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Unseen Ecosystems: Microbial Life in Soil, Water, and Air

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ABSTRACT

Microorganisms are vital, yet unseen, components of the biosphere, crucial for nutrient cycling, environmental stability, and ecosystem sustainability. They are distributed across major reservoirs like soil, water, and air, and their presence and characteristics are indicators of environmental quality and potential health impacts. This study aimed to isolate and profile microorganisms from representative environmental samples. Soil, water, and air samples were collected aseptically from different locations. Standard microbiological methods, utilizing nutrient agar media, were employed for isolation. Inoculated plates were incubated for 24 hours at relevant temperatures, after which visual colonies were observed. Individual colonies were then morphologically characterized based on size, shape, margin, elevation, color, and texture. Gram staining was performed to identify cell wall properties and microscopic morphology. The findings revealed abundant microbial growth across all sample types, underscoring their widespread environmental distribution. Soil samples exhibited the highest colony diversity, displaying varied pigmentation and textures. Water samples showed moderate but distinct colony types, while air samples had comparatively fewer colonies. Gram staining confirmed the presence of both Gram-positive and Gram-negative bacteria, with cocci and bacilli forms observed microscopically. This research collectively supports the universality and variability of microorganisms in soil, water, and air, highlighting their ubiquitous, albeit invisible, presence in our surroundings.

Keywords: Microbial diversity, soil health, water quality, microbial isolation

1. INTRODUCTION

Microbial pathogens represent a significant and persistent threat to global public health, with transmission occurring through multiple environmental pathways that vary in epidemiological significance and transmission efficiency. Understanding the ecological origins and transmission mechanisms of disease-causing microorganisms is fundamental to developing effective prevention and control strategies. This study examines three primary environmental exposure routes - waterborne, soilborne, and airborne pathways - through which pathogenic microorganisms reach human populations and pose public health risks[14, 20]. Waterborne microorganisms constitute a major public health concern, particularly in regions where water treatment infrastructure and sanitation systems are inadequate[19]. These pathogens originate

from contaminated water sources receiving human and animal waste, including sewage, agricultural runoff, and fecal material[12]. Major waterborne pathogens such as Escherichia coli, Salmonella, and Cryptosporidium demonstrate remarkable persistence within water distribution systems, enabling prolonged contamination and recurring outbreaks in vulnerable populations[1,15]. Communities lacking adequate water treatment capacity face disproportionate exposure risks and increased susceptibility to waterborne disease transmission. Soilborne microorganisms occur naturally within soil ecosystems but present significant health risks when they gain access to human hosts through direct contact, ingestion, or inhalation pathways. Pathogenic species including Bacillus and Clostridium bacteria, as well as fungal genera such as Aspergillus and Blastomyces, thrive under specific edaphic conditions and may produce severe infections, particularly among immunocompromised individuals[4,11]. Occupational groups such as agricultural workers and construction personnel face elevated exposure risks due to frequent soil contact [6]. Airborne microorganisms represent a highly efficient transmission route for respiratory pathogens, which disperse through aerosols and respiratory droplets released during coughing, sneezing, and speech by infected individuals[17]. These suspended particles traverse considerable distances in both indoor and outdoor environments, facilitating rapid dissemination of respiratory infections throughout populations[17].

2. MATERIALS AND METHODS

2.1 Materials

Six sterile 50 mL containers for collecting the soil and water were procured. Nutrient agar media was procured from HiMedia. Gram staining reagents were procured from ThermoFisher Scientific.

2.2 Media Preparation

In order to prepare nutrient agar plates, commercially obtained nutrient agar powder (28 g) was precisely weighed and dissolved in an estimated volume of 800 mL of distilled water in a 1 L conical flask. The solution was heated gently as it was stirred with continuous mixing until all the medium dissolved and the volume was adjusted to 1000 mL using distilled water. The flask was subsequently blocked with cotton or sealed with aluminum foil and sterilized in an autoclave at 121°C under 15 psi pressure for 15 minutes.

The sterilized medium was left to cool down to 45–50°C to avoid excessive condensation and ensure safe handling. About 20 mL of the molten nutrient agar was carefully poured into each sterile Petri plate under aseptic conditions without creating air bubbles. The plates were allowed to sit at room temperature until the medium solidified. The plates were then inverted to ensure that the condensation droplets did not fall on the agar and were stored at a temperature of 4°C before use.

2.3 Collection of Water Samples

Water samples from three different locations were collected **to capture environmental variability for comparison**. Sterile 50 mL containers were used to avoid outer contamination at every location. The containers were opened right before the collection and placed beneath the water surface to prevent contamination by surface debris.

