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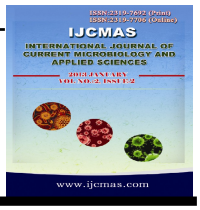

Original Research Article

A study on bacterial and fungal diversity in potted soil

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Soil microorganisms; Potted soils; Enumeration of soil; Microbiota of soil.

A B S T R A C T

Soil microorganisms play an important role in soil quality and plant productivity. The development of effective methods for Studying the diversity, distribution, and behavior of microorganisms in soil habitats is essential for a broader understanding of soil health. The present work aims to critically evaluate some central methods and procedures utilized in microbial ecological studies of pot soils in order to improve our understanding of the factors that affect the measurement results and to provide support for the design of experiments. Excluding plant roots, the majority of soil biomass is formed by microorganisms, the two largest groups being prokaryotic bacteria and eukaryotic fungi. In potted soil bacterial (including actinomycetes) and fungal biomass is estimated to be 8-10 and 6-10 species, respectively. Potted soils are a collective form of various kinds of soil with each their own composition and specific structure, especially for plants in pots and flower boxes. Potting soil is light in weight, unlike for example the heavier dark garden soil. Temperature and pH varies in potted soils. The highest plate count of potted soil sample was bacteria such has *Bacillus, Pseudomonas* was dominating and fungai such as *Cladosporium, A.flavs* were dominating of samples which were incubated at 20°C. The diversity of the total bacterial and fungal community was *Stretococcus, Actinomycets and S.pyogenes, S.coelicolor*. Progressively lower counts were obtained in fungi.

Introduction

Soil bacteria and fungi play pivotal roles in various biogeochemical cycles (BGC) (Molin and Molin, 1997; Trevors, 1998b; Wall and Virginia, 1999) and are responsible for the cycling of organic compounds. Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition (George et al., 1995; Timonen et al., 1996), plant health (Srivastava et al., 1996; Filion et al., 1999; Smith and Goodman, 1999), soil structure (Wright and Upadhyaya, 1998; Dodd et al., 2000) and soil fertility (Yao et al., 2000; O'Donnell et al., 2001). Our knowledge of soil microbial diversity is limited in part by our inability to study soil microorganisms. It has also been estimated that about 5000 bacterial species have been described (Pace, 1997, 1999).

Approximately 1%of the soil bacterial population can be cultured by standard laboratory practices. It is not known if this 1% is representative of the bacterial

population (Torsvik et al., 1998). An estimated 1,500,000 species of fungi exist in the world (Giller et al., 1997). But unlike bacteria, many fungi cannot be cultured by current standard laboratory methods (Thorn, 1997; van Elsas et al., 2000). Although molecular methods have been used to study soil bacterial communities, very little research has been undertaken for soil fungi (van Elsas et al., 2000). All organisms in the biosphere depend on microbial activity (Pace, 1997).

The diversity of physical characteristics of soil associate with aggregation at small scales means that soil can contain a large diversity of microorganisms in close proximity, and the chemical composition of soil is also highly heterogeneous in both vertical and horizontal dimensions (Dighton et al., 1997; Gallardo et al.,) Potting soil is a collective name for various kinds of soil with each their own composition and specific structure, especially for plants in pots and flower boxes.

Potting soil is light in weight, unlike for example the heavier dark garden soil. Potting soil is a common medium used in gardening because it is convenient to use. In addition, potting soil consists of amendments and nutrients needed for plants to thrive. Physical properties of the soil include water holding capacity, aeration, plasticity, texture, structure, density and colour etc.

Chemical properties refer to the mineralogical composition and the content of the type of mineral such as Kaolinite, illite and montmorillonite, base saturation, humus and organic matter content. The biological property refers to a content of extent and types of microbes in the soil which include bacteria, fungi, worms and insects.

Materials and Methods

Collection of sample

Soil Samples were collected in the month of September. In the present study soil sample is collected from various pot soil available in the college campus (Nirmala College for women, Coimbatore, Tamilnadu, India).The area lies in Latitude: 11°1′6″N Longitude: 76°58′21″E longitudes. Hence three different potted soils were selected for the present study. The selected potted soils were taken from cartions plant pot, rose plant pot, and Bonsai pot. The samples were collected from 4cm depth .Soil profile where most of the microbial activity takes place, and thus where most of the bacterial population is concentrated. Soil samples were collected (approx 100g) in clean, dry and sterile polythene bags using sterilized spatula.

Determination of Soil Temperature

The temperature of the soil at the three different sites was determined by the use of thermometer. The thermometer was inserted into the soil up to depth of 5cm and allowed to stay for 10minutes, after which the temperature reading was obtained.

Determination of soil pH

Soil pH is a measure of the acidity or alkalinity of a soil. The term pH applies to solutions, so the analysis must be conducted on a soil. The soil sample is mixed with water, allowed to equilibrate for at least an hour, and then the pH measured. Several factors affect pH measurement. Primary among these is the salt concentration of a soil (a salt is any molecule that, when placed in water, separates into positively and negatively charged components or ions). The salt concentration of a soil may vary with the season or with fertilizer application, and is generally greater immediately following fertilizer application than before.

