CZECH UNIVERSITY OF LIFE SCIENCES
FACULTY OF ENVIRONMENTAL SCIENCES
DEPARTMENT OF APPLIED ECOLOGY

ASSESSMENT OF MICROBIAL CONTAMINATION OF INDOOR AIR

BACHELOR THESIS

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Faculty of Environmental Sciences

BACHELOR THESIS ASSIGNMENT

Min Manwayk Sorn

Environmental Engineering

Thesis title

Assessment of microbial contamination of indoor air

Objectives of thesis

The main aim of the bachelor thesis is verification of a sedimentation technique for the assessment of microbial contamination of indoor air. The theoretical part of the work will cover the legislative framework of indoor air quality (IAQ) at the EU level, give an overview of the main types and factors affecting pollution in the indoor environment and describe currently available measurement techniques. The practical part of the work will be carried out on selected university premises through variable sedimentation settings.

Methodology

The bachelor thesis must follow the rules stated in the Methodological Guidelines for writing a bachelor's thesis at the Faculty of Environmental Sciences. As an experimental work, it will be structured as a comprehensive literature search, followed by a practical part conducted in accordance with Good Laboratory Practice (GLP) and related standards. Technically, the IAQ assessment will be carried out by the sedimentation technique at selected sites, with temperature and humidity considered as one group of factors and different agars and exposure time as another group of factors.

The proposed extent of the thesis

40-50 pages incl. appendixes

Keywords

microbial, contamination, premisses, sedimentation, agar, exposure, time, humidity

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Recommended information sources

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I hope this achievement makes you as proud as I am to be your child.

Abstract

This study evaluates the efficacy of the sedimentation technique for monitoring indoor microbial air quality within university facilities. The research investigated the influence of two cultivation media, Tryptic Soy Agar (TSA) and Nutrient Agar (NA), across exposure durations of 8, 12, and 24 hours. Sampling was conducted across diverse functional zones at CZU Prague, including classrooms, student lounges, and toilets. Statistical analysis using ANOVA and Welch's t-tests revealed that location was the primary driver of microbial variance, identifying toilets and high-traffic lounges as significant hotspots ($p < 0.05$). While both media performed comparably in low-load environments, TSA demonstrated superior recovery of diverse microbial communities in highly contaminated zones. Results indicate that the 8-hour exposure provides the most reliable volumetric estimation, as 24-hour sampling led to significant underestimation due to agar desiccation and inter-colony competition. These findings validate the sedimentation method as a robust screening tool for indoor environments and support the implementation of risk-based maintenance protocols and standardized 8-hour sampling exposure to ensure data integrity.

Keywords: microbial, contamination, premises, sedimentation, agar, exposure, time, humidity

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List of Abbreviation

α —Significance level

ANOVA — Analysis of Variance

AOAC — Association of Official Analytical Chemists

CFU — Colony-Forming Unit

CFU/m³ — Colony-Forming Units per cubic meter

CO_2 — Carbon Dioxide

CV — Coefficient of Variation

CZU — Czech University of Life Sciences Prague

df — Degrees of Freedom

EU — European Union

F — F-statistic (Fisher's ratio)

FES — Faculty of Environmental Sciences

GLP — Good Laboratory Practice

HSD — Honestly Significant Difference (Tukey's test)

HVAC — Heating, Ventilation, and Air Conditioning

hr — Hour

IAQ — Indoor Air Quality

IMA — Index of Microbial Air Contamination

ISO — International Organization for Standardization

MCEV II — Metabolic Center of Environmental Sciences II (Building)

MVOCs — Microbial Volatile Organic Compounds

NA — Nutrient Agar

P — Probability value (p-value)

PM2.5 — Particulate Matter less than 2.5 micrometers

SD — Standard Deviation

TSA — Tryptic Soy Agar

W — Shapiro-Wilk test statistic

1. Introduction

Indoor Air Quality (IAQ) has emerged as a fundamental pillar of environmental health, as European populations spend approximately 90% of their time within enclosed spaces such as residences, workplaces, and educational institutions. Within these environments, occupants are exposed to a complex mixture of pollutants, including chemical compounds and biological contaminants known as bioaerosols. These biological particles, which include bacteria, fungi, and viruses, originate from both outdoor infiltration and indoor sources such as human activity, ventilation systems, and damp building materials.

Exposure to elevated levels of microbial contamination is a documented public health concern, linked to respiratory irritation, allergic reactions, and the exacerbation of asthma. The urgency of monitoring these environments was further highlighted by the COVID-19 pandemic, which underscored the role of indoor spaces in the transmission of airborne pathogens. In educational settings, where high-density occupancy is common, maintaining hygienic limits is essential for the health and cognitive performance of students and staff.

In the Czech Republic, indoor environmental conditions are governed by national legislation, specifically Decree No. 43/2025 Sb., which establishes limits for biological indicators in spaces used for long-term occupancy. While various active volumetric sampling methods exist, the sedimentation (settle plate) technique remains widely utilized due to its technical simplicity and cost-effectiveness. However, the accuracy of this passive method is influenced by numerous methodological factors, including the choice of cultivation media and the duration of plate exposure.

This bachelor thesis aims to verify the efficacy of the sedimentation technique through an experimental study conducted at the Faculty of Environmental Sciences, CZU Prague. The work evaluates how different agar types, Nutrient Agar (NA) and Tryptic Soy Agar (TSA) and varying exposure times affect the quantification of microbial loads. By analyzing these variables across diverse functional areas, including classrooms, hallways, and toilets, standardized protocols for reliable indoor microbial monitoring and risk-based maintenance strategies were sought through the analysis of these variables.

2. Objective of the thesis

The main aim of the bachelor thesis was verification of the sedimentation technique for assessment of microbial contamination of indoor air. The theoretical part of the work covers the legislative framework of indoor air quality (IAQ) at the EU level, gives an overview of the main types and factors affecting pollution in the indoor environment and describes currently available measurement techniques. The practical part of the work was carried out on the selected university premises through variable sedimentation settings.

3. Literary Research

3.1 Indoor Air quality

Indoor Air Quality (IAQ) is an important component of environmental health because it directly influences human health, comfort, and productivity. People in Europe spend approximately 90% of their time indoors, particularly in residential buildings, workplaces, schools, and other public facilities (Settimo et al., 2020). For this reason, indoor environments can represent a significant source of exposure to pollutants. Indoor air pollution may originate from multiple sources, including building materials, ventilation systems, human activities, and outdoor air infiltration. Among the most important categories of indoor pollutants are particulate matter, chemical compounds, and biological contaminants such as bacteria, fungi, and other microorganisms.

3.1.1 Major Indoor Pollutants

Indoor environments have plenty of chemical, physical, and biological substances that come from building materials, furniture, cleaning products, electronics, and everyday activities. These substances all add to pollution over time (Shetty et al., 2023). Indoor air is often restricted, which means that pollutants can stay in the air for longer periods of time, especially in areas with little ventilation (Górski et al., 2024). This is different from outdoor air, which is constantly circulating. This situation is more serious because people spend most of their time inside, resulting in prolonged and intensified exposure levels, even though the sources of emissions seem small (Shetty et al., 2023). Moreover, extensive health assessments have demonstrated that pollution-related disease burdens persist at elevated levels worldwide, with indoor exposure serving as a significant and occasionally underappreciated factor in detrimental health effects (Fuller et al., 2022).

Biological particles present in indoor air are commonly referred to as bioaerosols. These particles include viable microorganisms, spores, fragments of microbial cells, and other biological material that may be transported in the air. Bioaerosols may originate from outdoor environments or from indoor sources such as damp building materials, ventilation systems, dust reservoirs, and human activity. Exposure to microbial contamination in indoor air has been associated with respiratory irritation, allergic reactions, asthma exacerbation, and other respiratory diseases (World Health Organization, 2009). High humidity, water damage, and insufficient ventilation are among the most important factors supporting microbial growth in buildings.

3.1.2 Factors Influencing Indoor Air Quality

This process is also influenced by mechanical systems. If filtration is not good enough or the systems fail to regulate; ventilation and air-conditioning systems may allow pollutants from outside into the building. This lets fine particles and gases circulate throughout occupied spaces (Maji et al., 2023). When these pollutants get inside, they can settle on surfaces and stay there for a long time, which means people are exposed to them for longer (Mihucz et al., 2022). In addition, outdoor bioaerosols and microorganisms can be transported indoors via air exchange, contributing to overall indoor environmental quality and causing potential health risks (Sigsgaard & Hoffmann, 2024; Saxena, 2019).

Modern construction methods also play a role because energy-efficient designs often lower the rate of air circulation, which can unintentionally lead to the buildup of pollutants in occupied spaces. Environmental reviews suggested that multiple indoor sources, long-term human occupancy, and limited pollutant removal mechanisms can make conditions where concentrations may be higher than those measured outdoors (Shetty et al., 2023). These traits help explain why it's not sufficient to just control pollution outdoors to keep populations healthy.

3.2 Influence of Indoor Air Quality on Human Health

The problem of indoor air quality is particularly relevant in public and educational buildings where large numbers of people spend extended periods of time. In such environments, inadequate ventilation may lead to increased concentrations of carbon dioxide, humidity, particulate matter, and airborne microorganisms. These conditions may contribute to symptoms such as headaches, fatigue, respiratory discomfort, and reduced cognitive performance (Settimo et al., 2020). For this reason, monitoring microbial contamination and maintaining adequate ventilation are essential measures for ensuring healthy indoor environments.

The COVID-19 pandemic proved very clear that indoor air pollution is a serious public health problem that may harm people. People spend a lot more time inside their homes, schools, and workplaces during lockdowns. This made them more likely to breathe in indoor pollutants like particulate matter, carbon dioxide, and volatile organic compounds (VOC). Various research has demonstrated that indoor air pollution persisted at significant levels during this timeframe, despite improvements in outdoor air quality, showing that indoor settings can accumulate pollutants and elevate health risks (Du & Wang, 2020).

The pandemic additionally revealed that indoor spaces that aren't well-ventilated may accumulate aerosols that transmit microbes, which makes it more likely that COVID-19 will spread and that people will inhale the virus (Braggion et al., 2024). This

circumstance showed that the quality of air that circulates indoors can directly affect people's health, not just by contributing to infections but also by causing symptoms such as migraines, eye irritation, and breathing problems (Agarwal et al., 2021). Also, the pandemic made indoor pollution worse in many buildings by causing inhabitants to use more home-cleaning products and minimizing outdoor ventilation (Domínguez-Amarillo et al., 2020). In general, the COVID-19 pandemic situation showed that polluted indoor air can make health problems worse. This confirms the need to monitor and treat indoor pollution in the air as a serious and avoidable harm to human well-being (Braggion et al., 2024).

3.3 Legislation and Standards for Indoor Air Quality

Indoor air quality is regulated and investigated through a combination of international standards and national legislation that aim to protect human health and ensure safe indoor environments. These regulatory frameworks define acceptable pollutant concentrations, establish monitoring procedures, and provide methodological guidance for assessing indoor environmental conditions.

3.3.1 International Standards for Investigation of Indoor Air Quality

Investigation and monitoring of indoor air quality are supported by international guidelines and technical standards that define standardized methods for sampling and evaluation of indoor pollutants. One of the most important frameworks for indoor air quality investigation is the ISO 16000:2012 series, which provides methodological guidance for measuring indoor air pollutants in buildings.

The ISO 16000:2012 series describe several key steps that should be followed when conducting indoor air investigations. According to ISO 16000-34, the indoor air assessment should begin with a preliminary inspection of the building, including identification of potential pollution sources, evaluation of ventilation systems, and assessment of environmental factors such as temperature and humidity. Based on this preliminary evaluation, a suitable measurement strategy is selected, including the choice of sampling locations, sampling methods, and monitoring duration.

Another important standard within the ISO 16000:2012 series is ISO 16000-19:2012, which focuses on the detection of mold contamination in buildings. This standard emphasizes the importance of visual inspection, moisture assessment, and sampling of air or surfaces when microbial contamination is suspected. The standard also recommends comparing indoor and outdoor microbial concentrations to identify potential indoor sources of contamination.

Microbiological contamination of indoor environments is further addressed in the ISO 14698:2003 series, which provides general principles for monitoring biological contamination in indoor environments. ISO 14698-1:2003 describes strategies for designing microbiological monitoring programs, including selection of sampling methods, frequency of monitoring, and evaluation of environmental factors that may influence microbial growth. ISO 14698-2:2003, focuses on interpretation of microbiological monitoring data and emphasizes the importance of trend analysis rather than relying solely on single measurements.

Although these standards were originally developed for controlled environments such as cleanrooms, the principles described in ISO 14698:2003 are also applicable to environmental monitoring in buildings. In particular, the standards highlight the importance of selecting appropriate sampling methods and considering environmental factors such as temperature, humidity, and human activity when evaluating microbial contamination of indoor air.

3.3.2 European Regulations and Guidelines

Policies pertaining to environmental health and air quality in Europe offer a crucial framework for controlling indoor and outdoor air pollution. Even though directives like the Ambient Air Quality Directive (2008/50/EC) of the European Union primarily address outdoor air quality, these laws nevertheless have a big impact on indoor environmental conditions. Improvements in outdoor air quality can indirectly lead to better indoor air conditions because outdoor pollutants can enter buildings through ventilation systems, open windows, and structural gaps. The significance of healthy indoor environments is emphasized by European organizations and research projects in addition to legislative directives, especially in public buildings like offices, schools, and healthcare facilities. These programs support guidelines for ventilation performance, biological contaminant and particulate matter monitoring, and regulation of humidity levels that could encourage microbial growth. (World Health Organization, 2009; European Collaborative Action, 1993)

Therefore, European guidelines offer a broad strategic framework for managing indoor environmental quality; however, individual member states usually implement these guidelines at the national level. These guidelines are modified by each nation based on its own building codes, public health priorities, and environmental circumstances. (Settimo et al., 2020). Consequently, national legislation represents the key tool for translating European principles into practical requirements for indoor environments used for long-term occupancy. These rules typically set hygienic limits for microbial contamination, define acceptable pollutant concentrations, and outline technical specifications for ventilation systems and building upkeep. In this regard, the Czech Republic has created laws that control indoor environmental conditions in buildings and guarantee that occupants' indoor environments are safe and healthy.

3.3.3 National Legislation for Indoor Air Quality in the Czech Republic

In addition to international standards, indoor air quality is regulated through national public health legislation. In the Czech Republic, hygienic requirements for indoor environments are defined by the Decree No. 43/2025 Sb., which establishes limits for chemical, physical, and biological indicators in indoor spaces used for long-term occupancy.

The decree defines requirements related to ventilation, indoor temperature, humidity, and concentrations of selected pollutants. Carbon dioxide (CO_2) concentration is used as an indicator of ventilation efficiency, and the regulation specifies that indoor concentrations should not exceed defined hygienic limits during normal occupancy. The decree also establishes limits for particulate matter ($PM_{2.5}$) and specifies acceptable levels of microbial contamination in indoor air.

According to the regulation, visible mold growth on interior surfaces is not permitted, and concentrations of airborne bacteria and mold spores must remain below specified hygienic limits. The Decree No. 43/2025 Sb. also requires that ventilation systems ensure adequate air exchange in occupied spaces to remove pollutants and control indoor humidity. Regular maintenance of ventilation systems is required to prevent accumulation of contaminants and microbial growth.

