

# Exploring the Microbial Load of Soil and Water Samples Using Dilution Plate Assay Method

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## Abstract:

This study assesses and compares the microbial load and community structure of soil and water samples of university campus area using the dilution plate assay methodology. The primary objective was to quantitatively and qualitatively characterize the viable microbial populations in each environment. Serial dilutions of both soil and water samples were prepared and subsequently plated on a variety of culture media to isolate viable bacteria and fungi. Colony-forming units (CFUs) were enumerated, and the resulting colonies were subjected to macroscopic and microscopic analysis, including Gram staining for bacterial morphology. The results revealed a stark contrast in microbial abundance with the soil sample exhibiting a significantly higher microbial load of approximately  $1.32 \times 10^6$  CFU/g, an order of magnitude greater than the water sample's  $4.5 \times 10^3$  CFU/ml. Qualitative analysis further demonstrated that the soil sample harbored a richer diversity of microorganisms including a wide array of filamentous fungi and various bacterial morphotypes. The findings align with the established ecological principle that soil, with its complex physical structure and abundant organic matter, provides a more favorable and nutrient-rich habitat for microbial communities than most aquatic environments. This study confirms the utility of the dilution plate assay as a foundational method for comparative microbial ecology and highlights the need for more advanced molecular techniques to fully elucidate microbial diversity and function in these complex ecosystems.

**Keywords** — Microbial Load, Dilution Plate Assay, Soil Microbes, Water Microbes, CFU, Fungi, Bacteria.

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## I. Introduction

### A. Background on Microbial Ecology

Microorganisms are ubiquitous and fundamental components of all ecosystems, playing a central role in maintaining environmental health and function [1,2]. Within soil, they are the principal drivers of global biogeochemical cycles, including the cycling of essential elements such as carbon, nitrogen, phosphorus, and potassium [3]. This intricate network of microbial activity, which includes processes like organic matter decomposition and nutrient transformation, is

powered by a vast and diverse community of bacteria, fungi, archaea, and protozoa. The sheer scale of this microscopic world is staggering; a single teaspoon of healthy topsoil is estimated to contain up to a billion individual microbial cells and thousands of different species [4]. The abundance and diversity of these soil-dwelling organisms are directly influenced by a range of factors, including soil organic matter content, nutrient availability, pH, and physical conditions such as porosity and aeration [5].

In contrast, aquatic environments support microbial communities with distinct ecological roles [6].

Microbes in water are critical for processes like bioremediation [7], where they degrade organic contaminants and pollutants by using them as energy sources. They can also serve as crucial indicators of water quality and potential contamination. For example, the presence of certain bacteria like

*Escherichia coli* or *Vibrio* species can signal potential public health risks [8]. While water ecosystems support significant microbial life, the unique physical and chemical properties of these environments, which are generally less nutrient-dense than soil, often result in a lower overall microbial load and a more specialized microbial community.

### B. The Dilution Plate Assay and Its Principles

The dilution plate assay is a cornerstone technique in microbiology for estimating the number of viable microorganisms in a sample [9]. The method's effectiveness stems from its core principle: a serially diluted sample is spread onto a solid growth medium, leading to the formation of distinct colonies from individual viable cells or cell aggregates. Each colony is counted as a Colony-Forming Unit (CFU), a proxy for the number of living microorganisms initially present in the diluted sample [10]. By multiplying the number of CFUs by the reciprocal of the total dilution factor and the volume plated, one can accurately estimate the microbial concentration in the original sample, typically expressed as CFU per gram (CFU/g) for solids or CFU per milliliter (CFU/ml) for liquids.

Despite its simplicity and statistical repeatability, the dilution plate assay has inherent limitations. It only provides a snapshot of the culturable fraction of the microbial community—a small subset of the total population [11]. The majority of microorganisms in a natural environment are not easily cultured under standard laboratory conditions due to complex growth requirements or a dormant state induced by nutrient limitation. Therefore, the results of this assay provide a comparative "index" rather than an absolute measure of the total microbial population.

### C. Study Objectives

The present study aimed to conduct a comparative analysis of the microbial loads in distinct soil and water samples using the dilution plate assay. The specific

objectives were:

- 1) to quantitatively compare the number of viable microorganisms in a soil sample and a water sample;
- 2) to qualitatively characterize the macroscopic and microscopic features of the predominant bacterial and fungal isolates from each sample;
- 3) to interpret the observed differences in microbial load and diversity within the broader context of microbial ecology and ecosystem function.

