
**ASSESSING THE ABUNDANCE OF SOIL MICROORGANISMS IN THREE SPECIFIC
GARDENS WITHIN BUSITEMA UNIVERSITY'S NAGONGERA CAMPUS**

BY

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CONSENT FOR SUBMISSION

I have satisfactorily read through the Dissertation and consent to its submission to the Department of Biology, Nagongera campus for the award of Bachelor of Science and Education of Busitema University.

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DECLARATION

I, Okot Brian Innocent, declare to the best of my knowledge that the study here forth is my original work, and has not been submitted to Busitema University or any other University or Institution before for the award of a degree or diploma.

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DEDICATIONS

To my relatives who stood for their self-support and beloved friends whose love and support has pushed me through.

ABSTRACT:

Introduction:

Soil microorganisms play a crucial role in ecosystem functioning, nutrient recycling, and plant health. This study investigates the abundance of fungi and bacteria in the soil of three different gardens: pine, cassava, and eucalyptus garden. Soil samples were collected from each garden and microbial abundance was assessed using plate counts. The abundance of soil microorganisms is critical to maintaining soil health, influencing nutrient cycling, organic matter decomposition, and plant productivity (Smith and Paul, 2020). This study aims to assess the abundance of soil microorganisms in different garden; that is to say pine garden, eucalyptus garden, and cassava garden. The findings suggest that gardens with greater plant diversity and organic amendments have a significantly higher microbial count (Brown and Thompson, 2021).

METHODS:

Three gardens in Busitema University Nagongera Campus were selected and soil samples were picked from them and taken to the biology laboratory for further investigation on the abundance of microorganisms like fungi and bacteria in each garden. The methodology undertaken was plate count technique and staining technique. The plate count technique is a widely utilized method for estimating the abundance of viable microorganisms in soil samples. This method is particularly useful in quantifying bacteria and fungi, which play essential roles in soil health and fertility.

RESULTS:

The results show significant differences in microbial abundance between the gardens, with the cassava garden having the highest bacteria abundance, followed by the pines garden with the higher bacterial abundance and lastly the eucalyptus garden with the least bacterial abundance. The pine garden with the highest fungal abundance, followed by the cassava garden with higher fungal abundance and lastly with eucalyptus garden with the least fungal abundance.

CONCLUSION:

From the study it was observed that cassava garden having the highest abundance of bacteria while pine garden having the highest fungal abundance that could be influenced by various factors, including soil composition, moisture levels, temperature, PH levels, and the presence of organic material.

CONSENT FOR SUBMISSION	Error! Bookmark not defined.
DECLARATION	Error! Bookmark not defined.
ACKNOWLEDGEMENT	iii
DEDICATIONS	iv
ABSTRACT:.....	v
Introduction.....	v
METHODS	v
RESULTS	v
CONCLUSION	v
CHAPTER ONE: INTRODUCTION	1
1.0 Background.....	1
1.1 Statement of problem	2
1.2 General objective	3
1.4 Specific objectives	3
1.5 Hypothesis.....	3
1.5.1 Null hypothesis	3
1.6.0 Significance of the study and anticipated outcome.....	3
1.6.1 Significance of the Study:	3
1.6 Anticipated Outcome:	4
1.7 Scope of the study	4
CHAPTER TWO: LITERATURE REVIEW	5
CHAPTER THREE: MATERIAL AND METHODS.....	7
3.0 Introduction	7
3.1 Experimental design:	7
3.2 Preparation of Potato Dextrose Agar (PDA) medium.....	7
3.3 Preparation of Tryptic Soy Agar (TSA) medium	7
3.4 Pouring of the medium.....	7
3.5 Gram Staining for Bacteria.....	8
3.5.1 Procedure.....	8
3.5.2 Microscopic observation	8
CHAPTER FOUR: RESULTS AND DISCUSIONS	9
4.0 Introduction	9

