STUDIES ON ECOLOGY OF EDAPHIC MICROBIAL POPULATIONS AND THEIR ACTIVITIES IN MAIZE FIELDS

(Abstract)

By

MISS MAMTAJ S. DKHAR

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY

To

DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES
NORTH-EASTERN HILL UNIVERSITY
SHILLONG-793014
INDIA

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Three maize fields differing in agricultural systems were selected for the present study. They were terrace land, valley land and jhum land agricultural systems. Analyses of various physico-chemical characters of the soils of all the three agricultural fields reveal that in general the nutrients followed a trend valley land > terrace land > jhum land. Both quantitative and qualitative assessment of soil microflora and their various activities viz., dehydrogenase and urease enzyme activities and carbon dioxide evolution measurement were carried out at monthly intervals for two crop cycles of maize (Zea mays L.). Results demonstrate that the microbial populations and their various activities also followed the trend valley land > terrace land > jhum land. Qualitatively, the composition of mycoflora was found to be almost similar in all the three agricultural fields. Penicillium chrysogenum, Trichoderma viride, Aspergillus niger and Mucor hiemalis were found to be of frequent occurrences in all the study sites. Pythium sp. was rarely obtained from the soil of jhum land agriculture whereas, this species was commonly isolated from the soil of valley land and terrace land agricultures. Zygorhynchus sp. and Paecilomyces sp. could be isolated only from the soil of terrace land agriculture. Phoma humicola and Endomyces sp. were restricted to valley land soil. Blakeslea sp., Cunninghamella echinulata and Gongronella sp. could be

isolated from the jhum land soil only.

Relationships between dehydrogenase and urease activities, carbon dioxide evolution, microbial population and physico-chemical characters of the soil were worked out. Statistically no significant correlation was obtained between dehydrogenase activity and microbial populations. However, a positive correlation was obtained between carbon dioxide evolution and fungal population. Urease activity was positively correlated with moisture content, bacterial population, organic carbon and total nitrogen.

Decomposition of maize litters in field condition was studied using litter bag method for a period of seven months. It was found that the rate of decomposition was largely influenced by the types of litter. The rate of decomposition of leaf was more than that of stem and root. The weight loss of all the litters followed Olson's (1963) negative exponental model. Both fungal and bacterial populations were minimum at the early stages of decomposition coinciding with the slow rate of decomposition at the time and attained maximum at the later stages of decomposition. Qualitatively, composition of mycoflora was almost similar in case of all the three types of litters. In decomposed litters, Trichoderma viride, Penicillium chrysogenum, Fusarium moniliforme, Absidia glauca and Mucor hiemalis were the most common fungal species. The pattern of change in cellulose, hemicellulose,

lignin, sugar and amino acid was almost similar for all the litters. Loss in sugar and amino acid was more rapid and the lignin content of the litters degraded at the slowest rate. Increment in the total nitrogen was noted in all the litters which declined towards the end of the experiment.

Under laboratory condition, the rate of litter decomposition by Trichoderma viride and Absidia glauca was very slow as compared to that under field condition. Fastest rate of decomposition was observed in the set amended with mixed culture of Trichoderma viride and Absidia glauca and slowest rate was recorded in case of Absidia glauca alone.

The study of the mycoflora of gut contents and casts of earthworm did not show difference in species composition. It was found that fungal numbers was maximum in fore-gut and exhibited a decreasing trend towards the distal region of the gut canal while the number of bacteria and actinomycetes increased towards the distal end of the gut canal. Penicillium chrysogenum, Fusarium moniliforme, Geotrichum candidum and Aspergillus niger were found to be present throughout the gut canal. Casts of the earthworm contained always higher number of microorganisms (fungi, bacteria and actinomycetes) than the surrounding soil.

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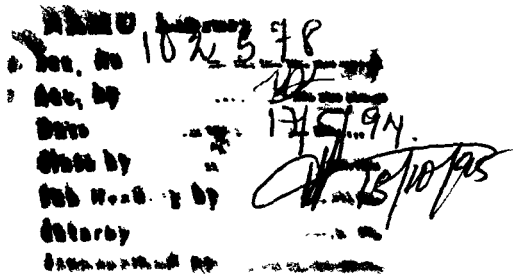

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I certify that the thesis entitled "Studies on Ecology of Edaphic Microbial Populations and their activities in Maize fields" submitted by Miss Mamtaj S. Dkhar for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

R.R. Mishra 28/7/83

Signature of the Supervisor

Place: Shillong

Date : 28th July, 1983

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I am greatly indebted to my parents who have been the constant source of inspiration for me.

