

Comparative Study of Soil Bacterial Communities in Pagoh Campus

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Abstract

This study conducted a comparative analysis of soil bacterial communities in two distinct locations within UTHM Pagoh Campus which are UTHM Wetland and Pagoh Residential College. The research aimed to investigate the influence of environmental factors, including pH, temperature, and moisture content, on microbial diversity and distribution. Soil samples were collected and analysed using a systematic approach, which included measurements of physical parameters, bacterial culturing, morphological examination, Gram staining, and bacterial identification. The study sought to identify the relationships between soil properties and bacterial composition, providing insights into the ecological dynamics of soil microbial communities in contrasting environments. The results showed distinct bacterial diversity where UTHM Wetland had three families of bacteria, while Pagoh Residential College had one. This is because, UTHM Wetland, characterized by acidic pH, high moisture content, and varied environmental conditions, supported a more diverse bacterial community. Families such as Enterobacteriaceae, Xanthomonadaceae, and Listeriaceae were prevalent, reflecting the wetland's rich and dynamic habitat conducive to microbial growth. In contrast, Pagoh Residential College, with its neutral to slightly alkaline pH, dry soil conditions, and more stable environment, exhibited reduced microbial diversity, with Moraxellaceae as the dominant bacterial family. These differences underscore the role of environmental parameters and land use in shaping microbial ecosystems. The study highlights the ecological importance of wetlands as reservoirs of microbial diversity and emphasizes the impact of land conversion and reduced moisture availability on microbial communities in Pagoh residential areas. Furthermore, conservation efforts should focus on protecting and restoring wetlands to maintain their ecological balance and biodiversity. This research contributes to the broader understanding of soil microbial communities, their environmental drivers, and the need for sustainable land management practices to ensure ecosystem health and resilience.

1. Introduction

Soil forms a critical component of terrestrial ecosystems, providing a medium for plant growth, a reservoir for water and nutrients, and a habitat for several microbial communities. Among these microorganisms are bacteria that perform several functions in nutrient cycling, organic matter decomposition, and maintenance of soil structure and fertility [1]. Despite their importance, the bacterial diversity within soils across the UTHM Pagoh Campus, particularly in ecologically distinct environments such as the UTHM Wetland and Pagoh Residential College, remains poorly studied. Understanding soil bacterial communities is very important because they not only affect ecosystem productivity but also serve as indicators of soil health and resilience. The lack of access to information on bacterial diversity in such areas reduces our ability to assess soil health and to understand how environmental factors, such as pH, temperature, and water content, impact microbial composition and activity [2].

These alterations in land use and human activities, especially urbanization and agricultural practices, are increasing this problem by altering the properties of soil and disturbing microbial ecosystems. Urbanization significantly reduces the alpha diversity of soil microbes, which in turn diminishes stability and weakens the interactions within microbial communities. This decline is associated with changes in soil properties and environmental conditions, highlighting the harmful impact of urbanization on soil quality and microbial diversity [3]. For instance, wetlands, which are recognized as biodiversity hotspots, are increasingly threatened by habitat loss and environmental degradation [4]. These disturbances can lead to reduced microbial diversity, impairing soil's ability to support plant growth, sequester carbon, and mitigate pollution [5].

A review of past research work puts forward the important role of soil microorganisms in maintaining ecosystem functions. Such factors as pH have a great influence on microbial diversity, with specific bacterial groups being favoured in acidic and alkaline soils [6]. Similarly, moisture content and temperature regulate microbial activity by affecting the availability of nutrients and oxygen in the soil [7]. However, much of the existing literature focuses on broader geographic scales, with limited emphasis on localized studies in tropical regions. This study seeks to fill this gap by using a combined approach of bacterial culturing coupled with Gram staining and morphological analysis to characterize bacterial communities in these two contrasting environments. The results increase our understanding of how environmental variables impact soil microbial ecosystems and highlight the ecological significance of wetlands as reservoirs of microbial diversity and critical components of sustainable land use practices. To address these concerns, this study set out two primary objectives which to conduct a comparative analysis of soil bacterial communities in two different locations within UTHM Pagoh Campus and to measure the pH, temperature and moisture content in soil that influence microbial community composition.