When health complaints occur or moisture problems are identified in buildings, microbiological investigation of indoor air may be necessary to identify potential sources of contamination. Standardized procedures for microbiological sampling and evaluation of indoor environments are described in national methodological guidelines for environmental microbiology (Klánová & Vrkoslavová, 2021). These procedures include several methods for sampling airborne microorganisms, including sedimentation techniques, which are commonly used for assessing microbial contamination of indoor air.

3.4 Microbial Contamination and Its Influencing Factors

Indoor air quality (IAQ) is strongly influenced by the presence and concentration of airborne microorganisms, collectively referred to as bioaerosols. Bioaerosols include bacteria, fungi, viruses, and their fragments or metabolic by-products, which are dispersed in the air as particulate matter and may be viable or non-viable (Rastmanesh et al., 2024). Exposure to these biological particles can influence human health and contribute to respiratory diseases, allergies, and other health problems (Hayleeyesus & Manaye, 2014; Hussain et al., 2022). Because individuals spend a significant portion of their time indoors, understanding the sources, types, and environmental factors

influencing microbial contamination is essential for effective indoor air quality management.

3.4.1 Major Microbial Agents in Indoor Air

Airborne microorganisms in indoor environments mainly consist of bacteria and fungi, while viruses are generally smaller and typically depend on human hosts for transmission and survival. Bacteria commonly detected in indoor air belong to the phyla Firmicutes, Proteobacteria, and Actinobacteria, including genera such as *Staphylococcus*, *Streptococcus*, *Micrococcus*, and *Bacillus*. Many of these bacteria originate from human skin, respiratory emissions, and other biological activities of occupants (Chen et al., 2020; Hayleeyesus & Manaye, 2014).

Fungal bioaerosols are also frequently reported in indoor environments. Common indoor fungal genera include *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria*, which are capable of remaining airborne for extended periods due to their spore-forming characteristics (Hayleeyesus & Manaye, 2014; Xu et al., 2017). These fungi are widely recognized as major contributors to allergic reactions, asthma, and symptoms associated with sick building syndrome. In addition, damp or poorly ventilated buildings often promote fungal proliferation, further increasing airborne fungal concentrations.

Indoor microbial communities are typically composed of a mixture of microorganisms originating from both indoor and outdoor sources. Outdoor air is a major contributor to indoor microbial populations, introducing microorganisms through ventilation systems, open windows, doors, and structural leakage. Seasonal variations in outdoor microbial abundance can therefore significantly influence indoor microbial composition (Xu et al., 2017; Els et al., 2020).

Human occupants represent another important source of indoor microorganisms. Daily activities such as breathing, speaking, coughing, walking, and skin shedding release microorganisms into the air. As a result, bacteria associated with human skin and respiratory systems often dominate indoor microbial communities, particularly in densely occupied spaces or poorly ventilated environments (Prussin & Marr, 2015). In addition to humans, pets and indoor plants can also introduce microorganisms associated with fur, soil, and plant surfaces (Mahnert et al., 2015).

Indoor surfaces and settled dust act as important reservoirs for microorganisms. Carpets, furniture, mattresses, and other materials accumulate microbial particles that can later become airborne through resuspension caused by human movement or cleaning activities (Leppänen et al., 2018). Moreover, inadequately maintained building systems such as heating, ventilation, air-conditioning, and plumbing may

facilitate microbial growth and contribute to the distribution of microorganisms within indoor environments (Prussin & Marr, 2015).

Exposure to airborne microorganisms can affect human health through inhalation, dermal contact, or deposition on surfaces. High concentrations of bioaerosols have been associated with respiratory symptoms, allergic reactions, asthma, and infectious diseases, particularly among vulnerable populations such as children, the elderly, and immunocompromised individuals (Prussin & Marr, 2015). Opportunistic pathogens such as *Staphylococcus*, *Acinetobacter*, and *Mycobacterium* species have been detected in indoor air and linked to respiratory or skin infections (Xu et al., 2017).

Fungal bioaerosols are particularly important in triggering allergic and inflammatory responses. Spores of *Cladosporium*, *Penicillium*, and *Aspergillus* are well-known allergens that may cause asthma, allergic rhinitis, and other respiratory disorders (Leppänen et al., 2018). Long-term exposure to damp or mold-contaminated environments has been consistently associated with chronic respiratory conditions and reduced lung function.

In addition to viable microorganisms, microbial fragments and metabolic products such as endotoxins and mycotoxins may also contribute to adverse health effects. These compounds can provoke inflammatory responses even when the microorganisms themselves are no longer viable (Prussin & Marr, 2015).

However, not all microbial exposure is detrimental. Recent research suggests that moderate exposure to diverse environmental microorganisms may support immune system development and reduce the likelihood of allergic diseases, particularly during early childhood (Leppänen et al., 2018; Mahnert et al., 2015). This dual role highlights the complexity of microbial exposure in indoor environments.

3.4.2 Factors Influencing Microbial Concentration

Indoor microbial concentrations are influenced by multiple environmental and operational factors that affect microbial survival, dispersion, and deposition. Among these, temperature, relative humidity, ventilation rate, particulate matter concentration, and human activity are widely recognized as key determinants of microbial dynamics in indoor air (Hiwar et al., 2021).

Environmental parameters influence microorganisms both directly and indirectly. Direct effects involve impacts on microbial metabolism, growth, and viability, whereas indirect effects involve modifications in airflow patterns, particle transport, and surface contamination processes (Stanaszek-Tomal, 2020).

Temperature is one of the primary environmental parameters affecting microbial behavior. Most indoor microorganisms are mesophilic organisms that thrive at moderate temperatures typically associated with human comfort. Consequently, indoor thermal conditions that support occupant comfort may also favor microbial survival in the air (Stanaszek-Tomal, 2020).

Elevated indoor temperatures have been associated with increased airborne bacterial concentrations in healthcare settings, suggesting that warmer conditions may enhance microbial viability (Hiwar et al., 2021). Although temperature does not directly generate microorganisms, it influences the persistence of existing microbes in the air. In hospital environments, prolonged airborne survival of microorganisms may increase the risk of infection (Capolongo et al., 2017).

Temperature also affects air density and circulation patterns. Warmer air tends to rise, altering airflow dynamics and microbial distribution within indoor spaces. Conversely, lower temperatures may promote the deposition of microorganisms on surfaces, which can later become airborne through resuspension caused by human activity (Hiwar et al., 2022). Seasonal variations in temperature also influence indoor microbial levels by affecting outdoor microbial concentrations that enter buildings through ventilation systems (Núñez & García, 2023).

Relative humidity is another critical environmental parameter that interacts closely with temperature to influence microbial survival. Many microorganisms, particularly fungi, require sufficient moisture to grow and reproduce, making humidity an important factor in microbial dynamics (Stanaszek-Tomal, 2020).

High relative humidity may promote microbial growth and increase airborne fungal concentrations. Persistent moisture in indoor environments can lead to condensation on walls, ceilings, and ventilation systems, creating favorable conditions for microbial proliferation (Capolongo et al., 2017). Once established, these microorganisms may become airborne and increase exposure risks.

Conversely, excessively low humidity may also negatively affect indoor air quality. Dry air conditioning can allow certain microorganisms to remain airborne for longer periods and may impair the protective function of the human respiratory tract. Low humidity can dry mucous membranes, increasing susceptibility to airborne infections (Capolongo et al., 2017).

Studies investigating the relationship between humidity and microbial concentration have produced mixed results. Some research suggests that relative humidity does not always show a strong direct correlation with airborne microbial levels, indicating that humidity often acts indirectly through interactions with other environmental variables such as temperature, ventilation, and particulate matter (Hiwar et al., 2021).

Air circulation and ventilation also play a crucial role in shaping microbial concentration in indoor environments. Ventilation systems regulate the exchange of indoor and outdoor air, influencing the introduction, dilution, and removal of microorganisms. Poor ventilation can lead to microbial accumulation, whereas adequate air exchange can reduce microbial concentrations.

Seasonal changes may further influence microbial levels through variations in outdoor environmental conditions. Warmer seasons often correspond with higher microbial concentrations due to increased microbial growth and outdoor source strength (Núñez & García, 2023; Chen et al., 2024). Indoor microbial communities frequently reflect these seasonal patterns.

Human occupancy and activity are additional important factors affecting microbial concentration. Activities such as walking, cleaning, and movement of occupants resuspend particles from indoor surfaces and settled dust, increasing airborne microbial levels. Higher occupancy density generally leads to greater microbial emissions from human sources (Capolongo et al., 2017). In healthcare environments, fluctuations in patient numbers, staff activities, and visiting hours can significantly affect airborne microbial load (Hiwar et al., 2021).

Overall, microbial concentration in indoor air is determined by the combined effects of environmental conditions, building characteristics, and human activities. No single microclimatic parameter can reliably predict microbial load independently. Instead, the interactions among temperature, humidity, ventilation, particulate matter, and occupancy patterns collectively shape microbial dynamics in indoor environments (Capolongo et al., 2017; Stanaszek-Tomal, 2020).

3.5 Methods for Assessing Microbial Contamination of Indoor Air

The assessment of microbial contamination in indoor air is an important component of indoor environmental quality monitoring because airborne microorganisms may influence occupant health and contribute to the spread of infectious agents, allergens, and microbial metabolites (Chawla et al., 2023). Microorganisms present in indoor air are usually associated with particulate matter such as dust particles, skin fragments, or droplets, which facilitates their transport through indoor environments and their subsequent deposition on surfaces (Chawla et al., 2023).

Sampling of microbial air contamination is generally divided into two principal approaches: active air sampling and passive air sampling. These approaches differ in their sampling principle, equipment used, and the form in which results are expressed. Active sampling involves the mechanical collection of a known volume of air and enables quantitative evaluation of viable airborne microorganisms. Passive sampling relies on gravitational settling of particles onto exposed culture media and therefore

reflects microbial deposition rather than airborne concentration (Pasquarella et al., 2000; Chawla et al., 2023). Because these approaches capture different aspects of microbial contamination, they are generally considered complementary methods for indoor air quality assessment (Chawla et al., 2023).

3.5.1 Active Air Sampling

Active air sampling is based on the controlled aspiration of a defined air volume through a sampling device that captures airborne microorganisms for subsequent cultivation or analysis. The collected microorganisms are typically deposited onto a solid culture medium, retained on a membrane filter, or collected in a liquid medium for further microbiological processing (Chawla et al., 2023).

The principal advantage of active sampling is that it enables quantitative evaluation of microbial contamination, since results are expressed as colony-forming units per cubic meter of air (CFU/m^3). This allows comparison of microbial contamination levels between different indoor environments and monitoring conditions (Pasquarella et al., 2000; Chawla et al., 2023).

Several types of active air samplers are used in microbial air monitoring, differing mainly in the physical principle used to capture airborne particles.

Impaction samplers are among most commonly used devices for microbial air sampling. In these samplers, air is drawn through a perforated plate containing multiple small holes or nozzles. As the air accelerates through these openings, airborne particles gain momentum and impact onto the surface of an agar plate positioned beneath the perforated lid (Scherwing & Arakel, 2021).

This method allows the direct cultivation of collected microorganisms and is widely applied for monitoring bacteria and fungi in indoor environments. The efficiency of impaction samplers is influenced by airflow rate, sampling duration, and the particle size collection efficiency of the device (Ljungqvist et al., 2012).

A key parameter describing sampler performance is the d_{50} value, which represents the particle diameter at which 50% of airborne particles are collected by the sampler. Most microorganisms in indoor air are associated with particles larger than 1 μm, and samplers with lower d_{50} values generally achieve higher collection efficiency for microbe-carrying particles (Scherwing & Arakel, 2021).

Filtration-based sampling involves drawing air through a membrane filter that retains airborne microorganisms. After sampling, the filter can be placed directly onto culture medium or processed further to recover viable microorganisms (Chawla et al., 2023).

Gelatin membrane filters are widely used in microbial air sampling because they provide high retention efficiency while preserving microbial viability. After sampling, gelatin filters can be dissolved in sterile liquid or transferred to culture media, which facilitates cultivation and further microbiological analysis (Scherwing & Arakel, 2021).

Liquid collectors, also known as impingers, capture airborne microorganisms in a liquid medium. During sampling, air is drawn through a sterile liquid that traps airborne particles containing microorganisms (Chawla et al., 2023).

This approach reduces desiccation stress and may improve the survival of sensitive microorganisms during sampling. The resulting liquid sample can subsequently be used for cultivation, microscopic analysis, or molecular methods (Chawla et al., 2023). However, impingers typically require more complex equipment and careful handling compared with solid-medium samplers.

3.5.2 Passive Air Sampling

Passive air sampling evaluates microbial contamination through the natural sedimentation of airborne particles onto exposed culture media. Most commonly used passive technique is the settle plate method, in which Petri dishes containing a selected agar medium are exposed to indoor air for a defined period (Pasquarella et al., 2000).

During exposure, airborne particles carrying microorganisms settle onto the agar surface due to gravitational forces. After incubation, the number of colonies that develop reflects the level of microbial deposition from the air (Pasquarella et al., 2000).

In contrast to active sampling, passive methods do not collect a defined air volume and therefore cannot determine microbial concentrations in CFU/m³. Instead, results are usually expressed as colony-forming units per plate and exposure time or converted to deposition rates such as CFU/m²/h (Pasquarella et al., 2000; Chawla et al., 2023).

The sedimentation plate method is technically simple, inexpensive, and requires minimal equipment, typically consisting of sterile Petri dishes containing culture medium and a timer to control exposure duration. Because of its simplicity, passive sampling is widely used for environmental monitoring in hospitals, laboratories, cleanrooms, and other indoor environments (Pasquarella et al., 2000).

To improve comparability between measurements, standardized exposure conditions have been proposed. One commonly used approach is the Index of Microbial Air Contamination (IMA) method, which recommends exposing settle plates for one hour

at a height of approximately one meter above the floor and at least one meter away from walls or obstacles (Pasquarella et al., 2000).

Although passive sampling provides useful information about microbial deposition onto surfaces, the method is strongly influenced by environmental conditions and air movement within the indoor environment (Chawla et al., 2023).

3.5.3 Factors Influencing Microbial Air Sampling

Both active and passive sampling results may be influenced by several environmental and methodological factors. Airflow and ventilation play a critical role in determining the distribution and transport of airborne microorganisms. Human movement, ventilation systems, and other sources of air turbulence may significantly affect particle suspension and sedimentation rates, particularly in passive sampling (Pasquarella et al., 2000; Chawla et al., 2023).

Temperature and relative humidity also affect microbial viability and sampling efficiency. Elevated humidity levels may support microbial survival, whereas low humidity can lead to desiccation stress and reduced recovery of viable microorganisms. In passive sampling, low humidity may additionally cause drying of agar surfaces during prolonged exposure (Chawla et al., 2023).

Another important factor is the duration of exposure or sampling. Longer sampling times typically increase colony counts but may also lead to overlapping colonies, agar desiccation, or changes in microbial viability, which may complicate accurate colony enumeration (Pasquarella et al., 2000).

The selection of culture media also affects microbial recovery. Non-selective media such as Tryptic Soy Agar (TSA) and Plate Count Agar (PCA) are commonly used for bacterial monitoring because they support the growth of a broad range of environmental bacteria. For fungal monitoring, Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) are frequently used because they promote the growth of yeasts and filamentous fungi (Chawla et al., 2023).

