MICROBIAL MAPPING IN AIR AND SURFACE SAMPLES COLLECTED FROM SCHOOLS

A Thesis Submitted to
the Graduate School of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Biotechnology

by
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June 2022
İZMİR

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest thanks to my dear supervisor, Assoc. Prof. Dr. Hatice Eser ÖKTEN for the motivation and courage she has given along with her scientific and academic contributions throughout my studies. In addition, I would like to express my eternal thanks to dear Dr. Ayşegül Yağmur GÖREN KARA for her valuable contribution to field work and the most importantly the joy she has put in this study.

I would like to thank my co-supervisor dear Assoc. Prof. Dr. Efe SEZGİN for his attendance, opinions, ideas, and knowledge that he has contributed for the whole period of my graduate studies. I would like to thank Prof. Dr. Aysun SOFUOĞLU for providing the necessary funding for my thesis project through the Scientific Research Projects of IZTECH (2021IYTE-1-0102). I would like to thank Prof. Dr. Çağlar Hüseyin KARAKAYA for his permission to use his laboratory and equipment. I would like to thank CFR, IzTech IRC for the usage of equipment and service. I would like to thank Letgen Biotechnology for service procurement.

I would like to thank dear Pelinsu EKMEKCİ with my deepest feelings for being by my side without waiting for any response, regardless of the time and place. Thank you for your precious friendship. And last but not least, thank you for your contributions to the field side of this study.

Last for the real part, I would like to thank my lovely parents dear Bünyamin and Neziha ALKAN for their eternal love and faith in me. Besides, I would like to thank my brothers, Alper Doruk and Mustafa Ufuk ALKAN for their supports and courage.

For the fictional part, I would like to thank the anime characters, Reigen Arataka (Mob Psycho 100), Bakugo (Boku No Hero Academia), Gintoki (Gintama), Rei-I Mayer (Ergo Proxy), Alphonse (FMA Brotherhood), Dio (Last Exile), Shion (Psycho Pass), and many others. I would like to thank Anıl Can Önder, Damla Yalçın, Mustafa Kara who I believe are cyborgs sent to observe and cover me. And lastly, I would like to thank Dark Kutlu even I do not know its purpose.

ABSTRACT

MICROBIAL MAPPING IN AIR AND SURFACE SAMPLES COLLECTED FROM SCHOOLS

Indoor environmental quality has gained attention in the past decades due to the increased periods of time spent enclosed in buildings. Parameters such as ventilation, water damage, type of paints used, use of carpets, etc. affect the indoor air quality significantly. With the rising concern regarding outbreaks and pandemic, it is important to investigate the microbial load and consortium structure in schools. This thesis aims at determining the microbial loads in three selected schools in the Balçova District of İzmir, Türkiye. Two sampling campaigns were conducted, one in the winter break (February 2022) and one in the spring semester (May 2022). Surface swab samples and settle-plate air samples were collected from pre-defined spots at schools. Surface swab samples and settle-plate air samples were examined via both classical microbial culture methods and modern DNA analysis methods. Via classical microbial culture methods, culture comparison from the database, fungi cultures were dedicated up to genus level. Via modern DNA analysis method, Sanger sequencing, bacterial cultures were dedicated up to genus level too. All the results indicated that three schools were hosting possibly dangerous genera of both fungi and bacteria. But, this also showed that genus-level discrimination is still not enough to utter exact indoor air quality from the perspective of indoor microbiota to the identification of possible health risks for occupants. For further studies, microbial mapping should discriminate up to species level to demonstrate exact indoor air quality and indoor microbiota vie high throughput sequencing methods.

ÖZET

OKULLARDA HAVA VE YÜZEY ÖRNEKLERİNDE MİKROBİYAL HARİTALANDIRMA

İç mekan çevre kalitesi, binalarda kapalı geçirilen zamanın artması nedeniyle son yıllarda önem kazanmıştır. Havalandırma, kullanılan boyaların türü, su hasarının olup olmaması, halı kullanımı vb. parametreler iç hava kalitesini önemli ölçüde etkiler. Salgınlar ve pandemi ile ilgili artan endişeler, okullarda mikrobiyal yükün ve konsorsiyum yapısının araştırılmasını önemli hale getirmektedir. Bu tez, Türkiye'nin İzmir ili Balçova ilçesinde seçilen üç okulda mikrobiyal yüklerin ve mikrobiyal konsorsiyumu oluşturan türlerin belirlenmesini amaçlamaktadır. Biri kış tatilinde (Şubat 2022) ve diğeri bahar döneminde (Mayıs 2022) olmak üzere iki örnekleme kampanyası yürütülmüştür. Okullarda önceden belirlenmiş noktalardan yüzey sürüntü örnekleri ve çökeltme plakası hava örnekleri toplanmıştır. Yüzey sürüntü örnekleri ve çökeltme plakası hava örnekleri hem klasik mikrobiyal kültür yöntemleri hem de modern DNA analiz yöntemleri ile incelenmiştir. Klasik mikrobiyal kültür yöntemleri ile veri tabanından kültür karşılaştırması yapılarak mantar kültürleri cins seviyesine kadar tahsis edilmiştir. Modern DNA analizi yöntemi, Sanger dizilimi ile bakteri kültürleri de cins seviyesine kadar tahsis edilmiştir. Tüm sonuçlar, üç okulun hem mantar hem de bakterilerin muhtemelen tehlikeli cinslerine ev sahipliği yaptığını göstermiştir. Ancak bu aynı zamanda, cins düzeyinde tespitin, iç mekan mikrobiyotası perspektifinden, kamu sakinleri için olası sağlık risklerinin belirlenmesinde iç mekan hava kalitesini tam olarak ifade etmek için yeterli olmadığını göstermiştir. Daha ileri çalışmalarda, mikrobiyal haritalamada, iç mekan hava kalitesini ve iç mekan mikrobiyotasını tam olarak göstermek adına yeni nesil dizileme yöntemleriyle tür seviyesine kadar ayrım yapılmalıdır.

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CHAPTER 1

INTRODUCTION

The need for mass production of goods induced the development of the modern factory system in the late 1700s England. Although it might seem like a simple change in mode and scale of production, the introduction of the modern factory system had such severe consequences that it was called a revolution, namely the first industrial revolution. It has not only caused the redistribution of wealth in the world, it also caused major changes in borders. Leaving most of these drastic changes and their consequences to researchers in the field of social sciences, we can say that one of the major outputs of the first industrial revolution was facilitation of and an unprecedented increase in urbanization (Encyclopedia Britannica). Catalyzed by industrialization, populations worldwide have since been shifting towards urban settlements. Average urbanization in world has changed from 33.6% to 56.2% between the years 1960 and 2020 (Figure 1.1.). The OECD countries, which included Türkiye, and the European Union also urbanized during the same time period, and their urbanization rates followed a similar trend. Amongst the countries that has experienced intense urbanization, Türkiye and China stood out with drastic increases. While in 1960's Türkiye, urbanization was merely at 31.5%, by 2020, it more than doubled, reaching 76.1%. China's case was even more significant, increasing from 16.2% (1960) to 61.4% (2020). When global trends were assessed, 68% of the world population was expected to live in urban areas, with 90% residing in Asia and Africa (UN, 2022). As a consequence of urbanization, an increase in the built environment has also been experienced. Common and individual spaces

Daily life of an average person includes working/studying, doing house chores, sleeping, shopping, eating-drinking, and enjoying leisure. When the time allocated to these activities were evaluated, it became obvious that today's societies spent most of their time indoors (Figure 1.2) (Ortiz-Espena, 2020). The National Human Activity Pattern Survey (NHAPS) conducted throughout U.S.A. with 9386 respondents revealed that average U.S. citizens spent 87% of their time indoors (Klepeis et al., 2001). Considering the total time spent for sleeping, house chores, elderly and child care,

eating/drinking, personal care, some leisure time as well as 75% of paid work time, an average person in Türkiye was calculated to spend more than 80% of his/her time indoors.

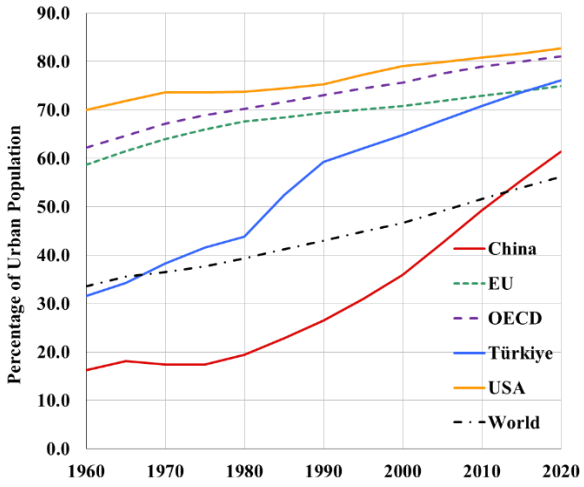


Figure 1.1. Time-dependent change in urban population in regard with total population. (Source: The World Bank, 2022.)

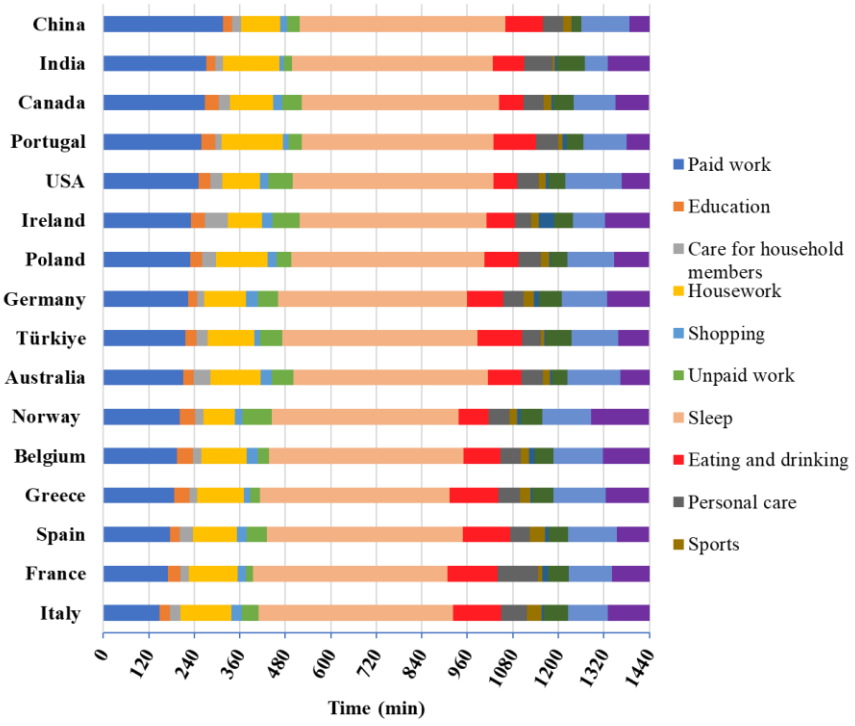


Figure 1.2. Country-based information on time spent for different activities (Source: Ortiz-Espena, 2020.)

Spending more time in buildings and indoor caused occupants to be more exposed to their indoor environment in manner of sharing microbial agents such as bacteria, fungi, and viruses (Feng, Shen, Nie, & Wu, 2021). While occupants are spending their time

indoors, they release aerosols into the environment, they touch to the shared objects, items, equipments, and surfaces. Aerosols that are capable of carrying microbial agents mentioned above are called bioaerosols. The size of bioaerosols vary from 20 nm to 100 μm (Brandl & Mandal, 2011) and because of their light weight, transferring of bioaerosols are quite easy via air before settling. Also, bioaerosols can easily reach deep into occupants' respiratory systems. When bioaerosols carrying bacteria, fungi, and viruses go deep into respiratory system of occupants, they may cause cold related illnesses, asthma, bacterial infections, and specifically last few years' hot topic COVID-19.

The virus that started the COVID-19 pandemic was SARS-CoV-2, a virus with moderate infectivity and known to be transmitted via bioaerosols. Although its infectivity has changed with different variants, its route of infection has not. In many cases, shared spaces such as shared breathing zones, shared rooms or even shared buildings have been the culprit. Katelaris et al. (2021) reported that an outbreak of COVID-19 that was caused by an infected chorister, who attended multiple services, infecting at least 12 people seated at close proximity (up to 15 m). A superspreading event regarding an infected individual attending a party at a bar with no mechanical ventilation was reported by Chau et al. (2021). Backtracking in the footsteps of patient 1, authors have traced back to 19 confirmed cases, of which 12 people were asymptomatic. Another superspreading event was reported by Miller et al. (2021), presenting inhalation of bioaerosols as the main cause of COVID contraction at Skagit Valley, Washington, USA. After an infected chorus member has attended a practice with 60 other people, 53 people were infected and 2 people eventually died. During the gathering several precautions were taken: the use of hand sanitizers was encouraged, hand shaking and hugging were forbidden, chairs were arranged in such a way to keep chorus members apart (0.75 m lateral, 1.4 m forward). The common ground of these studies were that people congregated to spend half an hour or longer in poorly ventilated indoor spaces. A 2021 study by Hwang et al. reported that in infected person (index patient) living on the 6^{th} floor of an apartment building in Seoul, South Korea might have infected her neighbors through the ventilation duct that connected different units' bathrooms. Following the days after the index patient was admitted to the hospital 9 more patients were admitted with symptoms such as cough, fever and sore throat.

Apart from the transmission via bioaerosols, SARS-CoV-2 is known to spread via contact with surfaces (fomite transmission). Liu et al (2021) have reported the stability of the virus on several environmental surfaces and in human excreta. They have prepared an

infectious titre of 10^6 50% tissue culture infectious dose (TCID50) per milliliter and spread 50 microliters of that titre on plastic, stainless steel, glass, ceramics, surgical mask, latex gloves, cotton cloth, wood and paper. Surfaces were listed in descending order according to stability of virus after 4 days: glass, stainless steel, plastic, surgical mask, ceramic, latex gloves, wood, paper, and cotton. The level of detection (LOD) was $10^{1.5}$ for the studied surfaces. Liu et al. (2021) have inoculated the feces and urine collected from healthy individuals (2 adults and 1 child) with the previously defined virus stock. In fecal samples, viability of SARS-CoV-2 has decreased quickly, 6 hours for adult 1 and 24 hours for adult 2. However, the viability was still measured in the child's feces after 2 days. Stability was more pronounced for the urine samples as it was observed for 3 days for the adults and 4 days for the child.

The everyday interactions with the microorganisms that dominate the anthroposphere govern the quality of life for humans. While a healthy gut microbiota may mean functional digestion, immune and metabolic systems, contraction of pathogens via different routes may cause diseases such as tuberculosis, measles, chicken pox, SARS, MERS, COVID-19 etc. In today's highly urbanized world, it is important to develop an understanding of the urban microbiome and its interaction with humans.

1.1. Urban Microbiomes

Urban microbiome is defined as the total microbial community that resides in the components of the urban landscape. These components can be listed as soil, air, sewage and buildings. Among these components, soil is known to be the richest in terms of microbial diversity with thousands of individual taxa, millions of species and billions of individual organisms (Fierer, 2017). Soil microbiome is directly affected by the land use and vegetation. Aerobiome is more dilute in terms of microbial load, and it is significantly affected from the dynamic conditions of the atmosphere. A recent multi-marker metabarcoding study by de Groot et al. (2021) revealed that the aerial microbial community composition was heavily dependent on meteorological conditions such as air pressure, wind direction and air temperature. They collected their samples on adhesive tapes via active sampling devices from the roof of a building at the campus of Wageningen University, the Netherlands. Aerobiome's prokaryotic portion was dominated by bacteria (95.7%) and the Firmicutes phylum (62.9%). The majority of the

eukaryotic portion was composed of fungi (98.9%), mostly belonging to Basidiomycota (80.5%) and Ascomycota (18.2%). There were also parasites and pathogenic microorganisms found in the microbiome. One of the most important indicators of urban development is the access to sanitary facilities in terms of water treatment. In developed urban settlements, polluted water, which is composed of human waste, chemicals and rainwater, is carried away from the buildings via pipelines. LaMartina et al. (2021) investigated the microbial diversities of the sewer microbiomes and their temporal change for 77 sampling sites across the USA and 12 sampling sites in Milwaukee, WI. They found that as the sewage traveled more in the sewer, the resident community of the sewer became more dominant. In other words, *Bacteroides*, which was the most dominant genus found in human stool by 53%, was only represented at 11% at the sewer system. Descending order of the abundance of genera constituting the microbial community of wastewater treatment plant influent were given as *Acinetobacter, Arcobacter, Bacteroides, Acidovorax, Aeromonas, Trichococcus, Flavobacterium, Lactococococcus, Prevotella_9, Faecalibacterium, Agathobacter, Alistipes and Parabacteroides*. The last components of the urban microbiome are the buildings, which are microbial reservoirs affected by humidity, temperature, light and aeration/climatization. Specifically, bacteria and fungi can survive indoors for long periods of time. Ng et al. (2019) revealed that the bacteria colonizing the AC unit was able to sustain viability using human skin squames as the carbon and energy source. Among the bacterial species that were used in this study, *Bacillus thuringiensi*s was the fastest one in ammonia production by emitting 35 ppm after 3 days of incubation, contributing to the urine-like smell coming from the AC unit. Fahimipour et al (2018) investigated the dust collected from houses in terms of microbial communities. They found that bacterial abundances of visible and ultraviolet communities were significantly lower than the abundance of communities left in the dark. Also due to sufficient amount of nutrients bacterial communities could survive on house dust even after 90 days, highlighting the resilience and survival skills of microorganisms.

Urban microbiome is affected by the level of urbanization. In a recent study, Parajuli et al. (2018) investigated the effects of different urbanization levels on the diversity of environmental microbiota. In total 56 sites were included in the study, 26 of them being urban sites and the rest being identified as rural sites. In the urban sites, the omission of gut microflora (from animals) was the most important finding. Another important finding was that urban sites hosted pathogenic bacterial families of Enterobacteriaceae, Streptococcaceae and Mycobacteriaceae more abundantly. The

authors concluded that due to increased urbanization, while people were exposed to less diverse microbiota, they were also exposed to pathogenic bacteria. The main implications of this situation being adverse effects on immune function and consequently developing allergies.

An important niche for urban microbiome is the public transit systems, which hosts millions of people every day. Perhaps the most extensive and comprehensive study to date was conducted by Danko et al. (2021) who sampled the public transportation system of 60 cities worldwide (none of the Turkish cities was included), collecting 4728 samples. They set out to tackle the ambitious mission of creating a global atlas of urban microbiome. Danko and colleagues found that the urban microbiome of different cities had a relatively conservative core, consisting of primarily bacteria belonging to the phyla Firmicutes, Proteobacteria and Actinobacteria and a universal eukaryotic representative, *Saccharomyces cerevisiae*. The core of urban microbiomes did not contain any viruses or archaea. On the other hand, city-specific variations in the microbiome were significantly affected by environmental factors such as climate. One interesting finding of the study was discovery of yet uncultured species. In summary, they observed 4424 taxa in total, 31 of which belonged to core taxa with more than 97% prevalence.