2. Materials and methods

This research was conducted at two different sites, namely the UTHM Pagoh Campus which are the UTHM Wetland and Pagoh Residential College as Fig. 1. These locations were selected to represent two different soil environments, which were characterized by different land use types, soil conditions, and environmental factors. The UTHM Wetland is characterized by high moisture content and acidic soil, while Pagoh Residential College shows drier conditions with neutral to slightly alkaline pH levels. To enable a good comparison, three sampling points were systematically chosen to account spatial variability at each site to capture the variability existing within the areas [2]. Soil samples weighing around 30 grams were collected from three different points at each site using sterile McCartney bottles to reduce the risk of contamination. The samples were then stored in a refrigerator at 4 °C for subsequent analysis [8]. The sampling process was conducted under stable weather conditions to minimize external variability in environmental factors like temperature and humidity, which could affect microbial activity and composition [5]. Previous research indicates that stable environmental conditions during sampling lead to more reliable data, as fluctuations can introduce biases in microbial diversity assessments [9].

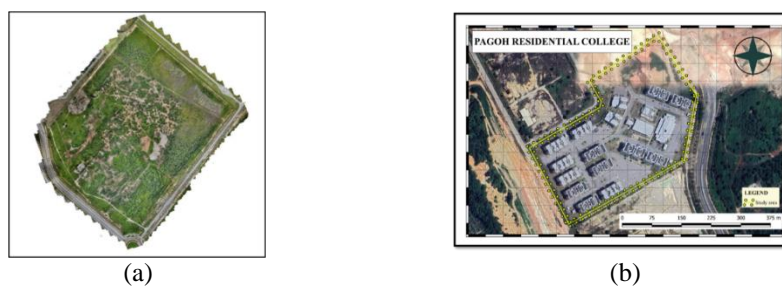


Fig. 1 a) Maps of the UTHM Wetland Conservation Research Station land area [10] (b) Maps of Pagoh Residential College

2.1 Measurement of Soil Physical Parameters

The physical parameters of the soil, such as pH, temperature and moisture content, were measured for all collected samples using an electronic 4-in-1 soil survey instrument. Soil pH was assessed because it is crucial for evaluating the soil's acidity or alkalinity. This measurement is crucial for understanding microbial diversity, as different microbial species thrive in specific pH conditions [6]. Soil temperature was measured because it plays a crucial role in regulating microbial activity and enzymatic processes, which in turn affects nutrient cycling and decomposition rates [11]. Soil moisture availability is vital for microbial metabolic processes, influencing nutrient diffusion and gas exchange within the soil matrix [7].

2.2 Bacterial Culture and isolation

To study the bacterial communities, present in the soil, nutrient agar was prepared as the culture medium according to established protocols. This medium supports the growth of various bacterial species, enabling the cultivation of diverse microbial populations [12]. Serial dilution techniques were used to reduce the concentration of bacterial cells in the soil suspension, making it easier to isolate individual colonies. To prepare the serial dilutions, aliquots of the soil suspension were added to sterile saline solution and mixed well. The diluted samples were then spread onto nutrient agar plates using sterile methods to avoid cross-contamination. The plates were incubated at 37°C for 24 to 48 hours to encourage bacterial growth, allowing for colony formation [13]. After incubation, colonies with unique morphological traits were chosen and streaked onto new agar plates to create pure cultures for additional identification and analysis [14].

2.3 Morphological Analysis of Bacterial Culture

Morphological analysis was performed on bacterial colonies to record their physical traits, such as size, shape, colour, texture, elevation, and margin. These characteristics offer valuable insights into bacterial taxonomy and their ecological roles [15]. Microscopic observation of pure bacterial cultures was performed under high magnification to identify key cellular traits such as cell shape, arrangement, and surface structures. The observed characteristics were compared with reference descriptions in scientific literature to classify the bacteria into specific groups [16]. This analysis played a crucial role in developing a foundational understanding of the microbial diversity found in each soil sample.

2.4 Gram Staining

The Gram staining technique is employed to classify bacteria into two main groups: Gram-positive and Gram-negative. This classification is based on the distinct structural differences in their cell walls. The process involves a series of staining steps, beginning with crystal violet as the primary stain, followed by iodine, which acts as a mordant to fix the dye. Ethanol is then used as a decolourizer to eliminate any excess stain, and finally, safranin serves as a counterstain. Bacterial smears from pure cultures are prepared on slides, and the staining procedure is meticulously controlled to ensure precise results [17]. The stained slides were examined using a light microscope at 100x magnification with oil immersion. Gram-positive bacteria showed a purple colour because they retained the crystal violet-iodine complex in their thick peptidoglycan layer, whereas Gram-negative bacteria appeared pink or red due to the counterstain binding to their thinner cell walls [18].