However, no single culture medium supports the growth of all airborne microorganisms, and culture-based methods therefore detect only a fraction of the total microbial population present in indoor air (Pasquarella et al., 2000).

3.5.4 Standards and Guidelines for Microbial Air Sampling

Several international and European standards provide guidance on microbial air monitoring and interpretation of sampling results. For example, EN 17141:2020

specifies performance requirements and validation procedures for microbiological air samplers used in controlled environments. EN 13098:2000 provides guidance on measurement strategies and exposure assessment for airborne microorganisms in occupational environments. Earlier standards such as ISO 14698:2003 established principles for biocontamination control and monitoring in cleanrooms and controlled environments (Ligugnana, 2021).

These standards primarily define performance criteria for sampling devices and monitoring strategies in regulated environments such as pharmaceutical production or cleanroom facilities. However, standardized monitoring procedures for general indoor environments, including public buildings or educational facilities, are less clearly defined and often rely on semi-quantitative assessment methods (Chawla et al., 2023).

3.5.5 Cultivation and Measurement Techniques

After bioaerosols are collected from indoor air using active or passive sampling methods, laboratory analysis is required to determine the presence, concentration, and characteristics of microorganisms. Various analytical techniques are used to evaluate microbial contamination, including culture-based methods, microscopic techniques, and molecular or biochemical detection methods. These methods enable the quantification and identification of airborne microorganisms and can also detect microbial metabolic by-products such as microbial volatile organic compounds (MVOCs) (Chawla et al., 2023).

3.5.5.1 Culture-Based Methods

Culture-based methods remain the most widely used approach for assessing microbial contamination in indoor environments. In these methods, airborne microorganisms collected during sampling are deposited onto suitable culture media and incubated under controlled laboratory conditions to allow viable microorganisms to grow into visible colonies. The colonies are then enumerated using the plate count method, which allows the quantification of culturable microorganisms present in indoor air (Kumar et al., 2022).

Several types of microbiological media are commonly used for the cultivation of indoor bioaerosols. General-purpose media such as nutrient agar, tryptic soy agar, blood agar, and casein soy peptone agar are frequently applied for bacterial cultivation, while potato dextrose agar, malt extract agar, rose bengal agar, and dicloran glycerol agar are widely used for fungal isolation and quantification (Chawla et al., 2023).

Environmental monitoring programs often use TSA agar for the cultivation of environmental bacteria and SDA agar for fungal detection and enumeration (Ligugnana, 2021). These cultivation techniques allow both quantitative and

qualitative evaluation of microbial contamination, providing information on microbial abundance and diversity in indoor environments.

Despite their widespread application, culture-based methods have certain limitations. Only microorganisms capable of growing under laboratory conditions can be detected using this approach, meaning that many airborne microorganisms that are viable but non-culturable may remain undetected (Chawla et al., 2023).

3.5.5.2 Microscopic and Identification Techniques

Microscopic techniques are also used for the identification and enumeration of microorganisms collected from indoor environments. After appropriate staining and sample preparation, microorganisms can be observed using bright-field, phase contrast, fluorescence, or electron microscopy, which allows the identification of microbial morphology and structures (Baloch et al., 2020).

In some cases, airborne microbial particles are collected directly onto glass slides, membrane filters, or semi-solid media and examined microscopically without prior cultivation. These methods are particularly useful for detecting fungal spores and other microbial particles that may not grow easily on culture media (Chawla et al., 2023).

3.5.5.3 Molecular and Advanced Detection Methods

Recent advances in analytical technologies have introduced molecular and spectrometric methods that complement traditional cultivation techniques. Molecular detection techniques such as polymerase chain reaction (PCR), sequencing, and mass spectrometry allow rapid and sensitive detection of microorganisms, including species that cannot be cultured using conventional laboratory techniques (Kumar et al., 2022). One widely used technique for microbial identification is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This method identifies microorganisms by analyzing the mass-to-charge ratio of microbial peptides and proteins and comparing the obtained spectral fingerprints with reference databases (Chawla et al., 2023).

Other emerging technologies include laser-induced fluorescence (LIF) and laser-induced breakdown spectroscopy (LIBS), which allow rapid and real-time detection of bioaerosols based on their optical signatures (Chawla et al., 2023).

3.5.5.4 Detection of Microbial Volatile Organic Compounds (MVOCs)

Microbial contamination can also be assessed indirectly by measuring microbial volatile organic compounds (MVOCs) produced during microbial metabolism. These

compounds are released by bacteria and fungi during growth and degradation of organic materials (Mentese et al., 2015).

More than 200 MVOCs have been identified in indoor environments, including alcohols, aldehydes, esters, and hydrocarbons. These compounds often contribute to the characteristic moldy odor associated with microbial growth in damp indoor environments (Mentese et al., 2015; Wanner et al., 1993).
Exposure to MVOCs has been associated with irritation of the eyes and respiratory tract, and in some cases may contribute to allergic reactions or asthma symptoms among building occupants (Mentese et al., 2015).

Overall, the assessment of microbial contamination in indoor environments typically involves a combination of analytical approaches. Culture-based methods provide quantitative information on viable microorganisms, microscopic techniques assist in morphological identification, and molecular methods enable rapid detection and characterization of microbial species. Additionally, MVOC analysis can provide indirect evidence of microbial activity and hidden microbial growth in indoor environments

4. Methodology

The primary objective of this study was the verification of a measurement technique, rather than a simple examination of the current IAQ situation. Thus, the study directly evaluated the efficacy of the sedimentation technique (passive air sampling) in selected indoor environments. The microbial deposition was assessed repeatedly with different exposure times, room usage, and cultivation media.

4.1 Study Area

The research took place in the MCEV II building of the Faculty of Environmental Sciences, CZU Prague, located at Praha-Suchdol, Prague, Czech Republic. This building is primarily utilized for educational and research activities.

The selected rooms represent different functional areas within the university building, including classrooms, laboratories, and offices. These spaces differ in occupancy levels, duration of use, and intensity of human movement, which might influence the concentration of airborne microorganisms.

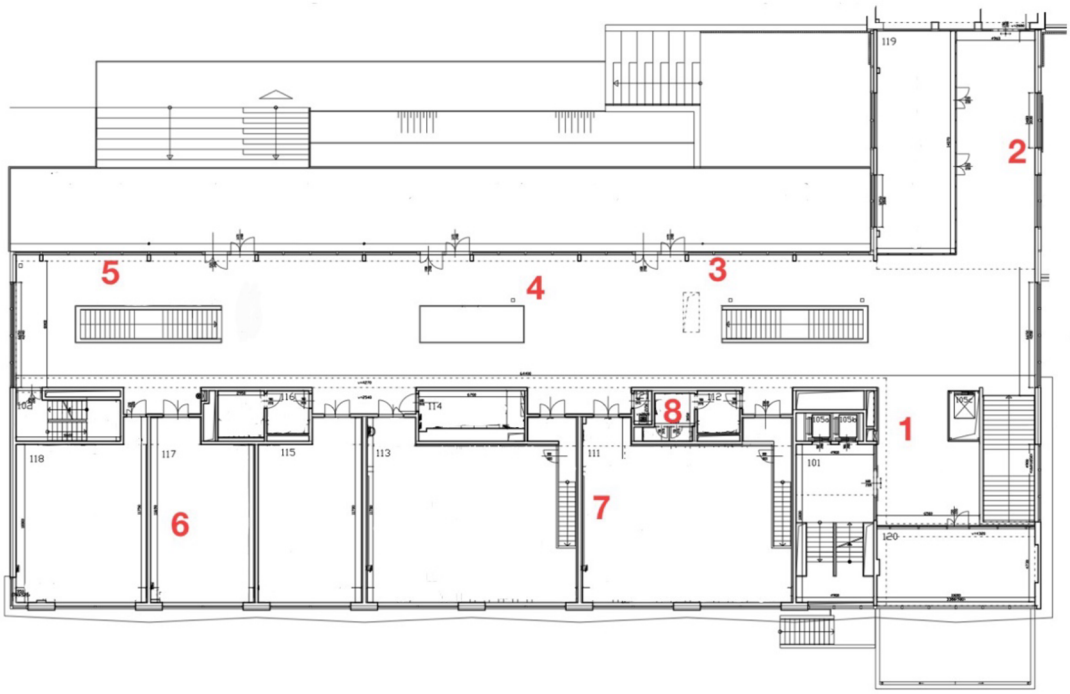


Figure 4-1.Floor plan of the MCEV II building (Faculty of Environmental Sciences, CZU Prague) indicating the spatial distribution of sampling sites

Table 4-1. Identification of sampling spot numbers and their corresponding functional locations within the university premises

Spot Number	Location
1	Corridor A
2	Corridor B
3	Student Lounge A
4	Student Lounge B
5	Student Lounge C
6	Classroom (Z117)
7	Lecture Hall (Z1)
8	Male Toilet

The selected rooms represent common functional spaces in the university building: a classroom, a lecture hall, a student lounge, a hallway, and a toilet. The classroom and lecture hall are used for teaching activities. The student lounge is used for studying and informal student activities. Hallways are used mainly for movement between rooms, and people stay there only briefly. The toilet is a small, enclosed space used periodically throughout the day.

The building is regularly used by students, teachers, and university staff. Occupancy varies during the day depending on class schedules and other activities. All studied rooms are supplied by a mechanical ventilation system, which provides controlled air exchange during the monitoring period.

4.2 Sampling Strategy

4.2.1 Instrumentation and Equipment

- Colony Counting - Cole-Parmer CC-200 Digital Colony Counter; 90 to 230 VAC; 50/60 Hz (Cole-Parmer, UK) Can count 0 to 999
- Q-Cell laboratory incubator (POL-LAB, Poland), [Q-Cell 240] INOX model, with a temperature range of +8 °C to +70 °C.
- Temperature and Humidity sensor HTC-1 Digital Thermometer (ECLIPSERA, Czech Republic)
- TSA agar 25ml – ø 85mm
- Nutrient agar 25ml – ø 85mm

4.2.2 Sampling Strategy

Sampling was carried out during two different seasons in two measurement campaigns. The first sampling was taken on September 6, 2025, which was late summer. The second sampling occurred on December 3, 2025, reflecting winter conditions. For every sampling event, measurements had been taken in four replicates per agar type, specifically utilizing Nutrient Agar (NA) and Tryptic Soy Agar (TSA). Three different exposure time of the sampling were used: 8, 12, and 24 hours.

Agar plates were set down at the chosen places 1-1.5m heights available in each sampling location. The placement was generally in line with typical indoor breathing zones, but there was no strict height standardization. This was done to reflect real indoor conditions and normal human exposure.

Agar plates were collected according to the assigned exposure times. All plates were removed at the same time to ensure identical incubation start times and consistent laboratory processing.

The occupancy conditions differed between the two sampling campaigns. During the first sampling, the sampling points were empty. During the second sampling, measurements were carried out while students were present during normal building use. During both sampling campaigns, windows remained closed and the mechanical ventilation system operated normally.

Airborne bacteria and fungi have been assessed by counting the number of colony-forming units (CFU) grown on culture media after passive air sampling and incubation. Microbial contamination was quantified as CFU per cubic meter of air (CFU/m³), based on the colony count on the exposed agar plates.

The following physical parameters were measured to describe the indoor environmental conditions during sampling:

- Air temperature, which is measured in degrees Celsius (°C),
- Relative humidity, shown as a percentage (%).

These two parameters were identified five minutes before the start of sampling in each room that was examined at. These measurements were used to convey the indoor environment at the time of exposure.

The samples were taken to the lab right after they were collected. They were kept in a controlled environment for 48 hours in 35 °C. To find out how many microbes there were, a colony counter is used to identify and record only colonies that could be seen. These totals were then used to determine the final airborne concentrations.

After incubation, the number of colonies grown on NA and TSA agars was counted. The results were converted and reported as CFU per cubic meter of air (CFU/m³), which is the typical method to measure the contamination of indoor air is with microbes.

4.3 Data Analyses

All collected data were organized and pre-processed using Microsoft 365 Excel version 2602 (Build 19725.20172) (Microsoft Corporation, USA). Excel was used for initial data management, including recording colony counts, organizing sampling information (room, agar type, and exposure time), and preparing datasets for further statistical analysis.

4.3.1 Calculation of Microbial Concentration

After incubation, visible microbial colonies grown on the agar plates were counted and recorded as colony-forming units (CFU). The sedimentation method provides results based on gravitational settling of airborne particles onto agar surfaces. Colony counts obtained from different exposure times and agar media were converted into estimated CFU/m³ values using the Omeliansky formula (Chawla et al., 2023).

The relationship of Omeliansky formula used in the study is shown below.

$$N=\frac{5ax10^4}{b\times t}$$

Where:

N – estimated microbial concentration in the air (CFU/m³)
a – number of colonies counted on the Petri dish
b – surface area of the Petri dish (cm²)
t – exposure time of the plate (minutes)

The calculated results of CFU/m³ concentrations were then used for further statistical analysis and comparison among sampling locations, agar types, and exposure durations.

4.3.2 Statistical Analysis

Statistical analyses and hypothesis testing were performed using RStudio version 2026.01.1+403 (Posit PBC, USA) with the R statistical environment. The analyses aimed to evaluate whether exposure time and type of agar medium significantly

influenced the number of colony-forming units (CFU) obtained from sedimentation sampling.

Prior to statistical analysis, the assumptions of normality and homogeneity of variances were evaluated. The Shapiro–Wilk test was used to assess the normality of the data distribution, while Bartlett's test was used to determine whether the variances among groups were equal.

For comparisons between two groups with normally distributed data and equal variances, an independent samples t-test was applied. When the assumption of equal variances was violated, Welch's t-test was used as it does not require homogeneity of variances.

For comparisons among more than two groups, one-way analysis of variance (ANOVA) was used when the data met the assumptions of normality and homogeneity of variances. When significant differences were identified, Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to determine which groups differed from each other.

When the assumption of equal variances was violated, Welch's analysis of variance (Welch ANOVA) was applied as a robust alternative. In this case, post-hoc comparisons were performed using the Games–Howell test, which is suitable for unequal variances and unequal sample sizes.

If the data did not satisfy the assumption of normality, the non-parametric Kruskal–Walli's test was used to assess differences among groups. When significant differences were detected in the Kruskal–Walli's test, post-hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction to control for Type I error.
All statistical analyses were conducted using R statistical software, and statistical significance was determined at a significance level of $p < 0.05$.

Two general hypotheses were tested in the study:

Hypothesis 1 – Effect of agar type

H_0 (null hypothesis): There is no statistically significant difference in microbial colony counts between the tested agar media.
H_1 (alternative hypothesis): There is a statistically significant difference in microbial colony counts between the tested agar media.

Hypothesis 2 – Effect of exposure time

H_0 (null hypothesis): Exposure time does not significantly influence the number of microbial colonies detected using the sedimentation method.

H_1 (alternative hypothesis): Exposure time significantly influences the number of microbial colonies detected using the sedimentation method.

Hypothesis 3 – Effect of location

H_0 (null hypothesis): Sampling location does not significantly influence the number of microbial colonies detected using the sedimentation method.

H_1 (alternative hypothesis): Sampling location significantly influences the number of microbial colonies detected using the sedimentation method.