The relationship between the quality of the indoor environment and human health has been investigated since the time spent indoors has increased significantly in the last decades. A study conducted in the late 1990s (Sudakin, 1998) reported on a building with water leakage and then shortly afterwards the office workers in the building began to suffer from upper and lower respiratory system issues. After the building was vacated due to severe building dampness and subsequent mold formation, microbial samplings were conducted from air and surface of building materials. High numbers of fungal and bacterial colonies were found in the samples collected from air ducts, ceiling tiles, carpets, HVAC unit, window sills etc. Fungal genera found predominantly in air, bulk, swab and liquid samples were *Penicillium, Phoma* and *Cladosporium*. Building's occupants have reported that they have suffered from fatigue, headaches, mold allergies, asthma and bronchitis while they were working in the building. Sahlberg et al. (2009) conducted a longitudinal study between years 1989 and 1997 in Sweden, investigating the how different exposures indoors have affected the symptoms related to sick building syndrome (SBS). 348 people participated in the study and they were asked about their smoking habits, allergies and symptoms such as eye irritation, nasal obstruction, sore throat, cough etc. The dwellings were also evaluated based on building age and type, moisture,

ventilation system, wall-to-wall carpeting, microbial growth, and odor problems. Results showed that number of smokers and asthma cases have decreased from 1989 to 1997. Also the indoor environment was improved during the 8-year study as water leakage in buildings was taken care of and consequently prevalence of mold formation and indoor moisture decreased. When symptoms were analyzed, smokers' symptom score was significantly higher than non-smokers' score, especially regarding the recurring mucosal symptoms.

One of the most important indoor spaces that was worth investigating in terms of microbial loads was schools. Since young individuals aged 5 to 17 spend a significant amount of their time in school buildings, studies on the quality of the environment in those buildings have been the topic of scientific research for many years. Kallvik et al. (2016) conducted a voice hoarseness study in Finland with 1857 children from ages 6 to 9. They have also evaluated the buildings of the schools that children attended in terms of moisture damage, which was associated with microbial growth. Their results showed that voice hoarseness and indoor air problems caused by moisture damage were significantly correlated, as the moisture damage became more severe, number of the children suffering from voice hoarseness increased. In a more recent study Fu et al. (2020) investigated the relationship between the indoor microbiome and asthma cases in junior high schools in Johor Bahru, Malaysia. They have collected floor dust from 21 classrooms and also evaluated the physical conditions. They have concluded that moisture damage in the buildings and visible mold has contributed to the increase in the asthma cases, Ascomycota being the dominant fungal phylum. In a 2021 study Fu et al. conducted a metagenomic study in the floor dust collected from 5 urban and 5 rural high schools in Shanxi province, China. 1332 students were asked to complete questionnaires on experienced symptoms related to unhealthy indoor environment and 944 of them participated. 46.3% of the urban students experienced SBS symptoms, while it was 36.8% for rural students. The most common symptoms were nasal, throat and tiredness. Abundance of Actinobacteria was found to be correlating with SBS symptoms. Norbäck et al. (2016) investigated the relationship between SBS symptoms in students and microbial load at vacuumed dust in schools in Malaysia. 8 secondary schools were selected for this study and 32 classrooms were vacuumed for dust. They analyzed the dust for three hydroxyl fatty acids (for detection of endotoxins), muramic acid (for detection of Gram-positive bacteria), ergosterol (for detection of total fungal load) and five fungal DNA sequences. Important results of the study included high concentrations of two

hydroxyl fatty acids with the implication of increased risk for rhinitis and dermal symptoms. Total fungal DNA and *Aspergillus* spp. and *Penicillium* spp. (*Asp*/*Pen*) DNA were found in all dust samples. The most abundant fungal species was *Aspergillus versicolor* with 87%, followed by *Stachybotrys chartarum*, and *Streptomyces* spp. Another important finding was the strong correlation between fatigue and high concentration of *A. versicolor* in the classrooms. They also found that as the amount of dust increased there were also increases in the rhinitis and ocular symptoms. Students mostly reported fatigue and headache as the main symptoms followed by rhinitis, irritated throat, ocular symptoms and dermal symptoms.

1.2. Motivation

The indoor environment is an important factor in determining the school children's academic performance. This thesis is one of the first studies conducted in Türkiye that sets out to map the microbial load in terms of abundance and diversity in selected schools within Balçova district of İzmir, Türkiye.

1.3. Thesis Overview

This thesis is composed of 5 chapters. The first chapter begins with giving an introduction on the brief history of industrialization and its facilitation of urbanization. Then the significant increase in the time spent indoors and its causes were mentioned. The time spent indoors brings out the importance of the indoor environment's quality in terms of exposure to volatile and semi-volatile organic chemicals and microbial load. Since this thesis is interested in determining the indoor microbial loads in schools, significance of microbial diversity is also included.

The second chapter endeavors to cover the related scientific literature and the gap that is needed to be filled. The chapter includes the microbial diversity in the built environment, epidemiology of the indoor microbiota and monitoring techniques that used for studying the indoor microbiota.

The third chapter covers the methodology that was followed in this thesis. Schools at Balçova district, which were selected as the study areas, were introduced. Preparation of the growth medium is conveyed along with sampling, storage and incubation

procedures. Due to economic constraints pooling needed to be done in the study. Then DNA extraction and quantification were conducted. As a preparation step to the DNA sequencing step, PCR was conducted with universal primer sets and the amount and purity of the amplicons were determined using DNA agarose gel electrophoresis. Also Sanger sequencing was conducted in this study through purchase of services.

The fourth chapter discusses the results obtained. Due to economic constraints only bacterial samples were sent to Sanger sequencing. Therefore, evaluation of fungal growth was done based on observations according to mostly color, shape, size, etc. Bacterial results were evaluated based on the performance of DNA extraction, purity and concentration. Lastly, the sequencing results were discussed.

The fifth chapter concludes the thesis study, while briefly mentioning the future studies that can be done.

CHAPTER 2

LITERATURE REVIEW

2.1. Microbial Diversity in the Built Environment

Microorganisms are everywhere on Earth, even in extraordinary places like deep seas, and highly acidic, alkali, salty, hot and cold environments. Therefore, it is not surprising that microorganisms can also be found abundantly in built environments. The advent of the molecular techniques in recent decades have enhanced our understanding and appreciation of the diversity of microbial consortiums found in different environments. Since microbiota is generally quite diverse, some of the microorganisms can be beneficial while others can be harmful (i.e. pathogens). Previous studies have demonstrated that humans and the type of their built environment (urban vs. rural) are significant factors in determining the microbial diversity. Humans interact with the surfaces and the indoor air, changing the microbial loads, diversity and abundance. Culture-dependent studies and studies done with advanced molecular techniques demonstrated some core microbiota for the indoor environment (Gilbert and Stephens, 2018). The most common fungal genera include *Cladosporium*, *Penicillium, Aspergillus* and *Stachybotrys* (in water damaged buildings), whereas the most commonly found bacterial taxa are *Corynebacterium, Staphylococcus, Lactobacillus, Streptococcus, Enterobacteriaceae, Acinetobacter, Sphingomonas, Mycobacterium, Methylobacterium, Bacillus* and *Pseudomonas*. It should be noted that since microorganisms lose viability in dry environments, the water availability in a given material (water activity) governs the viability of the microorganisms. Also, other important parameters that determine if a given surface is going to be the host for or hostile towards microorganisms are chemical composition, pH and the physical properties of surfaces. Therefore, water damage and consequent dampness in a building creates the perfect environment for microbial growth.

2.2. Epidemiology of Indoor Microbiota

In cases such as outbreaks and pandemics, as it had been in the focus of the entire world for the last three years, pathogens in the built environment gain unprecedented attention. In the indoor microbiome, bacterial, fungal and viral pathogens can be found. Bacterial pathogens such as *Legionella pneumophila* and *Mycobacterium tuberculosis* can be found while investigating the Legionnaires disease/Pontiac fever and tuberculosis cases, respectively (CDC, 2022). Pontiac fever has an incubation period of 36 hours, whereas it is 2-10 days for Legionnaires disease. Pontiac fever, as it can be understood from the name, causes fever, as well as headache and muscle aches. Legionnaires disease may cause death as patients suffer from pneumonia.

Cryptococcus neoformans, *Histoplasma capsulatum*, *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus terreus* can be listed among fungal pathogens found indoors. *C. neoformans* infection is generally seen at people with compromised immune systems. Especially HIV-related cryptococcal meningitis is still a problematic issue for the Sub-Saharan Africa, because it claims the lives of more than 162,000 people every year (CDC, 2022). *H. capsulatum* causes histoplasmosis, a disease with mild symptoms such as headache, fever, dry cough, and fatigue. It spreads as the soil contaminated with bat or bird droppings is disturbed, so it is generally common in the countryside (VDH, 2022). The *Aspergillus* spp. spread as the spores of the fungi are inhaled. While it may not cause any problems in healthy individuals, people with compromised immune systems may develop conditions ranging from allergies to invasive pulmonary aspergillosis. *A. fumigatus* infections have high mortality rates (Bandres et al., 2022).

Rhinovirus and influenza viruses can be transmitted by direct inhalation. Human rhinovirus may cause asymptomatic infections, upper respiratory system infections (common cold, acute otitis, rhinosinusitis), lower respiratory system infections (Croup, bronchiolitis, community-acquired pneumonia) and chronic pulmonary infections (asthma and chronic obstructive pulmonary disease) and cystic fibrosis (Jacobs et al., 2013). There are four types of influenza viruses (A, B, C, D), of which influenza A viruses present the highest in terms of public health. Seasonal influenza is known to spread fairly quickly in crowded indoor spaces, therefore, seasonal outbreaks occur. When an infected person talks, coughs or sneezes, in the absence of a mask, the infectious droplets can enter

the breathing range of other people, causing the spreading of the virus. Symptoms include headache, cough, fever, and nasal congestion (WHO, 2022).

2.3. Monitoring techniques for indoor microbiota

For microbial monitoring, traditional microbial culturing techniques were very common from the early 20th century to the beginning of 21st century. These techniques are still very common for isolation of single colonies of targeted pathogens, diagnosis of suspicious microbial agents, respiratory and skin illnesses causing fungi and bacteria. Traditional microbial monitoring starts with inoculation of a rich non-selective growth media for bacteria, fungi, or both of them together. After inoculation, growth media is put into certain and predetermined or specific temperature conditions for specific time periods. The incubation temperature can vary from 4°C to 38°C for both bacteria and fungi. Also, incubation time period mostly changes from 24 hours to 48 for bacteria or for slow growing bacteria up to 72 hours, and 2 days to 14 days for fungi. After the first inoculation, each culture was selected for culture isolation in selective media or rich media. This step may be replaced or followed by selective media inoculation with selected colonies to obtain targeted or suspected colonies and colony forming units (CFU). For the first step the mainly used rich non-selective media are blood agar, trypticasein soy agar, Sabouraud dextrose agar, potato dextrose agar. Adding antibiotics or antimycotic to these growth media makes them selective for only bacteria, only yeast, or certain types of bacteria or fungi. Other types of selective media are for some extraordinary conditions like pH spectrum, presence of sodium salts, chemicals, and dyes. As pH is an important parameter for biochemical reactions and so, metabolism, microbial growth is limited by pH condition. Even pH is a limitation, some kind of microorganisms are adapted to live and growth in extraordinary pH spectrums like >12.5 and <3.5. These conditions select highly alkaliphilic microorganisms above pH 12.5 and highly acidophilic microorganisms below pH 3.5. Another condition of presence of sodium salts. In the selective culture media, which contains an amount of sodium salt, only halophilic bacteria can live and grow. Adding chemical substances is used to inhibit Gram positive or Gram negative bacteria growth against each other. Dyes are used to inhibit bacterial growth. Most used dye to inhibit bacterial growth is crystal violet. Crystal violet inhibits growth of Gram positive bacteria but Gram negative. On the other hang Malachite green inhibits

growth of both Gram positive and Gram negative bacteria (Bonnet, Lagier, Raoult, & Khelaifia, 2020). The advantage of the traditional microbial culturing techniques is storing the isolated microorganisms, observation of colony properties, experimenting growth conditions and limits. But for monitoring, traditional microbial culturing methods requires time, labor, an expense of consumables. For microbial monitoring, beside traditional microbial culturing methods, the culture free advantage of modern molecular DNA analysis methods are involved in. Skipping culturing methods after sampling, saves time, labor, and cost. In modern molecular DNA analyzing methods, DNA molecules are directly isolated from the collected samples and prepared vie mostly PCR based methods depending on the further steps. One of the analyze way is Sanger sequencing but even it gives sequence result per sample, it is not a high-throughput sequencing (HTs) method. HTS methods give multiple sequence results per sample, and this is the biggest advantage of it for taxonomy and biodiversity analyses. With multiple sequences, more DNA sequence matches are obtained from the online DNA databases. To obtain the DNA sequence matches from DNA databases, several computer software and online tools, and bioinformatic methods are developed. With the help of all together taxonomy identifications are made more rapid and precise.

In this study both traditional and modern methods are combined. Even HTS methods are better than Sanger sequencing, it is chosen to make an initial combination of both traditional and modern molecular techniques to save time and cost.

Several studies have been conducted on assessment of microbial consortia and concentrations (bacterial, fungal, etc.) in indoor air using different analyses methods. For instance, Mucci et al. (2020) investigated the impact of air microbiota on human health in different indoor environments using next-generation sequencing (NGS) based metagenomics. Indoor biological monitoring was performed in recreational facilities, public restrooms, and offices by both active and passive sampling methods. The passive sampling method was utilized with tissue-based sampling (TBS) and index of microbial air contamination (IMA), while active sampling was operated using high-volume aerosol collection system (HAC) and surface air system (SAS). Results showed that while colonies could not be observed for HAC and TBS sampling protocols, SAS and IMA sampling procedures yielded 30±10 and 2±1 isolates, respectively. These observations verified the limited capabilities of culturing methods. Following the culturing step, DNA extraction and 16S rDNA sequencing were conducted. The metagenomics study yielded over 3.2 million sequence reads with identification of over 170 operational taxonomic

units (OTUs). While abundant taxa found in indoors were *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Paracoccus*, *Propionibacterium*, *Micrococcus*, the ones found in outdoors were *Bradyrhizobium*, *Delftia*, *Pseudomonas*, *Acinetobacter*, *Brevundimonas*. One of the most important results of this study was that the mode of sampling significantly affected identified species. Active sampling identified *Bradyrhizobium*, *Par*acoccus, *Delftia*, *Pseudomonas*, and *Acinetobacter* as the most abundant genera (11.6 to 4.4% of all sequence reads). On the other hand, passive sampling techniques showed *Staphylococcus* (7.4%), *Corynebacterium* (6%), and *Propionibacterium* (4.6%) as the abundant genera. The high-throughput sequencing (HTS) and quantitative polymerase chain reaction (qPCR) methods were performed to evaluate the fungal concentrations and consortia in personal air, outdoor, and indoor (An et al., 2018). The indoor air samples were collected using a 25 mm membrane air filter inserted air sampler with mini-pump and the air sampling devices were utilized at height of 1 m from the floor in the bedroom. The geometric mean fungal concentrations were 1.3×10^4, 9.3×10^4, and 1.4×10^4 GCN/m^3 for indoor, outdoor, and personal air, respectively. Based on the HTS, the detected total fungal genera were 493 and the 4 most dominant genera with their mean relative abundances values were *Pseudocercospora* (7.9 %), *Catenulostroma* (4.2 %), *Alternaria* (4.1 %), and *Fusarium* (3.6 %). HTS results also presented superior species abundance in personal than indoor air, demonstrating that humans are exposed to outdoor fungal species that are not in indoor air. On the other hand, the indoor: outdoor (I: O), personal: outdoor (P: O), and personal: indoor (P: I) fungi ratios were found to be 0.12, 0.15, and 1.2, respectively, indicating that expose to fungi is better signified by indoor than outdoor fungi concentrations. The fungi ratios, on the other hand, varied per taxon, demonstrating the difficulty of making generalizations human exposure to the complex kingdom Fungi. Overall, results showed that the HTS-qPCR method is efficient for measuring taxon-specific fungal exposure, which might be challenging to perform successfully using non-DNA-based methods.

Over the last decades, the researchers focused on monitoring and analysis of microbial concentrations and communities of indoor surfaces. For instance, the microbial surface contamination considering bacterial, fungal, and human cell concentrations were evaluated using adenosine triphosphate (ATP) and qPCR methods in school classrooms on 66 desk surfaces (Kwan et al., 2019). In sampling, the microbes and ATP were gathered from 2.5 × 2.5 cm areas of desk with cotton swabs with sterile 0.15 M NaCl and 0.1% Tween 20 solution for qPCR sampling and PocketSwab Plus rapid ATP swabs for

ATP sampling. Moreover, linear regression analysis was used to evaluate if the qPCR-measured bacterial, fungal, and human cell measures were independent variables and to analyze correlations between qPCR and ATP concentrations. Regression analyses revealed that the ATP method was an excellent predictor of bacteria (r = 0.7, P =.00001), fungi (r = 0.5, P =.01), human cells (r = 0.5, P =.05), and the qPCR sum (r = 0.7, P =.00002) for all microbiotas. Moreover, the microbial surface cleanliness of school desks was considered with ATP correlated qPCR concentration methods. When compared to qPCR, ATP measurements of cleaning effectiveness are 1.5-2 folds higher on average. Consequently, authors reported that the ATP methods is a promising technology for testing overall bacterial and fungus removal efficiency on school desks because of its cost effectiveness, ease of use, and quick results.

In a separate study, the indoor microbiota at a hospital foodservice (blast chiller, cold storage, food processing rooms, kitchen, and storage room) was studied using a 16S-based culture-independent HTS to explain the possible contamination sources during food cooking and preparation practices (Stellato et al., 2015). Food contact surfaces were sampled using a sterile cotton-tipped swab wet with sterile PBS and rubbed horizontally, vertically, and diagonally over a 100 cm^2 sampling region, turning the swab to ensure thorough contact with all areas of the swab tip and then DNA extraction of samples were performed by a Biostic Bacteremia DNA Isolation Kit. The metagenomics study yielded over 278 thousand sequence reads with average value of 3303 reads per sample. While abundant taxa obtained in storage area, kitchen, and tools were *Alicyclobacillus*, *A. johnsonii*, and *Chryseobacterium*, the ones observed in all pre-processing areas (knife, cutting board, workbench, sink, etc.) were *Alicyclobacillus*, *Acinetobacter johnsonii*, and *Acinetobacter*. Moreover, the *Paracoccus* specie was most abundant OTUs observed in both the pre-processing vegetables (80%) and pre-processing fish (60%). Overall, authors suggested that 16S-based culture-independent HTS method might be quite beneficial for future screenings of food related indoor environments to get a rapid map of the surface microbiota.

In the perspective of detecting indoor microbiota of public schools, there is a gap in the literature. Even there are some studies to detect indoor microbiota of public schools, mostly traditional microbial culturing methods are used. In a study, a school in Istanbul is monitored to detect microbiota. Bacteria were not identified in species or genus but colony forming units (CFU). On the other hand, fungi were identified by the traditional microbial methods. Total of thirteen fungi genus were detected. In their findings,

Penicillium spp., Aspergillus spp., Candida spp. were detected from the isolates (Sivri, Dogru, Bagcigil, Metiner, & Seker, 2020). Detecting in genus can only provide assumptions because not all the species in these genera are infectant. Some of them may cause skin and respiratory diseases and allergies while some of them are safe and used in industry. *Penicillium chrysogenum* is used to produce antibiotic penicillin (Houbraken, Frisvad, & Samson, 2011). Besides being pathogen and opportunistic pathogen some *Aspergillus spp.* are important for industry like organic acid production (de Vries et al., 2017). Also, *Aspergillus oryzae* is known to be used to produce alcoholic beverage called sake (Kitamoto, 2002). So, the importance of detecting species is making more precise assumptions of the danger of pathogens. Bacteria and fungi can be found anywhere and everywhere both indoor and outdoor except special places. So, finding bacteria in CFU is no sense but finding fungi in genus is enough to discriminate pathogens, allergens, and non-infecting fungi. It is known that *Streptococcus spp., Haemophilus spp., and Moraxella spp.* may infect respiratory tract and spread from person to person (Cappelletty, 1998). That is why HTS methods should be included in the scientific studies to obtain more specific data about the microbiota of places that concerns public health.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study Site

The study was determined to be done in three public schools within Balçova district of İzmir, Türkiye. Three schools were Asil Nadir Primary School, 80. Yıl Orhan Gazi Secondary School and, Ahmet Hakkı Balcıoğlu Vocational and Technical Anatolian High School.

