

**EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE
BACTERIAL LOAD OF STORED CD PLATES**

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APRIL, 2024

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY
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CERTIFICATION

This is to certify that this project work was carried out by Success Osaruonamen AGHO (Miss) with matriculation number **LSC1906797** in the Department of microbiology, Life Sciences, University of Benin, Benin City under the supervision of;

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(Head of Department)

Date

DEDICATION

I dedicate this work to God Almighty. For being my source of wisdom and guidance.

ACKNOWLEDGEMENT

To God Almighty, the great one and my king, who has never left my side. Thank you for your love and guidance. Thank you for always lighting my path and never letting me walk in darkness. I want to appreciate my amiable Supervisor, Dr. I. S. Obuekwe. Thank you for your guidance and supervision. Thank you for your corrections, it brought out the best outcome. My gratitude to the Head of Department, Prof. (Mrs.) F.I. Akinibosun and other members and staff of the department.

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ABSTRACT

A compact disc, sometimes referred to as a CD, is an optical digital medium that can hold various types of data, including documents, audio, photos, and video. Understanding the bacterial load in stored CD plates is crucial to ensuring the longevity and integrity of CDs. This study determined the impact of temperature and relative humidity on bacteria load of stored CD plates at different locations (laboratory, lecture theatre and office). Thirty-six (36) CD plates were purchased and positioned at these different locations (as opened, closed with perforations and burnt with short video clips and opened). Temperature and relative humidity were monitored with thermometer and hygrometer, while bacterial count and identification were based on standard procedures for four weeks. Results obtained showed a fluctuation in temperature every week, but generally within the range of room temperature (30°C - 37°C) conversely, relative humidity increased weekly in the studied locations. Bacteria counts of all CD plates studied increased with increase in humidity with time. Bacteria isolated from studied CD plates were *Bacillus mycoides*, *Pseudomonads aeruginosa*, *Staphylococcus aureus*, *Bacillus pumilus*, *Escherichia coli* and *Serratia marcescans*. Conclusively, relative humidity had a positive correlation with bacteria load of the studies CD plate, while temperature had little effect on bacterial counts.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

A compact disc, sometimes referred to as a CD, is an optical digital medium that can hold various types of data, including documents, audio, photos, and video. Understanding the bacterial load in stored CD plates is crucial to ensuring the longevity and integrity of CDs. The storage of compact discs (also known as CDs) has become a common practice in various industries, including music, software, and data archiving (Ashish, 2017). However, the process of storing CDs can lead to bacterial growth, which can cause disc degradation and ultimately render the disc unreadable. It has been established that bacteria and actinomycetes can grow on floppy and compact discs under ambient conditions (Khan *et al.*, 2008). The bacterial load on CDs grows over time, which suggests that extended storage can cause a sizable rise in the number of germs. Bacterial colonies on the surface of CDs cause a decrease in reflectivity and an increase in scattering, leading to reduced optical performance and potential data loss. Bacteria on CD plates that have been preserved are worth studying for a number of reasons. The integrity and usability of the data stored on the discs may be jeopardized by the presence of microorganisms on CD plates (Khan *et al.*, 2008). Data loss or corruption may arise from the breakdown of the CD surface caused by bacterial contamination. Maintaining the durability and dependability of recorded data requires an understanding of the variables, such as temperature and humidity, that lead to bacterial development on CD plates. Bacteria on CD plates may be harmful to your health, especially if you handle the CDs often and from different people. It's critical to comprehend how storage conditions can affect bacterial load and viability. This is because some strains of bacteria have the potential to cause allergies or diseases (Jatzlauk *et al.*,

2017). Furthermore, the influence of environmental conditions on the longevity of digital storage media such as CD and DVD technology is becoming more and more significant as the world becomes more dependent on them. By investigating how temperature and humidity affect the number of bacteria on CD plates that are preserved, we can better understand how to keep these types of media in a way that maximizes their useful life.

According to a study by Qiu *et al.* (2019), the storage conditions, including temperature and humidity, play a crucial role in the preservation of objects and the stability of the bacterial strains stored on them. The authors reported that different bacterial strains have different optimum growth temperatures and that low temperatures can cause bacterial cells to enter a state of dormancy, known as the viable but non-culturable (VBNC) state, which can lead to a significant reduction in bacterial load. High humidity levels can provide a favorable environment for bacterial growth while low humidity levels can inhibit bacterial growth (Dannemiller *et al.*, 2017). High humidity causes an object's surface to produce a biofilm, which is home to microorganisms and gives protection, enabling bacteria to live and grow under harsh conditions (Yannick *et al.*, 2014). Microorganisms shrivel and lose water in dry air, and when the relative humidity (RH) falls below 60%, microbial replication ceases (Beuchat *et al.*, 2013). Closets and other enclosed areas with high humidity and restricted air flow may promote the growth of bacteria (Bockmuhl, 2017). The enzymatic reactions that drive bacterial growth and survival are both temperature- and humidity-dependent (Wolkoff, 2018). Therefore, this study assessed the impact of temperature and humidity on CDs stored at various locations

1.2. Specific objectives of the study were to:

1. assess the temperature and relative humidity of three different locations where CDs were stored
2. enumerate, isolate and identify bacteria from CDs stored at these different locations
3. evaluate the impact of temperature and humidity on the bacteria load of CDs at these locations

CHAPTER TWO

LITERATURE WORK

2.1. CD Plates

Compact discs are portable storage devices that can be used to record, save, and play digital media, such as audio and video. A compact disc can be defined as a plastic memory device that resembles a disc. CDs are media that can be read optically (Allegrezza, 2015). Vinyl records have been replaced by compact discs (CDs). The CD is primarily made of plastic. The plastic has a circular form, and it is covered on one side with a reflective metal coating typically aluminum. Optic media allow for far denser data storage than magnetic media (such as hard drives). Optic media are substantially more durable. This covering is burnt with millions of pieces. Numerous data kinds, including audio, video, games, papers, and more, can be stored on CDs. To visualize the data, a laser beam scans them using a CD driver. The only thing that a CD lacks in comparison to an HDD or DVD is storage capacity, which is also significantly less than read-write speed. A CD has a maximum storage capacity of 700 MB. In the beginning, as CD was created in 1960 by American physicist James Russell. In 1980, Philips and Sony unveiled the compact disk (CD), which was eventually made available in 1982. He sought to make the record player better after realizing one day that the audio quality was really bad while listening to music. However, he came to understand that the recorder or rather, the needle's contact with the recorder is the cause of the music's low quality. Then it occurred to him that this could be prevented by reading the music on the disk with a light without really touching it. Subsequently, he began working with optic media to capture digital data. He discovered a method for putting media onto a photosensitive platter that had 1-micron-diameter bits of light and dark. Then, in 1966, he submitted an application for a patent, which was approved in 1970. Following this, he licensed

his patents to Sony and Philips for the use of optically readable media for recording. Finally, in 1982, Sony released the CDP-101, a CD player. This is the introduction to CDs. The CD is made up of an 80mm diameter, 1.2mm thick hole in the middle and a tiny, 120mm diameter disc with a density of 16,000 TPI (Palermo *et al.*, 2021). These are the smallest compact discs available. The disk is composed of a thin polycarbonate plate with a label attached to one side that usually contains data and a metal film formed of an aluminum alloy dispersed over a plastic coating that shields the metal surface. In contrast to a vinyl record, a CD begins to reproduce from the inside edge rather than the outside. The process of burning CDs begins in a factory where the primary disk is punctured by a very strong laser. A pattern is generated from this master disk, which will be used to store the duplicates. Unlike with the primary disc, copies are created using pressure rather than a laser. Two trillion pits are positioned in a single spiral that extends from interior to outside and spans a whole spiral of 6 km long CDs. The perforated sections are referred to as "pits," and the closed areas are called "soils." In contrast to a vinyl record, CD playback begins right away after the "lead-in" (innermost area). The spiral width of CD singles ranges from 0.5 to 0.83 microns, with a 1.6 micron gap between them. Following the holes, a 125 nm thin coating of acrylic is applied to the polycarbonate block, a clear layer is left on top for protection, and a label is printed on it, as seen in the picture. The CD spins rapidly as it reads the interior, then slows down as the head gets closer to the outer edge.

