

**COMPARATIVE MICROBIAL DIVERSITY IN A
FOREST AND AGROECOSYSTEM AT SITES-
CHANDA DEVI AND SULTAN NAGARI OF
DISTRICT NAINITAL**

Thesis

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By

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Certificate

This is to certify that the thesis entitled “**COMPARATIVE MICROBIAL DIVERSITY IN A FOREST AND AGROECOSYSTEM AT SITES-CHANDA DEVI AND SULTAN NAGARI OF DISTRICT NAINITAL**”, submitted in partial fulfillment of the requirement of the degree of **Master of Science** with major in **BOTANY**, of the College of Post-Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **MS. Shalini Sharma, ID No. 30672**, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation and sources of literature have been duly acknowledged.

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We, the undersigned, members of the Advisory Committee of Ms. **Shalini Sharma, ID No. 30672**, a candidate for the degree of **Master of Science** with the major in **Botany**, agree that the thesis entitled “**COMPARATIVE MICROBIAL DIVERSITY IN A FOREST AND AGROECOSYSTEM AT SITES-CHANDA DEVI AND SULTAN NAGARI OF DISTRICT NAINITAL**” may be submitted in partial fulfillment of the requirement for the degree.

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CONTENT

SL.NO.	TITLE	PAGE NO.
1	INTRODUCTION	
2	REVIEW OF LITERATURE	
3	MATERIALS AND METHODS	
4	RESULTS	
5	DISCUSSION	
6	SUMMARY	
	LITERATURE CITED	
	VITA	

1. Introduction

Soil is a living dynamic natural resource, which we've been using thanklessly since time immemorial. It basically corresponds to the top layer of earth's crust i.e. lithosphere. It has three major components-chemical, physical and biological. The biological component is the most vulnerable to soil management or agricultural practices. It includes- soil meso, macro, as well as micro fauna that are worst affected by human activities and thus can be used as a sensitive indicator for determining soil quality or soil health. Among the various biota (arthropods, nematodes etc.) present in soil, microorganisms are the smallest but are by no means of less importance.

The broad domain of microorganisms comprises of all organisms with a diameter of 1 mm or less. These include metazoan animals, protozoa, many algae, fungi, viruses and bacteria. Microorganisms are cosmopolitan in behavior and are

found almost everywhere; soil also serves as a major harbour for them. (Fig.1)

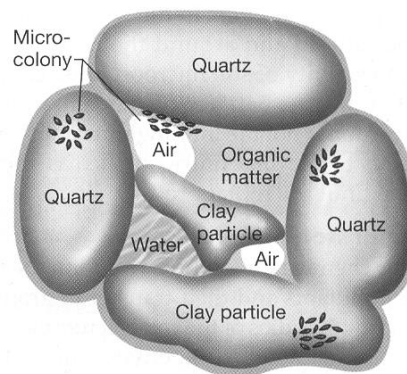


Fig 1: Soil as a habitat for microorganisms (Madigan *et al.*, 2003)

Microorganisms are found in large numbers in soil. The soil microflora consisting of bacteria, fungi, algae and actinomycetes is the most abundant. One gram of fertile soil contains billions of these microorganisms. The live weight of the microorganisms may be about 4000 kg per ha and may constitute about 0.01 to 0.4 per cent of the total soil mass (**Biswas and Mukherjee, 2002**).

Despite their small size microorganisms have attracted scientists, industrialists and environmentalists alike because of their impact on human affairs. They affect many aspects of our lives-be it agriculture, food, environment or other industries. (Fig.2). To describe in detail the various ways in which microorganisms affect human beings and their environment

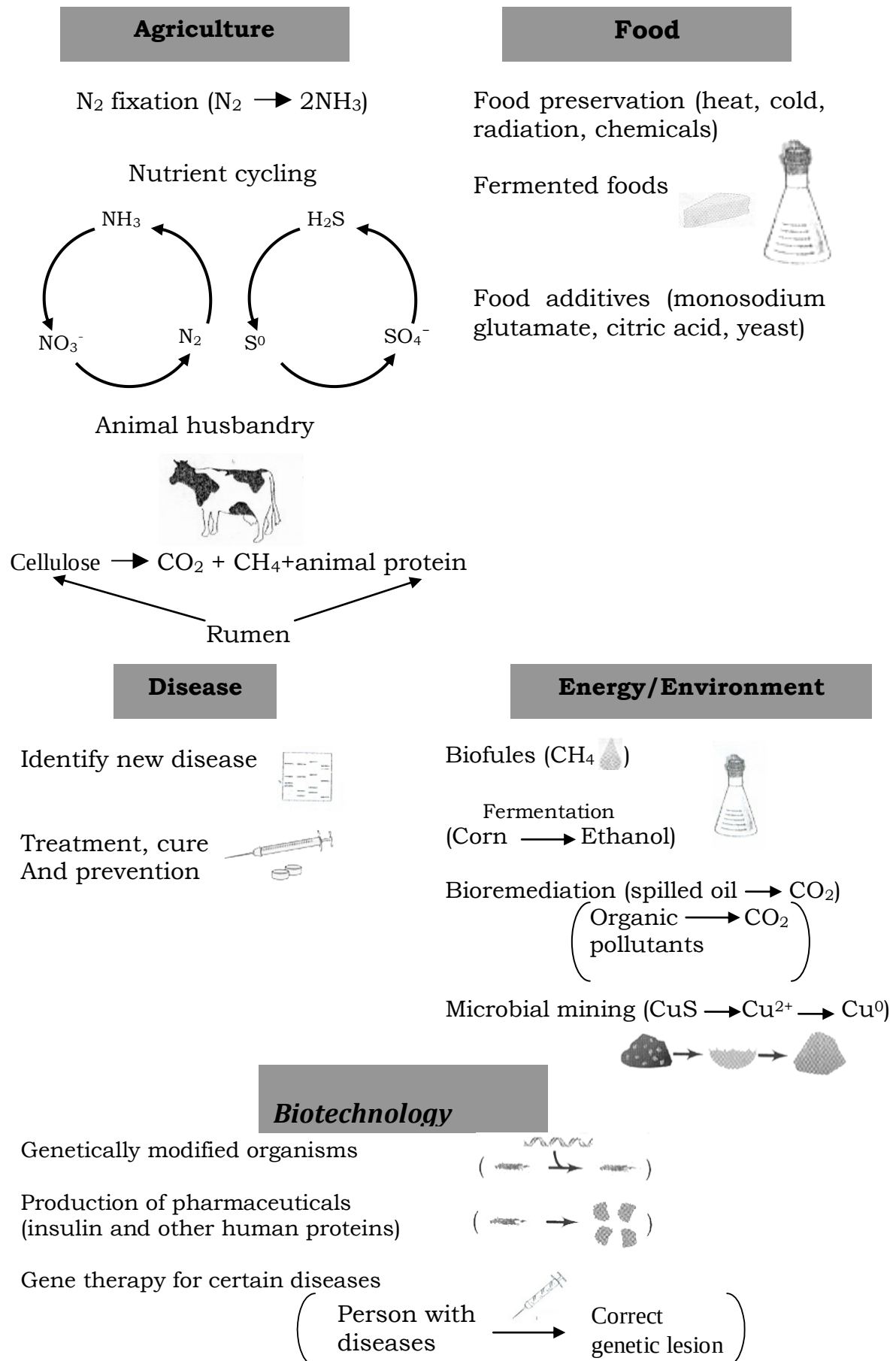


Fig 2: Impact of microorganisms on human affairs (Madigan *et al.*, 2003)

would be a difficult task, so the major functions of these microscopic organisms are described in brief over here:

The microorganisms increase the nutrient uptake of the plant by directly entering into a relationship with it, which is called symbiosis e.g., nitrogen fixers, mycorrhizae. They also play a crucial role in biogeochemical cycles and biodegradation i.e., decomposition of the organic matter entering the soil (e.g., plant litter) and therefore also in recycling of nutrients present in soil. Microorganisms alter nutrient solubility making the otherwise unavailable nutrients available to plants. They help in maintaining the soil structure by decomposing organic matter and forming soil aggregates and thus preventing soil erosion. Biocontrol and bioremediation are also beneficial aspects of microorganisms.

Thus the microorganisms dominate the earth's biosphere despite their small size and understanding the way they function would benefit us in many ways and in many fields but especially in enhancing as well as maintaining the quality of our soils. When the soil is taken into account, not only does the microbial population play an important role but also the diversity of microorganisms is important. Diversity is calculated in terms of population and also functions i.e. population diversity and

functional diversity. Functional diversity gives the total functions performed by microbial community in soil. It directly affects soil resilience. Thus, for maintaining soil fertility and hence sustaining agriculture production, preserving and conserving soil microbial diversity is of utmost importance.

Keeping the aforesaid fact in mind, the present study is being undertaken to find out the comparative relationship between soil microbial diversity of agriculture and forest soils in the Central Himalayan region of India (Uttaranchal state). Sixty-five per cent of total geographical area of the Central Himalayas is covered by forest canopy. Most of the forest land has been cleared for agricultural purposes to feed the people inhabiting these harsh conditions. Therefore much attention has not been given to agriculture system in terms of high input provided to fields. Traditional agriculture such as this is thus heavily dependent on the forests for the organic matter and nutrients (**Maikhuri *et al.*, 1997**). Most of agriculture on hills is based on small terraces where not much cultivation practices are followed. Eighty-five per cent of agriculture is rain fed with only about fifteen per cent irrigated. Thus modern agriculture has not penetrated the hills in any form, forests being the chief source of nutrients.

This change from forest land to agricultural land, might affect the soil nutrient dynamics bringing a change in the diversity of the micro and macro fauna and flora of the soil. Thus, studying soil microbial diversity in the hills of Central Himalayas where cultivated land has organic linkages with the forests would give us a clear picture about traditional form of agriculture as practiced in the hills and comparing the microbial diversity of forest and agriculture land would show how the microbial population is affected when a change from a natural ecosystem to a man made ecosystem occurs. With these points in mind, the present study was undertaken with the following objectives:

1. To study the comparative soil microbial diversity in agriculture and forest ecosystems in the hills of Central Himalayas.
2. To study soil health in the agriculture and forest ecosystems.

2. Review of Literature

“Nature to be commanded, must be obeyed.”

The above statement gives an apt description of our behavior as it should be towards ‘The Earth’. In the flow of the ongoing scientific progression the need to protect and preserve our natural wealth has been forgotten; whose after effects have now driven our attention towards the most essential but one of the less acknowledged resources like the soil.

2.1 Soil

2.1.1 A valuable natural resource

As early as 5000 B.C., the Vedas and the Upanishads as well as other ancient Indian documents mention soil as synonymous with land-the Mother-supporting and nourishing all life on earth **(Biswas and Mukherjee, 2002)**.

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. It is a critical resource for not only agricultural production and food security but also to the maintenance of most life processes **(CBD, 2001)**. Its importance also lies in the fact that it is a non-renewable resource. It's

regeneration through chemical and biological weathering requires millions of years (**Huber et al., 2001**). Infact certain heavily degraded soils are not even recoverable.

Thus, soil holds immense value for us as a natural resource and hence it is important to evaluate the effect of soil management practices on soil quality or soil health.

2.1.2 Soil health

The term soil quality describes the status of soil as related to agricultural productivity or fertility (**Singer and Ewing, 2000**). Soil health on the other hand is a somewhat new and comprehensive term and it broadens the definition of soil quality to include the biological or the living component of soil.

According to **Doran and Zeiss (2000)**, soil health is defined as “the continued capacity of soil to function as a vital living system within ecosystem and land use boundaries, to sustain plant and animal production, maintain or enhance quality of air and water environments, and promote plant, animal and human health”.

Pimm (1984) defined a healthy soil as being ‘resistant’ and ‘resilient’ where resistance is ability to resist changes resulting from imposed disturbances and resilience is potential to recover following disturbances or degradation. (Fig.3).

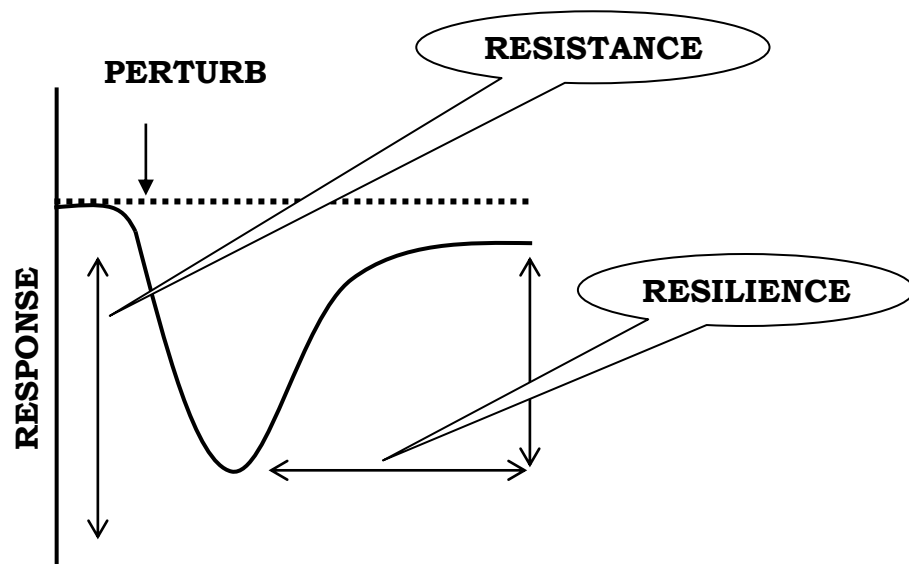


Fig 3: Graphical representation of the difference between resistance and resilience (Ritz *et al.*, 2003)

A healthy soil is alive and it responds and reacts to any kind of human activity and it also responds to the vagaries of nature. Thus, this relatively new term ‘Soil health’ was used to indicate the importance of the living component of soil and moreover to promote the fact that soils are alive. Today soil health is becoming an area of increasing interest for one and all due to the impact it has on sustainability and productivity of above ground plant communities **(Doran and Parkin, 1994)**.

Soil health faces various natural as well as anthropogenic pressures and this has a direct or indirect affect on various aspects of our lives **(Nielsen and Winding, 2002)**. (Fig.4).

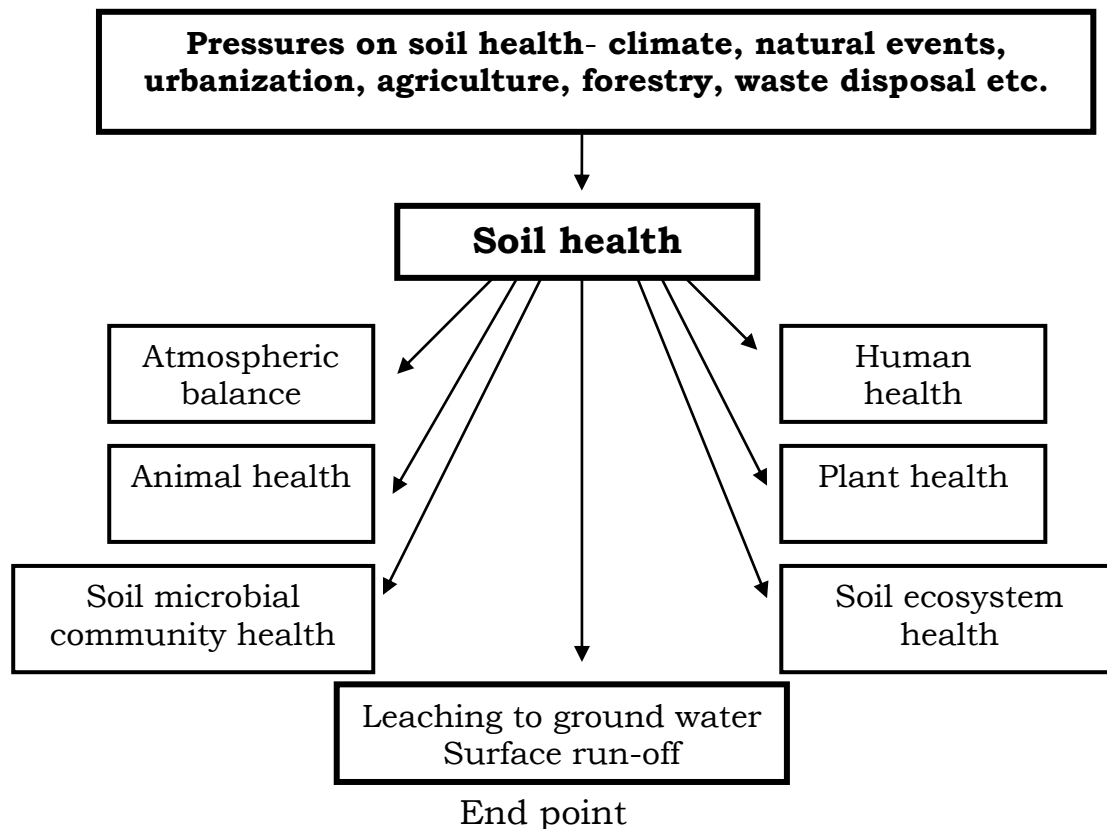


Fig 4: Pressures on soil health and the various end points they affect (Nielsen and Winding, 2002)

The objectives for studying soil health are different in various ecosystems (**Singer and Ewing, 2000**). In agriculture, soil may be managed to maximize production without adverse effects on the environment, whereas in a natural ecosystem, soil may be managed by a set of baseline values against which future changes in the system may be compared (**Karlen and Andrews, 2000**).

Agriculturists have realized that a good fertile soil is not just one that supports plant growth but is one that does so continually for a long stretch of time. In the light of the present statement, **Mader et al. (2002)** have described a fertile soil as

one that provides essential nutrients for crop plant growth supports a diverse and active biotic community, exhibits a typical soil structure, and allows for an undisturbed decomposition.

Today the realization that this non-renewable resource can be depleted easily and can also be preserved or improved by a little effort has led the world to pay, attention long due to the study of soil and soil health.

2.2 Micro-organisms

2.2.1 With soil as a Habitat

Although not apparent to the naked eye, soil is one of the most diverse habitats on earth and it contains one of the most diverse assemblages of living organisms (**Giller *et al.*, 1997**). It serves as a favourable habitat for the proliferation of microorganisms with the most extensive microbial growth, taking place on surface of soil particles. Even a small soil aggregate can have many different microenvironments and thus several different types of microorganisms may be present (**Madigan *et al.*, 2003**)

Number of microorganisms in soil habitats is normally much higher than those found in fresh water or marine habitats (**Mishustin, 1975**). Microorganisms are found in large numbers in soil. Usually between one to ten million micro organisms are

present per gm of soil -with bacteria and fungi being the most prevalent. Bacteria are infact estimated to be found in soil at populations of 10^4 - 10^9 cells g^{-1} soil (**Kennedy, 1999**). Thus the soil is home for a wide range of microorganisms.