Asil Nadir Primary School had a population of 274 male students, 256 female students age between 5- to 10-year-old including nursery class and 29 adult teaching, administrating and service personnel. Also, the school building and floor plans were given below in Figure 3.1..

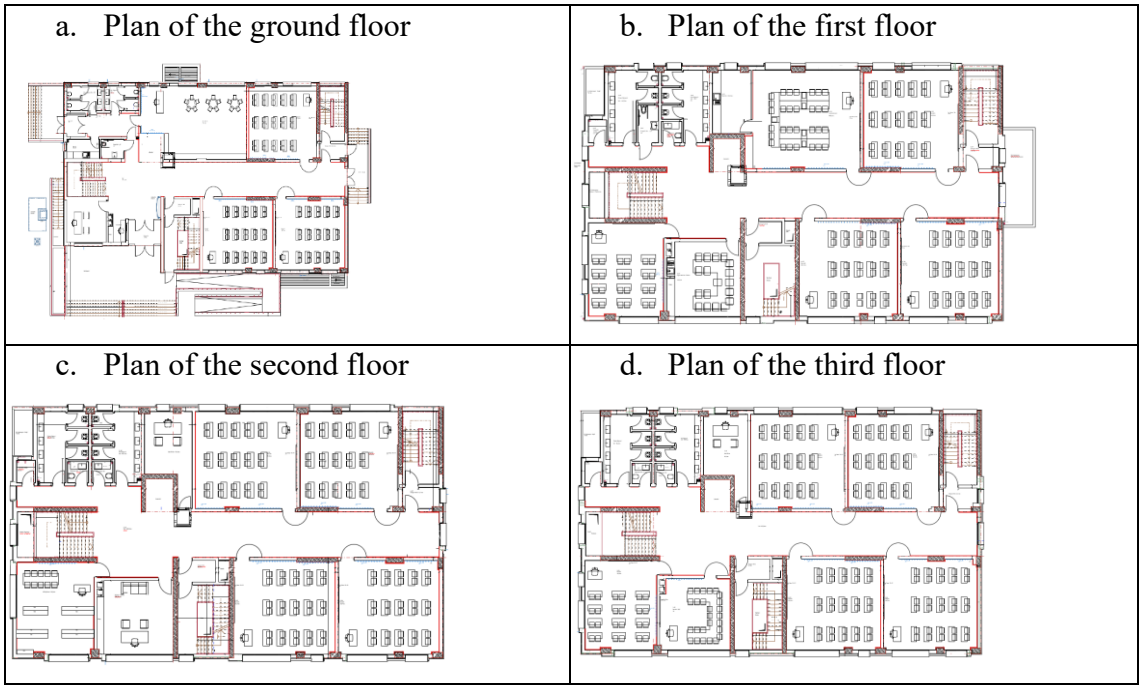


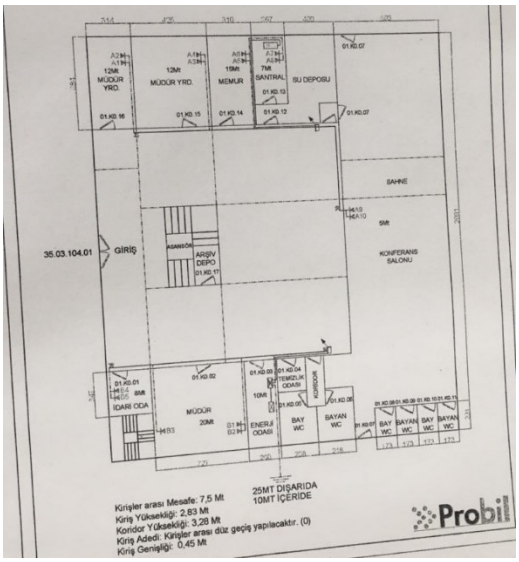
Figure 3.1. Plan of the Asil Nadir Primary School, ground floor (a), first floor (b), second floor (c), and third floor (d).

80. Yıl Orhangazi Secondary School had a population of 316 male students, 311 female students age between 10- to 14-year-old and 48 adult teaching, administrating and service personnel. Also, the school building and floor plans were given below in Figure 3.2..

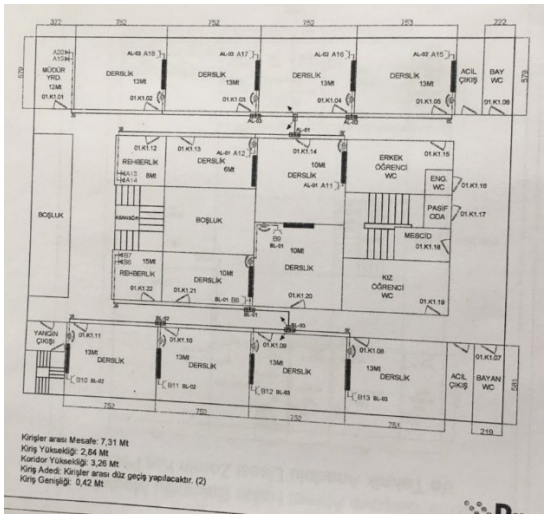
Figure 3.2. Plan of the 80. Yıl Orhangazi Secondary School, basement floor (a), ground floor (b), first floor (c), second floor (d), and third floor (e).

Ahmet Hakkı Balcıoğlu Vocational and Technical Anatolian High School had a population of 149 male students, 103 female students age between 14- to 18-year-old and 45 adult teaching, administrating and service personnel. Also, the school building and floor plans were given below in Figure 3.3..

a. Plan of the ground floor



b. Plan of the first floor



c. Plan of the second floor

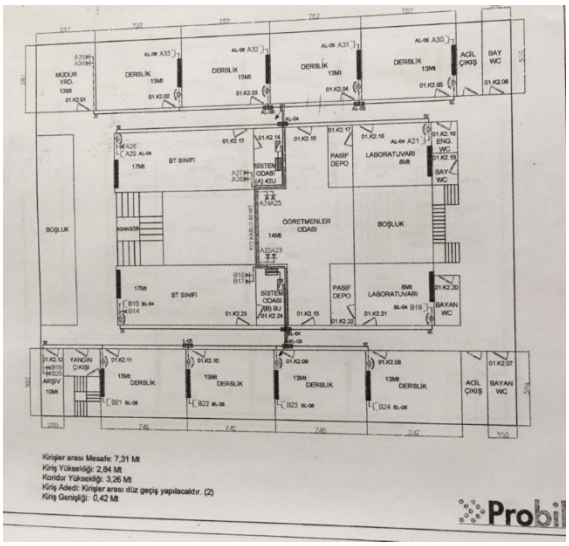


Figure 3.3. Plan of the Ahmet Hakkı Balcıoğlu Vocational and Technical Anatolian High School, ground floor (a), first floor (b), second floor (c).

3.2. Preparation of Growth Medium

Two types of non-selective rich nutrient growth media were selected. For bacteria Trypticasein Soy Agar (TSA) was selected. TSA contains soy proteins and casein as amino acid source and glucose as energy source. For preparation of TSA petri dishes, manufacturer's guide is followed. 40g of TSA powder was suspended in one liter of distilled water and dissolved by heating. Solution was boiled for one minute and sterilized by autoclave under the conditions; at 121°C for 15 minutes. When TSA solution was cooled to 45°C – 50°C, 10ml – 20ml of TSA was poured into each 90x17mm Petri dishes (Condalab, 2022). Petri dishes were sealed with parafilm to prevent air transfer in and out. The preparation process was repeated until enough number of media filled petri dishes were ready. For fungi Sabouraud Dextrose Agar (SDA) was selected. In SDA, dextrose is the carbon and so energy source. Peptone mixture in SDA provides aminoacid, minerals, vitamins, and nitrogen. To prepare SDA, manufacturer's guide was followed. 65g of SDA powder was suspended in one liter of distilled water and dissolved by heating. Solution was boiled for one minute and sterilized by autoclave under the conditions; at 121°C for 15 minutes. When SDA solution was cooled to 45°C – 50°C, 10ml – 20ml of SDA was poured into each 90x17mm Petri dishes (Condalab, 2022). Petri dishes were sealed with parafilm to prevent air transfer in and out. The preparation process was repeated until enough number of media filled petri dishes were ready. All the prepared TSA and SDA containing Petri dishes stored at dishes stored at 4°C.

3.3. Sampling, Storage, and Incubation

A protocol was signed with the Balçova Provincial Educational Directorate in order to conduct a sampling campaign of the microbial loads regarding three selected schools and sharing of collected data. The sampling campaigns were conducted twice, the first one being at the mid-academic year break (February 2022) and the second one in May 2022 while school children were present.

3.3.1. Surface Sampling

Surface sampling was done on the pre-determined surfaces from schools in Balçova province İzmir. Almost the same spots in all three schools; primary school, secondary school, and high school were selected. Sampling spots were listed in the Table 3.1. and Table 3.2.. Sampling was performed by using sterile swabs. Coverage of sterile swabs were opened just next to the sampling spot and sterile swabs were wet by dipping into distilled water. Following the wetting step, wet sterile swabs were smeared on the sampling surface vertically, horizontally, and diagonally as illustrated in Figure 3.4 (Texwipe, n.d.; DES, 2018). On float surfaces like student desk, corridor wall, teachers' meeting desk, etc. 10 x 10 cm square frame was used to keep equal sampling by cm^2. On little but float surfaces like door handle and window handle, 5 x 1 cm frame was used to keep equal sampling by cm^2. On shapeless or too little surfaces like bidet nozzle valve or toilet flush, frames were not used. While smearing, wet sterile swabs also were rotated to make cotton tip of the swabs fully covered by the sample. Surface sample covered swabs were smeared directly on both Sabouraud Dextrose Agar Media and Trypticasein Soy Agar Media to transfer the surface sample to the growth media to perform spread plate technique. After smearing swabs to the media, for background samples, cotton tips of the swabs were and put into 99.6% Ethyl alcohol in 2 ml Eppendorf tubes and broke. For actual samples cotton tips of the swabs were put into 1X Phosphate buffered saline (PBS) in 2 ml Eppendorf tubes and broke. Both TSA and SDA petri dishes were sealed with parafilm after inoculation, and each was put into lock pockets.

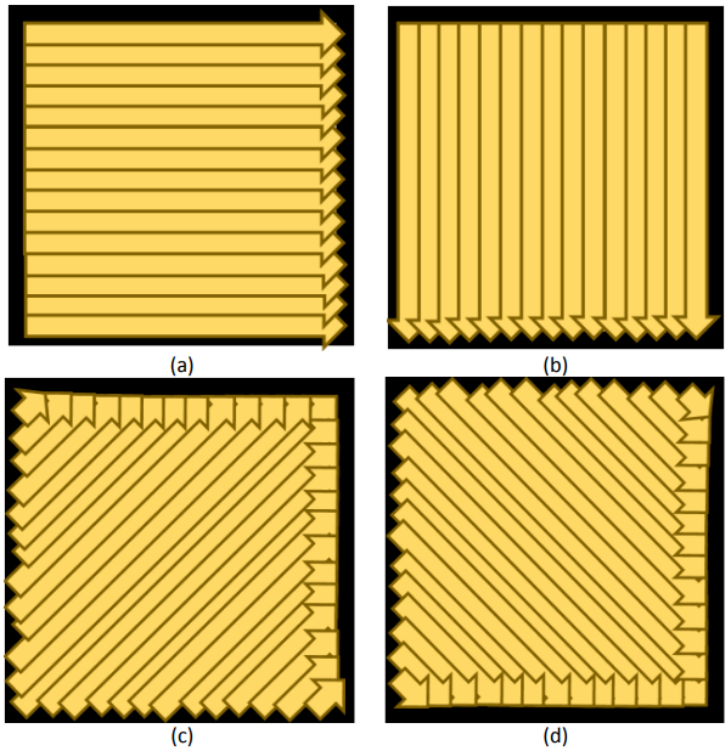


Figure 3.4. llustration of swab sampling directions in a determined area shown as black: Horizontally swabbing the area from left to right (a), Vertically swabbing the area from top to bottom (b), Diagonally swabbing the area from bottom left to top right (c), Diagonally swabbing the area from top left to bottom right (d).

Table 3.1. Background sampling campaign: sampling area identification, number of collected samples per school.

Sampling (Number/Area/Spot)			**Number of samples taken**	**Asil Nadir Primary School (1)**	**80. Yıl Orhangazi Secondary School (2)**	**Ahmet Hakkı Balcıoğlu Voc. and Techn. Anatolian High School (3)**
1	**Entrance**	Door inside handle	1	x	x	x
2		Door outer handle	1	x	x	x
3	**Corridor**	Wall	1	x	x	x
4	**Classroom**	Door inside handle	1	x	x	x
5		Door outer handle	1	x	x	x
6		Desk surface	1	x	x	x
7		Window handle	1	x	x	x
8		Teachers' meeting desk	1	x	x	x

(Cont. on next page)

Table 3.1. (Cont.)

9		Warm air heating unit	1	x	x	x
10	**Workshop**	Desk surface	1			
11	**Library**	Desk surface	1			x
12		Book cover	1			x
13	**Stairs**	Handrail	1	x	x	x
14		Wall	1	x	x	x
15	**Teachers' Lounge**	Teachers' meeting desk	1	x	x	x
16		Kitchen bench	1	x	x	x
55		Computer mouse	1	x	x	x
17		Door inside handle	1	x	x	x
18		Door outer handle	1	x	x	x
19	**Men's Bathroom**	Door inside handle	1	x	x	x
20		Door outer handle	1	x	x	x
21		Inner cabinet door inside handle	1	x	x	x
22		Inner cabinet door outer handle	1	x	x	x
23		Toilet flush	1	x	x	x
24		Bidet nozzle	1	x	x	x
25		Tap mixer	1	x	x	x
26		Towel rock	1	x	x	
27	**Girls' Bathroom**	Door inside handle	1			
28		Door outer handle	1			
29		Inner cabinet door inside handle	1			
30		Inner cabinet door outer handle	1			
31		Toilet flush	1			
32		Bidet nozzle	1			
33		Tap mixer	1			
34		Towel rock	1			
35	**Faculty Bathroom**	Door inside handle	1			
36		Door outer handle	1			
37		Inner cabinet door inside handle	1			
38		Inner cabinet door outer handle	1			
39		Toilet flush	1			

(Cont. on next page)

Table 3.1. (Cont.)

40		Bidet nozzle	1			
41		Tap mixer	1			
42		Towel rock	1			
43	**Computer Lab**	Keyboard	1	x	x	x
44		Computer mouse	1	x	x	x
45		Desk surface	1	x	x	x
46	**Dining Hall**	Desk surface	1			
47		Bench	1			
48		Food serving bench	1			
49	**Sports equipment**	Basketball	1	x	x	
50		Table-tennis bat	1	x	x	
51		Football	1	x	x	
52	**Science Lab**	Desk surface	1	x	x	
53		Equipment	1	x	x	
54	**Special-ed class**	Desk surface	1	x	x	x
56	**Nursery class**	Desk surface	1	x	x	
57		Toy	1	x	x	
	Total number of numbers		57	35	35	29

Table 3.2. Sampling campaign: sampling area identification, number of collected samples per school.

Sampling (Number/Area/Spot)			**Number of samples taken**	**Asil Nadir Primary School (1)**	**80. Yıl Orhangazi Secondary School (2)**	**Ahmet Hakkı Balcıoğlu Voc. and Techn. Anatolian High School (3)**
1	**Entrance**	Door inside handle	1	x	x	x
2		Door outer handle	1	x	x	x
3	**Corridor**	Wall	1	x	x	x
4	**Classroom**	Door inside handle	1	x	x	x
5		Door outer handle	1	x	x	x
6		Desk surface	1	x	x	x
7		Window handle	1	x	x	x
8		Teachers' meeting desk	1	x	x	x
9		Warm air heating unit	1	x	x	x
10	**Workshop**	Desk surface	1		x	

(Cont. on next page)

Table 3.2. (Cont.)

11	**Library**	Desk surface	1		x	x
12		Book cover	1	x	x	x
13	**Stairs**	Handrail	1	x	x	x
14		Wall	1	x	x	x
15	**Teachers' Lounge**	Teachers' meeting desk	1	x	x	x
16		Kitchen bench	1	x	x	x
17		Computer mouse	1	x	x	x
18		Door inside handle	1	x	x	x
19		Door outer handle	1	x	x	x
20	**Men's Bathroom**	Door inside handle	1	x	x	x
21		Door outer handle	1	x	x	x
22		Inner cabinet door inside handle	1	x	x	x
23		Inner cabinet door outer handle	1	x	x	x
24		Toilet flush	1	x	x	x
25		Bidet nozzle	1	x	x	x
26		Tap mixer	1	x	x	x
27		Towel rock	1		x	
28	**Girls' Bathroom**	Door inside handle	1	x	x	x
29		Door outer handle	1	x	x	x
30		Inner cabinet door inside handle	1	x	x	x
31		Inner cabinet door outer handle	1	x	x	x
32		Toilet flush	1	x	x	x
33		Bidet nozzle	1	x	x	x
34		Tap mixer	1	x	x	x
35		Towel rock	1		x	
36	**Faculty Bathroom**	Door inside handle	1	x	x	x
37		Door outer handle	1	x	x	x
38		Inner cabinet door inside handle	1		x	x
39		Inner cabinet door outer handle	1		x	x
40		Toilet flush	1	x	x	x
41		Bidet nozzle	1	x	x	x
42		Tap mixer	1	x	x	x
43		Towel rock	1		x	x
44	**Computer Lab**	Keyboard	1		x	x
45		Computer mouse	1		x	x

(Cont. on next page)

Table 3.2. (Cont.)

46		Desk surface	1		x	x
47	**Dining Hall**	Desk surface	1	x		x
48		Bench	1	x		x
49		Food serving bench	1	x		x
50	**Sports equipment**	Basketball	1	x	x	
51		Table-tennis bat	1			
52		Football	1		x	
53	**Science Lab**	Desk surface	1		x	
54		Equipment	1		x	
55	**Special-ed class**	Desk surface	1			x
56	**Nursery class**	Desk surface	1	x	x	
57		Toy	1	x	x	
	Total number of samples		57	42	52	47

3.3.2 Air Sampling

Air sampling is done on the pre-determined spots via settle plate air sampling method. Sabouraud Dextrose Agar Media and Trypticasein Soy Agar Media containing lid-open petri plates were put onto average one and half meters higher than the ground and let the air settle for average four hours. Background settle plate air sample spots and duration of being lid-open were shown in Table 3.3 and settle plate air sampling spots and duration of being lid-open were shown in Table 3.4. Both TSA and SDA petri dishes were sealed with parafilm after inoculation, and each was put into lock pockets.