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Place: Shillong

Date: 28th July, 1983

(Miss Mamtaj S. Okhar)

CONTENTS

GENERAL INTRODUCTION

North-Eastern region of India is unique in many respects such as, rich floristic composition, high annual precipitation, undulated topography and varying types of agricultural systems. This region experiences high rainfall of a monsoonic type which is followed by a dry winter and a brief summer. Maize (Zea-mays L.) is an important staple crop of this region. Sowing period of maize is generally during last week of April to first week of May. Normally harvesting is done in the month of August. Three types of agricultural systems are generally practised for the cultivation of maize. The systems commonly practised are slash and burn type of shifting agriculture (Jhum), valley land and terrace land agricultures. Shifting agriculture locally known as 'Jhum' is extensively practised by the tribal people of north-eastern hill region of India. It is the most primitive type of agricultural systems. The system consists of felling of the forests at various stages of development followed by drying and burning of the slash before cropping. One almost universal feature is the mixed cropping. There may, however, be a relatively high proportion of one crop in the mixture. Almost total reliance is placed entirely on the bush fallow for the maintenance of fertility, no fertilizers or manures are generally applied. Minerals added to the surface soil in the ash provide nutrients to the crops. The amount of the nutrients contributed

by the ash depends on the soil type, the nature and development of the vegetation and also on the degree of burning. Part of the ash is lost by erosion due to heavy rainfall and wind (DeBano and Conrad, 1976). Heavy loss of nutrients takes place during cropping, particularly in the absence of crop cover at the time of sowing (Ramakrishnan and Toky, 1981). Burning of plants also affects the microbial community of the ecosystem and the physico-chemical characteristics of the soil. Depending upon the degree of burning, the microbial population is completely or partially destroyed. In the sterile soil environment thus exposed for the colonization, the nature of microflora appearing therein will depend upon the physico-chemical status of the habitat. The soil being devoid of microbes in the beginning, no resistance is offered for the new colonizers. In this process a number of forms from different sources will try to colonize the substrate available without competition. The possibility sometimes exists for the introduction and establishment of certain pathogenic forms which may prove hazardous for the plant species growing in the area. Elimination of certain, otherwise, useful saprophytes may also be expected.

Valley land agriculture is the type of the system followed in plains of valleys. The soil of this system is generally rich in nutrients which are transported from adjacent hills through erosion. It possesses high water retention capacity.

Terrace land agricultural system is comparatively of recent introduction and therefore, relatively less proportion of land is under this type of system. The cultivation is done on terraces of the hill slopes. In this system, soil erosion is less as compared to that of the shifting agriculture.

Agricultural activities of man greatly modify the distribution of soil microbes. Hattori (1973) observed that ploughing affects the bacterial population markedly; the number may increase twenty to thirty times the normal level in a few days after ploughing. Microbial numbers again fall off to a normal level within a few weeks. Yen and Sheridan (1980) observed that soil types and vegetation cover are also important in determining the density and composition of the microflora in agricultural soils. Among various factors which influence the distribution and abundance of microorganisms in agricultural soils are organic matter, soil reaction, moisture, temperature, nitrogen, phosphorus and potassium contents, and also the nature and duration of cropping (Mishra and Kanaujia, 1972). Soil depth is another important factor which influences the microbial population (Selvaraj and Ramaswami, 1978). Formation of soil aggregates is promoted by cultivation and the soil becomes more favourable habitat for various microbes. Microbes require optimum moisture content and good aeration for maximum population.

For better understanding of the activity of microbes in

agricultural soils, the measurement of respiratory activity in relation to microbial biomass as well as the environmental factors are of great importance (Seto and Tazaki, 1975). Respiratory measurement by carbon dioxide evolution method has widely been used for estimating the general biological activity of soils (Wagner, 1975). Enzymatic activity is also considered to be an important indicator of the total biological activity and also the fertility of soil. Thus there appears to be a direct relationship between soil respiration, the number of microorganisms, and the enzymatic activity. Dehydrogenase enzymes are considered to play an important role in the initial stages of oxidation of soil organic matter by transferring hydrogen or electron from substrates to acceptors. This aspect has been studied by several workers since its introduction by Lenhard (1956). It is a sufficiently reliable method of measuring metabolic activity of the soil microorganisms.

