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Research Article

Isolation of Microbes from Landfill Soil of Gorakhpur, Uttar Pradesh, India

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Abstract

This study focuses on the isolation and characterization of microbes from landfill soil in Gorakhpur, India. Landfills are a major source of soil pollution, and understanding the microbial composition in these environments is crucial for assessing soil quality and developing remediation strategies. Microorganisms can degrade numerous of organic pollutants owing to their metabolic machinery and to their capacity to adapt to inhospitable environment. Thus, microorganisms are major players in site remediation. However, their efficiency depends on many factors, including the chemical nature and the concentration of pollutants, their availability to microorganisms, and the physio-chemical characteristics of the environment. The research identified a diverse microbial community in the landfill soil, with predominant bacterial representatives including Gamma-proteobacteria, firmicutes, and bacteroids. Gram staining revealed the prevalence of gram-positive bacilli, along with distinct fungal species. These findings emphasize the potential of microorganisms in degrading organic pollutants and transforming various compounds in landfill soil. By elucidating the microbial diversity in landfill sites, this study provides insights for sustainable waste management practices and environmental conservation efforts. One of the major cause of soil pollution are landfills. There are the sites designated for dumping rubbish, garbage, or other sorts of solid wastes. Because most of these waste materials are non-biodegradable, they heap in the landfills where they stay for years, impacting on soil quality and polluting the land. The aim of this study is to isolate and investigate the role of microorganisms in a particular landfill area of Gorakhpur, Uttar Pradesh and to identify the microbial community found in that particular area.

Keywords

Isolation, Site Remediation, Landfill Sites, Pollutants, Microbial Community

1. Introduction

Soil is nature's gift to mankind let us nurture it. The soil is the upper weathered and humus containing layer of the earth's crust which sustains plant life and contain numerous living organisms and their dead remains [1]. Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates as well as plants and algae. The soil performs a variety of key functions. They Provides food, fuel, and fiber needs of the world's population. Regulates the quality of the air and water. Provides food, fuel, and fiber needs of the world's

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population. Decomposes organic wastes. Recycles nutrients, and Acts as a sink for pollutants.

The soil is a complex environment colonized by an immense diversity of microorganisms [2]. Soil microbiology focuses on the soil viruses, bacteria, actinomycetes, fungi, algae and protozoa [3]. The surface layers of soil contains the highest number and variety of microorganisms because these layers receive the largest amounts of potential food sources from plants and animals. Microorganisms are found in large number in soil. Usually between 1to 10 million microorganisms are present per gram of soil- with bacteria and fungi being the most prevalent [4]. However, the availability of nutrients is often limiting for microbial growth in soil and most soil microorganisms may not be physiologically active in the soil at a given time. The microflora of a soil is an intimate part of the soil organic matter; in fact much of the colloidal portion of humus consists of living and dead microbial cells or their disintegrating residues. Different groups of organisms, other than viruses, constitute the microflora population of soil [5].

1. Soil Bacteria- They are the smallest and most abundant group, the number of which varies between 10^8 and 10^{10} cells per gram soil [6]. Example of some soil bacteria is *Agrobacterium, Arthrobacter, Bacillus, Alcaligens, Clostridium, Corynebacterium, Erwinia, Nitrosomonas, Nitrobacter, Pseudomonas, Rhizobium, Thiobacillus* etc. [7].

2. Soil Fungi- Fungi share a major part of total microbial biomass in most of aerated or cultivated soils because of their large diameter and extensive network of mycelium. However, population of soil fungi ranges from 2×10^4 to 1×10^6 propagules per gram dry soil and its number differs according to isolation procedure and composition of media. Example of some soil fungi is *Alternaria, Aspergillus, Cladosporium, Phytopthora, Sclerotium,* etc.

3. Soil Algae- Soil algae (both prokaryotes and eukaryotes) luxuriantly grow where adequate amount of moisture and light are present. The prominent genera are *Anabaena, Calothrix, Oscillatoria, Nostoc, Scytonema,* etc.

4. Actinomycetes- Population of actinomycetes in soil remains greater in grassland and pasture soil than in cultivated land. In temperate zone the number of actinomycetes ranges from 10^5 to 10^8 per gram soil. The important members of actinomycetes are: *Actinomyces, Actinoplanes, Micromonospora, Nocardia, Streptomyces,* etc.

5. Protozoa- About 1600 species of protozoa have been recorded from soil so far. Depending on soil, the number of protozoa varies from 10,000–10,00,000 individuals per gram dry mass of soil. Soil protozoa include naked amebae, testate amebae, flagellates, ciliates, microsporidia, and sporozoans.