Table 3.3. Background settle plate air sampling spots

Sampling Spots	**Number of samples taken**	**Asil Nadir Primary School/ Duration(min)**	**80. Yıl Orhangazi Secondary School/ Duration(min)**	**Ahmet Hakkı Balcıoğlu Vocational and Technical Anatolian High School Duration(min)**
Entrance	1	x/288	x/247	x/228
Outside	1	x/253	x/245	x/226
Teachers' Lounge	1	x/276	x/244	x/222
Men's Bathroom	1	x/275	x/242	x/224
Clasroom	1	x/278	x/246	x/226

Table 3.4. Settle plate air sampling spots

Sampling Spots	**Number of samples taken**	**Asil Nadir Primary School/ Duration(min)**	**80. Yıl Orhangazi Secondary School/ Duration(min)**	**Ahmet Hakkı Balcıoğlu Vocational and Technical Anatolian High School/ Duration(min)**
Entrance	1	x/220	x/183	x/202
Outside	1	x/223	x/173	x/200
Teachers' Lounge	1	x/216	x/209	x/206
Men's Bathroom	1	x/218	x/205	
Clasroom	1		x/213	x/226

3.3.3. Storage and Incubation

Cotton swab tip containing Eppendorf tubes stored in -24°C for long term storage (longer than two weeks). Inoculated Sabouraud Dextrose Agar Media containing petri dishes were stored at room temperature for two weeks for incubation (Bills, Christensen, Powell, Thorn, 2004) then stored at 4°C for later processes. Inoculated Trypticasein Soy Agar Media containing petri dishes were stored at 38°C for 48 hours for incubation (Moldenhauer, 2014) then stored at 4°C for later processes.

3.4. Homogenization and Pooling

Samples from the same groups and groups with similar properties were homogenized and pooled to lower the amount of sample processing while conserving the differentiating properties of the sample groups. Homogenization of the sample groups were shown in Table 3.5 and Table 3.6. Stored swabs were aseptically chopped with hand and put into 1 ml of 1X PBS containing Eppendorf tube of each group. Grown colonies and cultures of bacteria and if there were other microorganisms were transferred to the same Eppendorf tube of each group aseptically. Each group were mechanically damaged to make it homogenous by vortex at max level of the machine for average 30 seconds.

Table 3.5. Homogenized/pooled background sample groups. First and second numbers on the code representing sampling location and the school, respectively.

Homogenized/pooled Groups of Primary School		**Number of spots**	**Homogenized/pooled Groups of Secondary School**		**Number of spots**	**Homogenized/pooled Groups of High School**		**Number of spots**
H1B1	Entrance	5	H1B2	Entrance	5	H1B3	Entrance	5
	Corridor			Corridor			Corridor	
	Stairs			Stairs			Stairs	
H2B1	Classroom	6	H2B2	Classroom	6	H2B3	Classroom	6
H3B1	Teachers' lounge	5	H3B2	Teachers' lounge	5	H3B3	Teachers' lounge	5
H4B1	Men's bathroom	7	H4B2	Men's bathroom	8	H4B3	Men's bathroom	7
H8B1	Nursery class	2	H5B2	Computer lab	3	H5B3	Computer lab	3
			H8B2	Nursery class	1	H6B3	Library	2
						H7B3	Special-ed class	1

H: homogenized; B: background.

Table 3.6. Homogenized sample groups

Homogenized/pooled Groups of Primary School		**Number of spots**	**Homogenized/pooled Groups of Secondary School**		**Number of spots**	**Homogenized/pooled Groups High School**		**Number of spots**
H1	Entrance	5	H1	Entrance	5	H1	Entrance	5
	Corridor			Corridor			Corridor	
	Stairs			Stairs			Stairs	
H2	Classroom	6	H2	Classroom	6	H2	Classroom	6
H3	Teachers' lounge	5	H3	Teachers' lounge	5	H3	Teachers' lounge	5
H4	Men's bathroom	7	H4	Men's bathroom	8	H4	Men's bathroom	7
H5	Girls' bathroom	7	H5	Girls' bathroom	8	H5	Girls' bathroom	7
H6	Faculty Bathroom	5	H6	Faculty bathroom	8	H6	Faculty bathroom	8

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Table 3.6. (Cont.)

H7	Canteen	3	H8	Sports equipment	2	H7	Canteen	3
H8	Sports equipment	1	H9	Nursery class	2	H10	Library	2
H9	Nursery class	2	H10	Workshop Library	3	H11	Computer lab	3
H10	Library	1	H11	Computer lab	3	H12	Special-ed class	1

3.5. DNA isolation

Before PCR amplification, total DNA isolation was done on all homogenized samples. To perform total DNA isolation Hibrigen Bacterial DNA Isolation Kit (Hibrigen, Gebze) was used and manufacturer's protocol was followed. The isolation process was done in biological safety cabinet at Department of Molecular Biology and Genetics, Karakaya Lab. Total DNA isolates were stored in manufacturer's storage buffer at -20°C until quantification by NanoDrop spectrophotometer and DNA fragment amplification via PCR.

3.6. Quantification of DNA

Quantification of DNA was done by NanoDrop spectrophotometer. NanoDrop spectrophotometer measures the purity and concentration of DNA, RNA, and proteins in a very small volume like 0.5 µl to 2 µl of sample. For DNA samples, the measurement of the absorbance is set at 260, 280 and 230. DNA and RNA absorb at 260 nm, proteins mostly absorb at 280 nm, and carbohydrates mostly absorb at 230 nm wavelength. 260/280 and 260/230 wavelength ratios tell the purity of DNA and RNA. A measurement of pure DNA should result in about 1.8 and 2.0-2.2 in the order. Contaminants like proteins lowers 260/280 absorbance measurement and contaminants like carbohydrates lowers 260/230 absorbance measurement (García-Alegría et al., 2020).

For this study extracted bacterial and fungal sample DNA purity and concentrations were measured by using NanoDrop 8000 Spectrophotometer at IZTECH

Biotechnology and Bioengineering application and Research Center. Before measuring the concentration of the isolation samples, storage buffer was used to set background. The DNA purity and concentrations of Eight samples were measured at the same time in the order thanks to the capability of the device.

3.7. Polymerase Chain Reaction

All the total DNA isolates were amplified via Polymerase Chain Reaction (PCR). For biodiversity analysis, 16S ribosomal ribonucleic acid gene (V4-5) region was aimed for bacterial samples and internal transcribed spacer (ITS) gene was aimed for fungal samples. For PCR, primer sets for both 16S rRNA gene and ITS were listed below in the direction of 5' end to 3' end:

16S rRNA (V4-5) gene primer set:
Forward: 515f
5' – GTGCCAGCMGCCGCGGTAA – 3'
Reverse: 926r
5' – CCGYCAATTYMTTTRAGTTT – 3'
ITS gene primer set:
Forward: ITS1F
5' – CTTGGTCATTTAGAGGAAGTAA – 3'
Reverse: ITS2
5' – GCTGCGTTCTTCATCGATGC – 3'

Following PCR protocol was used for both isolated bacterial and fungal total DNA. Reagent mixture was composed of 13 µl RNase free PCR-grade water, 10µl 5' Hot master mix, 0.05 µl 10 µM forward primer, 1 µl 10 µM reverse primer, 1 µl template DNA in a total 25 µl volume of reaction. The set PCR amplification conditions to the 96-well thermal cycler were initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 60 s, extension at 72°C for 90 s, final extension at 72°C for 10 min, and 4°C hold (Walters et al., 2015).

3.8. DNA Agarose Gel Electrophoresis

DNA is negatively charged due to phosphate groups in its backbone. Phosphate groups contain oxygen, and the oxygen atom is negatively charged in the phosphate group. The backbone of DNA is composed of sugar and phosphate group chain for both strands and due to the high amount of negatively charged phosphate groups in DNA backbone, DNA molecules are negatively charged. This electrochemical property of DNA molecule is used in the method of DNA agarose gel electrophoresis (AGE). The designed tool for AGE is basically produces and directs a direct current (DC) from one end of the device to the other end. In the middle of the DC, agarose gel is placed. Agarose gel consists of an amount of agarose and Tris-acetate-EDTA (TAE) buffer. While making the gel, with the help of some supporting tools, wells are made at the one end of the gel to be a place for DNA placement for the method. In the DC, DNA molecules goes from negative to positive end of the device within agarose gel. Due to the different sizes of the DNA fragments, each fragment goes with different velocity. So, at the end of the progress, each DNA fragment is placed at different place of the gel. With the help of a DNA ladder which contains known DNA fragments in length, each progressed DNA fragment can be measured in length (Steward, 2022).

For this study, after PCR, AGE was done for each sample to see if PCR worked or not. To prepare 1% agarose gels with 8 + 1 wells, 0.3 g of agarose gel was suspended in 30ml of TAE buffer via heating in microwave oven and after letting it cool for about 10 minutes, 2.5µl of ethidium bromide (EtBr) was added to the solution before pouring to 8x7 cm gel tray. The solution was poured into the gel tray and 8 + 1 tooth comb was placed to make wells. When the agarose solution was cooled down and became solidified gel, it was ready to be used for AGE. Each AGE was done with 5 µl of DNA ladder in the first well next to the bonus well and 7 amplified DNA samples in the following 7 wells in the order of the sample number. The last two AGE runs were done with 8 samples instead of 7 by adding lowered volume of (3µl) ladder to bonus well. Each amplified DNA sample was mixed with loading dye in the ratio of 4(DNA):1(loading dye) and total volume of the mixture was 5 µl. For AGE, time was set to 1 hour and voltage was set to 80 V. The expected length of the fragments was about 500 bp.

3.9. Sanger Sequencing

All the PCR products were sent to be sequenced. The sequence method was determined as Sanger Sequencing. For Sanger sequencing, PCR products were sent to Letgen Biotech (Letgen, İzmir) via service procurement. Simply, Sanger sequencing aims to identify the sequence of targeted DNA fragments by primers. First double stranded DNA fragments become denatured to obtain single stranded DNA fragments. Single stranded DNA fragments are the target of primers. In the same batch, in addition to DNA fragments and primers, deoxynucleotide triphosphates (dNTPs; dATP, dTTP, dCTP, dGTP) and dideoxynucleotide triphosphates (ddNTPs; ddATP, ddTTP, ddGTP, ddCTP) were added. dNTPs were needed to form double stranded DNA and it continues until termination by a ddNTP. Each ddNTP carries a base specific fluorescent marker, such as red for tyrosine. Each fragment is separated in gel electrophoresis according to its size. Each fragment's fluorescent marker gives different signal and so from the order detected from the gel, the sequence of the fragment is understood (Solomon, 2018; Gomes, Korf, 2018).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Fungal Culture Growth

TSA and SDA culture media incubation was done as it was mentioned in Chapter 3. Growth of fungal and bacterial cultures were seen in all the cultures except the petri dish named SDA16B2 which refers to Sabouraud dextrose agar media inoculated at 80. Yıl Orhan Gazi Secondary School, Teachers' Lounge, Kitchen Bench. The observation was only growth positive and growth negative and done by eye of the observer. The results were photographed via Oppo A9 2020 Smartphone 48MP Ultra-Wide Quad Camera by the observer.

From the observations, many fungal cultures were seen mixed in all the plates. Some of the plates were not much mixed or were not mixed at all. With eye, only genus assumptions could be made. For the assumptions the reference images and descriptions from online fungi library, Mold Busters was used. To make better assumptions and especially diagnosis, the opinion of an expert of mold was required but it was not possible. The clearest assumptions were genus; *Aspergillus, Penicilium, Scopulariopsis, Candida, Alternaria, Trichosporon, Rhizopus, Absidia, Chrysosporium, Microsporum, Cladosporium, Stachybotrys, Wallemia, Eurotium, Microascus, Sporothrix, Mucor, Rhodotorula, Nigrospora, Arthrinium, Bipolaris, Chaetomium.* The most common point of these genus are they are found indoor so, both references and results of the culture observations were supporting each other. On the other hand, it is very important to be sure about the presence of these fungi by more precise methods like modern molecular methods or specific chemical tests as traditional microbial techniques because the other common point of these genera was that they are all causing airborne infections especially when the immune system weak (Bust Mold Inc., 2022). Even the immune system is not weak, continuous fungal exposure may cause long term serious health issues. The most common disease caused by fungi is allergy. Especially after cold seasonal times, when the immune system is weak, allergens of the fungi like spores, place and infect respiratory track. When the subject is urban and public places like schools, these airborne indoor

infectants spread among the children very fast. Combining with virulence factors in and after the cold seasons, this may cause a massive allergic reaction with flu-like symptoms among children. There are some examples of both allergic and more serious diseases directly affecting internal organs and may lead cancer. *Aspergillus spp.* produce and secretes aflatoxins and produces ochratoxins as secondary metabolites. Among ochratoxins, ochratoxin A is a known carcinogenic and toxic metabolite which may cause kidney failure (Bayman, Baker, Doster, Michailides, & Mahoney, 2002). Also, aflatoxins are very dangerous as they are carcinogenic, mutagenic, and immune system repressors (Amare & Keller, 2014). Besides allergic symptoms, *Rhizopus spp.* can cause mucormycosis which is caused by the mold itself when it becomes airborne. Mold affects lungs, skin and, stomach and requires medical treatment (Cheng et al., 2009). Again, *Eurotium* are dangerous because they produce aflatoxins (El-Kady, El-Maraghy, & Zohri, 1994). These are just some examples of the dangerous fungi, and these examples show the importance of detecting the danger in public and indoor places. That is why besides traditional microbial methods, modern molecular methods are needed to detection fungi in species level.

Table 4.1. Photography images of the culture growth of background SDA culture media inoculated by swaps of Asil Nadir Primary School.

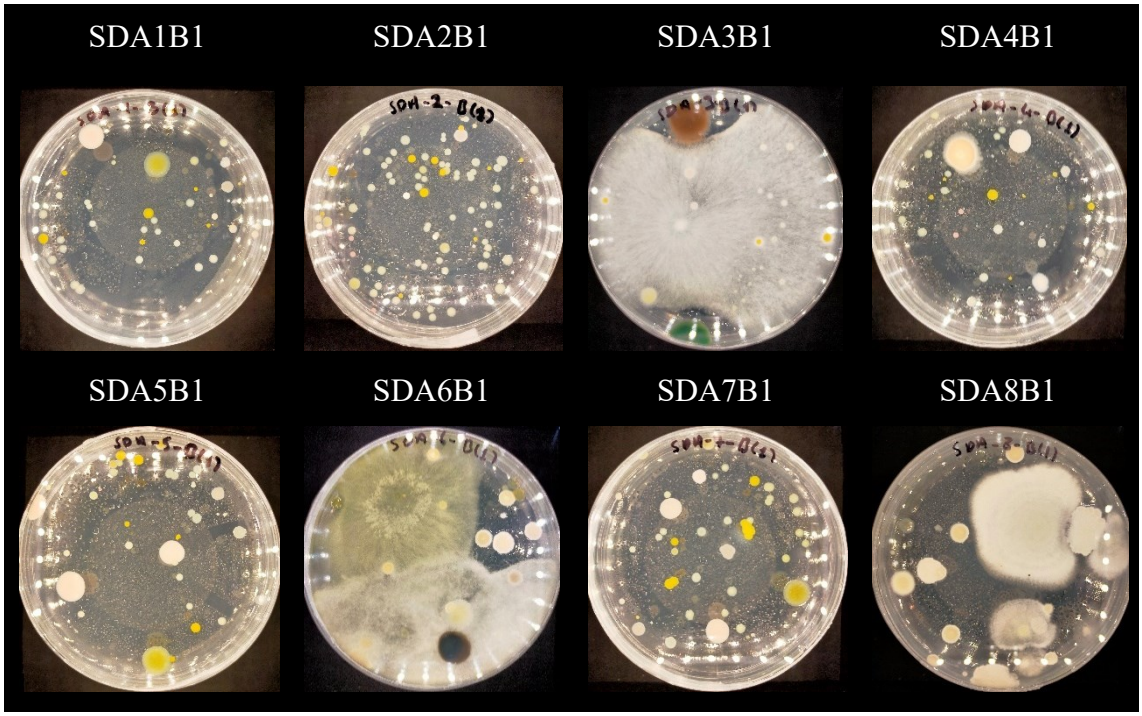

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Table 4.1. (Cont.)

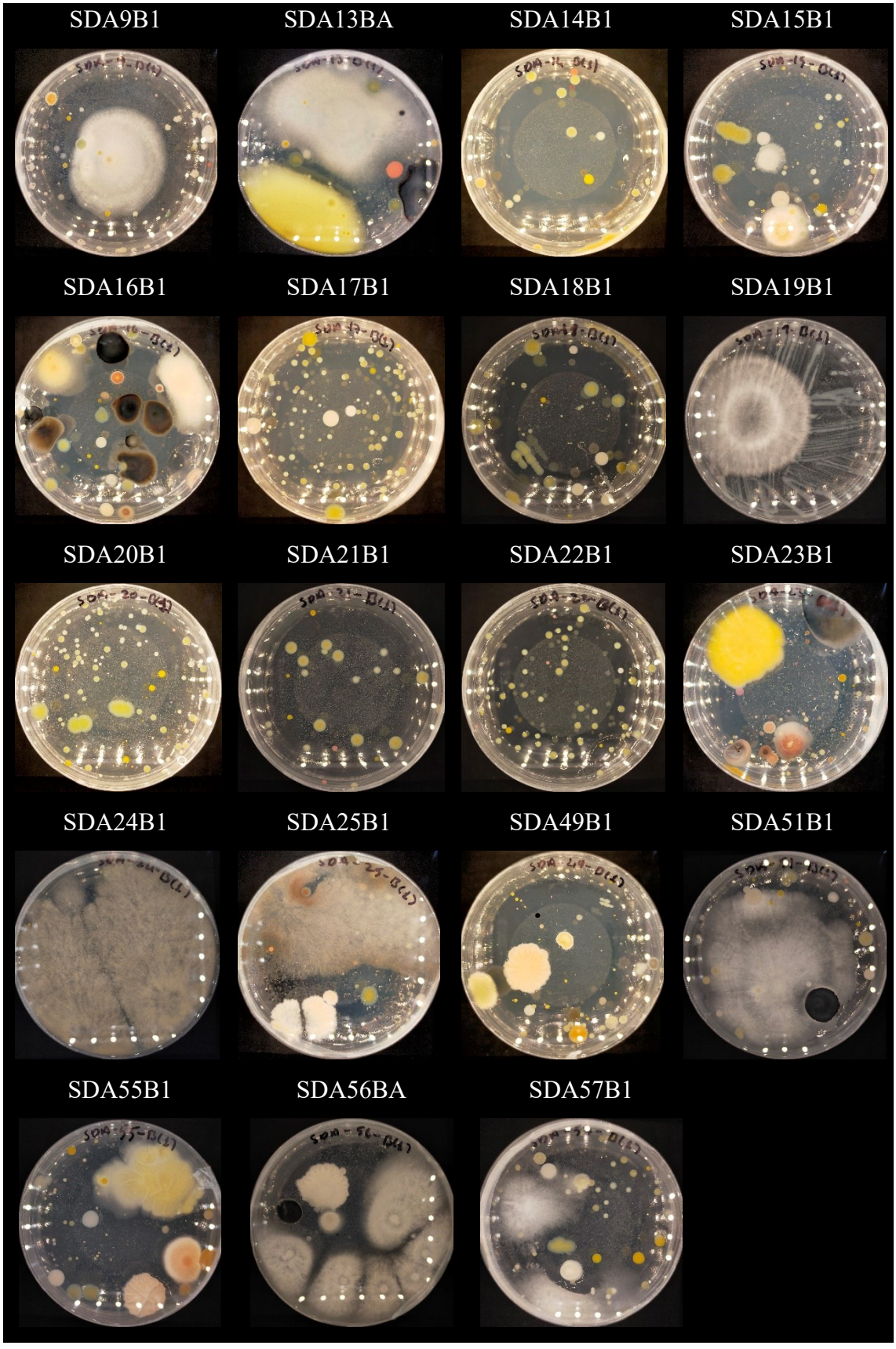

Table 4.2. Photography images of the culture growth of background SDA culture media inoculated by swaps of 80. Yıl Orhan Gazi Secondary School.

