

**STUDY OF MICROBIAL LOADS IN WATER AND AIR SAMPLES FROM
OGUN STATE DUMPSITE**

By

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF
BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED
SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN
STATE, NIGERIA.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN MICROBIOLOGY**

SEPTEMBER, 2022

DECLARATION

I hereby declare that the project report was written under the supervision of DR. ADEBAMI G. E. and it is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and the list of references provided. This researched project report has not been presented for the award of any degree.

EGBETOKUN Samuel Oluwaseyi

Signature/Date

CERTIFICATION

This is to certify that this research project titled “**MICROBIAL LOAD IN AIR AND WATER SAMPLES FROM OGUN STATE DUMPSITE**” was carried out by EGBETOKUN, Samuel Oluwaseyi, with Matriculation number 19010101032. This report meets the requirements governing the award of Bachelor of Science (B. Sc.) Degree in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

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(Supervisor)

Date

Dr. (Mrs.) C. I. Ayolabi
(Ag. Head of Department)

Date

DEDICATION

I dedicate this project work to God the Father, God the Son and God the Holy Spirit who saw me through and gave me strength for the completion of this great work. And also, to my entire family for their advice, never-ending support, care and constant prayers towards its successful completion.

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ABSTRACT

In this study, the settled plate method was used to measure the microbial loads in air and water samples taken from the Shagamu dumpsite in Ogun State. The analysis procedure comprises exposing sterile potato dextrose agar (PDA) and nutritional agar (NA) and NA to the air for 0 to 30 minutes. Additionally sampled and transported to the lab for study was water from a nearby river. Colonies that could be seen were counted and noted in water samples and exposed air plates. The isolates underwent morphological and biochemical characterizations. Testing the isolated bacteria for antibiotic sensitivity was also done. The colony count after 30 minutes of plates exposure for nutrient agar (NA) and (PDA) potato dextrose agar ranged from 27 – 400 cfu and 73 – 1400 cfu respectively. The highest counts was observed at 30 minutes of exposure while the lowest was at 0 minutes. The colony count after 48 hours of incubations ranged from 150×10^3 - 45×10^5 and 300×10^3 - 130×10^5 for the first and second wastewater sampling respectively. *Escherichia coli*, *S. aureus*, *Micrococcus* sp., *Pseudomonas* spp., and *Bacillus* spp., were the predominant bacteria. Antibiotics sensitivity testing carried out on the isolated bacteria showed that 27% were resistant to the selected antibiotics disk. In conclusion, our study's observations of the landfill waste disposal and its close vicinity to neighborhoods suggest that there may be a serious environmental health risk that could have a serious impact on people's health and safety. To maintain public safety and to control waste management, the government must enact policies.

Keywords: Air pollution, dumpsites, wastewater, bacteria, fungi, antibiotics resistance

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Each year, diseases linked to pollution and waste cause the deaths of almost 5.2 million people and approximately 4 million children. Any solution mixture, material, or item that is carried for reprocessing, dumping, elimination by incineration, or other ways of disposal is referred to as waste in general. Pollution is becoming a significant issue as a result of urban industry, social advancement, and population growth. The environmental effects of wastes can be terrible and damaging if they are not properly handled and disposed off. (Faith *et al.*, 2010).

In poor nations, waste management is typically associated with land disposal or waste discharge into bodies of water. In poor nations, waste management practices are illogical and annoying to the general people; as a result, pollution is the end outcome. When wastes are carelessly deposited on land, bacteria and fungi flourish because the waste's components provide them with nutrients for growth and the degradation of the organic waste's constituents. (Funmilayo *et al.*, 2021).

Solid waste management that is done well lessens negative effects on the environment, human health, and economic growth that enhances quality of life. Effective management of solid waste involves several different operations, including monitoring, collecting, transportation, processing, recycling, burning, landfilling, and composting. (Enerijiofi *et al.*, 2019). Remediation of polluted places is also covered. It is critical to note that microbial activity in solid wastes that have been dumped plays a critical role in the breakdown of organic waste and greenhouse gas emissions. Due to the abundance of varied organic matter and substrate complexity, landfills are characterized as microbial pools because different microorganisms thrive there. (Enerijiofi *et al.*, 2019).

All life forms require water in order to survive. The three most important elements for life are air, water, and food. A week without water, fewer than five minutes without air, and around a month without food are all that humans can endure. (Funmilayo *et al.*, 2021).

Water is used for drinking, bathing, treating illnesses, industrial processes, food processing, recreation, and other things. The cause of one-third of all intestinal infections worldwide is water borne illness.(Bikram *et al.*, 2021.). According to conventional wisdom, drinking water should be completely free of germs; however, packaged water, which is the subject of concern, is not. Around the world, a billion people lack access to clean drinking water, and 2.6 billion lack adequate sanitation facilities. As a result, 1.8 million people per year pass away from water-related diseases, with children under the age of five being infected at a rate of 90%, primarily in developing nations. (Taiwo *et al.*, 2012). Young children's growth and nutrition are negatively impacted by contaminated water supplies. Due to the increased demand for such water, the production of excellent water products is becoming more and more challenging. (Enerijiofi *et al.*, 2019).

Due to variations in sociological settings, varied climatic conditions, and other localized factors prevalent all over the world, the implementation of a universal standard for drinking water is not being adhered to to the letter. However, water treatments like filtration, coagulation, and chemical treatment can be applied to enhance the quality of the end product made from the original water. (Funmilayo *et al.*, 2021).

There are three categories of criteria for measuring water quality: physical, chemical, and microbiological. The presence of coliforms and other harmful organisms in drinking water that has undergone water treatment is a sign that the procedures have not been applied properly (Funmilayo *et al.*, 2021). Product information, odor, and look are all investigated physically, as well as the color, turbidity, and existence of any floating particles or foreign components (Weller BF, 2009). Studies on sachet water products in Nigeria have revealed the variables that contribute to water contamination, including cutting practices, poor vendor cleanliness, a filthy atmosphere, and disregard for WHO/NAFDAC rules. (Funmilayo *et al.*, 2021).

The MPN (Most Probable Number) approach, which assesses the mobility of water, can be used to quickly find coliform bacteria, one of the pathogens investigated by microbiological parameters that are linked to waterborne disease (Adewoye *et al.*, 2013). *Escherichia coli*, *Vibrio cholerae*, *Salmonella* species, viruses like hepatitis A, and protozoan parasites like *Giardia lamblia* are a few of the microorganisms that are considered to be contaminants. Nigeria reached a point where it was thought that sachet water was more likely to be contaminated than bottled

water, which prompted NAFDAC to declare a potential "gradual" statewide ban on sachet water so that producers could begin winding down or switching to bottle packaging. (Taiwo *et al.*, 2012).

When it comes to drinking water, there are numerous laws and standards. The National Agency for Food and Drug Administration and Control (NAFDAC), which was founded as a parastatal of the Federal Ministry of Health by Decree No. 15 of 1993, is responsible for monitoring such rules in Nigeria. Due to this, certain sachet water goods were improperly treated when being transformed to bottled items. Waterborne illness is the world's greatest source of morbidity and mortality, causing around 3.4 million deaths annually. (Oyedepi *et al.*, 2010).

According to the WHO standard (from which Nigeria derived the term "drinking water quality"), the amount of heterotrophic bacteria in drinking water should not exceed 10 cfu/ml, and there shouldn't be any coliform in 100 ml of water. This indicates that only a certain number of microorganisms are permitted to be present in drinking water. When heterotrophic bacteria are abundant in water products, it is a sign that improper manufacturing procedures were used during processing. Hepatitis A, cholera, typhoid, amoebiasis, botulism, shigellosis, legionellosis, severe acute respiratory syndrome, etc. are diseases linked to contaminated water. (Adewoye *et al.*, 2013)

The bacteria in the water can multiply to a point where they can become harmful due to how and where they were stored as well as during processing. This study's objective is to determine and evaluate the microbiological load of various water products offered in Ekiti State. (Funmilayo *et al.*, 2021).

1.2 Statement of Problem

Human health is seriously threatened by the effects of pollution, and landfills that are close to residential areas have been a substantial source of pollution itself. It has been proven that these pollutants, which include solid waste, air, and water coming into touch with people, can lead to illnesses and present ongoing risks. Therefore, it is important to understand the scope of the issue and what can be done to mitigate it, but regrettably, the government is doing little to address it.

1.3 Justification

The two necessities of life that are used the most widely are water and air. In order to provide varied environments with quality water, it is important to confirm the microbiological activity of the water supply. Water that is devoid of pathogens and other organic pollutants that can be tracked to its source using its geographic surroundings is ideal for human consumption and health. To ascertain the safety of human life nearby, it is crucial to analyze the microbial activities in both water and air through microbial load activities.

1.4 Aim and Objectives of the study

This study aims at investigating the microbial load in water and air samples from selected dump sites in Ogun State, Nigeria.

