* was found mostly in the building Y with the mold *Absidia* (> 25% *Ulocladium*, > 40% *Absidia*). Despite the presence of irritation and inflammation of the airways and possible skin reactions, it remains a low pathogenic mold such as mold presented previously.

Finally, concerning the mold found in the minority in our analyzes (*Geotrichum, Aureobasidium pullulans, Cladosporium, Paecilomyces, Scopulariopsis, Oidiodendron*), they can cause allergies whose resulting fever and asthma. For *Cladosporium*, this species is recognized as that can cause rhinitis and allergic asthma in humans, as well as hypersensitivity pneumonitis as *Aureobasidium pullulans* and *Paecilomyces*. As for *Geotrichum* and *Scopulariopsis*, they may be responsible for skin lesions but also of lung infection, sinusitis ... This effect is particularly strong among immunodeficient persons.

The presence of *Phialophora* in our analyzes is suspected and is a surprise. Indeed, *Phialophora* is very rare mold of indoor air and it is a species of aggressive mold, which can cause skin infections and subcutaneous but also responsible and chromoblastomycosis phaeohyphomycosis.

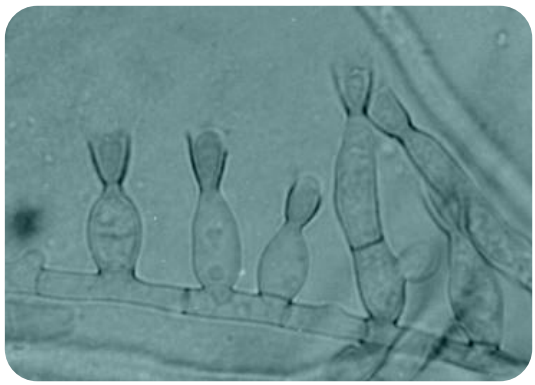


FIGURE 27. Microscopic observation of *Phialophora*

Finally, *Actinomycete* is the most present bacteria in this study. They are a group of gram-positive bacteria. They resemble fungi because they are adapted to life on solid surfaces and they can produce mycelium and dry spores like most fungi. Actinomycete spores are known to be important air contaminants in occupational environments and as indicators of mold problems in buildings. They are responsible of harmful effect on human health as allergic alveolitis and other severe health effects. Because of the stimulattion of lung macrophage reactions, inflammation and tissue injury can be observed. Actinomycete spores are more difficult to aerosolize cause of thier smaller size than fungal spores.

With the results, several things were observed. Based on the European Union mold exposure standards, a vast majority of the analyzed pieces have a concentration < 50 CFU/m^3 (Building J, Fitness room, P_1, Mt_1, Staff room and A240) ie, a very low risk of contamination.

However, we can also see an overrun of this first level of contamination on different sampling areas. Indeed, concerning the seem-like yeast, the areas P_2, P_3 and Y_2 obtain a higher level of CFU/m^3. Thus, 88,3 CFU/m^3, 381,6 CFU/m^3 and 81,3 CFU/m^3 are obtained for the three different areas. Concerning *Actynomycetes*, areas P_3 and Mt_2 also exceeded the 50,0 CFU/m^3 value. The concentrations obtained are thus 67,1 CFU/m^3 for P_3 and 53,0 CFU/m^3 for Mt_2. Finally, for molds, four sampling zones exceed this threshold value. A concentration of 95,4 CFU/m^3 for the P_3 zone ; 95,5 CFU/m^3 (malt

2% agar) and 62,9 CFU/m^3 (DG-18 agar) for Y_1 ; 83,3 CFU/m^3 (malt 2% agar) and 77,7 CFU/m^3 (DG-18 agar) for Y_2 ; Finally, 83,3 CFU/m^3 (malt 2% agar) and 77,8 CFU/m^3 (DG-18 agar) for Y_3 are observed. This excess value can have a significant impact on the percentage of contamination as well as on human health.

One of the fundamental steps in prevention of the propagation of mold is to reduce dust. In fact, mold can be attached to them and thus spread into the indoor air. A good cleaning allows a significant reduction of the risk of contamination. In case of demolition or repair of a structure which has been contaminated by mold, this may have the effect of multiplication by 1 million dust concentration [25]. It is imperative to manage the proper dust removal to ensure the health of other occupants.

An element who significantly reduces contamination and proliferation of mold is to reduce humidity. Although a breakdown is generally ideal for an excessive humidity in the house, it is possible to observe, in case of malfunction, an aggravation of the phenomenon. Mechanical ventilation (simple or double flow, hygroadjustable…) must be optimum and must effectively filter the air to remove moisture from air change can contain mold spores attached or not. From a regulatory point of view, every hour, the half-of the total air volume of the rooms must be renewed [26].

