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Research Article

THE DETERMINATION OF MICROORGANISMS IN INDOOR AND OUTDOOR ATMOSPHERE IN THE Y.T.U. DAVUTPASA CAMPUS

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ABSTRACT

Air can contain a number of foreign matters and microorganisms in various sizes, which may be harmful to human health besides essential substances necessary for respiration such as oxygen. The air quality in terms of microbiology in indoor and outdoor environments plays an important role in determining the possible risks and the precautions to be taken.
The aim of this study is to examine the microorganisms in indoor and outdoor air in the Davutpasa campus of Yildiz Technical University. The active and passive sampling methods were used in nine different indoor and outdoor atmospheric environments.
The best results were obtained by active sampling and membrane filtration methods. In the samples taken in March, the highest bacterial colony was detected in the wastewater laboratory at 35 °C. The highest bacterial and fungal colonies were found in the wastewater laboratory and in the middle garden, respectively. In the samples taken in April, the highest the number of bacteria at 20 °C was in the microbiology laboratory while the number of fungi showed differences according to the type of cultivation methods. In the gram staining, gram positive bacteria belonging to Sarcina sp, gram negative Monococcus sp., gram positive *Diplococcus sp*., gram positive Tetracoccus sp., gram-negative actinomycetes (branched bacteria), gram-negative Vibrio sp. and miscellaneous fungi have been observed.
As a result, it has observed that there are various bacteria and fungi in the indoor and outdoor air we breathe. The number of microorganisms has changed according to the environment conditions.
Keywords: Microorganisms, indoor, outdoor, atmosphere.

1. INTRODUCTION

The atmosphere is a large biospheric layer that integrates many living and non-living things with providing living space to creatures. The most important feature of it is to provide oxygen for animals and carbon dioxide for photosynthesis of plants. Living things cannot live without oxygen and die very quickly in an oxygen-free environment. For this reason, fresh air and air pollution have vital importance. Air pollution can also occur in the excess of microorganisms as well as when the gases and particulate matter in the atmosphere are normally in excess. The amount of microorganisms in indoor and outdoor atmospheric environment varies due to many

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environmental factors. Outside environment microorganisms are generally less than in the inner environment, which is the biggest reason to disperse in the atmosphere. Outside environmental microorganisms originate from the flora and fauna of the earth which treat the natural wastes as the main origin. Secondary sources are found in solid and liquid wastes in residential areas, and are mixed into the atmosphere through air by developing in various workplaces and habitats. Bes ides drinking water and food, air is one of the microbiological contamination routes. Microorganisms can be transported into indoor environment by people, especially with their shoes or clothes, as they can come from heating, ventilation and cooling systems, from doors, windows, wall openings and plumbing pipes.

The growth of microorganisms in the indoor environment influence humidity, temperature, the presence of nutrient (dirt, wood, paper, paint, etc.), the amount of oxygen and light. The most common microorganisms in the indoor environment are fungi and bacteria. The spores produced by the fungi can be mixed into the air; some fungi can also produce toxic substances such as mycotoxins or volatile organic compounds. The most common fungal species that can cause disease in the indoor environment are *Penicillium sp., Aspergillus sp*., and *Alternaria sp*.

Similarly, some bacteria can produce endotoxins and volatile organic compounds, which are poisonous substances. Studies have shown that high amounts of airborne micros are caused by asthma and allergic rhinitis [1], hypersensitivity pneumonitis [2] and patient building syndrome [3]. However, the health problems they create are not limited to allergic diseases alone; bioaerosols and their by-products are known to cause infection [4] and toxic effects [5]. In the case of pathogenic biological objects and non-biological compounds having the same sources and coexistence of the fine dusts in the indoor environment with bioaerosols, asthma crises and allergic are triggered. So, in studies of human health air quality, non-biological contaminant concentrations are often measured along with biological concentrations [5-7].