2.2.2 Their major hub- The Rhizosphere

The rhizosphere (region of soil directly influenced by plant roots) is a major hub of microorganisms (**Bowen and Rovira, 1976**). Infact microbial populations are hundred times higher in rhizosphere than in the surrounding root-free soil (**Woldendorp, 1978**). (Fig.5).

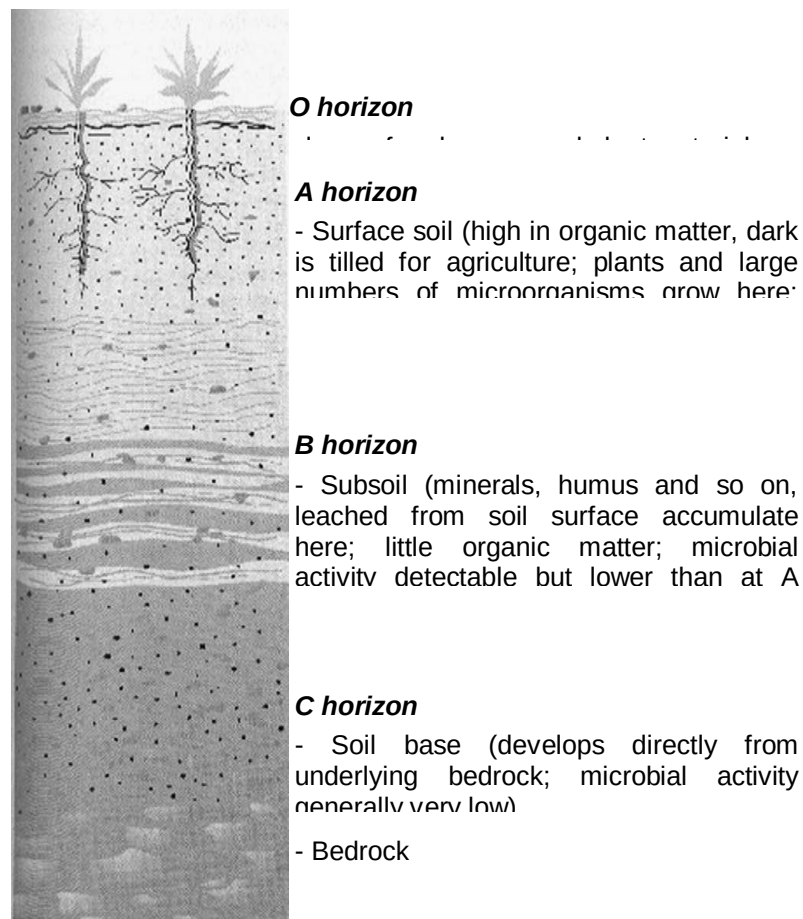


Fig 5: Soil profile: Showing areas of high microbial activity (Madigan *et al.*, 2003)

This might be due to the beneficial relationship soil microorganisms' share with plant roots. Their interaction fulfills nutritional requirements of both the plant and associated microorganisms. Plant roots help to a great extent in establishing microbial colonies. It is the structure of the plant root system that contributes to the establishment of the rhizosphere microbial population (**Bowen, 1980**).

Microbes increase recycling and solubilization of mineral nutrients, help in synthesis of vitamins and various plant hormones. They also show antagonistic effect against potential plant pathogens (**Alvarez *et al.*, 1995**).

Microorganisms in the rhizosphere influence availability of mineral nutrients to plants. They increase the availability of phosphate through solubilization of materials that would otherwise be unavailable to plants.

On the other hand, microbes also provide nutrients in the rhizospheric area which are taken up by the plant roots or by the germinating seed (**Rouatt and Katznelson, 1961**). Even the decaying root systems serve as source of nutrients for the surrounding microorganisms (**Swinnen *et al.*, 1995**).

2.2.3 Soil microbe functions

Soil organisms are an integral part of agricultural and forest ecosystems; and play a critical role in maintaining soil health,

ecosystem functions and production (**CBD, 2001**). They are essential for the functioning and sustainability of all natural ecosystems but are frequently ignored due to their small size and consequent methodological difficulties in detecting cells and assessing their activity (**Prosser, 2002**).

Microorganisms perform a plethora of functions in the soil. According to **Nannipieri and Badulucco (2003)**, 80-90 % of the processes in soil are reactions mediated by microbes. They have a central role in ecosystem processes like bio-geochemical cycling of nutrients and biodegradation (**Prosser, 2002**). They are key players in the cycling of nitrogen, sulphur and phosphorus, and the decomposition of organic residues. Thereby, they affect nutrient and carbon cycling on a global scale (**Pankhurst et al., 1997**).

The main mineralization activity is performed by soil microbial communities (**Neely et al., 1991**). The microbes decompose dead plant and animal organic matter. The organic residues are hence converted to biomass or mineralized to CO₂, H₂O, mineral nitrogen, phosphorous and other nutrients (**Bloem et al., 1997**). Thus, it is evident that if it were not for the decomposition done by microorganisms, ecosystems would be flooded by unwanted debris and energy would also remain

trapped in dead plant and animal organic matter. The microbial biomass, a labile fraction of soil organic matter (**Jenkinson and Ladd, 1981**), acts as a temporary sink for plant-available nutrients (immobilization) and also as a source (mineralization) (**Singh et al., 1989**).

The production and release of extra-cellular polysaccharides and other cellular substances into the soil, microorganisms help in maintaining soil structure, as these materials function as cementing agents for stabilizing soil aggregates. Thereby, they also affect water holding capacity, infiltration rate, erodibility and susceptibility to compaction (**Elliott et al., 1996**).

Microorganisms also carry out nitrogen fixation (utilization of nitrogen gas (N₂) as a source of nitrogen) and sometimes this occurs in association with certain plants, which is called symbiosis. As far as is currently known, no eukaryotic organisms fix nitrogen (**Madigan et al., 2003**).

It can, therefore be said that due to the central role of microorganisms in ecosystem processes including biogeochemical cycling of nutrients and biodegradation, ignoring any aspect of their ecology would be both unwise and dangerous (**Prosser, 2002**).

2.2.4 As indicators of soil health

Identification of indicators of soil health is one of the challenges faced by the scientific world. As defined by **Pankhurst *et al.* (1997)**, soil health indicators are measurable surrogates for environmental processes that collectively indicate whether the soil is functioning normally.

Microorganisms possess the ability to give an integrated measure of soil health; this cannot be done by other physical/chemical measures and/or by analysis of diversity of higher organisms (**Nielsen and Winding, 2002**).

According to **Sun *et al.* (2004)**, changes in soil microbial community structure and diversity may reflect environmental impact. Extensive studies demonstrated perturbation of microbial community equilibrium by changes in environmental conditions and soil management practices (**Smit *et al.*, 2001**). Similar conclusions were given by an earlier work of **Giller *et al.* (1997)**. According to them ecosystem function may be significantly impaired by loss of soil biodiversity, if not inevitably, then at least within a limited range and this damage cannot be fully undone.

The accurate assessment of the function and diversity of soil microbial community is an essential element for evaluating impact of various management decisions on agricultural, urban or

reclamation sites and for studying the stability of native ecosystems (**Tate et al., 2002**).

Microbial characteristics are used as potential indicators of soil health, even if soil health depends on a complex of physical, chemical and biological properties (**Kennedy and Papendick, 1995**). The rationale behind this is the role of microbes in cycling of carbon and nitrogen and their sensitivity to change (**Nannipieri et al., 1990**).

Microorganisms respond quickly to environmental stress and in fact it was observed that changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation (**Pankhurst et al., 1995**). Soil microorganisms hence can act as suitable soil health indicators.

2.3 Biodiversity

2.3.1 It's meaning and relevance

Biodiversity is a shortened term for biological diversity (**Giller et al., 1997**). Biodiversity can be simply defined as the number of species present in the system (**Nannipieri et al., 2003**). The communities of species or the biota and their associated biological, chemical and physical processes i.e., abiotic factors, collectively called ecosystems make the biosphere

functional or drive the biogeochemical processes of the biosphere **(Odum, 1975)**.

In the present scenario, the diversity in species or the biodiversity is suffering as a result of the activities of human expansion or anthropogenic activities **(Ehrlich and Wilson, 1991; Soule, 1991)**.

As of today, biodiversity measurement and conservation has become a general concern due to various factors. According to **Naeem et al. (1994)**, loss of biodiversity, in addition to loss of genetic resources, loss of productivity, loss of ecosystem buffering against ecological perturbation and loss of aesthetic and commercially valuable resources, may also alter or impair the services that ecosystems provide i.e., declining biodiversity would alter the performance of ecosystems.

Vitousek et al. (1997) have also observed that the two most dramatic ecological trends of the past century are human induced changes in biotic diversity and alterations to the structure and functioning of ecosystems. A similar concern was voiced by **Chapin et al. (1997)**. According to them, apart from the ethical, aesthetic, and economic concerns raised by the declining biodiversity, the biotic changes will also influence ecosystem processes sufficiently to alter the future state of the world's ecosystems and the services they provide to humanity.

The combination of adequate biotic diversity and heterogeneity is considered to be necessary for long term ecological functioning and resilience of ecosystems (**Bengtsson, 2002**).

Higher biodiversity within a community is thought to reduce the variations in functional activities of the communities, thus reducing the risk of loss of functions after extreme environmental conditions (**Ekschmlt et al., 2001**).

If functionally similar species have different environmental sensitivities then stability of ecosystems increases on the other hand if functionally different species have different sensitivities than this makes the ecosystem susceptible, rather vulnerable to change (**Chapin et al., 1997**).

Thus, diversity has a direct impact on ecosystem processes and eventually on the human being as such. **Chapin et al., (1997)**, shows this figuratively (Fig.6).

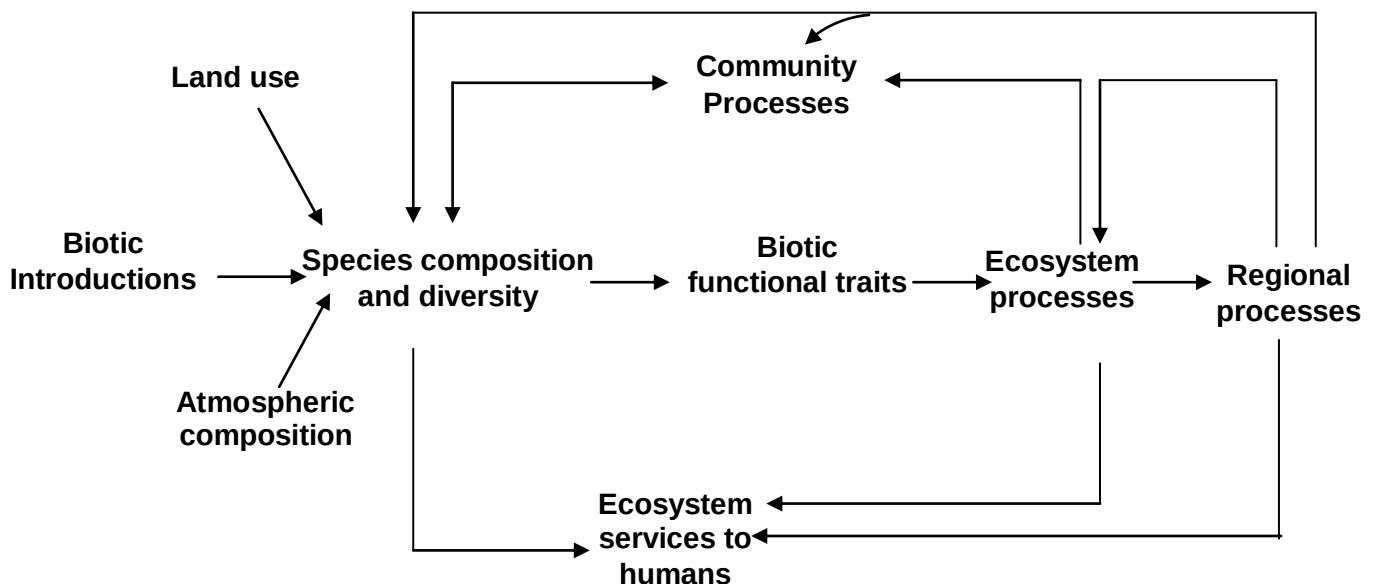


Fig 6: Effect of diversity on ecosystem processes and human affairs

2.3.2 Meaning and relevance in relation with micro-organisms

The diversity of the microbial population is more extensive than that of any other group of organisms in the world (**Kennedy and Gewin, 1997**). Bacteria, fungi, actinomycetes, algae and protozoa are enclosed in the broad spectrum of microorganisms. And even within these specific groups diversity is observed (**Biswas and Mukherjee, 2002**).

Microbial diversity is a general term used to include genetic diversity, that is, the amount and distribution of genetic information, within microbial species, diversity of bacterial and fungal species in microbial communities, and ecological diversity, that is, variation in community structure, complexity of interactions, number of trophic levels, and number of guilds (**Nannipieri *et al.*, 2003**).

According to **CBD report (2001)**, soil biodiversity per se may not be a soil property that is critical for the production of a particular crop but it is a property that may be vital for the continued capacity of the soil to support that crop. Thus improved management of soil biota would help in maintaining soil quality and soil health.

Tate *et al.* (2002), have a similar viewpoint. According to them the importance of a diverse, resilient soil microbial

community in the development of functional, sustainable land based ecosystems can not be ignored. According to **Giller *et al.* (1997)**, biodiversity not only ensures the multiplicity of functions of soil organisms but also maintains the functions in the face of perturbations. Today the study of microbial diversity has attained greater relevance as now it can be applied to “fingerprint” soils (**Kennedy and Gewin, 1997**). Here, it is observed that not only climate and geography but also management practices influence microbial heterogeneity (**Wander *et al.*, 1995**).

2.3.3 Factors affecting microbial diversity

Microbial diversity in soil may be affected by various factors as depicted figuratively by **Kennedy and Gewin, (1997)**. (Fig.7)

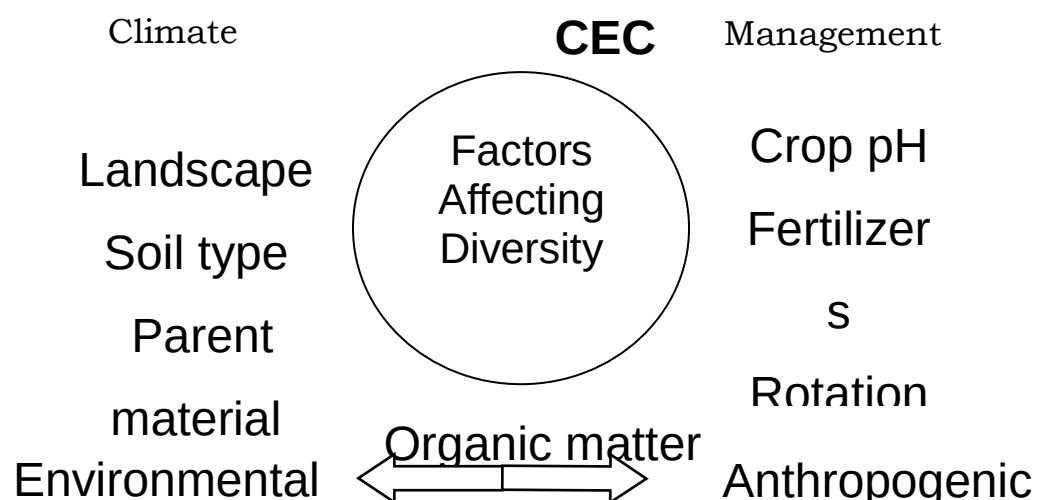


Fig 7: Factors affecting diversity

Microbial diversity and function respond to a multitude of characteristics that are best exemplified by the diversity of soils themselves **(Kennedy and Gewin, 1997)**.

Plant community and their above ground ecology can impact not only nutrient availability but also microbial function **(Christensen, 1989)**. Plants exert a strong influence on the composition of microbial communities in soil through rhizodeposition and decay of litter and roots. As a result of co-evolution, microbes and plant species show a strong link **(Brimecombe et al., 2001)**. Not only the soil microbial communities but the key ecosystem processes that they mediate are also significantly altered by plant species richness **(Zak et al., 2003)**. **Schlöter et al. (2003 a)** also observed that in agriculture ecosystems interactions between diversity of primary producers (plants) and of decomposers (microbes and mesofaunal communities), the two key functional groups that form the basis of all ecosystems have major consequences on functioning of the ecosystem.

Individual plant species may affect soil biota as each plant species differs in both quantity and quality of resources they return to soil **(Wardle et al., 2004)**.

Kennedy and Gewin (1997) were of the opinion that physical position of the microbes can also affect the level of

diversity and function. Climate and parent material are also factors that affect microbial diversity (**Wander *et al.*, 1995**).

Conservation tillage and legume-based crop rotations support diversity of soil microbial communities and may affect the sustainability of agricultural ecosystems (**Lupwayi *et al.*, 1998**).

Another important factor that affects microbial biodiversity comprises of tillage, crop rotation and fertilizer, pesticide use etc. **Lahav and Steinberger (2001)** also observed that human intervention greatly affects soil biota.

2.3.4 Culturable as well as functional diversity

During studies concerning diversity, it was found that the functional characteristics of component species are at least as important as the number of species *per se* for maintaining essential processes (**Bardgett and Shine, 1999**) i.e., a minimum number of species are needed for stable ecosystem functioning whereas a large number of species is essential for maintaining the dynamics of the ecosystems in a changing environment (**Loreau, 2001**). This is the concept of total diversity being different from functional diversity. Functional diversity and culturable diversity are vastly different from each other. Functional diversity includes the magnitude and capacity of soil inhabitants that are involved in key roles, such as nutrient cycling, decomposition of various

compounds and other transformations (**Zak et al., 1994**) whereas culturable diversity is independent of function and is usually determined by culturability and isolation of species and it may represent only a fraction of bacteria present and active in soil (**Kennedy, 1999**).