The significance level of $\alpha = 0.05$ was used for all statistical tests. Results were considered statistically significant when the p-value was lower than this threshold.

5. Results

5.1 Environmental conditions during sampling

The environmental conditions recorded during the sampling periods are detailed in Appendix A Table A-2 and A-5. During the first sampling campaign (late summer), the indoor temperature remained stable, ranging from 22.7°C to 24.6°C, with relative humidity levels between 44% and 48%. In the second sampling campaign (winter), temperatures were slightly lower and more varied, ranging from 20.3°C to 24.1°C, while the relative humidity was significantly lower, ranging from 22% to 32%. Despite the seasonal shift in baseline humidity, these values showed minimal variation within each specific sampling event. Therefore, the variations in microbial counts were primarily attributed to location and occupancy rather than indoor climatic fluctuations, and no further correlation analysis between environmental factors and microbial counts was conducted.

5.2 Results of Sampling 1

Sampling 1 was conducted across eight sampling spots using two types of agar media and three exposure times (8, 12 and 24 hr). Microbial contamination was expressed as (CFU/m³).

Mean microbial concentrations varied considerably between sampling spots and exposure times. Values ranged from 0 to 367.14 CFU/m³. The highest concentrations were generally observed at 12-hour exposure, where several spots showed markedly elevated values compared to 8-hour and 24-hour exposure (Figure 5-1).

Substantial variability was observed in multiple sampling spots. In several cases, the standard deviation was comparable to or exceeded the corresponding mean values. The coefficient of variation (CV) exceeded 40% in most sampling spots and reached values above 100% in some cases, indicating high dispersion of measurements and limited repeatability under the initial sampling conditions (Figure 5.2).

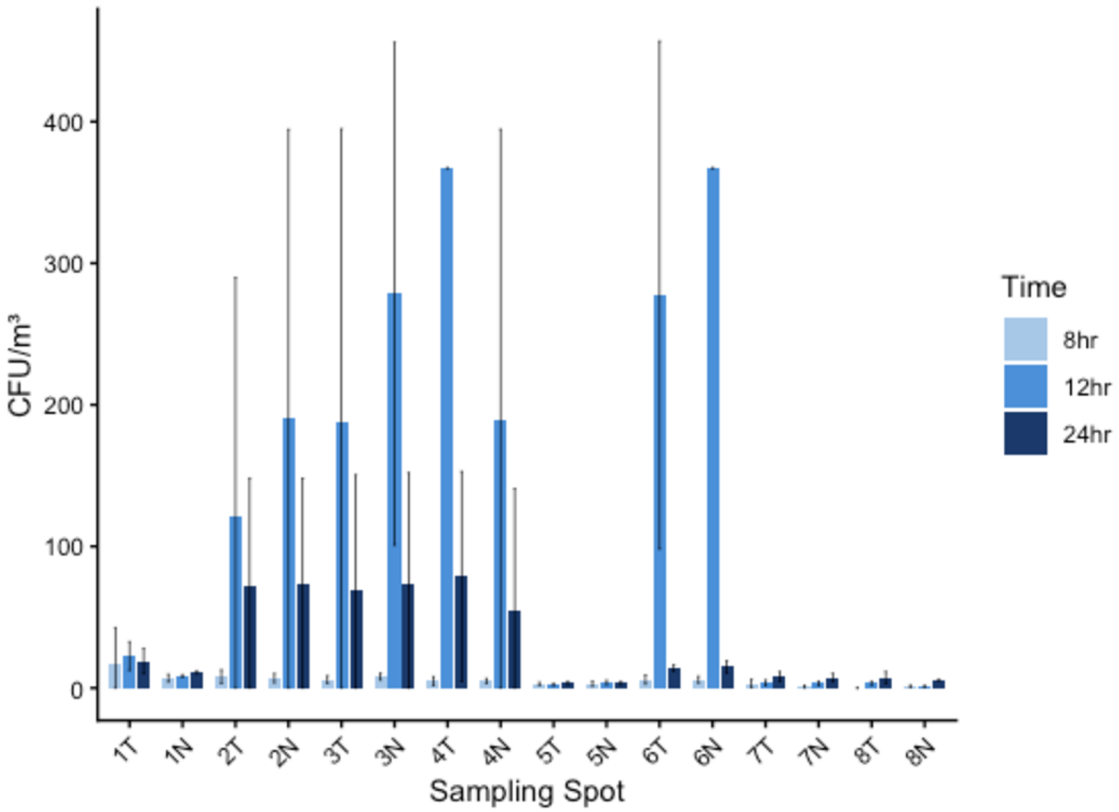
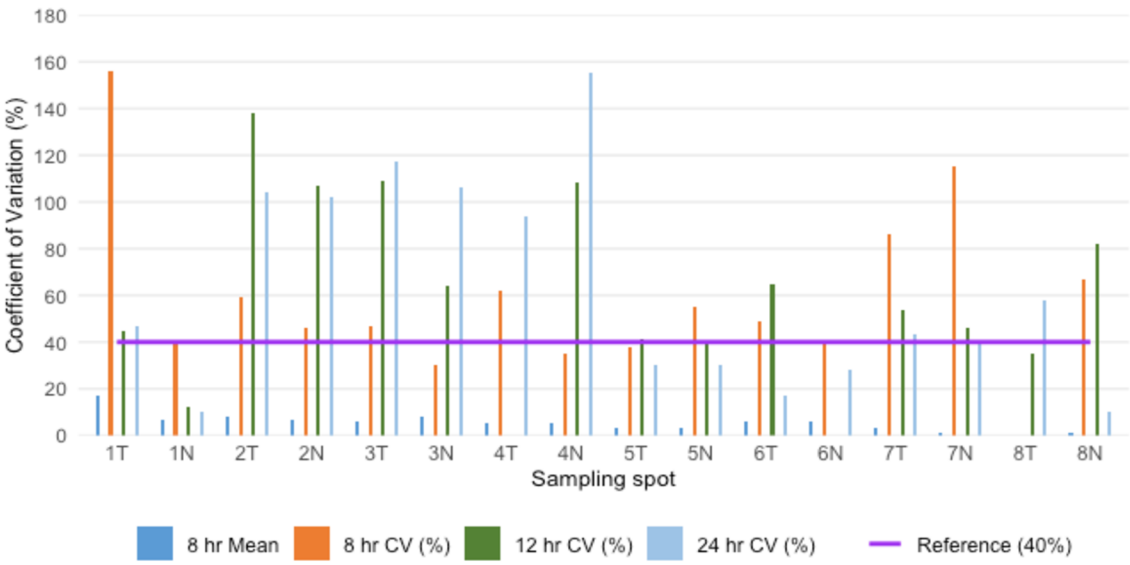


Figure 5-1.Comparison Mean and Standard Deviation of Sampling 1 Microbial Contamination across 8 spots



Spot Number – Location:
1 = Corridor A
2 = Corridor B
3 = Student Lounge A
4 = Student Lounge B
5 = Student Lounge C
6 = Classroom (Z117)
7 = Lecture Hall (Z1)
8 = Male Toilet

Figure 5-2.Variability of Sampling 1 Microbial Contamination

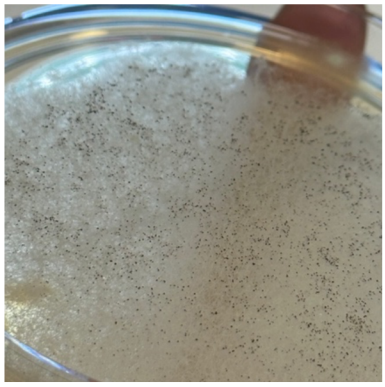


Figure 5-5 (a)

Figure 5-4 (b)

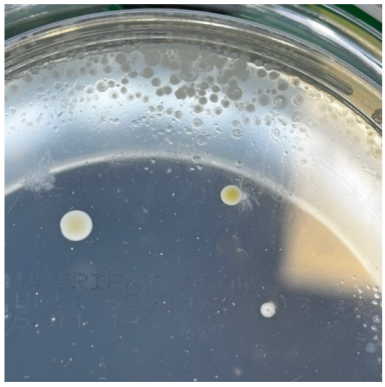


Figure 5-3 (c)

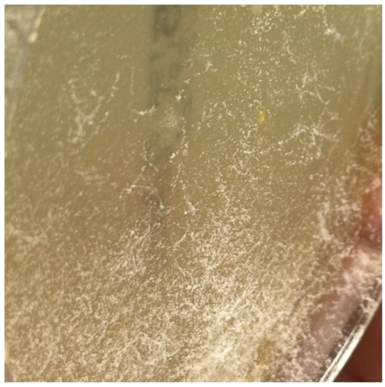


Figure 5-6 (d)

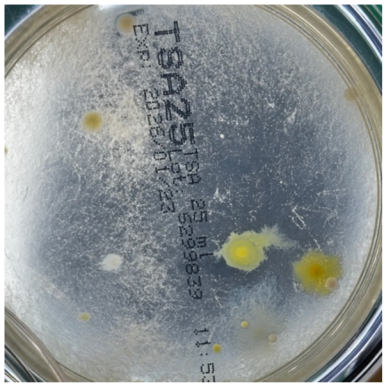


Figure 5-7 (e)

Figure 5-8 (f)

Figure 5-9(a-f). Morphological diversity and growth patterns of microbial colonies on TSA and Nutrient Agar plates (Sampling 1)

5.3 Adjusting Methodology

On the results of Sampling 1, the substantial variability and limited repeatability of measurements were observed. Elevated standard deviations and high coefficients of variation indicated that procedural factors may have influenced the stability of the results. Therefore, some adjustments to the cultivation and evaluation process were implemented prior to Sampling 2.

The sampling setup and exposure conditions remained unchanged. However, the cultivation procedure was modified. During Sampling 2, Petri dishes were incubated in an inverted position (agar side up, lid down), in contrast to the previous orientation. This adjustment was introduced to minimize potential condensation effects and surface disturbances that could influence colony distribution and growth patterns.

In addition, colony counting was refined by clear distinguishing between bacterial colonies and fungal growth. Bacteria and fungi were evaluated separately in order to improve clarity of interpretation and reduce potential bias associated with combined microbial counts.The purpose of these adjustments was to enhance the accuracy and reproducibility of microbial assessment while maintaining comparability between Sampling 1 and Sampling 2.

5.4 Results of Sampling 2

Sampling 2 was performed in identical sampling locations and exposure times. Microbial concentrations ranged from 0 to 371.73 CFU/m³ across all sampling spots. The highest mean concentration was recorded at spot 8T (371.73 CFU/m³ at 8-hour exposure), followed by spot Toilet - 8N (254.24 CFU/m³ at 8-hour exposure). Lower concentrations were observed in spots Classroom 6T and 6N, where values remained below 4 $CFU \cdot m^{-3}$ across all exposure times.

Compared to Sampling 1, exposure times showed more consistent patterns across sampling spots. In most locations, 8-hour and 12-hour exposures yielded higher concentrations than 24-hour exposure, although differences were moderate.
Standard deviation values were generally lower than those observed in Sampling 1. The coefficient of variation (CV) ranged predominantly between 2% and 20%, indicating improved repeatability of measurements. Only sampling spots with very low microbial concentrations (e.g., 6T and 6N) exhibited elevated CV values due to small absolute differences between replicates.
Overall, variability was substantially reduced compared to Sampling 1, where CV frequently exceeded 80–100%.

5.4.1 Descriptive Statistics

Microbial contamination levels varied considerably among sampling locations and exposure times. Across all measurements, the total microbial concentration ranged from 1.84 $CFU \cdot m^{-3}$ to 371.73 $CFU \cdot m^{-3}$ (Appendix XX). The highest microbial levels were observed in the toilet area, while the classroom exhibited the lowest contamination levels. In most locations, microbial concentrations decreased with increasing exposure time from 8 to 24 hours (Figure 5-4).

Compared with Sampling 1, lower variability between replicate measurements was observed, indicating improved consistency of the optimized cultivation method. Figure 5-5 illustrates the mean microbial contamination levels measured at exposure times of 8, 12 and 24 hr across all sampling locations. In general, the highest concentrations were observed after 8 hr exposure, while the lowest concentrations were typically recorded after 24 hr exposure.

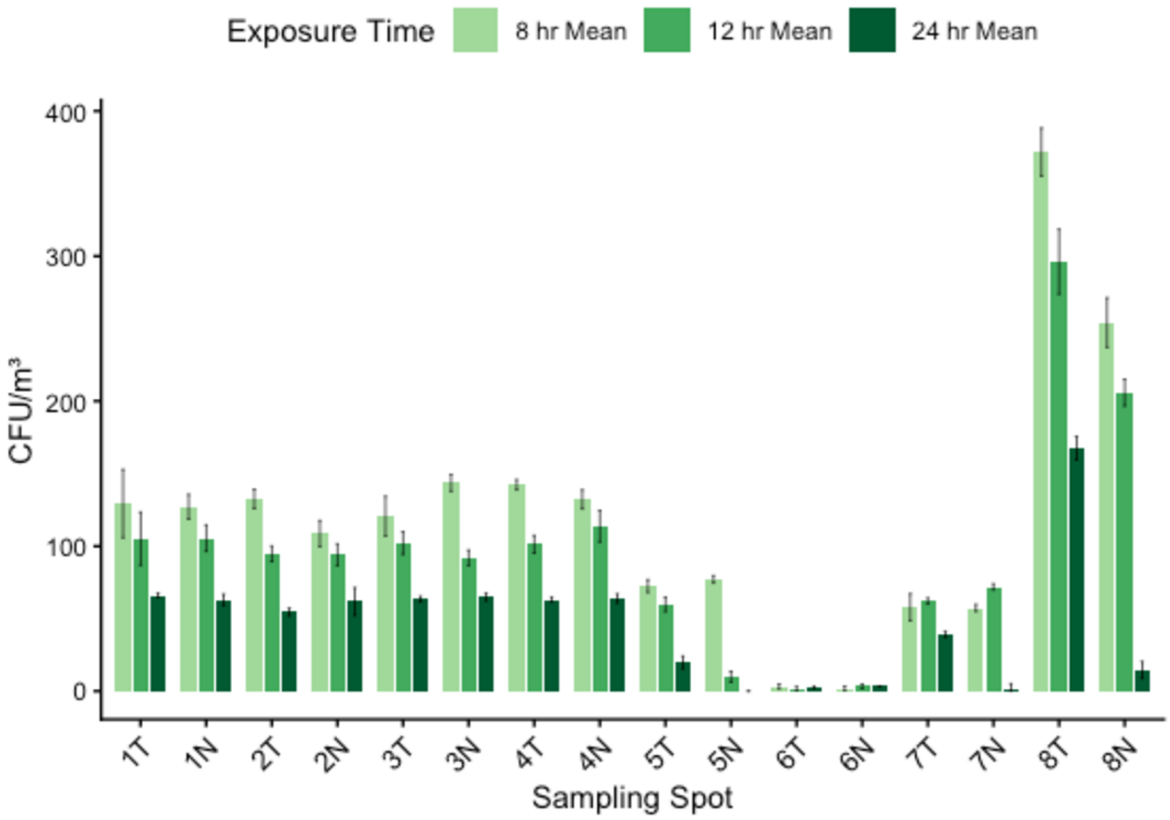


Figure 5-10. Comparison Mean and Standard Deviation of Sampling 2 Microbial Contamination across 8 spots