## II. Materials and Methods

### D. Sample Collection and Preparation

A soil sample was collected from roadside, and a water sample was obtained from a local pond. For the soil sample, 50 g of material was suspended in 100 ml of a sterile 10 mM phosphate buffer in a dilution bottle. The suspension was vigorously shaken for 30 minutes on a reciprocating shaker to dislodge and homogenize microorganisms, followed by a 15-minute settling period to allow heavier soil particles to sink. The water sample was processed directly, as per standard protocol for low-biomass liquid samples [9,10].

### E. Serial Dilution and Plating

A ten-fold serial dilution series was prepared for both the soil and water samples. This was achieved by transferring 1 ml of the initial suspension into 9 ml of sterile buffer, resulting in a

10<sup>-1</sup> dilution. This process was repeated sequentially to create subsequent dilutions up to 10<sup>-6</sup> for the soil sample and up to 10<sup>-3</sup> for the water sample.

To culture the isolated microorganisms: Nutrient agar medium was prepared by dissolving 14 g of nutrient agar powder in distilled water, followed by sterilization in an autoclave at 121 °C for 15 minutes. The sterile medium was then poured into Petri plates under aseptic conditions and allowed to solidify before inoculation. All platings were performed in triplicate to ensure data reliability and statistical reproducibility.

### F. Incubation and Colony Counting

All inoculated plates were incubated at 25°C for 48 to 72 hours, a temperature range that supports the growth of a wide variety of mesophilic microorganisms [1].

Following incubation, plates with a colony count between 30 and 300 were selected for CFU enumeration, as this range is considered statistically reliable for quantitative analysis. The CFU/g for soil and CFU/ml for water were calculated using the standard formula [13] :

$$\text{CFU/g (or mL)} = \frac{\text{Dilution Factor} \times \text{Volume Plated (mL)}}{\text{Number of Colonies}}$$

#### G. Morphological and Microscopic Analysis

Representative colonies were selected from the plates for further characterization. Macroscopic observations included an assessment of colony size, shape, color, texture (e.g., cottony, fuzzy, smooth, velvety), and elevation. For bacterial isolates, a Gram stain was performed to differentiate between Gram-positive and Gram-negative bacteria based on cell wall composition, and to observe cellular morphology and arrangement. Fungal isolates were mounted with lactophenol cotton blue stain and examined microscopically to visualize key structures such as hyphae, conidiophores, and sporangia, which are essential for genus-level identification.

### III Results

#### H. Quantitative Assessment of Microbial Load

The quantitative analysis of the plated samples demonstrated a significant difference in microbial load between the soil and water environments. The soil sample showed a considerably higher concentration of viable microorganisms. As summarized in Table 1, the bacterial load was estimated to be  $1.32 \times 10^6$  CFU/g, which falls within the range considered indicative of healthy soil [14]. Fungal enumeration yielded a count of  $3.5 \times 10^5$  CFU/g, a value consistent with typical microbial populations in soil. In stark contrast, the water sample showed a markedly lower microbial load, with a bacterial concentration of  $4.5 \times 10^3$  CFU/ml and a minimal fungal count of only  $1.0 \times 10^1$  CFU/ml. This more than thousand-fold difference in microbial concentration underscores the disparate ecological conditions of the two environments

Table 1: Microbial Load of Soil and Water Samples

Sample	Microorganism	Dilution Plated	Number of Colonies (Average CFU)	Calculated Microbial Load
Soil	Bacteria	10 <sup>-5</sup>	132	$1.32 \times 10^6$ CFU/g
	Fungi	10 <sup>-4</sup>	35	$3.5 \times 10^5$ CFU/g
Water	Bacteria	10 <sup>-2</sup>	45	$4.5 \times 10^3$ CFU/ml
	Fungi	10 <sup>-1</sup>	1	$1.0 \times 10^1$ CFU/ml

#### I. Qualitative Analysis of Colony Morphology Fungal Observations

The fungal isolates from the soil sample exhibited diverse and characteristic morphologies on SDA plates. The most prevalent colonies were characterized by their white, fuzzy, cotton-like appearance, which is a classic indicator of the rapid, mycelial growth of filamentous fungi. Based on these macroscopic features, these isolates were tentatively identified as members of the genera

*Rhizopus* and *Mucor*. Other colonies displayed a dense, velvety texture and were shades of grey or green, a morphology consistent with *Penicillium*. Additionally, some colonies showed a velvety texture with characteristic concentric rings and colors ranging from white to black, indicative of the genus

*Aspergillus*. The formation of concentric rings in fungal colonies reflects a pattern of radial growth and branching of hyphae, which expands outwards from the point of inoculation in a structured manner[15].