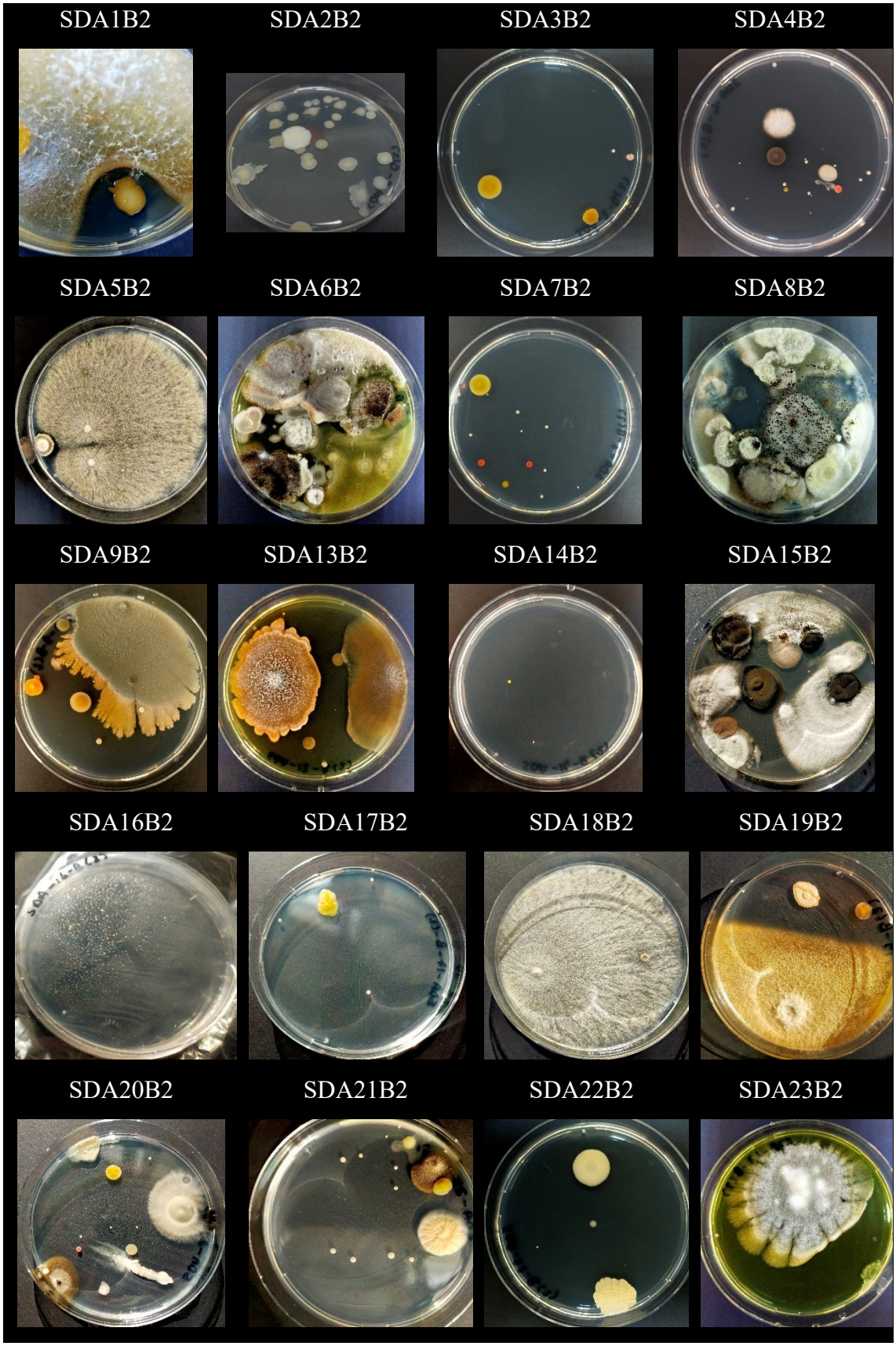

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Table 4.2. (Cont.)

Table 4.3. Photography images of the culture growth of background SDA culture media inoculated by swaps of Ahmet Hakkı Balcıoğlu Vocational and Technical High School.

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Table 4.3. (Cont.)

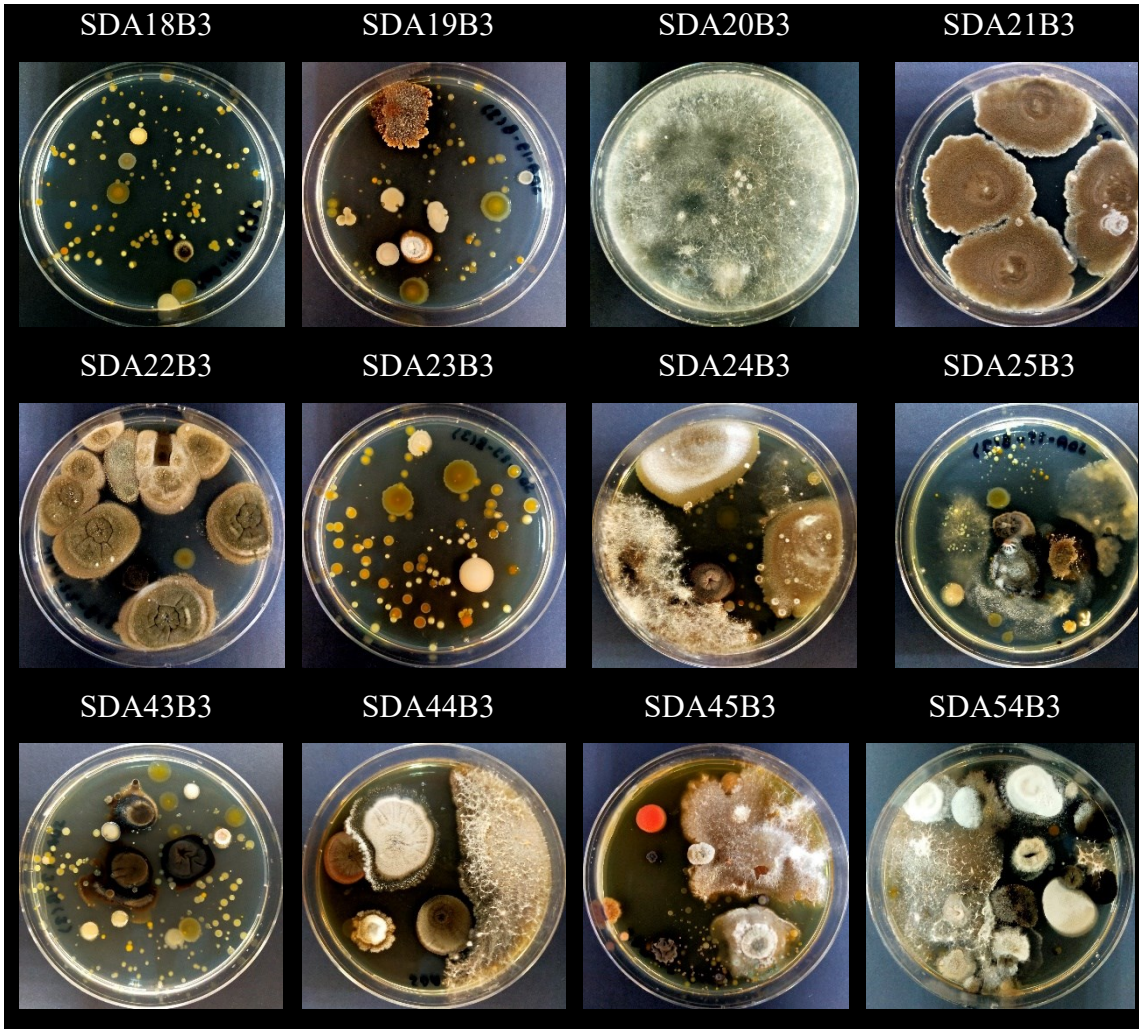
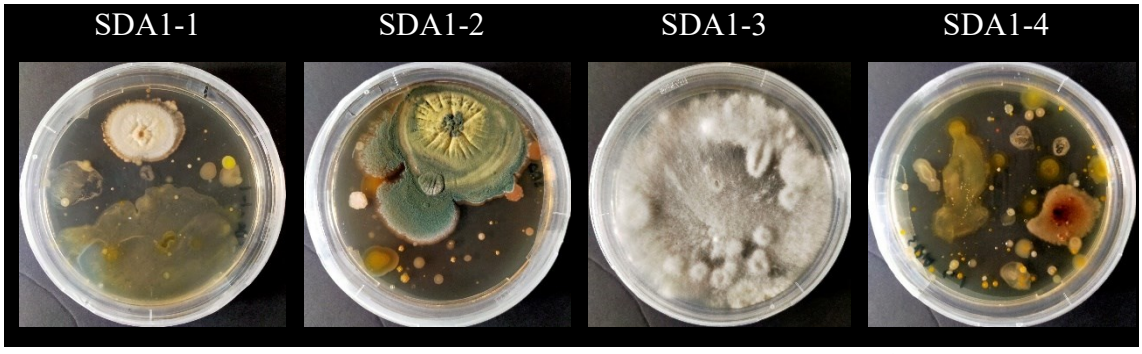


Table 4.4. Photography images of the culture growth of SDA culture media inoculated by swaps of Asil Nadir Primary School.



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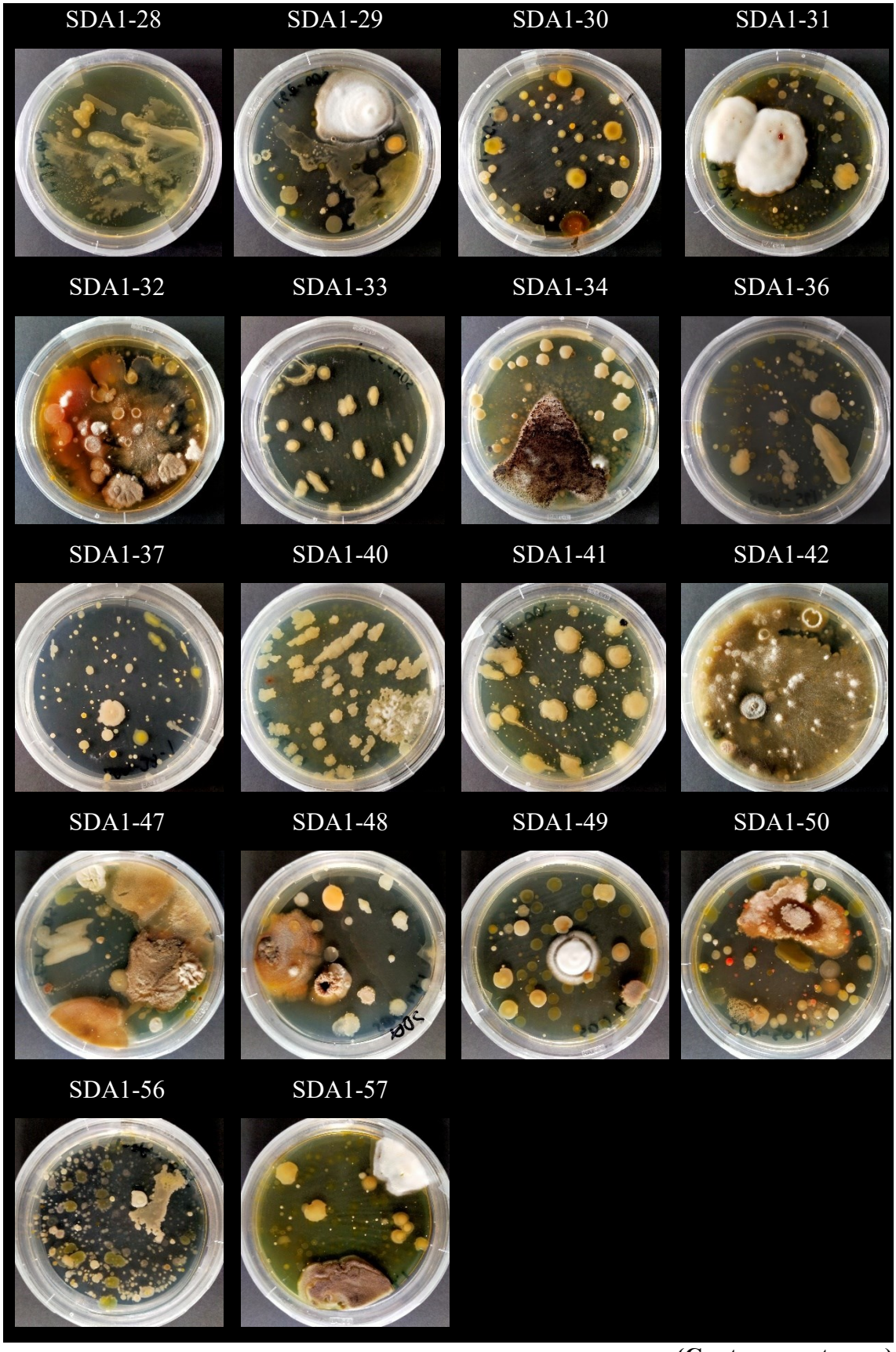

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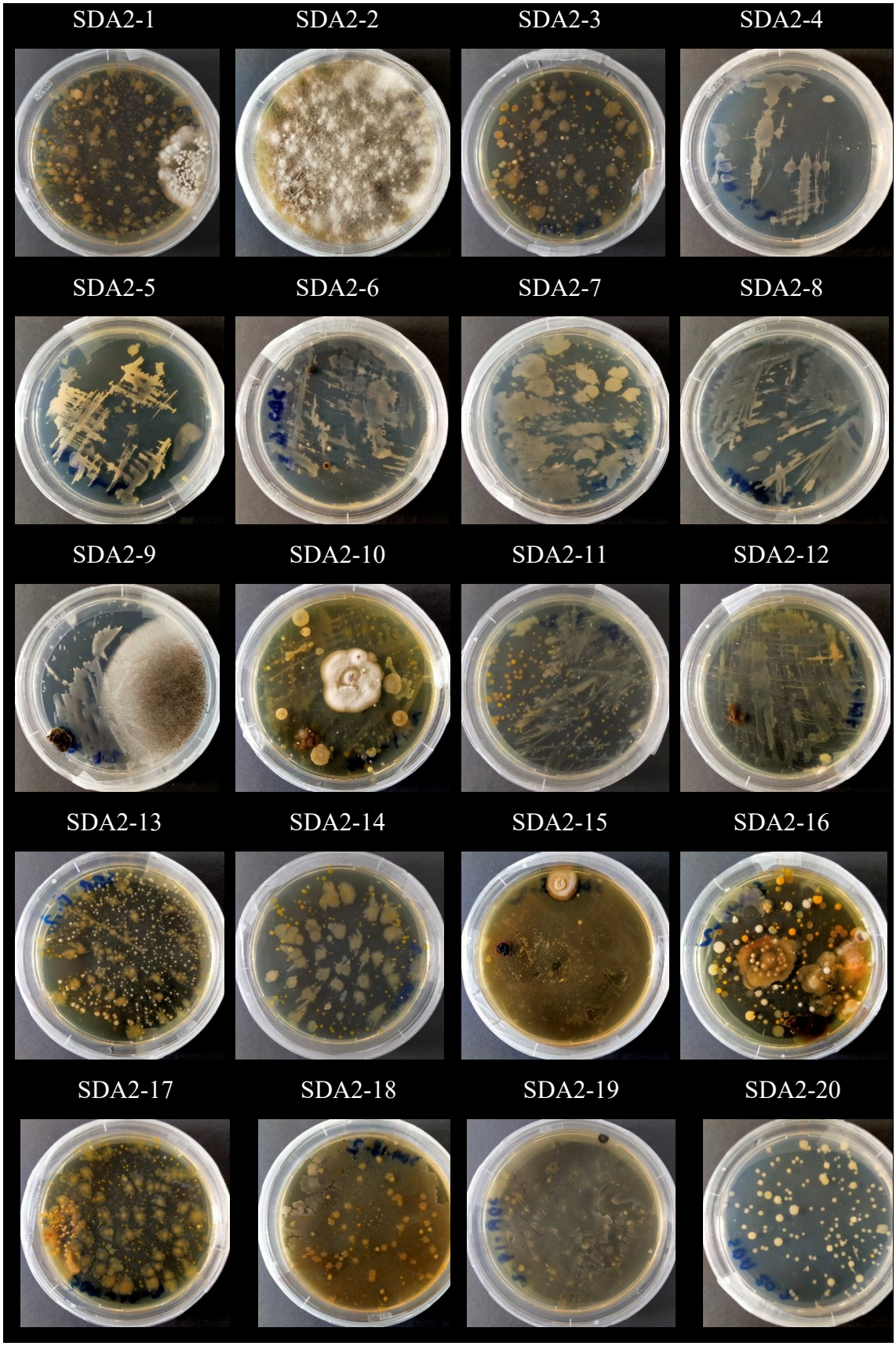
Table 4.5. Photography images of the culture growth of SDA culture media inoculated by swaps of 80. Yıl Orhan Gazi Secondary School.



