INDOOR-OUTDOOR RELATIONSHIP FOR AIRBORNE BACTERIA, FUNGI AND PARTICLES IN THE SELECTED AREA IN UPPER SILESIA, POLAND

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A MSc THESIS SUBMITTED TO DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY, INSTITUTE OF HEALTH SCIENCE, SCHOOL OF GRADUATE STUDIES OF JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF MASTER OF SCIENCE DEGREE IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY

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DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been fully acknowledged.

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ABSTRACT

Nowadays people spend their time more indoor than outdoor. Their activity makes them exposed to many environmental pollutants that affect their health. The microbes are normally present in both indoor and outdoor environments. Bioaerosols are associated with these dust events and can promote infectious diseases, allergy, asthma, and neurological diseases among vulnerable populations. The aim of this study was to investigate the total concentration of indoor-outdoor relationship of airborne bacteria and fungi, to find the indoor/outdoor ratio in the selected area of Upper Silesian, Poland. The study was conducted in Gliwice a typical city of upper Silesian, Poland. The measurement was done using Anderson six stage with aerodynamic diameter ranges for each stage is > 7µm, 7 - 4.7 µm, 4.7 - 3.3µm, 3.3-2.1µm, 2.1-1.1 µm, and 1.1- 0.65 µm with air flow latest 28.83 l/min for 10 minutes.

The highest median concentration value of bacterial measured in indoors of the locker room was (4802 CFU/m^3) and the lowest concentration level in the classroom was (2943 CFU/m^3). Comparing with the outdoor air (median: 261 CFU/m^3); the indoor air concentration was three times higher than the outdoor bacteria. The highest fungi levels concentration was (median: 491 CFU/m^3) in the corridor. The lowest fungi level concentration was observed in both locker room (median: 300 CFU/m^3) and in classroom (median: 276 CFU/m^3).

The indoor/outdoor ratios of bacterial and fungal concentrations was (1.2 min and 78.1 max) and (0.3 min and 1.6 max) in the primary school respectively. Size distribution bacterial aerosol distribution is unimodal. In indoor the peak appeared at stage 4 (2.1 – 3.3 µm) and 5(1.1 – 2.1 µm). Species of airborne gram-positive bacteria was the most abundant and it was 95.5%. Identified species are *Micrococcus spp, Staphylococcus spp, Bacillus spp, Corynebacterium spp, Pseudomonas spp.*

Keywords: indoor/outdoor ratio, Bioaerosols, size distribution,

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LIST OF ACRONYMS AND ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
API	Analytical Profile Index
CFU	Colony forming unit
Gem mean	Geometric mean
I/O	Indoor /Outdoor ratio
Max	Maximum
MEA	Malt extracted agar
Med	Median
Min	Minimum
SD	Standard deviation
S*pp*	Species
TSA	Tryptic soy agar
TSP	Total suspended particles

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CHAPTER ONE

1. BACKGROUND INTRODUCTION

Nowadays people spend about (70 - 90 %) of their time indoors: in houses, offices, schools, colleges, hospitals, and laboratory than outdoor. Their activity makes them exposed to many environmental pollutants that affect their health. Microbes are normally present in both indoor and outdoor environments (Gorny et al., 1999). The magnitude of microbes in a particular area depends upon the presence of water and other nutrient sources for their growth and physiological activities in that particular environment where they can develop widely. Therefore, microbes and particles move in into buildings over the doors, windows, air conditioners and also by people entering from outside (Roda et al., 2010).

Bioaerosol is as an artificially produced or naturally gathering of biological particles dispersed into the air or in another gaseous phase. The bioaerosol is a kind of aerosol having insignificant parts of biological origin or biologically active substances, which are in living organisms, and is responsible for cause reactions such as infections, allergies, toxic reactions and others (Gorny et al., 1999). Bioaerosol are a colloidal suspension, formed by droplets and particles of solid matters in the air whose components can contain or have attached to the virus, fungi spores, and conidia, bacterial endospores, plant pollen and fragments of plants tissues. Bioaerosols are associated with these dust events and can promote infectious diseases, allergy, asthma, and neurological diseases among vulnerable populations (Makut et al., 2014).

A number of studies have been conducted in a variety of environments to assess levels of bioaerosol and their by-products (e.g. endotoxin and 44 1, 3-β-d-glucan). Bacteria can grow fastly than those of slowest growing fungi. Even if the houses are more comfortable and clean residents houses off and the indoor concentrations of Aspergillus and Penicillium were usually higher than that of outdoor concentration (Yassin and Almouqatea, 2010). Bioaerosols are falsely produced or actually, arising particles of natural beginning suspended noticeable all around. Particles can exist in the airborne state as single cells or as clusters of microorganisms as little as 1 to 10 μm in size, and non-suitable bioaerosol particles cover a wide size range (Pastuszka et al, 2000).

In developing countries, population explosion along with widespread industrialization coupled with urbanization has resulted in dense urban centers with poor air quality. In addition to the poor ambient air quality, people in developing countries can be exposed to high concentrations of indoor air pollution. In recent years a number of studies have been undertaken to investigate the level of bioaerosol in indoor and outdoor. A study made by Hayleeyesus et al., (2015) in Jimma University, Ethiopia, bio-aerosols contamination in dormitory rooms was 511- 9960 CFU/m^3 for bacterial and 531- 6568 CFU/m^3 for fungi. This indicating that there are an excessive airborne bioaerosols contaminant, which is human possession generate a noticeable concentration enhance of bacterial pollution levels.

Other study was showed that bacteria and fungi in the indoor air of Jimma University libraries ranged between 367-2595 CFU/m^3. The average fungi density found in the indoor air of libraries did look to follow the same trend with bacterial density. Therefore this commonly associated with clinical manifestations of allergy, rhinitis, asthma, and conjunctivitis (Hayleeyesus and Manaye, 2014).

According to Zhu et al., (2003) experimental study of indoor and outdoor airborne bacterial concentrations in Tempe, Arizona, USA was shown the most governing bacteria which are overreaching up to 90% of them were Gram-positive bacteria from the measured population. The outdoor air is the main source of fungi commonly occurring in indoor environments and people spending their long stay in indoor have more contacts with the indoor fungi. Therefore the concentrations of fungal bioaerosols during rain were approximately seven times those in non-rainy days (Heo et al., 2014).

Because of some range on the climate, geography and ventilation system of the building normal fungus of an indoor environment more available than that of the local outdoor air. Outdoor fungi enter the indoor environments not only through open windows and doors but also through crashes in the structures and air conditioning system with blowing like other outdoor airborne particles. Fungi are also carried indoors on clothing and shoes of occupants, and on the fur of pets (Pastuszka, 2016).

The typical major of fungal spores in air, indoor and outdoor of domestic dwelling, most of the indoor/outdoor ratios were encompass to 1 and not dependent on the family of homes. Breathable fungal particles were found in higher levels than any others (between 79 to 98% of the entire). The concentrations observed, as well as the fungal species recognized in the separate houses, seem to be a threat to residents of all the groups of residence studied assumed that they are related to different health pathologies (Ponce and Lopez, 2011). The study made by Yassin and Almouqatea, (2010) on an assessment of the airborne bacteria and fungi in the indoor and outdoor was shown a significant difference between bacteria and fungi of indoor and outdoor media liabilities were 55% and 25% respectively. *Cladosporium* has the maximum median value of culturability. Microbial occurrences and indoor air quality in the houses of the more affluent residents' houses and the indoor concentrations of *Aspergillus* and *Penicillium* were largely higher than the outdoor concentration.

Aydogdu et al., (2010) found the result of indoor and outdoor airborne bacteria in child day-care centers in Turkey report of occupational exposure to bioaerosol contaminating high concentrations of bacteria and fungi. The instance at employment location of agricultural, composting and waste management, can precedence to respiratory diseases including allergies and infections. The bacterial and fungal concentrations both indoors and outdoors in Portugal indicates that the indoor/outdoor ratios for the observed fungi concentrations were around 1 and for bacteria concentrations, higher than 2 (Madureira, et al., 2015).

According to Rajasekar and Balasubramanin, (2011), the level of indoor airborne bacteria was powerfully affected by occupation levels whereas the indoor fungal concentration was dependent on relative moisture. As for the outdoor surrounding, there was an opposite relationship between temperature and airborne bacterial and fungal concentrations.

Study provided evidence that exposure to airborne bacteria and fungi increase with relation to the moisture of indoor and outdoor environments. A compressive study shows that gram-positive bacteria were dominated in the indoor air of the restaurants in which *Micrococcus* and *Bacillus species* were the most repeatedly founded bacteria species (Chan, et al., 2009). Research conducted by Sunar et al., (2015) stated that the mean concentration of indoor microbes at all complained situation is higher than that of their respective buildings outdoors. It is intimated that the microorganisms were imported from outdoors and encloses indoors due to zero air movement observed in a complained range. The majority of the indoor airborne bacteria and the fungal population are derived from outdoor sources (Mentese et al., 2009) and (Pastuszka et al., 2005).

Allergies and bronchial asthma are the main effects of bioaerosols on human health which is caused by bacteria, fungi or pollens. Separately from bioaerosols, there are other airborne dust particles acting as carriers of allergens, supporting in spreading and sustaining bioaerosols in the air (Jiřík et al; 2016). Airborne bacteria and fungi may be equally important in hospitals, in food factories and even in office, school buildings and other working environments. For example, high levels of airborne fungal spores in the workplace (shops) may have a significant negative effect on product shelf life, and airborne microorganisms may also be a contributory factor in incidents of so-called 'sick building syndrome' (Pastuszka et al., 2005).

1.1 STATEMENT OF THE PROBLEM

The attention in bioaerosol exposure has increased over the last few decades. The impact from bioaerosol exposure is increasingly significant in both the occupational and residential indoor environment because a biological agent causes wide range of adverse health effects with major public health impact, including contagious infectious diseases, acute toxic effects, allergies, and cancer. Indoor air pollution can be as much worse than that of outdoor air, it can cause a wide range of health problems. Mold, mildew, fungi, bacteria, viruses, microorganisms, chemical fumes, organic odors, dust pollen and other floating particles are potential threats in many households (Nevalainen, et al., 1992). Study conducted in the nursery school in Gliwice on bacterial aerosol in the indoor and outdoor environment specify that the indoor environment is more pollutant than that of outdoor. The level of culturable bacteria in indoor was around 3000 CFU/m^3 it was six to eight times higher than outside(Bragoszewska et al., 2016).

Certain human pathogens appear to be significant causes of infection from indoor air, infecting otherwise healthy individuals. On the other hand, airborne bacteria and fungi may be especially hazardous in clinics and hospitals where they may be the major factor in the increasing morbidity from respiratory diseases. Some bacteria such as *Streptococcus pyogenes, Neisseria meningitidis, Corynebacterium diphtheria* and *Mycobacterium tuberculosis* are known to be transmitted predominantly by airborne droplets from infected people, and they may cause nosocomial infection (Pastuszka, 2005). Exposure to bioaerosols should be controlled in such indoor environments like kindergartens and elementary schools.

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1.2 SIGNIFICANT OF THE STUDY

Prognosis of the adverse health effects due to the exposure to air pollutants indoors can be prepared using the dose-effect or dose-risk relationship. However, in the next step, when we want to reduce the health risk, it should be known which kinds of emission sources are the most important; these located indoors or outdoors. The ratio I/O describing the concentration level in the indoor versus the outdoor air seems to be very useful for this purpose. Unfortunately, the relation between the range of the I/O ratio and the contribution of specific pollutants outdoor-origin present indoors is still unclear. Besides, it is probably highly related to the local factors. To extend the knowledge in this field the aim of this study was to find the I/O ratio, size distribution and respirable ratio in the selected area of Upper Silesia. We believe that obtained results compared to the literature data could be also used in the classification of the healthy or non-healthy buildings, as well as in the summarized health impact assessment in the studied areas.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 GENERAL INTRODUCTION TO BIOAEROSOLS

Bioaerosols, meaning airborne particles derived from microbial, viral, and related agents, come in a wide variety of sizes, shapes, and classifications. Bioaerosols can cause two basic conditions: infections and allergies. Infections are generally the result of multiplication and growth of microbes inside humans while allergies are the result of exposures to antigens. Not all infectious organisms cause pathogenic diseases in humans, but those that can are of concern (Pastuszka, 2016).

Bioaerosols can exist in both viable (living) and nonviable states. Viable microorganisms such as bacteria, fungi, yeasts, and molds originate from sprays or splashes of media, from the agitations of dust, and from sneezes and coughs of which only the small particles (<10m) remain in the air long enough to travel any distance. Examples of nonviable agents that are occasionally sampled include pollens and insect parts. Grains, clusters of cells, and skin scales are many larger-sized particles than bacteria and viruses. Spores, which can be formed by fungi and certain bacteria, can be both viable and nonviable and are capable of causing disease in both forms (Pastuszka et al, 2000).

