Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; Sp 10(3): 17-20
Received: 13-03-2021
Accepted: 15-04-2021

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Comparative study of soil microflora of different ecosystem in Meerut region

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Abstract
Diversity in soil microflora directly affects plant growth and development by providing it with sufficient mineral nutrients. In the present study for comparative examination of plant performance in different ecosystems, soil samples were collected from four different locations within Meerut and labelled according to the site of collections as CAEHS (urban area), Sakoti (industrial area), Garh road (polluted area), and Samaypur (rural area). The total bacterial and fungal counts of the soil samples were estimated using standard spread plate technique. A pH strips was used to monitor soil pH while isolates were identified by their cultural, morphological and biochemical characteristics using established procedures. Bacterial counts were in the order of 10^3-10^7 cfu/gm of soil, while fungal counts were in the order of 10^3-10^7 cfu/gm of soil. The highest bacterial count was observed in Sakoti (industrial area), while the lowest value was seen in CAEHS (urban area). Similar bacterial and fungal species were encountered in the different sampling location in course of this study, but their occurrences and level of predominance were different. Spore forming bacilli species dominated the bacterial isolates while Aspergillus spp was the most dominate fungus across the different sampling locations. A near neutral pH was observed across the sampling sites. Bacterial and fungal abundance were typical of an environment with high species richness and functional diversity.

Keywords: bacteria, ecosystem, fungi, plant performance, top soil

Introduction
Soil plays a vital role in growth and development of the plant, also provides shelter and nutrients. Soil particles and microbes present in soil influences the growth of plant. Soil is the region on the earth's crust where geology and biology meet, the land surface that provide a home to plant, animal, and microbial life (Pelczar *et al.*, 1993) [5]. Soil contains microscopic/microflora such as bacteria, fungi, algae, protozoa, and viruses as well as macroscopic/macroflora such as earth worm, nematodes, mites, and insects, apart from this root systems of plants. There are variety of microorganism present in soil depends on different types of environmental factors: type and amount of nutrients available, moisture content, degree of aeration, temperature, pH etc. (Prescott *et al.,* 1999) [6]. The presence of Bacterial and fungal isolates available in soil plays a vital role in various biochemical pathways responsible for the recycling of organic compounds (Wall and Virginia, 1998) [11]. Microorganism also influence above ground ecosystem by contributing to plant health, plant nutrients, soil structure, and soil fertility (O'Donnell *et al.,* 2012) [4]. The highest number of microorganism were present in soil habitat in comparison to fresh water and marine habitats (Atals and Bartha, 1998) [1]. Fungi is present as free living organism or in mycorrhizal association with plant roots under the soil at depth of approx 10 cm from top and 30cm below. Microbial activities boost plant growth by manipulating the hormonal signaling of plants (Verbon and Liberman 2016) [10], by repulsion of pathogenic microbial strains (Mendes *et al.,* 2013) [3] and by increasing the bioavailability of soil-borne nutrients (Van der Heijden *et al.,* 2008) [9]. Soil microbes also metabolize recalcitrant forms of soil-borne nutrients to liberate these elements for plant nutrition. In natural ecosystems, most nutrients such as N, P, and S are bound in organic molecules and are therefore minimally bioavailable for plants. In order to access these nutrients, plants are dependent on the growth of soil microbes such as bacteria and fungi, which possess the metabolic machinery to depolymerize and mineralize organic forms of N, P, and S. The contents of these microbial cells are subsequently released, either through turnover and cell lysis, or via protozoic predation (Bonkowski, 2004; Richardson *et al*., 2009) [2]. This liberates inorganic N, P, and S forms into the soil, including ionic species such as ammonium, nitrate, phosphate, and sulfate that are the preferred nutrient forms for plants (Van der Heijden *et al*., 2008) [9].