2.2. Types of CD Plates

2.2.1. CD-ROM

ROM stands for memory that can only be read. These kinds of CDs include content that can only be read; it cannot be changed. This CD can be played on any regular Compact Disc player. This media was first widely utilized in the pre-recorded music industry. Since then, nevertheless, it

has been used to distribute data, licenses, computer software, and instructional materials. Large volumes of data may be stored on these CDs because they have a 650 MB storage capacity.

2.2.2. Recordable CD (CD-R)

Content can be written to this media using an external CD drive or a CD drive that is connected to a computer. A dye is applied to a new CD so that light shining on it will cause it to change color. The nature of this dye is photosensitive. A CD writer changes the color of the dye by shining specific laser beams on it while writing or burning data. Any standard CD player may read these CDs. Reading data through pits and lands in a typical CD is analogous to sensing a shift in hue while reading data. The physical dye layer has a shelf life; as time passes, its properties could decrease and errors could result. The quality and storage conditions of a CD determine its lifespan. On any specific CD, recording may only be done once; it cannot be changed or overwritten. The CD's unburned portion can be updated with new information.

2.2.3. Rewritable CD (CD-RW)

This medium uses a metallic alloy instead of CD-R dye. During CD writing, the metallic alloy layer's characteristics are altered by the laser beams. The reflectivity of the alloy can be utilized to monitor any changes in its crystalline or amorphous qualities, which are important for reading. Bypassing the lower intensity laser and reading CDs burned in this manner, most CD players can read them without affecting their attributes. This CD allows you to store new data and wipe existing info as often as you'd like. The writing procedure involves first melting the metallic layer with laser beams, after which the data is saved. It's crucial to remember that writing on this medium requires more time than writing on CD-R.

2.2.4. DVD

A digital videodisc can hold up to 4.7GB of content, which is six times more than a standard CD. DVDs are widely used in high-quality film releases and are thought to be significantly better than VCDs and VHS tapes. Special features on DVDs include commentaries, deleted sequences, and interactive menus. Data can be stored on both the top and bottom of it. Aside from that, it stores data in multiple layers. The dimensions and technology employed are identical to those of standard CDs; however, DVDs have a distinct advantage due to their many internal layers and sides.

2.2.5. Mini CD

The Mini CD has a width of about 3 inches and has a data storage capacity of 210 megabytes, or up to 24 minutes of music. Most CD players are compatible with mini CDs.

In addition to being frequently used for single song recordings, it was also employed in business and advertising.

2.3. Application of CD Plates

2.3.1. Advertising

Everybody has once received a free CD offer, whether it comes from a store, appears on the front of a magazine or newspaper, or is sent to them directly. When it comes to software promotion, CDs are an excellent marketing tool because they can have both information about the program and the software itself. This eliminates the need for an expensive printed brochure and saves a ton of money. The brochure's distribution expenses are also avoided, in addition to the cost of

publishing it. The global market for CDs is quite well-established. We can guarantee that anybody can listen to or see an advertising campaign on CD because almost every home has a device that can play the disc, such as a computer, CD music player, or even DVD video player. It is far less expensive to include the entire catalog on a CD disk if a business, such one that sells building supplies or electrical components, has a large product inventory. Instead of sifting through page after page of irrelevant products, the user may conduct intelligent searches for the things they need and discover this information quickly. The substantial cost savings on paper and printing that come with having the entire product catalog on CD are a major advantage. CD provides an alternative to hundreds of catalogue pages.

2.3.2. Educational CDs

A affordable and portable format for storing data is CD. They are highly well-liked at universities and schools as a result. Instructors can distribute recorded lectures on CDs to their students; in fact, they can record an entire semester or term's worth of coursework and provide it to them. Students can then easily store and transport their assignments by copying them onto a CD. Rewriteable discs allow students to make several uses of the same disc, resulting in multiple economies of scale from the investment in a single disc.

2.3.3. Office use

As mentioned before, CDs make excellent archival and storing media. Any type of file format can be stored and backed up on a disc, including designs, databases, spreadsheets, contracts, emails, backups, images, video, and audio files. Another usage for the CD in the office is as a business card; a rectangular CD specifically designed for this function has been produced. The disc has a print on top that looks just like a standard business card, but when inserted into the

computer, it functions just like a standard CD or data storage device. There are countless options. For instance, a graphic design firm may save their whole portfolio on their business card. Similarly, an animation or filming studio could copy their showreel movie on the CD. A construction or architectural firm may have numerous images of their structures or interior designs saved on the card; as a result, the user has instant access to samples of your work in addition to your contact information.

2.3.4. CDs for bands and Musicians

The CD is the perfect format for the release of music, whether a small quantity of promo CDs or a full CD pressing of most recent album. For CD duplication job, a large selection of CD cases are available, with the majority of cases starting at 100 units. The short run CD duplication work can appear just as distinctive and individualized as the music on the CDs thanks to the large selection of bespoke casings.

2.3.5. CDs for church and other faiths.

A great tool for sharing the gospel or ministry message is a CD. A CD can hold hours' worth of spoken word content if the audio is preserved in MP3 format, or up to 78 minutes of audio in total. This implies that numerous sermons, discourses, and preachings can fit onto a single disc, allowing listeners to enjoy the spoken word while working out or relaxing in their house or vehicle.

2.4. Microbial contamination of stored CD plate

Compact discs, or CDs, that are stored should be free of microbes if you want to save your digital data for a long time (Lee *et al.*, 2012). Bacteria, fungi, and mold are examples of microorganisms that can grow on the disc's surface and inside its layers, perhaps damaging the

disc and wiping out data (Iraci, 2017). These are caused by Inadequate storage conditions with factors like temperature, relative humidity and exposure to contaminated environments, might give birth to this problem.

2.4.1. Relative Humidity

Humidity affects microbial growth, as high humidity levels can provide a favorable environment for the growth of microbes, while low humidity levels can inhibit microbial growth. Humidity affects the survival of microorganisms, as low humidity levels can cause microbial cells to dehydrate, leading to cell death. High humidity levels can prevent bacterial cells from drying out, leading to increased microbial survival. Microorganisms shrivel and lose water in dry air, and when the relative humidity (RH) falls below 60%, microbial replication ceases (Beuchat *et al.*, 2013). Humidity affects microbial metabolism, as enzymatic reactions that drive microbial growth and survival are humidity-dependent. High humidity levels can accelerate enzymatic reactions, leading to increased microbial growth, while microbial metabolism is gradually suppressed when the relative humidity equilibrium drops, making the environment unfavorable for growth (Beuchat *et al.*, 2013). Optimal storage conditions for CDs and DVDs should be maintained to minimize bacterial contamination. Keeping CDs and DVDs in places with low humidity (less than 50%) and low heat (below 25°C/77°F) is a good condition to consider (Khan *et al.*, 2008)

2.4.2. Temperature

Numerous investigations have shown that temperature is a key factor in microbial growth (Montville and Matthews, 2001). Based on the range of temperatures at which they can thrive, microbes can be loosely categorized. Its minimal growth temperature is the lowest temperature at

which the organism can endure and proliferate. Its maximal growth temperature is the highest temperature at which growth is possible. Mesophiles are suited to temperate climates; ideal growing temperatures falling between room temperature (about 20 °C) to 45 °C (Schiraldi and De Rosa, 2015). Pathogens such as *Escherichia coli*, *Salmonella spp.*, and *Lactobacillus spp.*, as well as the typical human microbiota, are mesophiles, given that the human's body core temperature is 37°C(98.6°F) (Herrera, 2001). Psychrophiles, Psychrotolerant, another name for them, means they like colder climates, ranging from a high of 25 °C to a low temperature of roughly 4 °C (Clarke *al et.*,2013). They can be found in a wide range of temperate natural settings. They are also to blame for food that has been refrigerated going bad. The species recovered from Atlantic such as *Moritella profunda sp.* and *Moritella abyssi sp.* are regarded as severe cold-loving psychrophiles (Xu *et al.*, 2003). Psychrophiles are a type of bacterium that may develop at temperatures as low as 0 °C, have an optimal growth temperature of about 15 °C, and often cannot survive at temperatures higher than 20 °C. They live in cold climates that are always there, like the deep ocean waters (Georges and Gerday, 2003). Psychrophiles are significant decomposers in cold areas because they are active at low temperatures. Thermophiles are organisms that can grow at temperatures between 50 °C and 80 °C, which is their optimal range. At room temperature, they do not proliferate. Thermophiles are found in hot springs, geothermal soils, and artificial settings like garden compost piles, where microorganisms decompose leftover food and plant matter. *Geobacillus* species and *Thermus aquaticus* are two examples of thermophiles. The hyperthermophiles are found further up on the severe temperature scale. They thrive in temperatures ranging from 80 °C to a maximum of 110 °C (Gupta *et al.*, 2014), with some extreme specimens being able to withstand temperatures beyond 121 °C, which is the autoclave's average temperature. With projected temperatures of 340 °C (Li *et al.*,

2014), the hydrothermal vents at the ocean's bottom serve as a perfect illustration of harsh settings. Temperatures above 100 °C are ideal for the development of microbes isolated from the vents. Two notable instances are the archaea *Pyroboilus* and *Pyrodictium*, which can withstand autoclaving and thrive at 105 °C.