Microbial population and their frequency can vary according to the environment in which they are growing [8]. So to understand and estimate the types of microbes found in any particular area, their isolation must be performed preceding their detection.

Isolation of soil microflora:

Microbial isolation from the soil is the basic need to study the quality of soil, composition of microorganisms in it and their impact on soil's nutrient level. With the help of identification of microbial communities found in any particular area of soil, it can be studied that how the population and community residing in that particular area affects the nature of soil including its nutrient level, level of pollutants, whether the soil is good for agricultural use or not, etc [9].

There has been a rapid rise in the soil pollution over the last two decades caused by both natural and anthropogenic activities. All soils, whether polluted or unpolluted contain a variety of compounds (contaminants) which are naturally present [10]. Such contaminants include metals, inorganic ions and salts (e. g. phosphates, carbonates, sulfates, nitrates), and many organic compounds (such as lipids, proteins, DNA, fatty acids, hydrocarbons, PAHs, alcohols, etc.). These compounds are mainly formed through soil microbial activity and decomposition of organisms (e. g., plants and animals). When the amounts of soil contaminants exceed natural levels, pollution is generated. Some microorganisms have the astonishing naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e. g. oil), polyaromatic hydrocarbons (PAHs), radionuclides and metals.

Microorganisms can degrade numerous of organic pollutants owing to their metabolic machinery and to their capacity to adapt to inhospitable environment [11]. Thus, microorganisms are major players in site remediation. However, their efficiency depends on many factors, including the chemical nature and the concentration of pollutants, their availability to microorganisms, and the physiochemical characteristics of the environment [12]. One of the major cause of soil pollution are landfills [13]. There are the sites designated for dumping rubbish, garbage, or other sorts of solid wastes [14]. Because most of these waste materials are non-biodegradable, they heap in the landfills where they stay for years, impacting on soil quality and polluting the land [15]. The aim of this study is to isolate and investigate the role of microorganisms in a particular landfill area of Gorakhpur, Uttar Pradesh and to identify the microbial community found in that particular area.

2. Materials and Methodology

2.1. Sample Collection

Having made the site visit in the month of April (2022) for the purpose of research, from landfill site of Gorakhpur (Uttar Pradesh, 330C, 26.70N, 83.30E). The morphological composition of the landfill includes mixed waste comprised debris glass, plastics, metals, textiles, household wastes, residue from garden maintenance, etc. Collected the soil sample after removing the waste materials from the surface. Sample was taken from 15-20 cm depth with the help of stick and was brought to the laboratory in sterile plastic bag and stored at

room temperature for microbial study.

2.2. Cultivation of Microbes

Microbial analysis of soil was done by media preparation, serial dilution, streaking and spreading method. Further identification was performed by gram's staining followed by microscopic examination.

2.3. Media Preparation

The media used were Luria Bertani (LB) and Potato Dextrose Agar (PDA).

Preparation of LB (Luria Bertani) Media: (400ml) 400ml of distilled water was taken in a conical flask. 8g of LB (Luria Bertani) broth was weighed. Stirred properly to dissolve the content. 6g of agar was weighed and added to it. Then it was autoclaved for 15 minutes at 121 ℃ at 15 lbs pressure. Preparation of PDA (Potato Dextrose Agar) media. 0 *after spreading.*

2.4. Pure Culturing Results

A. Pure culture obtained from LB streak plates shows 4 different colonies with different morphologies.

Table 1. *Colonies obtained after pure culturing.*

No. of colonies	Colour	Texture
Colony 1	Orange	Moist
Colony 2	Diffused white	Mucoid
Colony 3	Pure white	Dry
Colony 4	Yellow	Moist

Figure 1. *Pure cultures on LB Agar plates.*

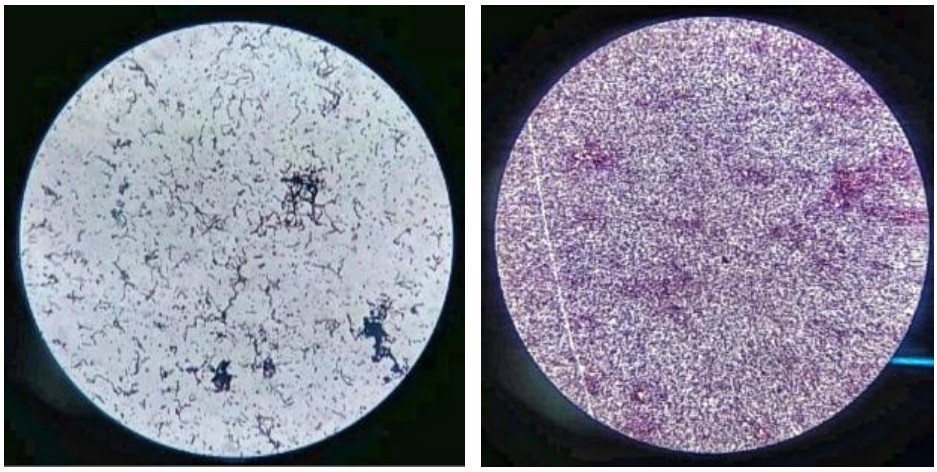
B. Pure culture obtained from PDA spread plates shows two different bacterial colonies and two identical fungal colonies.