In natural settings, these microbial nutrient transformations are key drivers of plant growth, and can sometimes be the rate-limiting step in ecosystem productivity (Schimel and Bennett, 2004) [8]. The objective of this study is to find the comparison of soil microflora available in different ecosystem of Meerut Region.

Materials and Methods: In the present work, we isolated heterotrophic bacteria and fungi from top soil sample collected from four different Meerut regions i.e. CAEHS, Sakoti, Garh road, Samaypur with a view to understanding the microbial flora that are found in the different top soil of this community.

Collection of Sample

The soil sample used for this work were collected from four different locations in Meerut region. The samples were labelled according to the site of collection as CAEHS (urban area), Sakoti (Industrial Area), Garh Road (Polluted Area), and Samaypur (Rural Area). Randomly located at distance of 5m X 5m were used to attain four sub samples of the topsoil (7.5 cm depth) using an auger of 8.5cm diameter.

Determination of Soil pH (Method)

Determination of Soil pH is only essential for examining the fertility of soil. Soil pH play a major role in the growth of plants. It is the major parameter to be examined for evaluating soil functioning beyond plant nutrient supply as it maintains soil properties to support plant growth and their biochemical processes Soil pH is controlled by the leaching actions of Ca, Mg, K, & Na. For the measurement of pH, 1 gram of soil was dissolved in 1 ml distilled water and measured with help of pH meter.

Serial Dilution of Soil Samples and culturing

The soil samples were collected in a sterilized container from different locations. The soil samples were serially diluted by taking 10 test tubes labelled from 0-9 containing 9 mL autoclaved distill water. 1 gram of soil sample was dissolved in 1 mL of autoclaved distilled water in separate test tube from which 1mL was transferred to test tube with dilution factor 10^{-1} and so on. 100µL sample from dilution factor 10^{-3},10^{-4}, 10^{-5} were spreaded on NAM (nutrient media agar) plates for the growth of bacteria and incubated at 37 °C for 24 hours and for fungal growth the sample was spreaded on PDA (Potato dextrose agar) and incubated at 28 °C for 48 hours.

Sub-Culturing of Isolates

After 24 hours and 48 hours of incubation for bacteria and fungi subculturing of obtained colonies was accomplished by picking up the colonies with help of sterile loop and streaking on NAM and PDA respectively and incubated in the same manner.

Colony Counting

After incubation period, the obtained colonies on the plates of bacteria and fungi were counted on colony counter. And the data was recorded.

Morphological Characterization

For morphological characterization two staining were performed for identification of bacteria and fungi.

A. Gram's staining for bacteria: Dr. Hans Christian gram's developed gram's staining in 1884 on the basis of colour. In this staining, the bacteria are classified into two groups on the basis of colour that bacteria are gram positive (purple color) and gram negative (pink color). We used four reagent in this staining such as crystal violet (primary stain), gram's iodine (mordent dye), decolorizer, and safranin (counter stain). A smear was made on a clean slide and heat fixed, 1-2 drop of crystal violet for 60 seconds were added and washed by distilled water dropwise. Then 1-2 drop of gram's iodine for 60 seconds were added and washed by distilled water. Decolorizer or ethanol for 20-30 seconds were added and washed by distilled water. Then Safranin was added for 60 seconds and washed. Extra water was removed by tissue paper and left the slide to dry. Examine the slide under microscope at 100X.

B. Cotton blue staining for Fungi: In lactophenol cotton blue staining, we identify the structure and spore of fungus. On a clean sterile glass slide 1-2 drops of lacto phenol cotton blue solution was taken and the fungal specimen using the sterile needle was added and covered carefully with a clean sterile coverslip without making air bubbles to the stain. The slide was examined at 45X to observe for fungal spores and other fungal structure.