2.4.3. Exposure to contaminated environment

CDs may become contaminated if they are kept next to other objects, including books or documents. This is because bacteria from these surfaces may spread to the CDs, especially if the CDs are not closed (Mahbubar *et al.*, 2008).

2.5. Microorganisms associated with the contamination of CD plate

Compact disc contamination is frequently linked to microorganisms such as fungi, viruses, and bacteria (Palermo *et al.*, 2021). These microbes can come into contact with CDs in a number of ways, including through handling them with unclean hands, coming into contact with contaminated surfaces, or storing them in an unhygienic environment. Commonly present on surfaces, bacteria such as *Escherichia coli*, *Bacillus* species, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* can readily infect CDs. If these bacteria get into contact with broken skin or mucous membranes, they may degrade the disc's surface and provide a health danger. Mold and yeast are two more typical fungi that contaminate compact discs. Since CDs are frequently kept in damp, dark places, they can serve as the perfect habitat for the growth of fungi. On the disc's surface, species like *Aspergillus*, *Penicillium*, and *Candida* can proliferate and lead to disc material degradation, discoloration, and odor. Viruses are less prevalent, but they can still contaminate compact disks (Khan and Karim, 1996), particularly if they come into touch with human fluids or respiratory droplets. For a brief length of time, viruses such as the influenza,

rhinovirus, and norovirus can potentially survive on CD surfaces. If these viruses are touched and subsequently spread to mucosal membranes or the face, there is a danger of transmission.

2.6. Bacteria associated with the contamination of CD plate

The accumulation of dust, debris, and other particles on the disc's surface gives bacteria something to consume and creates an environment that is conducive to their growth. Since bacteria are tiny creatures, they can quickly attach themselves to a CD's surface, particularly if the disc is not kept in a dry, clean environment (Shirahama *et al.*, 1996). Compact disc contamination can be linked to various forms of microorganisms. The common human skin bacteria *Staphylococcus* can be transferred to CDs by handling or contact. They are able to last in a variety of environments, and if they come into contact with broken skin, they may result in skin infections or other health problems. Spore-forming bacteria, or *Bacillus* bacteria, can withstand harsh environments. They have the ability to contaminate CDs and survive for long stretches of time, which may eventually lead to surface deterioration. *Pseudomonas* bacteria are frequently connected to moisture and water. *Pseudomonas* species can colonize the surface of CDs subjected to high humidity or water damage, which can accelerate disc destruction. Certain bacteria have the ability to produce acidic byproducts and enzymes that could deteriorate the compact disc's components. This may result in physical harm to the disc's surface, such as pits, scratches, or other flaws that may impair playback and readability. Compact discs may contain bacteria that could be harmful to the health, particularly if the discs come into contact with cuts or mucous membranes. If present on the disc surface, pathogenic bacteria such as *Escherichia coli* or *Staphylococcus aureus* may spread to a person's skin and cause infections or diseases. Compact disc bacteria have the potential to cause cross-contamination. The germs on the contaminated disc can move quickly to other regions and possibly cause more contamination if it

comes into touch with hands, objects, or other surfaces. Certain types of bacteria can discolor the disk surface or give off offensive odors. For instance, mold development might cause the CD to smell musty and develop obvious fungal patches, which can detract from the CD's aesthetic appeal and possibly render it unplayable (Khan and Karim, 1996).

2.7. Prevention and Control strategies

Maintain a temperature range of 15°C to 25°C (59°F to 77°F) with a relative humidity of no more than 60%. CDs should not be kept in moist or humid spaces like bathrooms or basements. CDs should be kept in their original cases or in pristine storage cases that are made to keep them safe from dirt, moisture, and other elements. Avoid placing CDs in direct sunlight since this might damage the disc and encourage the growth of microorganisms. Wash your hands before handling CDs to prevent bringing dirt or bacteria onto the disc. Use archival-quality sleeves and labels when removing CDs from their cases in order to prevent contamination. Indicators of microbial contamination include warping, discolouration, and mold growth on your CDs. If you find any problems, clean the discs right away and take care of the storage circumstances that are causing the issue. Appropriately make sure to properly dispose of a badly contaminated CD if the data cannot be recovered in order to stop the spread of bacteria. You may guarantee the longevity of your priceless digital data and drastically lower the chance of microbial infection of stored compact disks by taking these procedures.

2.8. Effects of Temperature on the bacterial load of stored CD plate

The subject of how macromolecules and metabolic processes adapt to harsh settings is an intriguing one. Numerous effects of very low temperatures are observed in cells. Ice crystal formation damages and depletes membrane fluidity (Ratkowsky *et al.*, 2005; Chintalapati *et al.*,

2004). Diffusion and chemical reactions slow down significantly. Proteins can denature and become too stiff to catalyze processes (Phadtare, 2004). Heat causes proteins and nucleic acids to become denatured, which is the other end of the temperature range. In membranes, increased fluidity hinders metabolic processes (Li *et al.*, 2005). The damaging effects of heat on bacteria can be used in practical ways, such as steam sterilization, pasteurization, and cremation of inoculating loops. When psychrophile proteins are compared to comparable proteins from mesophiles, they are often more flexible, include more hydrophobic residues, and have fewer secondary stabilizing links. It is typical to find antifreeze proteins and solutes that lower the cytoplasm's freezing temperature. To improve fluidity, the lipids in the membranes are often unsaturated. Compared to growth rates at moderate temperatures, these are substantially slower. Mesophiles and even thermophiles may withstand cold temperatures in the right circumstances (Timer *et al.*, 2007). For long-term storage as stocks, liquid cultures of bacteria are combined with sterile glycerol solutions and frozen at -80 °C. After being freeze-dried, or lyophilized, cultures can be kept as powders in sealed ampules and reconstituted with broth as needed.

2.9. Effects of Relative humidity on the bacteria load of stored CD plate

The quantity of moisture in the air, or relative humidity, can have an impact on the development of bacteria on CD plates. Bacteria can only reproduce when there is water present, therefore they frequently flourish in moist conditions (Dannemiller *et al.*, 2017). Elevated relative humidity fosters an atmosphere conducive to bacterial growth, resulting in a higher bacterial burden on CDs that have been stored. High humidity causes an object's surface to produce a biofilm, which is home to microorganisms and gives protection, enabling bacteria to live and grow under harsh conditions (Yannick *et al.*, 2014). High humidity levels can accelerate enzymatic reactions, leading to increased bacterial growth, while microbial metabolism is gradually suppressed when

the relative humidity equilibrium drops, making the environment unfavorable for growth (Beuchat *et al.*, 2013). Spores and the length of a microorganism's growth stagnation period are impacted by relative humidity (Tang *et al.*, 2015; Esbelin *et al.*, 2018). Microorganisms' development and reproduction are influenced by Relative Humidity (Moat *et al.*, 2002). Microbial cells typically contain 70-90% water. Research has shown that some bacteria may endure longer in high humidity environments, which may increase the danger of contamination on CD surfaces.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1. Materials

The materials used for this experiment were compact discs, sterile distilled water, petri dishes, sterilized swap stick, streak loop, thermometer, hygrometer, autoclave, micropipette and pipette tips, glass slides, Whatman paper, Bunsen burner, Computer with software for data analysis, saline water, Agar (Nutrient agar, MacConkey agar, citrate agar, bile aesculin-azid agar, tryptic sugar iron), reagents (KOH reagent, Indole reagent and oxidase reagent).