The increase use of urea as a fertilizer has led many workers to study its fate in soil and its relationship with the nature of soil. Urea added to soils as fertilizer or as animal urine is hydrolyzed enzymatically by soil urease and the resulting release of ammonia coupled with increased pH may lead to several problems including damage to germinating seedlings (Gasser, 1964; Hutchinson and Viets, 1969). In order to overcome these problems such researches are required to collect sufficient information

concerning the distribution of urease activity in soil profiles and the factors affecting such activity.

Considerable attention has been paid to the influences of crop residues and their decomposition products on crop plants and their diseases. The rate of decomposition and the substances released may be affected by changing the nature of the soil environment by agricultural systems. The dead bodies of plants and animals provide substrate for the growth of the soil micro-organisms. The microorganisms during decomposition release minerals which are locked up in the body tissues of the plants and animals. The minerals thus released, are again utilised by living organisms. Inspite of a great deal of work on the decomposition of plant parts by microorganisms (Hudson and Webster, 1958; Webster and Dix, 1960; Witkamp, 1966a; Mishra, 1979), the succession of fungi and bacteria on decaying plant parts in relation to micro-climate and biological competition has not been properly investigated in Indian agriculture.

Earthworms are known to exert beneficial effect to the soil and they help in maintaining the soil fertility. The pioneer work of Darwin (1881) established the importance of earthworms in the fertility of soil. Earthworms improve aeration, water retaining capacity and nutrient status. They also enhance decomposition by mechanical breakdown and are chief agents for the crumb structure and mull formation in soils (Satchell, 1967;

Edwards and Lofty, 1972). In many ecosystems, such as pastures, crop fields, the earthworms constitute a large percentage of soil faunal biomass. There exists an interaction between earthworm and the soil microflora. Earthworms may affect the soil microbial community by consuming the latter as their food. Their tunnel making habit helps to keep soils open and porous thereby making favourable habitat for microbes. They graze over soil microflora and the grazing activity perhaps enhance the growth of microflora in soil by preventing senescence (Dash et al., 1979). Earthworms ingest food material at one place and lay casts at another place. Casts act as foci from which soil microorganisms can spread into surrounding soil (Lofty, 1974; Dash et al., 1979).

For proper management and economic exploitation of agricultural lands, knowledge on the microbial population and its various activities is of fundamental requisite. The decomposition study of the crop residues is important for better understanding of the organic matter dynamics and this may be exploited for the rapid decomposition and quick nutrient cycling. The importance of earthworms and their casts in soil fertility, their influence on growth of microflora in agricultural soils will give better understanding of their activity. Keeping these in view, the entire work has been carried out under the following heads:

1. Physico-chemical characters of three maize field soils differing in agricultural systems.

2. Soil microflora and their activities.

a) CO_2 evolution from soils.

b) Dehydrogenase and urease activities measurements.

3. a) Rate of decomposition of leaf, stem and root litters of maize under field condition.

b) Decomposition ability of certain dominant fungi under laboratory condition.

c) Estimation of cellulose, hemicellulose, lignin, sugar and amino acid of the decomposing litters.

4. Study of microflora in gut contents and the casts of earthworm.

STUDY AREA AND CLIMATE

Three maize fields differing in agricultural systems were selected for the present study. They were (1) Terrace land agriculture at Upper Shillong (altitude 1600 m., Latitude $25^{o}34^{"}$N and longitude $91^{o}52^{"}$ E) (II) Valley land agriculture at Polo (Shillong) (altitude 1350 m., latitude $25^{o}34^{"}$ N and Longitude $91^{o}52^{"}$ E) and (III) jhum land agriculture at Burnihat which is about 90 Km north of Shillong (altitude 100 m., latitude 26^{o}N and longitude $91^{o}50^{"}$ E). The soil of all the three agricultural systems is red laterite under red loam or brown loam soil type.

The climate of Shillong is cool with winter temperature going down to 4-5° C in the month of January. The lower temperature of winter results into frost which can be seen sometime early in the morning during December, January and February. The maximum temperature goes upto 25.6^{o}C in the month of May. The average maximum temperature is 21.03^{o}C and minimum temperature is 13.45^{o}C. The rainfall is spread over all the month except November, December and January when it is either nil or very less. The average annual rainfall is 214.3 mm. Similarly the average humidity is very high ranging in between 61.0 to 89.9 in a diurnal cycle.

The typical summer season is not found at Shillong. However, based on the meteorological condition the year can be divided into the following seasons:

Spring season: The period from the middle of February upto middle of April covers the spring season which experiences very

high wind velocity with less humidity and moderate temperature.