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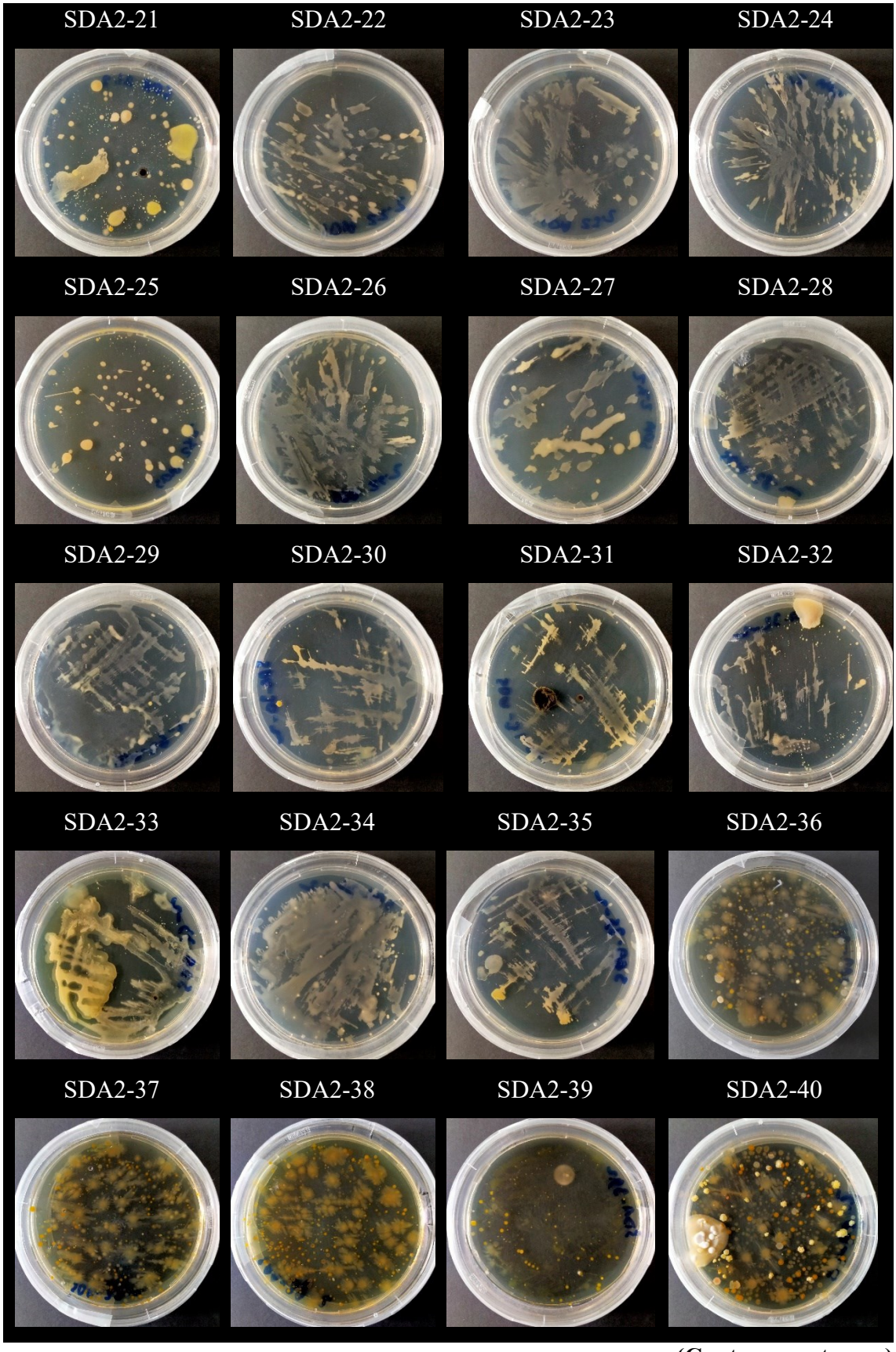

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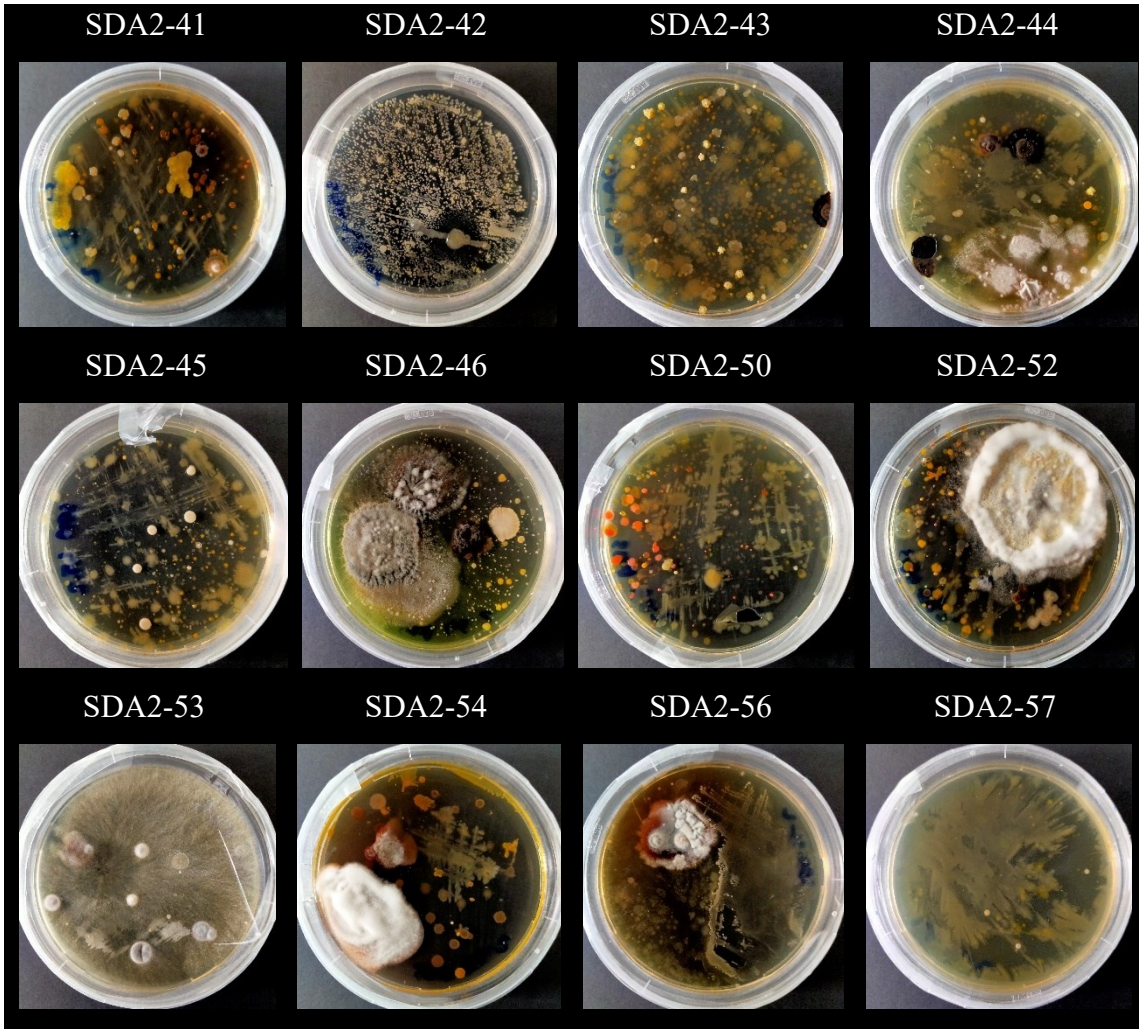
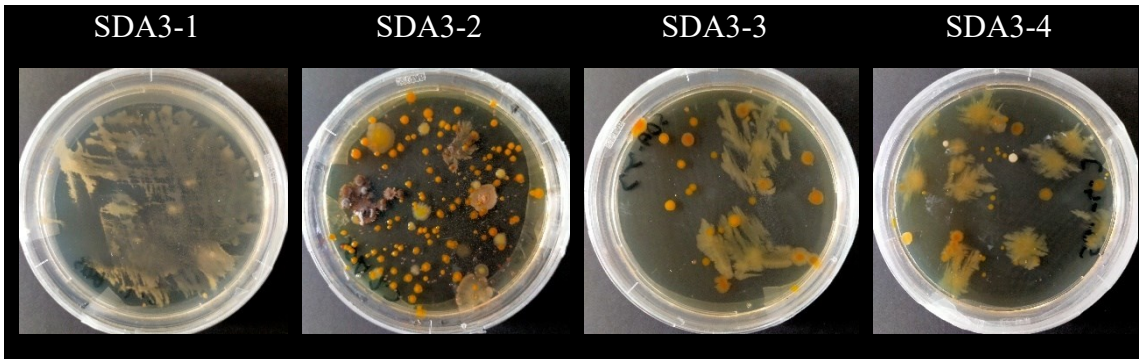


Table 4.6. Photography images of the culture growth of SDA culture media inoculated by swaps of Ahmet Hakkı Balcıoğlu Vocational and Technical High School.



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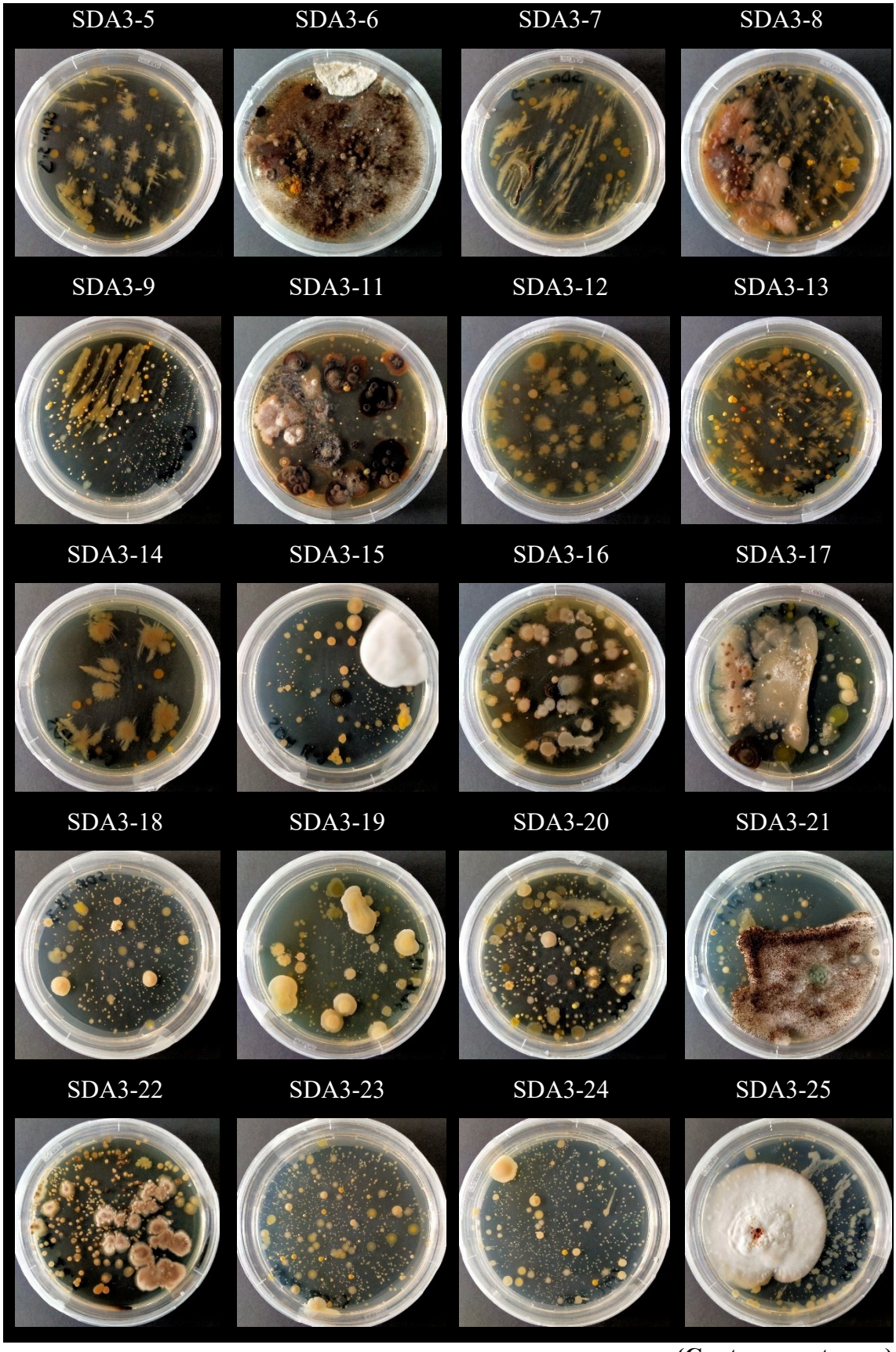

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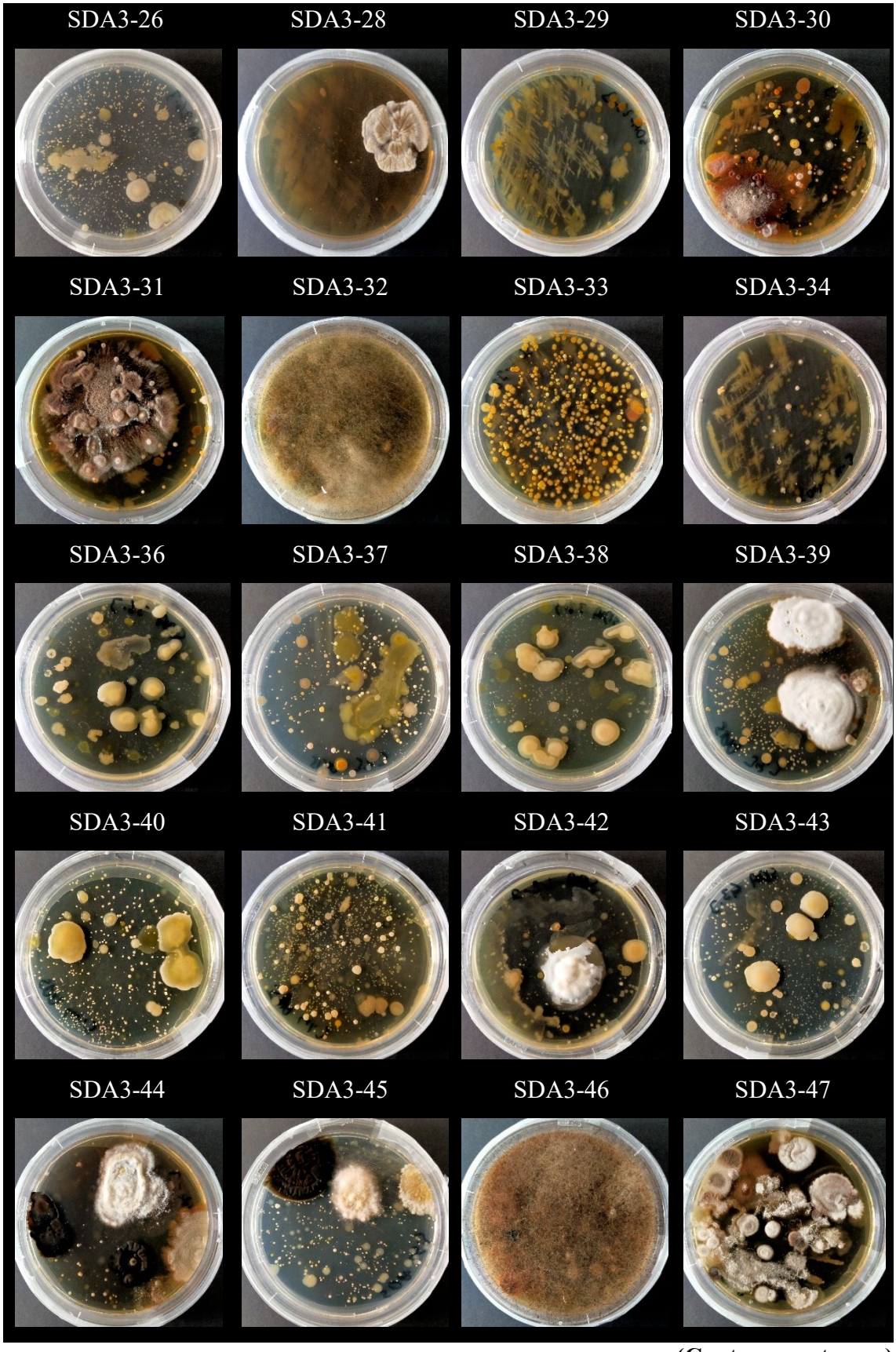

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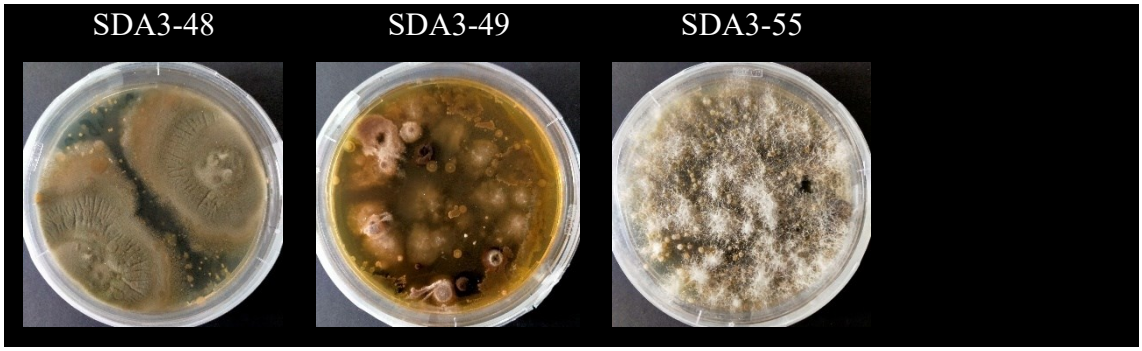


Table 4.7. Photograhy images of settle-plates from background samples at Asil Nadir Primary School

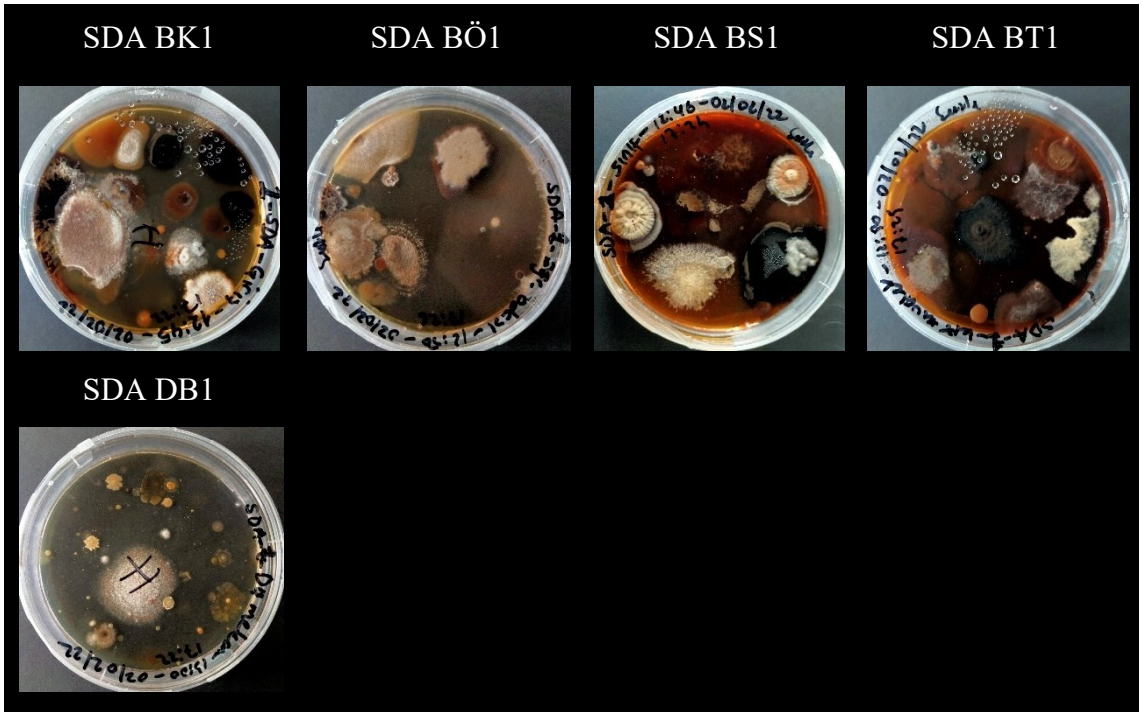


Table 4.8. Photograhy images of settle-plates from background samples at 80. Yıl Orhan Gazi Secondary School