Cell fragments may be present in indoor and outdoor air and are also examine as bioaerosol; for example, airborne particles of rotten microbial, plant, and animal material; wood and grain dusts; the dung and dried body parts of arthropods; and particles of larger creature skin, saliva, faeces, and urine (Ruzer and Harley, 2005).

Bioaerosol levels and the characterization of both indoor and outdoor environments have grown a significant issue due to their unfavorable soundness effects. Exposure to bacteria and fungi, as well as their metabolites and fragments, can overspread infections, hypersensitive diseases and toxic reactions in humans (Hospodsky et al., 2012). Bioaerosols express, approximately, all biologically begin aerosols and can be found both indoors and outdoors. The most elaborate Bioaerosols are the airborne bacteria and fungi. Health impacts of certain fungi appearance, such as *Penicillium* species, *Aspergillus* species, *Mucor* species and *Rhizoptts* species, are generally related to allergy, infection, irritation, and toxicity (Mentese et al., 2009).

Common sources of bioaerosol include soil, water, vegetation, and living organisms, and the generation mechanisms include wind action, anthropogenic activities, active release processes, and several others. Various industrial processes have been shown to generate bioaerosol, for example, wastewater treatment, composting, and land application of biosolids (Pastuszka et al, 2000).

The composition of bioaerosol may vary depending on their source and generation mechanism, and range in complexity from a single biological substance or microorganism, to aggregates and mixtures of such, and even more complex composite particles containing the aforementioned constituents in combination with other biological and non-biological materials including mucous residues, skin flakes, and soil and dust particulates (Ruzer and Harley, 2004).

2.2 BACTERIA AND THEIR CHARACTERISTICS

Bacteria are ubiquitous prokaryotic single-cell organisms, involving an abundance of species. They can be found everywhere like in the dust and on the surfaces of every house. Bacteria are prokaryotic organisms which replicate asexually by cell division. Furthermore, bacteria generate endospores that are resisting to environmental stresses and that may be activated for duplicate under proper environmental circumstances (ICOP IAQ, 2010).

Bacteria may also remain in an inactive condition in order to ensure outliving. Bacteria can be categorized into distinct groups, such as gram negative and gram positive bacteria, depending on their ability to retain Gram stain, and into rod-shaped bacteria or spherical cocci based on morphological criteria. Bacterial cells are typical of the size range from 1 to a few micrometers. Water is life-threatening for bacterial growth and they require higher water activities than most of the fungi. The temperature and nutrient demands are generally met in most indoor environments (Pastuszka, 2016).

2.2.1 INDOOR BACTERIA AND THEIR SOURCE

The most frequently associated with airborne bacteria is Mycobacterium tuberculosis which is a move by droplet nuclei. The organisms such as *Staphylococcus aureus*, *Micrococcus* species, *alpha-hemolytic Streptococci* and *Gram-negative rods*, *Streptococcus pyrogens*, *Neisseria meningitides*, *Corynebacterium diphtheria* and *Mycobacterium tuberculosis* are understood to be transmitted principally by airborne droplets from contaminating people and they may cause nosocomial infection (Jaffal et al., 1997). As well as Staphylococcus is the most commonly occurring bacteria, followed by Micrococcus, which together contributed 58-78% of total bacteria concentration (Pastuszka et al., 2005).

The main sources of bacteria in the indoor environment are outdoor air, people, and indoor bacterial growth. Sources of airborne bacteria in the built environment are not well known but include humans, pets, soils, and plants, both as direct sources and indirectly from dust perturbations. Bacteria may happen in the air as single cells and as totals or attached to different particles (Pastuszka, 2016).

Usually, bacteria penetrate into buildings through the doors, windows, and air conditioners and also by people in going from outside. The type of species and amount of organisms present impend on the viscosity, temperature, lighting, and food available in that specific environment. Among the microorganisms present in the indoor environment, some species of microorganisms if present beyond the limit can cause serious health problems (Sheik et al., 2015). Another important origin of indoor airborne bacteria may be human oral and respiratory liquid discharge via coughing, sneezing, talking, and respiration or the direct shedding of skin-associated microbiota (Hospodsky et al., 2012). The majority of the indoor airborne bacteria and the fungal population are derived from outdoor sources (Bragoszewska et al., 2016).

2.2.2 OUTDOOR BACTERIA AND THEIR SOURCE

Natural outdoor bacterial aerosols come from vegetable, soil, and water. Bacteria are common on or in all of this origin. Every leaf is colonized with a population of bacteria that possibly is existence to the healthy life of the vegetable. Most bacteria are most likely discharge from leaf surfaces, and the fundamental mechanisms for release are possibly droplet splash and wind (Hospodsky et al., 2012).

Although as yet unmeasured, bacterial clouds released during rainfall are likely to be equivalent to the clouds produced by the fungi. Droplets descent into water causes bubbles that scavenge bacteria from the liquid and introduce the cells into the air when the bubbles burst (Pastuszka, 2016). Outdoor microbial concentrations exchange accordingly to the season and time of day, and these variations is also reflected in indoor air. The maximum concentrations are by and large detected in summer (Hospodsky et al., 2012).

2.2.3 BACTERIAL CONCENTRATION

The airborne bacteria to which community are open daily infrequently reason human illness, although some bacteria are agents of hypersensitivity, infectious, or inflammatory diseases. Endotoxin, a compositional of the outer membrane of Gram-negative bacteria, has been acknowledging as a health risk in various occupations and associated with asthma severity (Pastuszka, 2016).Airborne bacteria in the indoor surrounding are the confirmed or presumed generative agents of several infectious diseases, and their components are associated with the development and exacerbation of chronic respiratory illness including asthma (Hospodsky et al., 2012).

2.3 FUNGI AND CHARACTERISTICS OF FUNGI

Fungi are saprophytic or parasitic eukaryotic organisms which they are abundant in the human environment, most live in the soil on the decaying matter also they are common causes of damage to crops, foodstuffs, fabrics and building materials. Fungi (yeasts) help in making wine, beer, bread and for other purposes. The majority of fungi are obligate aerobes and can be grown in the laboratory on simple culture media. Fungal-related diseases can be divided into two types: mycosis and mycotoxicosis. Mycosis represents a variety of toxic effects, including dermatitis, hypersensitivity pneumonitis, and some systemic diseases that result from an infection by the organisms themselves. Mycotoxicosis is produced by metabolites of various fungi and causes diseases such as toxic aleukia and yellow rice disease (Mentese and Mentagel 2009).

Particular conformational components of fungal cells have been recognized as biologically active agents and are therefore interesting from the health point of view. Beta-(1->3)-D-glucans are systematically constituents of fungal cells and powerful agents contributing to the health consequence linked with fungal exposures. Another key thing to remember in the conformational ingredient of fungal cells, ergosterol, which is the common sterol for all fungi, it is interesting as a chemical marker in the assessment of entire fungal biomass, but its possible role in health hazard of fungal exposures is weakly understood (Pastuszka, 2016).

2.3.1 INDOOR FUNGI AND THEIR SOURCE

Not only bacteria but also airborne fungi influence by Climate change in their concentrations both outdoors and indoors. In cold climates, outdoor and indoor concentrations are typically low during the wintertime when there is snow overspread on the ground. Fungi may also originate from contamination in special construction structures, e.g. the crawl space. Fungi are distributed in the building due to an under-pressure inside the building source by mechanical ventilation, which is a normal situation in cold climates (Hospodsky et al., 2012). The common origin of indoor fungi is also place or areas in the construction structures where wetness can be collected for one reason or another (Pastuszka, 2016).

2.3.2 OUTDOOR FUNGI AND THEIR SOURCE

Important sources of environmental fungi are all natural environments that provide surface and substrate for their growth, which is organic material and enough moisture. The more vegetation, the more organic cycle of nutrients and the more fungi those do most of the decaying work of organic material (Pastuszka, 2016).

2.3.3 FUNGAL CONCENTRATION

Depending on the season the concentration of fungi is different, in cold climates when there is snow overspread on the ground and low outdoor concentrations, the indoor concentrations of fungi are often higher than outdoors pointing to the presence of indoor sources, Fungi that contain known allergens are, e.g., *Cladosporium, Aspergillus, Alternaria*, and *Fusarium species* (Hospodsky et al., 2012).

They have irritating potential but may also supply defensive performance against respiratory allergies likewise to bacterial endotoxin. Harmful not only to the material needs of humans but also to individuals. Fungal diseases are primarily infections or allergies, but they may also be caused by eating foodstuffs contaminated by fungal toxins (mycotoxins). Fungi may be

very common, but they do not cause widespread or dangerous epidemics and they rarely kill, However Fungi that contain known allergens are, e.g., *Cladosporium, Aspergillus, Alternaria*, and *Fusarium* species (Pastuszka, 2016).

2.4 AIRBORNE PARTICLES

Airborne particulate or particulate matter is very resembling to smoke in that it consists of small solids and/or liquids suspended in air; however, the origin of the suspended substances are not necessarily the rise of burning organic substances. Dust, sand, abraded material from tires and brakes, salt sprays, and even small water droplets like fog are some of the other constituents. PM is usually the terminology used from a regulatory compliance perspective and may be further subdivided into size related classifications such as PM10, PM2.5 (Jacobson and D.G, 2009).

When examine human exposures to airborne pollutants, especial subject is the exposure to airborne particles, and especially to its finer fractions, classified as ultrafine particles (often defined as smaller than 0.1μm), sub-micrometre particles (smaller than 1μm) or particular matter fraction (mass concentration of particles with aerodynamic diameter smaller than 2.5μm). Smaller particles have a higher chance of insight into the deeper parts of the respiratory tract and also enclose higher levels of trace elements and toxins, such as the polycyclic aromatic hydrocarbons and mutagens (ACGIH, 1984).

2.4.1 INDOOR AIRBORNE PARTICLES AND THEIR SOURCE

Particulate material in the indoor environment appears from a broad kind of origin, depending on the type of activities and processes taking place. Some activities such as sweeping and washing of floors have potential to produce dust through mechanical attrition of solid materials in the indoor environment. Not only the above mentioned, combustion of fuels such as biomass, cooking gas and liquid fuels (Kerosene) for domestic heating, cooking and

lighting purposes are examined as anthropogenic sources of indoor air pollution (Onabowale and Owoade, 2015).

In the indoor domestic environment, the origin of airborne particles includes the re-entrainment of existing particles through activities such as sweeping or dusting, indoor emissions from cooking or cigarette smoking, and transportation from outdoors by leakage through the walls, windows, doors and ventilation systems (Keuken et al., 2013).

2.4.2 OUTDOOR AIRBORNE PARTICLES AND THEIR SOURCE

The contribution of outdoor air to the amount of particulate matter concentration in domestic air depends, in addition to the particle fraction, in especial on the ventilation behaviour of the room user, the tightness of the construction envelope, the dust deposition rates indoors, and the resuspension effects in the room and the coagulation behaviour of the particles. The ventilation behavior itself is spontaneously dependent to an abundant extent on the season and the meteorology (Onabowale and Owoade, 2015).

2.4.3 AIRBORNE PARTICLES CONCENTRATION

Depending on their size distribution airborne particles has adverse health effects, for example, airborne ultrafine particles have been associated with adverse health sign, and more powerfully than coarser particles because of the efficiency of the former to penetrate deeper into the respiratory area and to translocate to other organs. As the same way Also, the ultrafine particle portion has a high course material content, particularly concerning health-relevant species with a high oxidative stress potentially (Viana et al., 2014). Experiencing to high levels of particulate matter is associated with cardiopulmonary and respiratory diseases and lower life (Jacobson and D.G, 2009).

2.5. ADVERSE HEALTH EFFECTS OF BIOAEROSOL

Most bacteria or bacterial agents are not very potent allergens, with the exception of the spore-forming actinomycetes described above. Bacterial cell wall components, such as endotoxin and peptidoglycans (most prevalent in Gram-positive bacteria), are agents with important pro-inflammatory properties that may induce respiratory symptoms. The effects of peptidoglycans are assumed to be very similar to those observed with endotoxin exposure (Pastuszka, 2005).

In most situations, exposure occurs to complex mixtures of toxins and allergens (and chemicals) and a wide range of potential health effects have to be considered. Three major groups of diseases associated with bioaerosol exposure can be distinguished: 'infectious diseases', 'respiratory diseases' and 'cancer'. Infectious and Respiratory diseases are most common; however, valid incidence or prevalence data for most diseases caused by biological agents are lacking (Mentese and Mentagel 2009).

The major part of research that links outdoor biological particles to health are allergological study connected with pollen and fungal allergens. Otherwise, health effects of exposures to airborne biological agents have mainly been studied in either occupational settings or in indoor environments. Most health effects connected with bioaerosol are various respiratory conditions and skin reactions. Mechanism infections, irritation symptoms and allergic diseases to toxic or immune toxic reactions and other conditions with less evident pathophysiology (Pastuszka, 2016).