Rainy season: The rainy season extends from the middle of April to the middle of October. However, the early period of the rainy season is a bit warm representing summer, while the later period of the season is comparatively cool. Due to high rainfall the humidity is also high during rainy season.

Winter season: Winter season starts at the end of October and continues upto middle of February. The average lower temperature during winter is 6°C and maximum is 22°C. The rainfall is very low.

The climate of Burnihat remains hot and humid during May to September. The average maximum temperature is 33.6°C and minimum temperature is 11.5°C. The rainy season extends from May to September with an average annual rainfall of 132.1 mm.

The climate of the year may be divided into the three main seasons:

Summer season: It starts from April to June with high wind velocity and dry and warm weather.

Rainy season: It extends from July to September and is characterized by high temperature and heavy rainfall.

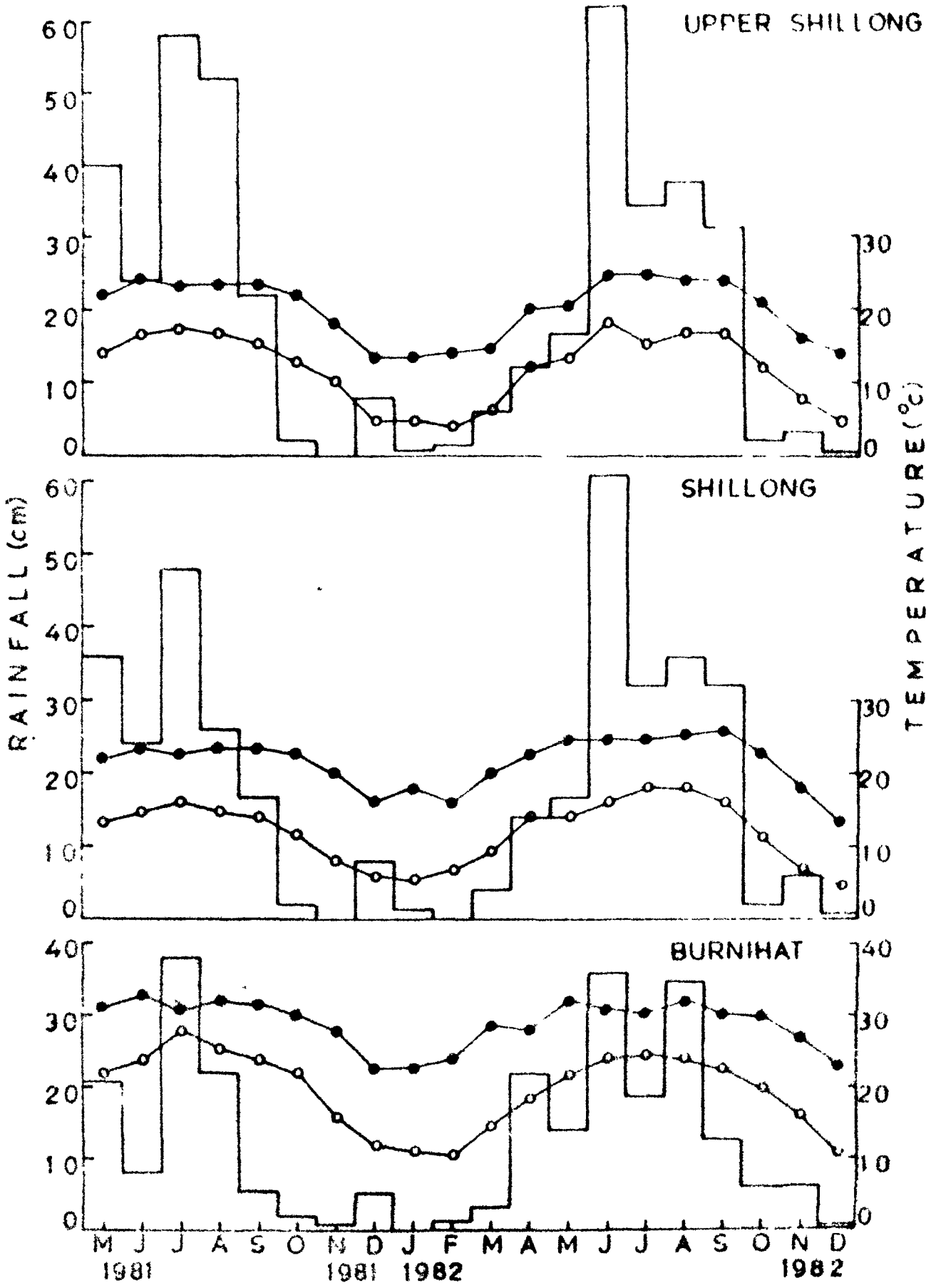
Winter season: There is a mild winter season during December to February. The temperature drops to 11.5°C in the month of January and occassional showers are recorded during this period.