Figure 2. *Pure culture on PDA Agar plates.*

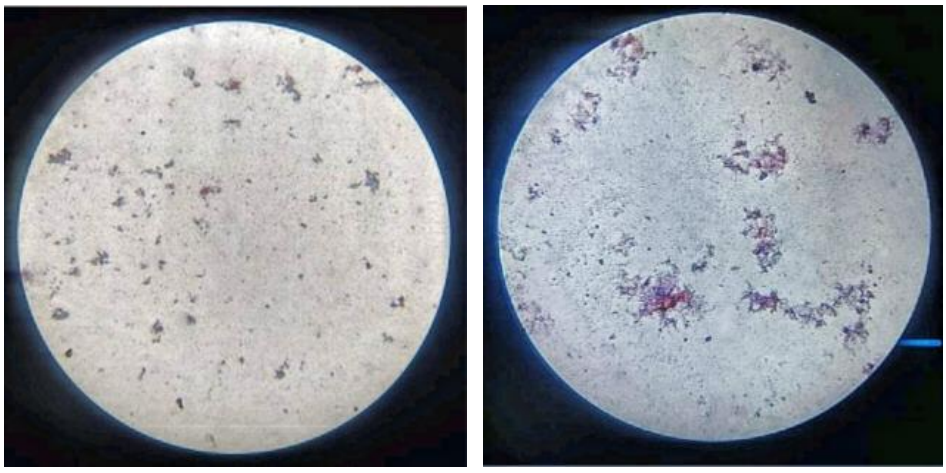
C. Observation and results of Gram's staining:

Table 2. *Slides obtained after Gram's staining.*

S. No.	Colour of bacterial colony	Shape observed	Appearance	Gram's class
1	Orange	Bacilli	Purple	Gram positive
2	White	Irregular	Purple	Gram positive
3	Diffused white	Spherical	Purple	Gram positive
4	Yellow	Irregular	Purple	Gram positive



A. Yellow colony B. Orange Colony



C. Diffused white colony D. White colony

Figure 3. *Showing microscopic view of Gram's stain slides of different pure colonies (A, B, C, D).*

D. Result and observation of Antibiotics Susceptibility test:
a) Agar well diffusion method-
Observed the zone of inhibition around the wells of culture spread plates.

Table 3. *Showing antibiotics and their zone of inhibition.*

S. No.	Selected antibiotics	Antibiotic conc.	Bacterial culture conc.	Zone of inhibition
1.	Streptomycin	5mg/ml	0.1ml	2.6cm
2.	Cefotaxime	5mg/ml	0.1ml	3.5cm
3.	Ceftriaxone	5mg/ml	0.1ml	3.1cm
4.	Distilled water	_____	0.1ml	0.0cm

Result- Highest zone of inhibition is observed around Cefotaxime antibiotic suggesting that the bacterial culture is more susceptible to it as compared to other two.

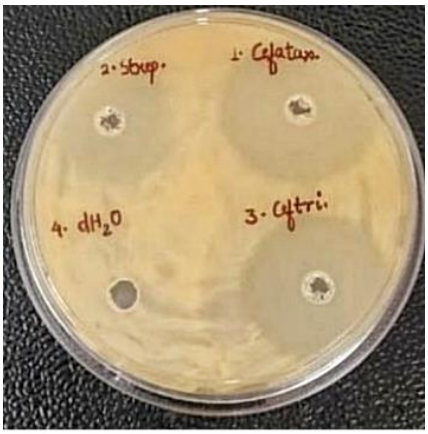


Figure 4. *Showing Zone of inhibition of different antibiotics against bacterial culture.*

Observed the zone of inhibition by using different concentration of Cefotaxime antibiotics.