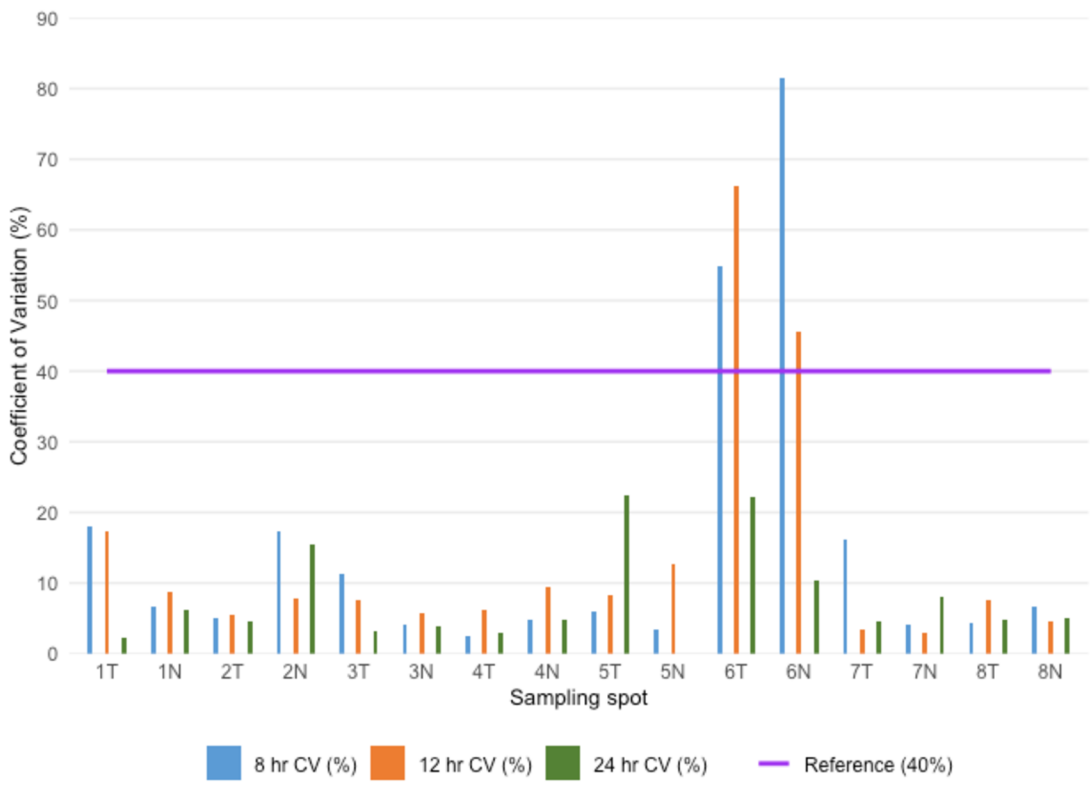


Figure 5-11. Variability of Sampling 2 Microbial Contamination

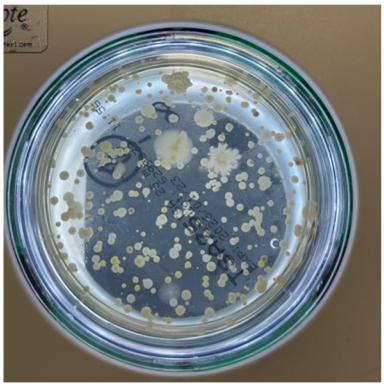


Figure 5-6 (a)

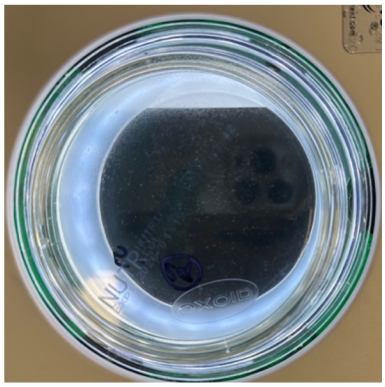


Figure 5-6 (b)

Figure 5-6 (c)

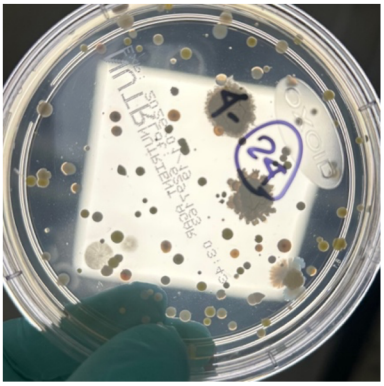


Figure 5-6 (d)

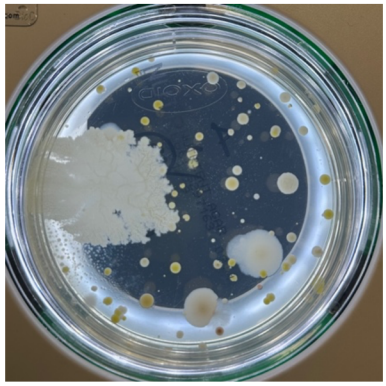


Figure 5-6 (e)

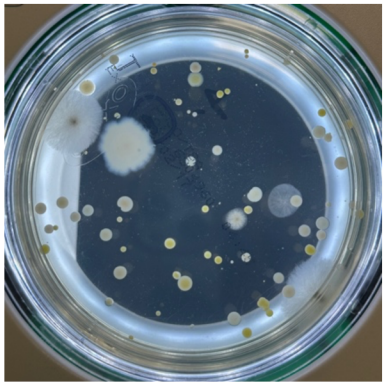


Figure 5-6 (f)

Figure 5-12.(a-f). Morphological diversity and growth patterns of microbial colonies on TSA and Nutrient Agar plates (Sampling 2)

In order to ensure consistency in colony enumeration and statistical analysis, microbial growth was differentiated based on morphological characteristics into bacterial and fungal (mould) colonies. Bacterial colonies were identified as small, circular, smooth-edged, and typically opaque or slightly pigmented formations. In contrast, mould colonies were distinguished by their larger size, filamentous or fuzzy appearance, irregular edges, and often radial growth patterns with aerial hyphae.

This distinction was essential for accurate counting, as mould colonies tend to expand rapidly and can obscure bacterial colonies, potentially leading to underestimation or overlap effects. Therefore, during enumeration, bacterial and fungal colonies were counted separately, and areas of confluent growth were carefully interpreted to minimize bias in the statistical evaluation.

5.4.2 Statistical Analysis

Statistical analysis was conducted using replicate plate counts obtained during Sampling 2 to evaluate the effects of agar type and exposure time on microbial contamination levels. Prior to statistical testing, assumptions of normality and homogeneity of variance were assessed.

5.4.2.1 Effect of Agar Type

Table 5-1. Statistical comparison of microbial counts obtained using TSA and Nutrient agar at different locations and exposure times.

Test	Location	Time	Test used	p-value	Significance	Interpretation
1	Corridor A	8 hr	Student's t-test	0.8601	No	No significant difference between agars
2	Corridor A	12 hr	Student's t-test	0.9772	No	No significant difference between agars
3	Corridor A	24 hr	Student's t-test	0.1908	No	No significant difference between agars
4	Corridor B	8 hr	Student's t-test	0.05465	No	No significant difference between agars
5	Corridor B	12 hr	Student's t-test	0.8977	No	No significant difference between agars
6	Corridor B	24 hr	Student's t-test	0.1965	No	No significant difference between agars
7	Student Lounge A	8 hr	Student's t-test	0.02077	Yes	Significant difference between agars
8	Student Lounge A	12 hr	Mann–Whitney U	0.1059	No	No significant difference between agars
9	Student Lounge A	24 hr	Mann–Whitney U	0.4596	No	No significant difference between agars
10	Student Lounge B	8 hr	Student's t-test	0.03455	Yes	Significant difference between agars
11	Student Lounge B	12 hr	Student's t-test	0.09071	No	No significant difference between agars
12	Student Lounge B	24 hr	Student's t-test	0.6857	No	No significant difference between agars
13	Student Lounge C	8 hr	Student's t-test	0.1209	No	No significant difference between agars
14	Student Lounge C	12 hr	Student's t-test	7.52e-05	Yes	Significant difference between agars
15	Student Lounge C	24 hr	Mann–Whitney U	0.02107	Yes	Significant difference between agars
16	Classroom	8 hr	Student's t-test	0.2778	No	No significant difference between agars
17	Classroom	12 hr	Mann–Whitney U	0.2059	No	No significant difference between agars

18	Classroom	24 hr	Mann–Whitney U	0.1692	No	No significant difference between agars
19	Lecture Hall	8 hr	Welch's t-test	0.9294	No	No significant difference between agars
20	Lecture Hall	12 hr	Student's t-test	0.0005672	Yes	Significant difference between agars
21	Lecture Hall	24 hr	Student's t-test	0.2255	No	No significant difference between agars
22	Toilet	8 hr	Student's t-test	6.08e-05	Yes	Significant difference between agars
23	Toilet	12 hr	Student's t-test	0.0002896	Yes	Significant difference between agars
24	Toilet	24 hr	Student's t-test	4.08e-05	Yes	Significant difference between agars

To evaluate whether agar type affected microbial recovery, pairwise comparisons between TSA and Nutrient agar were performed for each sampling location and exposure time. Depending on the data distribution and variance equality, Student's t-test, Welch's t-test, or Mann–Whitney U tests were applied.

Overall, most comparisons showed no statistically significant difference between the two agar types ($p > 0.05$). However, statistically significant differences were observed in several locations. Significant differences between agar types were detected in Student Lounge A (8 hr), Student Lounge B (8 hr), Student Lounge C (12 and 24 hr), Lecture Hall (12 hr), and all exposure times in the Toilet location (8, 12 and 24 hr).

These results suggest that although both agar types generally produced comparable results, TSA agar occasionally supported higher microbial recovery in environments with higher contamination levels, such as the toilet and some student lounge areas.

5.4.2.2 Effect of Exposure Time

Table 5-2. Statistical comparison of microbial concentrations across different exposure times

Test	Comparison Group	Assumptions	Statistical test	Overall p-value	Significance	Post-hoc significant comparisons*
2.1	TSA – Corridor A	Normal, unequal variance	Welch ANOVA	0.007897	Yes	8 vs 24hr, 12 vs 24hr
2.2	Nutrient–Corridor A	Normal, equal variance	ANOVA	2.55e-06	Yes	All pairs significant
2.3	TSA – Corridor B	Normal, equal variance	ANOVA	1.77e-08	Yes	All pairs significant

2.4	Nutrient– Corridor B	Normal, equal variance	ANOVA	0.00184	Yes	8hr vs 24hr, 12hr vs 24hr
2.5	TSA– Student Lounge A	Normal, unequal variance	Welch ANOVA	0.0005458	Yes	8hr vs 24hr, 12hr vs 24hr
2.6	Nutrient– Student Lounge A	Normal, equal variance	ANOVA	6.70e-09	Yes	All pairs significant
2.7	TSA– Student Lounge B	Normal, equal variance	ANOVA	3.02e-09	Yes	All pairs significant
2.8	Nutrient– Student Lounge B	Normal, equal variance	ANOVA	1.21e-06	Yes	All pairs significant
2.9	TSA – Student Lounge C	Normal, equal variance	ANOVA	1.54e-07	Yes	All pairs significant
2.11	Nutrient–Student Lounge C	Not normal	Kruskal-Wallis	0.005976	Yes	8hr vs 24hr
2.12	TSA – Classroom	Normal, equal variance	ANOVA	0.345	No	None
2.13	Nutrient – Classroom	Normal, equal variance	ANOVA	0.196	No	None
2.14	TSA – Lecture Hall	Normal, unequal variance	Welch ANOVA	3.16e-05	Yes	12hr vs 24hr
2.15	Nutrient – Lecture Hall	Normal, equal variance	ANOVA	2.45e-07	Yes	All pairs significant
2.16	TSA – Toilet	Normal, equal variance	ANOVA	1.12e-07	Yes	All pairs significant
2.17	Nutrient – Toilet	Normal, equal variance	ANOVA	1.29e-07	Yes	All pairs significant

*Note: *(column "post-hoc significant comparisons" includes only statistically significant pairwise differences ($p < 0.05$))*

To investigate the influence of exposure duration on microbial contamination measurements, comparisons among the three exposure periods (8, 1 and 24 hr) were performed for each agar type and location. Depending on assumption testing results, one-way ANOVA, Welch ANOVA, or Kruskal–Wallis tests with subsequent corresponding post-hoc test were applied.

Statistically significant differences between exposure times were observed in most sampling locations. In particular, the significant differences were detected in Corridor A, Corridor B, Student Lounge A, Student Lounge B, Student Lounge C, Lecture Hall, and Toilet environments for both TSA and Nutrient agar ($p < 0.05$).

Post-hoc comparisons indicated that microbial counts measured after 24 hours were generally significantly lower than those obtained after 8 or 12 hours. This trend suggests that prolonged exposure may lead to colony competition or overgrowth effects that reduce the number of individually countable colonies.

In contrast, no significant differences between exposure times were observed in the classroom locations, where microbial concentrations were consistently low.

5.4.2.3 Effect of Location

The effect of sampling location (Corridor A, Student Lounge C, and Toilet) on total microbial load (CFU) was evaluated across six independent tests (Tests 3.1–3.6).
All sampling locations were initially analyzed. However, these three locations were selected for detailed statistical comparison as they represent distinct levels of microbial contamination based on their mean CFU values: high (Toilet), moderate (Corridor A), and low (Student Lounge C). This selection allows for clearer interpretation of location-based differences in airborne microbial load.

Normality of residuals was assessed using the Shapiro–Wilk test. All datasets showed no significant deviation from normality ($p > 0.05$), indicating that the assumption of normality was met.

Table 5-3. Statistical comparison of microbial concentrations across different location

Test	Group	Assumptions	Statistical test	Overall p-value	Significant	Post-hoc significant comparisons*
3.1	TSA – 8hr	Normal, equal variance	ANOVA	2.61e-09	Yes	All pairs significant
3.2	TSA – 12hr	Normal, equal variance	ANOVA	2.25e-08	Yes	All pairs significant
3.3	TSA – 24hr	Normal, equal variance	ANOVA	8.02e-11	Yes	All pairs significant
3.4	Nutrient – 8hr	Normal, unequal variance	Welch ANOVA	3.39e-05	Yes	All pairs significant
3.5	Nutrient – 12hr	Normal, equal variance	ANOVA	5.44e-10	Yes	All pairs significant
3.6	Nutrient – 24hr	Normal, unequal variance	Welch ANOVA	<0.001	Yes	All pairs significant

*Note: *(column "post-hoc significant comparisons" includes only statistically significant pairwise differences ($p < 0.05$))*

Homogeneity of variances was evaluated using Bartlett's test. Variance homogeneity was satisfied in Tests 3.1, 3.2, 3.3, and 3.5 ($p > 0.05$), while unequal variances were

detected in Tests 3.4 and 3.6 ($p < 0.05$). Accordingly, one-way ANOVA was applied when assumptions were met, and Welch's ANOVA was used when variance homogeneity was violated.