The data of temperature and rainfall during the study period (May 1981 to December 1982) has been presented in Fig.I.

Fig. I Data of the temperature and rainfall during the period of 1981-82.

Histograms: rainfall; Closed circle: maximum temperature; Open circle: minimum temperature.

Fig I


UPPER SHILLONG
SHILLONG
BURNIHAT
RAINFALL (cm)
TEMPERATURE (°C)
0
10
20
30
40
50
60
M J J A S O N D J F M A M J J A S O N D
1981
1981 1982
1982

CHAPTER I

PHYSICO—CHEMICAL CHARACTERS OF MAIZE FIELD SOILS

INTRODUCTION

Soil is the natural medium for the growth of plants and microorganisms. It is formed by the gradual weathering of the parent rock material. The nature and chemical composition of the parent rock have a direct bearing on the fertility of the soil. The physico-chemical characteristics of a soil vary in time and and space. Topography, type of parent rock, climate and the living organisms affect the nature and properties of soil. The fertility of soil depends not only on total nutrient content but also on the qualitative and quantitative composition of micro-organisms inhabiting the soil. Temperature to some extent influences the growth of plants indirectly through its effect on the microbial population of the soil. It may even change the pH of a soil which in turn affects the growth of microorganisms responsible for the conversion of organic forms into inorganic ones. The distribution of soil microbial population is also determined by the crop and type of agricultural practices.

Among various physico-chemical properties of a soil temperature, pH, moisture content, organic carbon, nitrogen, phosphorus and potassium play important role in regulating the population and activity of soil microbes.

Present chapter deals with the monthly and depth-wise variation of various physico-chemical characteristics of three

maize field soils differing in agricultural systems.

MATERIALS AND METHODS

Soil samples from each study site were collected from three depths (0-10 cm, 10-20 cm and 20-30 cm) and the following investigations were carried out:

Temperature was recorded at three depths.

Determination of pH and Moisture Content:

The soil of 10 g was diluted in 50 ml distilled water and stirred for 15 minutes on a mechanical shaker. The pH was read in a electric digital pH meter. For the determination of moisture content, 10 g of soil was dried in a hot air oven at 105°C for 24 hours and the weight was taken. The percentage moisture content was calculated as follows:

$$\% \text{ moisture content} = \frac{\text{Loss in weight on drying (g)}}{\text{Initial sample weight}} \times 100$$

Three replicates were maintained for each determination.

Determination of Organic Carbon:

Walkley and Black's (1934) rapid titration method was followed for the determination of organic carbon. To 1 g of sieved soil (through 0.2 mm sieve) in a 500 ml conical flask 10 ml of $K_2Cr_2O_7$ (1N) and 20 ml of conc. H_2SO_4 were added and left for 30 minutes. The mixture was then diluted with 200 ml

distilled water and then 10 ml of phosphoric acid (85%) was added. Finally it was titrated with $FeSO_4$ (1N) using diphenylamine as an indicator. The percentage organic carbon was calculated according to the formula given below:

$$\% \text{ organic carbon} = \frac{V_1 - V_2}{W} \times 0.003 \times 100$$

where, V_1 = volume of $K_2Cr_2O_7$

V_2 = volume of $FeSO_4$

W = weight of soil (g)

Determination of Nitrogen:

Kjeldahl's method was followed for the determination of nitrogen (Jackson, 1973). To 10 g of sieved soil in a 300 ml digestion flask, 10 ml distilled water was added to moisten the soil. 20 g of Na_2SO_4 mixed catalyst (1kg Na_2SO_4 + 20 g copper sulphate + 3 g mercuric oxide + 1 g selenium dioxide) and 35 ml of conc. H_2SO_4 were added. The digestion was carried out on a digestion unit. At the end of the digestion when the colour of the solution turned greenish yellow, the heating was stopped and the flasks were allowed to cool. The content was diluted with distilled water. The solution was then ready for the determination of ammonium content by distillation.