4.1 Research design: experimental	9
Plate 1: Shows the petri dish of pine garden soil sample cultured using TSA medium.....	10
Table 1: Results of pine garden soil sample cultured using TSA medium.	10
Plate 2: Shows petri dish of eucalyptus garden soil sample cultured using TSA medium	10
Table 2: Results of eucalyptus garden soil sample medium cultured using TSA medium.	11
Plate 3: Shows petri dish of cassava garden soil sample cultured using TSA medium.	11
Table 3: Results of cassava garden soil sample cultured using TSA medium.	12
Plate 4: Shows petri dish of pine garden soil sample cultured using PDA medium.	12
Table 4: Results of pine garden soil sample cultured using PDA medium.	12
Plate 5: Shows petri dish of eucalyptus garden soil sample cultured using PDA medium.	13
Table 5: Results of eucalyptus garden soil sample cultured using PDA medium.	13
Plate 6: Shows petri dish of cassava garden soil sample cultured using PDA medium.	13
Table 6: Results of cassava garden soil sample cultured using PDA medium.	13
DISCUSSION OF RESULTS.....	14
CHARPTEr SIX: CONCLUSION AND RECOMMENDATION	16
6.0 CONCLUSION	16
6.1 RECOMMENDATION	16
CHAPTER SEVEN: REFERENCES:.....	17

CHAPTER ONE: INTRODUCTION

1.0 Background

The health and productivity of terrestrial ecosystems rely heavily on the intricate interactions between plants, soil, and microorganisms inhabiting the soil. The soil contains a wide range of microorganisms whose population changes constantly with diurnal and seasonal fluctuations (Sylvia, Fuhrmann, Hartel, and Zuberer, 2005). These Soil organisms influence the physio - chemical properties of the soil and aid in decaying and decomposition of organic matter hence transformation of mineral nutrients in the environment (Brady and Weil, 2017). Soil microorganisms perform numerous important ecosystem services in the soil including improved soil structure and soil aggregation, recycling of soil nutrients, and water cycling, organic matter decomposition, and plant health maintenance (Van Elas, Jansson, and Trevors, 2007) Soil microorganisms form micro aggregates in the soil by binding soil particles together with their secretions. These micro-aggregates are building blocks for improving soil structure. Improved soil structure increases water infiltration and water- holding capacity of the soil (Six, Bossuyt, Degryze, and Denef, 2004). Fungi are the major decomposers in the soil and aid in nutrients turnover, binding together of soil particles as well as delivering water and nutrients to plant roots (Dighton, 2003).

Understanding the abundance and diversity of these microorganisms within specific environments is crucial for effective ecosystem management and sustainable agricultural practices. (Bardgett and van der putten, 2014).

This research aims to assess the abundance of soil microorganisms in three distinct gardens, that is to say pine, cassava, and eucalyptus garden situated within Busitema University's Nagongera campus. By focusing on these specific locations, we can explore potential variations in soil microbial communities influenced by different garden management practices, soil properties, and environmental conditions. (Strickland and Rousk, 2010).

The selected gardens represent diverse habitats within the campus, each subjected to unique management approaches and environmental factors. By conducting comprehensive soil sampling

and microbial analysis, we seek to quantify the abundance and diversity of soil microorganisms, including bacteria, and fungi, in each garden. (Six et al, 2006).

The findings of this research hold significant implications for sustainable land management practices, ecosystem conservation, and agricultural productivity within the Busitema University campus. By elucidating the dynamics of soil microorganisms in these specific gardens, we can inform decision-making processes aimed at enhancing soil health, promoting biodiversity, and improving crop yields in agricultural systems. (de Graaff et al, 2011).

Soil fertility is an essential component of sustainable agriculture and ecosystem health. (Bardgett and van der Patten, 2014).

Understanding the relationship between the bacterial (B) and fungal (F) populations in soil is crucial in determining soil health and fertility. The B: F population ratio is an important metric used to evaluate the status of the soil microbial community and its ability to sustain plant growth. B: F ratios are generally indicative of healthy of ecosystems, as fungi are important for nutrients cycling and carbon sequestration, while bacteria are involved in nutrient mineralization and decomposition processes. (Strickland and Rousk,2010). Therefore, monitoring B: F ratio can provide valuable insights into the functioning of soil microbial communities, and aid in developing

1.1 Statement of problem

Microbial diversity in gardens is heavily influenced by the type of vegetation present, organic matter content, and human activities. For example, gardens with diverse plant species tend to support more varied microbial communities due to different types of organic matter and root exudates that each plant species provides (Van Der Heijden, Bardgett, and Van Straalen, 2008).

Soil pH, moisture, and nutrient levels are typically found to be significant drivers of microbial community composition. For instance, slightly acidic soils (pH 6-7) are often associated with higher bacterial diversity, while more alkaline soils may favor fungal growth (Rousk et al., 2010).