A one-way ANOVA revealed a statistically significant effect of location on total CFU in Tests 3.1, 3.2, 3.3, and 3.5 ($p < 0.001$). Similarly, Welch's ANOVA indicated significant differences among locations in Tests 3.4 ($F = 230.99$, $p < 0.001$). In Test 3.6, extreme heterogeneity of variance resulted in instability of the Welch ANOVA output; however, subsequent post-hoc analysis remained valid.

Post-hoc comparisons were conducted using Tukey's HSD test for ANOVA and Games–Howell test for Welch's ANOVA. Across all tests, pairwise comparisons demonstrated statistically significant differences between all locations ($p < 0.05$).
Especially:

1. The Toilet consistently exhibited significantly higher CFU counts compared to both Corridor A and Student Lounge C,
2. The Student Lounge C showed significantly higher CFU than Corridor A.
3. The Corridor A consistently had the lowest microbial load.

6. Discussion

The main aim of the bachelor thesis was verification of the sedimentation technique as a valuable or non-valuable tool for assessment of microbial contamination of indoor air.
The transition from Sampling 1 to Sampling 2 was primarily driven, as I believe, by the need to mitigate experimental error caused by moisture accumulation. In Sampling 1, the high Standard Deviation (SD) and Coefficient of Variation (CV) were likely artifacts of spreader colonies. When plates are incubated right-side up, moisture condenses on the lid and drips onto the agar surface, causing motile bacteria or adjacent colonies to merge into a single, unquantifiable mass (Sanders, 2012).

By adopting an inverted incubation strategy in Sampling 2, the methodology aligned with international standard such as AOAC protocols, which mandate that Petri dishes be stored and incubated "bottoms up" to ensure that any condensation remains on the lid and away from the growing microbes (Sanders, 2012; USGS, 2006). From my point of view, this adjustment preserved the isolation of Colony Forming Units (CFUs), leading to more consistent and reproducible data.

6.1 Effect of Agar Type on Microbial Recovery

It is evident that the results demonstrate that **agar type did not significantly influence microbial recovery in most sampling locations and exposure times**, indicating that

both Tryptic Soy Agar (TSA) and Nutrient Agar (NA) are generally comparable for routine airborne microbial assessment. Therefore, the null hypothesis (H_0) was not rejected, indicating that agar type did not significantly influence microbial colony counts. However, the occurrence of statistically significant differences in specific environments, particularly student lounges and the toilet, suggests that agar composition plays a conditional role depending on environmental microbial load and diversity.

One explanation for the overall similarity between agar types, I think, lies in the inherent limitations of culture-based methods. It is widely recognized that only a small fraction of environmental microorganisms is cultivable under standard laboratory conditions, often less than 1–2% of the total microbial community (Tomazin et al., 2024). As a result, both TSA and NA capture only a limited portion of airborne microorganisms, which reduces observable differences between media, particularly in low-contamination environments such as corridors and classrooms.

Airborne microbial communities are also strongly influenced by environmental factors such as occupancy, emission sources, and particulate matter, which determine both abundance and diversity (Ruiz-Gil et al., 2020). In relatively clean environments, microbial loads tend to be low and dominated by easily culturable organisms, making, from my point of view, differences between media negligible. This explains the predominance of non-significant results across most locations in this study.

In contrast, significant differences observed in high-occupancy and high-contamination environments (e.g., toilet and student lounges) can be attributed to the more complex microbial composition in these settings. Such environments typically harbor a broader diversity of microorganisms, including fastidious and metabolically demanding species, which may require richer media for optimal growth. TSA, being a nutrient-rich medium, likely enhances the recovery of these organisms compared to NA. Previous studies have shown that culture media composition influences the detectable diversity of airborne microorganisms, particularly in complex environments (Tomazin et al., 2024).

Another important factor influencing microbial recovery is the efficiency of air sampling and post-collection viability. Microbial air samplers depend on both physical collection efficiency and biological recovery efficiency. It has been reported that air samplers may collect between approximately 22% and over 99% of airborne microorganisms, depending on design and operational conditions (Whyte et al., 2007). However, even when particles are successfully collected, cell damage caused by impaction and dehydration can reduce capturability, making media composition critical for recovery.

Furthermore, airborne microorganisms are subjected to environmental stress during aerosolization, including desiccation and oxidative stress, which can reduce their

ability to grow on standard media. Studies have demonstrated that aerosolization can significantly decrease cultivability while cells remain viable (Zhen et al., 2020). Under such conditions, nutrient-rich media such as TSA may better support the recovery of stressed or injured cells, explaining the significant differences observed in certain locations and exposure times.

The consistent significance observed in the toilet location across all exposure times further supports this interpretation. This environment likely contains higher concentrations of human-associated microorganisms and organic matter, resulting in increased microbial diversity and metabolic variability. In such conditions, the enhanced nutrient composition of TSA provides a more suitable growth environment, leading to higher detectable microbial counts compared to NA.

6.2 Influence of Exposure Time on Microbial Counts

Exposure time demonstrated a statistically significant effect ($p < 0.05$) across nearly all sampled environments, including corridors, student lounges, lecture halls, and toilets. Most tests (2.1–2.11, 2.14–2.17) showed significant differences between exposure durations, with post-hoc analyses frequently indicating either all pairwise differences significant or specifically between 8, 12 and 24hr exposures. Therefore, the alternative hypothesis (H_1) was confirmed, indicating that exposure time significantly influences microbial colony counts. This supports the principle that passive air sampling is inherently time-dependent, as longer exposure allows greater sedimentation of airborne microorganisms onto agar surfaces. Such findings align with previous studies, which describe settle plates as cumulative samplers that reflect microbial deposition over time rather than instantaneous airborne concentrations (Napoli et al., 2012).

In contrast, the classroom environment (Tests 2.12 and 2.13) showed no statistically significant differences ($p > 0.05$) between exposure times. This suggests that in relatively low-contamination or clean environments, increasing exposure duration may not significantly increase colony counts due to the limited availability of airborne microorganisms. Similar observations have been reported in controlled environments, where passive sampling becomes less sensitive at low microbial loads (Karigoudar et al., 2020).

Despite the overall trend of increased microbial recovery with longer exposure, the results indicate that 24hr exposure does not produce a proportional increase in colony counts compared to shorter durations. While many comparisons showed significant differences involving the 24-hour samples, the increase was not linear. This can be attributed to confluent growth, where colonies merge due to overcrowding on the agar surface, leading to underestimation of actual microbial numbers. Additionally, nutrient depletion and spatial competition limit further colony development over extended

exposure periods. Previous studies have shown that microbial recovery on agar surfaces is affected by factors such as surface density and competition, which can reduce observable colony counts (Stewart et al., 1995).

Furthermore, prolonged exposure introduces environmental stress factors, particularly desiccation of the agar medium. Extended exposure to air causes moisture loss, increasing osmotic stress and reducing the viability of sensitive microorganisms, especially those deposited early during sampling. Environmental conditions such as temperature and relative humidity further influence microbial survival, with unfavorable conditions reducing cultivability over time (Tang, 2009).

Overall, these findings confirm that while longer exposure times enhance microbial detection in most environments, extended durations such as 24 hours may introduce methodological limitations, including colony overlap, nutrient exhaustion, and microbial inactivation. Therefore, moderate exposure times (e.g., 8–12 hours) may provide a more accurate and reliable representation of airborne microbial contamination in indoor environments.

6.3 Spatial Variation of Microbial Contamination

The present results clearly indicate that sampling location was the most influential factor governing airborne microbial contamination, as all statistical tests (3.1–3.6) demonstrated highly significant differences ($p < 0.001$), with post-hoc analysis confirming that all pairwise comparisons were significant. Therefore, the alternative hypothesis (H_1) was confirmed, indicating that sampling location significantly influences microbial colony counts. This consistent pattern strongly supports the concept of spatial heterogeneity, where each indoor environment exhibits a distinct microbial signature. Such findings are well aligned with previous aerobiological research, which emphasizes that airborne microbial communities are highly variable and influenced by environmental conditions, emission sources, and human activity (Ruiz-Gil et al., 2020).

One of the primary drivers of this spatial variability is human occupancy and activity level. Indoor air is recognized as a major reservoir and transmission medium for microorganisms originating from occupants, surfaces, and environmental sources (Napoli et al., 2012). Areas such as corridors and student lounges, which experience continuous human movement and interaction, showed significantly higher microbial loads compared to classrooms. This can be explained by the process of activity-driven aerosolization, where microorganisms attached to skin cells, dust, and droplets are continuously resuspended into the air through walking, talking, and other movements. Previous studies have demonstrated that occupant density and movement are key determinants of airborne microbial concentrations, contributing to elevated bioaerosol levels in high-traffic environments (Ruiz-Gil et al., 2020).

In contrast, classrooms function as relatively controlled environments, often characterized by structured occupancy, lower activity intensity, and more stable ventilation conditions. These factors reduce the resuspension of particles and result in comparatively lower microbial counts. Environmental parameters such as ventilation rate and air exchange further modulate this effect, as improved airflow can dilute airborne microorganisms and reduce their accumulation. The importance of these parameters has been widely documented, with temperature, humidity, and ventilation identified as critical factors influencing airborne microbial survival and distribution (Tang, 2009).

A particularly notable finding in this study is the consistently high microbial load observed in the toilet environment, which was significantly different from all other locations. This can be attributed to the well-documented toilet plume or toilet turbulence effect, where flushing generates bioaerosols containing microorganisms that can remain suspended in air and later settle onto surfaces. In addition, toilets represent environments with high moisture content and organic matter, conditions that favor microbial survival and persistence. Anthropogenic sources such as waste handling and sanitation activities have been identified as major contributors to airborne microbial emissions in indoor environments (Ruiz-Gil et al., 2020). These combined factors explain why toilets act as microbial hotspots within built environments.

Another important aspect contributing to spatial variability is the influence of environmental stressors on microbial survival. Airborne microorganisms are subjected to environmental conditions such as temperature, relative humidity, and radiation, all of which can affect their viability during transport and deposition. Generally, moderate environmental conditions enhance microbial survival, whereas extreme temperatures or low humidity can reduce viability (Tang, 2009). However, indoor environments with stable microclimates—such as lounges or toilets—may provide conditions that prolong microbial persistence, thereby increasing measurable colony counts.

Additionally, methodological factors related to air sampling must be considered when interpreting spatial differences. The use of passive sampling (settle plates) reflects the sedimentation of particles over time, which is directly influenced by local air turbulence and particle concentration. Studies comparing active and passive methods have shown that passive sampling is particularly sensitive to environmental activity and can capture variations in microbial deposition across different locations (Napoli et al., 2012). Furthermore, the efficiency of microbial recovery can be affected by sampling stress, such as impaction forces, which may reduce cultivability and influence measured counts (Stewart et al., 1995). Despite these limitations, the consistency of significant differences across all tests suggests that the observed spatial patterns are robust and not merely methodological artifacts.

Overall, the findings confirm that indoor air microbial contamination is highly location-dependent, driven by a complex interaction of human activity, environmental conditions, and source strength. High-traffic and high-moisture environments act as hotspots, while controlled spaces exhibit lower contamination levels. These results are consistent with previous studies highlighting the importance of spatial variability in bioaerosol distribution and reinforce the need to consider location-specific factors when assessing indoor air quality and microbial exposure risks (Ruiz-Gil et al., 2020; Tang, 2009; Napoli et al., 2012).

6.4 Implications for Indoor Air Monitoring

The primary methodological limitation of this study lies in the use of passive (sedimentation) sampling, which inherently captures only larger bioaerosol particles that settle under gravity while underrepresenting smaller, respirable particles capable of remaining suspended for prolonged periods. As a result, this method does not fully characterize the total airborne microbial population but rather reflects only the fraction that deposits onto surfaces (Napoli et al., 2012). This gravitational dependency introduces a systematic bias, particularly in environments where fine aerosols dominate airborne transmission dynamics. In addition, under conditions of high microbial load, such as toilets and densely occupied lounges, the sedimentation technique is prone to coincidence error, where excessive particle deposition leads to overlapping colonies on agar surfaces. This masking effect reduces count accuracy and can significantly underestimate true microbial concentrations, especially when surface loading exceeds countable limits (Stewart et al., 1995).

Furthermore, environmental factors such as airflow patterns, ventilation efficiency, and indoor air mixing strongly influence particle settling behaviors, introducing additional variability into passive sampling outcomes. Air movement can either enhance or inhibit deposition rates, meaning that measured microbial loads may reflect airflow conditions as much as actual contamination levels. Variability in sampling height, as encountered in this study due to spatial constraints, may further affect deposition efficiency and compromise comparability between locations.

Despite these inherent limitations, the findings of this study provide important practical insights for optimizing the application of sedimentation-based monitoring. The results clearly support the standardization of exposure duration, identifying an 8–12 hr period as the most reliable sampling window. This duration represents a balance between sufficient particle deposition and minimal methodological distortion. Prolonged exposure (e.g., 24 hr) introduces additional biases associated with agar desiccation, nutrient depletion, and inter-colony competition, all of which can suppress microbial growth and reduce recovery efficiency. Such effects are well recognized in

culture-based sampling, where extended exposure can negatively impact microbial viability and distort colony enumeration (Stewart et al., 1995).

Overall, while passive sedimentation sampling remains a simple, cost-effective, and practical tool for routine monitoring, particularly for assessing surface contamination risk, it should be interpreted with caution. Its dependence on gravitational settling, susceptibility to environmental influences, and limitations in capturing fine aerosols highlight the need for standardized protocols and, where possible, complementary sampling approaches to achieve a more comprehensive assessment of indoor bioaerosol dynamics.

7. Conclusion

The bachelor thesis successfully verified the sedimentation technique as a valuable tool for assessment of microbial contamination of indoor air, taking into account impacts of exposure time and the media type. The primary finding was that the 8hr exposure window provided the most accurate and highest recovery of environmental microbes. Extending the exposure to 24 hr led to a paradoxical decrease in CFU recovery, likely due to agar desiccation and inter-colony competition on the agar surface, which inhibited the growth of later-settling microorganisms.

Both Tryptic Soy Agar (TSA) and Nutrient Agar (NA) proved effective for general monitoring. However, TSA showed superior recovery in high-load areas such as toilets and lounges, suggesting it is better suited for identifying diverse and nutritionally demanding microbial communities. The study successfully identified microbial hotspots (Toilet, Student Lounges) and clean zones (Classroom). These findings provide a clear baseline for targeted cleaning protocols and ventilation improvements in high-traffic areas. The transition from Sampling 1 to Sampling 2, specifically the implementation of inverted incubation; significantly improved data precision. This adjustment reduced moisture-related counting errors, leading to a lower Coefficient of Variation (CV) and more reliable CFU/m3.

To build upon these results and address the limitations of passive sampling, future studies should use active air sampling to compare passive sedimentation results with volumetric measurements. This would result in better capturing of smaller bioaerosol particles. Furthermore, expansion of monitoring of these locations across different seasons (summer vs. winter) is necessary to determine if environmental factors like temperature, humidity and HVAC usage, influence microbial load in indoor air throughout the year.