3.2. Samples collection and Preparation

Thirty-six (36) CD plates were purchased and positioned at three different locations with different temperature and relative humidity which were determined with thermometer and hygrometer; Laboratory (36°C, 13%), Lecture theatre (37°C, 12%) and office (37°C, 12%). Out of these CDs, twelve (12) were opened, another 12 were closed (wrapped in plastic with perforations for air) and 12 were burnt with short video clips and opened. This is to check for the integrity of the stored information after it has been exposed at different temperature and humidity conditions. The discs were stored for four weeks, and three each were removed from each location per week for swabbing and bacterial isolation.

3.3. Preparation of Culture Medium

The medium used was prepared according to the manufacturer's instructions. The medium used was Nutrient Agar. Twenty-eight (28) g of nutrient agar was measured and dissolved in 1 liter of distilled water in a conical flask covered with aluminum foil. It was mixed thoroughly until the

agar dissolved and was sterilized in an autoclave at 15psi for 15mins at 121°C. The medium was left to cool after sterilizing to 45-50°C.

3.4. Bacterial Isolation

Nine (9) vile bottles of saline water were autoclaved at 121°C for 15 mins. After cooling, the vile bottles were labelled appropriately according to the sample codes in the location placement; LTO, LTC, LTB, LBO, LBC, LBB, SOO, SOC and SOB. Swabbing process was used for samples collection from the different CDs. This involved moistening the sterile swab by dipping it into saline water, ensuring that the swab was saturated without excessive dripping. The swabbing was thorough, covering the area of the disc systematically. After swabbing, the swab was carefully returned to the vile bottle containing saline water with the right sample code labelling. After each collection, the vile bottles were sealed securely to prevent leakage during transport.

Pour plate method was used for culturing. Eighteen (18) sterile petri dishes were labelled appropriately (9 samples in duplicates for accurate results). Using a micropipette and pipette tips, 0.1ml of the samples were carefully inoculated into the petri dishes. The prepared nutrient agar was poured into the plates containing the samples and swirled carefully. Then left to solidify. The plates were inverted and incubated for 24 h. After which petri dishes were observed for growth and colonies counted. The cultural characteristics; colony size, shape, margin, color and elevation were observed.

3.5. Isolation and Pure Culture

The growing colonies picked from the plates were sub cultured on fresh nutrient agar plate to continue the growth process and maintain the culture.

3.6. Bacterial Identification

3.6.1. Differential Culture

This process involves using differential media to identify bacteria. This method allows for the differentiation of bacteria based on their ability to grow in specific conditions and utilize particular nutrients, leading to the identification of different bacterial species. Differential media contains substances that enable differentiation of bacteria based on their metabolic properties, such as fermentation of specific sugars or production of certain enzymes. The differential media used were; Bile-aesculin-azide Agar, MacConkey Agar, Citrate Agar and Tryptic Sugar Iron Agar.

3.6.2. Preparation of differential medium

Bile-aesculin-azide Agar is a selective and differential medium that contains bile salts and the compound esculin, which inhibit the growth of many Gram-positive and Gram-negative organisms except for the group D streptococci and enterococci. The presence of a black color on BAA agar indicates that the bacteria being tested are able to ferment glucose and produce aesculinase. It was prepared by measuring 64.5 g of BAA agar in 1 liter of distilled water in a conical flask covered with aluminum foil. It was then mixed thoroughly until the agar dissolved and was sterilized in an autoclave for 15 mins at 121°C. The medium was left to cool to 45-50°C.

Citrate Agar is a differential medium used in distinguishing between bacterial strains based on their ability to utilize citrate as their sole carbon source. Bacteria that can use citrate will turn the PH indicator in the agar from green to blue. It was prepared by measuring 24.28 g of Citrate agar into 1 liter of distilled water in a conical flask covered with aluminum foil. It was then mixed

thoroughly until the agar dissolved and was sterilized in an autoclave for 15 mins at 121°C. The medium is left to cool to 45-50°C.

MacConkey Agar is a differential medium used to differentiate between lactose fermenting and non-lactose fermenting bacteria. Lactose-fermenting bacteria, such as *Escherichia coli* will grow pink colonies on MacConkey agar while non-fermenting bacteria will grow colorless colonies. It was prepared by measuring 52 g of MacConkey Agar in 1 liter of distilled water in a conical flask covered with aluminum foil. It was then mixed thoroughly until the agar dissolved and was sterilized in an autoclave for 15 mins at 121°C. The medium was left to cool to 45-50°C.

Tryptic Sugar Iron Agar is also a differential medium that contains various sugars and a sulfur source to assess a bacterium's ability to ferment these sugars and produce hydrogen sulfide (H₂S). Based on the fermentation patterns and H₂S production, TSI allows for visual differentiation between bacterial strains through color changes and precipitate formation. It was prepared by dissolving 64.5 g of TSI agar in 1 liter of distilled water in a conical flask covered with aluminum foil. It was then mixed thoroughly until the agar dissolved and sterilized in an autoclave for 15 mins at 121°C. The medium is left to cool to 45-50°C.

After all the agar needed for differential culturing were prepared, they were all poured on Petri dishes and left to solidify. Using Streak method, the colonies in the sub cultured plates were re-streaked on the solidified differential plates and incubated for 24 h. The Tryptic Sugar Iron Agar which is quite different from the rest differential plating are poured into test tubes and left to solidify. With a streak loop, the colonies were carefully dipped into the solidified test tube.

3.6.3. Reading Differential media

The presence of a black color on BAA agar indicated that the bacteria being tested were able to ferment glucose and produce aesculinase. Bacteria that used citrate turned the pH indicator in the agar from green to blue. Lactose-fermenting bacteria grew pink colonies on MacConkey agar while non-fermenting bacteria grew colorless colonies. The presence of red coloration of the test tubes showed that the pH of the bottom was acidic, while yellow coloration means the pH was alkaline. Black coloration on some corners meant Hydrogen sulphide was produced. Bubbles in the test tube also showed the presence of gas.

3.7. Biochemical tests

3.7.1. KOH (Potassium hydroxide) test

KOH can be used to identify certain types of bacteria called Gram-positive cocci. Slime, a viscous, gelatinous substance is produced by these bacteria when tested. A drop of KOH solution was added to a glass slide. Isolates were picked with inoculating loop and smeared on the slide. A slimy texture gives a positive result.

3.7.2. Indole test

Indole test is used to identify certain bacteria that can break down the amino acid, tryptophan and produce indole as a by-product. A Whatman paper was drenched with indole reagent and left to dry a little. The isolate was smeared on the filter paper. A green color change after 10s gave a positive result.

3.7.3. Oxidase test

Oxidase test is used to identify bacteria that produce the enzyme, oxidase. The enzyme is found in Gram-negative bacteria. A Whatman paper was drenched with Oxidase reagent (1% aqueous

solution of tetramethyl-p-phenylenediamine hydrogen chloride) and left to dry a little. The isolates were smeared on the filter paper. A green color change gave a positive result.

3.7.4. Data Analysis

The data were analysed using the SPSS package version 21.0. All data are mean of replicates.

The mean, range and standard deviation of each parameter was determined.