Studies have shown that microbial populations can fluctuate significantly with the seasons. During wetter, warmer seasons, microbial activity and abundance typically increase due to favorable conditions for growth (Lipson and Schmidt, 2004)

Additionally, assessing soil microorganisms at different times throughout the year could uncover seasonal fluctuations and offer insights into the temporal dynamics of microbial populations. Furthermore, investigating how environmental variables such as soil pH, moisture content and nutrient levels affect microbial abundance could deepen our understanding of the factors that shape soil microbial communities within campus (Smith and Johnson, 2018).

1.2 General objective

To determine the different soil microorganisms found in the selected three gardens.

1.4 Specific objectives

To identify the soil microorganisms in the selected three gardens.

To compare the abundance of soil microorganisms in the selected three gardens.

1.5 Hypothesis

1.5.1 Null hypothesis

The soil from the selected gardens has similar soil microorganisms

The abundance of soil microorganisms is the same in the three selected gardens within Busitema University's Nagongera Campus.

1.6.0 Significance of the study and anticipated outcome

1.6.1 Significance of the Study:

Understanding the abundance and diversity of soil microorganisms can inform sustainable land management practices within the university campus, promoting soil health and ecosystem resilience. Soil microorganisms play crucial roles in nutrient cycling and plant health. Insights gained from this study can aid in optimizing agricultural practices within the campus, leading to improved crop yields and soil fertility. Soil microorganisms contribute to ecosystem functioning and support above-ground biodiversity. By assessing their abundance, this research can contribute to conservation efforts within the campus by highlighting areas of high microbial diversity that may correlate with overall ecosystem health. The research provides an opportunity for students and researchers to engage in hands-on learning and gain practical experience in environmental science and microbiology. It can serve as a platform for further research and academic exploration in related fields.

1.6 Anticipated Outcome:

The study is expected to identify specific microbial taxa that are particularly abundant or scarce in the soil of the selected gardens, providing insights into the microbial community composition within the campus.

By analyzing the relationship between soil microbial abundance and environmental factors such as soil composition and land use practices, the research may reveal the drivers shaping microbial communities in the study area.

Based on the findings, recommendations can be made for land management strategies to enhance soil health and biodiversity within the campus, potentially improving ecosystem services and supporting sustainable development goals.

The research contributes to the growing body of literature on soil microbiology and urban ecosystems, adding to our understanding of microbial ecology in university campus environments and providing a basis for future research endeavors.

1.7 Scope of the study

This research will be conducted in the Busitema University Biology Laboratory, soil samples shall be collected from three specific gardens, that is to say the pine garden, cassava garden, and eucalyptus garden within Busitema university's Nagongera campus. The collected soil samples will be taken to the Science laboratory for examining the abundance of soil microorganisms in the samples using culture method and microscopy techniques.

CHAPTER TWO: LITERATURE REVIEW

Microbial communities in soil consist of a very broad range of organisms in different physiological states. These are frequently termed as active, viable, living, dormant, passive, dying, dead and so on, states (Johnsen et al., 2001) and are often difficult to differentiate among (Rousk et al., 2009). These terms can be summarized as four physiological states of microorganisms. The first three are living states. The first is the active state of the microorganisms. The active microorganisms are involved in the ongoing utilization of substrates and associated biochemical transformations. The second is the potentially active microorganisms. This part is

in physiological alertness (De Nobili et al., 2001; Raubuch et al., 2010) and can switch to utilization of substrates within minutes to a few hours. The last state of living microorganisms is the dormant state. It does not contribute to ongoing processes currently but can contribute under altered circumstances. The fourth state of microorganisms in soil is dead (including lysed cells and microbial residues), but also quantified by some methods and does not directly contribute to any ongoing processes. Dead microbial biomass does, however, affect the turnover of Carbon and nitrogen as a source of easily available substrates. All these parts of total microbial biomass are crucial for evaluating soil functions and comparing treatments, environmental conditions, land use, and management practices. However, only active microorganisms are involved in the ongoing processes and consequently, all processes should be related to the mass of active microorganisms driving biogeochemical elements cycling in soil.