The container was filled to about 90 percent of the capacity with little space left to minimize oxygen exchange and exposure on the outside. Containers were securely closed and marked with the date, location of sampling, and time. All samples were taken to the laboratory in a timely manner and under controlled conditions in order to reduce microbial changes prior to analysis.

2.4 Collection of Soil Samples

Three environmental sites were sampled to collect soil samples **representative of diverse ecological conditions**. The collection was done in sterile 50 mL containers to ensure the integrity of the sample. About 10–20 grams of soil were taken off the surface of the soil at sites—specifically, the top layer of 2–5 cm of soil—with a sterile spatula, since **this upper stratum exhibits the highest microbial metabolic activity**.

Caution was observed to prevent the inclusion of debris, stones, leaves, or other non-soil materials. The soil that was collected was placed straight into the sterile container and closed immediately. The samples were well labeled with the location, date, and time of collection, and then transported to the laboratory for microbiological analysis.

2.5 Air Sampling

There were three sampling sites chosen for airborne microbials to determine variation in airborne microorganisms. The settle plate technique was used using sterile agar plates. A sterile agar dish was opened in one of the sampling sites and left exposed to the air for a standard time period of 15 minutes to enable airborne microorganisms to be deposited on the agar surface as a result of gravitation.

The plate was placed carefully at a height close to human breathing height and was not subjected to direct disturbances in the airflow to provide representative samples. After exposure, the agar plates were covered, sealed, and marked with the location of the sampling, date, and the time of exposure. The plates were then taken to the laboratory where they were incubated and assessed for microbial growth.

2.6 Serial Dilution

In the case of soil samples, 0.5 g of soil was aseptically weighed and placed in the first tube holding 5 mL of sterile distilled water. The solution was vortexed to produce a homogeneous suspension representing the initial dilution (regarded as 10^{-1}). After this, 0.5 mL of the suspension was moved to a second tube containing 4.5 mL of sterile diluent to create a 10^{-2} dilution, giving a final volume of 5 mL. This tenfold serial dilution was repeated subsequently until a final dilution of 10^{-6} was achieved. At each step of the process, homogeneity was provided through proper mixing.

In the case of water, a 0.5 mL aliquot of the collected water sample was aseptically transferred into a tube containing 4.5 mL of sterile diluent to obtain a 10^{-1} dilution (total volume 5 mL). The tube was shaken and 0.5 mL of this dilution was then placed in another tube with 4.5 mL of diluent to give a 10^{-2} dilution. This process was continued until a final dilution of 10^{-3} was achieved. All dilution tubes were thoroughly mixed prior to plating.

2.7 Isolation of Organisms from Water and Soil Samples

From appropriate dilutions of water or soil samples, aliquots (0.1 mL) were aseptically pipetted onto sterile nutrient agar plates. The inoculum was spread evenly with the aid of a sterile L-shaped spreader to obtain isolated colonies. One or two minutes were allowed to pass to permit sample absorption, and then the plates were incubated at 37°C in an inverted position over a period of 24 hours. After incubation, visible colonies formed on the agar surface were counted and characterized according to colony morphology.

2.8 Gram Staining

A thin smear of the bacterial culture was first prepared on a clean, grease-free glass slide using a sterile inoculating loop. The smear was air-dried and then heat-fixed by passing the slide gently through a flame 2–3 times to fix the cells onto the slide surface. The smear was then flooded with crystal violet solution (primary stain) and allowed to stand for 1 minute. Excess stain was gently rinsed off with distilled water.

Next, the smear was covered with Gram's iodine (mordant) for 1 minute to form a crystal violet–iodine complex, followed by gentle washing with water. The slide was then decolorized carefully using 95% ethanol or acetone–alcohol for approximately 10–20 seconds until the runoff became clear. Immediate rinsing with distilled water was performed to stop the decolorization process.

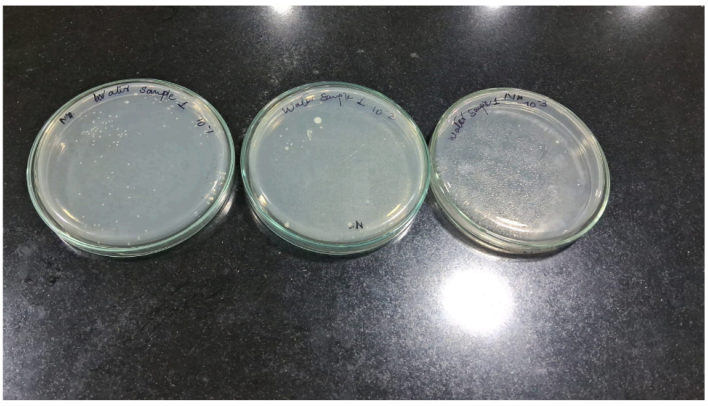
After decolorization, the smear was counterstained with safranin for 1 minute and then rinsed gently with water. The slide was blotted dry using bibulous paper and examined under a microscope using the oil immersion objective (100X). Gram-positive bacteria appeared purple or violet, retaining the crystal violet stain, whereas Gram-negative bacteria appeared pink or red, taking up the safranin counterstain.