Another effective method that can reduce humidity is the use of chemical or electric moisture absorbers. As regards the chemical absorbent, the air passes through a chemical cartridge or pellet, usually sodium chloride, which absorbs moisture and turns into water. For electric absorbent, it is more effective than the chemical absorber. The ambient air is sucked through a ventilation system, the air condenses and the water is collected in a recovery tank. A humidity of around 70% is ideal for mold growth, so it is necessary to reduce around 30%. The latest technologies to prevent mold growth and proliferations the optimization of the insulation and the use of anti-fungal treatment. Indeed, in both cases, mold will not have direct contact with the interior of the building. For anti-fungal, they will allow the establishment of the protective barrier against the mold [27].

Concerning the analysis made on the campus and the other building, all measures were achieving only been done once. It is possible that several factors such as temperature, humidity, air flow (not measured in our study), age of buildings, special events (leding

pipes, treatments ...) before and/or during measurements, or the construction materials used can have an impact on results. So, it is possible that the risk of contamination is much higher (or lower) cause of the state of the buildings, particularly for buildings Y.

In this building, after several hours in this building, fatigue and a strong sinus irritation and throat was felt, despite the mask. The entire buildings had a strong musty smell and a large number of wall and floors had inlaid molds. According to officials, these problems are due to water damage that has suffered the building. Indeed, some pipelines have exploded several years ago during the winter and thus degraded the quality of the insulation and integrity of the building. The cuisine of buildings Y is less affected by the overlay of mold in walls and floors.

However, it has a large number of windows with poor insulation. Moisture and mold can thus seep through these contact areas. The room ventilation system (and the building) is down, so there is a heavy accumulation of dust and particles in suspention in the air, can accommodate large quantities of mold spores or bacteria.

Regarding the building P, for the three analyzed rooms, the ventilation system is operational. One of the major problems of this building is the high concentration of dust in some sofas. A large cleaning is necessary to eliminate a maximun mold spores, dust related. This building has the same problem of insulation on the windows, so it is necessary, in case of remediation, change with double glazing to avoid contamination, but also energy losses. In the meeting room, often being occupied by students, the temperature and humidity is very important. It maybe good to establish a moisture reducer (electrical or chemical) to reduce the humidity of the room, so the possibility of contamintion by mold.

As regards the other buildings on campus as well as external buildings of the university, the air quality remains good despite the presence of some ubiquitous molds in small quantities and certain bacteria. No heavy treatment is to achieve. It is possible to store in these buildings as some plant species like *Hedera Heli*x or *Sansevieria trifasciata Laurentii*, which allow reductions toxins produced by microorganisms. Indeed, plant leaves produce negative ions, similar to many air purifying machines. Negative ions attach themselves to particles such as dust, mold spores, bacteria, and allergens.

FIGURE 28. Sansevieria trifasciata Laurentii & Hedera Helix

6 CONCLUSION

In this study, there was several different kinds of microbial air sample in buildings. Ubiquitous mold and bacteria in our indoor environment were observed. These have a significant impact on human health and can significantly degrade the integrity of a building. However, knowledge about indoor mold-related risks are very limited. One obstacle is related to the lack of standardization means for measuring the concentrations of each species of molds in housing.

This deficiency makes it difficult to compare existing studies using sample-and very different analysis. This difficulty is amplified by metrology mark in some global indicators studies (glucan, chitin ...) which do not allow to connect a pathology in one or more species data. Moreover, all the environment-related respiratory diseases are not clearly defined (sick building syndrome, for example).

Furthermore, interactions with chemicals (formaldehyde ...), organic (mycotoxins, endotoxins) or between microbial species are not likely to simplify the analysis of these complex environments. Finally, individual sensitivity plays an important role and makes the establishment of a dose / effect even more hypothetical. However, the progress of standardization of techniques, including the use of distributable techniques without exceptional expertise (qPCR) and the study of a growing number of cases in the future should provide further insights into the environmental risk to indoor mold.

Finally, some uncertainty errors in this analysis can happened during the sampling, the cultivation and during the indentification. So, it is possible to erroneous conclusions. In order, to refine the measures and results, it is necessary to carry out much analysis and spend more time on identifications with a more suitable material. The sampling and

analysis were made in winter time, like it is suggested. However, this kind ofmeasures will present the situation only during sampling time and it will need to make new measures/identifications. It’s possible to use the PCR identification the next time to obtain better identification.

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1) Correction Table

Liite 5. 6-VAIHEIMPAKTORIN PESÄKEMÄÄRIEN KORJAUSTAULUKKO (Andersen 10-800).