Recent studies stress the importance of gaining an understanding of the functions of microbial communities, as population diversity alone may not be adequate in maintaining ecosystem stability. Recognizing the diversity and the links within the key functional groups in a given system can lead to better ways to manage diversity and function and thus improve process stability (**Hulot et al., 2000**). **Cavigelli et al. (1995)** expressed a similar view. Functional diversity relates to our comprehension of soil systems therefore its study has become important in recent times.

According to **Zak et al. (1994)** functional diversity is a component of overall diversity in soil that provides a more practical and “ecologically relevant” measure of microbial diversity. It is different from species diversity as it concerns functions expressed *in situ* by microbes rather than species present in soils. It includes ability of soil microbes to participate in soil processes. Microbial functional diversity includes a vast

range of activities including nutrient transformations, decomposition, plant growth promotion and modification of soil physical processes (**Degens and Harris, 1997; Degens *et al.*, 2001**).

Soil community with high functional diversity has ability to accomplish a wide range of metabolic activities such as substance degradation. Functional diversity of a microbial community describes potential of community to degrade different organic compounds (**Zak *et al.*, 1994**).

Analysis of functional diversity and its dynamics in the environment is thus essential for understanding the biogeochemistry of soil systems (**Kelly, 2003**).

2.4 Methods to analyze diversity

An essential element for evaluating impacts of various management decisions on agricultural, urban, or reclamation sites and for studying the stability of native ecosystems is the accurate assessment of the function and diversity of the soil microbial community (**Tate *et al.*, 2002**). Several approaches are available for assessment of microbial diversity (**Atlas, 1984**).

Groups with distinctive morphological characteristics can be directly observed. But this method is useless when analyzing diversity in complex environments, like the soil when quantification of measures of diversity is needed (**Prosser, 2002**).

According to **Hill et al. (2000)**, two challenges faced by soil microbiologists were the development of effective methods to

- 1) Determine which microorganisms are present in soil, and
- 2) Determine microbial function *in situ*

Until recently, the major methods for assessing diversity were laboratory cultivation techniques where cultivation of organisms was done on solid media after dilution of their environmental samples. Post incubation, pure cultures obtained from isolated colonies were identified using well established phenotypic characters (**Prosser, 2002**) but now, the approach to analyze soil microbial communities has changed dramatically as new methods are available for assessment of microbial diversity (**Hill et al., 2000**).

Microbial diversity is measured by various techniques such as traditional plate counting and direct counts as well as the new molecular-based procedures and fatty acid analysis (**Nannipieri et al., 2003**).

2.4.1 Culture dependent methods to assess diversity

Dilution plating and culturing methods

Plate counts of culturable species provide a preliminary measure of diversity on the subsets of population (**Kennedy and Smith, 1995**). Plate counts offer the advantage of being a quick, inexpensive reliable method of obtaining data. These

measurements are useful in comparative studies of different soil types (**Dodds *et al.*, 1996**). This method can help in isolating and identifying species which are able to perform desired functions such as in biological control (**Kennedy *et al.*, 1991**). According to **Prosser *et al.* (2002)**, it is impossible to design growth media and cultivation conditions that are suitable for all members of community. Some species that are non-culturable or are unable to grow on particular media would be left out and it is also well established that plate counts estimate only 1-10% of overall soil microflora.

To justify this traditional method of diversity study, **Bakken (1997)** hypothesized that the culturable bacteria have an ecological significance in soil and are more important than that which appears from their small numbers.

Another widely used culture dependent method for analyzing soil microbial community is identification based on utilization of carbon sources or substrate utilization (**Konopka *et al.*, 1998**). “Fingerprints” of community structure can be obtained by substrate utilization patterns (**Zak *et al.*, 1994**). **Kennedy (1995)** also observed that substrate utilization can be used to differentiate species units based on ability of bacteria to utilize carbon substrates.

According to **Neilsen and Winding (2002)**, functional diversity of microbial populations in soil may be determined by expression of different enzymes by carbon utilization patterns or by extra cellular enzyme patterns.

Thus, culture dependent techniques are not ideal for diversity study because only a fraction of microorganisms are culturable (**Cavigelli et al., 1995**) and also because of their different media composition requirements. But still culture dependent techniques are used till today because of their various advantages. These methods are quick, inexpensive, isolate individual species (**Kennedy and Gewin, 1997**) and they also provide information about growth habit, development and potential function of microorganisms in soil habitats (**Hill et al., 2000**).

2.4.2 Culture independent techniques

Various methodological limitations hindered investigation of the relationship between microbial diversity and soil processes (**Kelly, 2003**), therefore new innovative, culture independent methods are now being used for study of diversity among micro-organisms.

The culture impendent approach has enabled us to obtain abundant information about the structures and functions of some rare microbial communities, which were not possible to explore

with only culture dependent strategies (**Nakamura et al., 2004**).

Soil enzyme activities

Soil enzyme activities indicate the potential of the soil to support biochemical process essential for the maintenance of fertility of the soil (**Dkhar and Mishra, 1983**). Microorganisms especially fungi and bacteria are the major source of soil enzymes (**Gobat et al., 2005**) therefore soil enzyme activities are often used as indices of microbial growth and activity in soils (**Frankenberger and Dick, 1983**).

According to **Dick et al. (1996)** soil enzyme activities are often closely related to soil organic matter, soil physical properties and microbial activity or biomass; ii) change much sooner than other parameters, thus providing early indications of changes in soil health, and iii) involve simple procedures. A similar observation was made by **Fraser et al. (1988)**. According to them any change in the soil influences its microbial community and hence may alter soil enzyme activities.

Soil enzyme can serve as indicators of soil health as disturbances of soil microbial activity as shown by changes in levels of metabolic enzymes, can serve as an estimate of ecosystem disturbance. **Kandalar et al. (1996)** had clearly shown this relation with soil polluted with heavy metals.

Fatty acid methyl esters (FAME) profiles

Methods involving biochemical components or structural components as biomarkers can be used to assess soil quality and quantify the diversity of microorganisms (**Ibekwe and Kennedy, 1999**).

Lipids are present in all cells and are characteristic of a species as well as being responsive to environmental conditions. Fatty acids can be used to fingerprint soils through FAMEs analysis (**Kennedy, 1995**).

According to **Cavigelli et al. (1995)** analysis of fatty acid methyl ester (FAME) profiles from soils is a rapid and inexpensive procedure that holds great promise in describing soil microbial community structure without traditional reliance on selective culturing, which seems to severely underestimate community structure. They further explain that the value of FAME profiles arises from the facts that there are a great number of different kinds of fatty acids in the lipids of microorganisms and that different organisms have different combinations of these fatty acid. For e.g., fungi have a longer chain length than what is found in bacteria. Many differences are found between fatty acids of Ascomycetes and Basidiomycetes (**Johnk and Jones, 1992**).

The fact that the information of the fatty acid composition of bacteria is coded in the nucleic acids of the chromosome and is not influenced by plasmid information makes the fatty acid profile a stable feature of a species and an acceptable taxonomic marker **(Suzuki *et al.*, 1993).**

For the analysis of microbial communities in soil, an interesting method is the direct extraction of fatty acids from soil samples and subsequent gas chromatographic analysis. In this way a surprisingly accurate quantitative and qualitative “snapshot” of the resident micro flora may be produced **(Kluepfel, 1993).**

After going through various methods of diversity study **Liesack *et al.* (1997)** reported that a combination of culture based and culture independent approaches is likely to reveal more information regarding the composition of soil microbial communities.

2.5 Agriculture ecosystem and forest ecosystem

The foremost thing in the mind of man since prehistoric times is the exploitation of nature and manipulating his surroundings such as to derive the maximum benefit for himself. In this process, he has changed the face of the earth, (many times for the better also) and various different environments and ecosystems have evolved as a result. One such man made

ecosystem is agriculture ecosystem. In the following pages an effort is being made to bring about a contrast between agroecosystem, a man made ecosystem and forest ecosystem, a natural ecosystem.

Agroecosystems have certain characteristics, or even unique features that distinguish them from natural ecosystems. Examples of such characteristic features include a high export of organic material and nutrients, a relatively insignificant litter layer and low plant-species diversity. The agroecosystem is unique in its high degree of human manipulation, e.g., cultivation, fertilization and biocide treatment (**Lowrance *et al.*, 1984**).

White, (1997) demonstrated that soil physical conditions are usually best under permanent grassland or forest and as soil is cultivated, these conditions deteriorate at a rate dependent of climate, soil texture and management. **Amezquita *et al.* (2000)** have found significant negative effects of continued cropping on the physical properties of soils.

In agroecosystems, a decline in soil organic matter occurs as a result of ploughing and cultivation. Ploughing leads to a decrease in populations of soil structure stabilizing fungi and earthworms (**White, 1997**). Whereas natural forests are recognized as sites of high biodiversity, where complex relationships between fauna, flora and microflora are maintained

due to the structural richness of the habitat and because anthropogenic disturbance by management is restricted (**Hackl et al., 2004**).

There is a man made homogeneity of both plant communities and the topsoil in agroecosystems. This is in direct contrast to natural ecosystems where there are marked variations in both (**Andren et al., 1988**).

Conventional agriculture as practiced today has had major environmental impacts, in particular with respect to soil degradation. Soil structure, fertility, microbial and faunal biodiversity have all suffered a decline (**Ariena and Aad, 2003**).

The human intervention in agroecosystems may affect soil biota, which is the “biological engine of the earth”, and is responsible for driving many fundamental processes such as nutrient cycling, maintenance of soil structure, and is implicated in many aspects of human, animal and plant health (**Ritz et al., 2003**). Human encroachment has negatively affected soil biota in agroecosystems and this has a crucial impact on system productivity and it’s maintenance (**Lahav and Steinberger, 2001**).

Today an understanding of agroecosystems is important as it would be the key to determine effective farming systems (**Mader et al., 2002**). Understanding role of soil biota, mainly microorganisms in natural ecosystems also provides insights into

the functioning of agroecosystems, and *vice-versa*, enabling scientists to manipulate systems in the interest of sustainability and the maintenance of the quality of the environment. The importance and potential of such studies is now starting to be more widely recognized by agriculturists (**Hawksworth, 1991**).

Thus, everything in nature is linked and for the preservation of one, a complete understanding of both man-made and natural ecosystems is needed.

2.6 Central Himalayas: Much to be explored

Central Himalayas is a relatively untouched area of India. Due to the extreme ecological conditions and inaccessibility, modern agriculture could not penetrate this region (**Maikhuri et al., 1996**). In this region, traditional form of agriculture is followed which is unique and complex in that-crop husbandry, animal husbandry and forests constitute interlinked production systems (**Maikhuri et al., 1997**).

Traditional agriculture suffered a setback in the wake of modern agriculture (**Thrupp, 1996**) but is still responsible for feeding the major chunk of people in the hills.

In traditional form of agriculture, crop yields are sustained with nutrient, water, energy and organic matter inputs from the surrounding forests (**Semwal et al., 1996**). Agriculture land is

mainly rain fed i.e. about eighty-five percent of the area (**Maikhuri et al., 1997**), and mainly traditional crops like millets, cereals etc., are grown.

Traditional agriculture relies upon the local i.e., easily available resources unlike modern agriculture which requires high input from outside. Thus, if modern agriculture makes a hold over the hilly regions, there is a high risk of the hills become dependent on outside world whereas traditional agriculture had made them self sufficient (**Sen et al., 1997**).

Despite its disadvantages, modern agriculture has its positives sides like the yields are higher. Therefore, an integrated system involving the advantages of both modern and traditional agriculture should be evolved so as to address the problems of mountain farming system. There is much to be explored about the traditional farming system and the problems existing in the hills - whether socio-economic or otherwise - can only then be overcome (**Maikhuri et al., 1997**).

3. Materials & Methods

The various materials required and the methods employed during the study are given below:

3.1 Site selection

The objective of the study was to compare microbial diversity in agriculture and forest ecosystems at two different altitudes. Thus, as per the requirement of the objectives such sites were to be selected where both a forest and an agriculture field were present at the same altitude.

These requirements were fulfilled at the two sites, namely-

- i) Chanda Devi: Altitude 850 m above sea level.
- ii) Sultan nagari: Altitude 550 m above sea level.

3.2 Site description

Site 1: Chanda Devi

- i) Agriculture ecosystem

Location: Village Amarpur, Chanda Devi

Owned by: Shri Vishnudutt Palariya

(Land being cultivated since 50 years)

Other factors: 1. System rainfed



Chanda Devi: Forest ecosystem



Soil sampling at Chanda Devi

2. Crop rotation – wheat and maize

3. FYM (Farm Yard Manure) was added

No Chemicals used.

4. Tillage- Man power.

ii) Forest ecosystem

Area : Half Acre

Dominant tree species: *Shorea robusta* and *Tectona grandis*

Site 2 : Sultan nagari

i) Agriculture ecosystem

Location: Near Haldwani (Golapar)

Owned by: Shri Saumjeet Tewari

(Land being cultivated since 50 years).

Other factors: 1. System irrigated

2. Crop rotation – wheat and maize

(Occasionally, mustard taken in place of wheat)

3. Chemical fertilizers (Urea, Di-ammonium phosphate) and fungicide (Bavistin) and weedicide (Pendimethylene) used.

4. Tillage –Bullocks.

ii) Forest

Dominant tree species: *Acacia catechu* and *Dalbergia sissoo*

Meteorological data

Precipitation: Annual rainfall 890 mm at both the sites.

Temperature: Chanda Devi- Min.15°C and Max.23°C



Sultan nagari: Forest ecosystem.

Sultan nagari-Min.18°C and Max.27°C

(During the time of investigation).

During the experimental time period i.e. November to April temperature was fluctuating throughout and a sudden fall in temperature was recorded in the month of February.

Collection and preservation of above ground flora

The above ground flora of both the sites (Chanda Devi and Sultan nagari) and for both the ecosystems (agriculture as well as forest) was collected. The procedure followed is given below—

The specimens were collected, identified and then they were kept in polythene bags, which were tied with rubber bands to make them airtight. Poisoning of specimen was done to prevent any pest or fungal attack, using a poisoning solution. (Appendix).

The specimens were now pressed using a table press. The newspapers containing the collected flora were changed every few days. The dried specimens were finally mounted on standard herbarium sheets by the help of an adhesive.

Soil sampling

Random soil samples were collected in a zig-zag manner, from both the ecosystems. Rhizospheric soil was collected from the upper 15 cm layer in all the cases (A Horizon) after removing one inch top layer. The collection of soil sample was done twice –

before and after winter- i.e., in November and April, respectively. Each site was divided in three blocks where three samples were taken and pooled and thus three replicate samples were obtained for each site.

The soil was finally divided into three parts for –

- i) Chemical analysis
- ii) Cultural diversity
- iii) Biochemical analysis

The analysis

- a) Soil chemical analysis
 - 1. pH- Glass electrode, pH meter (Elico)
 - 2. Organic carbon (%)- **Walkely and Black (1934)**
 - 3. Phosphorus- **Rao (1984)**
 - 4. Potassium- **Rao (1984)**
 - 5. Micronutrients- **Lindsay and Norvell (1978)**
- b) Percent soil moisture was calculated by the following formula

$$\text{Moisture content \%} = \frac{\text{Moist soil} - \text{dry soil} \times 100}{\text{Moist soil}}$$

Total cultural diversity

For total cultural diversity analysis, the dilution plating method was used. The steps involved are enumerated as follows:

- One gram soil was added to 0.8%, 9 ml saline blanks.

- For making further dilutions, 100 µl of the previous solutions was added to 900 µl saline.
- 100 µl of the 10⁻⁴ dilution was used for spread plating on the High Plate Count Agar media for bacteria (Appendix).
- For fungi pour plating of 10⁻³ dilution was done using the Potato Dextrose Agar media (Appendix).

Plates were then incubated in a BOD incubator at 28°C. Total bacterial counts were taken after one day and total fungal counts after three days. Cfu (Colony forming units) was calculated by the following formula

$$\text{Cfu} = \frac{\text{No. of cells per g of soil} \times \text{Dilution factor} \times 10}{\text{Dry wt. of soil}}$$

In addition log Cfu per g of soil was also calculated.

Functional analysis

Functional analysis was done for siderophore production, amylase, xylanase, protease, cellulase activity and phosphate solubilization.

Siderophore production (Schwyn and Neilands, 1987)

Spread plating was done on Chrome-Azurol S (CAS) plates (Appendix) and they were incubated at 28°C. An orange halo zone was observed indicating siderophore production.

Phosphate solubilization

Spread plating of soil dilutions were done on Pikovaskaya's agar plates (Appendix). A zone was seen around phosphate solubilizing colonies.

Amylase, Xylanase, Cellulase and Protease activity

Spread plating was done on respective media (Appendix) and following observations were made-

Amylase plates- After adding lugol's iodine a zone was seen around amylase producers.

Cellulase and Xylanase plates- After adding Congo red dye and then destaining with 0.2% NaCl solution zones were observed around cellulase and xylanase producers.