3. RESULTS

3.1 Isolation of Organisms from Water Samples

Microbial analysis of the three water samples demonstrated variable bacterial loads and colony morphologies.

A



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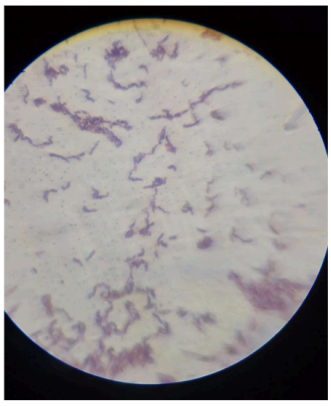


Figure 1. Isolation of organisms from water sample 1. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

A B

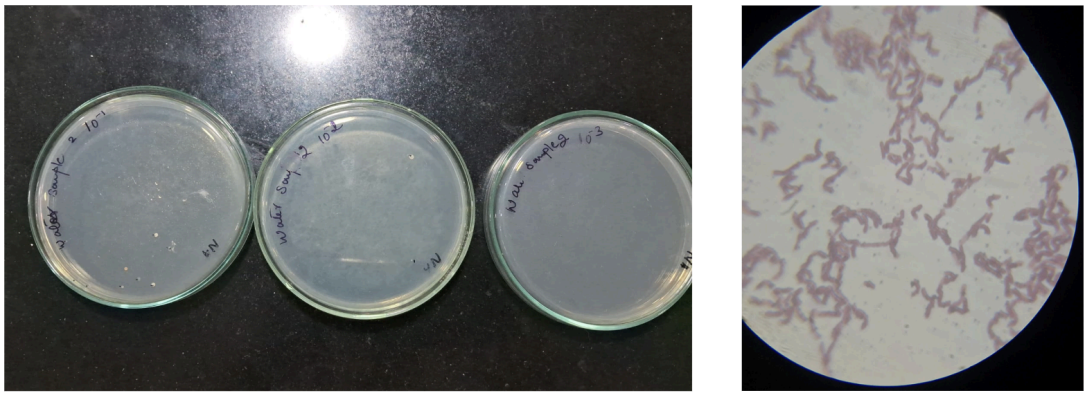


Figure 2. Isolation of organisms from water sample 2. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

A B

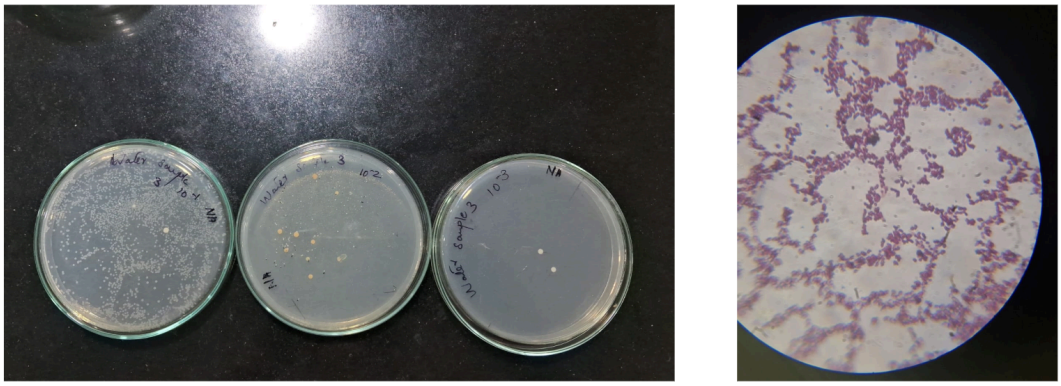


Figure 3. Isolation of organisms from water sample 3. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

(Note: Experimental photographs documenting plate growth and microscopy fields are referenced as Figures 1 through 3). Gram staining results reveal the presence of Gram-positive rods in water samples 1 and 2, and Gram-positive cocci in water sample 3.

Tables 1, 2, and 3 indicate the microbial load in water samples 1, 2, and 3, respectively. Microbial counts revealed a heavy microbial load in water samples 1 and 3, followed by water sample 2. Table 4 summarizes the macroscopic colony characteristics observed across the water samples.