1.5 Objectives of the study

- To isolate the microorganisms from the contaminated air and water samples
- To identify the organisms using morphological and biochemical characterizations
- To determine the antibiotics susceptibility test on the isolated bacteria

CHAPTER TWO

LITERATURE REVIEW

2.1 Waste management

With 182 million people, Nigeria's major difficulty with trash generation and disposal is that there are many difficulties to be solved. In Nigeria, residential, agricultural, and industrial sources account for the majority of the municipal wastes, which can be classified as liquid, solid, gaseous, and hazardous. The liquid and solid wastes, however, are the most challenging. The solid waste management problem is a complicated one that calls for technology, human resources, and financing. The liquid wastes are wastewater from municipal and industrial sources. To get the intended results, a lot of work needs to be placed into controlling wastes at all times during manufacturing, collection, transportation, treatment, and final disposal. (Adebayo *et al.*, 2018)

Numerous research have tried to compare and contrast the levels of microbial loads acquired from active and passive samplings, but the results have been inconsistent: in some situations, there was a strong association, while in others there was none (Igbeneghu *et al.*, 2014). Currently, air sampling procedures are not standardized, making it challenging to compare the findings of various studies (Anon, 1994). It has recently come to light that various active samplers exhibit high variability and produce a range of results when used simultaneously in the same location. (Weller, 2009). Sayer *et al.* did not find this correlation using the Andersen Active Sampler (Ogbonna, 2006), and Petti *et al.* showed that, at low air contamination levels, results provided by active Surface Air System sampler (SAS) and settle plates were not correlated. Whyte found a correlation between the active and passive method, comparing settle plates with the Active Casella Slit Sampler (Napoli *et al.*, 2010). In poor nations, the topic of waste management is typically associated with land disposal or discharge into aquatic bodies (Cilinskis and Zaloksnis, 1996). Such a waste management technique is illogical and annoying to the general population; as a result, pollution is the end outcome. When waste is dumped on land, it encourages the growth of bacteria and fungi, which use the waste's components as food for growth and break down the organic waste's organic components.

Different indoor environments have varying levels of bio-contamination, varying airflow, varying populations of people working there, and varying amounts of people wearing varying types of personal protective equipment, all of which have an impact on the results of the sampling and the comparison between methods. (Osazee *et al.*, 2013). Perdelli *et al.* also compared the SAS with the Index of Microbial Air Contamination during the surgical activity, when contamination is higher. Sampling can also be done in numerous ways. An ISO standard stated that it could be useful to examine bio-contamination before the operation begins, when the room is vacant, in order to assess the theater's performance capabilities, particularly its air systems (Igbeneghu *et al.*, 2014).

Water contains a variety of pathogens' bacteria, including parasitic worms called Helminthes, Spirochete, Rickettsia, Escherichia coli, Shigella, Salmonella, Enterobacter, gastroenteritis viruses, enteroviruses, Klebsiella, Citrobacter, and enteric hepatitis viruses. (Bharti *et al.*, 2003; Bosch, 2007). In addition to the aforementioned, many mold species, including the toxic and allergenic Aspergillus and Penicillium spp., are also discovered in drinking water (Hageskal *et al.*, 2006). These fungi are responsible for the negative health impacts as well as the taste and odor issues in drinking water (Dogget, 2000).

2.2 Microbes in dump sites

The bacteria in trash dumps get their nutrition from the waste components, which helps with the detoxification of complicated organic molecules (Osazee *et al.*, 2012). But some solid trash may also include a significant amount of organic contaminants that might linger in the environment (Williams and Hakem, 2016). The proximity of these dumpsites to residential buildings, which poses a security risk to the public, is one of the main issues with the indiscriminate dumping of waste. The mediators and catalysts for organic inputs, native soil organic matter change, and xenobiotic detoxification are soil enzymes. (Dick, 1997).

Since changes in soil management and land use, such as xenobiotic pollution, agriculture, de-vegetation, and revegetation, are reflected in the soil enzyme activity, soil enzymology is now of practical importance because it can predict changes in soil quality before they are detected by other soil analyses (Stephen, 2020). Additionally, since external and intracellular enzyme activity as well as those of culturable and unculturable organisms are evaluated throughout the process, the use of soil enzymes in the evaluation of soil quality has grown in value. (2012).

"Biotechnological Tools for Environmental Sustainability." This investigation's goal was to evaluate the microbiological population and physicochemical condition of soil from Benin City's municipal dumpsites. (Stephen, 2020).

2.3 Water borne microbes

According to Locking et al. (2001), contact with diseased farm animals or their excrement as well as polluted drinking and recreational water are the main sources of water-borne E. coli transmission. Wild and domestic animals grazing in water catchments have been linked to waterborne E. coli 0:157 illnesses and fecal pollution of water (Bharti et al., 2003). Pathogens in drinking water distribution systems have been found using molecular methods, particularly in systems with biofilms (Edberg et al., 1988). However, standard laboratory techniques that are used to measure spore formers, coliform, viable count, mold and yeast number, and other contaminants in drinking water, can still be used to establish the level of contamination. A common technique for determining the presence of coliform involves three successive steps: a presumptive test, a confirmed test, and a completed test. It has been used as a water quality monitoring technique for more than 80 years (Edberg et al., 1988).

Water contains a variety of pathogens' bacteria, including parasitic worms and enteroviruses as well as Spirochete, Rickettsia, Citrobacter, Noroviruses, enteric hepatitis and gastroenteritis viruses, Escherichia coli, Shigella, Salmonella, Enterobacter, and Klebsiella (Bharti et al., 2003; Bosch, 2007). Additionally, a variety of mold species, including the toxic and allergenic Aspergillus spp. and Penicillium spp., are found in drinking water. These fungi are also responsible for the unfavorable health consequences and the taste and odor issues in drinking water. (Dogget, 2000).

2.3.1 Disease associated with contaminated water

Hepatitis A, cholera, typhoid, amoebiasis, botulism, shigellosis, legionellosis, severe acute respiratory syndrome, etc. are diseases linked to contaminated water. Water can get contaminated not just during processing but also due to how and where it was stored, allowing the microorganisms to multiply to a hazardous level. (Anon., 1994.).

2.4 Role of microorganisms in the environment

In order to transform environmental natural resources into more palatable forms for consumption, a significant portion of man's activities in his environment include chemical synthesis. Man also causes pollution issues during the production of goods. Because of this, the easiest way to deal with environmental waste is to simply incorporate it back into the environment from which it originated. That approach makes use of microbes (usually yeasts, bacteria, or fungi) (Ezeonu *et al.*, 2012). The substrates that produce the desired industrial products, such as bioleaching (biomining), biodetergent, biotreatment of pulp, biotreatment of wastes (bioremediation), biofiltration, aquaculture treatments, biotreatment of textiles, biocatalysts, biomass fuel production, and biomonitoring, incorporate these microorganisms or their byproducts. (Adebayo *et al.*, 2018).

Microorganisms play important roles in the carbon and nitrogen cycles as well as other key functions, such as recycling the waste products and corpses of other organisms through the decomposition process. These functions are essential to humans and the environment in which they exist. Additionally, as symbionts, microorganisms play a significant role in higher-order multicellular creatures (Adebayo *et al.*, 2018).

Despite the difficulties of surviving in the atmosphere, many microbes can continue to exist even after long periods of time, even when exposed to UV, low moisture levels, and oligotrophic under harsh settings (Barea *et al.*, 2005). According to Askepidis *et al.* (2000), atmospheric mobility is a crucial route of microbial dispersal, and the spread of airborne plant and animal pathogens can have a considerable effect on ecosystems, human health, and agricultural productivity. In this study, airborne bacteria around a particular dump site in the Ado-Ekiti region were isolated, identified, and their level of antibiotic resistance was then determined. (Odeyemi, 2012).

2.5 Airborne Microbes

The term "airborne microbes" refers to biological airborne contaminants, also known as "bioaerosols," such as bacteria, viruses, or fungi, as well as airborne toxins that can be transmitted from one victim to another through contaminated air without physical contact, at the very least causing irritation. 2020 (Stephen). The wind carries microorganisms from garbage

dumps to the sky. Their ability to survive is mostly determined by their resistance, air pollution, weather, and time spent in the atmosphere (Marthi et al., 1990). An aerosol is the air we breathe. The facility's staff and nearby people may develop respiratory illnesses and other health issues as a result of airborne germs (Wouters et al., 2002; Douwes et al., 2003; Heldal et al., 2003; Curtis et al., 2006; Schrapp et al., 2010). According to the World Health Organization, air pollution is responsible for an estimated 2 million premature deaths each year, as well as many additional cases of heart disease, lung infections, and even cancer (Madhukar and Srikantaswamy, 2013). Wastes have been shown to contaminate air, groundwater, soil, and surface water, which causes extra issues for ecosystems, other species, and humans (Obire et al., 2002). When garbage is not adequately managed or disposed of, harmful compounds in solid waste and pathogenic microbes can be released into the environment (Wai-Ogosu, 2004; Ogbonna *et al.*, 2006). Due to its effects on people's health, air pollution has recently become a subject of great concern. Infections and significant respiratory problems are brought on by air pollution. (Patella, 2018). Additionally, the World Health Organization (WHO) revealed in 2018 that 9 out of 10 people daily breathe in air of low quality and that an estimated 3.8 million people die each year as a result of home air pollution. Due to how difficult it is to see and identify the contaminants, controlling air pollution has proven difficult throughout time (Adams, 2016). However, according to a prior study, air could support microbial and fungi colonies. The air may contain microbial particles including single spores, aggregated spores, mycelium, fungal spores, pollens, bacterial cells, virus particles, and other biological components. Bioaerosols are these microscopic particles, and their presence in the air has a substantial impact on its quality. Heart-related diseases have an increased risk factor when there is poor air quality (Erqou, 2018).