Airborne endotoxins, lipopolysaccharide fragments of the cell of Gram-negative bacteria, produce acute toxic effects, bronchoconstriction, impaired lung function and, in the case of chronic exposure, airway remodeling (Jiřík et al., 2016).

Bacterial endotoxin is a well-known component of the outer membrane of gram-negative bacteria. It consists chemically of proteins, lipids, and lipopolysaccharide but the term "endotoxin" is often used to emphasize its immunotoxic properties. Bacterial endotoxins and glucans of fungi are normal constituents of bacterial and fungal cells and they have immunotoxic potential that is probably contributing to the health effects associated with exposures to biological particles and dust (Pastuszka, 2016). Another type of toxic compound linked with bacteria and fungi are microbial toxins that are produced as secondary metabolic products of these organisms. Toxins produced by fungi are called mycotoxins, but many species of both fungi and bacteria have the potential of producing highly bioactive and toxic secondary metabolites. Mycotoxins are fungal secondary metabolites that pose a potential health risk to humans and/or animals due to the unusual toxicity of many such metabolites (Pastuszka, 2016).

Health effects of particulate matter present in the air have been subject to many epidemiological and toxicological studies performed in the last 25 years, and are sufficiently well known. For particulate matter, inhalation is the only route of exposure that is of concern in relation to the direct effects of suspended particulate matter on human health.

Epidemiological studies have shown effects on total and respiratory mortality, both acute and long-term. Particulate matter has been found to be associated with hospital admissions, the prevalence of bronchodilator use, the prevalence of a cough, and acute lower respiratory symptoms. As effects have been observed down to background levels of particulate matter, the WHO provides guidelines in the form of dose-response relationship that makes it possible for individual regulatory bodies to select the appropriate level with which to protect the population (Pastuszka, 2016).

2.7 INDOOR-TO-OUTDOOR RATION (I/O) OF BIOAEROSOLS

Outdoor Air Quality influences the indoor air quality, means of penetration of air from outdoors to indoors, which mainly depends on the ventilation efficiency. The contribution of outdoor air to indoor air pollution depends on the concentration and composition of bioaerosols present in the outdoor air (Jiřík et al; 2016).

Some researchers showed that Even if the outdoor concentrations affect the indoor air. The indoor-to-outdoor (I/O) ratio will mostly be >1. This is due to several sources of indoor bioaerosols that contribute to the increase in indoor bioaerosol levels (Mentese and Mentagel 2009). Average I/O ratio for all indoor and outdoor fungi values for summer season was calculated as 0.60. Since the mean, I/O ratio for fungi is below 1.0 and the levels of fungi in indoor environments are relatively low in the summer that means there are no significant mold sources in these indoor environments (Mentese, et al., 2012).

CHAPTER THREE

3. OBJECTIVE OF THE STUDY

3.1 GENERAL OBJECTIVE

- To investigate the total concentration of indoor-outdoor relationship of airborne bacteria, fungi, and particles in the selected area in Upper Silesia, Poland

3.2 SPECIFIC OBJECTIVE

- To investigate the total concentration outdoor-indoor relationship of airborne bacteria and fungi
- To find the indoor/outdoor ratio of airborne bacteria and fungi in the selected area
- To analyze the size distribution of outdoor-indoor airborne fungi and bacteria
- To analyze respirable airborne bacteria and fungi

CHAPTER FOUR

4. METHOD AND MATERIALS

4.1 STUDY AREA

The study was conducted in Gliwice (50^{0}17’37.1’’N 18040’54.9’’E). Gliwice is a typical city in the industrial region of Upper Silesia, Poland (with 4.5 million people in the region) (Bragoszewska, et al. 2016). The measurement was carried out (from 17^{the} Marche to 02^{the} June 2017) in the laboratory of Politechnika Slaska University in the department of air pollution and Primary school the concentration level of airborne bacteria and fungi for the indoor-outdoor particle. A total suspended particle was done indoor and outdoor of the dormitory.

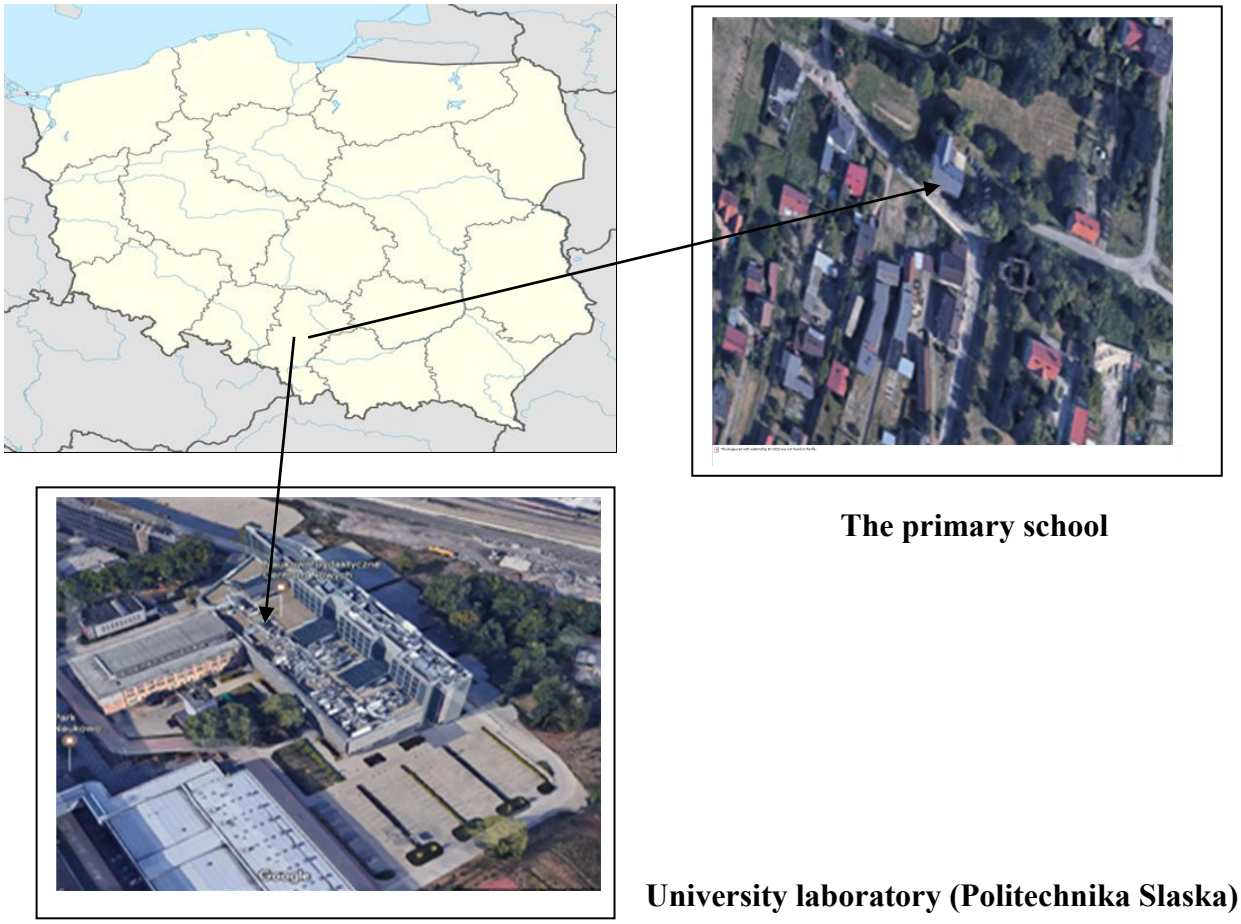


Figure 1. Locations of the sampling sites *(source: - https://www.google.pl/maps)*

4.2 STUDY DESIGN

A cross-sectional study was used to examine the indoor-outdoor relationship of airborne bacteria, fungi, and particles and assess the concentration of indoor-outdoor environments, including laboratories, and classrooms, corridors, gymnastic hall and locker room of primary schools in the Upper Silesia, Poland.

4.3 SAMPLING AND ANALYTICAL METHOD

Air samples were collected by six-stage Andersen cascade impactor which is the aerodynamic diameter ranges for each stage are stage 1 (> 7 µm), stage 2 (4.7-7 µm), stage 3 (3.3 – 4.7 µm), stage 4 (2.1-3.3 µm), stage 5 (1.1 - 2.1 µm) and stage 6 (0.65 – 1.1 µm). The air was a sample at constant a flow rate of 28.3 L/min for 10 minutes using a calibrated pump and impacted directly onto agar plates during the measurement. Anderson cascade impactor has a several-orifice, cascade impactor which is commonly used to measure the concentration and particle size distribution of aerobic bacteria and fungi in the ambient air. Air is drawn in through a circular orifice and then through a succession of six circular plates, each perforated with 400 hundred holes through the particles are onto the sterile medium in Petri dishes, with a diameter ranging from 1.8 mm in the first stage to 0.25mm in the sixth stage (Andersen, 1958).

Tryptic soy agar (TSA) was used for bacteria, with cycloheximide added to inhibit fungal growth. Duplicate sets of plates with each type of medium were taken at every sampling point, and the sample surface were disinfecting each time with a 70% ethanol solution. The Petri dishes was incubating 48 h at 36 ± 1^0C. Malt extract agar (MEA) was applied for fungi, which is chloramphenicol adds to inhibit bacterial growth and the petri dish was incubated at 26^0C for five days (Bragoszewska et al., 2016).

It is important to note that although direct measurement of the concentration of living airborne bacteria is extremely difficult, the commonly used substitute of the concentration of living microorganisms present in the air is the number of colonies forming units in the volume of air CFU/m^3. The next step was the identification of collected bacteria, which took place in two stages. The first stage involved an analysis of morphological and microscopic colonies of grown cells stained with Gram. In the second stage API, biochemical tests were carried out, which allowed the differentiation of the bacterial strains on the basis of their metabolic properties. Bacterial identification was based on morphology, Gram staining, and endospore formation. Bacteria were grouped as Gram-positive cocci, nonsporing Gram-positive rods, endospore-forming Gram-positive rods bacilli, and Gram-negative bacteria, according to their microscopic morphology (Bragoszewska et al., 2016).

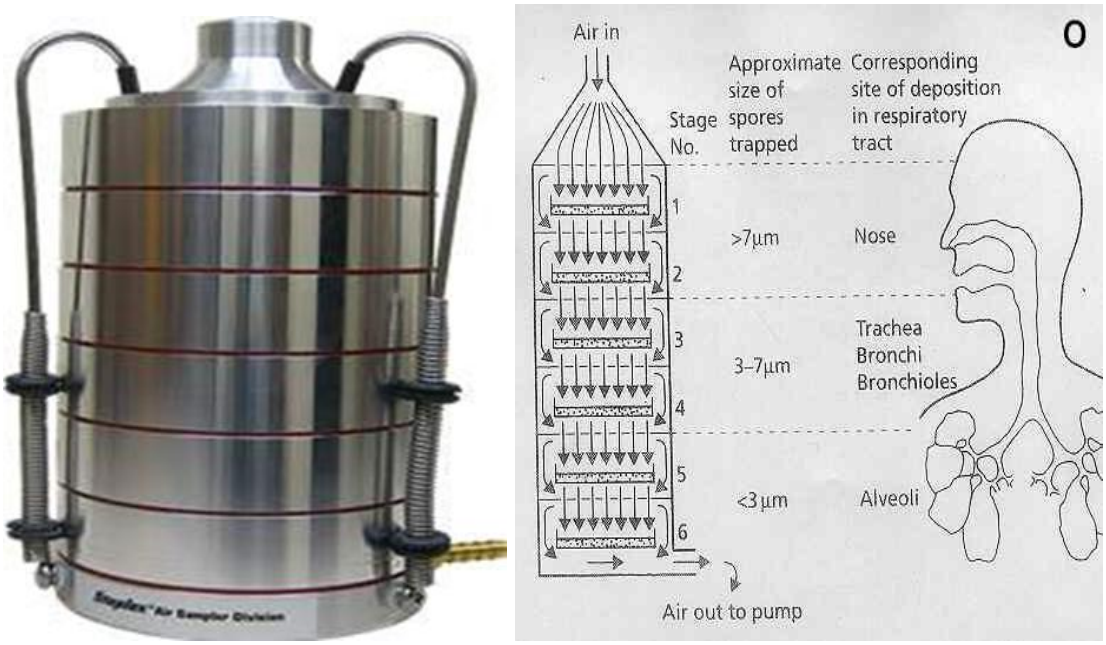


Figure 2. Six-stage Andersen Sampler

The second sampler is one stage impactor called air ideal operates at a sampling flow rate of 100 litters per minute and sampling time were 3 minutes. The advantage of using six-stage Andersen impactor is helped to get particle size distribution of the bioaerosol and very important in bioaerosol monitoring. Other is not time-consuming like that of the passive sampler.