Table 1: Colony Forming Units of Water Sample 1

Serial Number	DILUTIONS	CFU/0.1 ML	Number of CFU/0.1 ML	CFU/1ML
1	10^{-1}	105	105×10^{1}	1050×10^{1}
2	10^{-2}	23	23×10^{2}	230×10^{2}
3	10^{-3}	01	1×10^{3}	10×10^{3}
Final concentration				1.45×10^{4}

Table 1. The colony forming units of water sample 1
CFU: colony forming units

Table 2: Colony Forming Units of Water Sample 2

Serial Number	DILUTIONS	CFU/0.1 ML	Number of CFU/0.1 ML	CFU/1ML
1	10^{-1}	9	9×10^{1}	90×10^{1}
2	10^{-2}	2	2×10^{2}	20×10^{2}
3	10^{-3}	00	–	–
Final concentration				1.45×10^{3}

Table 2. The colony forming units of water sample 2
CFU: colony forming units

Table 3: Colony Forming Units of Water Sample 3

Serial Number	DILUTIONS	CFU/0.1 ML	Number of CFU/0.1 ML	CFU/1ML
1	10^{-1}	TNTC	–	–
2	10^{-2}	10	10×10^{2}	100×10^{2}
3	10^{-3}	2	2×10^{3}	20×10^{3}
Final concentration				1.50×10^{3}

Table 3. The colony forming units of water sample 3
CFU: colony forming units
TNTC: Too many to count

Table 4: Macroscopic Observations of Water Samples

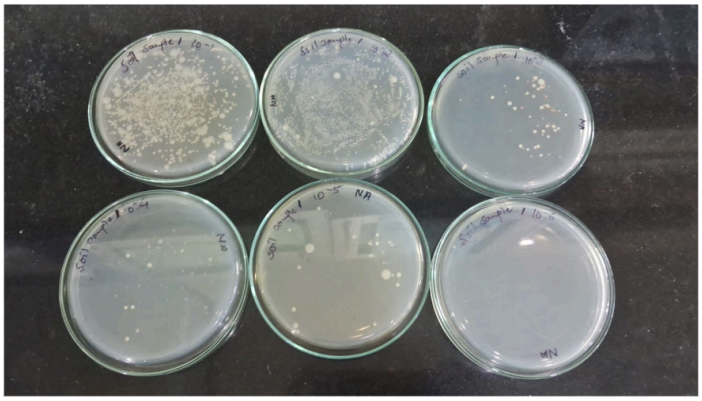
Sample	Water sample 1	Water sample 2	Water sample 3
Characteristics			
Size	Pinpoint	2mm	1mm
Shape	Circular	Circular	Circular
Colour	White	**Off- White**	Orange
Margin	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque
Consistency	Smooth	Smooth	Smooth
Gram's nature	Positive rods	Positive rods	Positive cocci

Table 4. The macroscopic observations of water sample

3.2 Isolation of Organisms from Soil Samples

Microbial analysis of the three soil samples demonstrated variable bacterial loads and colony morphologies.

A



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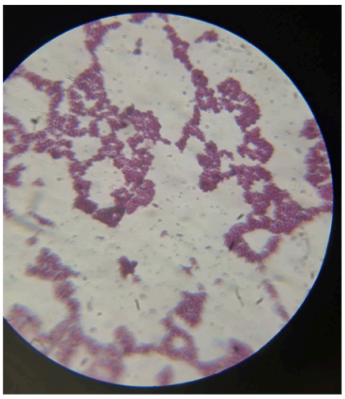

Figure 4. Isolation of organisms from soil sample 1. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

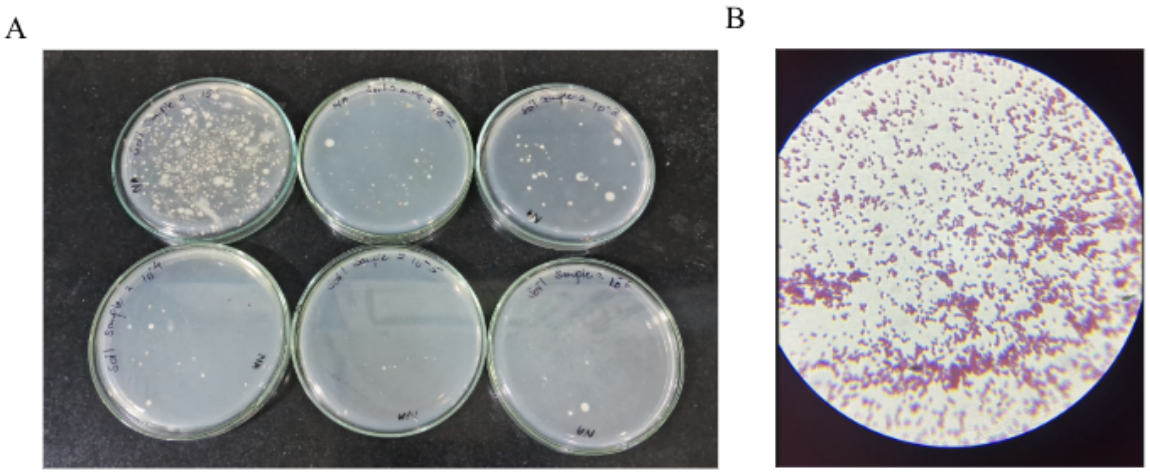

Figure 5. Isolation of organisms from soil sample 2. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