The presence of these kinds of bioaerosols outside is a major cause for concern because they can spread through a variety of processes over large regions and into enclosed spaces as well. Anaerobic bacteria can contribute to the conversion of organic waste into gases that can explode when they interact with other substances and migrate through the atmosphere (Molino, 2013). According to Maecka-Adamowicz et al. (2020), allergens can be produced by both high and low quantities of particular bacteria in the atmosphere. Respiratory issues are linked to fungus-produced allergens (Twaroch, 2013).

Furthermore, it is a prevalent practice in Ghana, Nigeria, and even in some regions of the regional capitals of the African continents to burn rubbish at landfills as a method of waste

management. Activities involving burning can dramatically worsen air pollution (Premakumara, 2018). Burning waste at landfills may release aerosols with primary biological origins in addition to greenhouse gases and other particulate matter, which could ultimately result in microbial air pollution. Sanitary landfills have been used for a while in most developed nations to reduce air pollution (Cointreau, 2006). Nevertheless, using designed landfills is a typical practice in developing nations. The properties of these landfills, which are crucial for effective waste management at these locations, are not sufficiently known. As a result, the majority of landfills in this country operate significantly below the advised criteria for sanitary practices. According to Thompson (2010), the size of the landfills in Ghana is insufficient because of the nearby population. In such circumstances, those people run the risk of breathing air that is of poor quality and may be polluted with pathogenic microbial bioaerosols. Therefore, it is crucial to have access to clean air in the neighborhoods or towns near landfills, to continuously monitor the hazards to biological and physical health, and to notify stakeholders, politicians, and the general public. (Thompson, 2010).

2.5.1 Air Floral

The term "air flora" refers to the microbial load, which includes vegetative spores and cells of bacteria, fungus, and algae, as well as protozoan cysts and viruses. The type and amount of microorganisms in the air over a specific area relies on the surface pollution source. Microorganisms from soil, plant and water surfaces, industrial and agricultural waste, sick animals, including humans, and other sources contaminate the air. Before settling back down on the surface of the earth, microbes can travel for several miles or only a few feet on dust particles, pollen grains, or droplets. (Adebayo *et al.*, 2018)

2.6 Role of airborne microbes from dump site in the environment

"Bioaerosols" are aerosols with biological origins, whereas aerosols are defined as airborne suspended particles. Nonbiological aerosols (such as dust, ash, heavy metals, sulfur oxides, and organic compounds) have been the focus of extensive research due to their negative effects on human health and natural ecosystems. (Weller *et al.*, 2009).

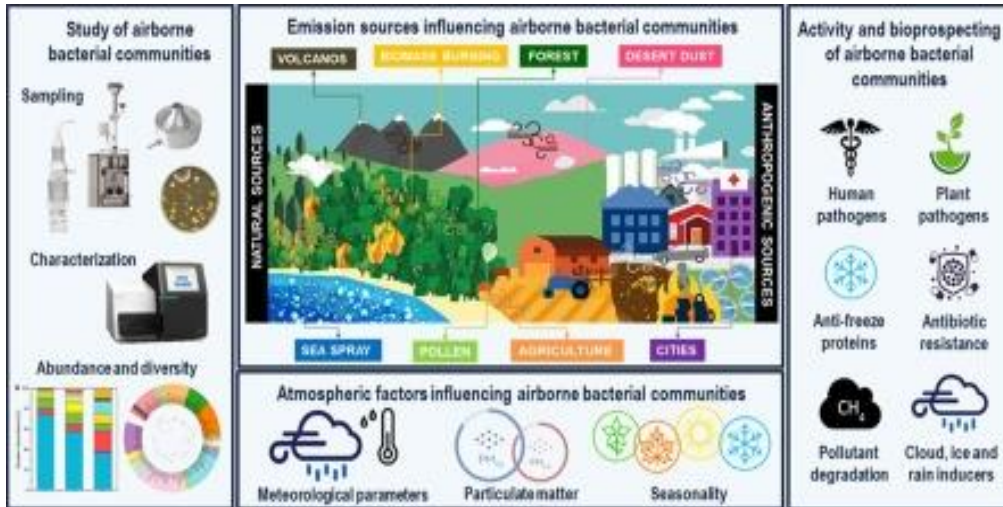


Figure 1.1: Airborne bacterial communities of outdoor environments: (Obire *et al.*, 2002).

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Dust plume motions have been well studied since they have both positive and harmful effects on the entire world. Due to their ties to their use in agriculture, they may aggravate or create health problems in humans, animals, and the transmission of infections, opportunistic bacteria, chemicals, and trace metals. The majority of the sand belt's dust-producing regions are in the Sahara, Australia, Asia (the Gobi Desert), South Africa, and arid South America (the Atacama Desert) (Tickell *et al.*, 2000).

The bacterial concentration can rise by an order of magnitude during dust events. The more prevalent taxa in desert dust plumes are members of the bacterial phyla Firmicutes (*Bacillales*), Actinobacteria (*Micrococcales* and *Corynebacteriales*), and Bacteroidetes (*Sphingobacteriales*, *Bacteroidales*, and *Flavobacteriales*). *Burkholderiales* and the pathogenic *Neisseriales* are the Proteobacteria orders that have a stronger correlation with dust plumes (Mgbakor, 2011). The well-known aquatic bacterial families *Synechococcales* and *Vibrionales*, for example, are known to concentrate in dust plumes (Gray, 2004).

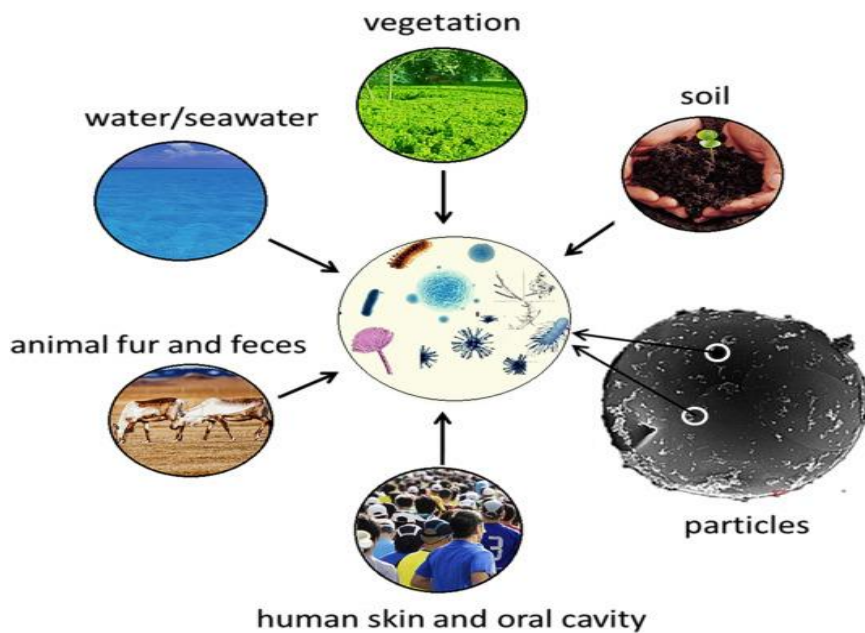


Figure 1.2: Main sources of airborne over particles source (Thompson *et al.*, 2010)

A wide range of sources, such as soils, plant leaves, waterbodies, and both natural and anthropogenically damaged habitats, can produce different taxa of airborne bacteria. These sources are schematized in Fig. 1.2. The primary environmental sources of airborne bacteria are terrestrial settings like soil and plant leaf surfaces. They are followed by marine environments, where bacteria on the water's surface are aerosolized through sea spray by crashing waves or strong winds, and these environments typically occur in deserts and other arid climates. (Sooryamoorthy, 2013).

Additionally, as bacteria are typically attached to suspended particles, the kind of source (natural or anthropogenic) impacts not only the makeup of bacterial communities but also the size of the suspended particles in the air and, as a result, the residence period of bioaerosols.

Important sources of bioaerosols include the aerosol emissions from urban activities (such as hospitals, homes, pet waste, construction, and transportation), agricultural activities (such as livestock and farming), and waste treatment facilities (such as compost, landfills, and wastewater) (Douwes, 2003). When substances are released into the environment, they can either

be attributed to a single source (such as waste treatment facilities and agricultural operations) or a variety of sources (such as urban activities) (Faith, 2010). Transcontinental dissemination is also possible for aerosolized microbes. Recent investigations have raised the idea that transoceanic aerosols may contain pathogens responsible for human disease, even if not all transported organisms may have an impact on human health. In densely populated locations, where controlling aerosolized germs has proven difficult or impossible, analytical techniques that can survey these people are of relevance for both public health and national defense (Odeyemi, 2012).

The effects that have been felt over time in every place and the challenges associated with finding specific solutions for one or more source types are extremely relevant, even though this classification can change depending on the scale used. The aforementioned movements and aeration processes are intimately related to aerosolization from anthropogenic sources. geographical spread. Estimates of bacterial fluxes in the atmosphere are considerably less than those in anthropogenically disturbed agroecosystems and are also substantially lower (Omalu et al., 2012). For instance, wind speeds above a certain threshold can aerosolize the predominant manure particles in fertilized fields and allow the detection of cow fecal bacterial markers in the near-surface atmosphere (Curtis et al., 2006). Additionally, studies have shown that the aeration and agitation systems in wastewater treatment plants have high levels of airborne microorganisms. (Ezeonu *et al.*, 2012).