Protease plates – Zones was observed around protease producing colonies

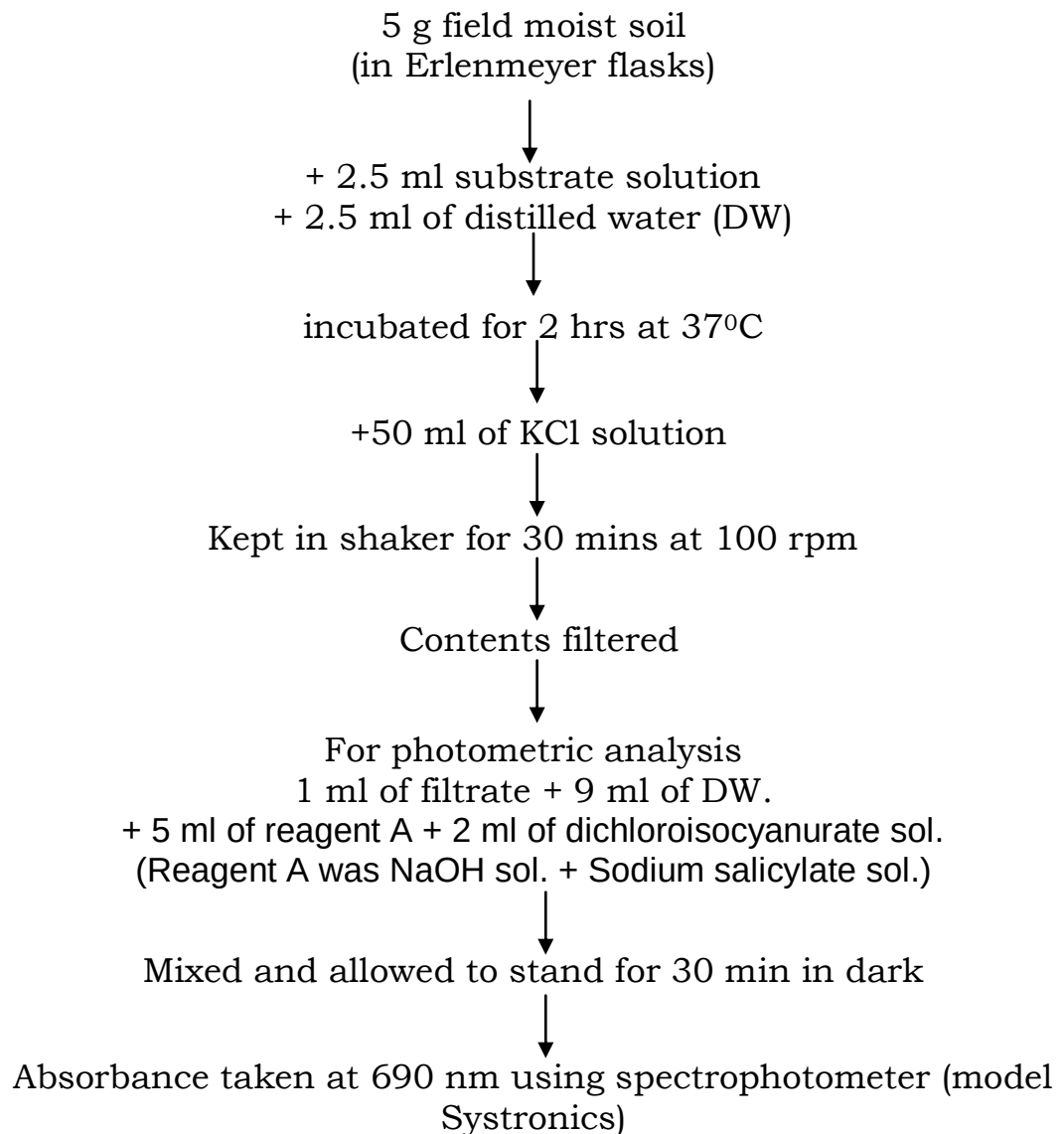
Biochemical analysis

1. Urease activity - **Kandeler and Gerber (1998).**
2. Dehydrogenase activity - **Thalmann (1968).**
3. Acidic and alkaline phosphomonoesterase activity -

Tabatabai and Bremner (1969).

Urease

Procedure



Calculation for urease

Level (μg) of N was calculated from calibration curve-

$$\text{Urease activity} = \frac{S \times 10 \times 100 \times 55}{5 \times \% \text{ dm}} = \mu\text{g N g}^{-1} \text{ dm } 2\text{h}^{-1}$$

S - mean value of samples ($\mu\text{g ml}^{-1}$)

10 - dilution factor

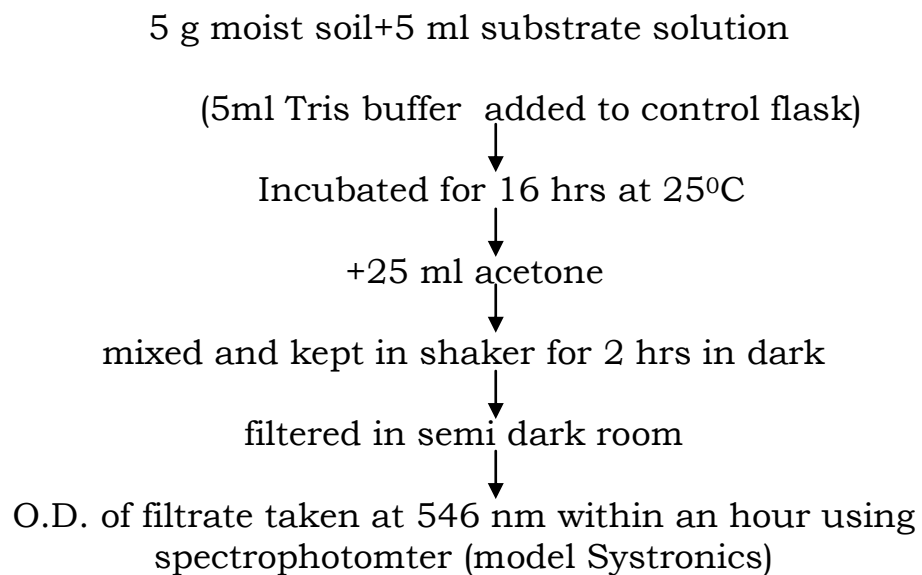
55 - volume of extract (ml)

5 -initial soil weight (g)

100%- dm- factor for soil dry matter

Dehydrogenase activity

Procedure



Calculation of dehydrogenase

The level (µg) of Tri-phenyl formazon (TPF) in the filtrate were determined from calibration curve

$$\text{Dehydrogenase activity} = \frac{S \times 100}{5 \times \% \text{ dm}} = \mu\text{g TPF}^{-1} \text{dm} 16\text{hr}^{-1}$$

S- mean value of samples (µg TPF)

5-initial soil weight (g)

100% dm⁻¹- factor for soil dry matter

Acid and Alkaline Phosphomonoesterase activity

Procedure

1 g soil+1 ml desired substrate solution + 4 ml corresponding working buffer (according to acidic or alkaline phosphomonoesterase activity study)



Only 4 ml working buffer added to control



Flasks kept on shaker briefly, stopper was used to cover flasks



Incubated for 1 hr at 37°C



+1 ml of CaCl₂+4 ml of NaOH solution



+90 ml DW



thoroughly shaken and then filtered



O.D. taken at 400 nm using spectrophotometer (model Systronics)

Calculations

Level of para-nitrophenol (pNP) in samples and control was calculated from calibration curve.

Phosphomonoesterase activity

$$\frac{S \times 10 \times 100}{\% \text{ dm}} = \mu\text{g pNP g}^{-1} \text{ dm h}^{-1}$$

S - mean value of samples (μg pNP)

10 - factor for dilution of the extract

100% ⁻¹dm - factor for soil dry matter

Fatty Acid Methyl Ester (FAMES) Cavigelli *et al.*(1995)

- Soil samples (5 g) were saponified by adding 5 ml of 3.25 M NaOH in CH₃OH.
- After thorough mixing, tubes were heated in a 100°C water bath for 5 min.
- Remixing was followed by heating on a water bath for an additional 25 min with intermittent mixing.
- Samples were methylated by adding 10 ml of 3.25 N HCl in CH₃OH, mixed and tubes were placed in a 80°C water bath for 10 min.
- Allowed to rapidly cool in ice water.
- FAMES were extracted by adding 1.5 ml of one part methyl tert-butyl ether in one part (v:v) hexane; tubes were shaken end over end for 10 min.
- Top organic phase was transferred by pipette to dry tube and was washed using 3 ml of dilute NaOH.
- The organic phase was transferred to a clean vial for Gas chromatograph (GC) analysis.

Samples (2-3 µl) of fatty acid methyl esters were analysed using GC (Perkin Elmer; Model No. Autosystem SL). The chromatogram obtained showed the presence of fatty acid in samples as amount in percentage. Retention time of the individual fatty acid methyl ester was also obtained.

Data analysis

Arithmetic mean of various cfu values was calculated.

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n}}$$

Where $\bar{x}$ = arithmetic mean

x = value of variable

n = no. of observations

Shannon Weaver Index (1949)

The amounts of fatty acids as expressed in percentage were used to calculate diversity indices. Shannon Weaver Diversity Indices within a sample was calculated according to the formula:

$$H^1 = -\sum p_i \ln (p_i)$$

Where p_i = relative abundance of a given FAME peak

$\ln$ = natural logarithm.

Richness equal to number of peaks was calculated.

Evenness was calculated as per the formula

$$\text{Evenness} = \frac{\text{Diversity}}{\text{Richness}}$$

Principle component analysis (PCA) was performed using SPSS 7.5 version.

4. Results

The present study was carried out with the aim of comparing soil microbial diversity in forest and agriculture ecosystems. The sites chosen were in the hills of Kumaoun, in the newly formed state of Uttaranchal. The two sites were present at different altitudes—

Site 1 (Chanda Devi) was present at 850 m and Site 2 (Sultan nagari) was present at an altitude of 550 m. The investigation was carried out to study the microbial diversity at both the ecosystems (agriculture and forest) present at each site.

The results of the investigation are given as follows--

1 The above ground diversity observed at the two sites:

Site 1: Chanda Devi

i) Agriculture ecosystem

Weed species: *Oxalis* spp., *Pergularia*, *Boerhaavia*, *Vicia faba* and *Ageratum conyzoides*.

ii) Forest ecosystem

Dominant tree species: *Shorea robusta* and *Tectona grandis*

Other flora under trees: *Achyranthus aspera*, *Murraya paniculata*, *Desmodium gyrans*, *Lantana indica*, *Cassia fistula*, etc.

Plate 1:

(A) *Parthenium hysterophorus* L.

(B) *Murraya koenigii* (L) Spreng.

(C) *Polygonum cerrulatum*

(D) *Dalbergia sissoo* Roxb.

(E) *Cannabis sativa* L.

(F) *Asparagus racemosus* Willd.

(G) *Solanum nigrum*

(H) *Amaranthus viridis* L.

(I) *Euphorbia hirta* L.

(J) *Ageratum conyzoides* L.

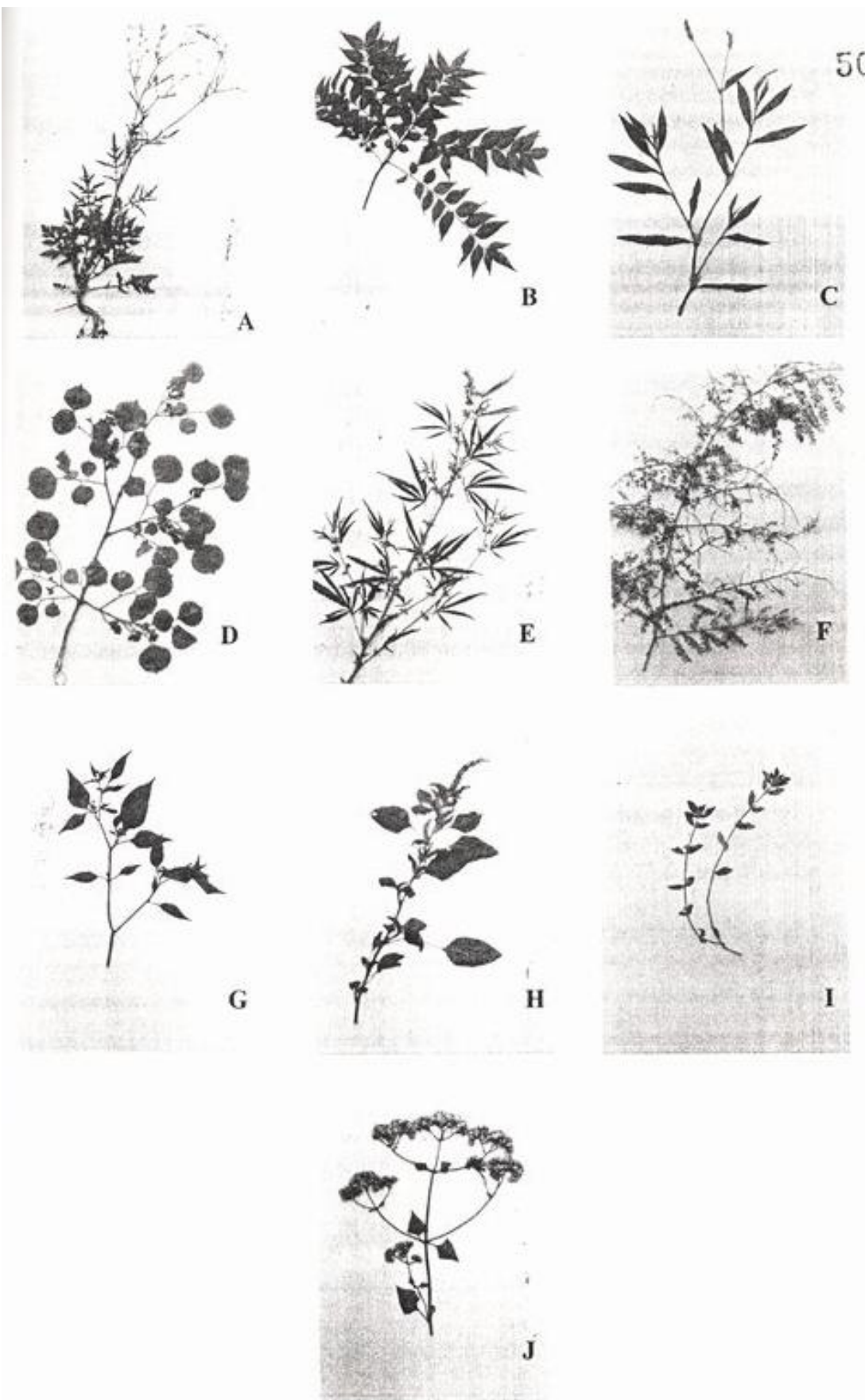


Plate-1: Above ground flora collected

Site 2: Sultan nagari

i) Agriculture ecosystem

Weed species: *Amaranthus*, *Commelina*, *Ageratum conyzoides*, *Sida cordifolia*, etc.

ii) Forest ecosystem

Dominant tree species: *Acacia catechu* and *Dalbergia sissoo*

Other flora: *Lantana indica*, *Euphorbia hirta*, *Amaranthus*, *Zizyphus*, *Solanum nigrum*, *Murraya paniculata* and *Clerodendrom viscum*.

The herbaria of the above ground flora collected from both the ecosystems of the two sites is presented in Plate 1.

Chemical analysis

The mean values of chemical parameters in the different ecosystems present at the two sites is given in Table 4.1.

The pH of all the sites was near neutral i.e. 7.0 and 7.1 for Chanda Devi forest and agroecosystem respectively and a slight increase was observed at the Sultan nagari site with a pH of 7.2 and 7.3 in the forest and the agroecosystem respectively. The organic carbon % was highest in the Sultan nagari forest (1.16%), followed by the agricultural land at Chanda Devi (1.13%). The forest at Chanda Devi had the lowest organic carbon (0.65%) and the agroecosystem at Sultan nagari had 0.72% organic carbon. Electrical conductivity was maximum at the Chanda Devi forest

Table 4.1: Chemical analysis of soil

Sites/ Ecosystems	pH	E.C.	O.C. %	P (kg/ha)	K (kg/ha)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
Chandadevi Forest	7.0	0.32	0.65	9.0	171	0.681	0.399	8.30	3.16
Chandadevi Ag	7.1	0.27	1.13	54.0	385	2.377	0.608	13.94	2.716
Sultannagari Forest	7.2	0.28	1.16	18.0	171	1.062	0.335	10.53	3.126
Sultannagari Ag	7.3	0.23	0.72	13.5	171	0.743	0.330	12.13	2.660

and minimum at Sultan nagari agriculture land, values being 0.32 and 0.23 respectively.

The available Phosphorus (P) and Potassium (K) were maximum at the Chanda Devi agroecosystem 54.0kg/ha and 385kg/ha respectively. Minimum value of available P was observed at Chanda Devi forest (9.0kg/ha). The forest at Sultan nagari was richer in Phosphorus than the corresponding agroecosystem, values being 18.0kg/ha and 13.5kg/ha respectively whereas at the same site Potassium had same values (171kg/ha) in both the ecosystems.

The micronutrients were present in a balanced amount at the ecosystems of both the sites. The highest amount of Zinc, Copper and Iron was found at the Chanda Devi agriculture site whereas the minimum values for Zinc and Iron were observed at the forest ecosystem of the same site (Table 4.1). Manganese was maximum at the Chanda Devi forest and minimum at Sultan nagari agroecosystem (3.16 ppm) and (2.66 ppm) respectively.