Most airborne bacteria exhibit optimum growth in mesophilic (20-35 °C) conditions. Bacteria produce some toxins that are their own metabolites, both intracellular and extracellular. These toxins include gases called microbial volatile organic compounds (MVOC). Bacteria can be found in the indoor environment in the range of 10-10000 CFU/m^3 depending on ambient conditions. The general method used to identify bacteria in the ambient air is to sample "live (cultivable) organisms". Bacteria sampled by this method are about 10% of total airborne bacteria [3-5]. *Staphylococcus*, *Streptococcus* and *Micrococcus* species in the group called Gram positive coccus are spread from the human skin and from the mucous membranes of the upper respiratory airway system. As *Penicillium*, *Aspergillus* and *Cladosporium* spores cling to each other and then enlarge their size, so they fall down faster. The particle diameter varies directly proportional with the rate of collapse. However, the spores can be re-suspended into the air by the movement of the air stream or by an external motion from where they collapse. Fungi can also survive by creating spores in difficult environmental conditions.

Fungi are nutrient-specific; that is, there are certain substratum requirements to be able to reproduce some species of fungi. Mesophilic fungi are at an optimum reproductive rate between 20-35 °C. However, some types of fungi are resistant to extreme humidity and temperature conditions. Fungi emit to environment the toxins called mycotoxin when they are restricted by the nutrient. *Penicillium* and *Aspergillus* species which are abundant in indoor air, generally produce mycotoxin. Aflatoxin is a mycotoxin [8], which is considered to be important today, produced by a species of Aspergillus. Considering the types of fungi isolated from indoor and outdoor environment, it is seen that there is a clear difference in terms of spores and structure. For example, *Cladosporium*, *Alternaria* and *Aureobasidium* species are more common in the outdoor environment [8]. Due to the compounds it contains, air is not a convenient medium for the development of microorganisms. The microorganisms in the air come from the earth or decaying organic wastes through the wind. Therefore, the air is not a permanent environment, but an environmental condition. The microbial load of air depends on the amount of shielding dust and human activities in the environment.

According to the examination s made, the air on the fertile soil contains more microorganisms than the air on the sandy slurry. Again, the air of an irregularly dirty room contains more microorganisms than the air of a regularly clean room. Again, the air of a field without bare plants compared to the air of meadow or forest covered area; a terrestrial area air contains more microorganisms than a marine area air. The humidity of the air also affects the quantitative state of microorganisms. A humid and rainy atmosphere contains less microorganisms than a dry, rainless atmosphere. In winter the air contains fewer microorganisms than dry air in summer [9].

The aim of this study is to get information about the microbiological quality in indoor and outdoor air at Yildiz Technical University – Davutpasa Campus.

2. MATERIALS AND METHOD

2.1. Sampling Sites

This work was carried out within the YTU Davutpasa Campus. The two outdoor and five indoor locations were selected as sampling points in 1 March and 1 April 2017. Sampling locations are:

- Central garden (O.B.)
- The entrance of Civil Engineering Faculty (I.G.)
- Environmental engineering, solid waste and reactor laboratory (K.A.L./KAR)
- Environmental engineering, microbiology research and student laboratory (M.A.L / M.O.L)
- Environmental engineering, biotechnology laboratory (B.L.)
- Environmental engineering, waste water laboratory (A.L.)
- Environmental engineering, drinking water laboratory (I.S.)

2.2. Sampling Methods

Passive and active air samplings methods were performed to determine the most suitable for sampling. The active sampling system consists of a vacuum pump and a Drechsel bottle.

In this method, the air of the medium to be tested was passed through a sterile liquid (200 mL of saline water) for 20 minutes by vacuum. The amount of air passing through the system was measured as 225 L/h by flowmeter. That is, 75 L of air has adsorbed in the liquid phase. Thus, the microorganisms in the air have passed through the liquid phase (Fig 1). The samples were taken from a height of 1-1.5 m. Spread plate cultivation method was planted to 1 mL to small ones and 3 mL to large ones of the ready fungal and bacterial medium from samples taken with sterile injector/pipettes. Homogenous spreading of the samples was provided by shaking.

The sedimentation method was used as the passive sampling system. 3 mL of purified and disinfected water was placed in the ready-made fungal and bacterial media to make the pads dehydrated before the caps were opened little. The airborne particles fell into petri dishes that remained open for 20 minutes depending on their mass and under the influence of gravity.