Indoor/ outdoor Airborne Particle size and number concentration measurements were conducted using Universal Air Sampling Pump (Pump Models224-PCMTX4) and deluxe pump with I.O.M. sampler. Design flow rate is 2.0 l/min, giving a 50% sampling efficiency (cut point) at 100µm (micron) particle size (Bragoszewska et al., 2016 and ACGIH, 1984).

Figure 3. In the left is Air IDEAL sampler and in the right is Universal Air Sampling Pump

Figure 4. Data collection on the left in primary school and on the right in laboratory

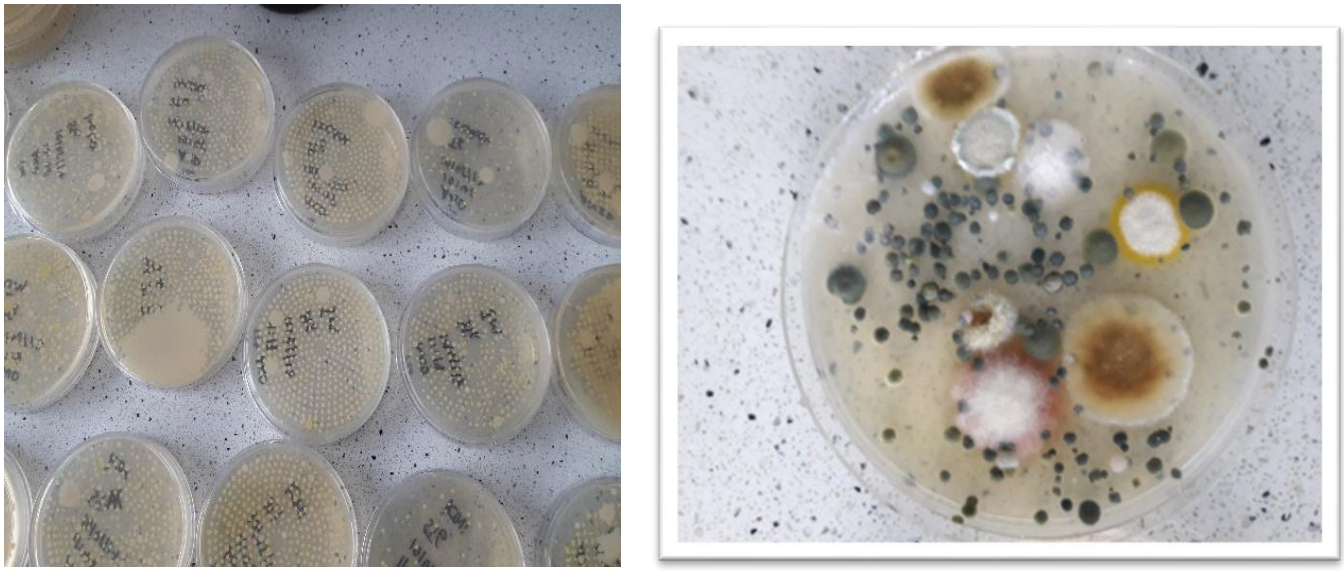


Figure 5. Number of colonies for both bacteria (left) and fungi (right)

Figure 6. Measuring the TSP in Laboratory (left) and collecting data outdoor for TSP (right)

Figure 7. Outdoor data collection

Colony forming unit calculation

Bioaerosol, such as bacteria and fungi, can grow and replicate to form a colony after being collected on a nutrient CFU or solid surface. Counts of these colonies are expressed as "colony-forming units," or CFU. For bioaerosol analyses, the term "CFU" is used to quantify numbers of viable microbes, with the concentration of bioaerosols defined as CFU per unit volume (CFU/m^3) of air sampled. During the sampling period t, the volume of sampled air can be calculated by the following equation:

V = Q * t, Where V is the volume of sampled air in m^3, Q is the flow rate of the sampling system in m^3 /min, and t is the sampling time in minutes. The average concentration of bioaerosols is determined by the following equation:

$\boldsymbol{C = \frac{N}{Q * t}}$, Where **C** is the average concentration of bioaerosol in CFU/m^3, **N** is the number of viable bioaerosol particles collected on the impaction substrate, in CFU (Nevalainen et al., 1992).

After sampling, the loaded filters were again desiccated and reweighed to determine the final weight. The concentration of the Total Suspended Particulate (TSP) in the air was determined from the difference in weight of the filter paper after and before sampling, the duration of sampling and the flow rate (Gokhale, 2009).

TSP (µg/m^3) = $\frac{Final\ weight\ (mg) - Initial\ weight\ (mg)}{Flow\ rate\ (m3/min) * sampling\ period\ (min)}$ * 100

4.4. QUALITY CONTROL

Quality control was practiced throughout the analyses to avoid any interference and minimize the risk of error. The bioaerosol analyses were continuously performed on the basis of the PN-EN12322 standard (2005), which recommends an adequate number of culture media from each series in order to test the microbial contamination. Sterility was ensured by incubating the culture medium at a temperature appropriate for the method used for at least 3 days (>72 h). The standard PN-EN 12322 does not specify how often the culture media must be controlled for sterility or the temperature to incubate them. Therefore, the sterility testing was based on another standard, ISO 11133 (2014). The sampling (Andersen Impactor) and laboratory (laminar flow cabinet, autoclave, incubators, and microscope) equipment are regularly checked and have current certificates.

4.5 STATISTICAL ANALYSIS

Statistical analyses were performed using statistical software Stata 12 for the size distribution of each stage of the sample was done for the bacterial and fungi concentration of indoor and outdoor environments. Calculate the arithmetic mean, geometric mean, maximum, minimum and I/O ratio for each sampling place with Microsoft Excel 2010.

CHAPTER FIVE

5. RESULTS

Airborne bacteria and fungi at the indoor and outdoor of the laboratory and primary school were sampled during the study. The results are summarized in Appendix 1 which shows the counted data from each sampling place. The daily concentration of total airborne bacteria and fungi were represented in figure (8) below. The concentration of bacterial and fungi presented in figure (8) shows the difference in the concentration of bacteria and fungi with day time difference (morning, mid-day and afternoon) in the indoor and outdoor of the laboratory.

It can be seen (figure 8) that bacterial concentration ranged between 120 and 535 CFU/m^3 with the peak value at outdoor in afternoon and the lowest was indoor air. At the same time fungi concentration ranged between 91 and 368 CFU/m^3 with the peak value at outdoor and lowest at indoor.

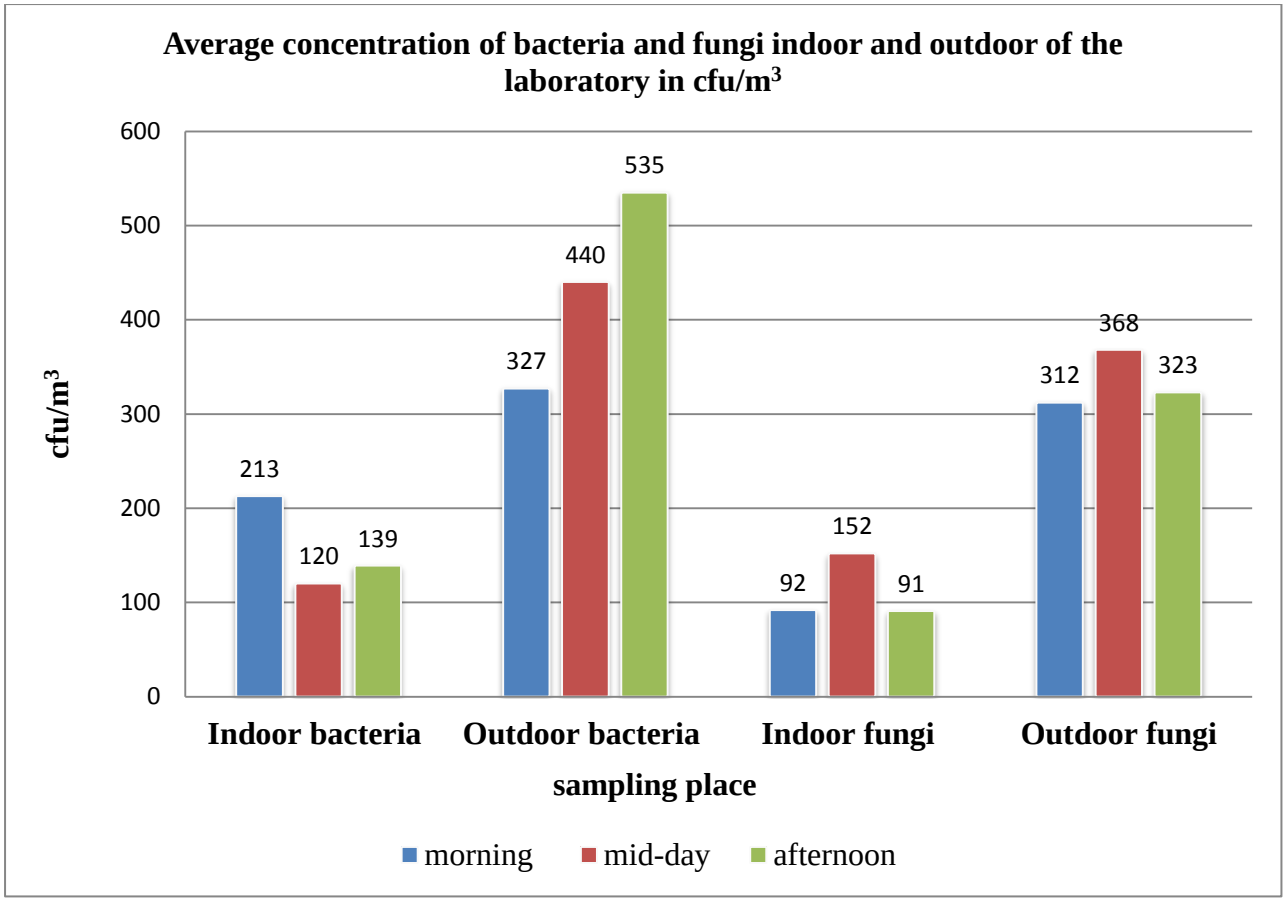


Figure 8. Average concentration of bacteria and fungi indoor and outdoor of the laboratory within a measurement day difference

The concentration of bacteria fluctuated throughout a day. The concentration indoors was higher in the morning but decline in afternoon and was lowest at the mid-day. In outdoor the airborne bacterial level was highest in the afternoon and decline in the mid-day being lowest in the morning. The indoor concentration of fungi was highest in the mid-day in the laboratory. In every case, the concentration indoors was the same like outdoors. However, the total concentration of both bacteria and fungi in the outdoor and indoor environment was high. Since the outdoor concentration in both bacteria and fungi is relatively very higher than the indoor.

5.1 THE TOTAL BACTERIAL AND FUNGI CONCENTRATION OF INDOOR AND OUTDOOR AIR LABORATORY

Bioaerosol levels were determined for both indoor and outdoor environments of the laboratory. Table 1**,** shows the averaged value of the total bacteria and fungi concentration (CFU/m^3) and the indoor/outdoor ratio (I/O) bacteria and fungi.

Table 1. Bacteria and fungi aerosol in indoor and outdoor of the laboratory.

		N	Min	Max	Mean	±SD	Median	Geom. mean
Bacteria	indoor	9	64	424	161	112.0	117	137
	outdoor	9	155	827	434	222.8	396	386
Fungi	indoor	9	21	371	112	102.3	92	85
	outdoor	9	113	615	334	181.7	371	281
I/O ratio	fungi	9	0.1	1	0.4	0.33	0.2	0.3
	bacteria	9	0.1	1	0.5	0.32	0.4	0.4

As shown in table 1. The total bacterial concentration level was varied on large scale in indoor and outdoor of the laboratory. The highest total bacterial concentration level was measured at the laboratory of outdoor (median: 396 CFU/m^3) and lowest level were measured in an indoor environment of the laboratory (median: 117 CFU/m^3). The concentration of total fungi as mentions in the table (1) was 21 to 371 CFU/m^3 in indoor and 113 to 615 CFU/m^3 at outdoor of the laboratory. The highest average fungi levels were (334 CFU/m^3) measured in the outdoor of the laboratory. The lowest average fungal level was (112 CFU/m^3).

5.1.1 THE INDOOR-TO-OUTDOOR (I/O) RATIO OF THE LABORATORY

Bioaerosol sampling is conducted indoor environment; outdoor bioaerosol sample should be taken for comparison of the indoor and outdoor strengths. The indoor /outdoor ratio (I/O) shows us where the source of bioaerosol exits. If this ratio is >1, there is a difference between outdoor and indoor bioaerosol sources although the source indicated in the indoor environment.