#### J. Bacterial Observations

Microscopic examination of the bacterial isolates from both samples revealed a variety of cellular morphologies and arrangements. From the soil sample, spherical, cocci-shaped bacteria were observed in irregular, grape-like clusters, a hallmark arrangement of *Staphylococcus* species. Rod-shaped bacteria, or bacilli, were also abundant, appearing either singly or in short chains, a common morphology for

*Bacillus* species. Furthermore, Gram-staining confirmed

the presence of Gram-negative bacteria with curved or spiral shapes, presumptively identified as

*Pseudomonas* or *Vibrio*-like organisms, both of

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Fig: 2  
 microbial

growth observed on nutrient agar extract medium

	<i>Pseudomonas</i> spp.	
<b>Fungi</b>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Rhizopus</i> , <i>Fusarium</i> , Yeasts	<i>Candida</i> , <i>Aspergillus</i>
<b>Other</b>	Actinomycetes, Protozoa	Algae, Algae

Soil samples showed higher microbial load compared to water samples due to rich organic matter and nutrient availability.

Fungal counts were lower than bacterial counts in both soil and water.

Fig: 3 microbial colonies formed on boiled milk agar extract medium

Fig:1 soil extract agar promotes diverse microbial growth.



Fig: 4 microbial colonies formed on milk agar extract medium



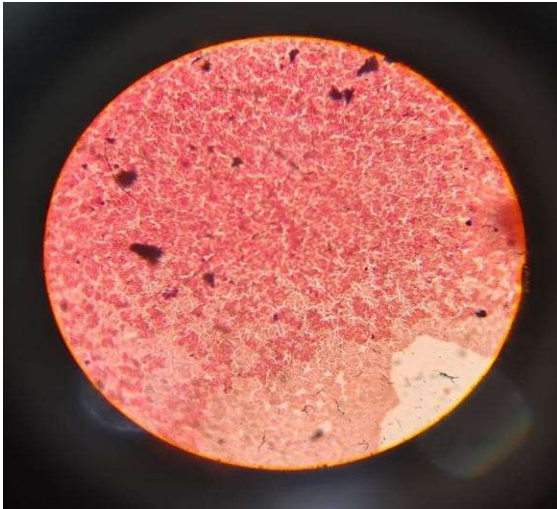


Fig: 6 mixed culture showing bacillus subtilis and aspergillus niger

Fig:7 showing presence of e.coli and candida albicans indicating contamination



Fig: 8 comparative microbial growth on unboiled and boiled agar media with different extracts.

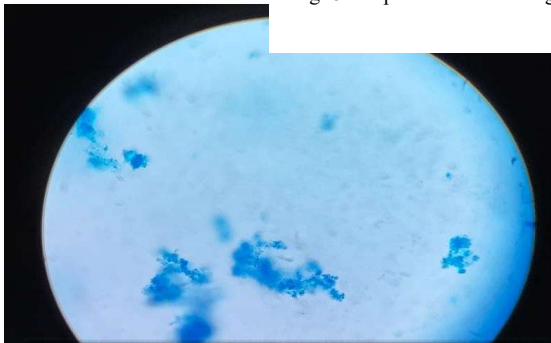


Fig: hypa



between the soil and water samples. The soil sample demonstrated a microbial concentration that was over three orders of magnitude higher than that of the water sample. This profound difference is a direct result of the which al niche geneous nt, soil surface organic source. ular, is a well-documented determinant of microbial biomass and activity. The fact that the soil sample in this study

supported a richer variety of fungi, including *Aspergillus* and *Penicillium*, and a greater presence of filamentous actinomycetes, further supports the hypothesis that these organisms thrive in the terrestrial, organic-rich habitats that soil provides.