2.6.1 Diseases Associated with Contaminated Air in Dump Site

Globally, and especially in the developing world, air pollution has been a significant problem. Microbiological air pollution may be the result of improper waste management and disposal. In developed nations, landfills are located distant from residential areas; in underdeveloped nations, the converse is true.

Serious respiratory problems could be brought on by air pollution. In 2018, the World Health Organization (WHO) claimed that 9 out of 10 people daily breathe in air of low quality and that an estimated 3.8 million people die each year as a result of household air pollution. Due to how difficult it is to see and identify the contaminants, controlling air pollution has proven difficult throughout time. (Oyedeki, 2010).

Particulate matter and greenhouse gases are the air pollutants most frequently thought to be related to air pollution. A variety of chemicals in the atmosphere make it difficult for bacteria to live there since those parameters, including nutrient requirements and optimum oxygen levels, cannot be met. But according to a prior study by Smets et al., air may include bacterial and fungi communities. The air may contain a variety of microbial particles, including single spores, spores in aggregate form, pollens, bacterial cells, viral particles, mycelium, fungal spores, and other biological materials. Bioaerosols are these microscopic particles, and their presence in the air has a substantial impact on its quality. Poor air quality is associated with increased risks of heart-related diseases (Kanthé *et al.*, 2012).

Because a significant quantity of CH₄ and CO₂ are produced during the decomposition of trash that is dumped in landfills, landfills play a significant role in the world's anthropogenic greenhouse gas (GHG) emissions. Landfill operation is typically associated with leachate from the landfill contaminating surface and groundwater, a strong odor, loud, unsettling noise from landfill bulldozers, and bioaerosol emissions; volatile organic compounds (Erqou et al., 2018). The degree of odors present at a landfill site can be affected by the storage of leachate in open lagoons. The presence of many harmful contaminants coming from landfill operations has raised concerns among locals who live close to waste sites. Litter, dust, excessive rodents, unforeseen landfill fires, etc. are some additional contaminants linked to the deposition of trash on landfills. The type and quantity of garbage placed, the age of the landfill, and the climatic conditions of the landfill sites are all factors that affect the by-product or emissions from landfills. Several gaseous pollutants, persistent organic pollutants (including dioxins, polycyclic aromatic hydrocarbons), heavy metals, and particle matter are frequently produced by complex chemical and microbiological interactions inside landfills. (Madhukar *et al.*, 2013).

Humans that continuously breathe in CH₄ may experience loss of coordination, nausea, vomiting, and death due to excessive concentrations of the gas. When introduced, acidic gases like nitrogen dioxide, sulphur dioxide, and halides have negative impacts on human health and the environment. Studies have revealed that symptoms such nose and throat irritation, bronchoconstriction, dyspnea, and respiratory infections are common when nitrogen dioxide and sulphur dioxide are inhaled or consumed by people, especially in asthmatic patients (Douwes et al. 2003). Patients who have asthma may experience episodes due to these effects. Additionally,

frequent human exposure to NO₂ makes people more susceptible to respiratory illnesses. Additionally, when these acidic gases get in contact with the atmosphere's moisture, they have a tendency to acidify it and cause acid rain to fall. Sulfur dioxide negatively affects plant development and productivity, according to Phadi et al. Additionally, exposure to heavy metals by inhalation or ingestion puts people at risk for lung cancer, asthma, ataxia, paralysis, vomiting emphysema, and impaired lung function. Heavy metal contamination has been demonstrated to cause conditions like high blood pressure and anemia (Hagerty et al., 1973). Additionally, heavy metals have a neurotoxic effect on the neurological system when in high concentrations, which results in neuropathies with symptoms like memory loss, sleep issues, rage, exhaustion, head tremors, blurred vision, and slurred speech. Additionally, it can harm the kidneys by increasing the likelihood of developing stones or nephrocalcinosis, renal malignancy, and early tubular dysfunction (Bharti et al., 2003). Lead exposure in humans can harm the glutamate system, the N-methyl-D-aspartate system, and the dopamine system (NMDA). The management of waste has been intimately linked to biological risks. The creation of bioaerosols and biological agents like fungi, bacteria, and volatile substances (including endotoxins, (1-3)-glucans, and mycotoxins) can be caused by the breakdown of waste items in the landfill, vehicle exhaust fumes, and suitable weather conditions (Wouters et al. 2002). Bioaerosol exposure has been linked to a number of respiratory conditions that can lead to airway inflammation. Studies have indicated that trash handlers and landfill workers face higher occupational risks than other workers. Communities that are closest to garbage facilities have reported cases of cancer and other respiratory problems. Endotoxins, which are found on the cell walls of Gram-negative bacteria, are the most potent pro-inflammatory substance in bioaerosols (Bikram et al., 2021).

Bioaerosols, which are constantly present in the atmosphere and play significant roles in practically all ecological units, are immensely variable and complicated. Airborne biological particles known as bioaerosols include bacteria, viruses, pollen, fungi, parasite eggs, and cells. These airborne bacteria are largely present in the atmosphere as a result of their natural origins in things like forests, marine environments, and desert dust. The components of bioaerosols studied by aerobiologists include (1) living organisms—including fungi, bacteria, microalgae, lichens; (2) biological components—including spores, pollen, viruses, mycotoxins, endotoxins, exotoxins, proteins, (1-3)- β -D-glucans, and (3) non-biological particles, organic dust, animal fragments/excreta (Douwes, 2003). Bioaerosols play a crucial role in the interaction, nucleation,

and health of ecosystems and have a biogeochemical relationship with the atmospheric, marine, and terrestrial environments (Molino, 2013).

Many pathogenic bioaerosols are produced at work by biological processes in the waste industries, such as wastewater treatment, solid waste composting, and landfilling (Bikram et al., 2021). Many harmful bioaerosols are released into the atmosphere as a result of the sophisticated biological and physical processes used in waste treatment plants to treat waste materials in a controlled setting (Bikram et al., 2021).

Several hazardous microorganisms, including the brand-new coronavirus SARS-CoV-2, are found in wastewater treatment plants. 41 antibiotic-resistant bacteria were found downwind of an MSW treatment plant, according to a recent study. Numerous studies have demonstrated that working around bioaerosols at places like landfills, wastewater treatment facilities, and composting operations can result in a number of allergy, inflammatory, and infectious disorders (Bikram et al., 2021).

People will therefore have to live close to landfills, which could cause an epidemic illness outbreak linked to bioaerosols (Hagerty et al., 1973). A landfill site may have a thousand times higher concentration of bioaerosols than any office building. (Bharti *et al.*, 2003).

2.7 Use of microorganisms in waste management

The aerobic biological treatment systems are home to a variety of microorganisms, including bacteria, fungus, algae, protozoa, rotifers, and other higher animals. The chemical properties of the industrial waste, the environmental constraints of the specific waste system, and the biochemical properties of the microorganisms will all play a role in determining whether any or all species of microbes may flourish in a given industrial waste disposal system (Omalu, 2012). Every microorganism that develops in a specific industrial waste disposal system affects the system's overall qualities, both positive and negative.

If the waste treatment system is to be optimally designed and run, it is crucial to understand the contributions made by each type of organism to the overall stabilization of the organic wastes. (Ezeonu *et al.*, 2012).

2.7.1 Bacteria

The fundamental biological components of aerobic waste treatment systems are bacteria. Bacteria's complex biochemical makeup allows them to digest the majority, if not all, of the organic chemicals contained in industrial wastes (Adebayo *et al.*, 2018) mandatory microbes. All aerobic waste treatment systems contain facultative microorganisms. Any species' ability to compete for a share of the system's organic material is a prerequisite for its ability to grow (Oyedeji *et al.*, 2010).

In aerobic waste treatment systems, microorganisms serve as the fundamental biological components. Most, if not all, of the organic chemicals found in industrial wastes can be metabolized by bacteria due to their complex biochemical makeup (Adebayo *et al.*, 2018) obliged microbes. In all aerobic waste treatment systems, facultative microorganisms are present. Any species' capacity for competitive acquisition of a portion of the system's available organic material determines how quickly it can reproduce (Oyedeji *et al.*, 2010).

The majority of the bacteria eventually die and lyse after the organic substrate is depleted. Other bacteria can thrive because the bacteria's biological components are released. Secondary predomination will happen since all biological therapy systems are typically overdesigned as a safety measure. The ability of the bacteria to flocculate is by far the most significant trait, after their metabolic traits. For full stabilization, all aerobic biological waste treatment systems rely on the separation of the microorganisms from the liquid phase through flocculation.

Initially, it was believed that *Zoogloea ramigera*, a single bacterial species, was responsible for flocculation, but more recent research has revealed that numerous other bacterial species are also capable of flocculation. All bacteria may be able to flocculate in specific environmental conditions, according to a theory. The primary influences on flocculation are the energy level and surface charges of the bacterium. It has been demonstrated that the electrical surface charge on bacteria cultured in diluted organic waste systems is less than the 0.020 volt threshold charge for auto-agglutination. This means that when two bacteria approach one another, Brownian

movement generates enough energy to overcome the electrical forces that repel them, allowing the Van der Waal forces of attraction to take control and hold the two bacteria together. If the system's energy level is high enough to allow the bacteria to grow and move quickly, autoagglutination won't happen. When bacteria lack the energy of motility to overcome the Van der Waal forces, autoagglutination, also known as flocculation, takes place. Some of the bacteria lyse and die after floc formation has begun. The remaining bacterial cell is mostly composed of polysaccharide and is insoluble. As the floc ages, more polysaccharide accumulates and less active bacteria are enmeshed in it (Nahar et al., 2011).