3 Total bacterial and fungal counts

In general the bacterial population was more in the month of April at both the sites except in Sultan nagari forest ecosystem where they were more in November (Fig 4.1). There was no significant difference between agroecosystems in both the sites for

Plate 2:

Total cultural diversity

- (A) Total fungal diversity on a PDA plate
- (B) Total bacterial diversity on HPCA plate
- (C) Total fungal diversity on PDA plate
- (D) Total bacterial diversity on HPCA

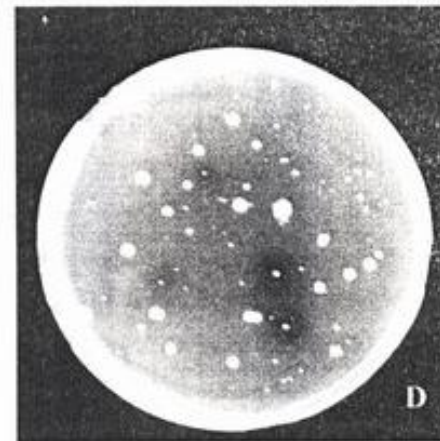
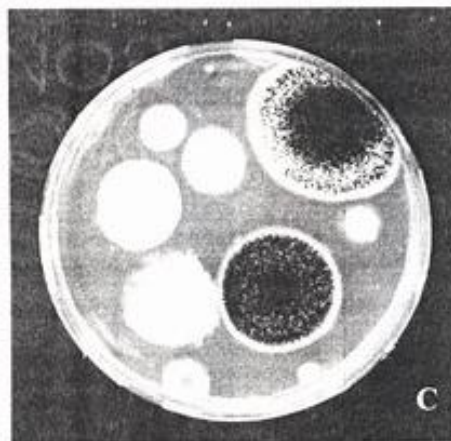
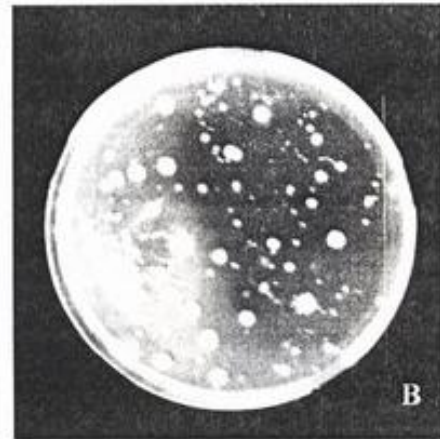
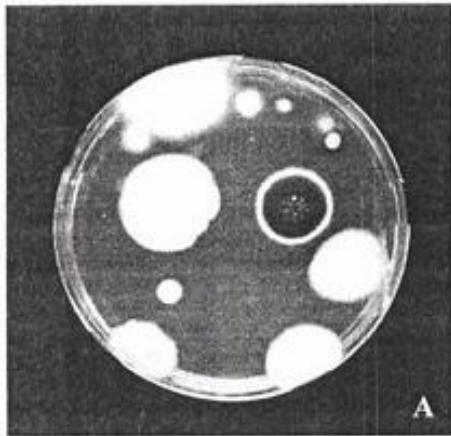


Plate-2: Total cultural diversity

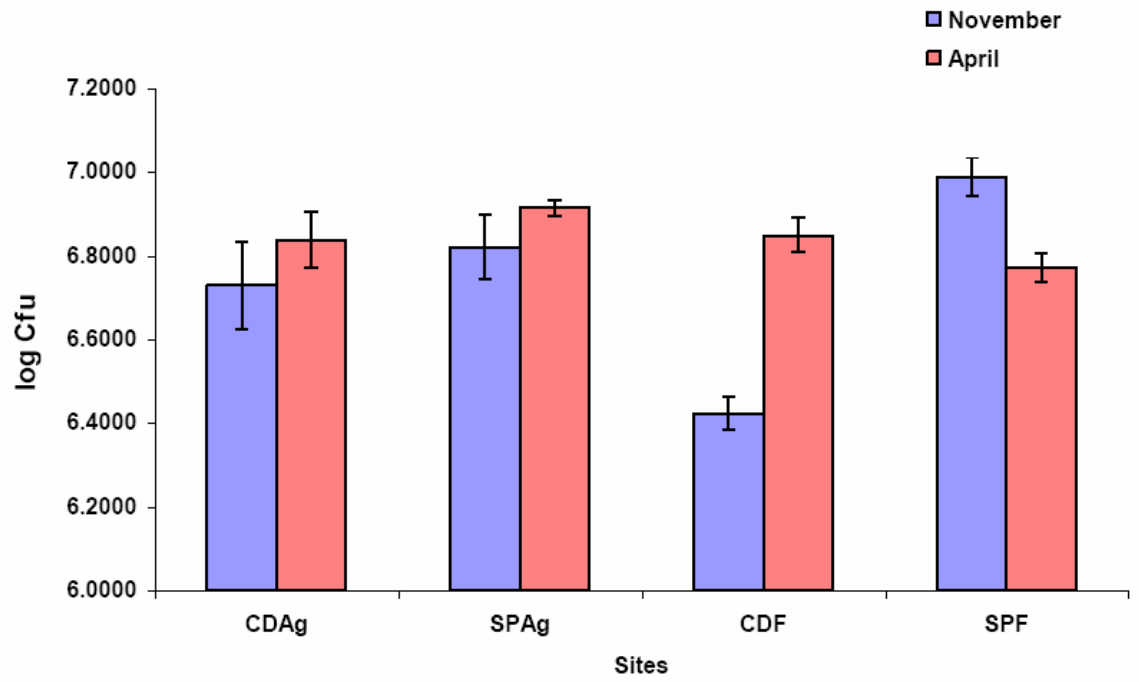


Fig. 4.1: Bacterial Diversity

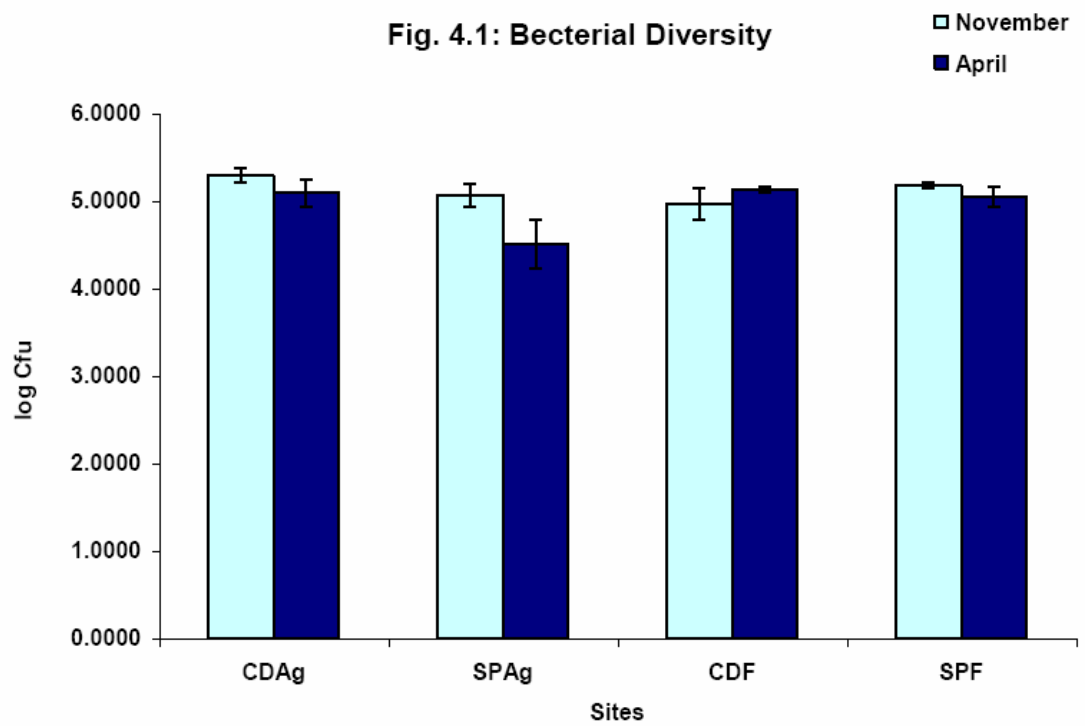


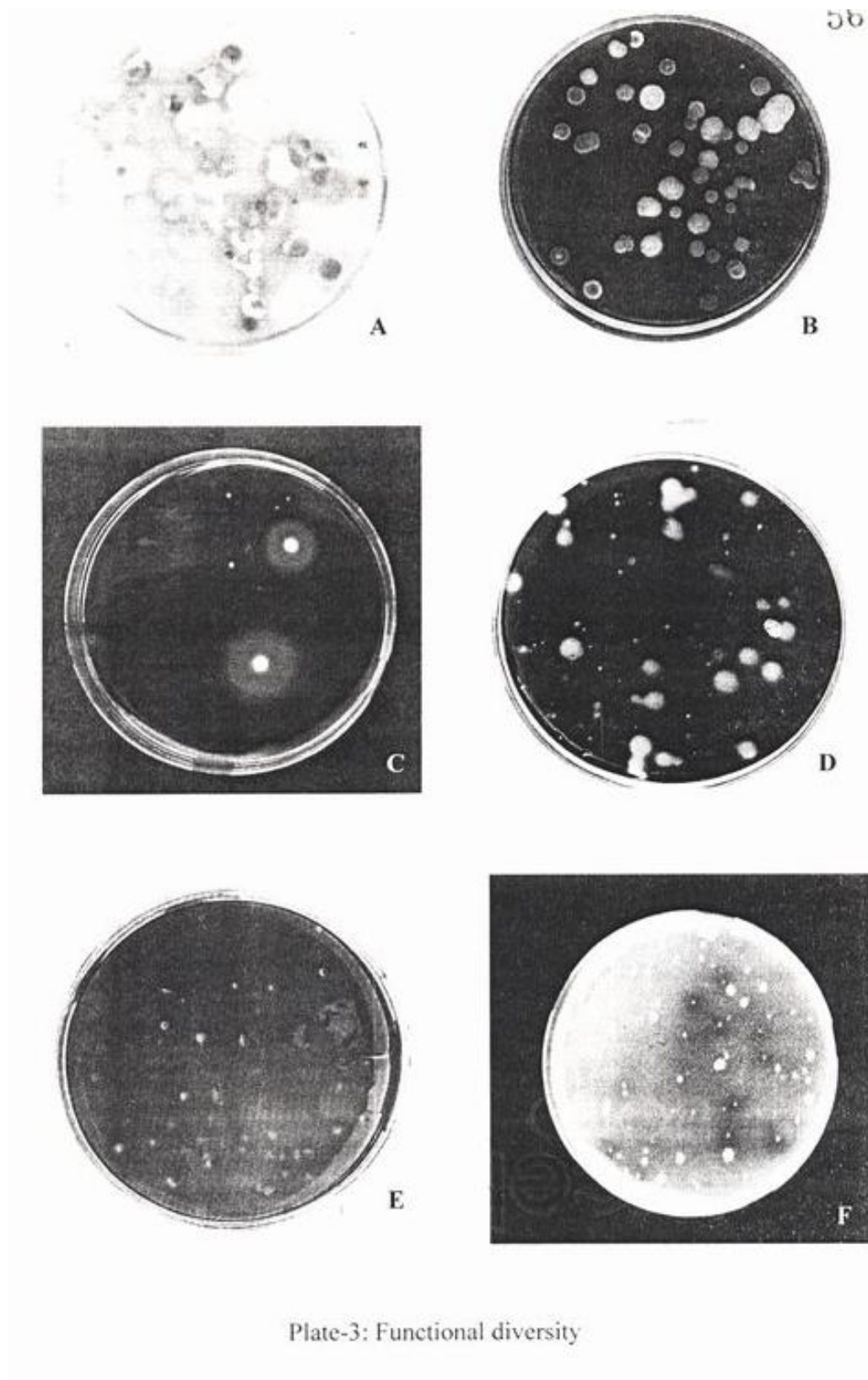
Fig. 4.2: Fungal Diversity

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

Plate 3:

Functional diversity

- (A) Protease plate**
- (B) Xylanase plate**
- (C) Siderophore plate**
- (D) Amylase plate**
- (E) Cellulase plate**
- (F) Piko plate**



both the seasons, however between the forest ecosystems there was a significant difference between the sites and for both the seasons (Fig 4.1).

A reverse trend was observed in case of fungal populations (Fig 4.2), the seasons did not make any significant difference except in the Sultan nagari agroecosystem where the fungal population in April reduced significantly from November. (Fig 4.1 and 4.2, Plate 2).

Xylanase producers

The bacterial population of xylanase producers was found to be significantly more at Chanda Devi site compared to Sultan nagari agroecosystem (Fig4.3). At Chanda Devi the agroecosystem showed more Xylanase producing bacteria than the forest ecosystem. A reverse trend was observed at the Sultan nagari site where the forest was richer in bacteria in both the seasons, population of fungi was also more as compared to agroecosystem. Fungal populations were more or less stable i.e. no significant differences were observed in the populations in both the seasons at the Chanda Devi site while at the Sultan nagari site the fungal xylanase producers were found to be significantly higher in the month of November in the forest but the difference was non-significant in April. (Fig 4.3 and Fig 4.4).

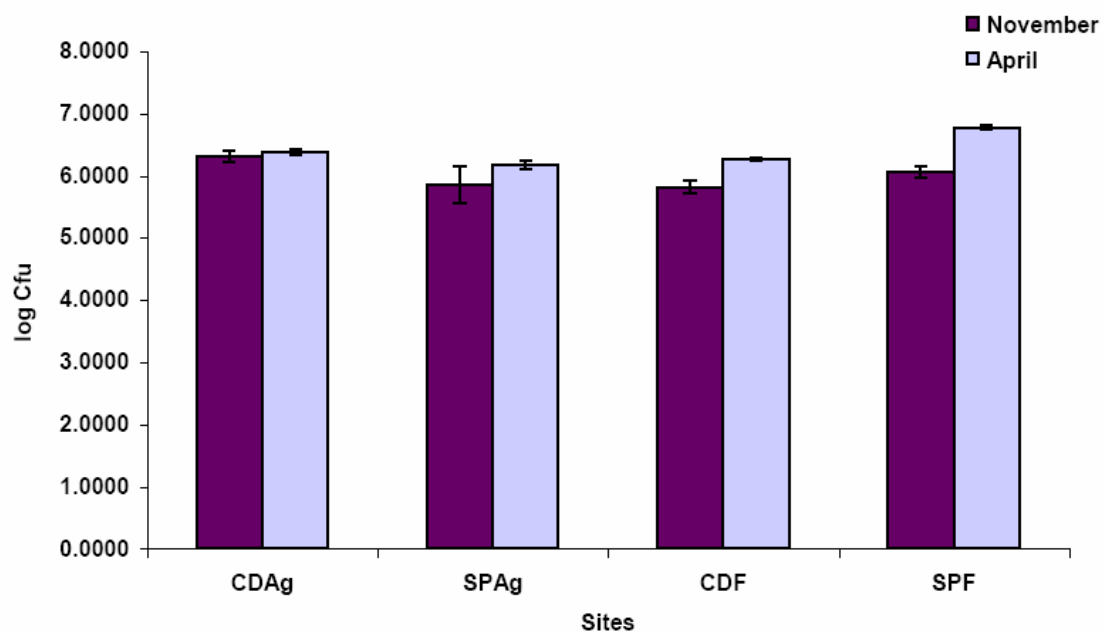


Fig. 4.3: Xylanase producers (Bacteria)

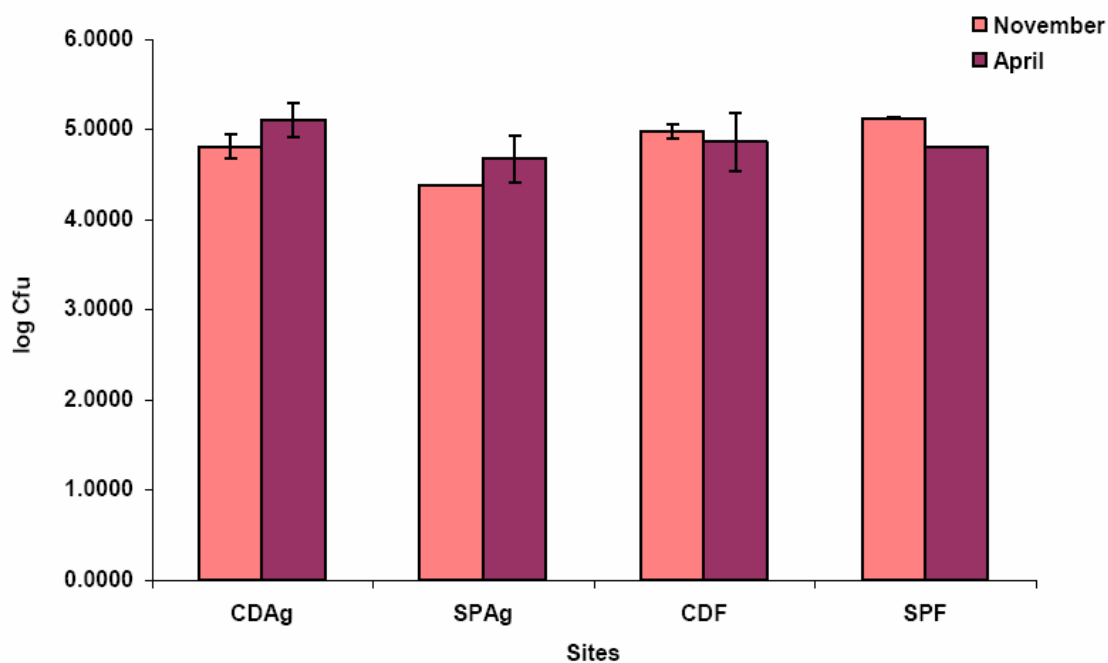


Fig. 4.4: Xylanase producers (Fungi)

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

Protease Producers

The protease producing bacteria and fungi are depicted in Fig 4.5 and Fig4.6, respectively.

Greater protease producing bacterial populations were observed in November but as compared to the month of April the results were non-significant at the Sultan nagari agriculture ecosystem. Comparing the two agroecosystems the bacterial populations were higher at Chanda Devi agroecosystem.

The fungal populations were less in the month of November in agroecosystems than the forest ecosystems present at the same altitudes at the two sites, whereas in April they were more in the agroecosystem. The fungal populations were stable for both the seasons at both the sites in the agroecosystems (Fig 4.5 and 4.6).

Amylase producers

Bacterial amylase producers were greater in number at both the sites in the agroecosystems for both the seasons than the forest ecosystem. Comparing the two sites Chanda Devi agriculture and forest ecosystem had greater bacterial population in November and less in April.

