



Rozšíření výuky v laboratořích TZB o mikrobiální hodnocení kvality vnitřního vzduchu

Řešitel:

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Projekt:

Finanční podpora inovačních projektů akademických pracovníků ČVUT zaměřených na podporu výuky a vzdělávání pro rok 2024

Výstupy projektu:

Typ výstupu	počet
Podklady pro přednášky / semináře / cvičení v CZ/EN (včetně elektronických)	2
Elektronická učebnice / skripta / studijní materiály / dokumentace pro podporu výuky v CZ/EN	1
Vzdělávací / inovativní opory pro zefektivnění a inovaci různých forem výuky (audio/video nahrávky, VR/AR apod.) v CZ/EN	1

Podklady pro přednášky / semináře / cvičení v CZ/EN (včetně elektronických):

Dokumentace pro podporu výuky v EN část 1.

Laboratory Exercise - Quantitative Assessment of Microbial Indoor Air Quality

Part 1: Collection and cultivation of microbial samples

The exercise is used to determine microbial contamination of air and surfaces, bacterial and fungal contamination will be examined in more detail. Students will first learn about microbiological sampling methods, then take samples from the air and surfaces of the area under investigation, and finally prepare samples for culture. In a subsequent exercise, they will be introduced to and practice quantitative assessment of the samples collected.

Necessary equipment:

- Aeroscope with impactor
- Sticks with saline solution
- Petri dishes with agar medium
 - TSA agar (tryptone soy agar) - bacterial contamination
 - Sabourad's soil – fungi contamination
- Incubator
- Colony counter
- Autoclaves
- Disinfection - denatured alcohol in a spray bottle and Savo
- Protective equipment (laboratory coat and disposable gloves)

Theoretical part:

Methods of microbiological sampling

Methods of microbiological sampling can be divided according to several different criteria, the basic one being whether the sampling is qualitative or quantitative

Qualitative assessment means qualitative analysis dealing with the demonstration of the presence of a specific target group of microorganisms

Qualitative assessment of a sample means the determination of the number of viable micro-organisms in a given sample

A further possible subdivision is the method of sampling

Passive monitoring, passive sampling, or the fallout method, is sampling carried out using sedimentation petri dishes (Figures 1 and 2).

These dishes with nutrient medium (most commonly TSA agar (Tryptic Soy Agar) or Sabourad's soil) are placed in the area to be monitored. The placement is done in such a way that they are not tampered with during normal operation and do not contaminate the user area. After a period of time (10-30 min for teaching purposes) of exposure to air, the petri dishes are closed and transported to the laboratory. The method is suitable for quantitative microbiological assessment and is therefore suitable for long-term monitoring of specific facilities.

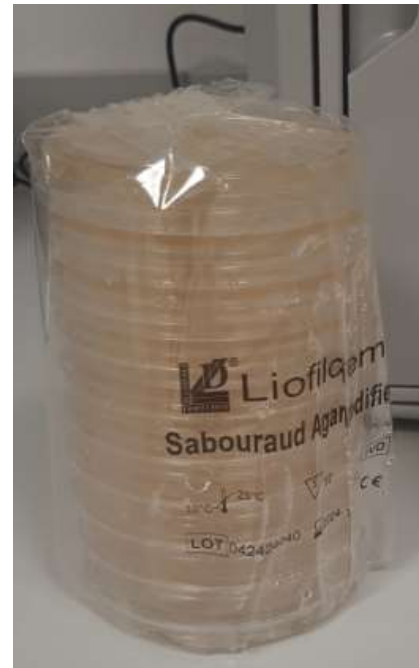


Figure 1 - Petri dishes with TSA agar Figure 2 - Petri dishes with Sabourad soil

Active sampling is a direct physical intervention into the area under investigation - i.e. taking swabs from surfaces, or taking air with a specialized instrument - an aeroscope

- Surface swabbing is used to capture the microbiome found only on the surface of the element being analysed. For the detection of microorganisms, it is advisable to use as large an area as possible for swabbing, i.e. (0.1m^2 - 0.3m^2), for the purpose of the exercise for the determination of the number of microorganisms, an area of 0.01m^2 (i.e. $10\text{cm} \times 10\text{cm}$) is sufficient
 - Swabbing is normally performed **using sterile instruments** (sterile swab, swabbing swabs, or sponge, Figure 3). This tool is used to wipe the marked area with swabs across the entire area and applied perpendicularly to each other and to the culture medium.