As cultivation techniques, standard plate cultivation and membrane filtration methods have been preferred. The half of samples planted by the spreading plate method and membrane filtration method. The samples planted by passive sampling were subjected to incubation at 20 °C and the other half at 35 °C for 2-5 days. At the end of these methods, microorganism colonies in breeding petri dishes were counted and the number of fungi and bacteria per m^3 were determined.

2.3. Mediums

Peptone dextrose agar and standard plate count agar medium was used to determine the colony counties of fungi and bacteria. Streptomycin and Rose-Bengal added to prevent excessive bacteria-inhibiting and fungal growth, respectively. The samples were incubated and followed

microorganism growing at 4ºC, 20ºC, 25ºC and 35ºC for ten days. The colonies formed on the culture medium as a result of the incubation were counted. Preliminary investigations have shown that there was little to reproduce at 4 °C and 35 ± 2 °C. Ongoing studies have determined that breeding at 20 and 25 °C, but optimal breeding is 20 ± 2 °C. It has been determined that the temperature suitable for natural outdoor conditions was 20 ± 2 °C and the subsequent studies were carried out at this temperature.

Figure 1. Active and passive sampling systems.

Total fungal and bacterial counts were made at 20 ± 2 °C, but cultivations at 4 °C, 25 °C and 35 °C were also included in the study with the approach that different species may occur at different temperatures in species determination.

Dichloran Rose Bengal Chloramphenicol Agar (DRBC) and Sabouraud Dextrose Agar (SDA) media were used for the production and counting of fungi, and Standard Plate Count Agar (SPCA, PCA) medium for bacterial counting. Plate count agar medium was used in two different forms. The first one was used suitable ready-made mediums according to prepared membrane filtration method and colony counting method, and the second one was prepared according to the formulation from standard plate agar medium and the sterilized medium was poured into petri dishes and then cooled. After frosted, plantations were made.

After the medium was removed from the autoclave, 50 mg/L Nystatin was added to prevent reproduction of the fungi to the SPCA mediums and 50 mg/L Streptomycin Sulfate was added to prevent reproduction of the bacteria to the DRBC and SDA mediums. After mixing, they were pour into petri dishes. The total number of microorganisms in the cubic meter of the air sampled using the amount of air passing through the reactor was determined by counting the colonies formed as a result of incubation in all media.

2.4. Analyzes

Colony counting methods were used to determine the number of microorganisms in the air, and pure cultures were examined by purifying the colonies formed in species or gender determination studies. For identification of the species or groups, after previous procedures (morphology of bacterial and fungal colony, staining reaction, cultural characteristics, metabolic tests, biochemical properties, etc.), a biological characterization using API Staph and API 50 CH, was carried out [10-12].

3. RESULTS

3.1. Microbial Colony Determination

3.1.1. Sampling Method

In the samples taken in March, some samples were used both in active and passive sampling and at different incubation temperatures. In passive samples, there were little microorganisms. So, the results of colony counting for active samples are generally shown in Table 1.

Table 1. Colony count results for March

Location	CFU/m^3			
	Bacteria (35°C)	Fungi (35°C)	Bacteria (20°C)	Fungi (20°C)
A.L.(active)	12913	0	22523	6006
M.A.L.(active)	601	0	1502	601
B.L.(active)	0	0	7808	3303
M.O.L.(active)	2102	0	0	0
O.B.(passive)	6	0	7	8
O.B.(active)	-	0	18619	28529
K.A.L.(active)	0	0	0	0
K.A.R.(active)	0	0	601	5105

In March samples, bacterial colonies at 20 °C showed more growth than at 35 °C. When we looked at the fungus colonies, it was observed that there was a certain colony formation at 20 °C and no colony at 35 °C. More colonies formed at 20 °C. This shows that microorganisms live at an ambient temperature of 20°C. When we compare active and passive samplings, the number of colonies obtained after active sampling is higher than in the passive method. An example has given in Figure 2. One of the reasons for the higher detection by active sampling is that this method is more reliable because air is drawn by vacuum pump. Since there is no air extraction in passive sampling, the microorganisms in the air do not have enough contact with the medium. Therefore, fewer microorganisms are detected. The passive sampling was performed in only one studying area (central garden-OB). The aim is to compare with active sampling. Since very low number of microorganisms was detected, it was not carried out in other study areas.