Chanda Devi again showed more population in November and lesser in April when the two sites were compared (for both the

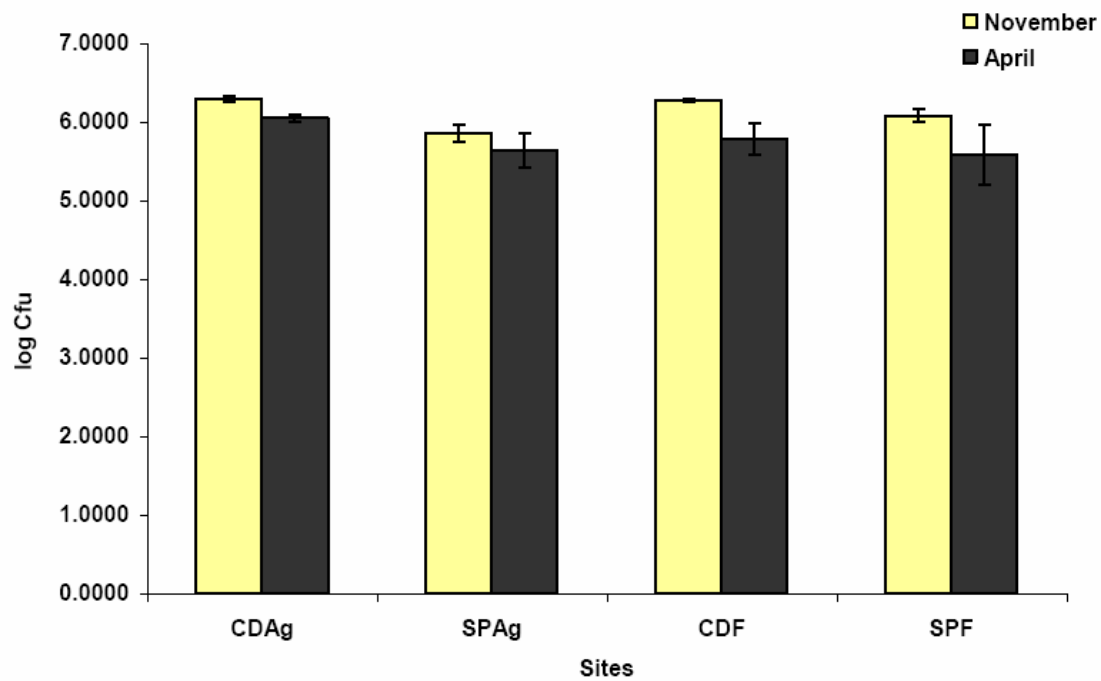


Fig. 4.5: Protease producers (Bacteria)

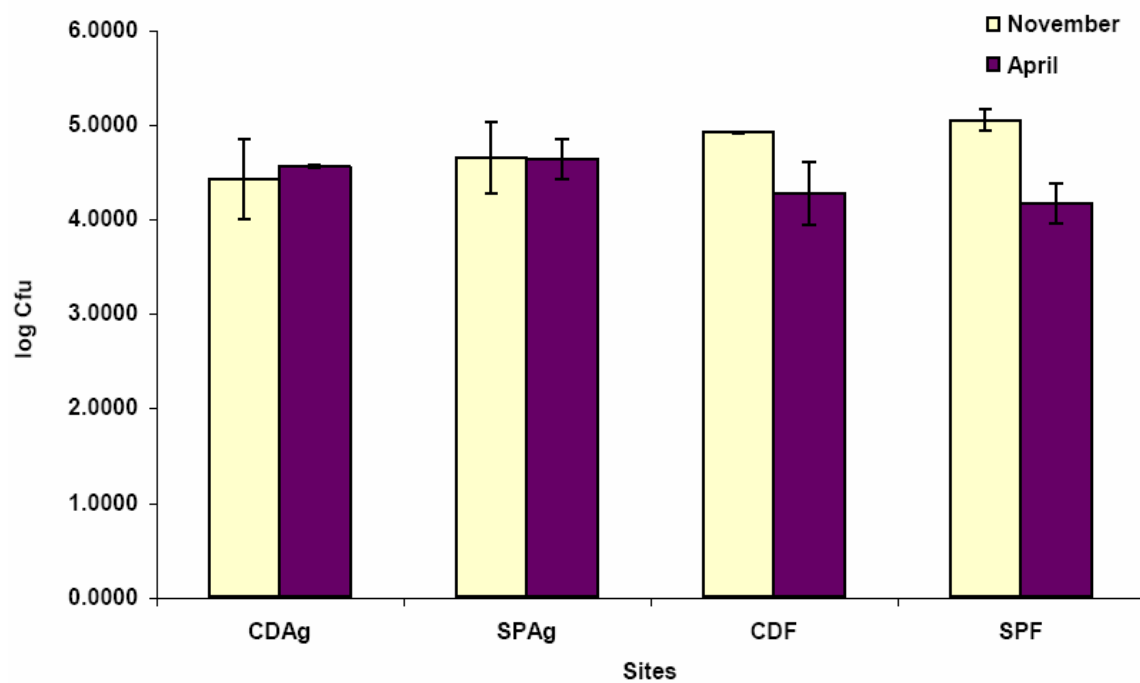


Fig. 4.6: Protease producers (Fungi)

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

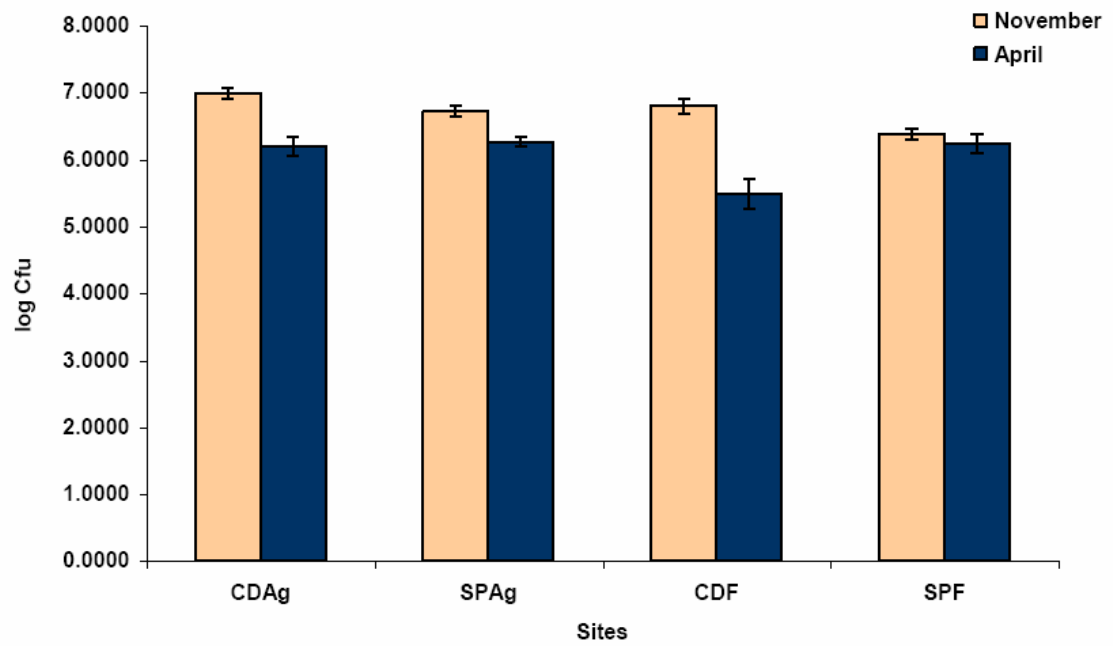


Fig. 4.7: Amylase producers (Bacteria)

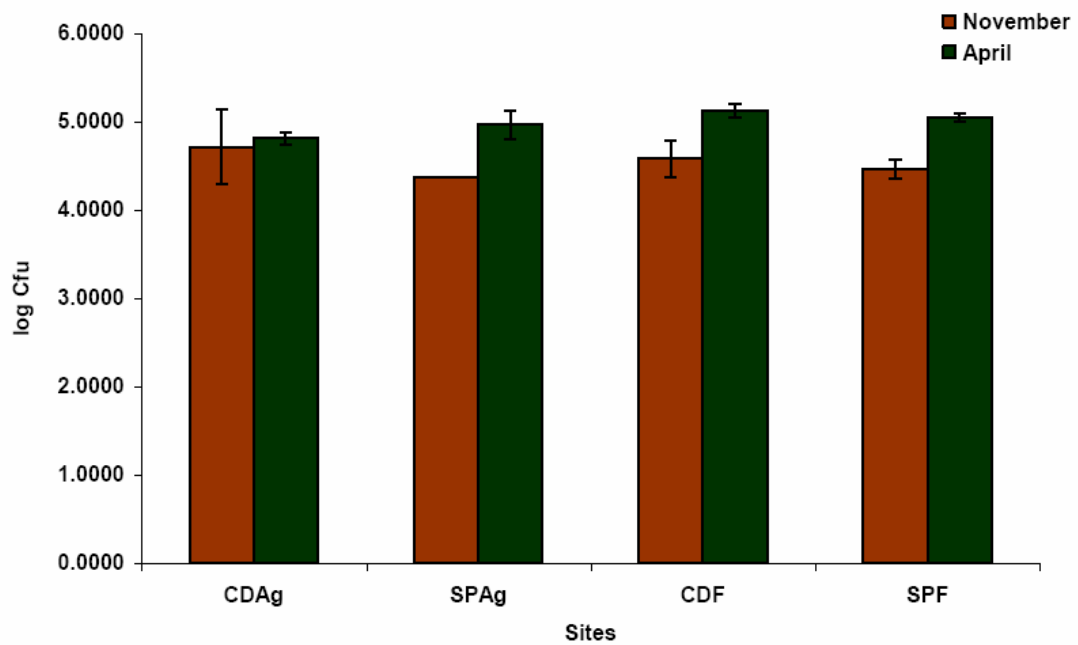


Fig. 4.8: Amylase producers (Fungi)

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

ecosystems). Although there were slight variations in fungal populations at both the sites, the differences were non-significant. (Fig 4.7 and 4.8).

Cellulase producers

The diversity in cellulase producing bacteria and fungi is shown in Fig. 4.9 and Fig. 4.10, respectively. At both the sites, the agroecosystems had more cellulase producing bacteria than the forest ecosystem at corresponding altitudes; however, results for the month of April were non-significant. When both the sites were compared, the Sultan nagari ecosystems were richer in bacteria in November and the results were non-significant in April.

The fungal populations again showed stability and the results were non-significantly different from each other both, when sites were compared and also when the ecosystems were compared.

P-Solubalizers and Siderophore producing bacteria

Forests sites were richer in Phosphate-solubalizing bacteria as well as fungi for both the seasons for both the sites. When both the sites were compared Chanda Devi was richer in P-solubalizing bacteria than the Sultan nagari site for both the ecosystems however the results for forests were non-significant. The fungal P-solubalizers were greater in agroecosystem of Chanda Devi when compared to Sultan nagari. However when the forest ecosystems

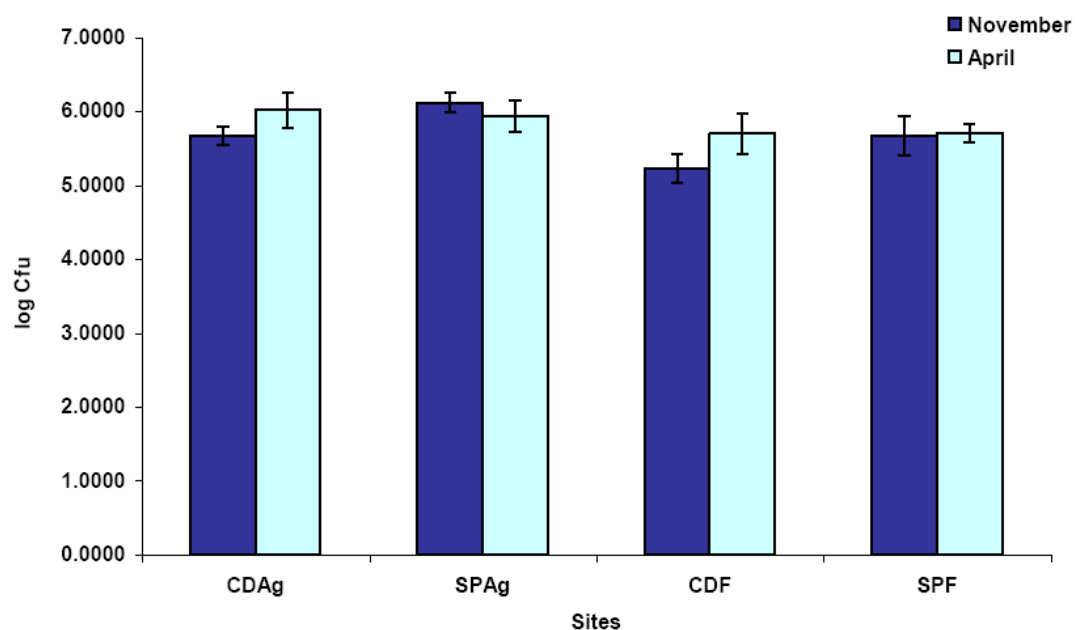


Fig. 4.9: Cellulase producers (Bacteria)

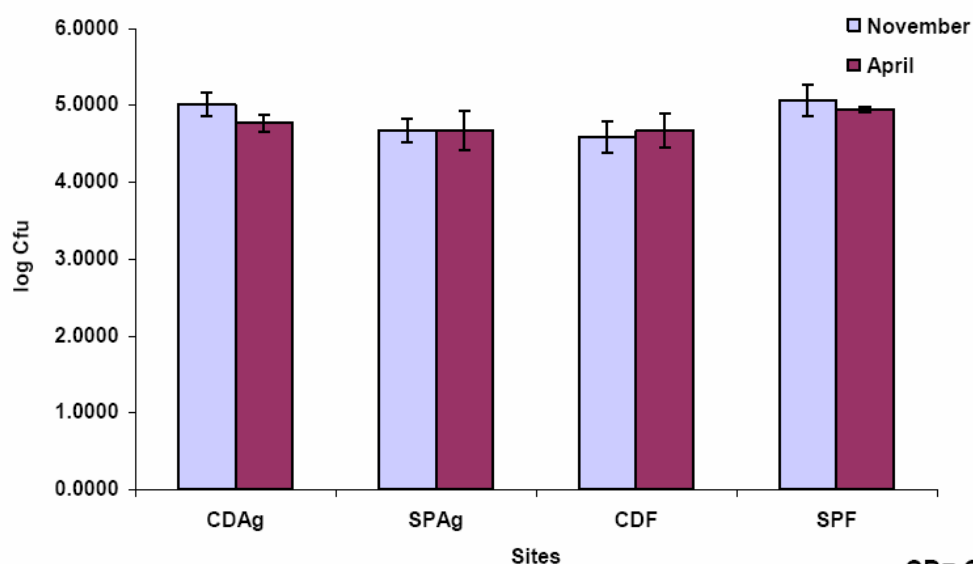


Fig. 4.10: Cellulase producers (Fungi)

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

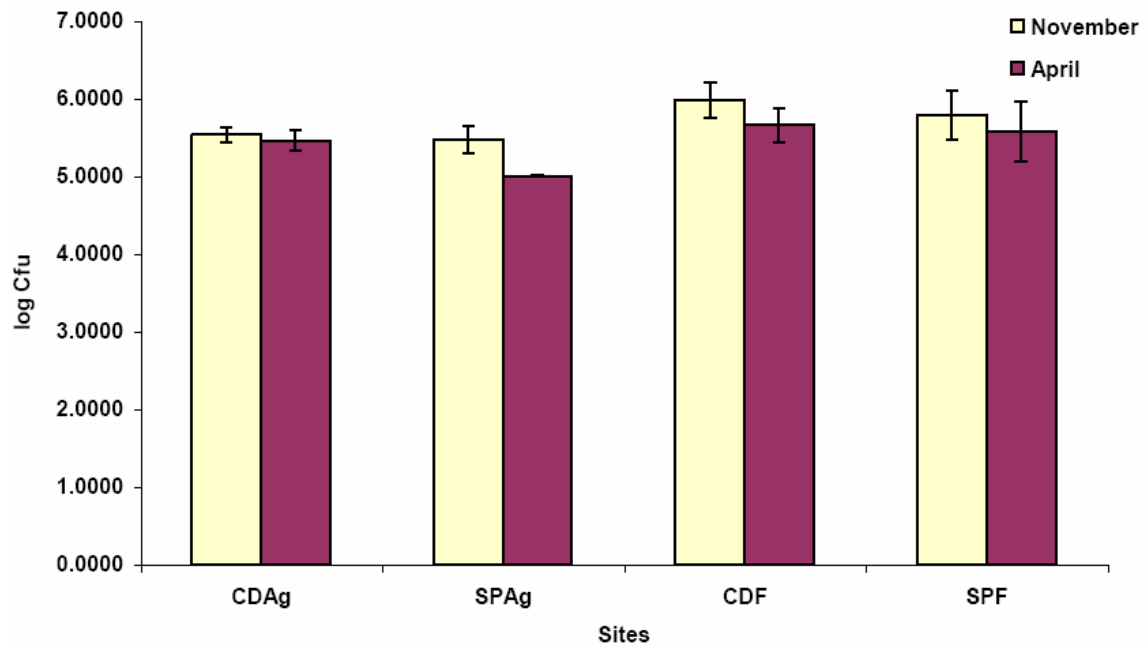


Fig. 4.11: P-Solubalizers (Bacteria)

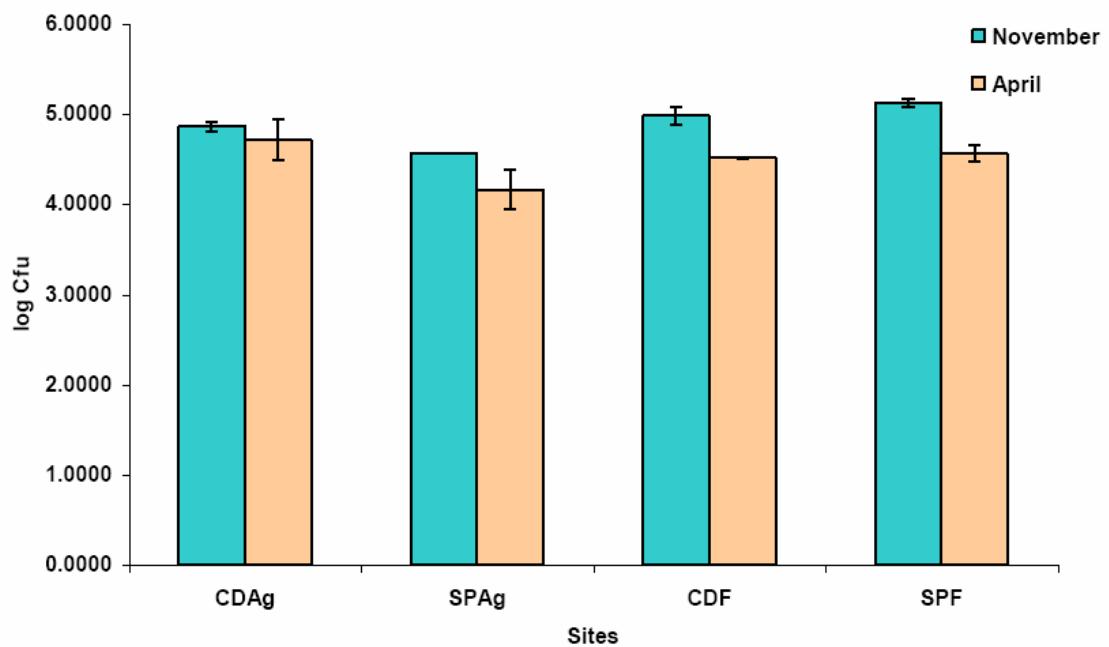


Fig. 4.12: P-Solubalizers (Fungi)

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

of the two sites were compared the results were non-significant. (Fig 4.11 and Fig 4.12).

Siderophore producing bacteria were more in the month of November than in April at both the sites for both the ecosystems. (Fig 4.13).

Cultural diversity in November and April

(Fig 4.14 and Fig 4.15 respectively). Both the agriculture sites had more cultural diversity in November, whereas a reverse trend was seen in favour of forests in April. Sultan nagari agroecosystem was richer in microbial population in November but results were non-significantly different from Chanda Devi agroecosystem. Whereas in April, Chanda Devi had more microbial population than the Sultan nagari agroecosystem. The forests at both the sites when compared to the agroecosystems had more cultural diversity in April (but results were non-significantly different from one another).