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Appendix

Table A.1. Raw Data of Microbial Colony Counts (CFU) Sampling 1

Agar	Location	Time	Total CFU 1	Total CFU 2	Total CFU 3	Total CFU 4
Unit		(hr)	CFU	CFU	CFU	CFU
TSA	Corridor A	8	1	2	3	30
TSA	Corridor A	12	10	24	13	27
TSA	Corridor A	24	12	41	44	28
Nutrient	Corridor A	8	2	3	5	5
Nutrient	Corridor A	12	8	7	6	7
Nutrient	Corridor A	24	21	17	18	17
TSA	Corridor B	8	2	3	5	8
TSA	Corridor B	12	300	9	77	12
TSA	Corridor B	24	40	300	92	43
Nutrient	Corridor B	8	4	3	6	2
Nutrient	Corridor B	12	16	300	300	7
Nutrient	Corridor B	24	29	86	300	64
TSA	Student lounge A	8	5	2	3	2
TSA	Student lounge A	12	300	7	9	300
TSA	Student lounge A	24	18	300	20	116
Nutrient	Student lounge A	8	6	3	4	4
Nutrient	Student lounge A	12	300	300	300	10
Nutrient	Student lounge A	24	19	300	123	41
TSA	Student lounge B	8	3	5	1	2
TSA	Student lounge B	12	300	300	300	300
TSA	Student lounge B	24	300	77	119	20
Nutrient	Student lounge B	8	2	3	4	2
Nutrient	Student lounge B	12	300	300	12	8

Nutrient	Student lounge B	24	19	20	22	300
TSA	Student lounge C	8	2	1	2	1
TSA	Student lounge C	12	2	2	3	1
TSA	Student lounge C	24	8	4	5	6
Nutrient	Student lounge C	8	1	2	1	3
Nutrient	Student lounge C	12	3	5	2	3
Nutrient	Student lounge C	24	6	8	5	4
TSA	Classroom	8	2	3	6	3
TSA	Classroom	12	300	7	300	300
TSA	Classroom	24	28	20	24	20
Nutrient	Classroom	8	5	3	2	3
Nutrient	Classroom	12	300	300	300	300
Nutrient	Classroom	24	25	21	34	18
TSA	Lecture Hall	8	3	0	1	3
TSA	Lecture Hall	12	1	5	3	3
TSA	Lecture Hall	24	14	9	9	21
Nutrient	Lecture Hall	8	1	0	1	0
Nutrient	Lecture Hall	12	3	3	1	4
Nutrient	Lecture Hall	24	7	17	15	9
TSA	Toilet	8	0	0	0	0
TSA	Toilet	12	3	2	4	2
TSA	Toilet	24	13	12	20	3
Nutrient	Toilet	8	0	1	1	1
Nutrient	Toilet	12	0	1	1	2
Nutrient	Toilet	24	10	10	9	8

Table A-2. Environmental Conditions during Sampling 1

Agar	**Location**	**Time**	**Temperature**	**Humidity**
Unit		(hr)	°C	%
TSA	Corridor A	8	23,2	46
TSA	Corridor A	12	22,9	47
TSA	Corridor A	24	23,6	46
Nutrient	Corridor A	8	23,2	46
Nutrient	Corridor A	12	22,9	47
Nutrient	Corridor A	24	23,6	46
TSA	Corridor B	8	23,2	48
TSA	Corridor B	12	22,7	47
TSA	Corridor B	24	23,3	45
Nutrient	Corridor B	8	23,2	48
Nutrient	Corridor B	12	22,7	47
Nutrient	Corridor B	24	23,3	45
TSA	Student lounge A	8	23,5	46
TSA	Student lounge A	12	23,5	47
TSA	Student lounge A	24	24,1	45
Nutrient	Student lounge A	8	23,5	46
Nutrient	Student lounge A	12	23,5	47
Nutrient	Student lounge A	24	24,1	45
TSA	Student lounge B	8	23,5	46
TSA	Student lounge B	12	23,5	46
TSA	Student lounge B	24	24,3	47
Nutrient	Student lounge B	8	23,5	46
Nutrient	Student lounge B	12	23,3	46
Nutrient	Student lounge B	24	24,3	47
TSA	Student lounge C	8	23,3	46
TSA	Student lounge C	12	23,5	45
TSA	Student lounge C	24	24,4	44
Nutrient	Student lounge C	8	23,3	46
Nutrient	Student lounge C	12	23,5	45
Nutrient	Student lounge C	24	24,4	44
TSA	Classroom	8	23,3	46
TSA	Classroom	12	23,5	45
TSA	Classroom	24	24,3	45
Nutrient	Classroom	8	23,3	46
Nutrient	Classroom	12	23,5	45
Nutrient	Classroom	24	24,3	45
TSA	Lecture Hall	8	23,5	47
TSA	Lecture Hall	12	23,4	46
TSA	Lecture Hall	24	24,6	45

Nutrient	Lecture Hall	8	23,5	47
Nutrient	Lecture Hall	12	23,4	46
Nutrient	Lecture Hall	24	24,6	45
TSA	Toilet	8	23,4	45
TSA	Toilet	12	23,3	46
TSA	Toilet	24	24,4	44
Nutrient	Toilet	8	23,4	45
Nutrient	Toilet	12	23,3	46
Nutrient	Toilet	24	24,4	44

Table A-3. Calculated Concentrations into CFU/m^3 and Mean, Standard Deviation and CV of Sampling 1

Agar	**Location**	**Time**	**Total CFU 1**	**Total CFU 2**	**Total CFU 3**	**Total CFU 4**	**Avg CFU per spot**	**Standard deviation**	**Coefficient of Variation**
Unit		(hr)	CFU/m^3	CFU/m^3	CFU/m^3	CFU/m^3			
TSA	Corridor A	8	2	4	6	55	17	25,74	156%
TSA	Corridor A	12	12	29	16	33	23	10,12	45%
TSA	Corridor A	24	7	25	27	17	19	8,93	47%
Nutrient	Corridor A	8	4	6	9	9	7	2,75	40%
Nutrient	Corridor A	12	10	9	7	9	9	1,00	12%
Nutrient	Corridor A	24	13	10	11	10	11	1,16	10%
TSA	Corridor B	8	4	6	9	15	8	4,86	59%
TSA	Corridor B	12	367	11	94	15	122	168,03	138%
TSA	Corridor B	24	24	184	56	26	73	75,36	104%
Nutrient	Corridor B	8	7	6	11	4	7	3,14	46%
Nutrient	Corridor B	12	20	367	367	9	191	203,89	107%
Nutrient	Corridor B	24	18	53	184	39	73	74,92	102%
TSA	Student lounge A	8	9	4	6	4	6	2,60	47%
TSA	Student lounge A	12	367	9	11	367	188	206,32	109%
TSA	Student lounge A	24	11	184	12	71	69	81,06	117%
Nutrient	Student lounge A	8	11	6	7	7	8	2,31	30%
Nutrient	Student lounge A	12	367	367	367	12	278	177,45	64%
Nutrient	Student lounge A	24	12	184	75	25	74	78,08	106%

TSA	Student lounge B	8	6	9	2	4	5	3,14	62%
TSA	Student lounge B	12	367	367	367	367	367	0,00	0%
TSA	Student lounge B	24	184	47	73	12	79	74,04	94%
Nutrient	Student lounge B	8	4	6	7	4	5	1,76	35%
Nutrient	Student lounge B	12	367	367	15	10	190	204,91	108%
Nutrient	Student lounge B	24	12	12	13	184	55	85,57	155%
TSA	Student lounge C	8	4	2	4	2	3	1,06	38%
TSA	Student lounge C	12	2	2	4	1	2	1,00	41%
TSA	Student lounge C	24	5	2	3	4	4	1,05	30%
Nutrient	Student lounge C	8	2	4	2	6	3	1,76	55%
Nutrient	Student lounge C	12	4	6	2	4	4	1,54	39%
Nutrient	Student lounge C	24	4	5	3	2	4	1,05	30%
TSA	Classroom	8	4	6	11	6	6	3,18	49%
TSA	Classroom	12	367	9	367	367	277	179,29	65%
TSA	Classroom	24	17	12	15	12	14	2,34	17%
Nutrient	Classroom	8	9	6	4	6	6	2,31	39%
Nutrient	Classroom	12	367	367	367	367	367	0,00	0%
Nutrient	Classroom	24	15	13	21	11	15	4,25	28%
TSA	Lecture Hall	8	6	0	2	6	3	2,75	86%
TSA	Lecture Hall	12	1	6	4	4	4	2,00	54%
TSA	Lecture Hall	24	9	6	6	13	8	3,47	43%
Nutrient	Lecture Hall	8	2	0	2	0	1	1,06	115%
Nutrient	Lecture Hall	12	4	4	1	5	3	1,54	46%
Nutrient	Lecture Hall	24	4	10	9	6	7	2,91	40%
TSA	Toilet	8	0	0	0	0	0	0,00	0%
TSA	Toilet	12	4	2	5	2	3	1,17	35%
TSA	Toilet	24	8	7	12	2	7	4,27	58%
Nutrient	Toilet	8	0	2	2	2	1	0,92	67%
Nutrient	Toilet	12	0	1	1	2	1	1,00	82%
Nutrient	Toilet	24	6	6	6	5	6	0,59	10%

Table A-4. Raw Data of Microbial Colony Counts (CFU) Sampling 2

Agar	**Location**	**Time**	**CFU 1 (CFU)**		**CFU 2(CFU)**		**CFU 3(CFU)**		**CFU 4(CFU)**	
Unit		(hr)	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
TSA	CorridorA	8	8	81	11	50	7	62	11	52
TSA	CorridorA	12	9	66	18	85	15	57	13	81
TSA	CorridorA	24	13	97	10	95	11	99	7	100
Nutrient	CorridorA	8	9	65	8	61	9	62	10	53
Nutrient	CorridorA	12	12	68	12	78	10	70	12	83
Nutrient	CorridorA	24	16	89	15	84	11	86	12	99
TSA	CorridorB	8	7	70	10	63	8	62	9	60
TSA	CorridorB	12	8	73	10	71	9	67	10	62
TSA	CorridorB	24	9	75	12	81	11	81	11	77
Nutrient	CorridorB	8	3	49	4	45	10	60	9	57
Nutrient	CorridorB	12	8	63	10	63	12	72	10	70
Nutrient	CorridorB	24	9	70	13	97	17	97	13	88
TSA	StudentLoungeA	8	7	59	7	49	7	60	6	68
TSA	StudentLoungeA	12	10	68	8	70	10	79	10	79
TSA	StudentLoungeA	24	11	95	12	93	10	89	15	90
Nutrient	StudentLoungeA	8	9	69	12	68	7	67	8	73
Nutrient	StudentLoungeA	12	10	66	9	63	10	62	12	69
Nutrient	StudentLoungeA	24	12	99	10	95	10	97	12	89
TSA	StudentLoungeB	8	6	69	9	70	7	70	10	69
TSA	StudentLoungeB	12	8	73	11	78	7	70	11	73
TSA	StudentLoungeB	24	15	89	10	92	11	89	12	95
Nutrient	StudentLoungeB	8	8	64	5	72	5	65	7	62
Nutrient	StudentLoungeB	12	7	73	9	89	11	84	9	90
Nutrient	StudentLoungeB	24	9	88	10	97	11	95	11	97
TSA	StudentLoungeC	8	6	31	5	37	6	35	6	32
TSA	StudentLoungeC	12	9	40	8	41	5	39	9	45
TSA	StudentLoungeC	24	10	20	9	15	7	28	12	29
Nutrient	StudentLoungeC	8	4	36	5	38	7	36	7	35
Nutrient	StudentLoungeC	12	4	22	3	24	1	19	3	22
Nutrient	StudentLoungeC	24	0	0	0	0	0	0	0	0
TSA	Classroom	8	0	2	0	1	1	2	1	0
TSA	Classroom	12	0	1	1	0	0	1	1	2
TSA	Classroom	24	2	4	2	2	1	3	2	2
Nutrient	Classroom	8	0	1	0	2	0	0	0	1
Nutrient	Classroom	12	1	2	1	0	1	3	2	1
Nutrient	Classroom	24	1	5	2	4	3	2	3	2
TSA	LectureHall	8	2	36	1	27	4	23	4	29
TSA	LectureHall	12	5	48	6	43	6	44	8	43
TSA	LectureHall	24	7	54	8	56	8	55	10	58

Nutrient	LectureHall	8	5	28	6	25	7	24	6	24
Nutrient	LectureHall	12	10	47	10	49	12	49	11	47
Nutrient	LectureHall	24	14	50	17	55	16	58	13	50
TSA	Toilet	8	25	177	29	179	24	166	30	180
TSA	Toilet	12	37	199	40	218	30	189	39	216
TSA	Toilet	24	44	223	49	230	40	220	45	245
Nutrient	Toilet	8	21	126	20	110	21	125	19	112
Nutrient	Toilet	12	30	140	32	129	27	137	37	141
Nutrient	Toilet	24	32	157	37	142	32	149	40	160

Table A-5. Environmental Conditions during Sampling 2

Agar	**Location**	**Time**	**Temperature**	**Humidity**
Unit	Location	(hr)	°C	%
TSA	CorridorA	8	21,1	29
TSA	CorridorA	12	21,2	29
TSA	CorridorA	24	22,3	23
Nutrient	CorridorA	8	21,1	29
Nutrient	CorridorA	12	21,2	29
Nutrient	CorridorA	24	22,3	23
TSA	CorridorB	8	20,8	27
TSA	CorridorB	12	20,8	24
TSA	CorridorB	24	21,6	25
Nutrient	CorridorB	8	20,8	27
Nutrient	CorridorB	12	20,8	24
Nutrient	CorridorB	24	21,6	25
TSA	StudentLoungeA	8	21,3	30
TSA	StudentLoungeA	12	20,5	26
TSA	StudentLoungeA	24	20,3	25
Nutrient	StudentLoungeA	8	21,3	30
Nutrient	StudentLoungeA	12	20,5	26
Nutrient	StudentLoungeA	24	20,3	25
TSA	StudentLoungeB	8	21,3	30
TSA	StudentLoungeB	12	20,4	26
TSA	StudentLoungeB	24	21,8	29
Nutrient	StudentLoungeB	8	21,3	30
Nutrient	StudentLoungeB	12	20,4	26
Nutrient	StudentLoungeB	24	21,8	29
TSA	StudentLoungeC	8	24,1	25

TSA	StudentLoungeC	12	20,5	28
TSA	StudentLoungeC	24	22,1	22
Nutrient	StudentLoungeC	8	24,1	25
Nutrient	StudentLoungeC	12	20,5	28
Nutrient	StudentLoungeC	24	22,1	22
TSA	Classroom	8	21,1	29
TSA	Classroom	12	21,5	25
TSA	Classroom	24	22,7	26
Nutrient	Classroom	8	21,1	29
Nutrient	Classroom	12	21,5	25
Nutrient	Classroom	24	22,7	26
TSA	LectureHall	8	20,8	31
TSA	LectureHall	12	21,7	25
TSA	LectureHall	24	22,2	23
Nutrient	LectureHall	8	20,8	31
Nutrient	LectureHall	12	21,7	25
Nutrient	LectureHall	24	22,2	23
TSA	Toilet	8	21,3	32
TSA	Toilet	12	20,8	30
TSA	Toilet	24	21	30
Nutrient	Toilet	8	21,3	32
Nutrient	Toilet	12	20,8	30
Nutrient	Toilet	24	21	30

Table A.6.1. Calculated Concentrations into CFU/m^3 and Mean, Standard Deviation and Coefficient of Variant of Sampling 2