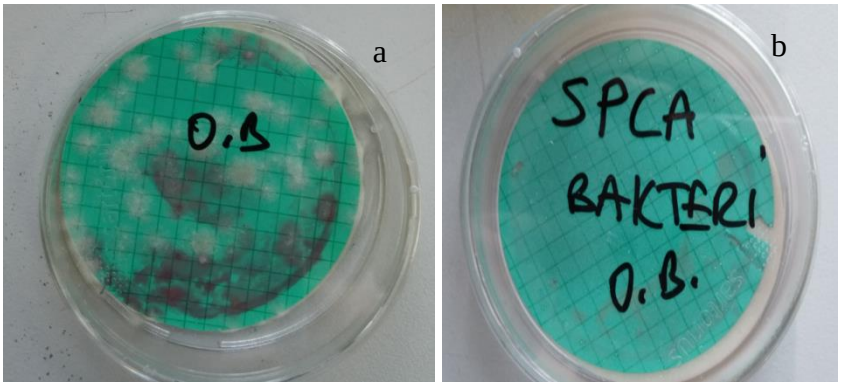


Figure 2. Active (a) and passive sampling (b)

The reasons for the counting of fewer colony in the passive samplings may be due to lack of volume, wind speed, aerodynamic effects, and only the capture of large particles. The formation of higher colony in the active sampling indicates that sufficient air volume is provided, i.e., more representative and accurate measurements are provided [13]. The colony count results of the samples in April are the following Table 2. With the first studies, it was determined that there was little reproduction at 35 ± 2 °C and the temperature suitable for natural outdoor conditions was determined to be 20 ± 2 °C and subsequent studies were carried out at this temperature.

Table 2. Colony count results for April

Location	CFU/m^3			
	Bacteria (20°C)	Fungi (20°C) DRBC	Fungi (20°C) SDA	Bacteria (20°C) PCA
A.L. (active)	901	0	0	0
O.B.(active)	1201	0	0	0
O.B.(passive)	8	0	0	0
M.A.L. (active)	105105	0	0	30030
K.A.R. (active)	601	18018	0	1001
B.L. (active)	24024	3003	0	1001
I.S. (active)	0	9009	3003	0
I.G. (active)	3303	12012	15015	6006

Analysis of the samples in April showed that bacterial colonies at 20 °C were higher than fungal colonies (Table 2). The growth of fungi is highly related to environmental conditions. While the temperature and high humidity are prerequisites for the growth of mesophilic fungi species, the presence of adequate nutrients in the fungi may also be important [14]. The average temperature of the air in March is 15.6 °C, whereas the average temperature in April is 22.4 °C. It is known that the level of moisture lead to the formation of fungi by increasing microbial activity [15]. The high amount of moisture in rainy weather in March explains why the fungus colonies are in excess.

In the first sampling in the wastewater laboratory, the bacterial colony at 20 °C is 22523 while in the second sampling; this number drops to 901 at 20 °C. In the first sampling, the fungus colony at 18°C is 6606, while the fungus colonies at the second sampling are 0, which is not present. The low numbers of colony count in the second sampling are considered to be unable to breed bacteria and fungi due to the long aeration of the environment.

In the first sampling, the colony number of bacteria at 20 °C in the microbiology laboratory is 1502, while in the second sampling it is 105105 at 20 °C. Also, in the second sampling, the colony number of bacteria in the PCA medium is 30030. In the first sampling, the fungus colony at 20°C is 601; while in the second sampling at 20 °C the fungus colonies are 0. It is known that the level of moisture increases microbial activity and leads to the formation of fungi. In the second sampling, the reason for not being able to reproduce the fungus is considered as the lack of sufficient moisture.

In the biotechnology laboratory, the number of bacterial and fungal colonies is 0 at 35 °C in the first sampling. In the first sampling, the number of bacteria at 20 °C is 7808 while in the second sampling it is 24024 at 20 °C. In the first sampling, the fungus colony was 3303 at 20 °C, while the second sampling had 3303 fungus colonies.