2.7.2 Fungi

In the stabilization of organic wastes, fungi are crucial. Similar to bacteria, practically every form of organic chemical included in industrial wastes can be metabolized by fungi. The fungi have the potential to outnumber the bacteria, but they only do so in exceptional environmental circumstances (Funmilayo et al., 2021). The majority of the fungi present in industrial wastes are filamentous, which makes them undesirable since they do not easily form a tight, compact floc and are prone to settling. Because of this latter factor, significant efforts are made to change the environment so that filamentous fungi predominate rather than bacteria (Funmilayo et al., 2021). Low oxygen tensions, low pHs, and low nitrogen concentrations favor filamentous fungus over bacteria. A limited oxygen supply or a high organic load that causes the demand to outweigh the supply leads to low oxygen tension. Under low oxygen conditions, metabolism stalls with the creation of organic alcohols, aldehydes, and acids rather than continuing to carbon dioxide and water. If there is not enough buffer in the system, organic acids will cause the pH to drop to a level that is more fungi-friendly (Kanthé, 2012). As a result, it is clear that low oxygen tension and pH may be connected. While few bacteria can grow well enough to compete, many fungi thrive at pH 4 to 5. Compared to bacteria, fungi need less nitrogen per unit mass of protoplasm. The fungi can produce more active masses of protoplasm from the wastes than the bacteria can, and they therefore predominate in nitrogen-deficient wastes. While fungi range from 5 to 6% nitrogen, bacteria often range from 10 to 12% nitrogen. Fungi will be present in a typical environment and will help to stabilize the organic debris. However, the fungus are secondary and will not take over. (Funmilayo *et al.*, 2021).

2.7.3 Algae

The third type of biological plant that contributes to the overall stabilization of organic wastes is algae. Like bacteria and fungi, algae do not need to metabolize organic substances in order to produce energy for synthesis (Kanthé, 2012). Instead, they primarily use the inorganic waste components, such as ammonia, carbon dioxide, phosphate, magnesium, potassium, iron, calcium, sulfate, sodium, and other ions, to form protoplasm. Since they do not use the same waste materials, it is conceivable for algae and bacteria to co-dominate. The bacteria break down the waste's organic components, releasing some of the inorganic elements that the algae use (Taiwo et al., 2012). The bacteria use the oxygen that the algae release during protoplasm synthesis to completely aerobically stabilize the organic matter. In the absence of sunlight, algae must produce their own energy through the metabolism of organic materials, just like bacteria and fungus do. Normally, this organic material originates from food that has been stored inside the cell, but in some algae species, it can also come from organic waste (Kanthé, 2012).

2.7.4 Viruses

These are biopolymer-based particles that can multiply and assemble into new virus particles inside active prokaryotic or eukaryotic cells. Because they can infect and degrade bacterial cultures in the environment, pathogenic viruses (also known as bacteriophages) must be removed, retained, or destroyed during water and wastewater treatment. Additionally, bacteriophages can be used to detect specific microbial pollution of waste in the environment. (Aberé *et al.*, 2011).

2.7.4 Protozoa

The simplest creatures that can be found in waste disposal systems are protozoa. By integrating a study of pure culture protozoa (Gram, 1953, unpublished observations) with the natural observations in diverse biological treatment systems, the role that protozoa play in stabilizing organic wastes has only lately been defined (Taiwo et al., 2012). This study demonstrated that the protozoa were not the main purification process, but rather were responsible for lowering the amount of free-swimming bacteria, assisting in the production of a cleared effluent. In biological waste disposal systems, the succession of protozoa has long been seen, but the causes of this succession were unknown (Tickell, 2000). The same reasons that affect any biological species' dominance also have an impact on protozoa's succession. The primary elements that affect the

predominance of the protozoa are the type of food and the level of food competition (Tickell, 2000). In aerobic waste treatment systems, the sarcodina are only occasionally detected because they cannot find enough food to outcompete bacteria and other biological forms. The Phyto-Mastigophora are able to consume soluble organics, which allows them to live a bit longer than the Sarcodina, but they cannot outcompete the bacteria and are quickly replaced. Because they can use the bacteria for food rather than competing with them for it, the Zoo-Mastigophora dominates the Phyto-Mastigophora. (Ezeonu *et al.*, 2012). But the free-swimming Ciliata, which have a better method for getting the bacteria and other food components, overtake the Zoo-Mastigophora. There are progressively fewer Ciliata that are free to swim as the system becomes more stable. The free-swimming, high-energy Ciliata is replaced by the low-energy stalked Ciliata. However, the stalked Ciliata quickly lose their ability to get sufficient energy and the system becomes so stable that they disappear (Ezeonu *et al.*, 2012).

A reliable indicator of the stability of the biological waste treatment system is the succession of protozoa. The same numerical population exists at two different and distinct stages of purification, making attempts to link protozoa numbers to the amount of stability unsuccessful. Both at low levels of purification (20–40%) and high levels of purification (75–95%), there are few free-swimming Ciliata. For each given system, the relative protozoa types and quantities can be used to estimate the system's approximate efficiency, which is 10%.

The protozoa are more vulnerable to harmful chemical substances than bacteria or fungus because they have more complicated metabolic processes. Regular monitoring of the protozoa in systems with poisonous organic chemicals can be utilized as a measure of the toxic concentration and a warning of potential toxicity to the bacteria that are in charge of stabilizing the wastes (Ezeonu *et al.*, 2012). The presence of protozoa may also signify a deficiency in certain vital nutrients, such as nitrogen or phosphorus. Deficits in nutrients will result in a decrease in the number of species overall as well as any single species. (Funmilayo *et al.*, 2021).

2.8 Microbial waste management

In general, biodegradable and non-biodegradable solid waste can be generically categorized. The solid wastes produced that could be broken down by microorganisms and do not pose serious long-term pollution sources are referred to as biodegradables (biowastes). They include wastes of

plant origin, wastes of animal origin (such as feces, carcasses, droppings, and poultry waste products), and wastes of paper (Ezeonu et al., 2012).

Even if certain types of solid trash are quickly broken down by microorganisms, they nonetheless emit a foul smell and disturb the aesthetics of the environment more than non-biodegradable solid wastes do. (Ezeonu *et al.*, 2012). They may also serve as a favorable environment for the growth of pathogenic bacteria that could easily contaminate supplies of fresh water and food in Nigeria's urban centers. However, microbes cannot degrade non-biodegradable solid wastes (Omalu, 2012).

This suggests that additional methods of treatment, such as landfilling, recycling, and incineration, are used to dispose of them. Examples of this group of solid wastes are solid wastes of metallurgical and smelting industries (abandoned vehicles, motor cycles, vehicle part and scrap metals, iron, zinc, aluminum sheets and other metals, machine parts); solids waste of construction industries (sand, gravel, bitumen wastes, concrete and waste building materials); solid waste of plastic industries (plastic buckets, cable insulators, tyres, chairs, tables, cellophane bags, plastic bottles, cutleries, sachet water containments, etc.) and glass products (Omalu, 2012)

2.8.1 Solid waste management

The proper management of solid waste contributes to economic growth, increased quality of life, and the reduction or elimination of harmful effects on the environment and human health (Adebayo et al., 2018). Composting, which is the controlled aerobic decomposition of organic waste materials by the activity of tiny invertebrates and microbes, is the most widely employed biological solid waste management method. Composting is a process that breaks down organic waste (food, plants, and paper) into compost, which is then reused in landscaping and agricultural uses. The most popular composting methods include in-vessel composting, vermin composting, static pile composting, and window composting. (Adebayo *et al.*, 2018).

2.8.2 Wastewater treatment: activated sludge and trickling filter

Microorganisms play a crucial role in wastewater treatment, which makes use of diverse mixed microbial populations that may break down any substance that is given to them (Oyedepi et al., 2018). The two main goals, according to Waites et al., are to eradicate all dangerous bacteria found in the sewage, notably the ones that cause the water-borne diseases cholera, dysentery, and

typhoid. The second goal is to convert the majority of the methane and carbon dioxide present in waste water into final effluent (outflow), which can then be safely released into the environment. Additionally, microbial processes can be used in the bioremediation of habitats contaminated by man-made xenobiotic chemicals and the breakdown of these substances in waste streams. (Oyededeji *et al.*, 2018).