The soil serves as a reservoir for many microbial communities of plants and herbs which can be producing, carbon dioxide and nitrogen cycle. The microorganisms play major role in soil ecosystem. Microbial composition and functioning changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control (Stefani's et al., 2013). Soil is an oligotrophic medium for the growth of fungal growths are extremely limited for most of the time and readily available are present for short periods in a limited zone. For most of the time, fungi are either dormant, or they metabolize and grow very slowly utilizing a range of organic molecules. The fungi distribute organic matter away from the roots. In general, the concentration of microbes is greatest close to the surface of roots (rhizosphere) and hyphae of arbuscular mycorrhizal fungi (mycorrhizosphere), where exudates are extraordinarily important

source of organic energy entering from soils. Genetic studies have shown that fungi are more closely related to animals than to plants. Fungi have 80 percent or more of the genes as humans (Ratnakumar et al., 2015; Dick, 2009 and Kirk, 2004). Fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as bio fertilizers and many other ways. Fungal biotechnology has become an integral part of the human welfare (Karthikeyan et al., 2014). Fungus benefits most plants by suppressing plant root diseases and fungi promote healthier plants by attacking plants pathogens with fungal enzymes. Fungi also use antagonism to reduce competition by producing antibodies, which suppress other microorganisms from growing. They produce many vitamins which promote plant growth. Beneficial fungi also form protective webs and nets around roots and leaves to protect the host plants (Lowenfels and Lewis, 2006; Sylvia et al., 2005). Fungus also protects plants by supplying a protective health to supply both water and phosphorus to the plant roots during droughts (Magdoff and VanEs, 2009).

CHAPTER THREE: MATERIAL AND METHODS

3.0 Introduction:

The method used to assess the abundance of microorganism in the three specified gardens in Busitema University Nagongera Campus is plate culturing method and staining technique.

3.1 Experimental design:

The research employs an experimental design, where soil samples were collected and subjected to microbial culturing techniques using two types of agar media: Potato Dextrose Agar (PDA) for fungi and Tryptic Soy Agar (TSA) for bacteria.

3.2 Preparation of Potato Dextrose Agar (PDA) medium.

19.5g of potato dextrose agar was suspended in 500ml of distilled water in the sterilized glass beaker. Distilled water was added little at time while stirring using sterilized rod to ensure dissolution. The mixture was then transferred into a clean sauce pan and heated using a heat source to ensure complete dissolution. After the mixture boiling was removed from the heat source and filled in the Duran bottle for autoclaving at temperature of 121°C for 15 minutes. Allow the medium to cool and add 1% chloramphenicol solution was added to the medium for preventing bacterial growth, before pouring into Petri plates. The plates were incubated for 24 hours at 25 temperatures until the colonies were formed.

3.3 Preparation of Tryptic Soy Agar (TSA) medium

20g of Tryptic Soy Agar was suspended in 500ml of distilled water in the sterilized glass beaker. Distilled water was added little at time while stirring using sterilized rod to ensure dissolution. The mixture was then transferred into a clean sauce pan and heated using a heat source to ensure complete dissolution. After the mixture boiling was removed from the heat source and filled in the Duran bottle for autoclaving at temperature of 121°C for 15 minutes.

3.4 Pouring of the medium

TSA medium was poured in the clean petri dishes, which were nine in number. Petri dishes containing poured TSA medium were left open to stand for 10 minutes for the medium to partially solidify. After partial solidifying, using inoculating loop, different soil samples made by serial dilution were picked using the inoculating loop and used to transfer the inoculum by

streaking culture plates (petri dishes). Then, the petri dishes were covered with other petri dishes then sealed using Para film. The sealed petri dishes with inoculum were kept at room temperature in the laboratory cupboard for 48 hours. After 48 hours, observations were made. Colony count was done on each petri dish.

3.5 Gram Staining for Bacteria

3.5.1 Procedure

Take a clean, grease- free slide. Label the slide by indicating the medium name and sample name.

Add a drop of distilled water to the center of the glass slide. Then, add inoculum from the bacterial culture with a sterilized inoculating loop on the glass slide.

After that, mix the inoculum with a drop of distilled water to make a thin film by uniformly rotating the inoculating loop until a thin bacterial film is formed. After smear preparation, pass the prepared slides over the Bunsen burners flame at least three times. Then, allow the slide to air dry.

Flood crystal violet solution over fixed smear. After 30 – 60 seconds, pour off the Crystal Violent solution and rinse with gentle running water.

Flood the Gram's Iodine solution over the smear. Leave the iodine solution for 30 – 60 seconds and pour off the excess iodine and rinse with gentle running water.

Shake off the excess water over the smear. Add a few drops of decolorizing solution (ethanol) and shake gently and rinse with distilled water after 5 seconds.

Rinse the smear with distilled water to wash decolorizes. Shake off the excess water over the smear

Pour safranin stain over the smear. Leave for 30 – 60 seconds and wash with gentle running water and air dry the smear.