CHAPTER FOUR

RESULTS

Temperature and relative humidity of three different locations (laboratory, Lecture theatre and office) were determined as well as bacterial load of different stored CD plates in these locations for four (4) weeks. The temperature and relative humidity of each location were measured before the samples were taken in all the weeks as shown in Figure 1 and 2. Figure 1 shows a little decrease in temperature every week, except the lab storage location which decreased in the second week, then increased in the third and fourth week. Figure 2 shows an increase in the relative humidity of the studied locations at week 2 and week 4. Temperatures were within the range of 30°C-37°C. Tables 1a, 1b, 1c and 1d, shows the bacteria count, mean and standard deviation of the CD plate samples stored at the laboratory, with the fourth week having the highest bacteria count. Tables 2a, 2b, 2c and 2d, shows the bacteria count, mean and standard deviation of the CD plate samples stored at the lecture theatre, with the fourth week showing the highest bacteria load. Tables 3a, 3b, 3c and 3d shows the bacteria count, mean and standard deviation of the CD plate samples stored at supervisor's office, with the third week having the highest bacteria load. The mean data of the bacteria load of the CD plate samples in the studied location for the four weeks period are shown in Tables 1e, 2e and 3e. Table 1e shows that there is increase in bacteria load as time progresses, with the open CDs having the highest bacteria load, which is due to the increase in the humidity every week. Table 2e shows that there is a decrease in the bacteria load at the second week, due to the little decrease in humidity. Table 3e shows that there is a decrease in the bacteria load on the fourth week, which correlate with the decrease in humidity at the fourth week. Table 1f, 2f and 3f shows the morphological characteristics and the biochemical test of the CD plate samples of each location and possible

bacteria such as; *Bacillus mycoides*, *Pseudomonads aeruginosa*, *Staphylococcus aureus*, *Bacillus pumilus*, *Escherichia coli* and *Serratia marcescans* where identified.

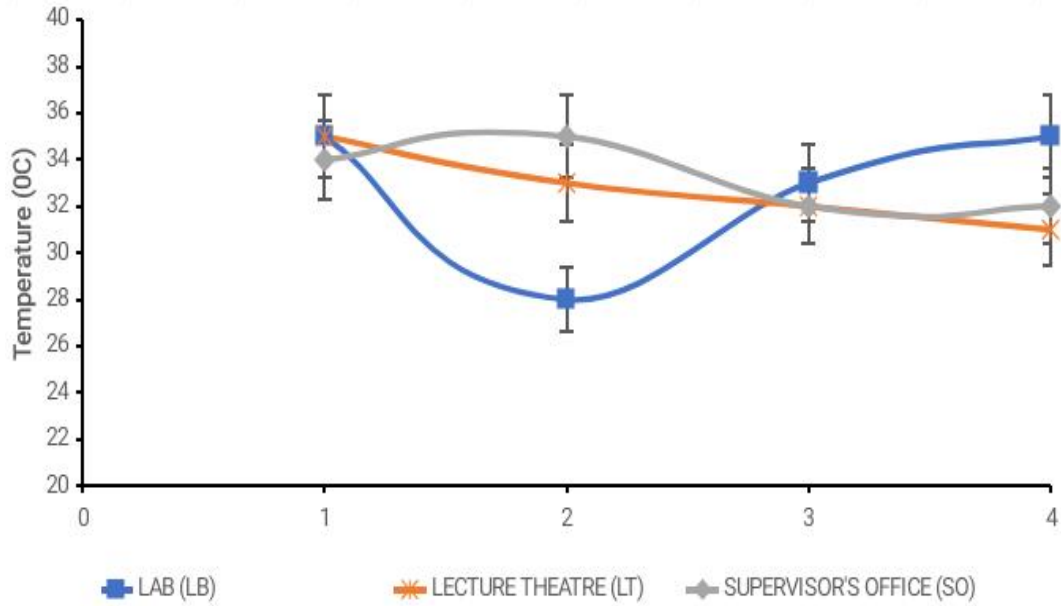


Figure 1: Temperature of studied locations at four weeks

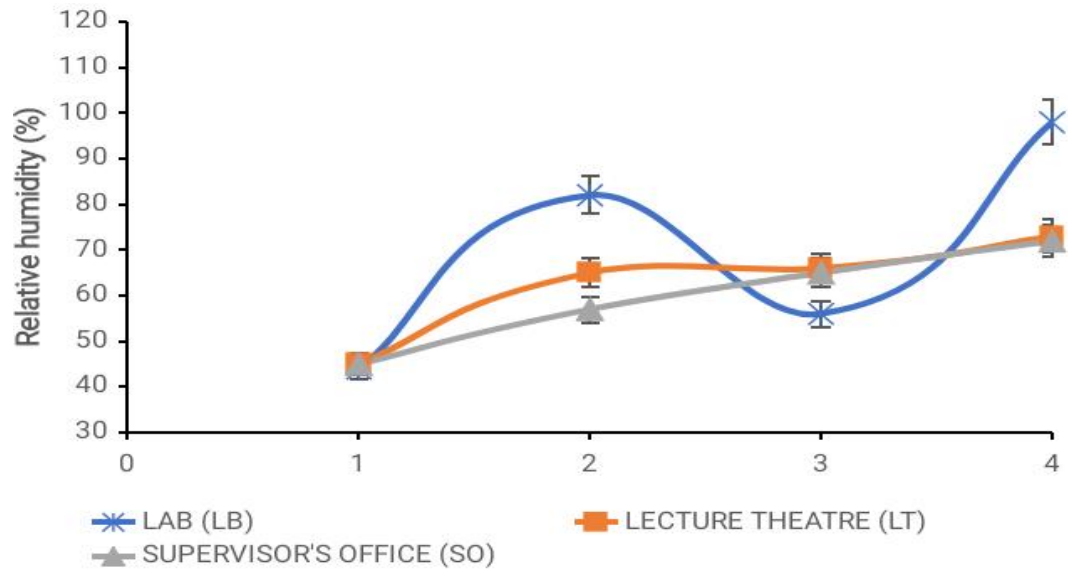


Figure 2: Relative humidity of studied location at four weeks

TABLE 1a: Bacterial count of CDs placed at the Lab at week 1

Sample Code		log10 Cfug(C1)	log10 Cfug(C2)	Mean	SD
LBO 1	Open CD	3.91	3.88	3.89	0.02
LBO 2	Open CD	3.66	3.49	3.58	0.12
LBC 1	Closed CD	3.04	3.08	3.06	0.03
LBC 2	Closed CD	3.20	3.20	3.20	0.00
LBB 1	Burned CD	3.41	3.52	3.47	0.07
LBB 2	Burned CD	3.38	3.40	3.39	0.01

TABLE 1b: Bacterial count of CDs placed at the Lab at week 2

Sample Code		log10 Cfug(C1)	log10 Cfug(C2)	Mean	SD
LBO 1	Open CD	3.99	3.85	3.92	0.10
LBO 2	Open CD	3.67	3.73	3.70	0.04
LBC 1	Closed CD	2.60	2.60	2.60	0.00
LBC 2	Closed CD	2.30	2.48	2.39	0.12
LBB 1	Burned CD	3.54	3.58	3.56	0.03
LBB 2	Burned CD	3.54	3.63	3.59	0.06

TABLE 1c: Bacterial count of CDs placed at the Lab at week 3

Sample Code		log10 Cfu/g(C1)	log10 Cfu/g(C2)	Mean	SD
LBO 1	Open CD	707.11	4.49	4.48	4.48
LBO 2	Open CD	1414.21	4.45	4.42	4.44
LBC 1	Closed CD	282.84	3.96	3.94	3.95
LBC 2	Closed CD	141.42	3.86	3.85	3.85
LBB 1	Burned CD	1414.21	4.51	4.53	4.52
LBB 2	Burned CD	3535.53	4.48	4.54	4.51

Table 1d: Bacterial count of CDs placed at the Lab at week 4

Sample Code		log10 Cfu/g(C1)	log10 Cfu/g(C2)	Mean	SD
LBO 1	Open CD	4.51	4.53	4.52	0.02
LBO 2	Open CD	4.56	4.49	4.52	0.05
LBC 1	Closed CD	4.48	4.32	4.40	0.11
LBC 2	Closed CD	4.31	4.43	4.37	0.09
LBB 1	Burned CD	4.51	4.53	4.52	0.02
LBB 2	Burned CD	4.49	4.46	4.48	0.02

Table 1e: Mean data on the bacteria load of lab CD plate samples.