Enzyme Diversity

A trend similar to that of cfu diversity was observed in November where both agriculture sites showed more enzymatic activity than the forest. Sultan nagari agroecosystem had the highest enzyme diversity in November whereas Chanda Devi agroecosystem had the highest diversity for the month of April. (Fig. 4.16 and Fig. 4.17)

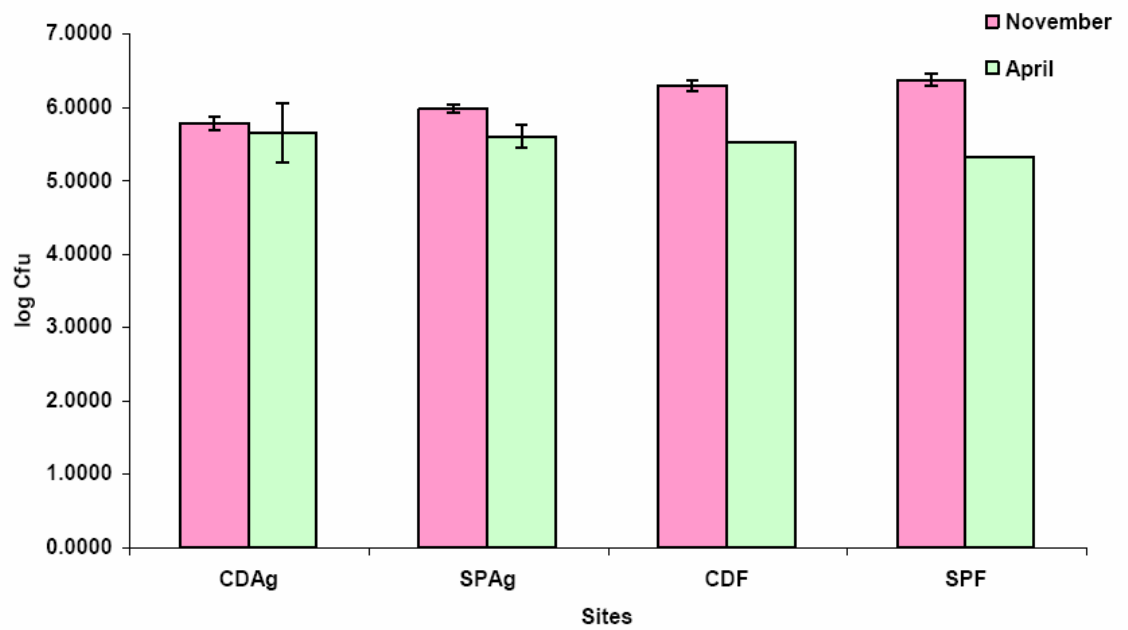


Fig. 4.13: Siderophore producing bacteria

CD= Chanda Devi
SP= Sultan nagari
Ag = Agriculture
F= Forest

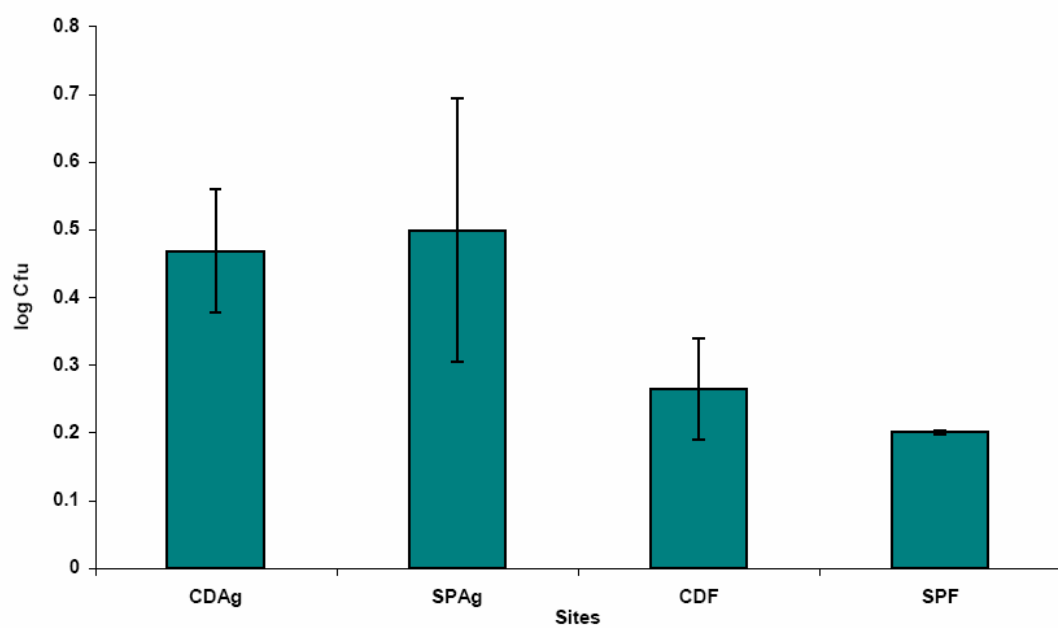


Fig. 4.14: Cultural diversity in November

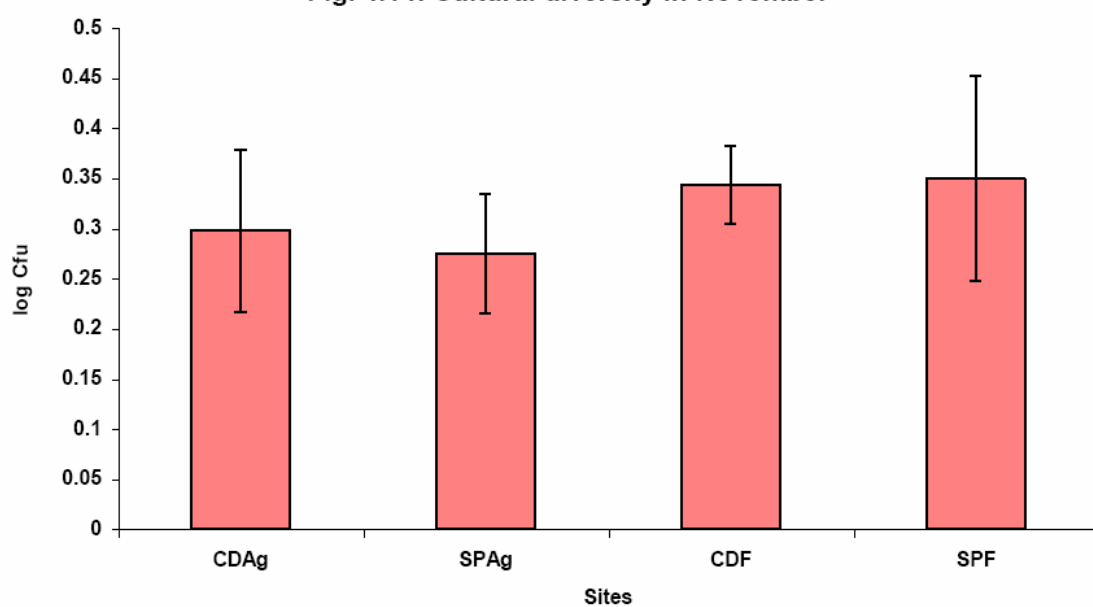


Fig. 4.15: Cultural diversity in April

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

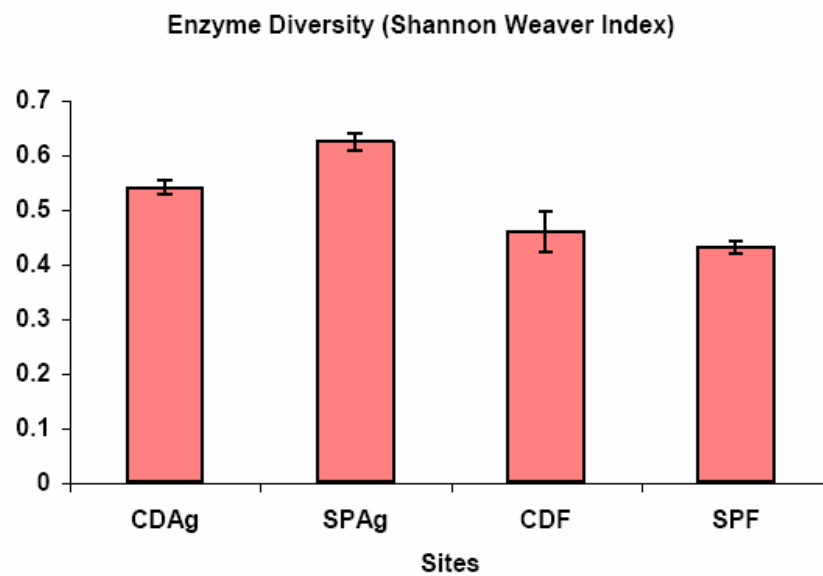


Fig. 4.16: Enzyme Diversity in November

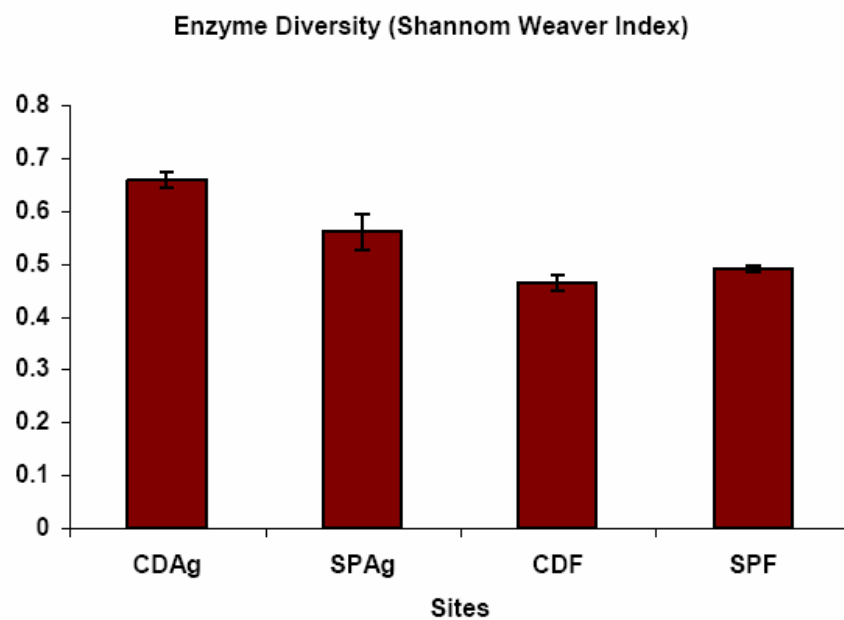


Fig. 4.16: Enzyme Diversity in April

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

As compared to the forests more enzymatic activity was observed in the agroecosystems; but a decline in the enzyme activity was observed at the Sultan nagari agriculture site in the month of April.

Dehydrogenase and Urease activity

For both the seasons dehydrogenase activity was more in forest ecosystems at both the sites. In April activity was comparatively more than the month of November. Chanda Devi showed the least dehydrogenase activity for both the seasons (Fig 4.18). Whereas urease activity was observed to be more in Chanda Devi agriculture site in April as compared to the forest at same altitude. In November whereas urease activity was less in the Chanda Devi agroecosystem it was found to be high in Sultan nagari agroecosystem. April had more urease activity but for the exception of Sultan nagari agroecosystem. (Fig 4.19).

Acid and Alkaline phosphomonoesterase

Maximum acid as well as alkaline phosphomonoesterase activity was observed in Sultan nagari forest (Fig 4.20 and 4.21 respectively) in the month of April. Chanda Devi agroecosystem had more acidic phosphomonoesterase activity than the forest at same altitude in April as well as in November. Whereas Sultan nagari forest had more activity of the same enzyme than its corresponding agroecosystem in April.