25 ml of 4% boric acid was taken into a 100 ml conical flask. To this, 2 drops of mixed indicator (0.5 g bromo-cresol green + 0.1 g methyl red dissolved in 100 ml alcohol) were

added. The flask was placed at one end of the condenser fitted with a receiver tube. The tube was dipped in the boric acid in the flask. 10 ml of digestion solution was poured into the distillation flask and then 80% of NaOH was poured drop by drop till the solution became brown in colour. The released ammonia was absorbed in 4% boric acid and titrated with N/14 HCl. The percentage nitrogen was calculated as follows:

$$\% \text{ nitrogen} = (T - B) \times N \times \frac{1.4}{S}$$

where, T = Sample titration

B = Blank titration

N = Normality of acid

S = weight of sample

1.4 is a constant factor.

Determination of Available Phosphorus:

Molybdenum blue method (Allen, 1974) was followed for the determination of available phosphorus. The available phosphorus was first extracted in Ammonium flouride extraction solution. The extract was prepared by mixing 15 ml of ammonium flouride solution (37 g/1000 ml) with 25 ml of HCl (0.5N) and 460 ml distilled water. 4 g sieved soil was taken in a 100 ml conical flask to which 14 ml extraction solution was added and stirred for 15 minutes on a mechanical shaker. The extract was filtered through Whatman No. 44 filter paper. 2 ml of this aliquot was diluted with 5 ml distilled water. To

this, 1 ml of ammonium molybdate and 2 ml of freshly prepared stannous chloride were added and left for 20 minutes to develop the colour. The optical density was recorded at 660 nm in a spectro photo meter. A calibration curve from the standards was used to determine phosphorus in the aliquot. The percentage available phosphorus was calculated as follows:

$$\% \text{ phosphorus} = \frac{C \text{ (mg)} \times \text{Solution Volume (ml)}}{10 \times \text{Aliquot (ml)} \times \text{Sample weight (g)}}$$

Determination of Exchangeable Potassium:

Potassium was extracted in Ammonium acetate solution (pH 7) which was prepared by mixing 575 ml of glacial acetic acid with 600 ml of ammonia solution and diluted to 10 litres with distilled water. The pH was adjusted to 7 ± 0.05 with the help of acetic acid and ammonia solutions. To 5 g of sieved soil, 125 ml of extraction solution was added. It was allowed for constant stirring for one hour and then filtered through Whatman No. 44 filter paper. The exchangeable potassium was read in a flame photometer (Allen, 1974) and converted into known unit through standard graph. The percentage potassium was calculated according to the following formula:

$$\% \text{ potassium} = \frac{C(\text{ppm}) \times \text{Solution volume (ml)}}{10^4 \times \text{Sample Weight (g)}}$$

RESULTS

Temperature:

Maximum temperature was recorded in jhum land soil and the minimum was recorded in the soil of terrace land. In terrace land soil the maximum temperature could be observed in the month of August (1981) and July (1982) and minimum was recorded in the month of May (1981) and September (1982), the range being in between 16-25^{o}C. In case of valley land soil the temperature gradually increased reaching to its maximum in the month of September (1981) and August (1982) and minimum was observed in May (1981) and September (1982). The temperature ranged in between 18-30^{o}C. In case of jhum land it was maximum in the month of August and minimum was recorded in the month of May during the study period of 1981 and 1982, the range being in between 28-42^{o}C. In all the cases, the temperature decreased along with soil-depth (Fig. 1.1).

pH:

pH of the soil was mostly acidic at all the study sites. It was found to be more acidic in case of terrace land soil, the range being 3.5-6.5, while in case of valley land soil it was in between 4.5-7.9. In case of jhum land pH varied between 5.0-7.0. It did not exhibit marked variation along depth (Fig. 1.2).

Fig. 1.1 Monthly variation in temperature (^{o}C) of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·1