As Table (1) shows the I/O ratio for both bacteria and fungi counts. The indoor/outdoor ratio of bacteria and fungi in the laboratory, therefore, ranged from 0.1 to 1 and 0.1 to 1. The I/O ratio for fungi and bacteria counts varied from 0.1 to 1 and 0.1 to 1 respectively. The I/O ratio of bacteria and fungi at the laboratory, where below 1. Since the mean value, I/O ratio for fungi is around 0.4 and in the same way for bacteria, the mean is 0.5.

5.2 THE BACTERIAL AND FUNGI CONCENTRATION IN PRIMARY SCHOOL

Bioaerosol levels were determined for both indoor and outdoor environments of primary school. Table 2, shows the averaged value of the total bacteria and fungi concentration (CFU/m^3) and the indoor/outdoor ratio (I/O) bacteria and fungi.

Table 2. Bacteria and fungi aerosol in indoor and outdoor of primary school.

Bacteria		**Min**	**Max**	**Mean**	**±SD**	**Med**	**Gem mean**
Locker room		4138	11032	6657	3803.092	4802	6030
In corridor		1936	4389	3499	1358.187	4173	3285
In class room		2300	4071	3105	896.5	2943	3020
Gymnastic hall		2360	6070	4292	1859.747	4445	3993
out door		53	3343	1219	1842.376	261	359
Fungi							
Locker room		92	5018	1803	2785.925	300	517
In corridor		113	5173	1926	2818.617	491	660
In class room		230	3569	1358	1914.632	276	610
Gymnastic hall		233	5809	2132	3185.229	353	782
out door		258	6820	2497	3744.63	413	899
I/O ratio	***bacteria***	1.2	78.1	23.2	0.459036	17.2	10.9
	fungi	0.3	1.6	0.8	21.86576	0.7	0.7

5.2.1 THE BACTERIAL CONCENTRATION IN PRIMARY SCHOOL

The table (2) above shows the concentration of bacteria and fungi which collected from the primary school of indoor (in the locker room, in the corridor, in classroom and gymnastic hall) and outdoor of the school environments.

The result from primary school shows that concentration of bacteria and fungi are significantly different from indoor to the outdoor. The concentration of bacteria indoors was three times higher than outdoor concentration.

As can be seen in table (2) the concentration of bacteria depending on their sampling places was differ in locker room (4138 to 11032 CFU/m^3), in the corridor (1936 to 4389CFU/m^3),in the classroom (2300 to 4071CFU/m^3), the gymnastic hall (2360 to 6070 CFU/m^3) and in the outdoor was between 53 to 3343 CFU/m^3. The average of bacterial concentration in the indoor air was 6,657 CFU/m^3 in the locker room, 3,999 CFU/m^3 in the corridor, 3,105 CFU/m^3 in the classroom, 4,292 CFU/m^3 while in the gymnastic hall and in the outdoor was 1,219 CFU/m^3.

5.2.2 THE FUNGI CONCENTRATION AT PRIMARY SCHOOL

The concentration of total fungi as indicated in table (2) the average was from 1358 to 1803 CFU/m^3 in the indoor of primary school. The outdoor average concentration 2497 CFU/m^3, this means the outdoor concentration is higher than the indoor concentration. As seen in the table (2) the fungi level was varied on the large value within indoor sampling place. In locker room (92 to 5018 CFU/m^3), in the corridor (113 to 5173CFU/m^3), in the classroom (230 to 3569 CFU/m^3), the gymnastic hall (233 to 5809 CFU/m^3) and in the outdoor was between 258 to 6820 CFU/m^3.

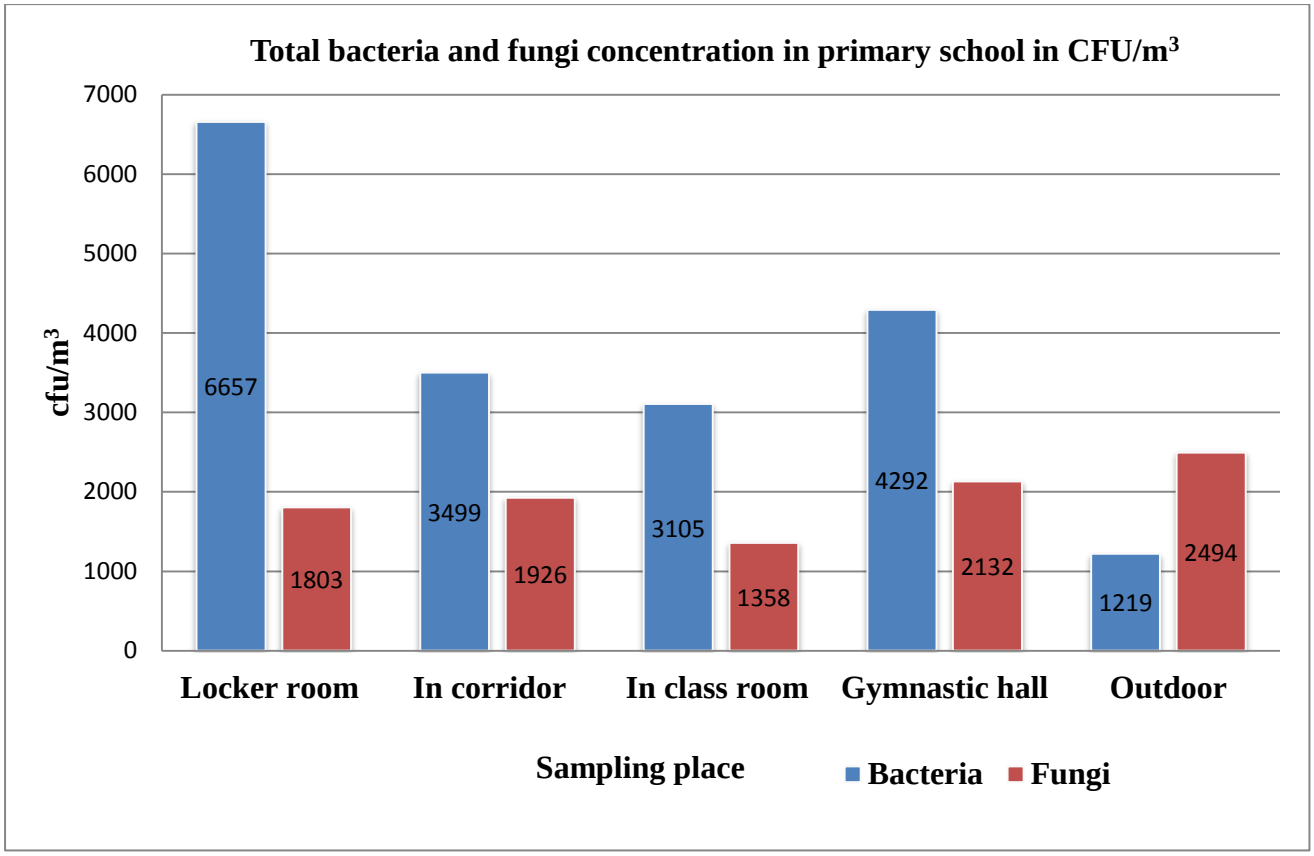


Figure 10. Average of total bacteria and fungi concentration of locker room, in the corridor, in the classroom, gymnastic hall and outdoor of primary school.

As shown from figure (10) the total bacterial concentration level compared to the total concentration of fungi; the level of total bacterial concentration is higher than the fungi concentration of indoor air while at the same time outdoor concentration level of fungi is higher than bacteria concentration.

5.2.3 THE INDOOR-TO-OUTDOOR (I/O) RATIO OF BACTERIA AND FUNGI

The indoor-outdoor (I/O) ratios of bacterial and fungal concentrations were range from 1.2 to 78.1 and 0.3 to 1.6 in the primary school respectively. Figure (11) shows the average I/O ratios for both bacteria and fungi counts, computing for each sampling place.

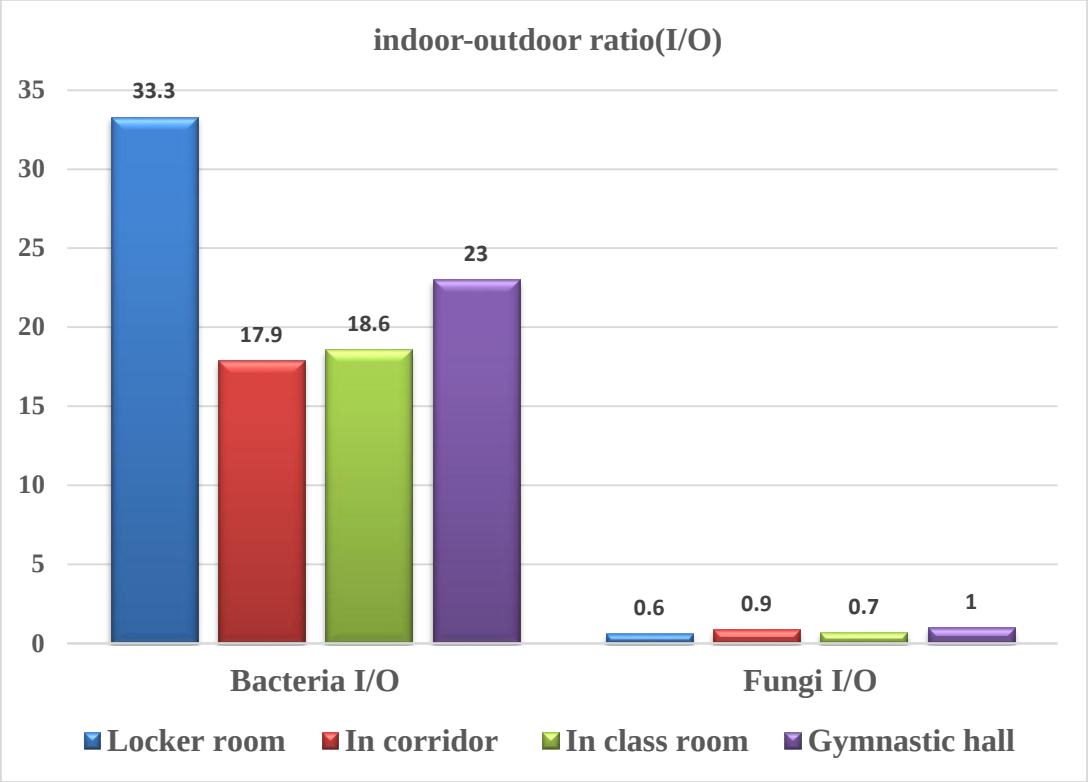


Figure 11. Average of indoor/outdoor ratio (I/O) for primary school

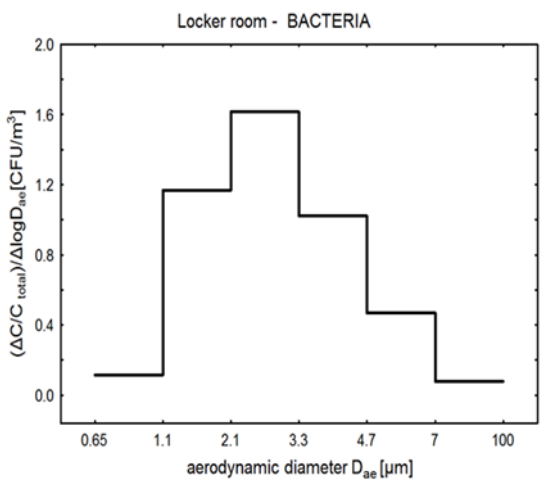
The I/O ratios for the bacteria counts were higher than 1, ranging between 17.9 and 33.3 in average. The highest I/O ratio for bacteria count was observed in the locker room (33.3) and gymnastic hall (23.0) in average. The I/O ratio for fungi counts varied 0.6 to 1.0 and these were not greater than one (>1) on average. The highest I/O ratio for fungi count was observed in the corridor (1.6) and gymnastic hall (1.4). The I/O ratio of the locker room and classroom; which were 0.6 to 0.7 on average respectively, were below 1.

5.3 SIZE DISTRIBUTION OF AIRBORNE BACTERIA AND FUNGI

The size distribution of airborne bacteria and fungi in six different fractions according to their aerodynamic diameter during the different sampling place (in locker room, corridor, in class, gymnastic hall and outdoor) in primary school are summarized below.

From size distribution, bacterial aerosol distribution is unimodal for all of the measurements in the atmosphere of indoor and outdoor environments. In the (indoor) locker room, in the corridor, and in the classroom, the peak appeared at stage 4; where airborne bacterial size was in the ranged of 2.1- 3.3 µm. In the gymnastic hall, the peak appeared at both the stage 4 and 5; where airborne bacterial size was in the range of 1.1 – 2.1 µm and 2.1 – 3.3 µrespectively.