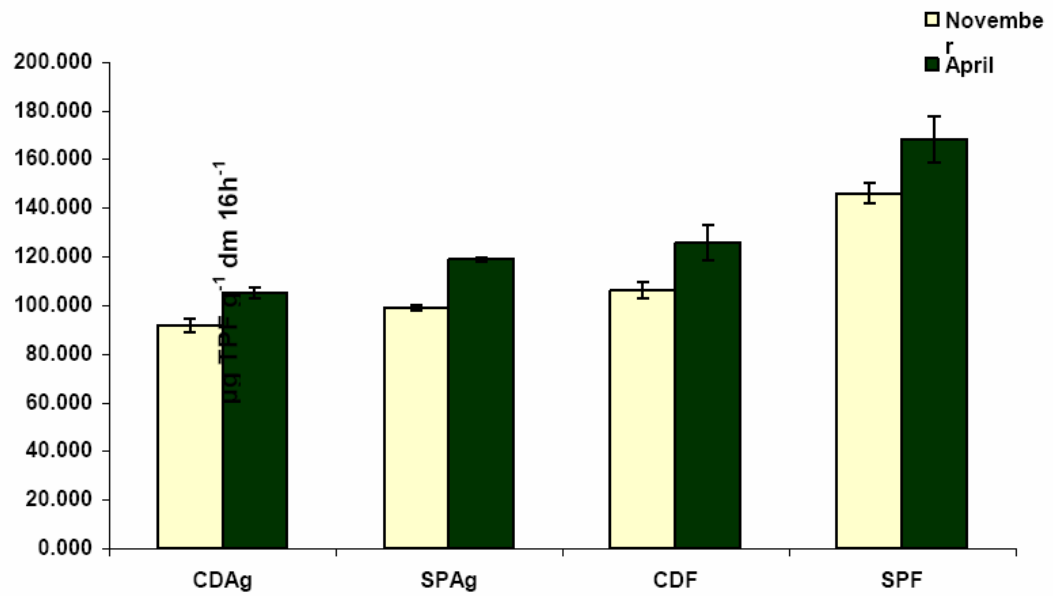


Fig. 4.18: Dehydrogenase Activity at different sites in two season

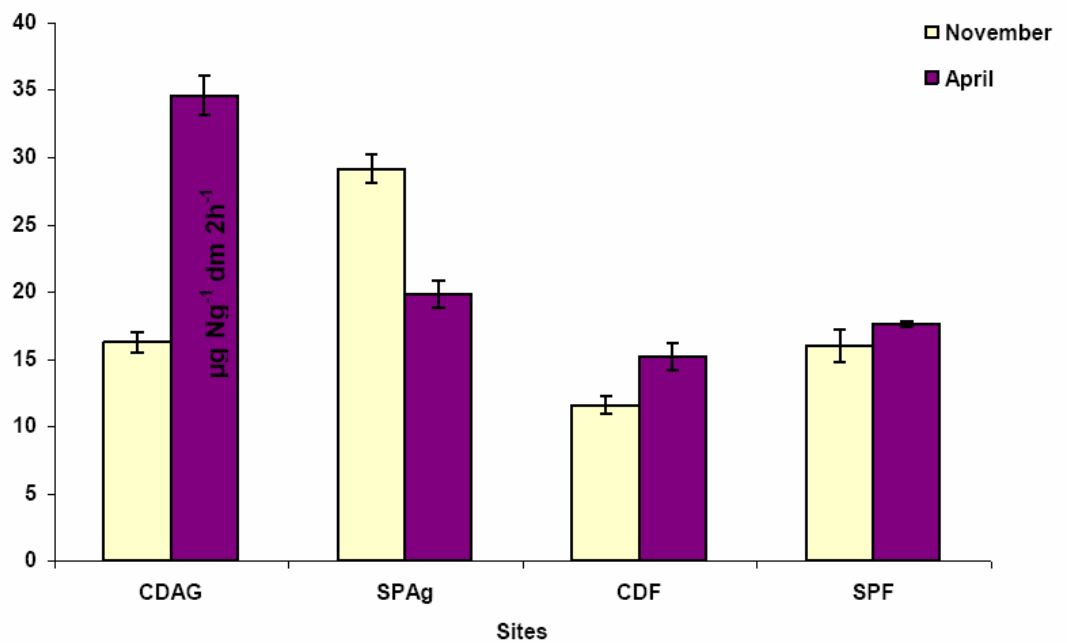


Fig. 4.19: Urease Activity at different sites

CD= Chanda Devi
 SP= Sultan nagari
 Ag = Agriculture
 F= Forest

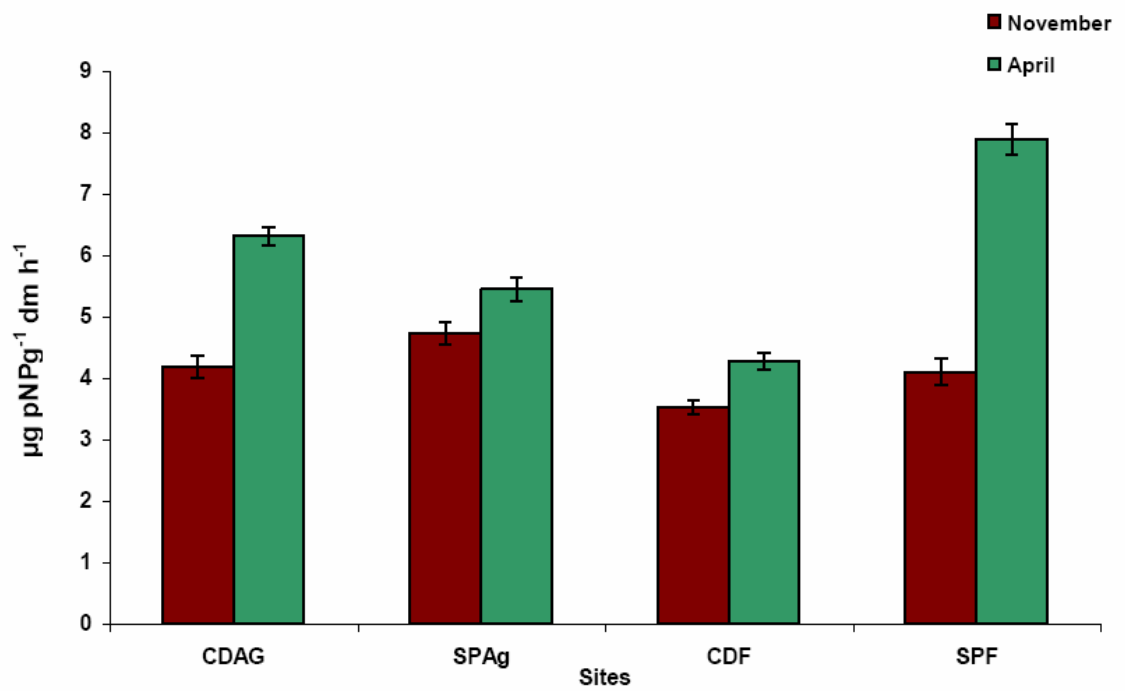


Fig. 4.20: Acid Phosphomonoesterase at different sites

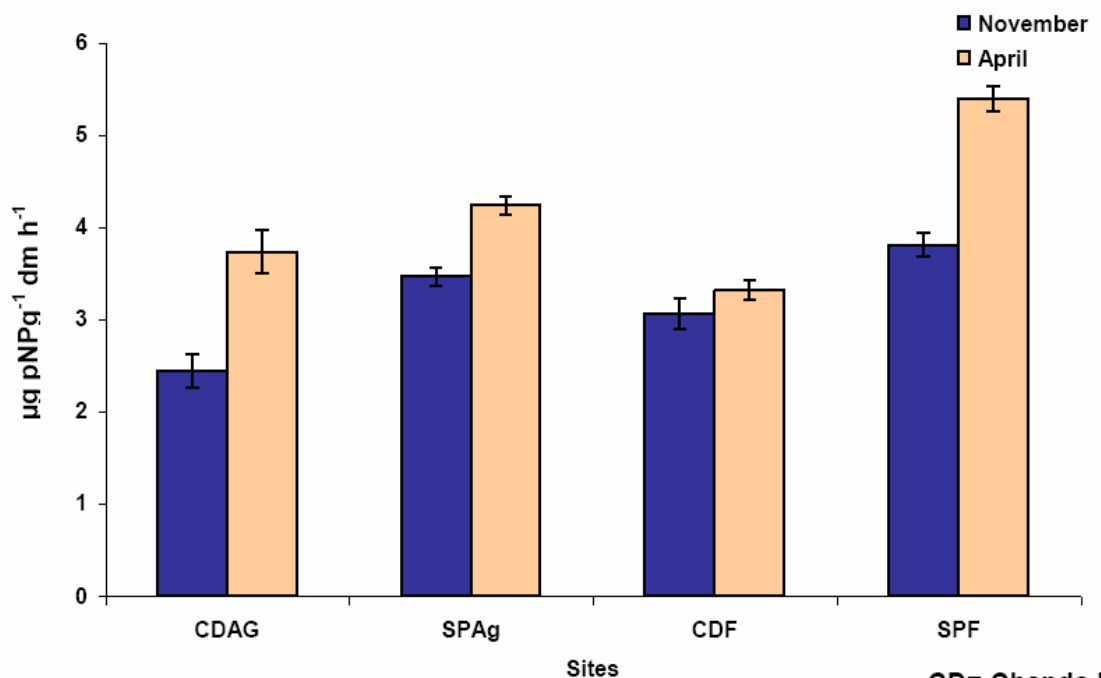


Fig. 4.21: Alk Phosphomonoesterase at different sties

CD= Chanda Devi
SP= Sultan nagari
Ag = Agriculture
F= Forest

For alkaline phosphomonoesterase Chanda Devi agriculture land had more activity than the forest for April whereas in November the reverse occurred.

FAMEs

The results for the fatty acid methyl esters profile are tabulated in Table 4.2. Evenness, Richness and Diversity indices are given.

Maximum richness and diversity was observed in Sultan nagari agroecosystem (2.708 and 1.569 respectively) in the month of November. Maximum evenness was seen in Chanda Devi forest (0.983) in the month of April (Table 4.2).

The least value of richness was observed at Chanda Devi forest as well as Sultan nagari forest in April (Table 4.2).

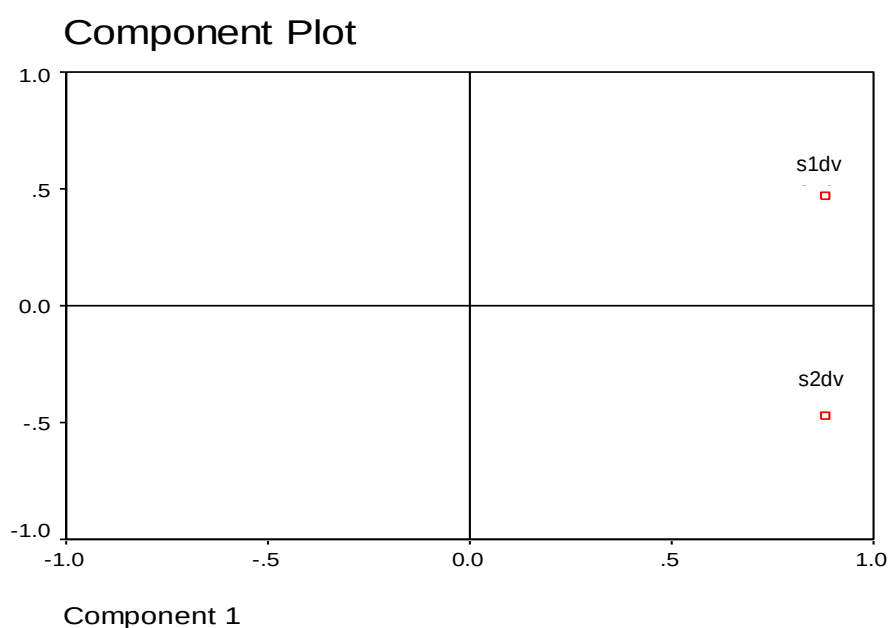


Fig. 4.22: Principal Component Analysis

PC I	77.91%
PC II	22.09%

Table 4.2: Shannon Weaver Indices to study FAMEs samples

	Diversity	Richness	Evenness
Nov. S1 Ag	1.107	2.079	0.532
Nov. S1F	1.168	1.386	0.843
Ap S1 Ag	0.644	1.946	0.331
Ap S1 F	1.080	1.099	0.983
Nov S2 Ag	1.569	2.708	0.580
Nov S2 F	1.124	1.792	0.627
Ap S2 Ag	0.837	1.792	0.467
Ap S2 F	0.760	1.099	0.692

S1= Chanda Devi

S2= Sultan nagari

Ag= Agriculture

F= Forest

Ap= April

Nov= November

5. Discussion

The present study was done with the objective of comparing soil microbial diversity in forest and agroecosystem at two different altitudes in the hills of Kumaon. The results obtained are discussed as follows-

The culture dependent techniques employed proved the agroecosystem at Chanda Devi to be richer in microbial diversity than the forest present at the same altitude. Whereas the Sultan nagari forest was richer in microbial diversity than the agricultural land at the corresponding altitude (550 m). The results indicate that the traditional agriculture practiced in hills (organic manuring, low tillage and no chemical use) might have made the soil richer in nutrients and hence as a result of abundant substrates the microbes could thrive better in the agricultural land at Chanda Devi (850 m).

The results of the culture dependent techniques were in accordance with the chemical analysis results. The organic carbon % was higher in the Chanda Devi agroecosystem as compared to the forest and this could have resulted in an increase

in microbial population. **Kennedy and Gewin (1997)** also observed that carbon serves as a substrate for microorganisms and they need sources of energy and carbon to carry out their functions.

The amount of phosphorus and potassium was also found to be greater in Chanda Devi agroecosystem than the forest. Thus showing that traditional agriculture practices have made the fields nutritionally balanced which resulted in high microbial diversity.

Similar results in favour of organic farming were obtained by **Mader et al, (2002)**. They reported a strong and significant increase in microbial diversity in an organically managed system.

The decrease in microbial population in the natural or conserved ecosystem at Chanda Devi forest might be due to unavailability of substrates for microbes as also observed in the chemical analysis results.

In the month of November an overall increase in microorganism population was observed (Fig 4.14). A possible explanation for this might be the rhizospheric influence on microbial activity. As in November, sowing of wheat was done and as microbial activity could be high during initial plant growth stages, therefore microbial populations could have showed an increase (Fig 4.14 and 4.15).

At the Sultan nagari site the forest was richer in organic carbon as compared to the agroecosystem. Similar were the results of plating done to get total microbial counts i.e. more population as well as diversity was observed in the forest of Sultan nagari.

The Sultan nagari agroecosystem as affected by weedicide (Pendimethylene) and Bavistin (fungicide) and chemical fertilizer use (Urea, di-ammonium phosphate) was microbially less diverse than the forest at the same altitude. Ploughing done in the agricultural land might also be reason for decrease in soil organic matter and thus reduced microbial diversity.

White (1997), made a similar observation. According to him ploughing and cultivation is usually accompanied by a decline in soil organic matter (SOM).

A particularly marked observation was made regarding fungal communities in all the ecosystems. The fungal populations were more or less stable and no major variation in population was observed in both the parameters; either season or land management practices.

The results strongly indicate that the fungal communities are stable as compared to bacteria, which showed a decline or increase in populations in response to the various parameters

studied. Results similar to this were observed by **Hagn *et al*, (2003)**. The only difference being that they had employed culture independent (i.e. molecular) techniques for their study. They also observed that fungal population in soil are overall stable and are not easily susceptible to land management changes. That the bacterial communities are easily influenced by seasonal changes was observed by **Schlöter *et al*, 2003**.

Phosphate solubilizers were more in the forest sites for both seasons, similar results were obtained for the alkaline phosphomonoesterase activity. A possible reason for this might be high amount of bound phosphates present in forest soils. Due to the presence of abundant specific substrate the specific microbes might be present to a greater degree in forest soils.

Both amylase and xylanase producing microbial population flourished in the agroecosystems, wherever the results varied they were mainly non-significantly different from the agriculture land.

The agriculture ecosystem had a greater share of cellulase producing bacteria than the forest, which could be due to the lack of particular substrate for cellulase enzyme in the forest. In agriculture land the manure added might be more easily degradable than the plant leaf litter present in forests i.e., farmers provide readymade substrate to microbes in the form of manure.

In April overall cultural diversity in microbes was less than that in November, but the reverse was observed in the case of enzyme diversity.

Enzyme diversity also showed that agroecosystems in hills of Kumaon had greater microbial diversity similar to the colony forming units observations. A contradiction to the cultural diversity results was the greater enzyme diversity in Sultan nagari agroecosystem. **Dick (1992)**, observed that use of inorganic fertilizers could increase soil biological activity because of an increased plant biomass production which on incorporation might increase soil biological activity. Infact he reported that there is little evidence suggesting that repeated applications of inorganic fertilizers could suppress production of certain soil enzymes involved in cycling of nutrients.

The cultural diversity results of Sultan nagari showed forests to be richer, but the enzyme diversity results were in confirmation with **Dick(1992)**.

These contradictions between enzyme activity results and cultural analysis might have arisen due to the fact that only a fraction of microorganisms in nature are cultivable. (**Cavigelli et al.,1995**)

Dehydrogenase activity which is a reliable index of microbial activity in soil (**Stevenson, 1959**), was found to be more in forest

soils, reason might have been more organic content in the forest due to litter fall.

Urease activity was high in agriculture ecosystems of the two sites. A possible explanation for this might be the addition of differential nitrogenous fertilizers and manure providing direct substrate to the microbes. Sultan nagari showed high urease activity which could be a result of urea being added to soil.

Forest showed high acidic and alkaline phosphomonoesterase activity which is a result similar to cultural phosphate solubalizers.

FAMES

The FAMES analysis showed results, which varied from the results obtained from cultural analysis. This clearly indicates the differences arising between culture dependent and culture independent techniques.

Diversity and richness showed maximum values in Sultan nagari agroecosystem and maximum evenness was observed in Chanda Devi forests.

In few of the samples for e.g. Chanda Devi forest(April) and Sultannagari forest(April), the results could not be found satisfactory due to low recovery of methyl esters.

6. Summary

The hilly areas of the newly formed state of Uttarakhand are untouched by modern agriculture. The following investigation was carried out with a view of comparing this traditionally managed agriculture ecosystem with the natural or conserved ecosystem. The estimation of soil health of the agricultural land as well as the forest at altitudes of 850 m and 550 m were also part of the aim of the investigation. Culture dependent techniques (dilution plating for total cultural population study as well as plate enzyme assays) and culture independent techniques (Soil enzyme activity, FAMEs profiles) were employed to study the microbial diversity. The major results of the investigation were-

1. The results of chemical analysis show the nutritional richness of agroecosystem over forest ecosystem at the traditionally managed Chanda Devi site. Here the maximum Potassium and Phosphorus values were observed (385 kg/ha and 54 kg/ha, respectively). Organic carbon % was also high (1.13%) as compared to the forest ecosystem (0.65%).

2. Agroecosystem at Chanda devi was comparatively richer in microbial diversity than the forest.
3. The forest at Sultan nagari was richer in microbial diversity than the agricultural land. The chemical analysis results for the forest ecosystem were-Phosphorus (18.0 kg/ha), Potassium (171 kg/ha) and organic carbon % (1.16%). The agroecosystem comparatively was poor in these nutrients (Table 4.1).
4. The month of November exhibited more microbial activity than April for both the sites.
5. Agroecosystems were richer in cellulase, xylanase and amylase producing microbes with a few exceptions.
6. P-solubilizing microbes were more in the forest ecosystem, whereas an uneven trend was observed for siderophore producing bacteria.
7. Enzyme diversity was observed more in agroecosystems than forest. However when individual enzymes were assessed, activity of the enzyme dehydrogenase was more in the forest in contrast to urease, which was more in the agricultural sites. At Sultan nagari acidic and alkaline phosphomonoesterase showed higher substrate utilization in the forest ecosystem. In April higher enzyme activity for

dehydrogenase, acidic phosphomonoesterase and alkaline phosphomonoesterase was observed as compared to the month of November.

8. FAMES profiles showed maximum richness in (Sultannagari) agricultural land.

The preliminary results from the present study indicate that traditional agriculture as followed in the mountains is favourable for the microorganisms who are important indicators of soil health. This proves that the soil is nutritionally rich in the hilly region and integration with modern agriculture (minimum tillage, judicious fertilizers use) might prove beneficial in solving the food problems of the people inhabiting these regions. Therefore further research is needed to understand the behavior of microbial population where molecular techniques can help to evaluate the diversity in a much precise manner.

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*** original not seen**

Appendix I

Poisoning solution used for herbaria specimen preservation:

Magnesium chloride	32 g
Alcohol	500 ml

Media for bacterial diversity gL⁻¹

High Plate Count Agar (HPCA)

Peptone	3.00
Soluble casein	0.50
Dipotassium phosphate	0.20
Magnesium sulphate	0.05
Iron (III) chloride	0.001
Agar	20.0
D.W	1L
Nystatin (antifungal)	75µg/ml
pH (at 25° C)	7.0±0.2

Media for fungal diversity

Potato dextrose agar (PDA) gL⁻¹

Potato extract	20.0
Dextrose	20.0
Agar	20.0
D.W	1L

pH	7.0±0.2
Streptomycin (antibacterial)	75µg/ml

Basal Media for cellulase, xylanase, amylase, protease production gL⁻¹

Yeast extract	0.5
Potassium nitrate	0.5
Sodium chloride	2.0
Dipotassium phosphate	2.0
Magnesium sulphate heptahydrate	0.05
Calcium carbonate	0.02
Ferrous sulphate heptahydrate	0.01
Agar	20
D.W	1L
pH	7.0±0.2

Basal Media was amended for

Xylanase – 1% xylan

Cellulase – 0.5% CMC

Amylase – 0.7% starch

Protease – 1% skimmed milk

Media for siderophore production

Basal Media (Nutrient Agar) gL⁻¹

Beef extract	3.0
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Peptone	5.0
Agar	20.0
D.W	1L
pH	7.0±0.2

Chrome Azurol's CAS agar medium

a. Dye solution

Chrome – azurol's	60.60 g
D.W.	50 ml

b. 1mM FeCl ₃ .6H ₂ O in	10 ml
10 mM HCl	10 ml

c. Hexadecyl trimethyl ammonium bromide 72.90 mg

d. D.W. 40 ml

Solution (a) was added to solution (b) and the resulting solution was added to solution (c). Dark blue solution was autoclaved and added to nutrient agar (autoclaved) before pouring. (200 ml Nutrient Agar+100ml dye)Recheck pH (pH7.0)

Media for phosphate solubilization gL⁻¹

Pikovskaya's Agar

Yeast extract	0.50
Dextrose	10.0
Calcium phosphate	5.00

Ammonium sulphate	0.50
Potassium chloride	0.20
Magnesium sulphate	0.10
Manganese sulphate	0.0001
Ferrous sulphate	0.001
Agar	20.0
D.W	1L
pH	7.0

Reagents for enzymatic activity

Urease

- Substrate solution – (79.9mM urea solution)
- Potassium chloride solution (2M)
- Sodium hydroxide solution (0.3M)
- Sodium salicylate solution (1.06M)
- Sodium dichloroisocynurate solution (39.1 mM)
- Standard stock solution (1000 $\mu\text{g NH}_4^+ \cdot \text{N} \cdot \text{ml}^{-1}$)

Dehydrogenase

- Tris buffer (0.1M, pH as per pH of soil i.e. 7.6)
- Substrate solution as per soil texture (2% TTC)
- Standard stock solution (10 mg TPF ml^{-1})
- Working standard solution (0.1 mg TPF ml^{-1})

Acid and alkaline phosphomonoesterase

- Substrate solution (p-nitrophenyl phosphate hexahydrate solution, 115 mM) in corresponding working buffer.
- Modified universal buffer solution. Tris (hydroxymethyl) aminomethane + Maleic acid + Citric acid monohydrate + Boric acid in 1M NaOH + D.W. (Store at 4° C).
- Working buffer solution for acid phosphomonoesterase (pH 6.5)
- Working buffer solution for alkaline phosphomonoesterase (pH 11)
- Calcium chloride solution (0.5M)
- Sodium hydroxide solution (0.5M)
- Working standard (20µg p-nitrophenol ml⁻¹)
- Standard stock solution (1 mg p-nitrophenol ml⁻¹)

VITA

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Abstract

Soil microorganisms are one of the most prominent biological factors that affect 'soil health' and thus above ground productivity. Any change in their population can be used to predict changes in not only the soil but also the ecosystem. Thus an estimation of microbial count and activity can help us in gaining a better understanding of various ecosystems.

The present study was undertaken to compare soil microbial diversity in a forest and an agroecosystem in the hilly areas of Kumaon. Both cultural and culture independent methods have been employed to study the microbial population.

The experimental results revealed greater enzyme activity as well as greater total viable counts (TVC) in the agriculture ecosystems. These results indicate that the traditional agriculture or low input agriculture as practised in the hills allow the soil microbial community to flourish.

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