3.5.2 Microscopic observation

Immersion oil was added onto the slide smear for clear observation

A slide was placed on the stage for microscopic observation

The lowest power objective lens was used for the best magnification.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.0 Introduction:

Microorganisms play crucial role in various ecosystems, including soil environment where they influence nutrient cycling, organic matter decomposition, and plant growth. Soil is a complex matrix, often contain vast microbial communities that include bacteria and fungi

4.1 Research design: experimental

The research employs an experimental design, where soil samples were collected and subjected to microbial culturing techniques using two types of agar media: Potato Dextrose Agar (PDA) for fungi and Tryptic Soy Agar (TSA) for bacteria. experimental processes involve the following steps:

The results show significant differences in microbial abundance between three selected gardens, indicating that type of vegetation and garden management practices have a profound impact on soil microbial communities. The pine garden had the highest fungal abundance, which may be attributed due to the acidic soil conditions and the presence of organic matter. The cassava garden had the highest bacterial abundance, which may be attributed due to high nutrient content and moisture levels in the soil. The eucalyptus garden had the lowest microbial abundance of both fungi and bacteria compared to pine and cassava gardens, which may be attributed to the allelopathic properties of eucalyptus, which can inhibit microbial growth.

Gram-negative brown diplobacilli were seen through microscopic observation from cassava, pine gardens using Tryptic Soy Agar (TSA) medium as seen from plate 1 and plate 2 and bacteria could be *pseudomonas spp*, *Enterobacter spp*, *Klebsiella spp*, *Escherichia coli* (E. coli) and other gram-negative bacteria

Gram-negative cream bacilli were seen through microscopic observation from pine garden using Tryptic Soy Agar (TSA) medium seen from plate 1 and bacteria could be *pseudomonas*, *Burkholderia*, *Acinetobacter*, *Alcaligenes* and *Chromobacterium*.

Gram-negative brown streptobacilli were seen through microscopic observation from eucalyptus garden using Tryptic Soy Agar (TSA) and bacteria could be novel streptobacilli that appear pink.

Plate 1: Shows the petri dish of pine garden soil sample cultured using TSA medium.



Table 1: Results of pine garden soil sample cultured using TSA medium.

Morphological feature	Colony 1	Colony 2
Form	Circular	Irregular
Margin	Entire	Undulate
Elevation	Raised	Flat
Opacity	Opaque	Opaque
Colour	Brown	Cream

Plate 2: Shows petri dish of eucalyptus garden soil sample cultured using TSA medium



Table 2: Results of eucalyptus garden soil sample medium cultured using TSA medium.

Morphological feature	Colony 1	Colony 1*
Form	Circular	Irregular
Margin	Entire	Undulate
Elevation	Raised	Flat
Opacity	Opaque	Opaque
Colour	Brown	Brown

Plate 3: Shows petri dish of cassava garden soil sample cultured using TSA medium.



Table 3: Results of cassava garden soil sample cultured using TSA medium.

Morphological feature	Colony 1	Colony 2	Colony 2*
Form	Circular	Irregular	Filamentous
Margin	Entire	Undulate	Filiform
Elevation	Flat	Flat	Flat
Opacity	Opaque	Opaque	Opaque
Colour	Brown	Cream	Cream

Plate 4: Shows petri dish of pine garden soil sample cultured using PDA medium.



Table 4: Results of pine garden soil sample cultured using PDA medium.

Morphological features	Colony 1	Colony 1*	Colony 2*
Form	Filamentous	Rhizoid	Circular
Margin	Filiform	Undulate	Entire
Elevation	Convex	Flat	Raised
Opacity	Translucent	Opaque	Opaque
Colour	White	White	Cream

Plate 5: Shows petri dish of eucalyptus garden soil sample cultured using PDA medium.



Table 5: Results of eucalyptus garden soil sample cultured using PDA medium.

Morphological feature	Colony 1	Colony 1*	Colony 1#
Form	Filamentous	Circular	Irregular
Margin	Filiform	Entire	Lobate
Elevation	Convex	Crateriform	Flat
Opacity	Translucent	Opaque	Opaque
Colour	White	White	White

Plate 6: Shows petri dish of cassava garden soil sample cultured using PDA medium.



Table 6: Results of cassava garden soil sample cultured using PDA medium.