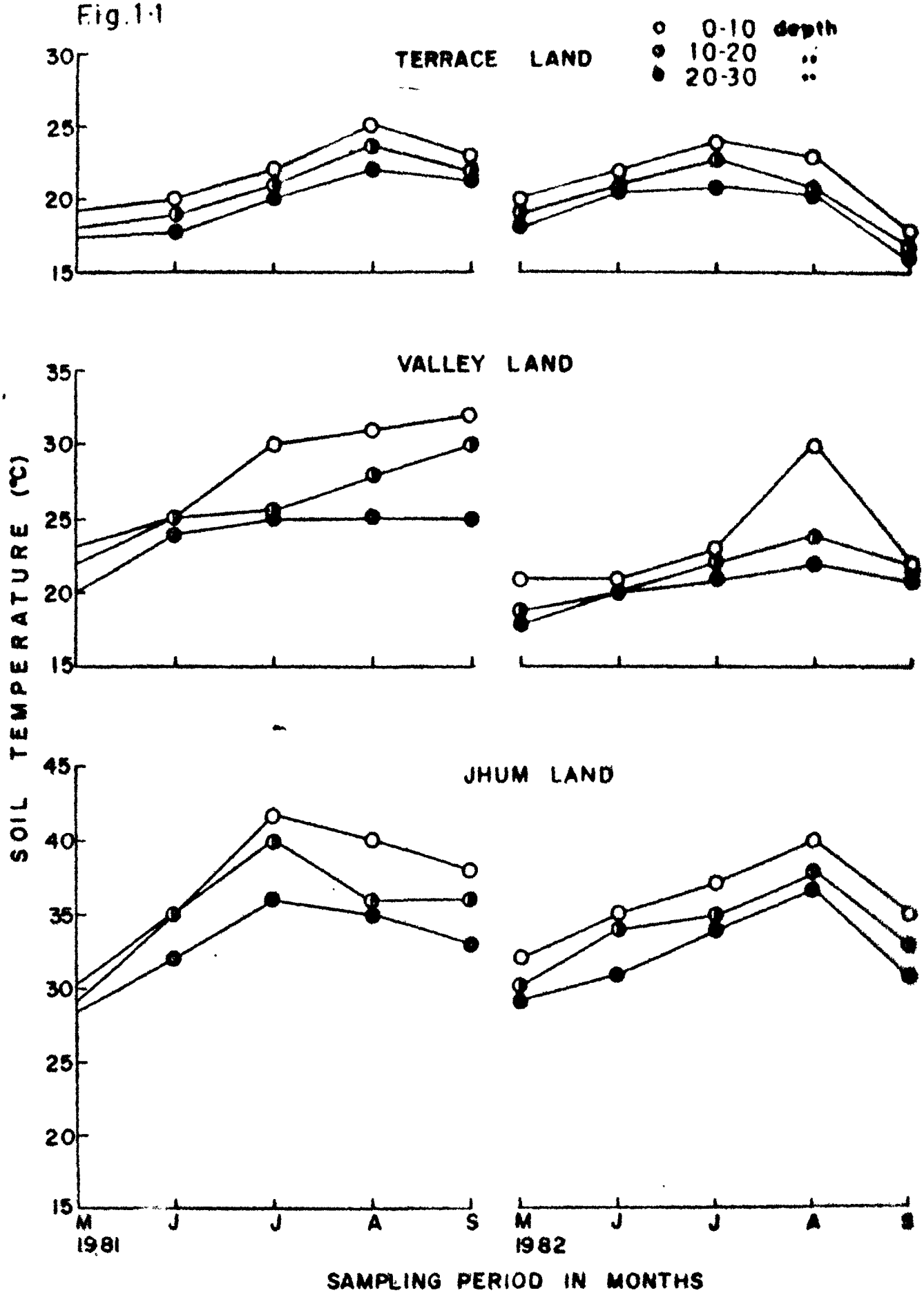
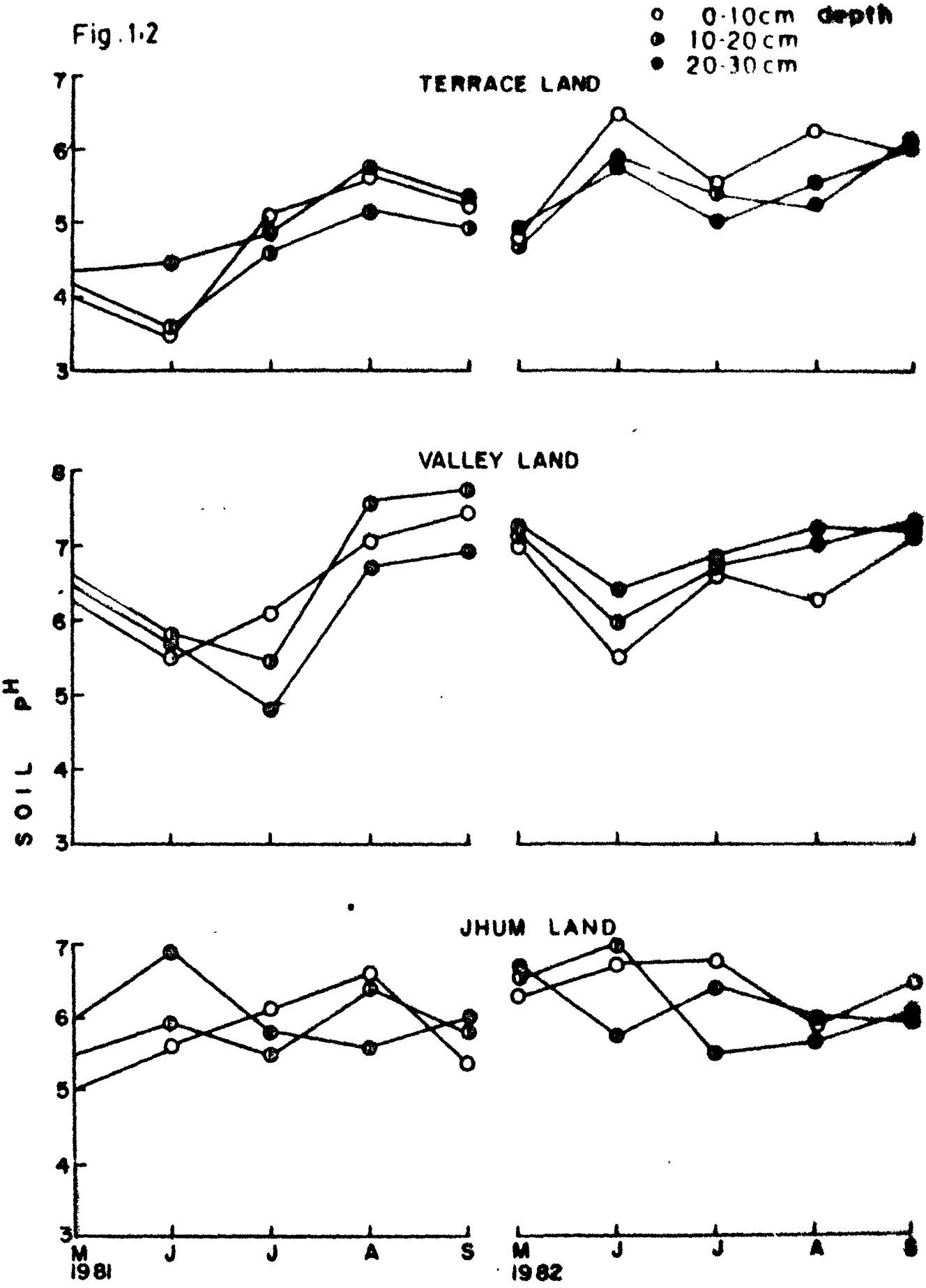