2.5 Bacterial Identification

Bacterial identification involved a mix of observing morphological traits, analysing Gram-staining results, and cross-referencing scientific literature, textbooks or online databases. The characteristics of the colonies, such as size, shape, colour, and margin, were matched with the cellular features seen under the microscope. These traits were then compared to reference data from trustworthy sources to pinpoint the possible bacterial strains in the samples. This approach shed light on the dominant bacterial groups and their ecological functions, enhancing our understanding of the bacterial communities in UTHM Wetland and Pagoh Residential College.

2.6 Data Analysis

The data collected from microbial analyses and soil parameter measurements were analysed to identify patterns and differences between the two study sites. The study evaluated the statistical correlations between soil parameters like pH, temperature, and moisture content, as well as microbial diversity, to understand how they influence the composition of bacterial communities [2]. The analysis emphasized the influence of environmental conditions on microbial ecosystems and laid the groundwork for understanding the ecological roles of bacteria in these diverse settings. By combining these insights, this study enhanced our overall comprehension of the relationships between soil characteristics and microbial activity in tropical soil ecosystems.

3. Result and Discussion

3.1 Physical parameters of soil

Table 1 shows the physical parameters of soil which are pH, moisture content and temperature of soil in UTHM Pagoh Campus between UTHM Wetland and Pagoh Residential College.

Table 1 Physical parameters of soil

| No. | Sample | Habitat | pH | Temperature (°C) | Moisture content |
|-----|--------|---------------------------|-----|------------------|------------------|
| | W1 | UTHM Wetland | 5.0 | 22 | Wet |
| | W2 | | 4.8 | 21 | Wet |
| | W3 | | 5.0 | 22 | Wet |
| | C1 | Pagoh Residential College | 7 | 21 | Dry |
| | C2 | | 6.8 | 23 | Dry |
| | C3 | | 6.7 | 23 | Dry |

Based on Table 1, the average pH in the UTHM Wetland is 4.9, which is considered acidic. The sample from W2, located at the central point of the UTHM Wetland area, recorded the lowest pH value of 4.8. Additionally, the average temperature in the UTHM Wetland is 21.7 °C, with the lowest temperature of 21°C observed in sample W2. Overall, the moisture content in the UTHM Wetland is categorized as wet using an electronic 4-in-1 soil survey instrument that simply states it as wet or dry [19]. In Pagoh Residential College, the average pH is 6.8, which is considered slightly acidic to neutral. The highest pH recorded was 7 in sample C1, which is neutral, while the lowest pH of 6.7 was observed in sample C3. The average temperature was 22.3°C, with the lowest temperature of 21°C recorded in C1. Overall, the moisture content in Pagoh Residential College is categorized as dry.

3.2 Morphological Analysis of Bacterial Culture

Table 2 shows the morphological analysis of the bacterial colonies between UTHM Wetland and Pagoh Residential College according to the size, colour, form, margin and shape.

Table 2 Morphological analysis

| No. | Sample | Habitat | Size | Colour | Elevation | Form | Margin | Shape |
|-----|--------|---------------------------|----------|-----------|-------------|-----------|------------------|-------|
| | W1 | UTHM Wetland | Small | Whitish | Convex | Round | Serrate/undulate | Rod |
| | W2 | | Small | Yellowish | Raised | Circular | Entire | Rod |
| | W3 | | Small | Whitish | Whitish | Irregular | Undulate | Rod |
| | C1 | Pagoh Residential College | Moderate | Whitish | Crateriform | Circular | Entire | Rod |
| | C2 | | | | | | | |
| | C3 | | | | | | | |

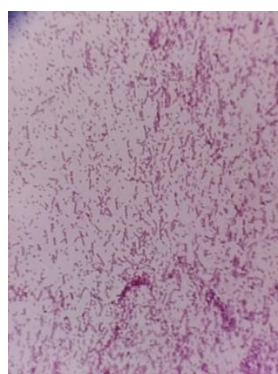


Fig. 2 The rod-shaped bacteria in W2

Based on Table 2, the samples from UTHM Wetland are generally smaller in size compared to those from Pagoh Residential College, where the samples are of moderate size. In terms of colour, some samples from UTHM Wetland showed a whitish to yellowish hue, while all samples from Pagoh Residential College were whitish. Regarding elevation, the samples from UTHM Wetland exhibited varying elevations which sample W1 was convex, W2 was raised, and W3 was flat, whereas the elevation in Pagoh Residential College was crateriform. As for the form, each sample from UTHM Wetland displayed a different shape where W1 was round, W2 was circular, and W3 was undulate, while all samples in Pagoh Residential College were circular. In terms of margin, the samples from UTHM Wetland had different types which W1 had a serrate/undulate margin, W2 had an entire margin, and W3 had an undulate margin. In contrast, all samples from Pagoh Residential College had an entire margin. Additionally, based on the shape of the bacteria from both sites was rod-shaped as Fig. 2.