HUOM!

vain vaiheiden 3 - 6 pesäkemäärät korjataan only stages 3-6 correc

LP = laskettu pesäkemäärä calculated colonies cfu

KP = korjattu pesäkemäärä corrected " cfu

LP	KP	LP	KP	LP	KP	LP	KP	LP	KP	LP	KP	LP	KP	LP	KP	LP	KP	LP	KP
1	1	41	43	81	91	121	144	161	206	201	279	241	369	281	485	321	649	361	931
2	2	42	44	82	92	122	146	162	208	202	281	242	372	282	488	322	654	362	942
3	3	43	45	83	93	123	147	163	209	203	283	243	374	283	492	323	659	363	952
4	4	44	47	84	94	124	148	164	211	204	285	244	377	284	495	324	664	364	963
5	5	45	48	85	96	125	150	165	213	205	287	245	379	285	499	325	670	365	974
6	6	46	49	86	97	126	151	166	214	206	289	246	382	286	502	326	675	366	986
7	7	47	50	87	98	127	153	167	216	207	292	247	384	287	506	327	680	367	998
8	8	48	51	88	99	128	154	168	218	208	294	248	387	288	508	328	686	368	1010
9	9	49	52	89	101	129	156	169	220	209	296	249	390	289	513	329	692	369	1023
10	10	50	53	90	102	130	157	170	221	210	298	250	392	290	516	330	697	370	1036
11	11	51	55	91	103	131	159	171	223	211	300	251	395	291	520	331	703	371	1050
12	12	52	56	92	105	132	160	172	225	212	302	252	398	292	524	332	709	372	1064
13	13	53	57	93	106	133	162	173	227	213	304	253	400	293	527	333	715	373	1078
14	14	54	58	94	107	134	163	174	228	214	306	254	403	294	531	334	721	374	1093
15	15	55	59	95	108	135	165	175	230	215	308	255	406	295	535	335	727	375	1109
16	16	56	60	96	110	136	166	176	232	216	311	256	409	296	539	336	733	376	1125
17	17	57	61	97	111	137	168	177	234	217	313	257	411	297	543	337	739	377	1142
18	18	58	63	98	112	138	169	178	236	218	315	258	414	298	547	338	746	378	1160
19	19	59	64	99	114	139	171	179	237	219	317	259	417	299	551	339	752	379	1179
20	21	60	65	100	115	140	172	180	239	220	319	260	420	300	555	340	759	380	1198
21	22	61	66	101	116	141	174	181	241	221	322	261	423	301	559	341	766	381	1219
22	23	62	67	102	118	142	175	182	243	222	324	262	426	302	563	342	772	382	1241
23	24	63	69	103	119	143	177	183	245	223	326	263	429	303	567	343	779	383	1263
24	25	64	70	104	120	144	179	184	246	224	328	264	432	304	571	344	786	384	1288
25	26	65	71	105	122	145	180	185	248	225	331	265	434	305	575	345	793	385	1314
26	27	66	72	106	123	146	182	186	250	226	333	266	437	306	579	346	801	386	1341
27	28	67	73	107	125	147	183	187	252	227	335	267	440	307	584	347	808	387	1371
28	29	68	75	108	126	148	185	188	254	228	338	268	443	308	588	348	816	388	1403
29	30	69	76	109	127	149	186	189	256	229	340	269	447	309	592	349	824	389	1438
30	31	70	77	110	129	150	188	190	258	230	342	270	450	310	597	350	832	390	1476
31	32	71	78	111	130	151	190	191	260	231	345	271	453	311	601	351	840	391	1518
32	33	72	79	112	131	152	191	192	262	232	347	272	456	312	606	352	848	392	1565
33	34	73	81	113	133	153	193	193	263	233	349	273	459	313	610	353	857	393	1619
34	36	74	82	114	134	154	194	194	265	234	352	274	462	314	615	354	865	394	1681
35	37	75	83	115	136	155	196	195	267	235	354	275	465	315	620	355	874	395	1754
36	38	76	84	116	137	156	198	196	269	236	357	276	468	316	624	356	883	396	1844
37	39	77	86	117	138	157	199	197	271	237	359	277	472	317	629	357	892	397	1961
38	40	78	87	118	140	158	201	198	273	238	362	278	475	318	634	358	902	398	2127
39	41	79	88	119	141	159	203	199	275	239	364	279	478	319	639	359	911	399	2427
40	42	80	89	120	143	160	204	200	277	240	367	280	482	320	644	360	921	400	*)

*) kvantitatiivinen raja (noin 2628 hiukkasta) ylitetty

Korjaus ottaa huomioon sen, että yhdestä keräimen silviälevyn reiästä on kulkeutunut useampi kuin yksi hiukkanen. 181