	Duration (Weeks)			
	1	2	3	4
Open CD	3.76	3.82	4.32	4.36
Closed CD	3.10	2.52	3.47	4.21
Burned CD	3.41	3.52	4.32	4.29

Table 1f: Morphological characteristics and biochemical tests of lab CD plate samples

Morphological					
Elevation	Flat	Flat	Flat	Flat	Raised
Margin	coarse	irregular	Undulate	Undulate	Smooth
Color	milk white	off-white	Cream	Cream	Cream
Shape	concave	concave	Irregular	Irregular	Irregular
Size	large	large	Large	Large	Small
Gr. diff. agar	BCA	BCA	EMB	EMB	MSA
Colour	Straw	Straw	Pink	green	Yellow
Staining					
Gram stain	+	+	-	-	+
cell type	Rod	Rod	Rod	Rod	Cocci
Arrangement	disperse	disperse	disperse	disperse	clusters
Color	purple	purple	pink	pink	Purple
Spore staining	+	+	-	-	-
Biochemical					
KOH String Test	-	-	+	+	-
Catalase	+	+	+	+	+
Indole	-	-	-	+	-
Citrate	-	+	+	-	+
Oxidase	-	-	-	-	-
Motility	+	-	+	+	-
Urease	-	-	-	-	+
Glucose	+	+	+	+	+
Sucrose	-	-	+	-	+
Lactose	-	-	+	+	+
Mannitol	-	+	-	-	+
Gas formation	-	-	-	+	-
H ₂ S formation	-	-	-	-	-
TSI (Slant/Butt) reaction	K/A	K/A	A/A(K*)G*	A/AG	A/A*
Esculin Hydrolysis	+	+	+	-	-
Identity	<i>Bacillus mycoides</i>	<i>Bacillus pumilus</i>	<i>Enterobacter aerogenes</i>	<i>E. coli</i>	<i>Staphylococcus aureus</i>

Key

Positive - (+) Negative- (-) Gas- (G)

Alkaline- (K) Acidic- (A) Hydrogen sulphide- (H₂S)

Table 2a: Bacterial count of CDs placed at lecture theatre at week 1

Sample Code		log ₁₀ Cf _u /g(C1)	log ₁₀ Cf _u /g(C2)	Mean	SD
LTO 1	Open CD	3.18	3.20	3.19	0.02
LTO 2	Open CD	3.38	3.48	3.43	0.07
LTC 1	Closed CD	2.78	3.00	2.89	0.16
LTC 2	Closed CD	3.11	2.95	3.03	0.11
LTB 1	Burned CD	2.85	2.90	2.87	0.04
LTB 2	Burned CD	3.41	3.41	3.41	0.00

Table 2b: : Bacterial count of CDs placed at lecture theatre at week 2

Sample Code		log ₁₀ Cf _u /g(C1)	log ₁₀ Cf _u /g(C2)	Mean	SD
LTO 1	Open CD	2.95	3.00	2.98	0.03
LTO 2	Open CD	3.23	3.26	3.24	0.02
LTC 1	Closed CD	2.78	2.70	2.74	0.06
LTC 2	Closed CD	2.48	2.48	2.48	0.00
LTB 1	Burned CD	3.70	3.68	3.68	0.01
LTB 2	Burned CD	3.54	3.49	3.52	0.04

Table 2c: : Bacterial count of CDs placed at lecture theatre at week 3.

Sample Code		log ₁₀ Cf _u /g(C1)	log ₁₀ Cf _u /g(C2)	Mean	SD
LTO 1	Open CD	3.58	3.56	3.57	0.02
LTO 2	Open CD	3.54	3.57	3.56	0.02
LTC 1	Closed CD	4.09	4.12	4.10	0.02
LTC 2	Closed CD	4.33	4.28	4.30	0.04
LTB 1	Burned CD	4.46	4.51	4.48	0.03
LTB 2	Burned CD	3.26	3.48	3.37	0.16

Table 2d : Bacterial count of CDs placed at lecture theatre at week 4.

Sample Code		log ₁₀ Cf _u /g(C1)	log ₁₀ Cf _u /g(C2)	Mean	SD
LTO 1	Open CD	3.88	4.05	3.97	0.12
LTO 2	Open CD	3.86	3.67	3.76	0.13
LTC 1	Closed CD	3.15	2.90	3.02	0.17
LTC 2	Closed CD	2.90	2.85	2.87	0.04
LTB 1	Burned CD	4.10	4.08	4.09	0.01
LTB 2	Burned CD	4.11	4.00	4.06	0.07

Table 2e: Mean data on the bacteria load of lecture theatre CD plate samples.

	Duration (Weeks)			
	1	2	3	4
Open CD	3.31	3.11	3.56	3.86
Closed CD	2.96	2.61	4.20	2.95
Burned CD	3.14	3.60	3.92	3.48

Table 2f: Morphological characteristics and biochemical tests on lecture theatre CD plate samples

Morphological					
Elevation	Raised	Raised	Flat	Flat	Raised
Margin	Entire	smooth	coarse	irregular	Entire
Color	lemon	Cream	milk white	off-white	Cream
Shape	Circular	Irregular	concave	concave	Circular
Size	Medium	Small	large	large	Medium
Gr. diff. agar	PCA	MSA	BCA	BCA	EMB
Colour	green	Yellow	Straw	Straw	opaque
Staining					
Gram stain	-	+	+	+	-
cell type	rod	Cocci	Rod	Rod	rod
Arrangement	disperse	clusters	disperse	disperse	disperse
Color	pink	purple	purple	purple	pink
Spore staining	-	-	+	+	-
Biochemical					
KOH String Test	+	-	-	-	+
Catalase	+	+	+	+	+
Indole	-	-	-	-	-
Citrate	+	+	-	+	+
Oxidase	+	-	-	-	-
Motility	+	-	+	-	+
Urease	+	+	-	-	-
Glucose	-	+	+	+	+
Sucrose	-	+	-	-	+
Lactose	-	+	-	-	+
Mannitol	-	+	-	+	+
Gas formation	-	-	-	-	-
H ₂ S formation	-	-	-	-	-
TSI (Slant/Butt) reaction	K/K	A/A*	K/A	K/A	K/A (*A/A)
Esculin Hydrolysis	-	-	+	+	-
Identity	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus mycoides</i>	<i>Bacillus pumilus</i>	<i>Serratia marcescens</i>

Key

Positive - (+) Negative- (-) Gas- (G)

Alkaline- (K) Acidic- (A) Hydrogen sulphide- (H₂S)

Table 3a: Bacterial count of CDs placed at Supervisor’s office at week 1

Sample Code		log10 Cfug(C1)	log10 Cfug(C2)	Mean	SD
SOO 1	Open CD	2.85	2.78	2.81	0.05
SOO 2	Open CD	3.08	3.15	3.11	0.05
SOC 1	Closed CD	3.08	3.26	3.17	0.12
SOC 2	Closed CD	2.48	2.48	2.48	0.00
SOB 1	Burned CD	2.78	2.78	2.78	0.00
SOB 2	Burned CD	2.78	2.78	2.78	0.00

Table 3b Bacterial count of CDs placed at Supervisors office at week 2

Sample Code		log10 Cfug(C1)	log10 Cfug(C2)	Mean	SD
SOO 1	Open CD	2.95	3.00	2.98	0.03
SOO 2	Open CD	3.15	3.11	3.13	0.02
SOC 1	Closed CD	2.90	3.20	3.05	0.21
SOC 2	Closed CD	3.48	3.45	3.46	0.02
SOB 1	Burned CD	2.90	2.85	2.87	0.04
SOB 2	Burned CD	2.00	2.00	2.00	0.00

Table 3c: Bacterial count of CDs placed at the Supervisor's office at week 3

Sample Code		log ₁₀ Cf _u /g(C1)	log ₁₀ Cf _u /g(C2)	Mean	SD
SOO 1	Open CD	4.18	4.14	4.16	0.03
SOO 2	Open CD	4.34	4.33	4.34	0.01
SOC 1	Closed CD	4.30	4.38	4.24	0.08
SOC 2	Closed CD	4.06	4.11	4.09	0.04
SOB 1	Burned CD	4.51	4.46	4.48	0.03
SOB2	Burned CD	4.49	4.51	4.53	0.03

Table 3d: Bacterial count of CDs placed at the Supervisor's office at week 4

Sample Code		log ₁₀ Cf _u /g(C1)	log ₁₀ Cf _u /g(C2)	Mean	SD
SOO 1	Open CD	3.43	3.18	3.30	0.18
SOO 2	Open CD	3.23	3.08	3.15	0.11
SOC 1	Closed CD	3.00	2.95	2.95	0.03
SOC 2	Closed CD	2.00	2.30	2.15	0.21
SOB 1	Burned CD	3.11	3.18	3.15	0.04
SOB 2	Burned CD	3.45	3.04	3.24	0.29

Table 3e: Mean data on the bacteria Supervisor's office CD plate samples.