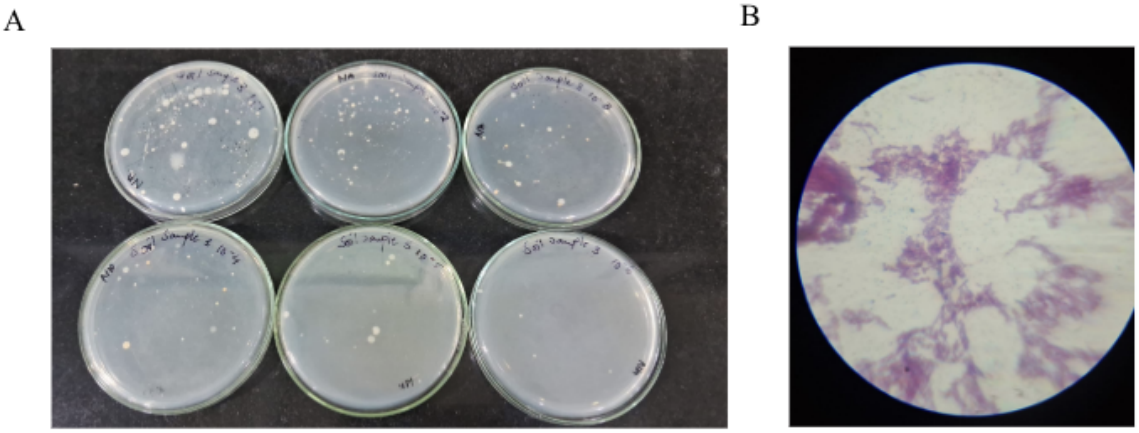

Figure 6. Isolation of organisms from soil sample 3. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

(Note: Experimental photographs documenting soil plate growth and corresponding microscopy fields are referenced as Figures 4 through 6). Gram staining results reveal the presence of Gram-positive cocci, Gram-positive rods, and Gram-negative short rods in soil samples 1, 2, and 3, respectively.

Tables 5, 6, and 7 indicate the microbial load in soil samples 1, 2, and 3, respectively. Microbial counts revealed a heavy microbial load in soil sample 2, followed by soil samples 1 and 3. Table 8 summarizes the macroscopic colony characteristics from soil samples 1, 2, and 3.

Table 5: Colony Forming Units of Soil Sample 1

Serial Number	DILUTIONS	CFU/0.1 ML	Number of CFU/0.1 ML	CFU/1ML
1	10^{-1}	TNTC	TNTC	–
2	10^{-2}	TNTC	TNTC	–
3	10^{-3}	64	64×10^{3}	640×10^{3}
4	10^{-4}	52	52×10^{4}	520×10^{4}
5	10^{-5}	22	22×10^{5}	220×10^{5}
6	10^{-6}	2	2×10^{6}	20×10^{6}
Final concentration				**1.9×10^{7}**

Table 5. The colony forming units of soil sample 1
TNTC: Too many to count
CFU: colony forming units

Table 6: Colony Forming Units of Soil Sample 2

Serial Number	DILUTIONS	CFU/0.1 ML	Number of CFU/0.1 ML	CFU/1ML
1	10^{-1}	TNTC	TNTC	–
2	10^{-2}	84	84×10^{2}	840×10^{2}
3	10^{-3}	36	36×10^{3}	360×10^{3}
4	10^{-4}	33	33×10^{4}	330×10^{4}
5	10^{-5}	15	15×10^{5}	150×10^{5}
6	10^{-6}	8	8×10^{6}	80×10^{6}
Final concentration				**1.97×10^{7}**

Table 6. The colony forming units of soil sample 2
CFU: Colony forming units
TNTC: too many to count

Table 7: Colony Forming Units of Soil Sample 3

Serial Number	DILUTIONS	CFU/0.1 ML	Number of CFU/0.1 ML	CFU/1ML
1	10^{-1}	TNTC	TNTC	TNTC
2	10^{-2}	76	76 x 10^{2}	760 x 10^{2}
3	10^{-3}	30	36 x 10^{3}	300 x 10^{3}
4	10^{-4}	23	23 x 10^{4}	230 x 10^{4}
5	10^{-5}	16	16 x 10^{5}	160 x 10^{5}
6	10^{-6}	4	4 x 10^{6}	40 x 10^{6}
Final concentration				**1.17 x 10^{7}**