At outdoor environment the peak appeared at stage 4; where airborne bacterial size was ranged of 2.1- 3.3 µm. The ratio of respirable concentration opposed to total concentration was 0.76 in the locker room, 0.86 in the corridor, 0.80 and in the classroom, 0.75 in the gymnastic hall and 0.56 at the outdoor environment.



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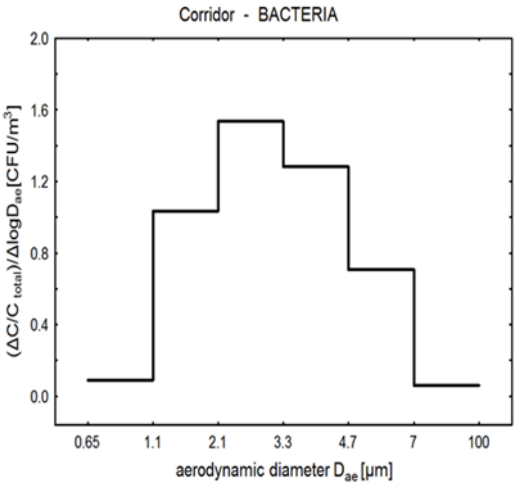

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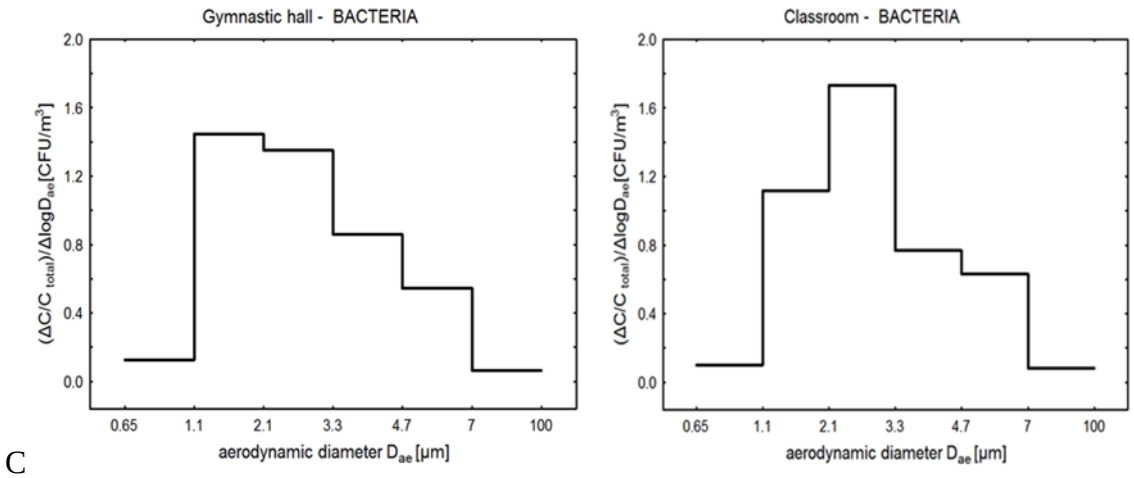

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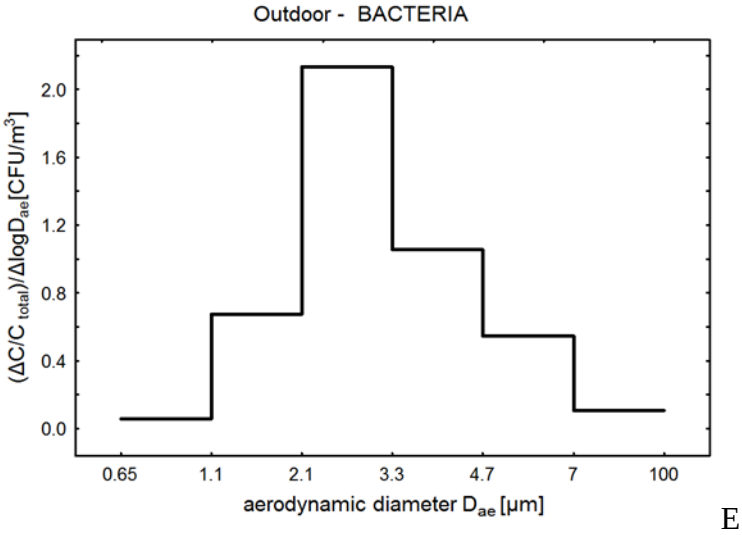
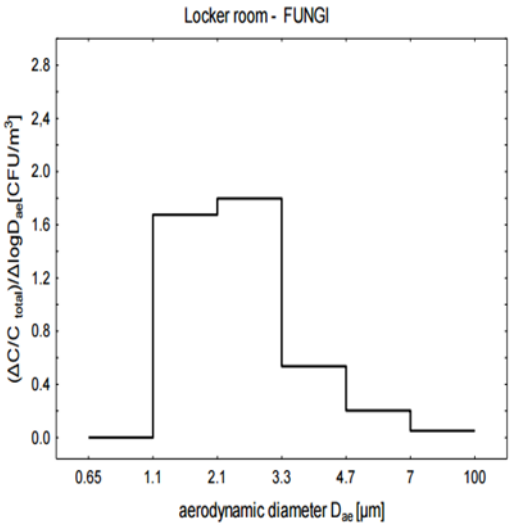


E

Figure 12. The size distribution of bacteria depending on their aerodynamic diameter in primary school (A) in the locker room,(B) in the corridor (C) in the classroom (D) in the gymnastic hall and (E) outdoor.

Fungi aerosol distribution is unimodal for all of the measurements in the atmosphere of indoor and outdoor environments. In the (indoor) locker room the peak appeared at both the stage 4 and 5; where airborne fungi size was in the range of 1.1 - 2.1 and 2.1 - 3.3 µm respectively.

In both corridor and classroom the peak appeared at stage 4; where airborne fungi size was in the range of 2.1 – 3.3 µm. At gymnastic hall the peak appeared at stage 5; where airborne fungi size was in the ranged of 1.1 - 2.1 µm. In the outdoor environment the peak appeared at stage 4; where airborne bacterial size was ranged of 2.1- 3.3 µm. The ratio of the respirable concentration of fungi opposed to total concentration was 0.79 in the locker room, 0.66 in the corridor, 0.85 and in the classroom, 0.73 in the gymnastic hall and 0.69 at the outdoor environment.



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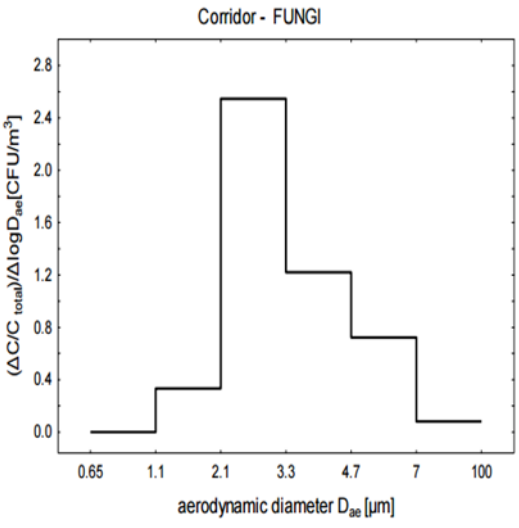

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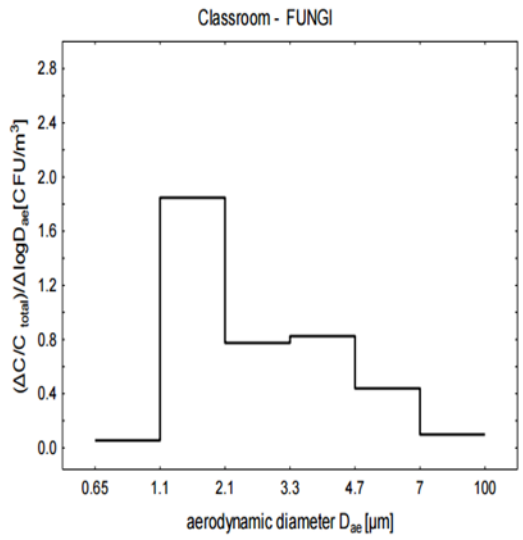

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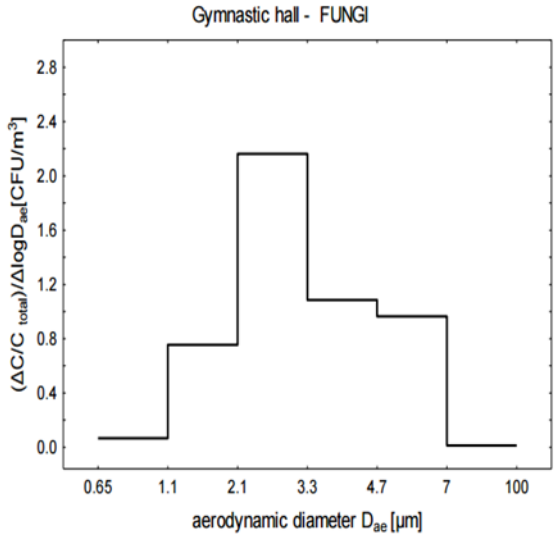

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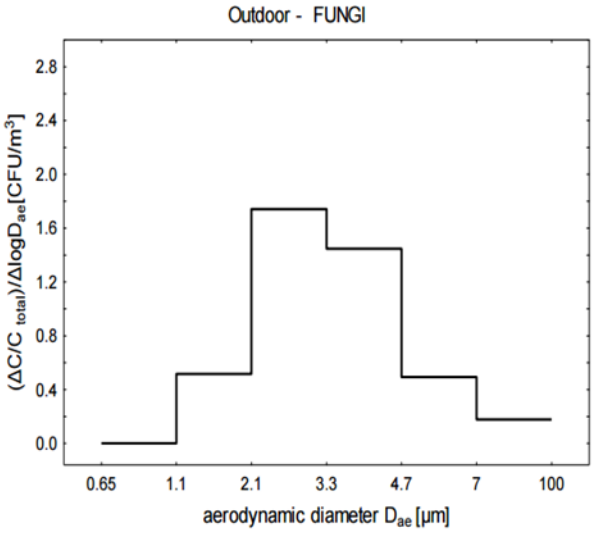

E

Figure 13. The size distribution of fungi depending on their aerodynamic diameter in primary school (A) in the locker room,(B) in the corridor (C) in the classroom (D) in the gymnastic hall and (E) outdoor.

5.4 SPECIES IDENTIFICATION

The observed bacteria are listed in both species and genera from indoor and outdoor. From this four group of bacteria were identified: gram-positive cocci, sporing gram-positive rods (family *Bacillaceae*), non-spring gram-positive rods and gram-negative rod.

Table 3. Most prevalent bacteria genera and species identified in the indoor of primary schools.

BACTERIA	Percentage of Genera/Species in Total Bacteria Concentration (%)
	Air Ideal
Gram-positive cocci, including:	**67**
Micrococcus sp.	26
Kocuria rosea	24
Staphylococcus lentus	12
Staphylococcus epidermidis	5
Sporing Gram-positive rods, family *Bacillaceae*, including:	**18**
Bacillus subtilis	11
Bacillus cereus	7
Non-sporing Gram-positive rods, including:	**15**
Corynebacterium auris	15

The observed bacteria species/genera according to the indoor sample *Micrococcus species, Staphylococcus, kocuria species, Bacillus species* and *Corynebacterium species* are the dominant species in the indoor environment in the table (3). The indoor environment of gram-positive cocci is ranked *Micrococcus species* (26%), *Kocuria rosea* (24%), *Staphylococcus lentus* (12%) and *Staphylococcus epidermidis* (5%). A spore-forming gram-positive rod (family *Bacilluacea*) including *Bacillus subtilis* (11%) and *Bacillus cerves* (7%). For non-sporing gram-positive rod are *Corynebacterium auris* (15%).

Table 4. Most prevalent bacteria genera and species identified in the outdoor of primary schools

BACTERIA	Percentage of Genera/Species in Total Bacteria Concentration (%)
	Air Ideal
Gram-positive cocci, including:	**29**
Micrococcus sp.	17
Kocuria rosea.	12
Sporing Gram-positive rods, family *Bacillaceae*, including:	**34**
Bacillus subtilis	22
Bacillus cereus	12
Nonsporing Gram-positive rods, including:	**28**
Corynebacterium auris	18
Corynebacterium striatum	10
Gram-negative rods, including:	**9**
Pseudomonas sp.	9

The outdoor airborne bacteria species observed are listed in the table (4) the dominant species are *Bacillus species, Corynebacterium* and *Micrococcus species*. For the outdoor environment of gram-positive cocci are ranked *Micrococcus species* (17%), *Kocuria rosea* (12%). Sporing gram-positive rods (family *Bacilluacea*) including *Bacillus subtilis* (22%) and *Bacillus cerevs* (12%). Non-aspirin gram-positive rods *Corynebacterium auris* (18%) and *Corynebacterium stratum* (10%). Gram-negative rods including *Pseudomonas species* (9%). Airborne Gram-positive bacteria were most abundant, with more than 95.5 % of the measured population in both indoor and outdoor while gram-negative bacteria about 4.5 % of total population.