The ultimate objective of wastewater treatment is to create microbes from the carbon and energy in the wastewater and remove those microorganisms from the water by settling (Napoli *et al.*, 2010). It's crucial to consider the connection between the source of carbon and the supply of energy for microbes. While energy must be supplied from sources outside the cell in order for synthesis to take place, carbon serves as the fundamental building block for cells. For growth, microorganisms need specific nutrients. The basic nutrients of abundance in normal raw sewage are carbon (C), nitrogen (N), phosphorus (P), with the ratio of C:N:P ratio approximately equal to 100:10:1.36. In addition to C, N, and P, trace amounts of sodium (Na), Potassium (K), magnesium (Mg), iron (Fe), and many others are required (Ezeonu *et al.*, 2012). The majority of these nutrients are present in typical municipal sewage. The majority of nutrient insufficiency issues arise in areas with high levels of industrial waste. When the right nutrients are lacking, the metabolism malfunctions and a type of bacterial fat (slime) starts to build up surrounding the cell. The cell's activity slows down because it is unable to make enough enzymes and because essential nutrients are unable to properly permeate the slime layer. The sludge won't settle, which slows down BOD removal (Napoli *et al.*, 2010).

Semerci (2016) defines activated sludge as a combination of microorganisms that come into touch with and break down biodegradable substances (food) from wastewater. As a result, the process of activating sludge is biological. Microorganism development needs to be carefully managed if the activated sludge process is to be effectively regulated. This entails managing the environmental factors that influence those microbes. It is observed that approximately 95% of the biomass in activated sludge is made up of bacteria. These single-celled organisms feed on biodegradable substances like proteins, carbohydrates, lipids, and a variety of other components to proliferate in the wastewater. Compressed air is used in activated sludge as a source of oxygen and for mixing. The fundamental method relies on diffused air at 6 to 8 psi to maintain the waste, oxygen, and microbes well mixed at all times and to provide oxygen to the bacteria. After

stabilizing the organic wastes, the bacteria flocculate and settle, leaving a clean supernatant with 10 to 15 ppm BOD. In order to maintain a high microbe population in the aeration tank, the settled microorganisms are partially returned to the head end of the tank as seed and the remaining portion is discarded to anaerobic digestion for further stabilization (Adebayo *et al.*, 2018). The trickling filter, on the other hand, is a widely used technique for secondary wastewater treatment. It consists of a filter bed with a highly porous media (gravel, plastic, etc.) that has a layer of microorganisms on the surface that causes a slime layer to form. The microorganisms in a trickling filter system cling to the medium in the bed and create a biofilm on top of it. The microorganisms consume and eliminate pollutants from the wastewater as it moves through the media. Sewage is sprayed through permeable media (a bed of rocks, molded plastic, gravel, and ceramics, among other things) in a trickling filter (Ezeonu *et al.*, 2012). The media must be both large enough to allow air to reach the bottom and small enough to optimize the surface area accessible to microbes. Because air may move through the media and the aerobic microorganisms in the slime layer can oxidize organic substances cascading over the surface into carbon dioxide and water, a biofilm of aerobic microorganisms develops on the media. This treatment method is less effective than activated sludge systems since it only eliminates 80 to 85% of BOD. They are easier to operate and do not have problem with toxic sewage (Adebayo *et al.*, 2018).

2.9 Recent advances in microbial waste management

The review of recent scientific developments in the useful application of microbes to environmental management and biotechnology is informed by recognition of the polluting effects of soil erosion, unwanted sediment migration, chemical fertilizers and pesticides, and improper treatment of human and animal wastes on the environment around man (Omalu *et al.*, 2012). Around the world, these negative events have led to significant environmental and societal issues, for which we must search outside of traditional physical and chemical technologies for remedies. Notably, developments in sustainable environmental cleanup techniques that are more adaptable to waste management systems include biological processes, including biotechnological tools. Biodegradation techniques including bioremediation, biostimulation, bioaugmentation, and phytoremediation are examples of such biotechnological technologies. (Igbeneghu *et al.*, 2014).

2.9.1 Antimicrobial Susceptibility Testing

Just knowing what kind of organism you are is insufficient. You should also be aware of the antimicrobial agents to which your organism is vulnerable. There are various ways to figure this out. Dilution testing is used to quantitatively determine the least antimicrobial agent concentration (in mg/ml) to prevent or eradicate the bacteria (Igbeneghu et al., 2014). This is accomplished by adding directly to an agar pour, a broth tube, or a micro-broth panel two-fold dilutions of the antimicrobial agent. The Minimum Inhibitory Concentration is the lowest concentration that prevents the organism from exhibiting visible growth. In Europe, the agar pour method is the standard test method. In North America, the broth dilution technique is more used. The E test (AB Bio disk) is a plastic strip that has been impregnated with antimicrobial chemicals in a gradient concentration. On an inoculated plate's surface, the strip is applied immediately. The strip where the growth inhibition intercepts the disk is where the MIC is read from. These strips cost a fair amount (Igbeneghu et al., 2014).

However, many doctors only need to be aware of the drugs that the pathogen is susceptible, intermediately sensitive to, or resistant to. The standardized procedure for determining antimicrobial susceptibility is the Kirby-Bauer agar diffusion method, which has a large body of research behind it. 6 mm-diameter disks of white filter paper are impregnated with known concentrations of antibacterial substances. The agent's name and concentration are encoded on each disk. For instance, AM-10 on the disk stands for 10 g of ampicillin. The Disk Zone Diffusion Diameter Chart includes the code (Bosch, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Samples use for the analysis were collected from Shagamu landfill in Ogun both in the South-west part of Nigeria.

3.2 Materials and Reagents

The following equipment and materials were used for the experiment: Volumetric flask, weighing balance, cotton wool, Filter paper, Funnel, Dropper, Test tubes, Test tube racks, Beaker, Measuring cylinder, Autoclave, Incubator, Spirit lamp, Water bath, Erlenmeyer flask, Nose mask, Hand gloves, Distilled water, Nutrient Agar, Potato Dextrose Agar, McCartney bottle, spatula, dropper, spreader, Petri dishes, glass slides, Microscope, Antibiotic Susceptibility Disc.

3.3 Culture Media and Reagents

The media used during the experiment were: Potato Dextrose Agar (PDA), Nutrient Agar (NA), Mueller Hinton Agar, Simmons Citrate Agar, MR-VP Broth. The reagents used during the experiment include: Lactophenol blue, ethanol, normal saline, Safranin, Methyl Red, Iodine, Crystal Violet, Bromo-Cresol Purple, Hydrogen Peroxide, Sodium Hydroxide (NaOH), Hydrochloric Acid (HCL).

3.4 Air sampling

Air sample was done using petri dishes each which were already prepared with PDA (Potato Dextrose Agar) and NA (Nutrient Agar). A total of 14 samples were collected from two different points to show the diversity and extent of microbial load in the air from areas close to landfills. The isolates were collected from different locations in and around the dump. These locations included the dump site A and B. 20 ml of nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The plates were then sealed and labeled appropriately and taken to the dump site where each was exposed for about five minutes at the selected locations for 30mins altogether. The plates were afterwards covered and taken into the laboratory for incubation at 37°C for 48hrs. This procedure was repeated for two days (Morning and afternoon).

Comment [Ma5]: space

3.5 Preparation of Nutrient Agar

According to the manufacturer's instruction, Nutrient Agar medium was prepared using 28.0 g of nutrient agar was measured on a weighing balance into a sterile conical flask; 1000 ml of distilled water was dispensed into the conical flask. 7 g of nutrient agar was measured in 250 ml of water. Swirling was done to the solution in the conical flask to dissolve the medium. The solution was then boiled in the water bath to ensure homogenization after mixing has been properly done. After boiling, the medium was autoclaved for 15 mins at 121 °C. Immediately after autoclaving, the medium was poured into the plates after serial dilution was done. The medium was allowed to solidify in this position.

3.6 Preparation of Potato Dextrose Agar

Potato Dextrose Agar (PDA) was used for the cultivation of fungi. PDA is a general-purpose medium for the cultivation of yeast and mold that can be supplemented with antibiotics to inhibit bacteria growth. 39 g of Potato dextrose Agar was suspended in 1000 ml distilled water, heated to boiling to dissolve the medium completely and sterilize by autoclaving at 121°C for 15 minutes and the pressure of the Autoclave was allowed to reduce to zero and the resulting mixture was well agitated before dispensing in a sterile Petri's dish (Adebayo *et al.*, 2018)

3.7 Serial Dilution

Serial dilution was performed for isolation of organisms from wastewater samples. 1 ml of wastewater was measured out and transferred into a sterile test tube with 9 mL of distilled water. Five-fold serial dilutions were prepared aseptically. The sterile test tubes were taken and labeled per dilutions ranging from 10^{-1} to 10^{-5} . 1ml of the respective wastewater sample was weighed and added to the first dilution blank of 9.0 mL of distilled water. 1.0 mL of the first dilution blank (10^{-1}) was added to the second dilution blank (10^{-2}). The tubes were shaken and serial dilution was done till the last tube dilutions (10^{-5}). 0.1 mL of the diluents was inoculated using the pour plate method in a disposable petri-dish with Potato Dextrose Agar (PDA) and Nutrient Agar. Then the plates were left for agar to set, and after that it was inverted and incubated at 37°C for 18-24 hours for NA and PDA was not inverted and incubated at 25°C for 48-92hours.

3.8 Pure culture Technique

This is a technique used to isolate a single species of microorganism from the mixed culture. This is used for both bacteria and fungi. Samples taken from the resulting colonies and a pure culture is grown on a new plate so that the organism could be identified. The pure culture of bacteria is then preserved onto agar slants for further studies and preservation.

3.9 Macroscopic and Microscopic Examination of Isolated Fungi

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium.