Result

Collection of Sample

The soil sample used for this work were collected from four different locations in Meerut region

Table 1: Location of Soil Samples

S. No.	Region	Area
1.	CAEHS	Urban area
2.	Sakoti	Industrial area
3.	Garh road	Polluted area
4.	Samaypur	Rural area

Determination of Soil pH

The pH values ranged from 6.80-7.58. The soil pH in Sakoti was higher than Garh road and Samaypur. However, difference in these soil pH values of the different sampling location were not observed to be statistically significant.

Serial Dilution of Soil Samples and culturing

After serial dilution of soil sample, the spreaded plates were incubated for the growth of bacteria at 37°C for 24 hours and for fungal growth the plates were incubated at 28°C for 48 hours. Good bacterial and fungal growth was obtained on plates that were processed for further subculturing.

Subculturing

Sub cultured plates were subjected for colony counting.

Colony Counting

After incubation period, the obtained colonies on the plates of bacteria and fungi were counted on colony counter. And the data was recorded. (Table 5)

Table 2: Colony count of different location samples

S. No.	Different sample locations	Bacterial colony	Fungus colony
1.	**CAEHS**		
	3	3×10'3	3×10'3
	5	2×10'5	2×10'5
	7	11×10'7	2×10'7
2.	**Samaypur (Rural Area)**		
	3	29×10'3	3×10'3
	5	5×10'5	2×10'5
	7	6×10'7	1×10'7
3.	**Sakoti (Industrial Area)**		
	3	11×10'3	4×10'3
	5	4×10'5	1×10'5
	7	32×10'7	4×10'7
4.	**Sisoli (Polluted Area)**		
	3	3×10'3	4×10'3
	5	23×10'5	2×10'5
	7	--	2×10'7

Total bacterial count
The mean total bacterial count (TBC) of each soil sample ranged from colony forming units (CFU) per gram of soil. Although there were difference in the averages total bacterial counts of the different sampling locations, these differences were not statistically significant. However, highest counts were observed in Sakoti, and lowest counts observed in CAEHS.

Total fungal counts
The mean total fungal counts (TFC) of each soil sample ranged from highest count were observed in Sakoti, lowest counts were observed in Samaypur. Differences in the average total fungal counts of the sampling locations were not statistically significant.

Morphological Characterization

Table 3a: Gram's staining for bacteria

S. No.	Locations	Gram staining	Species	Morphology (in broth)	Morphology (in solid media)
1.	Urban area	+	*Baccilu s* and *coccus*	Pellicle	smooth
2.	Industrial area	+	*Baccilus, coccus* and spore forming bacteria	Pellicle and diffused	Smooth
3.	Rural area	+	*Baccilus* and spore forming bacteria	Pellicle and diffused	Undulate
4.	Polluted area	+	*Baccilus*	Pellicle	smooth

Table 3b: Cotton blue staining for fungi

S. No.	Locations	Fungal Staining	Morphology	Suspected organism
1.	Urban area	Lactophenol staining	Conidiophores, conidia, globular vesicle observed	*Aspergillus sp.*
2.	Industrial area	Lactophenol staining	Conidiophores, conidia, globular vesicle observed	*Aspergillus sp.*
3.	Rural area	Lactophenol staining	Sporangium and sporangiospores with rhizoids were observed	*Rhizopus sp.*
4.	Polluted area	Lactophenol staining	Conidiophores, conidia, globular vesicle observed	*Aspergillus sp.*

Conclusion
The metabolic activities of soil microbiota increases the access of soil-borne nutrients for better plant growth and development. In the present study, we evaluated soil sample from different areas of Meerut region for assessment of microbiota. Soil is the major determinant of the microbial community associated with plant roots.
Microbial community composition facilitates in dissecting mechanisms involved in construction of nutritional network and supporting the better plant growth. Similar bacterial and fungal species were encountered in the different sampling location in course of this study, but their occurrences and level of predominance were different. Spore forming *bacilli* species dominated the bacterial isolates while *Aspergillus* species was the most dominating fungus across the different sampling locations. This study will help in determining the plant growth performance in soil of different ecosystem within the same region.

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