5.5 AIRBORNE PARTICLES

Airborne particulate matter represents a complex mixture of organic and inorganic substances, covering a wide range of diameters, from <0.1 µm and up to some 100 µm. Mass and composition tend to divide into two principal groups: coarse particles larger than 2.5 µm in aerodynamic diameter, and fine particles smaller than 2.5 µm in aerodynamic diameter. In our studies inhalable particulate mass dust particles having a 50% cut-point of 100 µm. These dust particles are hazardous when deposited anywhere in the respiratory tract. Depending on our measurements we found the total suspended particles were 234.4 µg/m^3 in the indoor and 53.4 µg/m^3 at outdoor was obtained.

Table 5. Total suspended particulates

Sampling place	Particle mass concentration	Time
Indoor	234.4 µg/m^3	32h:56min
Outdoor	53.4 µg/m^3	30h:51min

CHAPTER SIX

6. DISCUSSION

From the average results the peak value of bacteria 213 CFU/m^3 and outdoor 535 CFU/m^3 and for fungi indoor 152 CFU/m^3 and outdoor 368 CFU/m^3. The mean concentration of total bacteria in indoor air was lower than the maximum exposure limit of 500 CFU/m^3. While the mean concentration of total fungi indoor and outdoor was slightly lower than the maximum exposure limit of 1000 CFU/m^3 (ICOP IAQ, 2010).

So from this, the maximum concentration of airborne bacteria indoor reached high at morning and outdoor at afternoon, while the maximum concentration of airborne fungi reached at mid-day for both indoor and outdoor. This result is supported by Zhu et al., (2003) conducted in Arizona, the USA according to his results the concentration of outdoor airborne bacteria were highest in morning, but declined in the afternoon and reached the lowest point in the evening.

Results from other study show that the average number of the highest bacterial concentration of air has been recorded in the laboratory (320 CFU/m^3) Sheik, *et al* (2015). A study that conducted by Mentese and Mentagel (2009) in Ankara Turkey in laboratory shows (median: 66 CFU/m^3) of bacteria and in office (median: 146 CFU/m^3) and the average is 307 CFU/m^3. Slightly lower than the maximum exposure limit of 1000 CFU/m^3 (ICOP IAQ, 2010).

The average lowest bacterial concentration of air has recorded in office rooms is (61 CFU/m^3) (Sheik, et al 2015). So our results agree with those literatures. The lowest fungi levels were (median: 112 CFU/m^3) observed in indoor of the laboratory. When it comparing to the results from college applied medical sciences by Sheik, et al (2015).

The highest average fungi concentration of air was (460 CFU/m^3) in the laboratory and the lowest average concentration of fungi was (146 CFU/m^3) in the laboratory. From this, we can understand that the average of our result (112 CFU/m^3) lower the maximum exposure limit of ICOP IAQ (2010).

The mean value of bacteria was 0.5 while the fungi 0.4, the room can be treated as a healthy building. For non-moldy homes in multi-story buildings during the summer in Poland, with a geometric mean concentration of 225 CFU/m^3 were reported by Pastuszka et al., (2016). Our result (53 to 11032 CFU/m^3) were exceeded from a study conducted in Poland urban nursery school by Bragoszewska, et al. (2016) total concentration level of bacterial aerosol concentration in the indoor was ranged from 2500 to 3000 CFU/m^3. This results supported by Pastuszka et al., (2000) it was found that its typical level of bacteria bioaerosols indoor is about 10^3cfu/m^3 in the indoor.

The higher concentration of bacterial level was measured in indoor of the locker room (median: 4802 CFU/m^3) and the lowest concentration level in the corridor (median: 2943 CFU/m^3). Comparing with the outdoor; the indoor total concentration is three times higher than outdoor bacteria. These results indicate the significant part of the indoor emission source and agree with data found in rural nursery Poland, the maximum level of viral-bacterial aerosol in indoor was 2600 CFU/m^3, means higher three times than outdoor (Bragoszewska, et al. 2016) and ICOP IAQ (2010) significantly higher than the maximum exposure limit 500 CFU/m^3.

Another study in child day care center in Portugal, show that the indoor microenvironment with the highest bacteria concentration (median: 3870 CFU/m^3), whereas the lowest concentration was (median: 222 CFU/m^3) Madureira, et al (2015).

Our results agree with a study conducted in Ankara Turkey, the bacteria level was measured in kindergarten (median: 1078 CFU/m3) (Mentese and Mentagel 2009). In the study conducted in Poland a topical bacteria level where found as 10^3 CFU/m^3 and in the dwelling and 10^2 CFU/m^3 in office (Pastuszka, 2000). The lowest fungi level was observed in both locker room (median: 300 CFU/m3) and in the classroom (median: 276 CFU/m^3). Such results also confirmed by Bragoszewska, et al., (2016) who found that the fungi levels were 1549 CFU/m^3 with indoor concentration which is lower than the outdoor concentration. According to Madureira, et al (2015), the study showed that the indoor fungal concentration (median: 415 CFU/m^3) and the lowest was (median: 180 CFU/m^3) in child day care center in Portugal.

In a study conducted in Portugal, in urban and rural primary schools using passive and active method average fungal level of both indoor and outdoor was 2248 and 2072 CFU/m^3 respectively (Canha, et al 2015). Makut, et al., (2014) show that the concentration of fungi in different location range from 4.71 x10^2 to 4.60 x 10^3 CFU/m^3 for this he used open plate sedimentation method. Other study conducted by Ponce, et al., (2011) in Mexico found that the indoor and outdoor fungi concentration ranges from 264 to 17788 CFU/m^3 and 123 to 5771 CFU/m^3 respectively. The highest fungi levels were measured in the outdoor of the environment (max: 6820 CFU/m^3). To support our results of in indoors rooms it is true that the concentration of bacteria differs in unlike location range from 2.8 x 10^3 to 6.4 x10^3cfu/m^3 (Makut et al., 2014).

The highest I/O ratio for bacteria count was observed in the locker room (33.3) and gymnastic hall (23.0) in average. The I/O ratios have been reported in the range of 1.63–141.73 for bacteria (Mentese et al. 2009). Since the I/O ratio for airborne bacteria was higher than 1 in the entire sampling site place, their major sources might be any activity done in such

environments: the presence of human and pets, movement of children, and dying room (Mentese et al., 2009).

Since the I/O ratio was >1 in this place, their major source might be any activity done in the corridor and depends on occupant's activity. The small influence of human activity on fungal levels also confirmed (Bragoszewska, et al. 2016). The I/O ratio of the locker room and classroom; were 0.6 to 0.7 on average respectively, were below 1. Since this means I/O ratio of fungi is around 1 and the level of fungi was relatively low or not molds room.

Our result is supported by Mentese et al. (2009), the I/O ratio of two sampling were cafeterias (0.73) and high school classrooms (0.96), were below 1.00 and the level of fungi was relatively low. Comparing the obtained average values of I/O ratios with the research of Bragoszewska, et al., (2016) there was no significant fungal source in the indoor environments. The bacterial and fungal concentrations both indoors and outdoors in Portugal indicates that the indoor/outdoor ratios for the observed fungi concentrations were around 1 and for bacteria concentrations, higher than 2 (Madureira, et al., 2015).

As particle size determines the fate of particles on or in the human body which those effects person exposure risk, it is necessary to understand the size distribution of aerosol particle. It is known information that the potential health risk caused by exposure to airborne bacteria and fungi also related to the concentrations of respirable airborne bacteria and fungi. The respirable ratio of bacteria and fungi is defined as the sum of the third stage (3.3–4.7 μm), fourth stage (2.1–3.3 μm), fifth stage (1.1–2.1 μm), and sixth stage (0.65–1.1 μm), with respect to the total concentration of bacteria or fungi (Bragoszewska, et al., 2016). Those small particles more prevalent indoor air, these respirable particles mostly can be deposit in either the trachea, bronchial, or alveolar region of the lungs (Pastuszka, et al., 2016 and Bragoszewska, et al., 2016).

According to Bragoszewska, et al., (2016) the indoor contamination with airborne bacteria can be suspected if the indoor respirable /total ratio is higher than the outdoor ratio. The large microorganisms (>3.3 µm) was lower than their smaller forms. Such distribution of the particles aerodynamic diameters indicates the additional emission from a human organism (the increased emission during breathing).

The indoor respirable concentration is more than the outdoor; this indicates that the respirable airborne bacterial concentration is highest in the indoor environment. This confirms that the studied bacterial aerosol indoors was dominated by fresh bacteria emitted from human organisms similar results were obtained (Bragoszewska, et al., 2016). Also in our study, an airborne fungus was 0.79 in the locker room, 0.66 in the corridor, 0.85 and in the classroom, 0.73 in the gymnastic hall and 0.69 at the outdoor environment. At the indoor, the larger bacteria appeared to settle on the ground, while the smaller bacteria remained suspended in the air. Therefore, smaller bacteria such as *Micrococcus species*, which were the dominant bacterial species in the indoor air, could potentially penetrate the alveoli and cause disease (Bragoszewska, et al., 2016).

Our result shows that gram-positive cocci were dominant in the indoor of air this is supported by a study conducted by Bragoszewska et al, (2016) and Pastuszka et al., (2005). Other study showed that *Micrococcus species*, *Staphylococcus* and *Bacillus* are the dominated genera in the indoor environment of primary school (Mentese and Mentagel 2009). Research conducted in Upper Silesia, Poland, reported those gram-positive cocci: including *Staphylococcus* and *Micrococcus* were the dominant bacteria in indoor (Pastuszka, 2005). The study conducted in Poland shows that the most dominated species are *Micrococcus species* and *Staphylococcus epidermidis* (Pastuszka, 2005).

A similar study also conducted by Chan, et al., (2009) bacteria of indoor was a gram-positive bacteria in which *Micrococcus* and *Bacillus species* was the most abundant. *Staphylococcus* (39.16%), *Bacillus* (18.46%), *Corynebacterium* and *Micrococcus* (7.2 & 21%) were dominated among the genera identified in the study of in daycare center (Aydogdu et al., 2010). According to our results, the dominant one in the outdoor environment is the *Micrococcus*, *Bacillus*, and *Corynebacterium* this result also supported by the finding of Aydogdu et al., (2010).

From our results, the highest percentage of bacteria are non-sporing gram-positive rods of *Bacillus species.* Gram-negative rod found outdoor only *Pseudomonas species.* Airborne Gram-positive bacteria were most abundant, with more than 95.5 % of the measured population in both indoor and outdoor while gram-negative bacteria about 4.5 % of total population. So this is similar to other research in Gliwice, Poland by Bragoszewska (2016) airborne gram-positive bacteria were 90 % of the measured population and gram-negative bacteria were 10 % of outdoor samples in study school.

Other observation in Edirne, Turkey show that airborne Gram-positive bacteria were the most abundant, accounting for more than 95% of the measured population while Gram-negative bacteria were present in less than 5% of samples in child day-care centers. The concentration of total suspended particles in the indoor was 234.4 $\mu g/m^3$ with a sampling period of 32 hours and 56 min while the outdoor was 53.4 $\mu g/m^3$ with the sampling period of 30 hours and 51min. The concentration of total suspended particles in residential, rural and other areas is 200 $\mu g/m^3$ with an average period of 24 hours (Gokhale, 2009). The total suspended particle was the different in concentration only between indoor and outdoor. However, the concentration of solid particles (total suspended particles) obtained results indicated that the concentration levels of total suspended particles were below the recommended value.

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATION

The concentration of bacteria fluctuated throughout a day. The indoor concentration of fungi was highest in the mid-day in the laboratory. In every case, the concentration indoors was the same like outdoors. However, the total concentration of both bacteria and fungi in the outdoor and indoor environment was high. The concentration of bacterial and fungal aerosol in the laboratory room was extremely low. The indoor/outdoor ratio of bacteria and fungi in the laboratory, therefore, ranged from 0.1 to 1 and 0.1 to 1. The mean value of bacteria was 0.5 while the fungi 0.4, the room can be treated as a healthy building.

Large fluctuations in airborne bacteria and fungi levels were observed in both indoor and outdoor. The average of bacterial concentration in the indoor air was 6,657 CFU/m^3 in the locker room, 3,999 CFU/m^3 in the corridor, 3,105 CFU/m^3 in the classroom, 4,292 CFU/m^3 while in the gymnastic hall and in the outdoor was 1,209 CFU/m^3. The indoor /outdoor ratio of bacteria ranged from 1.2 to 78.1 and the mean value was 23.2. In the locker room show 33.3, in corridor 17.9, 18.6 in classroom and 23 at the gymnastics hall. This indicated that the main emission indoors was related to the activity of the students and was emission from human organism.