3.3 Gram Staining of Bacterial Culture

Table 3 shows the colour of gram staining of the bacterial colonies.

| No. | Sample | Habitat | Colour staining | Gram |
|-----|--------|---------------------------|-----------------|----------|
| | W1 | UTHM Wetland | Pink | Negative |
| | W2 | | Pink | Negative |
| | W3 | | Purple | Positive |
| | C1 | Pagoh Residential College | Pink | Negative |
| | C2 | | | |
| | C3 | | | |

Based on Table 3, the samples from UTHM Wetland displayed pink to purple staining, with sample W3 showing a purple hue, indicating a positive Gram reaction. In contrast, the samples from Pagoh Residential College exhibited pink staining, which indicates a negative Gram reaction.

3.4 Bacterial identification

Table 4 shows the bacterial identification in UTHM Pagoh Campus between UTHM Wetland and Pagoh Residential College using scientific journals.

| No. | Sample | Habitat | Bacterial family | Notable genera | References | |
|-----|--------|---------------------------|--------------------|---|-----------------------------|------------|
| | W1 | UTHM Wetland | Enterobacteriaceae | <i>Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus</i> | [20], [21] | |
| | W2 | | | Xanthomonadaceae | <i>Xanthomonas, Xylella</i> | [22], [23] |
| | W3 | | | Listeriaceae | <i>Listeria</i> | [24], [25] |
| | C1 | Pagoh Residential College | Moraxellaceae | <i>Acinetobacter, Moraxella, Psychrobacter</i> | [26], [27], [28] | |
| | C2 | | | | | |
| | C3 | | | | | |

3.5 Discussion

Some physical parameters of the soil samples from UTHM Wetland and Pagoh Residential College suggest very different environmental conditions, which might have effects on microbial diversity and distribution. The pH values of the wetland samples, including W1, W2, and W3, lie between 4.8 and 5.0, hence a slightly acidic environment. Soils from Pagoh Residential College, including C1, C2, and C3, have pH values between 6.7 and 7.0, which shows neutral to slightly alkaline conditions. However, the acidic conditions in the wetlands might favor bacterial families such as Enterobacteriaceae and Listeriaceae, which are associated with habitats with rich organic material and moderate acidity [20]; [24]. On the other hand, neutral to alkaline conditions in the college of residence might host genera like *Acinetobacter* of the family Moraxellaceae, mostly presenting in drier less acidic soils [29]. The moisture content further emphasizes the differences between the two environments.

This would suggest that the wetland samples fall under the "wet" class, which is in line with keeping high levels of moisture retention that would nourish bacteria such as *Xanthomonas*, which prefer water-abundant environments [22]. The residential college samples fall into the "dry" class, corresponding with the presence of *Acinetobacter*, which is capable of withstanding low-moisture conditions [27]. Moreover, only minor variations are seen in temperature data between 21–23°C and hence this parameter will likely play a secondary role in determining microbial diversity since both the habitats fall within the optimal range of bacterial growth. Further, morphological and Gram staining analyses clearly indicate the influence of environmental conditions on bacterial characteristics. The UTHM Wetland samples contained mostly small, rod-shaped bacteria with different colony forms, including irregular and undulate types, which indicated the diverse and dynamic conditions of the wetlands. Previous study shown that the main phyla found in constructed wetlands are Proteobacteria, Bacteroidetes, and Firmicutes, with Proteobacteria typically being the most dominant [30];[31].