Table 7. The colony forming units of soil sample 3
CFU: Colony forming units
TNTC: too many to count

Table 8: Macroscopic Observations of Soil Samples

Sample	Soil sample 1	Soil sample 2	Soil sample 3
Characteristics			
Size	5mm	3mm	6mm
Shape	Circular	Circular	Circular
Colour	Orange	**Off White**	**Off White**
Margin	Entire	Wavy	Entire
Opacity	Opaque	Opaque	Opaque
Consistency	Smooth	Smooth	Smooth
Gram's nature	Positive cocci	Positive rods	Negative short rods

Table 8. The macroscopic observations of Soil sample

3.3 Isolation of Organisms from Air Samples

Microbial analysis of the three air samples demonstrated variable bacterial loads and colony morphologies.

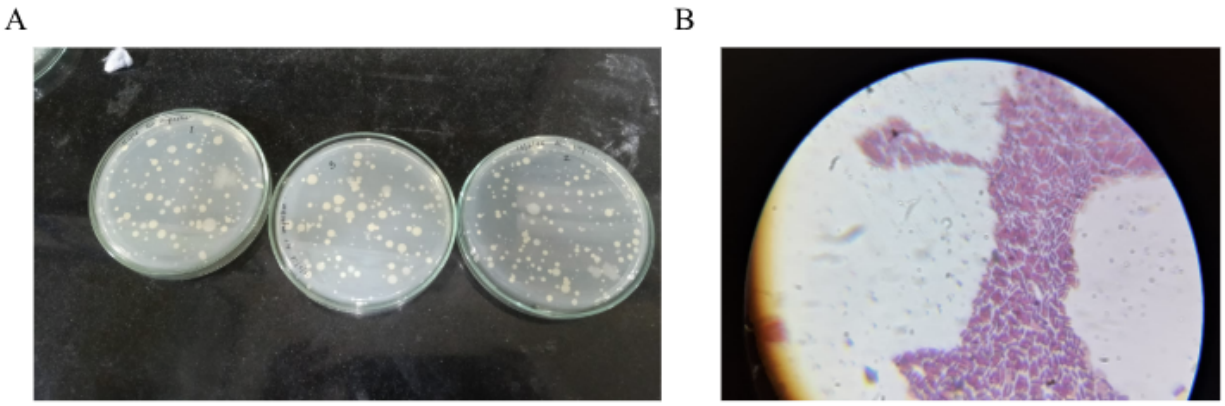

Figure 7. Isolation of organisms from air sample 1. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

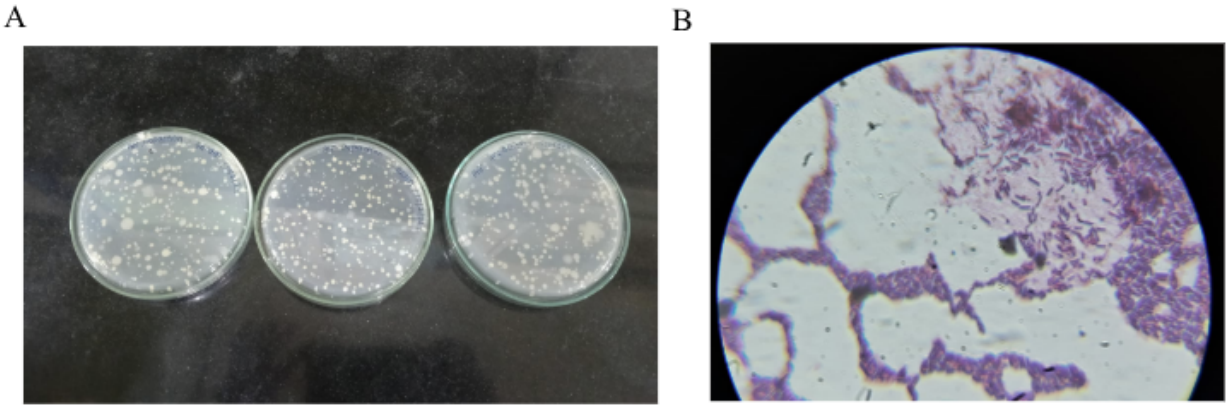

Figure 8. Isolation of organisms from air sample 2. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