3.10 Biochemical characterizations of the bacterial isolates

3.10.1 Gram Staining

The gram stain is fundamental to the phenotypic characterization of bacteria. A smear was made on a glass slide and allowed to air dry. The crystal violet which is the primary stain was flooded on the fixed culture for 1 minute; the stain was washed off with water. Iodine solution was added to the smear for 1 minute and was poured off; then was rinsed with water. A few drops of ethyl alcohol (decolorizer) were added and rinsed with water immediately after 10 seconds and finally safranin which is the secondary stain was added for 45 seconds and washed off, then the smear was left to air dry. After the drying of the slide, it was observed under the microscope using 100x magnification lens. Gram staining was done to find reactions of the bacterial isolates to Gram reagents. Gram stain helps in distinguishing and classifying bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria (Olutiola *et al.*, 2000).

3.10.2 Simmons Citrate test

A 2.14g aliquot of Simmons citrate agar was dissolved in 500ml of distilled water gently homogenized using magnetic stirrer while swirling gently to dissolve the medium completely. Afterwards, the medium was sterilized by autoclaving at 121°C for 15minutes and allowed to cool at 50°C and poured in sterile test tubes. The tubes were then stabbed with a loopful of each isolate into each test tube and then transferred to the incubator and incubated at 37°C for 24hours. After 24hours, the tubes were observed (Olutiola *et al.*, 2000).

3.10.3 Starch Hydrolysis

Using a sterile technique, a single streak inoculation of organism was made and then inoculated into the center of labeled plate. The inoculated plates were then incubated for 48 hours at 37°C. After incubation, the surface of the plates was flooded with iodine solution with a dropper for 30 seconds. Then the excess iodine was poured off and examined for the clear zone around the line of bacterial growth (Abera *et al.*, 2011).

3.10.4 Methyl Red test

0.1g of methyl red was dissolved in 300 ml of ethyl alcohol, 95% with sufficient distilled water to make 500 ml. The prepared solution was store at 4 to 8 degrees Celsius in a brown bottle.

3.10.5 Voges-Proskauer test

0.1ml of 6% alpha naphthol was added to 1ml of MR-VP broth with two drops of 40% KOH (Potassium Hydroxide). The resulting mixture was added to the broth, shaken together in a test tube and allow to oxidize.

3.9.6 Catalase test

The reagent used for the catalase test was hydrogen peroxide. The pure culture was smeared on a sterile slide using a sterilized inoculating loop. Then, a drop of hydrogen peroxide was dropped on the smear. The result was then observed. The presence of oxygen bubbles indicated the presence of catalase and the absence of the oxygen bubbles indicated the absence of catalase.

3.10.7 Sugar Fermentation

A weight of 1 g peptone, 0.1g of NaCl, 1g of the fermentable sugar (Glucose, Sucrose, Maltose, Lactose and Galactose) and a pint of bromocresol purple was measured into a conical flask and the 100ml of distilled water was added, homogenized, dispensed to 9 test tubes. Inverted Durham tubes were placed in each test tube, covered with corks and sterilized for 15 minutes. Afterwards, each isolate was inoculated into each test tube respectively and incubated at 37°C. After 24hours, the results were observed (Olutiola *et al.*, 2000).

3.11 Antibiotic Susceptibility Test

The bacterial isolates were tested for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method. A suspension of each isolate was prepared in peptone water to match 0.5

McFarland turbidity standards in order to standardize the inoculum. The standardized inoculum of each isolate were then inoculated in triplicates onto the surfaces of plain Mueller-Hinton agar plates and Septrin (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Amoxicillin (30 µg), Ciprofloxacin (10 µg) Augmentin (30 µg), Gentamycin (10 µg), Perfloxacin (30 µg), Ampiclox (30 µg),Zinacef (20 µg) Erythromycin (15 µg) and Streptomycin (30 µg) discs were placed aseptically and incubated at 37°C for 24 h; after which the zones of inhibition were measured and compared with the standards of the Clinical and Laboratory Standards Institute guidelines (Weller, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Colony count

Nine (9) morphologically different bacteria were isolated from Shagamu dumpsite located in Ogun. Table 4.1 and Table 4.2 shows the colony counts for the air sampling and wastewater samples respectively. In table 4.1, the colony count at the end of 30 minutes of plates exposure for nutrient agar (NA) and potato dextrose agar (PDA) ranged from 27 – 700 cfu and 73 – 1400 cfu respectively. The highest count was observed at 30 minutes of exposure while the lowest was at 0 minutes. Table 4.2 shows the colony count for the serially diluted wastewater samples from the dumpsite. At the end of 48 hours of incubations, the counts ranged from 150×10^3 to 45×10^5 cfu/mL and 300×10^3 to 130×10^5 cfu/mL for the first and second wastewater sampling respectively. According to the activities of the highest heterotrophic fungi count (3.20 0.3 104 cfu/g), this result is in agreement with the findings of Oshoma et al. (2017) that the increased availability of biodegradable organic and inorganic substrates from the variety of municipal wastes continuously being dumped at these sites.

The evaluation of the microbiological loads in the air and water from the dumpsite in the Shagamu area of Ogun state, Nigeria, as well as the associated risks to people's health and quality of life. The high rate of counts is a sign that microbial activity and fecal wastes, which are also improperly discharged at the open dumpsite, are having an effect on the site. According to a study released in 2011, the activities of external factors, such as the point of collection, are responsible for some of the recorded levels of microbial contamination in dump sites, supporting earlier reports in which, using the passive sampling method, higher counts were discovered on settle plates with longer exposure times (Igbeneghu et al., 2011).

Comment [Ma6]: Where did you get 400?

Table 4.1: Total colony counts for the air samples

Sampling location	Sampling duration (minutes)	Bacterial plates (CFU/g)	Fungal plates (CFU/g)
	0	73	27
	5	756	180
	10	800	220
Shagamu dumpsite	15	1000	324
	20	1268	456
	25	1370	626
	30	1400	700

Table 4.2 Colony count for the wastewater samples

Sampling Location	Sampling durations	Serial dilution/Colony forming units	
		10^{-3} (cfu/ml)	10^{-5} (cfu/ml)
Shagamu dumpsite	First sampling	150×10^3	45×10^5
	Second sampling	300×10^3	130×10^5

4.2 Morphological Characteristics of the Isolates

Table 4.3 reveals the morphological characteristics of the isolates with color, shape, elevation, surface, opacity, consistency edge and sizes of the organisms been identified. The isolates are predominantly *Streptococcus*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Micrococcus* and *E. coli* in the samples examined from all waste dump soil was resistant to most antibiotics used. This is consistent with the findings of Mentese et al. (2008), who found that bacteria isolates could be essentially identified to a precise level through morphological examination based on the colors of the colonies that formed on the top and bottom of the bacterial cultures. The shape of the structures was used for additional verification through microscopic analysis. The identification of isolates up to the family or genus level can be accomplished through morphological examination, identification, and characterization of the isolates.

This present study identifies the microbiological quality of the air and in wastewater around dumpsites and pointed out the risk it poses to public health. The predominant fungi characterized from the air samples were *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., *Candida pseudotropicalis* and *Cladosporium* sp.; while *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus* and *Bacillus* species were the predominant bacterial species found in the dumpsite as reported by Mentese et al. (2008).

Tables 4.3 Morphological Characteristics of the bacterial Isolates

Isolates	Color	Shape	Elevation	Surface	Opacity	Consistency	Edge	Size
SAN1	Milky yellow	Irregular	Undulated	Rough	Transparent	Friable	Entire	Small
SAN2	White	Round	Entire	Smooth	Transparent	Friable	Entire	Large
SAN3	White	Irregular	Entire	Smooth	Transparent	Mucoid	Undulate	Large
SAN4	Yellow	Filamentous	Filamentous	Rough	Opaque	Viscid	Entire	Large
SAN5	Yellow	Round	Entire	Smooth	Opaque	Butyrous	Entire	Large
SDWN1	White	Filamentous	Flat	Rough	Transparent	Mucoid	Irregular	Small
SDWN2	Yellow	Round	Raised	Smooth	Opaque	Viscid	Entire	Large
SDWN3	Yellow	Round	Raised	Smooth	Opaque	Viscid	Entire	Large
SDWN4	Milky yellow	Irregular	Flat	Rough	Opaque	Friable	Entire	Large

4.3 Biochemical Characterization of the Isolate

Table 4.4 shows the prevalence of microbial loads in the dumpsite atmosphere. *Bacillus*, *Streptococcus*, *Pseudomona*, *Staphylococcus* and *Escherichia* are the most prevalent bacteria in the dumpsite, while *Bacillus subtilis*, *Micrococcus* sp., *E. coli* and *Bacillus* sp., are common microorganism in water collected in samples ogun dumpsite. *A. clavatus*, *Candida albicans*, *Geotrichum* and *phoma* were not distributed over a considerable extent. Chloramphenicol showed great susceptibility and strong resistance in the bacterial contaminants. Since these drugs are frequently used to treat human illnesses and in veterinary practice, the development of antimicrobial resistance by these bacteria to these drugs poses a significant challenge in both human and animal medicine. Concern should be expressed due to the presence of important bacteria for public health that were isolated in this study, which have been vividly described in

Comment [Ma7]: Are these discussion or your results. Because there's no citations. Pure nonsense

other studies. Because garbage is a key contributor to environmental contamination, the population's inappropriate waste disposal practices appear to be making matters worse. Unfortunately, the poor air quality in this area exposes people who go about their daily business there to a lot of diseases (Mentese et al., 2008).