The result may be an apparent pH drop up to one-half a pH unit. When samples are collected frequently or at various times of the year it may be noted that pH values tend to increase and decrease, seemingly at random. This can lead to questions regarding the reliability of soil pH measurements, but the fluctuations may be due to changes in soil salt levels and do not usually present a serious problem in the use of the analysis. Some laboratories measure pH in a dilute salt solution to mask salt-induced variations.

This method gives lower pH values for which the laboratory should provide interpretation guidelines. The pH is determined using a glass and reference electrode with a pH meter on a 1:1 suspension (5 gram scoop of soil to 5 milliliters water). Samples of mineral soils with pH values of less than 6.0 are analyzed.

Dilution of samples

The samples were processed using soil dilution plate method. One gram of soil sample was serially diluted with sterilized distilled water upto 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} 10^{-6}, 10^{-7} and 1 ml of each dilution was added to 20ml of nutrient agar medium in 90mm diameter sterile Petri dishes and then enumerated.

Isolation of microorganisms

Single separate colonies on the agar plates were selected at random according to standard medium and streaked on the nutrient. Agar plates were incubated for 24 hrs at ± 30°C. Code names were given to each of the isolated plates and stored at ± 40°C for characterization and identification by standard methods. Once colonies rose in the media, the sub culturing was continued until a pure isolate was obtained. Identification of microbes was done with the help of standard literature. For isolation of bacteria, Nutrient agar medium, were prepared and to differentiate between gram positive and gram negative bacteria Gram's staining was done. Likewise, 1ml of soil suspension was aseptically poured in potato dextrose agar media plates, were prepared for the isolation of fungi. The plates were gently rotated so as to spread the suspension on medium. The plates were incubated at ± 25° C for 4-5 days.

Microbial population count

Soil samples after serial dilution plates were incubated to 48 hrs to grow the microbial colonies properly. Colony forming units (cfu) were counted by using a colony counter.

Bacterial and Fungal Enumeration

Advances in microbial methods have demonstrated that microorganisms globally are the dominating organisms both concerning biomass and diversity. Their functional and genetic potential may exceed that of higher organisms. Studies of bacterial and fungal diversity have been hampered by their dependence on phenotypic characterization of bacterial and fungal isolates. Molecular techniques have provided the tools for analyzing the entire bacterial and fungal community including those which we are not able to grow in the laboratory.

Results

Soil pH and temperature

Physico-Chemical Properties of soil sample of the different locations of potted soil with veration of the pH values ranged from 7.10 - 7.80. Temperature of the soil at the time of this investigation (summer season) revealed

that the soil environment had temperature range between 30°C and 38°C.

Table. 1 Soil pH and Soil temperature

Locations	Soil pH	Temperature
A	**7.4**	**35**
B	**7.6**	**32**
C	**8.0**	**37**

Isolation of bacteria

The sampling of potted soil, a total of 10 distinct strains of bacteria were recorded such as *Bacillus, Actinomycetes, and Staphylococcus and Streptococcus pseudomonas, Micrococus, proteus* and *Mycobacterium* (Table.2).

Table. 2 Isolation of bacteria and Fungi

S.no	Microorganisms	Site A	Site B	Site C
1	*Bacillus sp*	+	+	+
2	*Pseudomonas sp*	+	-	-
3	*Rhizobium sp*	-	+	+
4	*Staphylococcus aureus*	+	+	+
5	*Micrococcus lutes*	-	-	+
6	*Actinomycetes*	+	+	+
7	*Streptococcus*	+	+	+
8	*Proteus mirabilis*	+	-	-
9	*Mycobacterium*	-	-	+
10	*Aspergillus niger*	+	+	+
11	*Aspergillus flavus*	+	+	+
12	*Aspergillus terrus*	+	+	+
13	*Cladosporium*	+	+	+
14	*Fusarium*	-	+	-
15	*Streptomyces sp*	+	+	-
16	*Streptomyces pygones*	-	+	-

Isolation of fungi

The sampling of potted soil shows the type of fungi isolation. Distinct types of fungi were recorded from potted soil with *aspergillus sp* to be the dominant the least genera were *Streptomyces pygones* (Table.2).

Discussion

Microbial measurements for the present study were analyzed systematically and formal statistical analyses were employed to determine the amount of sample to be withdrawn from a homogenized soil that best represents the microbial community. All microbial measurements showed some difference among sample sizes examined, but a consistent pattern of acceptable results was obtained for the range of sample from 8-10 A number of methods are currently available for studies on soil microbial communities. General need to cultivate microorganisms from soil habitats to better understand their role in soil processes. The microbial studies can be utilized for the prevention of any pathogenic diseases caused by the microbes of this river. Future studies of soil microbial communities must necessarily rely on a combination of both culture-dependent and culture-independent methods and approaches. Only then will we be able to develop a more complete picture of the contribution of specific microbial communities to the overall quality and health of soils.

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