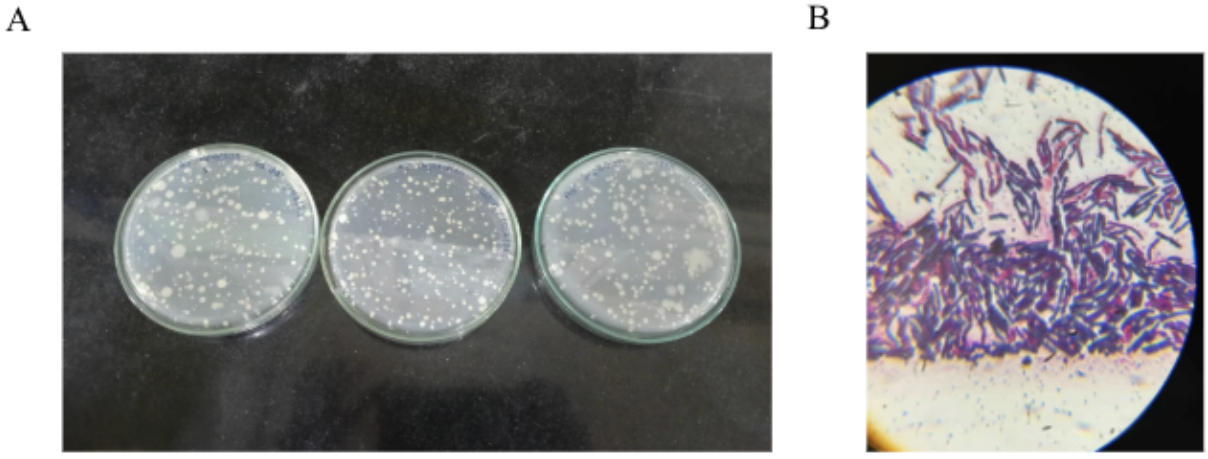

Figure 9. Isolation of organisms from air sample 3. A Growth of organisms on nutrient agar plates **B** Gram staining under light microscope

(Note: Experimental plates and microphotographs for air exposure samples are designated as Figures 7 through 9). Gram staining results reveal the exclusive presence of Gram-positive rods across all air samples.

Table 9 indicates the microbial load obtained via the settle plate technique for air samples 1, 2, and 3. Quantitative analysis revealed the heaviest airborne microbial settlement in air sample 1, followed by air samples 2 and 3. Table 10 summarizes the macroscopic colony characteristics observed from these air samples.

Table 9: Colony Forming Units of Air Samples (15-Minute Settle Plate Exposure)

Serial Number.	Plate 1	Plate 1	Plate 1	Average
Sample 1	366	361	434	TNTC
Sample 2	220	252	328	**266.7**
Sample 3	186	276	184	**215.3**

Table 9. The colony forming units of Air samples

Table 10: Macroscopic Observations of Air Samples

Sample	Air sample 1	Air sample 2 Colony 1	Air sample 2 Colony 2	Air sample 3 Colony 1	Air sample 3 Colony 2
Characteristics					

Size	0.1mm	0.2mm	1cm	0.2mm	0.3mm
Shape	Circular	Irregular	Irregular	Circular	Circular
Colour	White	**Off White**	**Off White**	Yellow	**Off White**
Margin	Entire	Entire	Filametous	Entire	Wavy
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat	Flat	Flat
Consistency	Smooth	Smooth	Hard	Smooth	Smooth
Gram's nature	Gram Positive rods	Gram Positive rods	Gram Positive rods	Gram Positive rods	Gram Positive rods

Table 10. The macroscopic observations of Air samples

4. CONCLUSION

This comparative study demonstrates a distinct gradient in cultivable microbial loads and phenotypic diversity across environmental reservoirs, confirming that structural and ecological parameters directly shape local microbial populations. Systematic culture-based isolation, phenotypic characterization, and Gram staining verified that viable microorganisms are ubiquitous across all soil, water, and air samples.

Quantitatively, the microbial load and morphological diversity peaked significantly within soil ecosystems, reached intermediate levels in aquatic samples, and were lowest within airborne environments. The discovery of both Gram-positive and Gram-negative bacterial architectures, presenting diverse cocci and bacilli morphologies, highlights the broad structural differences within environmental ecosystems.

These outcomes validate the role of soil as a primary, nutrient-dense reservoir capable of supporting highly complex microbial communities, while air acts predominantly as a transient, high-stress transport medium. Although culture-based assays reveal only a fraction of total microbial presence, they provide essential phenotypic baselines. Regular monitoring of these environmental signatures remains necessary to track ecosystem stability, detect potential public health shifting points, and manage vector mitigation pathways effectively.

5. DISCUSSION

5.1 Analysis of Reservoir Diversity and the Great Plate Count Anomaly

The experimental data demonstrates a clear stratification in bacterial abundance and morphological diversity across the evaluated mediums, with soil displaying the highest concentration of distinct colony forming units, followed by water and air. This variance aligns with environmental gradients, as soil offers a stable matrix with rich organic content and localized moisture micro-niches that support diverse cellular strategies.