Microbiology student laboratory, the first sampling is 0 at 20 °C fungal and bacterial colonies. The inability to breed bacteria and fungi is thought to be due to the long aeration of environment. No samples were taken from this place for the second sampling.

For solid waste laboratory, the number of bacterial and fungal colonies in the first sampling is 0 at 20 °C and 35 °C. Aerobic bacteria were not detected due to the presence of anaerobic bacteria in the medium. Therefore, no samples were taken from this place for the second sampling.

It has not detected bacteria colony in drinking water laboratory. Fungus colonies were observed as 12012 for DRBC and 15015 for SDA.

In the middle garden, in the first sampling, the fungus colony is 28529 at 20 °C and the bacterial colony is 18619 while the fungus colony at 20 °C is 0 and the bacterial colony is 1201 in the second sampling. When we look at the results of the first analysis, it is seen that there are more colonies counted as the result of active sampling in the middle garden. When the first sampling was made, the wind speed was quite high. Wind speed shows a positive correlation for both fungal and bacterial levels. It observed that the level of bio aerosol in the air increases as the wind speed increases.

In the Civil Faculty entrance, the number of bacteria is 3303 and 6006 while the number of fungi is 12012 and 15015 according to different nutrient medium.

When the results in Table 2 were examined, different amounts of colony counts were obtained in different nutrient mediums. For fungi we can say that DRBC and PCA mediums are more suitable than SDA mediums. While microorganisms are rarely released in the atmosphere, they are found on carriers that vary in volume and weight. These carriers are dust, mineral and organic coarse particles with a diameter of 10-200 μm. Its sources are vegetable fibers, animal tissue residues, feathers, pollen grains, skin epidermal fragments, and so on. According to their composition, it promotes the formation, revitalization or proliferation of the above-mentioned living creatures. They are not hanging in calm, stagnant weather; they go vertically to a solid surface level at a certain speed relative to their diameter. In windy environments where air currents occur, they are transported by horizontal or vertical currents depending on the situation [9].

3.1.2. Planting Method

The number of colonies cultured by membrane filtration at 20 °C is higher than the number of colonies obtained by sowing with spread plate. This shows us that more efficient results are obtained by membrane filtration method (Figure 3).

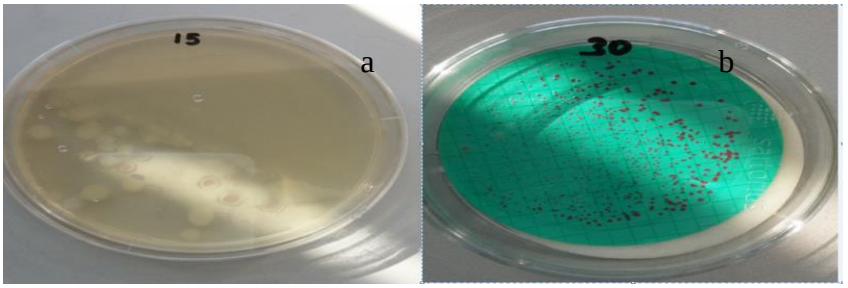


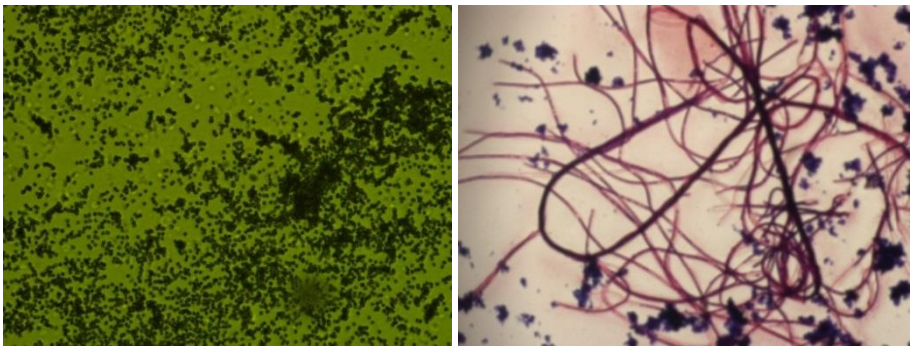
Figure 3. Membran filtration and spreading plate method (B.L-a, M.A.L-b)

3.2. Gram Staining

For identification of the species or groups, after previous procedures, morphology of bacterial and fungal colony, staining reaction, cultural characteristics, metabolic tests, biochemical properties, etc. were tested, and a biological characterization using API Staph and API 50 CH was carried out [10-12].