Morphological feature	Colony 1	Colony 1*	Colony 2
Form	Filamentous	Rhizoid	Irregular
Margin	Filiform	Filiform	Undulate
Elevation	Flat	Raised	Raised
Opacity	Transparent	Opaque	Opaque
Colour	White	White	Cream

The highest fungal abundance in the pine garden, attributed to acidic soil conditions and organic matter, suggests that maintaining these conditions is essential for promoting fungal growth. However, introducing a diverse range of organic amendments could further enhance both fungal and bacterial diversity, leading to more balanced soil microbial community.

The highest bacterial abundance in the cassava garden, linked to high nutrient content and moisture levels, indicates that these factors should be carefully managed. Regular soil testing and the application of organic fertilizers can help sustain high bacterial diversity. Additionally, considering crop rotation or intercropping with legumes may enhance nitrogen levels and further support beneficial bacteria such as *Enterobacter spp.* and *pseudomonas spp.*

The lowest microbial abundance in the eucalyptus garden, likely due to the allelopathic effects of eucalyptus, calls for interventions to mitigate these effects. Introducing organic amendments such as compost or bio char could help neutralize allelopathic compounds, promoting a healthier and more diverse microbial community. Additionally, incorporating plant species that are resistant to allelopathic effects might help boost microbial activity.

DISCUSION OF RESULTS

The pine garden exhibited the highest fungal abundance, which can be attributed to the acidic soil conditions and the presence of organic matter specific to pine ecosystems. Acidic soils are known to favor fungal growth, as many fungal species thrive in low pH environments. The organic matter from pine needles and other plant debris provides a continuous supply of nutrients that support fungal proliferation. Coniferous forests and pine-dominated areas often harbor diverse and abundant fungal communities due to specific soil chemistry and organic inputs (Van Der Heijden, Bardgett, and Van Straalen, 2008). The presence of gram-negative cream bacilli, including potential species like *pseudomonas*, *Burkholderia*, and *Actinetobacter*, further suggests a complex microbial ecosystem where both fungi and bacteria coexist, likely contributing to the overall soil health and nutrient cycling in the pine garden.

The cassava garden showed the highest bacterial abundance, which is likely due to high nutrient content and moisture levels in the soil. Cassava, being a root crop, produces significant amounts of root exudates that provide a rich source of carbon and other nutrients for bacterial

communities. This environment is conducive to the growth of various bacteria, including gram-negative brown diplobacilli, which could belong to species like *pseudomonas spp.*, *Enterobacter spp.*, *Klebsiella spp.*, and *Escherichia coli* (Raaijmakers et., 2009). The nutrient-rich and moist conditions in the cassava garden create an ideal habitat for these bacteria, which are known for their roles in nutrient cycling, soil fertility, and plant health. The high bacterial diversity observed may be indicative of a resilient soil ecosystem capable of supporting sustainable agricultural practices.

In contrast, the eucalyptus garden demonstrated the lowest microbial abundance for both fungal and bacterial populations. This reduced microbial activity can be attributed to the allelopathic properties of eucalyptus trees, which are known to release compounds that inhibit the growth other plants and microorganisms. Allelopathic chemicals, such as phenolics and terpenes, can have a detrimental effect on soil microbial communities, leading to less diverse and less active soil ecosystem (Mallik, 2008). The discovery of gram-negative brown streptobacilli, which may represent novel microbial strains, suggest that even in a suppressed microbial environment, some bacteria have adapted to survive, potentially by developing resistance to allelopathic compounds. However, the overall low microbial counts indicate that the allelopathic effects of eucalyptus are significant and likely contribute to a less fertile and biologically active soil environment compared to the pine and cassava gardens.

CHARPTER SIX: CONCLUSION AND RECOMMENDATION

6.0 CONCLUSION

The pine garden exhibits the highest fungal abundance, suggesting a conducive environment for fungal growth, potentially due to specific organic matter and P^H levels favoring fungal growth, potentially due to specific organic matter and P^H levels favoring fungal. The cassava garden shows the highest bacterial abundance, which may be attributed to its soil properties and root exudates that support bacterial community. The eucalyptus garden consistently demonstrates the lowest microbial counts in both fungal and bacterial populations, which could be due to allelopathic compounds released by eucalyptus trees, inhibiting microbial growth.

6.1 RECOMMENDATION

Given the significant difference in microbial abundance between the three gardens, it is recommended that future research focus on more detailed analysis of soil physical chemical properties like pH, organic matter content, moisture levels and their correlations with microbial population. The study suggests the vegetation type, such as pine cassava and eucalyptus, has profound influence in microbial composition of soil. further research should examine additional types of vegetation to better understand how different plants and their associated management practices contribute to soil health and microbial abundance.

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