However, a critical consideration when evaluating these results is the "Great Plate Count Anomaly." This established microbiological principle states that standard agar cultivation methods capture only a tiny fraction—frequently less than 1%—of total living environmental microorganisms. Because nutrient agar sets specific nutrient, moisture, and temperature conditions, many specialized, anaerobic, or fastidious environmental strains remain uncultivable on standard plates. Consequently, while the culture-based methods used here provide an accurate comparison of viable, fast-growing phenotypic traits, the true biological diversity within these samples is likely much higher than observed visually.

5.2 Comparative Ecological Dynamics

The topic of microbial diversity in soil is one of the most actively researched areas of environmental microbiology. Soil ecosystems possess highly sophisticated communities due to factors like moisture, nutrient gradients, and the structure of microhabitats. High-throughput sequencing studies of soil bacteria indicate that community diversity depends closely on soil physicochemical conditions and climate, showing deep diversity in mesic environments and a broad range of variation among biomes.

Microscale aqueous-phase processes select for distinct taxa by providing many isolated microhabitats, which explains the high colony variability found in the soil samples. Additionally, studies of saline soils emphasize the role of microorganisms in nutrient cycling and stress adaptation, highlighting the functional significance of these localized populations.

Microbial communities in aquatic environments are heavily affected by nutrient supply, organic matter input, and environmental stressors. The moderate diversity of cultivable microbes observed in the water samples reflects a well-documented subset of larger aquatic surveys. Extensive metagenomic profiles reveal that water habitats sustain complex assemblages of bacteria, algae, and protists that shift based on seasonal and geographical changes. These aquatic microbes perform crucial functions in biogeochemical cycles, including nitrogen and carbon transformations, directly affecting water quality and overall ecosystem productivity.

Microorganisms suspended in air occupy a highly challenging ecological niche, enduring harsh conditions such as severe nutrient scarcity, ultraviolet radiation, and desiccation. Culture-based approaches typically retrieve fewer airborne organisms because the atmosphere serves primarily as a transient dispersal pathway rather than a supportive growth medium.

Nevertheless, existing literature confirms that the atmosphere is an active microbial environment where specific bacterial taxa adapt structurally to survive long-distance transportation. Key taxa linked to human respiratory health and climate processes have been identified within these airborne communities, indicating that despite lower quantities, their public health relevance remains high.

5.3 Study Limitations

Several technical limitations should be noted when evaluating this study. First, relying entirely on culture-based isolation using nutrient agar leaves out non-cultivable or slow-growing species, meaning the data shows a targeted look at environmental microbes rather than a total census.

Second, the settle plate method used for air sampling is semi-quantitative and biased toward larger particles or aggregates that settle quickly due to gravity; it does not capture lighter aerosols that stay suspended in the breathing zone. Lastly, sampling was conducted at a single point in time, which means the datasets do not account for daily weather shifts, seasonal temperature changes, or local environmental disruptions that naturally alter microbial concentrations in the open air and surface water.

5.4 Practical Applications and Future Perspectives

The relevance of this research lies in its support of baseline environmental monitoring, ecosystem evaluation, and public health risk mapping. Monitoring environmental microbial communities is increasingly useful for tracing the origin and spread of antibiotic resistance. Recent studies focus heavily on soil, water, and air as major reservoirs and transmission pathways for antibiotic-resistant bacteria under anthropogenic pressures.

Practically, environmental microbiology offers wide applications across industries. In agriculture, understanding soil microbial composition can optimize farming practices to boost fertility, improve nutrient cycling, and support sustainable crop yields. In water resource management, microbial indicators guide water quality testing, wastewater treatment design, and pollution mitigation strategies. For public health, identifying airborne microbes and their survival limits helps refine indoor air quality assessments and pathogen surveillance models.

Future studies should expand beyond traditional culture methods by applying advanced molecular and metagenomic tools. High-throughput sequencing, 16S rRNA analysis, and functional gene profiling would allow for the identification of uncultivable strains, providing deeper insight into total community structures and metabolic capacities.

Long-term sampling designs would help evaluate seasonal and regional fluctuations across soil, water, and air ecosystems. Additionally, future work should explicitly test how changing environmental stressors—such as chemical pollution, urban runoff, and climate shifts—impact community structural shifts. Combining classic cultivation techniques with modern bioinformatics will improve ecosystem management models and benefit human wellbeing.

6. ACKNOWLEDGEMENTS

The author expresses sincere gratitude to parents and school teachers for their constant support, guidance, and encouragement throughout this research process.

7. CONFLICT OF INTEREST DISCLOSURE

There are no potential conflicts of interest to disclose.

8. FUNDING SOURCE STATEMENT

There are no funding sources or financial support instruments to disclose.

9. AUTHORS' CONTRIBUTION

TH conceptualized and designed the study, performed data collection, conducted the analysis, and interpreted the results. TH drafted the manuscript and approved the final manuscript for publication.

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