Agar	**Location**	**Time**	**Total CFU 1**	**Total CFU 2**	**Total CFU 3**	**Total CFU 4**	**Avg CFU (F)**	**Avg CFU (B)**
Unit		(hr)	CFU/m^3	CFU/m^3	CFU/m^3	CFU/m^3	CFU/m^3	CFU/m^3
TSA	CorridorA	8	163.4	112.0	126.7	115.6	17.0	112.4
TSA	CorridorA	12	91.8	126.1	88.1	115.0	16.8	88.4
TSA	CorridorA	24	67.3	64.2	67.3	65.5	6.3	59.8
Nutrient	CorridorA	8	135.8	126.7	130.3	115.6	16.5	110.6
Nutrient	CorridorA	12	97.9	110.1	97.9	116.3	14.1	91.5
Nutrient	CorridorA	24	64.2	60.6	59.4	67.9	8.3	54.8
TSA	CorridorB	8	141.3	134.0	128.5	126.7	15.6	117.0
TSA	CorridorB	12	99.1	99.1	93.0	88.1	11.3	83.5
TSA	CorridorB	24	51.4	56.9	56.3	53.8	6.6	48.0
Nutrient	CorridorB	8	95.5	89.9	128.5	121.2	11.9	96.8
Nutrient	CorridorB	12	86.9	89.3	102.8	97.9	12.2	82.0

Nutrient	CorridorB	24	48.3	67.3	69.8	61.8	8.0	53.8
TSA	StudentLoungeA	8	121.2	102.8	123.0	135.8	12.4	108.3
TSA	StudentLoungeA	12	95.5	95.5	108.9	108.9	11.6	90.6
TSA	StudentLoungeA	24	64.9	64.2	60.6	64.2	7.3	56.1
Nutrient	StudentLoungeA	8	143.2	146.9	135.8	148.7	16.5	127.1
Nutrient	StudentLoungeA	12	93.0	88.1	88.1	99.1	12.5	79.5
Nutrient	StudentLoungeA	24	67.9	64.2	65.5	61.8	6.7	58.1
TSA	StudentLoungeB	8	137.7	145.0	141.3	145.0	14.7	127.6
TSA	StudentLoungeB	12	99.1	108.9	94.2	102.8	11.3	89.9
TSA	StudentLoungeB	24	63.6	62.4	61.2	65.5	7.3	55.8
Nutrient	StudentLoungeB	8	132.2	141.3	128.5	126.7	11.5	120.7
Nutrient	StudentLoungeB	12	97.9	119.9	116.3	121.2	11.0	102.8
Nutrient	StudentLoungeB	24	59.4	65.5	64.9	66.1	6.3	57.7
TSA	StudentLoungeC	8	67.9	77.1	75.3	69.8	10.6	62.0
TSA	StudentLoungeC	12	60.0	60.0	53.8	66.1	9.5	50.5
TSA	StudentLoungeC	24	18.4	14.7	21.4	25.1	5.8	14.1
Nutrient	StudentLoungeC	8	73.4	78.9	78.9	77.1	10.6	66.5
Nutrient	StudentLoungeC	12	31.8	33.0	24.5	30.6	3.4	26.6
Nutrient	StudentLoungeC	24	0.0	0.0	0.0	0.0	0.0	0.0
TSA	Classroom	8	3.7	1.8	5.5	1.8	0.9	2.3
TSA	Classroom	12	1.2	1.2	1.2	3.7	0.6	1.2
TSA	Classroom	24	3.7	2.4	2.4	2.4	1.1	1.7
Nutrient	Classroom	8	1.8	3.7	0.0	1.8	0.0	1.8
Nutrient	Classroom	12	3.7	1.2	4.9	3.7	1.5	1.8
Nutrient	Classroom	24	3.7	3.7	3.1	3.1	1.4	2.0
TSA	LectureHall	8	69.8	51.4	49.6	60.6	5.0	52.8
TSA	LectureHall	12	64.9	60.0	61.2	62.4	7.6	54.5
TSA	LectureHall	24	37.3	39.2	38.5	41.6	5.0	34.1
Nutrient	LectureHall	8	60.6	56.9	56.9	55.1	11.0	46.4
Nutrient	LectureHall	12	69.8	72.2	74.7	71.0	13.2	58.7
Nutrient	LectureHall	24	39.2	44.1	45.3	38.5	9.2	32.6
TSA	Toilet	8	370.8	381.8	348.8	385.5	49.6	322.2
TSA	Toilet	12	288.8	315.7	268.0	312.1	44.7	251.5
TSA	Toilet	24	163.4	170.7	159.1	177.5	27.2	140.4
Nutrient	Toilet	8	269.8	238.6	268.0	240.5	37.2	217.1
Nutrient	Toilet	12	208.0	197.0	200.7	217.8	38.5	167.4
Nutrient	Toilet	24	115.6	109.5	110.8	122.4	21.6	93.0

Table A.6.2. Calculated Concentrations Mean, Standard Deviation and Coefficient of Variant of Sampling 2

Agar	**Location**	**Time**	**Avg CFU(Total)**	**Standard deviation**	**CV**
Unit		(hr)	CFU.m^-3	CFU.m^-3	
TSA	CorridorA	8	129.4	23.5	18%
TSA	CorridorA	12	105.2	18.3	17%
TSA	CorridorA	24	66.1	1.5	2%
Nutrient	CorridorA	8	127.1	8.5	7%
Nutrient	CorridorA	12	105.6	9.2	9%
Nutrient	CorridorA	24	63.0	3.9	6%
TSA	CorridorB	8	132.6	6.6	5%
TSA	CorridorB	12	94.8	5.3	6%
TSA	CorridorB	24	54.6	2.5	5%
Nutrient	CorridorB	8	108.8	18.9	17%
Nutrient	CorridorB	12	94.2	7.4	8%
Nutrient	CorridorB	24	61.8	9.6	15%
TSA	StudentLoungeA	8	120.7	13.6	11%
TSA	StudentLoungeA	12	102.2	7.8	8%
TSA	StudentLoungeA	24	63.5	2.0	3%
Nutrient	StudentLoungeA	8	143.6	5.7	4%
Nutrient	StudentLoungeA	12	92.1	5.2	6%
Nutrient	StudentLoungeA	24	64.9	2.5	4%
TSA	StudentLoungeB	8	142.3	3.5	2%
TSA	StudentLoungeB	12	101.3	6.2	6%
TSA	StudentLoungeB	24	63.2	1.8	3%
Nutrient	StudentLoungeB	8	132.2	6.5	5%
Nutrient	StudentLoungeB	12	113.8	10.8	9%
Nutrient	StudentLoungeB	24	63.9	3.1	5%
TSA	StudentLoungeC	8	72.5	4.4	6%
TSA	StudentLoungeC	12	60.0	5.0	8%
TSA	StudentLoungeC	24	19.9	4.4	22%
Nutrient	StudentLoungeC	8	77.1	2.6	3%
Nutrient	StudentLoungeC	12	30.0	3.8	13%
Nutrient	StudentLoungeC	24	0.0	0.0	0%
TSA	Classroom	8	3.2	1.8	55%
TSA	Classroom	12	1.8	1.2	67%
TSA	Classroom	24	2.8	0.6	22%
Nutrient	Classroom	8	1.8	1.5	82%
Nutrient	Classroom	12	3.4	1.5	46%
Nutrient	Classroom	24	3.4	0.4	10%
TSA	LectureHall	8	57.8	9.3	16%
TSA	LectureHall	12	62.1	2.1	3%
TSA	LectureHall	24	39.2	1.8	5%

Nutrient	LectureHall	8	57.4	2.3	4%
Nutrient	LectureHall	12	71.9	2.1	3%
Nutrient	LectureHall	24	41.8	3.4	8%
TSA	Toilet	8	371.7	16.5	4%
TSA	Toilet	12	296.2	22.2	8%
TSA	Toilet	24	167.7	8.1	5%
Nutrient	Toilet	8	254.2	17.0	7%
Nutrient	Toilet	12	205.9	9.2	4%
Nutrient	Toilet	24	114.6	5.8	5%

Table A.7. Statistical Analysis Result of Sampling 2 (Test for Agar)

Test	Group	Statistical test	Test statistic	df	p-value	Significant
1.1	CorridorA – 8hr	t-test	t = -0.18391	6	0.8601	No
1.2	CorridorA – 12hr	t-test	t = 0.02981	6	0.9772	No
1.3	CorridorA – 24hr	t-test	t = -1.4743	6	0.1908	No
1.4	CorridorB – 8hr	t-test	t = -2.3815	6	0.05465	No
1.5	CorridorB – 12hr	t-test	t = -0.13415	6	0.8977	No
1.6	CorridorB – 24hr	t-test	t = 1.4529	6	0.1965	No
1.7	StudentLoungeA – 8hr	t-test	t = 3.1128	6	0.02077	Yes
1.8	StudentLoungeA – 12hr	Mann–Whitney U	W = 2	—	0.1059	No
1.9	StudentLoungeA – 24hr	Mann–Whitney U	W = 11	—	0.4596	No
1.1	StudentLoungeB – 8hr	t-test	t = -2.7219	6	0.03455	Yes
1.11	StudentLoungeB – 12hr	t-test	t = 2.0136	6	0.09071	No
1.12	StudentLoungeB – 24hr	t-test	t = 0.42496	6	0.6857	No
1.13	StudentLoungeC – 8hr	t-test	t = 1.806	6	0.1209	No
1.14	StudentLoungeC – 12hr	t-test	t = -9.5516	6	7.52E-05	Yes
1.15	StudentLoungeC – 24hr	Mann–Whitney U	W = 0	—	0.02107	Yes
1.16	Classroom – 8hr	t-test	t = -1.1933	6	0.2778	No
1.17	Classroom – 12hr	Mann–Whitney U	W = 12.5	—	0.2059	No
1.18	Classroom – 24hr	Mann–Whitney U	W = 13	—	0.1692	No

1.19	LectureHall – 8hr	Welch t-test	t = -0.09546	3.37	0.9294	No
1.2	LectureHall – 12hr	t-test	t = 6.631	6	0.0005672	Yes
1.21	LectureHall – 24hr	t-test	t = 1.3506	6	0.2255	No
1.22	Toilet – 8hr	t-test	t = -9.9141	6	6.08E-05	Yes
1.23	Toilet – 12hr	t-test	t = -7.5045	6	0.0002896	Yes
1.24	Toilet – 24hr	t-test	t = -10.632	6	4.08E-05	Yes

Table A.8. Statistical Analysis Result of Sampling 2 (Test for Time)

Test	**Group**	**Statistical test**	**Test statistic (df)**	**p-value**	**Significant**	**Post-hoc comparisons (8hr, 12hr, 24hr)**
2.1	TSA – CorridorA	Welch ANOVA	F (2, 4.04) = 20.15	0.007897	Yes	8hr vs 24hr; 12hr vs 24hr
2.2	Nutrient – CorridorA	ANOVA	F (2, 9) = 74.22	2.55E-06	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.3	TSA – CorridorB	ANOVA	F (2, 9) = 233.1	1.77E-08	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.4	Nutrient – CorridorB	ANOVA	F (2, 9) = 13.75	0.00184	Yes	8hr vs 24hr; 12hr vs 24hr
2.5	TSA – StudentLoungeA	Welch ANOVA	F (2, 4.31) = 68.09	0.0005458	Yes	8hr vs 24hr; 12hr vs 24hr
2.6	Nutrient – StudentLoungeA	ANOVA	F (2, 9) = 290.4	6.70E-09	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.7	TSA – StudentLoungeB	ANOVA	F (2, 9) = 347.6	3.02E-09	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.8	Nutrient – StudentLoungeB	ANOVA	F (2, 9) = 88.41	1.21E-06	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.9	TSA – StudentLoungeC	ANOVA	F (2, 9) = 142.4	1.54E-07	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.1	Nutrient – StudentLoungeC	Kruskal–Wallis	χ^2 (2) = 10.24	0.005976	Yes	8hr vs 24hr
2.11	TSA – Classroom	ANOVA	F (2, 9) = 1.20	0.345	No	None
2.12	Nutrient – Classroom	ANOVA	F (2, 9) = 1.97	0.196	No	None
2.13	TSA – LectureHall	Welch ANOVA	F (2, 5.37) = 124.82	3.16E-05	Yes	12hr vs 24hr
2.14	Nutrient – LectureHall	ANOVA	F (2, 9) = 128.1	2.45E-07	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr

2.15	TSA – Toilet	ANOVA	F (2, 9) = 153.3	1.12e-07	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr
2.16	Nutrient – Toilet	ANOVA	F (2, 9) = 148.3	1.29e-07	Yes	8hr vs 12hr; 8hr vs 24hr; 12hr vs 24hr

Table A.7.9 Statistical Analysis Result of Sampling 2 (Test for Location)

Test	**Locations**	**Statistical test**	**Test statistic (df)**	**p-value**	**Significant**	**Post-hoc comparisons (locations)**
3.1	CorridorA, StudentLoungeC, Toilet	ANOVA	F (2, 9) = 359.1	2.61e-09	Yes	CorridorA vs StudentLoungeC; CorridorA vs Toilet; StudentLoungeC vs Toilet
3.2	CorridorA, StudentLoungeC, Toilet	ANOVA	F (2, 9) = 220.9	2.25e-08	Yes	CorridorA vs StudentLoungeC; CorridorA vs Toilet; StudentLoungeC vs Toilet
3.3	CorridorA, StudentLoungeC, Toilet	ANOVA	F (2, 9) = 783.8	8.02e-11	Yes	CorridorA vs StudentLoungeC; CorridorA vs Toilet; StudentLoungeC vs Toilet
3.4	CorridorA, StudentLoungeC, Toilet	Welch ANOVA	F (2, 4.42) = 230.99	3.39e-05	Yes	CorridorA vs StudentLoungeC; CorridorA vs Toilet; StudentLoungeC vs Toilet
3.5	CorridorA, StudentLoungeC, Toilet	ANOVA	F (2, 9) = 510.6	5.44e-10	Yes	CorridorA vs StudentLoungeC; CorridorA vs Toilet; StudentLoungeC vs Toilet
3.6	CorridorA, StudentLoungeC, Toilet	Games–Howe				

Figure A.1 to A.6. Photos of sampling spots

A.1 Student Lounge B

A.2 Toilet

A.3 Corridor A

A.4 Lecture Hall

A.5 Classroom

A.6 Student Lounge A

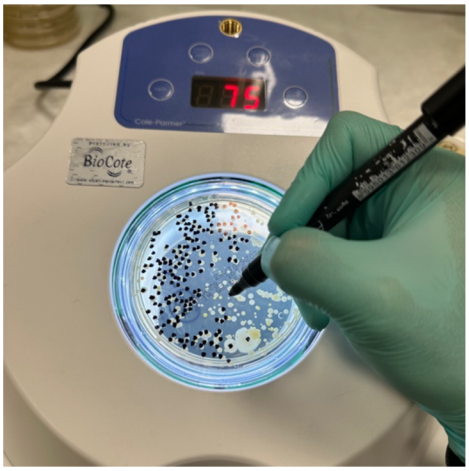

Figure A.7 colony counter

Figure A.8 Basket with Agar

Figure A.9 Digital Thermometer

Figure A.10 Laboratory Incubator

Figure A.11 and A12. Adjusting the cultivation techniques upside down in Sampling2

Figure A.11

Figure A.12