Studies done with gram and other staining methods have shown that gram-positive *Sarcina sp*, gram-negative *Monococcus sp*., gram positive *Diplococcus* and gram positive *Tetracoccus (Fig 4a)*, gram-negative Actinomycetes (branched bacteria), miscellaneous fungus and sporangium (Fig 4b), gram-negative *Vibrio sp*. are present. While Gram-negative actinomycetes (intercalating bacteria), gram-negative *Vibrio sp.*, gram negative *Monococcus sp*. were observed

in the indoor environment, gram-positive *Sarcina sp*, gram negative coccus, gram positive diplococcus, gram positive tetracoccus, gram-negative actinomycetes and miscellaneous fungus were determined in outdoor air (Civil Faculty entrance and the middle garden).



a) Bacteria b) Miscellaneous fungus and sporangium

Figure 4. The bacterial and fungal forms detected by microscopic studies.

4. DISCUSSION

The air which we breathe in the indoor and outdoor environment is important for our health. Air quality does not only relate the physical and chemical pollutants but also the microbiological pollutants. The microbial contamination in the indoor and outdoor air that we breathe is important to prevent some health problems. This study was carried out to determine some basic properties of microorganisms in different indoor and outdoor environments. To determine these, different sampling (active-passive), different temperature (20-35 °C), different time (March-April), different planting method (membrane filter method- spreading plate method) and different media (DRBC, SDA, PCA) was used. The most microorganisms were detected by active sampling methods at 20 °C as temperature, in membrane filtration technique. A higher colony was detected in March when the precipitation was high. DRBC and PCA agar have been found to be more suitable mediums for microbial growth. In indoor environments, fewer microorganisms have been observed that are cleaner and better ventilated conditions (such as drinking water laboratories). More microorganisms have been detected in biotechnology and microbiology research laboratories. Moisture and pH are important parameters for fungal life, and it is observed that there is little or no reproduction in this study because the solid waste laboratory examined does not contain much moisture. Factors affecting the presence of microorganisms in the outdoor environment are humidity, temperature, wind, etc. In the middle garden there are fewer microorganisms from the faculty entrance. The reasons of this are the lack of humidity, the open area of the wind.

The fungi were not detected in any environment when the highest the number of bacteria was found in the waste water laboratory at 35 °C in March. At 20 °C, the highest bacterial and fungal colonies were found in the wastewater laboratory and in the middle garden, which is the outdoor environment, respectively. In the samples taken in April, the highest the number of bacteria at 20 °C were in the microbiology laboratory, the number of fungi showed differences according to used medium type. The sampling sites where the highest fungal colony was detected according to DRBC, SDA and PCA agar were solid waste laboratory, faculty entrance and microbiology laboratories, respectively. In the result of gram staining methods, it is observed gram-positive Sarcina sp, gram-negative *Monococcus sp*., gram-positive Diplococcus and gram positive Tetracoccus bacteria types, gram-negative actinomycetes (branched bacteria), gram-negative *Vibrio sp*. and fungal miscellaneous.

As a result of this research, we have concluded that there are various bacteria and fungi in the indoor and outdoor environment that we breathe, and that the number of microorganisms is very influenced by environment conditions (temperature, humidity, wind/ventilation, etc.). Our recommendation for future studies is to observe both indoor and outdoor air quality in different media during 12 months.

NOTE: This paper submitted to 4th International Symposium on Environment and Morals 27-29 June 2018 (ISEM2018 Sarajevo / Bosnia and Herzegovina). However, there was no participation in the symposium and no presentations were made.

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