	Duration (Weeks)			
	1	2	3	4
Open CD	2.96	3.05	4.25	3.23
Closed CD	2.82	3.26	4.16	2.56
Burned CD	2.78	2.44	4.50	2.65

Table 3f: Morphological characteristics and biochemical tests on Supervisor's office CD plate samples

Morphological					
Elevation	Flat	Flat	Flat	Flat	Raised
Margin	coarse	irregular	Undulate	Undulate	smooth
Color	milk white	off-white	Cream	Cream	Cream
Shape	concave	concave	Irregular	Irregular	Irregular
Size	large	large	Large	Large	Small
Gr. diff. agar	BCA	BCA	EMB	EMB	MSA
Colour	Straw	Straw	pink	green	Yellow
Staining					
Gram stain	+	+	-	-	+
cell type	Rod	Rod	Rod	Rod	Cocci
Arrangement	disperse	disperse	disperse	disperse	clusters
Color	purple	purple	pink	pink	purple
Spore staining	+	+	-	-	-
Biochemical					
KOH String Test	-	-	+	+	-
Catalase	+	+	+	+	+
Indole	-	-	-	+	-
Citrate	-	+	+	-	+
Oxidase	-	-	-	-	-
Motility	+	-	+	+	-
Urease	-	-	-	-	+
Glucose	+	+	+	+	+
Sucrose	-	-	+	-	+
Lactose	-	-	+	+	+
Mannitol	-	+	-	-	+
Gas formation	-	-	-	+	-
H ₂ S formation	-	-	-	-	-
TSI (Slant/Butt) reaction	K/A	K/A	A/A(K*)G*	A/AG	A/A*
Esculin Hydrolysis	+	+	+	-	-
Identity	<i>Bacillus mycoides</i>	<i>Bacillus pumilus</i>	<i>Enterobacter aerogenes</i>	<i>E. coli</i>	<i>Staphylococcus aureus</i>

Key

Positive - (+) Negative- (-) Gas- (G)
 Alkaline- (K) Acidic- (A) Hydrogen sulphide- (H₂S)

CHAPTER FIVE

DISCUSSION

Thirty-six (36) CD plates were placed at three different locations; Laboratory, Lecture theatre and office. Out of these CDs, twelve (12) were opened, another 12 were closed (wrapped in plastic with perforations for air) and 12 were burnt with short video clips and opened. Subsequently, temperature and relative humidity were determined as well bacterial load for four (4) weeks. The observation of increased bacterial load over this four-week period suggests that CD plates stored at these locations (Lecture Theatre, Laboratory and Supervisor's office) serve as a favourable environment for bacteria growth. As time progresses, due to inadequate cleaning, fluctuation in temperature and high relative humidity effect, bacteria were able to accumulate on the surfaces of the CD plates. Certain bacteria species thrive in specific temperature ranges, and fluctuations in temperature can impact their growth rate (Gross *et al.*, 1995). This study observed a positive correlation between higher relative humidity level and increased bacterial counts of CD plates. Fluctuation in temperature were observed weekly (Figure 1) and were within the range 30°C - 37°C. This is the optimal temperature range for mesophilic bacteria. Conversely levels of humidity increased at week at weeks 2 and 4. This increase in humidity impacted bacteria load (Table 1e, 2e and 3e). And that is because high humidity is attributed to rise in bacteria growth. So, it's safe to say the effect of temperature on this study should not be emphasized, due to the stable temperature. The bacteria load was more affected by the relative humidity.

The CD plate samples from the lab had higher bacterial load in week 2 and 4 relative to other locations (Table 1e). That is because of the increase in the humidity levels (Figure 2). The temperature levels at those times were at room temperature which is also suitable for the growth

of bacteria (Pankowski *et al.*, 2016). The CD plate samples from the Lecture Theatre had high bacteria load at week 3 and 4 (Table 2e), that is also due to the increased relative humidity (Figure 2). Samples from my Supervisor's office also had the highest bacterial load at week 3 and 4 (Table 3e). It was observed that there was a high bacterial count under high relative humidity conditions. The temperature ranges (30°C - 37°C) was optimal for the growth of bacteria. Time (duration of study) also played a role because as it progressed, bacteria accumulated more on the surface of the CD plates in the studied locations. Again, the closed CDs had lower bacteria load as compared to the Open ones (Tables 1e, 2e and 3e). This could be because exposure to air allows for a greater diversity of bacteria to settle and colonize the surfaces, leading to a higher bacteria load. In contrast, a closed system (non-exposure to air) provided a more controlled environment where external contamination was limited, resulting in lower bacterial counts (Smith *et al.*, 2018).

Bacteria spp identified in the study were *Bacillus mycoides*, *Pseudomonads aeruginosa*, *Staphylococcus aureus*, *Bacillus pumilus*, *Escherichia coli* and *Serratia marcescans*. The presence of these diverse range of bacteria indicates that the conditions where these CD plates were stored were suitable for their growth. Also, the presence of pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus* (Ryan *et al.*, 2013) identified on the CD plates poses potential health risks when these surfaces come in contact with individuals.

Dust particles, dirt and microbial residues can interfere with the readability of the data stored on the disks. The CD plates that were burned were viewed on a computer system to check if the data stored were still valid. These discs with videos on them showed glitches when viewed. Hence, bacterial contamination on the burned discs lead to data corruption. That is probably due to the

enzymes or byproducts released by some bacteria that can be corrosive or damaging (Lear *et al.*, 2021) to the materials of the CD plates. This can result in chemical degradation of the disc surface, affecting the data stored on them. Also, certain bacteria species, especially those producing biofilms can create physical barriers (Vishwakarmar, 2020) on the CD surface that interfere with laser readability. Based on the identified bacteria and their growth preferences, it is essential to establish optimal storage conditions for CD plates to minimize bacterial contamination. This might include maintaining lower humidity levels, controlling temperature ranges and implementing regular cleaning schedule. Interventions such as implementing antimicrobial treatments on CD plates, enhancing ventilation to control humidity levels and establishing cleaning strategies are recommended. These can help reduce bacterial contamination and maintain a cleaner storage environment, in order to ensure data integrity and avoid potential health risk from contact with pathogenic bacteria on the surface of CD plates.

CONCLUSION

Relative humidity played a big role in the bacteria load of the CD plate samples, while the temperature had little effect on bacterial load. There is need for proper storage of CD plates under the right humidity levels and temperature to ensure the integrity of the stored data. From this study, the locations where these discs were stored are not under the right storage conditions. So, it is important to ensure a low humidity level at a constant temperature under a closed system (Closed CD plates) and regular cleaning to ensure the preservation of CD plates.

REFERENCE

- Allegrezza Stefano (2015). The Reliability of Optical Memories in the Long-term Preservation of digital documents. *Journal of Library and Information Science*. **6**(2): 101-125.
- Andrade Chittaranjan (2021) A student's Guide to the Classification and Operationalization of Variables in Conceptualization and Design of a Clinical Study. *Indian Journal of Psychological Medicine*. **40**(2).
- Beuchat R. Larry, Komitopoulou Evangelia, Beckers Harry, Betts P. Roy, Bourdichon François, Fanning Séamus, Josten M. Han, Kuile H. Ter Benno (2013). Low Water Activity Foods: Increased Concern as vehicles of food borne pathogens. *Journal of Food Protection*. **76**(1): 150-172.
- Bockmuhl DP. (2017). Laundry Hygiene: How to get more than clean. *Journal of applied Microbiology*. **122**(5): 1124-1133.
- Chintalapati S., Kiran MD., Shivani J. (2004). Role of Membrane Lipid Fatty Acids in Cold Adaptation. *Cellular and Molecular Biology*. **50**:631-642.
- Clarke Andrew, Morris G. John, Fonseca Fernada, Murray J. Benjamin, Acton Elizabeth, Price C. Hannah (2013). A low Temperature Limit for Life on Earth. *Public Library of Science*. **8**(6).
- Dannemiller K.C., CS. Wescher, J. Peca (2017). Fungal and Bacterial Growth in Floor just at elevated Relative Humidity Levels. *Indoor Air*. **273**:354-368.