#### M. Ecological Functions of Identified Microbes

The diverse array of microorganisms isolated from the soil and water samples play critical and distinct ecological roles. The filamentous fungi observed in the soil, such as *Aspergillus* and *Rhizopus*, are quintessential decomposers. They are masters at breaking down complex organic macromolecules like cellulose and lignin into simpler components that other organisms can then metabolize, thereby driving the flow of nutrients through the soil ecosystem [17]. The bacterial population, including genera like

*Bacillus* and *Pseudomonas*, is central to nutrient cycling, particularly the intricate nitrogen cycle [18].

Nitrogen-fixing bacteria convert atmospheric nitrogen into bioavailable forms for plants, while nitrifying and denitrifying bacteria facilitate the transformation of nitrogen compounds, ensuring the nutrient remains in a usable state within the soil.

The presence of actinomycetes, especially in the soil, is of particular significance. These filamentous bacteria are prolific producers of secondary metabolites and are the source of two-thirds of all known antibiotics used in clinical medicine [19]. The production of these compounds is not a random byproduct of metabolism but rather a sophisticated evolutionary strategy for survival in the highly competitive and densely populated soil environment. With billions of microbes competing for limited resources, the ability to produce antimicrobial compounds provides a distinct advantage, allowing these organisms to suppress the growth of competitors and secure their place in the community.

In the aquatic environment, the observed microbes, though fewer in number, are specialized for their habitat. The presence of genera like *Pseudomonas* and *Vibrio* is noteworthy, as many aquatic bacteria are key players in bioremediation [7]. They have the metabolic flexibility to degrade a wide range of organic and inorganic contaminants, either by utilizing them as a primary

energy source or by co-metabolizing them as a side reaction. The observation of algae, for example, is also ecologically important. While algae are crucial primary producers, their presence, especially when coupled with excess nutrients from agricultural or urban runoff, can lead to eutrophication and harmful algal blooms [20]. These blooms can rapidly consume dissolved oxygen upon their death, leading to a high biological oxygen demand (BOD) that creates hypoxic or "dead zones," which are lethal to aquatic life.

#### N. Methodological Limitations

While this study effectively demonstrated the significant differences in microbial load and diversity between soil and water samples, it is crucial to acknowledge the inherent limitations of the dilution plate assay. This method, by its very nature, can only account for a small fraction of the total microbial community—specifically, those microorganisms that are both viable and capable of growth under the specific laboratory conditions provided. The majority of microbes in a natural setting are non-culturable and may exist in a state of dormancy due to nutrient scarcity or environmental stress [11]. Consequently, the CFU counts presented here represent a valuable index for comparison but do not reflect the complete microbial biomass or the true extent of biodiversity in either environment. The microbial load and diversity are in fact far greater than what was quantitatively measured in this experiment. This limitation highlights the need for a more holistic approach to microbial ecology that moves beyond simple culturing techniques.

#### Conclusion

The results of this study unequivocally demonstrate that the soil sample contained a significantly higher microbial load and a greater diversity of viable culturable microorganisms than the water sample. This finding is a strong validation of the fundamental principles of microbial ecology which hold that soil's complex nutrient-rich matrix and provides a more fertile ground for microbial proliferation than a more transient aquatic environment. The dilution plate assay while providing a limited view

of the total microbial population also proved to be an effective tool for a preliminary and comparative assessment.

For future studies it is imperative to integrate more advanced methodologies to overcome the limitations of traditional culture-based assays. The use of molecular techniques such as quantitative Polymerase Chain Reaction (qPCR) and metagenomic sequencing would provide a more accurate and comprehensive assessment of total microbial biomass and the full genetic diversity of the community; including both culturable and non-culturable organisms [21]. Furthermore - expanding the study to include a wider range of soil and water types as well as a more detailed analysis of environmental variables like pH, temperature, and nutrient profiles, would provide a more nuanced understanding of the factors that shape these crucial microbial ecosystems.