Table 4. *Cefotaxime zone of inhibition at different concentrations.*

S. no.	Selected antibiotics	Antibiotic conc.	Bacterial culture conc.	Zone of inhibition
1.	Cefotaxime	5mg/ml	0.1ml	3.50cm
2.	Cefotaxime	2.5mg/ml	0.1ml	3.30cm
3.	Cefotaxime	1.25mg/ml	0.1ml	2.90cm
4.	Distilled water	_____	0.1ml	0.00cm

Result- Highest zone of inhibition is observed for 5mg/ml Cefotaxime concentration and lowest is observed for 1.25mg/ml Cefotaxime concentration.

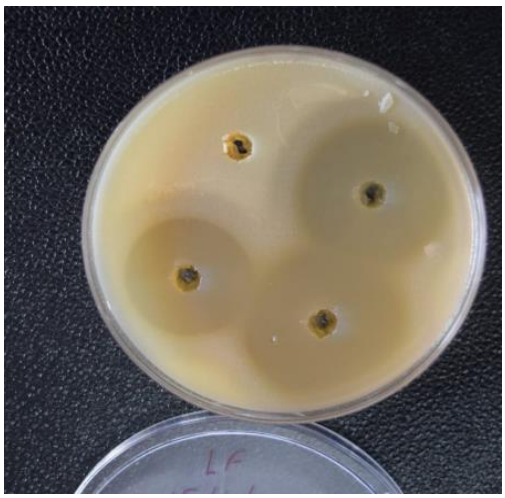


Figure 5. *Showing zone of inhibition at different concentration of Cefotaxime against bacterial culture.*

b) Agar disc diffusion method

Different concentration of Cefotaxime and Streptomycin are used and observed the zone of inhibition.

Table 5. Zone of inhibition obtained with disc diffusion method.

S. no.	Selected antibiotics	Antibiotic conc.	Bacterial culture conc.	Zone of inhibition
1.	Streptomycin	5mg/ml	0.1ml	3.5cm
2.	Cefotaxime	2.5mg/ml	0.1ml	3.2cm
3.	Distilled water	_____	0.1ml	0.0cm

Result- Streptomycin on doubling it's concentration gives slightly larger zone of inhibition as compared to Cefotaxime which was used half the concentration of Streptomycin.

3. Result and Discussion

Overall, the results of the study provide valuable insights into the microbial composition of landfill soil in Gorakhpur, emphasizing the importance of understanding the diversity and characteristics of microorganisms in such environments. These findings can inform future research on bioremediation strategies, waste management practices, and environmental conservation efforts in landfill sites. The key finding of the paper includes.

Microbial Diversity: The study revealed a diverse microbial community present in the landfill soil, with various bacterial colonies isolated using different methods. These colonies exhibited different morphology, including circular, irregular margins, bacilli, and spherical shapes.

Gram Staining: Upon subjecting the isolated bacterial colonies to gram staining and microscopic examination, the predominant strains were identified as gram-positive bacilli. This suggests the prevalence of certain bacterial species with specific characteristics in the landfill soil.

Bacterial Representatives: Regardless of the geographic area, the most predominant bacterial representatives in the landfill soil included Gamma-proteobacteria, firmicutes, and bacteroids. This highlights the presence of specific bacterial groups that play a significant role in the microbial composition of the landfill soil.

Fungal Species: In addition to bacterial colonies, the study also identified distinct fungal species in the landfill soil. This indicates the presence of a diverse microbial community comprising both bacteria and fungi, contributing to the overall ecosystem dynamics of the landfill site.

4. Conclusion

In conclusion, the study highlights the significant role of microbial communities in landfill soil remediation. By isolating and identifying various bacterial strains from the landfill site in Gorakhpur, we have observed a diverse array of microbial species, with predominant representatives including Gamma-proteobacteria, firmicutes, and bacteroids. These findings underscore the potential of microorganisms in degrading organic pollutants and transforming a wide range of compounds present in landfill soil, such as hydrocarbons, polyaromatic hydrocarbons, radionuclides, and metals. Understanding the microbial composition of landfill soil not only aids in assessing soil quality but also provides valuable insights for developing strategies to mitigate soil pollution and enhance environmental sustainability. Moving forward, further research on the metabolic capabilities of these microbial communities and their interactions within the landfill ecosystem could pave the way for innovative bioremediation approaches and sustainable waste management practices in landfill sites.

Abbreviations

PAH	Polyaromatic Hydrocarbons
DNA	Deoxyribonucleic Acid
PDA	Potato Dextrose Agar
LB	Luria Bertani

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Author Contributions

The first author Shalini Singh has done experimental work, the second author Dr. Deepa Srivastava has contributed in writing and editing manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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