Figure 3 - Scrubbing swabs and sponges

- Another option is to perform the swab using a Petri dish with agar medium

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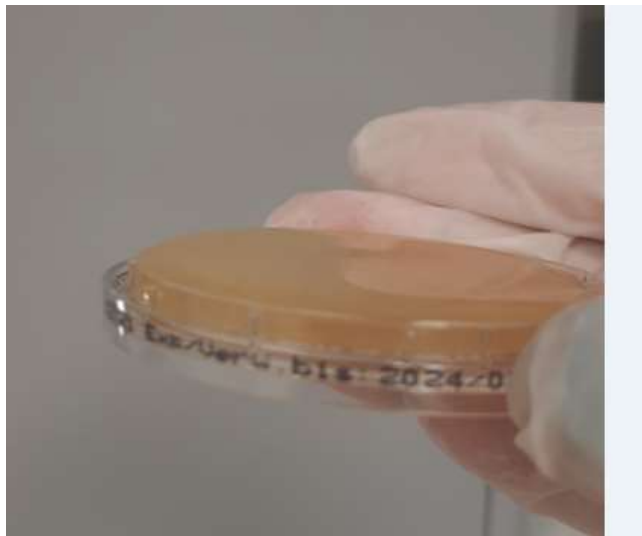


Figure 4 - Petri dish for wiping

- Collection of biological samples from the flowing medium is carried out with the help of sterile specialized instruments - aeroscope with impactor (Figure 5). It is necessary to take a precise amount of air. The impactor is a device for taking microbiological samples from flowing air, where air is vacuumed into the instrument by a fan, where the microorganisms are deposited on a petri dish with agar (Figure 6).



Figure 5 - Aeroscope for active air monitoring

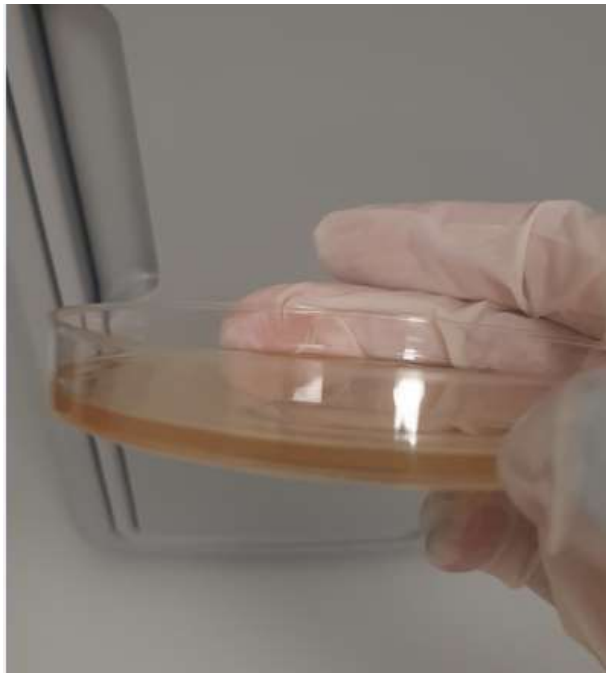


Figure 6 - Petri dish for insertion into the aeroscope

After sampling, the instruments used with the samples must be placed in a sterile environment, avoiding access to contaminants, water, direct sunlight and exposure to extreme temperatures. The samples thus prepared must be placed in an incubator (Figure 7) within 24 hours, where the microorganisms will be cultured.