- Eselin J., T. Santos, M. Hebraud (2018). Dessication: An environmental and food industry stress. *Food Microbiology*. **19**:81-88.
- Georges Feller and Gerday Charles (2003). Psychrophilic Enzymes: hot topics in cold adaptation. *Nature Reviews Microbiology*. **1**:200-208.
- Goodchild A., Saunders NF., Ertan H., Rafferty M., R. Curmi (2004). A Proteomic Determination of Cold Adaptation in the Antarctic Archean, *Methanococcoides burtonii*. *Molecular Microbiology*. **53**:309-321.
- Gross Trevor, Faull Jane, Ketteridge Steve, Springham Derek (1995). Microbial Growth. *Introductory Microbiology*. pp 31-61.
- Gupta G., S. Srivastava, Khare Sunil, Veeru Prakash (2014). Extremophiles: An overview of microorganisms from extreme environment. *International Journal of Agriculture Environment and Biotechnology*. **7**(2):371-380.
- Herrera Annavella Gaitan (2001). Mesophilic Aerobic Microorganisms. *Food Microbiology Protocols. Methods of Biotechnology*. **Vol 14**.
- Iracie Joe (2017). Longevity of Optical Disc Media: Accelerated Ageing Predictions and Natural Ageing Data. *International Journal for the Preservation of Library and Archival Material*. **38**(3): 273-393.
- Jatzlauk G., S. Bartel, H. Heine, M. Schloter, S. Krauss-Etschman (2017). Influences of Environmental Bacteria and their Metabolites on allergies, asthma and host microbiota. *Wiley Online Library*. **72**(12):1859-1867.

- Kim JS., J. Kaye, LK. Wright (2001). Moderating and Mediating Effects in Casual Models. *Issues in Mental Health Nursing*. **22**(1):63-75.
- Khan MR, Karim MA. (1996). Microorganisms Growing on Magneyc Tapes and Physiological Characteristics. *Bangladesh Journal of Botany*. **25**(2): 183-188.
- Khan Mahbubar, Mihir Lal Saha, Sanjida Binte Zuha (2008). Bacteria and Actinomycetes Growing on Floppy and Compact Discs under Ambient Conditions. *Bangladesh Journal of Botany*. **37**(1).
- Lee Kwang Young, Lim Dong Soo, Kim Ki Hyun, Cho Won Ik, Kim Young Joo (2012). The Need to Investigate the Longevity of the Information stored in Compact Discs. *Japanese Journal of Applied Physics*. **51**:8.
- Lear G., JM. Kingsbury, Franchinis V. Gambarini, SDM. Marday, JA. Wallbank (2021). Plastics and the Microbiome: Impacts and solutions. *Environmental Microbiology*. **16**:2.
- Li Sheng-Jin, Hua Zheng-Shuang, Huang Li-Nan, Li Joe, Shi Su-Hua, Chen Lin-Xing, Liang Jia-Liang, Liu Jun, Hu Min, Shu Wen-Sheng (2014). Microbial Communities Evolve Faster in Extreme Environments. *Scientific Reports*. **4**(6205);1-9.
- Li WF., XX. Zhou, P. Lu (2005). Structural Features of Thermoenzymes. *Biotechnology Advances*. **23**(4):271-281.
- MacKinnon DP. (2008). Introduction to Statistical Mediation Analysis: Mahway: *Lawrence Erlbaum Associates*. **18**:1-19.

- Mahbubar Rahman Khan, Mihir Lal Saha (2008). Bacteria and Actinomycetes Growing on Floppy and Compact Discs under Ambient Conditions. *Bangladesh Journal of Botany*. **37**(1): 7-14.
- Mishra P. Debi, Junhong Min (2010). Analyzing the Relationship between Independent and Dependent Variables in Marketing: A comparison of multiple regression with path analysis. *Innovative Marketing*. **6**(3).
- Moat A.G, J.U Foster, MP. Sector (2002). Microbial Physiology. *John Wiley and Sons*.
- Montville TJ, Matthews KR (2001). Principles which Influence Microbial Growth, Survival and Death in Foods. *Food Microbiology: Fundamentals and Frontiers*. **2**: 13-32.
- Palermo M. Ama, Antonio Gentile, Giuseppe Pellegrino (2021). *Heritage Science*. **9**:133.
- Pandey Anita, Dhakar Kusum, Kumar Bhavesh (2016). Temperature dependent lipase production from cold and pH tolerant species of Penicillium. *Annals of Microbiology*. **65**:809-816.
- Pankowski A. Jaroslaw, Puckett M. Stephanie, Nano E. Francis (2016). Temperature Sensitivity conferred by ligA Alleles from Psychrophilic Bacteria upon substitution in Mesophilic Bacteria and a yeast species. *Applied and Environmental Microbiology* **82**(6):1924-1932.
- Phadtare S. (2004). Recent Development in Bacterial Cold-shock Response. *Current Issues in Molecular Biology*. **6**:125-136.
- Qiu Yujia, Zhou Yan, Chang Yanyen, Liang Xinye, Zhang Hui, Lin Xiaorui, Wing Ke, Zhou Xiaojie, Luo Ziqiang (2022). The Effects of Ventilation, Humidity, and Temperature on

- Bacteria Growth and Bacteria Genera Distribution. *International Journal of Environmental Research and Public Health*. **19**(22):15345.
- Ratkowsky DA., Jolley, T. Ross (2005). Unifying Temperature Effects on the Growth Rate of Bacteria and Stability of Globular Proteins. *Journal of Theoretical Biology*. **233**:351-362.
- Ryan J. Kenneth, George C. Ray, Ahmad Nafees, Drew W. Lawrence, Lagunoff Micheal, Pottinger Paul, Reller L. Barth, Sterling R. Charles (2014). Pathogenesis of Bacteria infections. *Sherri's Medical Microbiology* pp 391-406.
- Schiraldi Chiara and De Rosa Mario (2015). Mesophilic Organisms. *Encyclopedia of Membranes*. pp 1-2
- Scotfield Vinicius, Jacques MS. Saulo, Guimaraes RD. Jean, Farjalla F. Vinicius (2015). *Frontiers in Microbiology*. **6**: 310.
- Shirahama H., M. Shiomi, M. Sakane, H. Yasuda (1996). Biodegradation of novel optically active polyesters. **29**(14): 4821-4828.
- Tang W., T.H. Kuehin, MF. Simcik (2015). Effect of Temperature, Humidity and Air Flow on Bacterial Growth Rate on Loaded Ventilation Filters. *Journal of Occupational and Environmental Hygiene*. **12**:525-537.
- Tankeshwar Acharya (2023). Psychrophiles, Mesophiles, Thermophiles. *General Microbiology*. **65**:809-816.
- Timer P., G. Mamo, EN. Karlsson (2007). Potential and utilization of thermophiles and Thermostable enzymes in biorefining. *Microbial Factories*. 1-8.

- Vishwakarmar (2020). Impact of Environmental Biofilms: Industrial Components and it's Remediation. *Journal of Basic Microbiology*. **60**:198-206.
- Wolkoff Peder (2018). Indoor Air Humidity, Air Quality and Health. *International Journal Of Hygiene and Environmental Health*. **221**(3):376-390.
- Xu Y., Y. Nogi, C. Kato, Z. Liang, HJ. Rugar, D. Dekegel, N. Glasdorff (2003). *Moritella Profunda sp. Nov.* and *Moritella abyssi sp. Nov.*, Two Psychropiezophilic Organisms Isolated from deep Atlantic Sediments. *International Journal of Systematic and Evolutional Microbiology*. **53**:533-538.
- Yannick DN., Tremblay Skander Hathrobi, Jacques Mario (2014). Bacterial Biofilms: Their Importance in Animal Health and Public Health. *Canadian Journal of Veterinary Research*. **78**(2): 110-116.