Table 4.4: Biochemical Characterization of the Isolate

Isolates	Gram Staining	Shape	Catalase	Methyl Red	VP Test	Starch Hydrolysis	Citrate Utilization	Glucose fermentation	Galactose fermentation	Sucrose fermentation	Fructose fermentation	Probable Isolates
SAN 1	+	Rod	+	+	-	-	+	A	A	A/G	A	<i>Streptococcus</i> sp.
SAN 2	+	Rod	+	+	-	-	+	A	-	A/G	A	<i>Bacillus</i> sp.
SAN 3	+	Rod	+	-	-	+	+	A/G	-	A/G	A	<i>Pseudomonas</i> sp.
SAN 4	+	Rod	+	+	-	-	+	A	A	A/G	-	<i>Staphylococcus</i> sp.
SAN 5	+	Rod	+	-	+	+	+	-	A	A/G	-	<i>E. coli</i>
SDWN 1	+	Rod	+	+	+	-	+	A	A	A/G	A	<i>Bacillus</i> sp.
SDWN 2	+	Rod	+	-	-	+	+	-	-	A/G	A	<i>Micrococcus</i> sp.
SDWN 3	-	Rod	+	+	+	-	+	A/G	A/G	A/G	A/G	<i>E. coli</i>
SDWN 4	+	Rod	+	-	+	+	+	-	-	A/G	A/G	<i>Bacillus</i> sp.

KEY: + positive test; - negative test; A= Acid Production; G- Gas Production

4.4 Antibiotics sensitivity testing using Gram positive antibiotics disk

Comment [Ma8]: Generally, poor discussion

Table 4.5: Shows antibiotics sensitivity testing using Gram positive antibiotics disk against bacterial isolates from the dumpsites. Streptomycin and Pefloxacin has 26mm and 22mm respectively against all isolates. Streptomycin was highly sensitive against Streptococcus and Bacillus found in the air samples around Shagamu dump sites. Streptomycin and Septrin has high susceptibility against Staphylococcus aureus found in SAN 4. There was a clear zone for *Pseudomonas* found in SAN 3 *Escherichia coli*. Erythromycin was highly sensitive against *Bacillus subtilis* and *Micrococcus* in all the dumpsites in Lagos. All isolates tested against Zinnacef in Lagos dumpsites showed resistance.

According to the results of the study, all of the antibiotics utilized exhibit high sensitivity to *Pseudomonas* in SAN 3, while Pefloxacin, Gentamycin, and Erythromycin exhibit high sensitivity to *Staphylococcus* in SAN 4. (Faith et al., 2010)

Table 4.6: Shows the sensitivity testing using Gram negative antibiotics disk bacteria against bacterial isolates from the dumpsites. Ciprofloxacin and Augmentin were the most effective against the isolates, having efficacy of 58.33% and 43.75% respectively. According to testing, every isolate was resistant to cloxacillin and amoxicillin (Faith et al., 2010). Based on the results, all of the bacteria employed in this experiment are either susceptible to each of the used microbial disks or are intermediate to them. When the proper amount is applied to the infection site, the antimicrobial agent inhibits the bacteria, making it a member of the vulnerable category (Bikram, 2021). In the intermediate category, the ability of the bacteria to respond may be less than that of the susceptible. (Even though all of the used bacteria are susceptible to all microbial disks, the diameter of the inhibitory disk varies. The findings in the table 4.6 is in agreement with (Stephen, 2020).

Table 4.5. Antibiotics sensitivity testing using Gram positive antibiotics disk against bacterial isolates from the dumpsites

Organism	PEF (mm)	CN (mm)	APX (mm)	Z (mm)	AM (mm)	R (mm)	CPX (mm)	S (mm)	SXT (mm)	E (mm)
SAN 1	22	20	14	14	12	19	18	26	20	18
SAN 2	23	15	15	18	19	13	22	22	20	18
SAN 3	Cz	Cz	Cz	Cz	Cz	Cz	Cz	Cz	Cz	Cz
SAN 4	Cz	Cz	18	17	20	21	22	26	19	Cz
SAN 5	Cz	19	18	13	Nz	Cz	Cz	Cz	Cz	Cz
SDWN 1	14	17	15	Nz	13	Cz	Cz	Nz	17	20
SDWN 2	Cz	Cz	Nz	Nz	Cz	Nz	Cz	Cz	Cz	17
SDWN 3	Cz	Cz	Nz	Nz	Nz	Cz	Cz	Nz	Cz	Cz
SDWN 4	Cz	Cz	16	24	Cz	Cz	Cz	Cz	Cz	Cz

Key; Nz- No Zone, Cz; Cleared Zone, PEF- Pefloxacin, CN- Gentamycin, APX- Ampiclox, Z- Zinnacef, A- Amoxicillin, R- Rocephin, CPX- Ciproflaxin, S- Streptomycin. SXT- Septrin, E- Erythromycin

Table 4.6. Antibiotics sensitivity testing using Gram negative antibiotics disk bacteria against bacterial isolates from the dumpsites

Organism	SXT (mm)	CH (mm)	SP (mm)	CPX (mm)	AM (mm)	AU (mm)	CN (mm)	PEF (mm)	OFX (mm)	S (mm)
SAN 1	17	20	22	21	15	Nz	18	17	20	19
SAN 2	17	16	Nz	25	Nz	Nz	Nz	19	19	16
SAN 3	Cz	Cz	Cz	Cz	Cz	Cz-	Cz	Cz	Cz	Cz
SAN 4	14	19	Cz	Cz	Cz	18	21	18	20	16
SAN 5	Cz	Cz	Cz	Cz	Cz	15	Cz	Cz	Cz	Cz
SDWN 1	18	Cz	Cz	Cz	Cz	14	19	25	19	18
SDWN 2	Cz	Cz	Cz	Cz	Cz	Nz	Cz	Cz	Cz	Nz
SDWN 3	19	19	13	22	Nz	Nz	Cz	Cz	Cz	Nz
SDWN 4	Cz	Cz	Cz	Cz	Cz	Cz	Cz	Cz	Cz	Cz

Key; Nz- No Zone, Cz; Cleared Zone, SXT- Septrin, CH- Chloramphenicol, CPX- Ciprofloxacin, AM- Amoxicillin, AU- Augmentin, CN- Gentamycin, PEF- Pefloxacin, OFX- Taivid, S- Streptomycin

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study found that bacterial contamination of the water and air around us is caused by inefficient waste management in our community, from the point of generation to the point of disposal. In general, bacteria are more likely than fungi to contaminate the air and water, which is particularly true given how dirty the area is around these dump sites. The open dump waste disposal method can be inferred to be one of the potential environmental quality issues that leads to air and soil pollution. It also lowers the quality of the air by releasing unpleasant odors, various gases derived from anaerobic decomposition, as well as occasionally burning, and it may be a source of microbial and toxic chemical pollution of the soils of the dumpsites. As it encourages the dispersion of bacterial infections into the air, whether as free suspended organisms or linked to particles, it also acts as a potential source of air pollution and contamination. When these pathogens are suspended in the air, they are less significant, but when they land on surfaces, they become a serious problem because they can lead to a variety of infectious diseases that can impair lung function and respiratory symptoms that can range from mild acute conditions that hardly interfere with daily life to severe chronic respiratory diseases, cancer, and other conditions that need specialized care. The Nigerian Ministry of Environment and other related regulatory authorities must therefore develop such thresholds for Nigeria that would direct policies about the microbiological quality of the environment for better health. Additionally, practically all of the microbe species discovered in this study (particularly those from the bacterial domain) are capable of inflicting deadly diseases on the hosts they infect. To ensure microbiological biological particles linked with bioaerosols, there should be adequate ventilation and proper sanitation at residences in addition to permitting reasonable distances between the dumpsites and neighboring residential areas.

5.2 RECOMMENDATIONS

From the findings of this study, the following are recommended:

- i. To mitigate the effect of pathogenic airborne microbes around environment inhabited by humans, with the need to ensure that refuse is dumped far away from residential settings so as to reduce the chance of exposure to these bioaerosols
- ii. Water samples from well and other surface water bodies should be subjected to treatments before they are used for any domestic activities.
- iii. Liquid wastes should be properly treated before their subsequent release and should be channeled through the sewers in view of the hazard they pose to the environment.
- iv. Foods should not be exposed around waste dumpsite environment because of the risk of cross-contamination to humans.
- v. There is need to periodically monitor the number of microorganisms within the air of waste dumpsite environments using the established threshold stipulated for microbial concentrations in air.

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APPENDIX

Table 4.9 Component of the Nutrient agar

Ingredient	Amount (g)
Yeast extract	2
Peptone	5
Sodium chloride (NaCl)	5
Agar agar	15
Beef extract	1
Distilled water	1 L

Composition of Simmons citrate agar

Composition	Amount (g)
Sodium chloride	5.0
Sodium citrate	2.0
Ammonium Dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.2
Bromothymol blue	0.08
Agar	15
Distilled water	1L

Composition of Starch Agar

Composition	Amount (g)
Peptone	5.0
Yeast extract	2.0
Beef extract	1.0
Sodium chloride	5.0
Agar	15
Distilled water	1L
Starch	1%