Quantitative evaluation of the samples taken

For quantitative evaluation, the microorganisms need to be cultured on the collected media. Cultivation takes place in an incubator (sometimes called a thermostat) at the set temperature required for cultivation for a precise period of time, depending on whether it is fungi or bacteria that are being assessed. Plates with TSA agar will be cultured in only one incubator and plates with sabouraud soil in the other.



Figure - Incubator for funghi cultivation



Figure 8 - Incubator for bacterial cultivation

Experimental part:

1. **Divide the students into teams of 4.**
2. **Preparation of petri dishes and thorough labelling (Figure 9) according to soil type, location and method of collection:**
 - a. Petri dish for fall-out
 - b. Petri dish for impactor suction
 - c. Petri dish for wiping



Figure 9 - Labeled petri dishes

3. **Placing petri dishes (Figure 10) for fallout for a set period of time (10 -30 min)**

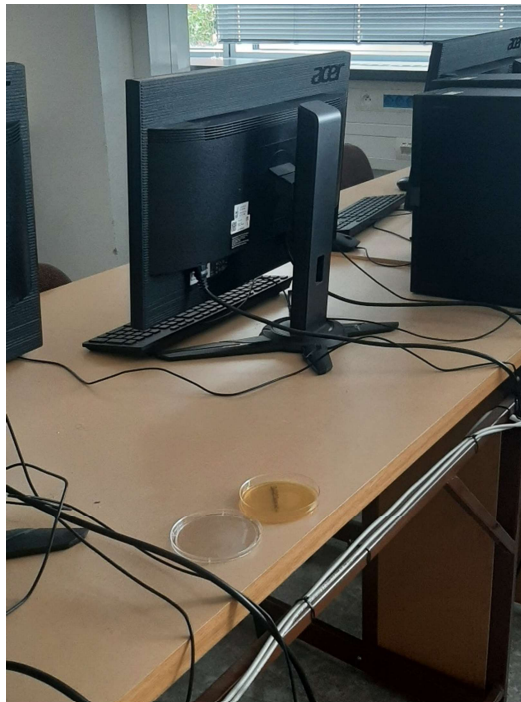


Figure 10 - Positioned Petri dish

4. **Collection of biological samples from the flowing air through the impactor with an aeroscope (fig. no. 11) see the chapter on working with the aeroscope**



Figure 11 - Air intake through the aeroscope

5. **Wipe specified surfaces of specified area (to be given by the teacher during the exercise**
6. **Sterilization of the aeroscope impactor - place in a sterilization bag and close, place in autoclave (15 minutes at 121°C) (Figures 12 and 13)**



Figure 12 - Autoclave



Figure 13 - Impactor placed in a sterilization bag for sterilization in an autoclave

- 7. Place the petri dishes with TSA agar in an incubator for 48 hours at $36^{\circ}\text{C} \pm 1^{\circ}$**



Figure 14 - Petri dishes in the incubator

- 8. Place petri dishes with sabourad soil in an incubator and incubate at $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 days**



Figure 15 - Controlling the incubator

Procedure of working with the aeroscope

Using an aeroscope with an impactor, take samples on two Petri dishes with agar - one for bacterial growth and the other for fungal growth. The flow rate and air volume should be adjusted according to the expected indoor air pollution (25 l/min, 50 l/min or 100 l/min).

Determination of the concentration of bacteria and mould in the indoor air is carried out in the indoor environment after 20 minutes of thorough ventilation and after a further one hour of closing the windows. In the case of air-conditioned rooms without ventilation, sampling in an unoccupied environment is required at the earliest 20 minutes after the cessation of activity.

Two air samplings shall be taken in the centre of the room in the inhalation zone at a height of 160 cm above the ground. The time between each sampling shall be a minimum of 10 minutes and a maximum of 30 minutes. Another location (location of the device, height above ground) may be chosen for the sampling depending on the purpose of the examination. This fact must be recorded in the measurement report.

Between each sampling, the upper part of the instrument is cleaned with a disinfectant-soaked cloth. Between air sampling in different interiors, the sampling head of the instrument is sterilised by autoclaving (15 minutes at 121°C).