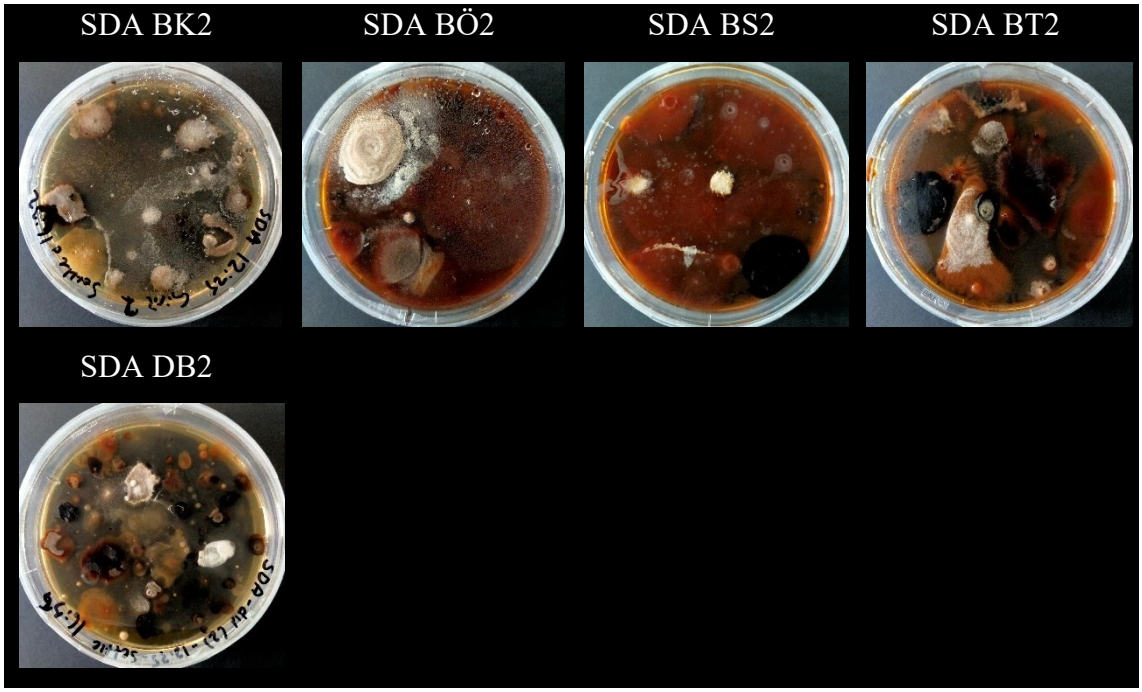


Table 4.9. Photograhy images of settle-plates from background samples at Ahmet Hakkı Balcıoğlu High School

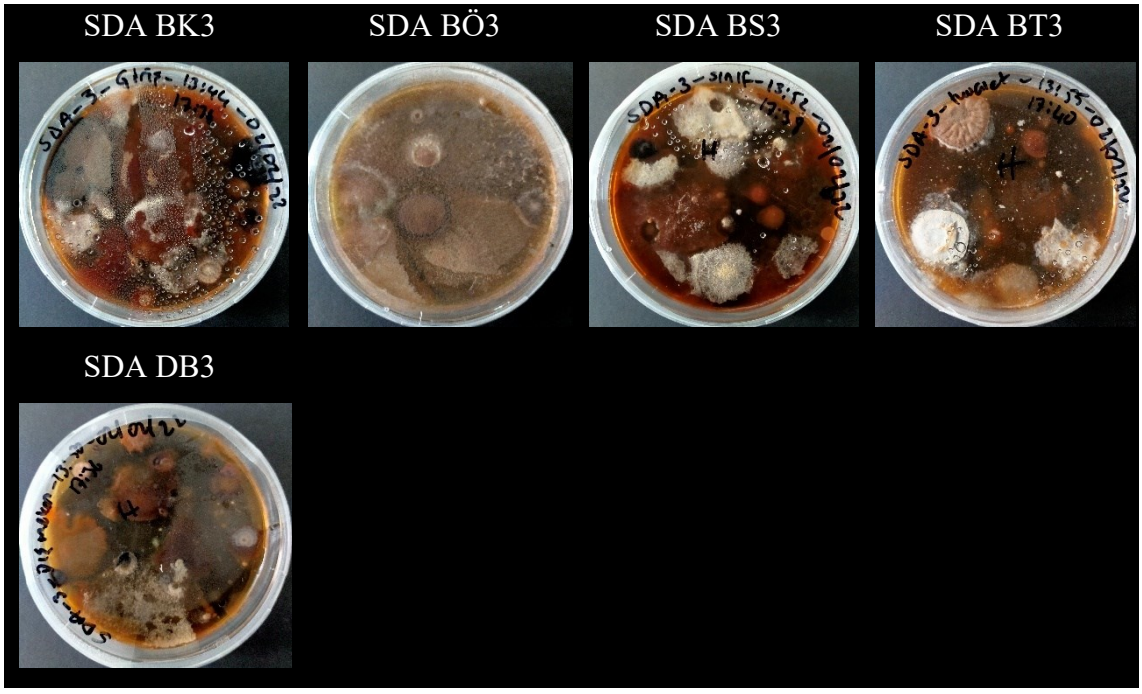


Table 4.10. Photograhy images of settle-plates from samples at Asil Nadir Primary School

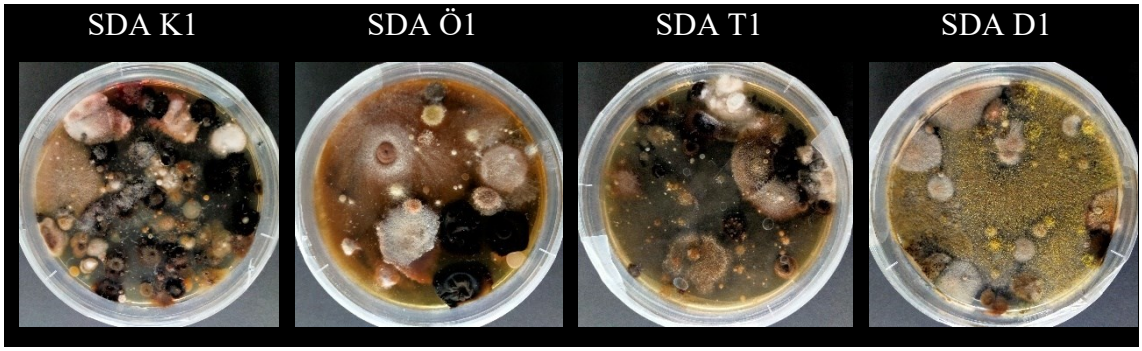


Table 4.11. Photograhy images of settle-plates from samples at 80. Yıl Orhan Gazi Secondary School

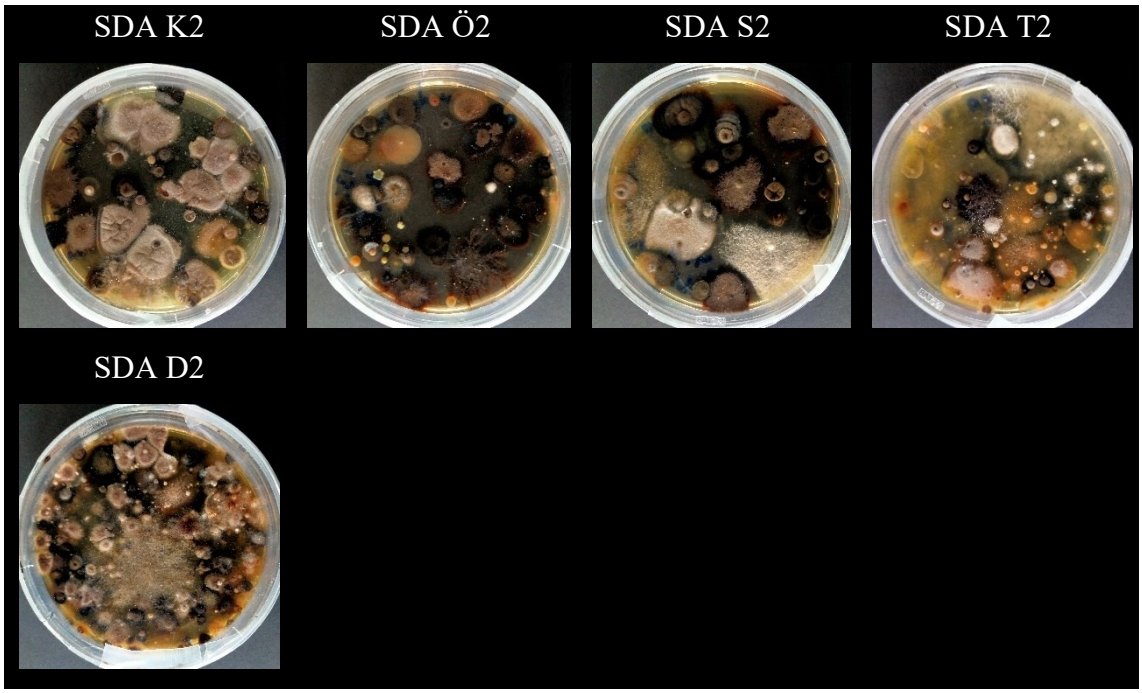
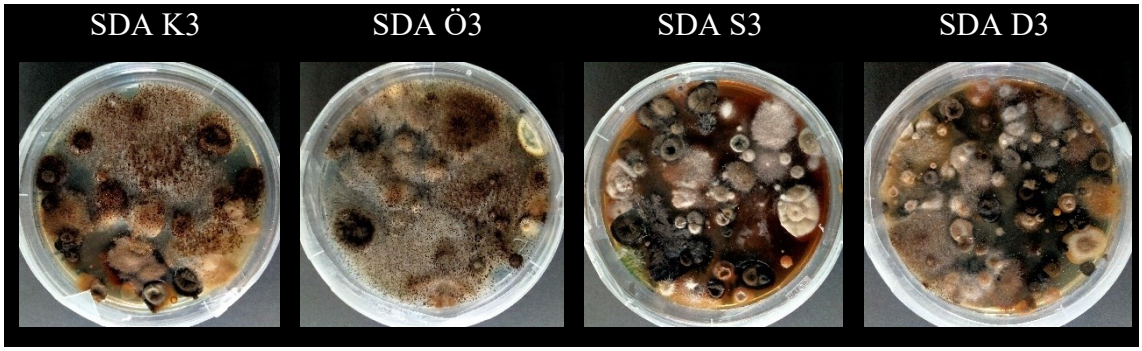


Table 4.12. Photograhy images of settle-plates from samples at Ahmet Hakkı Balcıoğlu High School



The another finding from the fungal cultures was that the fungal density changes depending on the sampling time. Background samples were taken while the schools were in semester holiday so there was no student. When the samples from the primary school were compared to their background, background samples seen less dense in both colony and diversity. Just before the background sampling, the school was freshly cleaned because the school administration was waiting for the visit of the governor of İzmir City. But some surfaces like computer equipment were known that they were not cleared regularly unlike bench, desk and bathroom surfaces. As seen from the images, all the cultures of computer equipment surface samples resulted in dense fungal growth for both samples and their backgrounds. For the other two schools, secondary and high schools, there was not an obvious difference in fungal growth dense. All the culture medias contained a diversity of fungal colonies. Even, regular cleaning protocols of public schools were done daily and weekly, the fungal presence could not be prevented. The main reason of it may be the moisture and the building materials of the public buildings. These three schools have no special air ventilation system. Only windows are the indoor source of the air refreshing. So, the molds placed on the indoor wall surfaces could be enhanced by the moisture and diverse via the seasonal air winds carrying other fungi to indoor. So, total of this shows additional air ventilation, conditioning or filtration systems to regular cleaning protocols are required for the indoor air and surface cleanliness.

4.2. DNA Isolation, Purity, and Concentration

NanoDrop spectrophotometer measurements showed the purity and the concentration of DNA isolated from the bacterial homogenized and pooled samples (Table 4.13, Figure 4.1). Some of the samples' DNA concentration were measured very high like above 70ng/µl and some measured very low like about 1ng/µl. Especially sample 5, 64, 67, 71 had very low concentrations. Most of the DNA concentrations were average and significant to be used in PCR. Even there was high and low values in concentrations, all the samples were amplified by PCR. Besides DNA concentration values, one important value was the absorbance ratios at 260/280 and 260/230. Pure DNA samples should have the value around 1.8 for 260/280 ratio and about 2.0 for 260/230 ratio but in this study, samples had average 1.575 absorbance ratio of 260/280 and average 0.562 absorbance ratio of 260/230 (Table 4.13). If the average ratio of 260/280 were lower than 1.8, it pointed to presence of proteins in the samples. Additionally, the absorbance value at 280nm proved and supported the presence of proteins in the isolated DNA. Even, there was protein presence for each isolate, average value of 260/280 was consistent when looked at the ratio 260/280 for each sample, except for sample 64, which had an absorbance ratio of 2.980. That was a very high value for the ratio, but when looked at the DNA concentration and the absorbance graph for sample 64, DNA concentration was very low so, the absorbance graph had almost a straight line with no peaks and very low values for all absorbance at 230, 260, and 280nm wavelength (Figure 4.1). On the other hand, the result of AGE for sample 64 also supported it by showing a very weak band (Table 4.14). Similar to sample 64, sample 5, 67, and 71 had low concentrations of DNA but, while sample 67 and 71 had weak AGE bands, sample 5 had a good and shiny AGE band matching the band 400-500bp of DNA ladder which meant it had expected DNA fragments in size (Table 4.14).

When looked at the 260/230 absorbance ratios, the values were quite lower than expected value which was 2.0. This meant, at the end of the DNA isolation procedure, phenol, chloroform, and carbohydrate contamination of the DNA isolate may possible. According to manufacturer's protocol, the Bacterial DNA isolation kit does not contain phenol/chloroform step. So, the most probable contaminants were carbohydrates. The explanation of it might be the presence of bacterial growth media in the homogenized and pooled samples. The growth media, TSA, was very rich in agar, so carbohydrate. During

the isolation, an irrefutable amount of carbohydrate contaminated the isolate as understood from the NanoDrop spectrophotometer absorbance values. But AGE of the PCR products showed that carbohydrate contamination did not affect PCR as it was obvious by the bands is expected size (Table 4.8).

Table 4.13. Isolated DNA concentration, absorbance, and absorbance ratio values.

Sample Number	Sample Name	Concentration ng/µl	A260	A280	260/280	260/230
1	B1H1	15.01	0.300	0.206	1.460	0.480
2	B1H2	76.02	1.520	1.070	1.420	0.430
3	B1H3	20.86	0.417	0.291	1.440	0.560
4	B1H4	23.9	0.478	0.335	1.430	0.380
5	B1H6	5.507	0.110	0.068	1.630	0.440
7	B1H8	11.66	0.233	0.177	1.320	0.420
8	B2H1	41.09	0.822	0.617	1.330	0.480
6	B2H2	16.99	0.340	0.263	1.290	0.330
9	B2H3	12.54	0.251	0.198	1.270	0.310
10	B2H4	34.93	0.699	0.461	1.520	0.480
11	B2H5	21.17	0.423	0.276	1.530	0.610
12	B2H6	26.27	0.525	0.390	1.350	0.360
13	B2H8	12.99	0.260	0.207	1.250	0.290
14	B3H1	18.85	0.377	0.273	1.380	0.250
15	B3H2	52.38	1.048	0.745	1.410	0.350
16	B3H3	51.63	1.033	0.773	1.340	0.280
17	B3H4	15.01	0.300	0.227	1.330	0.500
18	B3H5	42.08	0.842	0.610	1.380	0.390
19	B3H6	20.03	0.401	0.258	1.560	0.430
20	B3H7	7.416	0.148	0.122	1.210	0.280
21	1H1	22.59	0.452	0.298	1.520	0.540
22	1H2	55.39	1.108	0.686	1.610	0.560
23	1H3	127.7	2.555	1.732	1.470	0.700
24	1H4	59.69	1.194	0.725	1.650	0.690
25	1H5	35.43	0.709	0.517	1.370	0.400
26	1H6	45.59	0.912	0.563	1.620	0.640
27	1H7	14.75	0.295	0.215	1.370	0.460
28	1H8	28.36	0.567	0.437	1.300	0.510
29	1H9	16.32	0.326	0.222	1.470	0.540
30	1H10	37.43	0.749	0.617	1.210	0.460
31	2H1	101.7	2.034	1.223	1.660	0.690
32	2H2	30.86	0.617	0.349	1.770	0.750
33	2H3	36.53	0.731	0.442	1.650	0.790
34	2H4	25.69	0.514	0.332	1.550	0.500
35	2H5	23.31	0.466	0.304	1.540	0.560
36	2H6	110.3	2.206	1.355	1.630	0.690
37	2H8	37.72	0.754	0.467	1.620	0.650
38	2H9	64.06	1.281	0.826	1.550	0.530
39	2H10	109.8	2.196	1.351	1.630	0.600
40	2H11	52.83	1.057	0.696	1.520	0.570
41	3H1	107.2	2.144	1.233	1.740	0.840
42	3H2	157.3	3.146	1.893	1.660	0.590
43	3H3	61.32	1.226	0.780	1.570	0.630
44	3H4	57.28	1.146	0.708	1.620	0.650
45	3H5	68.4	1.368	0.894	1.530	0.610
46	3H6	104.9	2.099	1.257	1.670	0.780
47	3H7	51.81	1.036	0.638	1.630	0.690
48	3H10	61.5	1.230	0.737	1.670	0.800

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Table 4.13. (Cont.)

49	3H11	59.17	1.183	0.655	1.810	1.020
50	3H12	45.75	0.915	0.495	1.850	1.090
51	G1B	10.71	0.214	0.132	1.620	0.450
52	D1B	6.042	0.121	0.081	1.490	0.490
53	Ö1B	7.507	0.150	0.103	1.460	0.360
54	T1B	12.38	0.248	0.123	2.020	0.800
55	S1B	19.54	0.391	0.246	1.590	0.690
56	G2B	20.79	0.416	0.262	1.590	0.480
57	D2B	20.91	0.418	0.301	1.390	0.450
58	Ö2B	26.54	0.531	0.326	1.630	0.540
59	T2B	13.05	0.261	0.157	1.670	0.470
60	S2B	19.13	0.383	0.249	1.540	0.520
61	G3B	15.67	0.313	0.238	1.320	0.330
62	D3B	16.62	0.332	0.219	1.520	0.590
63	Ö3B	14.36	0.287	0.135	2.130	0.670
64	T3B	1.783	0.036	0.012	2.980	0.310
65	S3B	5.991	0.120	0.083	1.450	0.300
66	G1	55.2	1.104	0.682	1.620	0.600
67	D1	1.784	0.036	0.028	1.270	0.250
68	Ö1	7.984	0.160	0.089	1.800	0.540
69	T1	11.55	0.231	0.150	1.540	0.590
70	G2	12.11	0.242	0.150	1.610	0.860
71	D2	5.121	0.102	0.045	2.260	0.360
72	Ö2	16.91	0.338	0.213	1.590	0.610
73	T2	57.14	1.143	0.559	2.040	1.340
74	S2	27.33	0.547	0.324	1.690	0.650
75	G3	13.74	0.275	0.178	1.540	0.640
76	D3	12.32	0.246	0.132	1.860	0.850
77	Ö3	16.85	0.337	0.196	1.720	0.840
78	S3	16.94	0.339	0.212	1.590	0.670

Figure 4.1. Graphical visualizations of purity of the isolated DNA of bacterial homogenized and pooled samples.

4.3. Agarose Gel Electrophoresis

For DNA agarose gel electrophoresis, 11 AGE runs were done to cover all the PCR products including the negative control (NC). As can be seen in Table 4.8, PCR was successful for all DNA isolates except sample 64, 67, 71, and 79. Sample 79 was the negative control (NC) which was prepared by adding every gradient of PCR including DNA isolate storage buffer but any DNA isolate. Although NanoDrop spectrophotometer results for sample 64, 67, and 71 were not promising, there was still a possibility of DNA amplification as the DNA concentrations were not zero for these three isolates. However, it should be noted that there was no AGE band for these three DNA isolates.

The resolution and size of the AGE result images were different because of the visualizing device. The visualizing device was for common usage for Department of Molecular Biology and Genetics, İZTECH. As the results were obtained on different date

and time, the settings of the device were changed. Even all the results were measured by selecting auto UV exposure and getting best visuals by changing the saturation on the histogram in the user interface, the results were seen in different. But overall, this was not an obstacle to get the images were qualified enough to show the results.

Last six agarose gels had to wait overnight because of the unavailability of the visualizing device. This was resulted in degradation of DNA in agarose gel a bit. Degradation caused blurriness of DNA ladder and DNA bands, but they still had the enough resolution for discrimination of the bands.