Most of the bacteria in these samples were Gram-negative, as seen in *Escherichia* and *Xanthomonas*. In the samples from Pagoh Residential College, colonies were moderately-sized with forms that were rather consistent in being circular and Gram-negative-staining in *Acinetobacter*. These results give weight to environmental factors such as pH, moisture level, and structure of habitat in shaping microbial communities and their adaptation due to the ecological difference between the wetland and residential soil environments. Ecological significance of Gram-negative bacterial dominance where they play a crucial role in the decomposition of organic matter, contributing to nutrient cycling within ecosystems. For instance, certain Gram-negative bacteria play a crucial role in breaking down complex polymers like chitin and cellulose, which helps recycle carbon and nitrogen in the environment [32]. Other than that, these bacteria often exhibit a high degree of adaptability to various environmental conditions. In agricultural soils, a higher diversity of Gram-negative bacteria has been observed, which may be attributed to their ability to utilize a wide range of organic compounds and adapt to different soil environments [33]. Based on the result obtained (Table 1), the shape of the bacteria found in both sites were rod shaped. This is because, rod-shaped bacteria are commonly found in soil environments due to their efficient surface area-to-volume ratio, which enhances nutrient absorption and metabolic activity. Their shape also facilitates movement through soil pores, aiding in colonization and interaction with soil particles [26]. Other than that, the elongated shape of rod-shaped bacteria provides approximately 40% more surface area compared to spherical (coccus) bacteria of similar volume. This increased surface area allows for greater nutrient absorption and surface interactions, which are critical when surface contact is the primary means of nutrient uptake. This advantage gives rod-shaped bacteria an edge in nutrient-limited environments, enhancing their ability to survive and thrive in diverse ecological niches [34].

Next, based on the bacterial identification (Table 4), the total of bacterial families found for both sites are different. In UTHM Wetland, the bacterial family found are more than Pagoh Residential College. This is because the development of Pagoh Residential College in UTHM represents a land-use change. Previously, the land may have been used for agricultural, natural, or other purposes before being converted into a residential area to accommodate students. Land use change is currently one of the most important environmental changes and can alter soil environmental factors, nutrient conditions, and biological interactions, thereby affecting microbial communities. Similarly, the bacterial community in cultivated soil under intensive management was different from that in unmanaged soil [35]. For example, the abundance of Proteobacteria decreased significantly in plantations, orchards, sloping tillage, and paddy fields compared to primary forests. These changes in bacterial composition were linked to alterations in soil physicochemical properties, such as reduced soil organic carbon and nutrient availability following land-use change [36].

4. Conclusion

This study has successfully attained the objectives by carrying out a comparative analysis of soil bacterial communities at two different locations in UTHM Pagoh Campus which are UTHM Wetland and Pagoh Residential College. From the results obtained, it indicates that there were significant differences in bacterial diversity and composition between the two sites, which were influenced by environmental factors such as pH, temperature, and moisture content. The UTHM Wetland, having acidic pH and high moisture, supported a more diversified bacterial community, including families such as Enterobacteriaceae and Listeriaceae. In contrast, Pagoh Residential College, with a neutral to slightly alkaline pH and drier comparative soil, showed reduced diversity, where the family Moraxellaceae became the most dominant. These results indicate that environmental conditions are a must for determining the composition of soil microbial ecosystems.

Moreover, this study underlined the role of soil pH, temperature, and moisture content in influencing the composition of microbial communities. UTHM Wetland had suitable conditions for microbial habitation with its acidic and moist conditions, while the drier and more stable conditions found at Pagoh Residential College sustained a less diverse population of bacteria. This study thus implies that wetlands are important sources of microbial diversity, which should be preserved for sustainable land management practices toward maintaining soil health and ecosystem functionality. Effective wetland conservation needs a well-rounded strategy that involves community involvement, policy creation and scientific investigation. Adopting sustainable methods like

habitat restoration, pollution management, and careful resource utilization is crucial to protect wetlands and their important ecosystem services for the future generations [37]. Preservation of such wetlands, like the UTHM Wetland, should be concerned with conservation efforts since this ecosystem possesses high microbial diversity and ecological value. For example, utilize ecotourism to create alternative income opportunities while protecting wetland ecosystems [38]. The practices that are sustainable for the preservation of soil microbiological ecosystems balance include habitat restoration and controlled land use.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm their contributions to the paper as follows: **study conception and design:** Muhammad Shah Danial Jefry, Furzani Pa'ee; **data collection:** Muhammad Shah Danial Jefry; **analysis and interpretation of results:** Muhammad Shah Danial Jefry, Furzani Pa'ee; **draft manuscript preparation:** Muhammad Shah Danial Jefry, Furzani Pa'ee. All authors reviewed the results and approved the final version of the manuscript.

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