The general indoor-outdoor ratio for fungi ranged from 0.3 to 1.6 and the mean value was 0.8. In the locker room, I/O was 0.6, in corridor 0.9, in classroom 0.7 and 1.0 at the gymnastics hall. So, from this, we can conclude that the fungi ratio was relatively lower than 1. However, in some place, like a gymnastic hall, there was a relatively elevated level of fungi; this may it be coming from outdoor into indoor through the window.

The I/O ratios for the observed fungi counts were calculated as approximately around 1.0 and for the bacteria counts, they were higher than 1. A significant difference was found between indoor and outdoor bacteria concentrations. Therefore, increasing the ventilation rate by means of mechanical or natural systems can play a key role in improving the indoor air quality, especially in the school. Principally because of ventilation destroy particles including fungal spores from the supply air. From size distribution, for both bacterial and fungal aerosol distribution is unimodal for all of the measurements in the atmosphere of indoor and outdoor environments.

The ratio of respirable bacterial concentration, opposed to total concentration, was 0.76 in the locker room, 0.86 in the corridor and the classroom, 0.75 in the gymnastic hall and 0.56 at the outdoor environment. The indoor respirable concentration was higher the outdoor. From this we can conclude that the emission of respirable bacteria from students is suspended in the air. In the respirable fungi, was 0.79 in the locker room, 0.66 in the corridor, 0.85 in the classroom, 0.73 in the gymnastic hall and 0.69 was in the outdoor environment. The indoor respirable concentration is highly related to the outdoor respirable airborne fungi.

Such distribution of the particles indicates the additional emission from a human organism (the increased emission during breathing). Those small particles more prevalent indoor air, these respirable particles mostly can be deposit in either the trachea, bronchial, or alveolar region of the lungs. At the indoor environment, the larger bacteria appeared to settle on the ground, while the smaller bacteria remained suspended in the air. Therefore, smaller bacteria such as *Micrococcus species*, which were the dominant bacterial species in the air indoors, could potentially penetrate the alveoli and cause disease. It is known that the potential health risk caused by exposure to airborne bacteria and fungi is related mainly to the concentrations of respirable biological particles.

It can be concluded from species identification of airborne bacteria that Gram-positive bacteria were the most abundant and their contribution it was 95.5% of the total population. Gram-negative bacteria were only found in the outdoor environment and contributed 4.5% the total population of bacterial aerosol. The *gram-positive cocci species* which are identified as *Micrococcus species, Staphylococcus species* and sporting gram-positive rods (*Bacillus species)* and non-spring gram-positive rods were found *Corynebacterium species* in the studied indoor environment. In the outdoor environment *Micrococcus species, Bacillus species, Corynebacterium species* were found. Beside, gram-negative *Pseudomonas species* were present but only in the outdoor environment.

In the studied primary school, the exposure of students to airborne bacteria and fungi was slightly higher than in the school located in other European countries. The bacteria levels of primary schools were observed to be higher than in another environment in different countries. So our results in the primary school are indicating that the indoor air quality is not healthy or safe. Although, the health risk related to the exposure is low some improving of the ventilation is recommended. Increasing ventilation rates and use of low emission materials would contribute towards improving indoor air quality.

The indoor respirable concentration is more related to the outdoor respirable for airborne fungi. This should give a special attention for reducing the contamination to improve health and well-being of the children. Therefore, special attention should be given to the major sources of airborne bacteria and fungi in this primary school. Further investigations regarding to ventilation system, environmental characteristic and sources of biological pollutants would be important to provide information to the public health policies.

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Annexes

Annexe 1. Bioaerosol concentration was determined for both indoor and outdoor environments of laboratory. Shows the concentration of both fungi and bacteria of indoor and outdoor of six stage Anderson impactor (AND) and the single stage of Air Ideal concentration in colony forming unit per cubic meter (CFU/m^3).

MEA (fungi)		**TSA (bacteria)**	
AND CFU/m^3	*Air Ideal CFU/m^3*	*ANDCFU/m^3*	*Air Ideal CFU/m^3*
17/03/2017			
Indoor			
92	71	99	51
21	13	110	92
46	449	92	62
Outdoor			
403	355	396	439
117	156	247	727
124	220	827	170
24/03/2017			
Indoor			
106	77	424	282
371	292	187	142
110	67	120	57
Outdoor			
113	75	431	176
371	292	318	142
332	223	449	33

31/03/2017			
Indoor			
78	49	117	119
64	49	64	47
117	130	240	61
Outdoor			
420	418	155	326
615	792	756	210
512	587	329	244

Annexe 2. The indoor/outdoor ratio airborne bacteria and fungi in the laboratory

07/04/2017	**I/O bacteria**	**I/O fungi**
Indoor		
Locker room	78.1	0.7
In corridor	36.5	0.3
In class room	43.4	0.7
Gymnastic hall	44.5	0.6
Outdoor		
10/05/2017		
Indoor		
Locker room	18.4	0.4
In corridor	16	1.6
In class room	11.3	0.9
Gymnastic hall	23.3	1.4
Outdoor		
02/06/2017		
Indoor		
Locker room	3.3	0.7
In corridor	1.3	0.8
In class room	1.2	0.5
Gymnastic hall	1.3	0.9
Outdoor		

Annexe 3. The indoor /outdoor of concentration in colony forming unit measured by both Andersen and Air Ideal

MEA (fungi)		**TSA (bacteria)**	
AND CFU/m^3	*Air Ideal CFU/m^3*	*ANDCFU/m^3*	*Air Ideal CFU/m^3*
17/03/2017			
Indoor			
92	71	99	51
21	13	110	92
46	449	92	62
Outdoor			
403	355	396	439
117	156	247	727
124	220	827	170
24/03/2017			
Indoor			
106	77	424	282
371	292	187	142
110	67	120	57
Outdoor			
113	75	431	176
371	292	318	142
332	223	449	33
31/03/2017			
Indoor			
78	49	117	119
64	49	64	47
117	130	240	61
Outdoor			
420	418	155	326
615	792	756	210
512	587	329	244

Annexe 4. Bioaerosol concentration were determined for both indoor and outdoor environments of primary school in CFU/m^3 of minimum, maximum, mean, median and geometric mean

Bacteria	**07/04/2017**	**10/05/2017**	**02/06/2017**	Min	Max	Mean	Median	Geometric mean
Locker room	4138	4802	11032	4138	11032	6657	4802	6030
In corridor	1936	4173	4389	1936	4389	3499	4173	3285
In class room	2300	2943	4071	2300	4071	3105	2943	3020
Gymnastic hall	2360	6070	4445	2360	6070	4292	4445	3993
out door	53	261	3343	53	3343	1219	261	359
Fungi	07/04/2017	10/05/2017	02/06/2017					
Locker room	300	92	5018	92	5018	1803	300	517
In corridor	113	491	5173	113	5173	1926	491	660
In class room	276	230	3569	230	3569	1358	276	610
Gymnastic hall	233	353	5809	233	5809	2132	353	782
out door	413	258	6820	258	6820	2497	413	899

Annex 5. Each stage concentration in cfu/m^3 both in the laboratory and primary school.

Date 17/03/2017 in the laboratory

	MEA						**TSA**					
	IN	IN	IN	OUT	OUT	OUT	IN	IN	IN	OUT	OUT	OUT
AND	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**
Stage 1	7	0	0	28	18	46	18	14	14	117	106	4
Stage 2	0	4	28	78	32	28	7	14	11	60	42	32
Stage 3	21	7	64	141	42	25	0	7	11	85	25	4
Stage 4	49	11	530	124	25	21	21	35	21	67	46	4
Stage 5	14	0	205	25	0	0	14	32	35	35	28	0
Stage 6	0	0	0	7	0	4	39	7	0	32	0	4
Air ID												
P 1	80	23	483	360	173	183	83	87	80	397	530	7
P 2	83	0	407	350	133	260	7	67	107	573	923	0
P 3	50	17	457	0	160	217	63	123	0	347	0	503

Date 24/03/2017 in the laboratory

	MEA						**TSA**					
	IN	IN	IN	OUT	OUT	OUT	IN	IN	IN	OUT	OUT	OUT
AND	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**
Stage 1	7	39	4	0	39	14	71	28	25	102	53	102
Stage 2	7	25	7	21	25	32	49	32	7	88	60	124
Stage 3	25	106	18	4	106	120	67	18	11	81	88	74
Stage 4	60	110	42	46	110	102	102	46	32	113	85	60
Stage 5	7	92	39	35	92	57	92	49	35	21	25	67
Stage 6	0	0	0	7	0	7	42	14	11		7	21
Air ID	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**
P 1	73	297	53	67	297	270	240	113	60	163	110	43
P 2	83	287	80	83	287	177	270	197	30	147	117	23
P 3	73						337	117	80	217	200	

Date 31/03/2017 in the laboratory

				MEA						TSA		
	IN	**IN**	**IN**	**OUT**	**OUT**	**OUT**	**IN**	**IN**	**IN**	**OUT**	**OUT**	**OUT**
AND	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/m 3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**	**cfu/ m3**
Stage 1	4	7	11	60	71	78	46	60	21	32	173	67
Stage 2	0	4	7	60	99	60	18	0	88	35	117	28
Stage 3	7	49	60	269	184	314	4	0	102	11	170	39
Stage 4	42	4	25	4	205	60	25	0	14	39	170	0
Stage 5	18	0	11	28	42	0	25	4	11	35	110	21
Stage 6	7	0	4	0	14	0	0	0	4	4	18	173
Air ID												
P 1	53	63	130	327	920	496.7	143	53	57	490	253	357
P 2	40	47	153	483	713		120	53	70	340	230	213
P 3	53	37	107	443	743	676.7	93	33	57	147	147	163

Date 07/04/2017 in the primary school

	Fungi					bacteria				
	Locker room	In corridor	In class room	Gymnastic hall	out door	Locker room	In corridor	In class room	Gymnastic hall	out door
AND	**cfu/m^3**	**cfu/ m^3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**
Stage 1	18	11	32	4	85	375	134	223	177	4
Stage 2	11	14	21	39	35	336	237	251	223	4
Stage 3	25	21	35	39	92	650	382	272	311	0
Stage 4	106	57	42	99	141	1311	583	781	625	18
Stage 5	141	11	145	49	60	1357	562	721	958	21
Stage 6	0	0	4	4	0	110	39	53	67	7
Air ID										
P 1	130	83	143	137	170	1637	1050	1203	727	0
P 2	140	60	177	110	200	1227	1027	1137	990	43
P 3	153	100	153	117	230	1097	990	1050	1000	137

Date 10/05/2017 in the primary school

	fungi					**bacteria**				
	Locker room	**In corridor**	**In class room**	**Gymnastic hall**	**out door**	**Locker room**	**In corridor**	**In class room**	**Gymnastic hall**	**out door**
AND	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**	**cfu/m3**
Stage 1	7	60	11	28	53	622	216	208	594	32
Stage 2	4	46	7	39	25	357	226	343	739	25
Stage 3	7	110	67	124	46	841	710	495	1039	42
Stage 4	25	230	92	120	95	1138	1078	943	1244	110
Stage 5	49	46	53	42	32	1385	1456	894	1385	49
Stage 6	0	0	0	0	7	459	488	60	1074	4
Air ID										
P 1	107	357	243	177	243	963	887	897	783	210
P 2	117	297	183	360	183	1007	1017	867	1030	90
P 3	83	537	107	240	107	697	857	907	917	53

Date 02/06/2017 in the primary school

		Fungi					**Bactria**			
AND	Locker room cfu/m3	In corridor cfu/m3	In class room cfu/m3	Gymnastic hall cfu/m3	outdoor cfu/m3	Locker room cfu/m3	In corridor cfu/m3	In class room cfu/m3	Gymnastic hall cfu/m3	outdoor cfu/m3
Stage 1	431	643	247	792	926	1343	325	311	657	728
Stage 2	643	1187	297	855	1208	1929	424	283	495	813
Stage 3	1668	1244	905	1442	2184	1972	728	360	1110	481
Stage 4	1604	1583	1505	1958	2276	2000	1470	1173	1145	1018
Stage 5	664	502	615	749	198	2784	1293	1618	735	233
Stage 6	7	14	0	14	28	1004	148	325	304	71
Air ID										
P 1	2430	2020	2330	1220	2610	4100	1140	1660	1600	1550
P 2	2060	2560	2290	2430	2340	3590	1410	1870	2230	390
P 3	1900	1890	2220	2560	2140	3000	1820	1920	1520	1720