Sample 64 was TSA Background Bathroom Settle Plate of High School. Even microbial growth was seen on both SDA and TSA, probably the isolation step was failed for this sample. Sample 67 was TSA Outside Settle Plate of Primary School. This media showed no growth on the media. Additionally, the media was seen quite dry when its coverage was opened just before the homogenization process. The reason may be the season of the sample collection. It was the end of the spring and the beginning of the summer. These days were sunny and warm. Warm weather and sunlight might be the reason of the dryness of the culture media by affecting it during the sampling. Also, the incubation condition was 38°C for 48 hours. This may be the cont. of the dryness factors. Dryness of the media might prevent the culture growth, so, one option was during the passive sampling, no microbial agent landed to the media and another option was the media became too dry to provide growth conditions for microbial agents. Sample 71 was TSA Outside Settle Plate of Secondary School. The condition of the growth media inside the plate was very similar to sample 67. It was too dry when the coverage was opened just before the homogenization process. As the sampling conditions of sample 71 were quite similar to sample 67, the reasons of why no DNA isolated and no DNA amplified during PCR might be these two options; one option was, during the passive sampling, no microbial agent landed to the media and the other option was the media became too dry to provide growth conditions for microbial agents. The last option for the result for both sample 67 and 71 might be the failure of DNA isolation or PCR. This was a very least possibility because both DNA isolation and PCR were done at the same time for all samples at once and PCR was successful for 96.15% of DNA isolates.

Table 4.14. Agarose gel electrophoresis of PCR amplificated DNA samples.

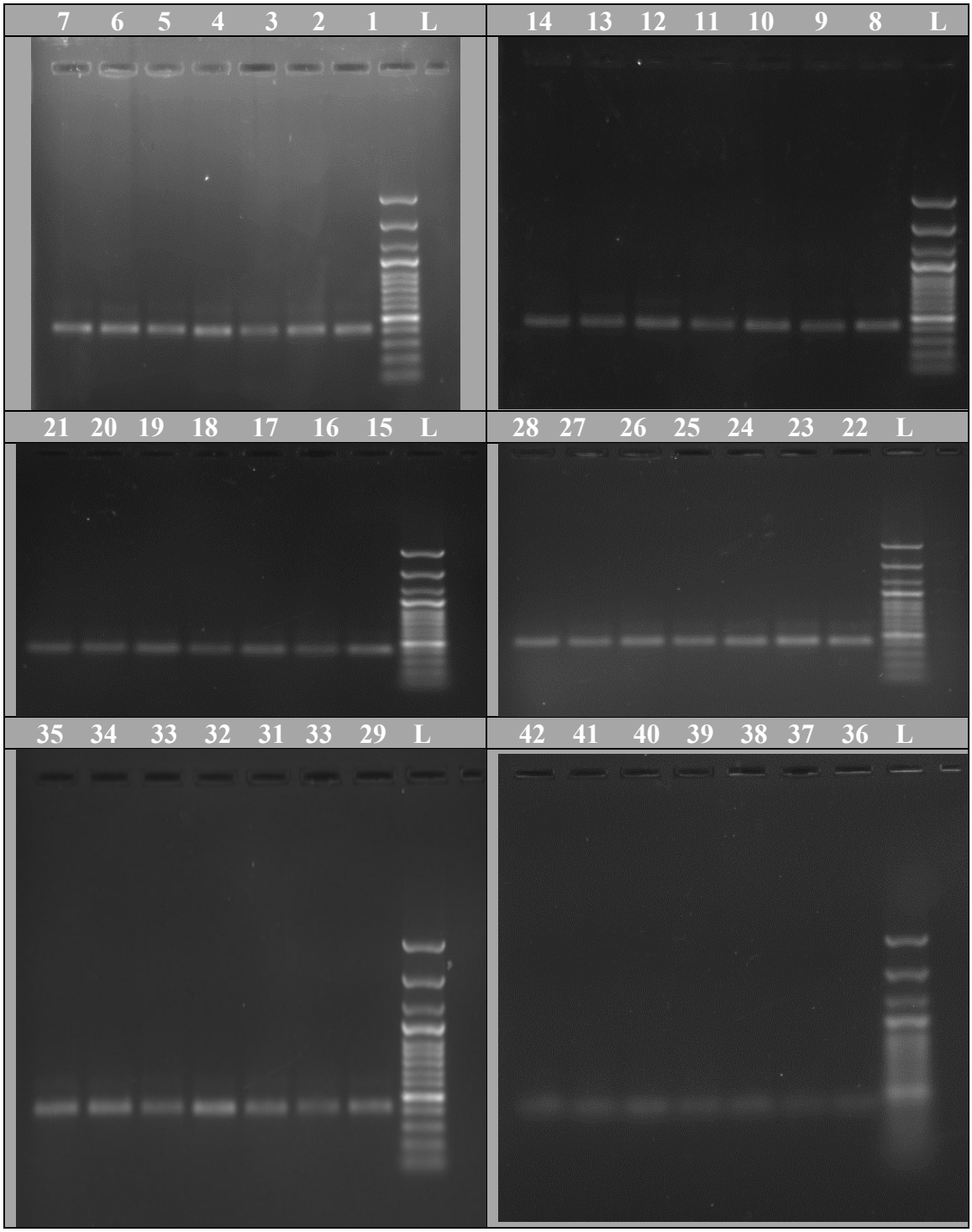

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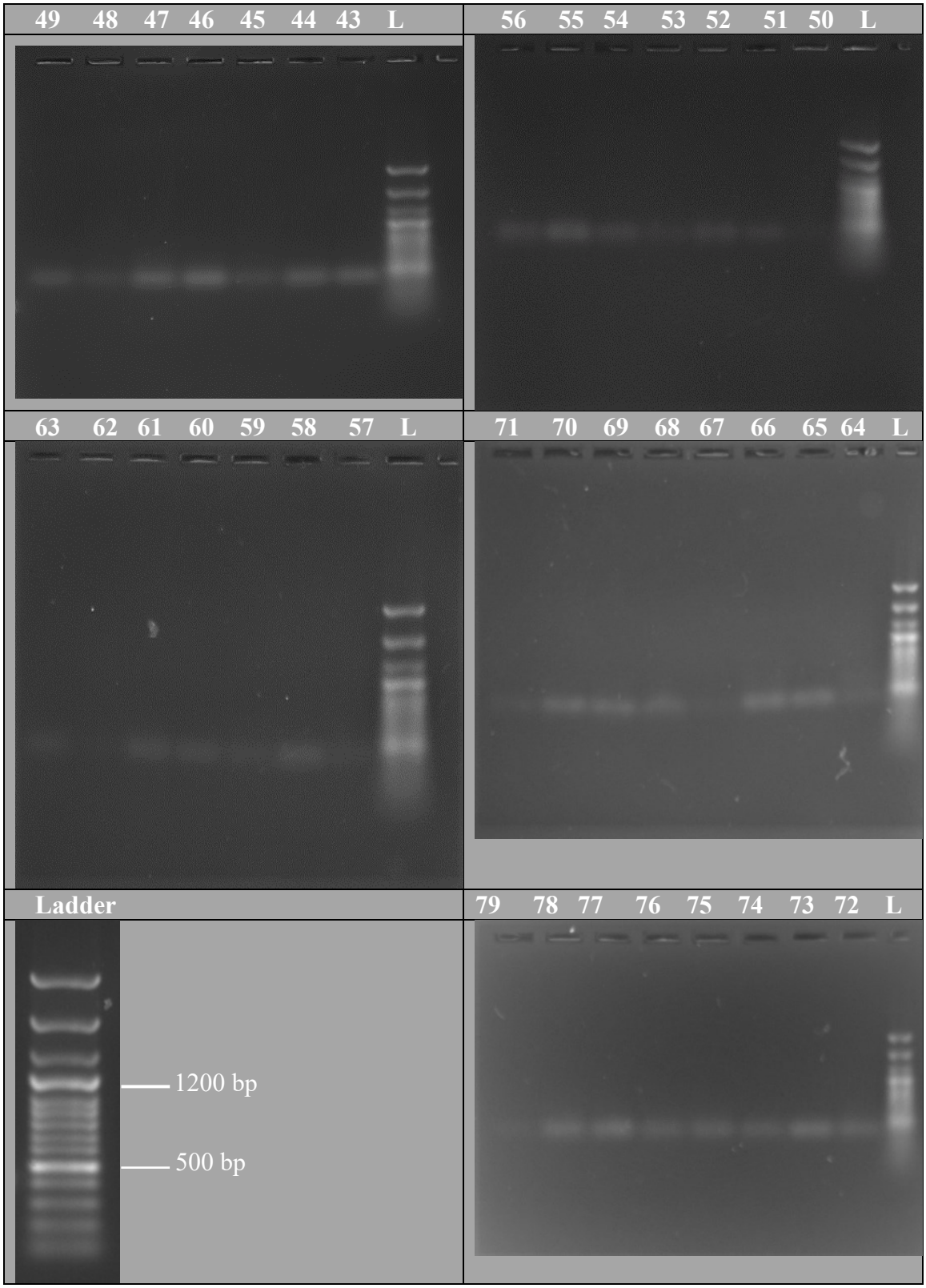
Table 4.14. (Cont.)



4.4. Sanger Sequencing and Taxonomy Analysis

The most possible 16S rRNA gene sequences were obtained via Sanger sequencing from the samples. Total of 72 samples were sent but 34 of 72 samples were resulted in failure in sequence. The reason of it may be the quality of the PCR products but the bands in AGE were quite clearly seen between the band size 400-500 bp. The subsequent reason may be the DNA washing step which was a sample preparation prior to Sanger sequencing and was done by Letgen Biotech (Letgen, İzmir) via inclusion service procurement of Sanger sequencing. The last reason may be the Sanger sequencing itself. Even the PCR products look right as 400-500 bp in size as expected fragment length for primer set, a similar step like PCR was done during Sanger sequencing with the primer set. The fragment products of Sanger sequencing should complete a sequence when they are aligned. If the DNA pool of PCR products were quite different, Sanger sequencing method also could not be able to bring the fragments together to complete a significant alignment. At the end, there were no potential to check these possible failures in the steps by replications because of the certain reasons like budget of the project.

The sequence results for 38 sample were screened directly in 16S ribosomal RNA sequence database via BLAST tool to see possible taxonomic unit matches to sequence data. Blast parameters were database: 16S ribosomal RNA sequences, program: highly similar sequences (megablast), maximum target: 100, threshold: 0.05, match/mismatch score: 1, -2.

Screening for 4 sample sequences in 38 sample sequences were resulted in no match. This was because of nonsignificant sequence of the sample. The common point of the no-match sample sequences was being short sequences compared to the others. Each sequence showed more than one target with the same E value and significant sequence match. Matched targets were the same in genus but some of them were not same in the species level of the organism. As the species level matches were uncertain, only genus level matches were given (Table 4.15). Genus matches were shown in pie chart to compare prevalence of genus at spots (Figure 4.2).

Table 4.15. 16S ribosomal RNA sequence database screen results in genus per significant sequence

Sample number	Genus	Sample number	Genus
1	*Pedobacter*	38	*Pseudomonas*
2	*Corynebacterium*	39	*Salmonella phage*
4	*Pedobacter*	40	*Pseudomonas*
9	*Micrococcus*	41	*Pseudomonas*
10	*Micrococcus*	42	*Pseudomonas*
14	*Sphingomonas*	43	*Burkholderia*
21	*Bacillus*	44-45	*Burkholderia*
23	*Comamonas*	46	*Burkholderia*
24-25	*Staphylacoccus*	47	*Bacillus*
26	*Burkholderia*	48	*Pseudomonas*
28-37	*Exiguobacterium*	49	*Pseudomonas*
29	*Burkholderia*	51	*Micrococcus*
31	*Pseudomonas*	54	*Pseudomonas*
32	*Burkholderia*	55	*Pseudomonas*
33	*Pseudomonas*	58	*Sphingobacterium*
34-35	*Burkholderia*	60	*Micrococcus*
36	*Pseudomonas*	65	*Staphylococcus*

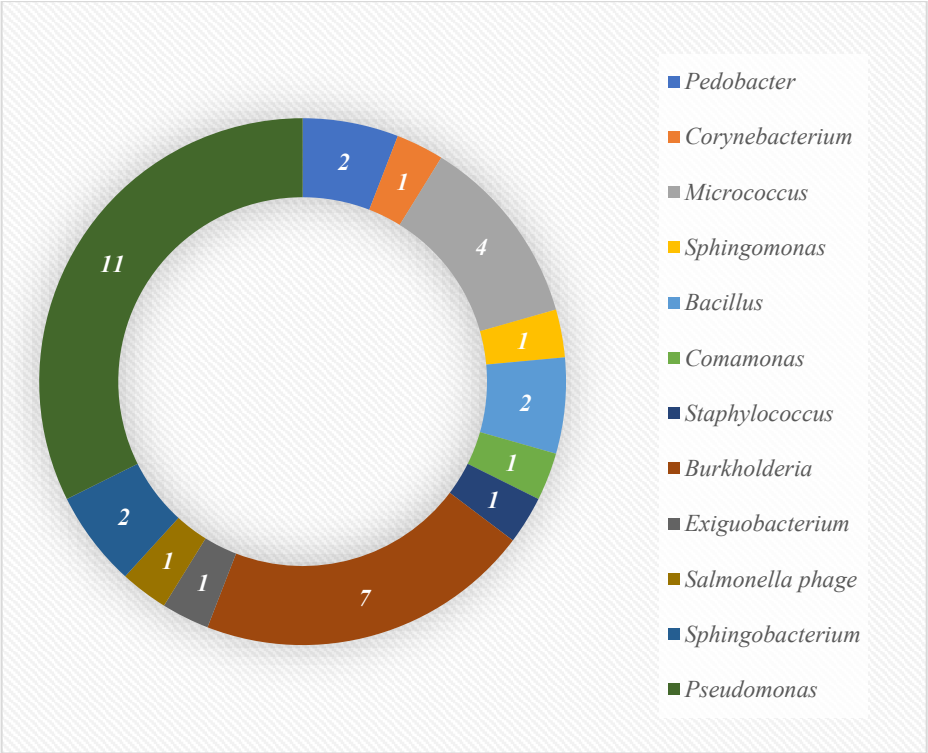


Figure 4.2. Genus's prevalence on spots shown as pie chart

The most common genera were *Pseudomonas, Burkholderia* and *Micrococcus.* The others were relatively less prevalence and equally seen in each other. *Pseudomonas sp.* are opportunistic pathogens which cause cystic fibrosis when the patients' immune system is weak (Elkin & Geddes, 2003). *Burkholderia sp.* are known opportunistic pathogen and pathogens for both animals and human. In human, they can cause pulmonary infection, melioidosis, and diabetes patients are susceptible to opportunistic pathogen *Burkholderia* (Woods and Sokol, 2006). *Micrococcus sp.* are rarely seen as opportunistic pathogen and infect when a patient suffers from suppressed immune system such as HIV (Smith, Neafie, Yeager, & Skelton, 1999). *Pedobacter sp.* are not dangerous, but they can live in extreme environments and some of them are known as antibiotic resistant superbugs (Viana, Caetano, Covas, Santos, & Mendo, 2018). Also, when looked from the perspective of this study, molecular methods such as DNA isolation and PCR were used. *Pedobacter sp.* were also considered to be DNA isolation and PCR method contaminants especially when working on bacterial subjects (Salter et al., 2014). But as the study shows, sample 64 and 71 were showed very less concentration of DNA after DNA isolation and their PCR products were not shiny in AGE under UV exposure. Besides it, the sequencing was also resulted in no sequence for these two samples.

Additional to this, negative control did not give a band in AGE. Total of it shows, there was no contamination in both DNA isolation and PCR steps of the study. *Sphingobacterium sp.* are known as non-pathogenic but there is a case report mentioned S*phingobacterium multivorum* was the causative agent of meningitis in an immunocompetent male patient (Abro, Rahimi Shahmirzadi, Jasim, Badreddine, & Al Deesi, 2016). *Sphingomonas sp.* are known as hospital related pathogens, but the infections caused by these agents are not life threatening and treated via antibiotics (Ryan & Adley, 2010). There is only *Bacillus cereus* shown as human health threat via food poisoning (Kotiranta, Lounatmaa, & Haapasalo, 2000). The most dangerous disease caused by *Corynbacterium* is diphtheria caused by *C. diphtheria* (Sharma et al., 2019). *Comamonas sp.* are very rare pathogen and there are second case reports showed *C. kestersii* caused an intra-abdominal infection and *C. testesteroni* caused acute appendicitis (Almuzara et al., 2013; Bayhan, Tanir, Karaman, & Ozkan, 2013). *Staphylococcus* infection can happen for anyone who has weak immune system. *Staphylococcus* can infect skin and open wounds via direct contact. Also, *Staphylococcus* can be placed on hospital devices and may infect patients via hospital device contact to open wounds or penetration of hospital devices (Foster, 1996). *Exiguobacterium sp.* are accepted as rare pathogens. Few case reports mentioned them as the causative agents of skin infection cases. In a case report of acute pneumonia,16S rRNA analysis showed that the causative agent was *Exiguobacterium sp.* (Chen et al., 2017).

Burkholderia, Sphingomonas, Corynbacterium, and Staphylococcus, were the most potentially dangerous agents among all found genera in the study. The study sites were public schools so especially pulmonary infectious *Burkholderia* and skin and open wound infectious *Staphylococcus* are the most possible infection causative agents as the children are less conscious and could not mind the infections when playing around in a hurry. When the infection way of *Staphylococcus* is considered, surface cleaning procedures become more important because they place onto surface of devices anywhere. They can be transferred from surfaces to the bodies of children and stalking for the weakening of their immune system or an open wound for the infection. On the other hand, *Corynbacterium diphtheria* should be considered. Even children are vaccinated in Türkiye, diphtheria can occur for both vaccinated and healed patients. So, children should become more conscious about sicknesses that make them cough and sneezing and also they should become more conscious about hand washing to prevent transfer of these agents trough hand contact from face and mouth to the others skin, hand and face.

CHAPTER 5

CONCLUSION

In this study, microbiota of three public schools were screened via both traditional microbial culture techniques and modern DNA analysis techniques to see diversity of indoor bacteria and fungi and potential health risks. At first, via the traditional microbial culture methods, bacterial and fungal cultures were constructed in two groups which were samples and their backgrounds. An exception occurred as on one SDA medium did not show microbial growth in it. Fungal microbial growth was similar for samples and their backgrounds except the situation for the secondary school that the fungal growth differences occurred because of the extra cleaning during the semester holiday. Bacterial growth was seen for all the media, and it was not possible to compare in the density of cultures but it was possible to compare in the DNA concentration and it was relatively higher for samples than their backgrounds. After traditional microbial growth, samples were homogenized and pooled in common groups for DNA isolation and PCR processes. This was sad because, homogenization and pooling process limited comparing the results. Also, HTS methods were more suitable for the biodiversity studies but again sadly Sanger sequencing method was chosen to process more samples compared to HTS because of the budged and currency problematics. After sequencing, bioinformatic results showed that some possible bacteria were on the surfaces of public schools may be dangerous for public health. Even it was Sanger sequencing, the study showed that, with modern DNA analysis techniques, microbiota can be detected, and time and labor consuming chemical and physical traditional microbial analysis methods can be skipped. Because Sanger sequencing showed the results in genus level while it is not possible by many of the classical methods except species selective growth media but again it requires many isolation steps consuming time and labor.

In conclusion, the study revealed that, the microbial screening of public indoor places can be improved via the modern DNA analysis methods and the study indicated the need of research in this area especially in Türkiye.

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