### References

1. Editorial: Microbial involvement in biogeochemical cycling and contaminant transformations at land-water ecotones, volume II - PMC, <https://pmc.ncbi.nlm.nih.gov/articles/PMC12907369/>
2. Ecological Role of Bacteria Involved in the Biogeochemical Cycles of Mangroves Based on Functional Genes Detected through GeoChip 5.0 | mSphere - ASM Journals, <https://journals.asm.org/doi/10.1128/msphere.00936-21>
3. Editorial: Microbial involvement in biogeochemical cycling and contaminant transformations at land-water ecotones - Frontiers, <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2024.1525521/full>
4. Systematic Review on the Role of Microbial Activities on Nutrient ..., <https://www.biorxiv.org/content/10.1101/2024.09.02.610905v1.full-text>
5. Ecological Perspectives on Microbes Involved in N-Cycling - PMC, <https://pmc.ncbi.nlm.nih.gov/articles/PMC4041230/>
6. The Potential Use of Actinomycetes as Microbial Inoculants and Biopesticides in Agriculture, <https://www.frontiersin.org/journals/soil-science/articles/10.3389/fsoil.2022.833181/full>
7. Use of soil actinomycetes for pharmaceutical, food, agricultural, and ..., <https://pmc.ncbi.nlm.nih.gov/articles/PMC9391553/>
8. Diverse Metabolic Capacities of Fungi for Bioremediation - PMC - NIH, <https://pmc.ncbi.nlm.nih.gov/articles/PMC4920763/>
9. Bacteria as Indicators of Environmental Pollution: Review - Scientific & Academic Publishing, <http://article.sapub.org/10.5923.j.ije.20140406.03.html>
10. Microbial Indicators and Their Use for Monitoring Drinking Water ..., <https://www.mdpi.com/2071-1050/12/6/2249>
11. High-Throughput Methods for Culturing Microorganisms in Very-Low ..., <https://pmc.ncbi.nlm.nih.gov/articles/PMC124033/>
12. The Effects: Dead Zones and Harmful Algal Blooms | US EPA, <https://www.epa.gov/nutrientpollution/effects-dead-zones-and-harmful-algal-blooms>
13. APHA Standard, Methods for Examination of Water and Waste Water, 16th Edition, American Public Health Association, Washington, DC, 1985. - References - Scientific Research Publishing - Scirp.org., <https://www.scirp.org/reference/ReferencesPapers?ReferenceID=373727>
14. Maximum likelihood estimators for colony-forming units - PMC - NIH, <https://pmc.ncbi.nlm.nih.gov/articles/PMC11371269/>
15. Serial Dilution | The Method - Ossila, <https://www.ossila.com/pages/serial-dilution>
16. Serial Dilution Problem Help - University of Vermont, <https://www.uvm.edu/~btessman/calc/serhelp.html>
17. Serial Dilution | Definition, Purpose & Calculation - Lesson - Study.com, <https://study.com/academy/lesson/serial-dilution-in-microbiology-calculation-method-technique.html>
18. What is the "Great Plate Count Anomaly," and does it mean microbes, in general, are considered colonial organisms? : r/microbiology - Reddit, <https://www.reddit.com/r/microbiology/>

[https://www.reddit.com/r/microbiology/comments/1p27ovy/what\\_is\\_the\\_great\\_plate\\_count\\_anomaly\\_and\\_does\\_it/](https://www.reddit.com/r/microbiology/comments/1p27ovy/what_is_the_great_plate_count_anomaly_and_does_it/)

19. The Great Plate Count Anomaly and the Unculturable Bacteria - ResearchGate, [https://www.researchgate.net/publication/274930416\\_The\\_Great\\_Plate\\_Count\\_Anomaly\\_and\\_the\\_Unculturable\\_Bacteria](https://www.researchgate.net/publication/274930416_The_Great_Plate_Count_Anomaly_and_the_Unculturable_Bacteria)
20. What is the "Great Plate Count Anomaly," and does it mean microbes, in general, are considered colonial organisms? : r/AskBiology - Reddit, [https://www.reddit.com/r/AskBiology/comments/1oxtgx5/what\\_is\\_the\\_great\\_plate\\_count\\_anomaly\\_and\\_does\\_it/](https://www.reddit.com/r/AskBiology/comments/1oxtgx5/what_is_the_great_plate_count_anomaly_and_does_it/)
21. Unraveling The Great Plate Count Anomaly by Using an Isolation Chip to Culture 'Unculturable' Organisms | Directory of scientists and professionals - Science.gc.ca Profiles