0-10 depth
10-20 ..
20-30 ..
TERRACE LAND
VALLEY LAND
JHUM LAND
SOIL TEMPERATURE (°C)
30
25
20
15
35
30
25
20
15
45
40
35
30
25
20
15
M
J
J
A
S
1981
M
J
J
A
S
1982
SAMPLING PERIOD IN MONTHS

Fig. 1.2 Monthly variation in pH of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·2


o 0-10cm depth
⊕ 10-20cm
● 20-30cm
TERRACE LAND
VALLEY LAND
JHUM LAND
SOIL pH
7
6
5
4
3
8
M
J
J
A
S
1981
1982
SAMPLING PERIOD IN MONTHS

Moisture Content:

Maximum moisture content was found in the soil of valley land closely followed by terrace land soil and the minimum was recorded in the soil of jhum land, the range being in between 25.3-38%, 20-36% and 12.5-22.0% respectively. Moisture content increased with increase in soil depth in all the three different agricultural soils (Fig. 1.3).

Organic Carbon:

Maximum organic carbon content was found in the soil of valley land followed by terrace land and minimum was found in jhum land soil. In case of valley land and terrace land soils it was found to be maximum in the month of June and thereafter it decreased while in case of jhum land soil, the maximum was recorded in the month of July (1981) and August (1982). The minimum organic carbon was found in the month of May in all the three agricultural soils. The trend of variation in organic carbon along the soil profile followed the usual decreasing trend in the soil of terrace land and jhum land while in case of valley land the organic carbon was slightly less in surface soil (0-10 cm) and remained unchanged or slightly increased at the deeper depths (Fig. 1.4).

Total Nitrogen:

Maximum nitrogen content was found in the soil of valley land and minimum was noted in jhum land soil. In the soil of valley land it was highest in the month of June during both the years,

Fig. 1.3 Monthly variation in moisture content of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·3

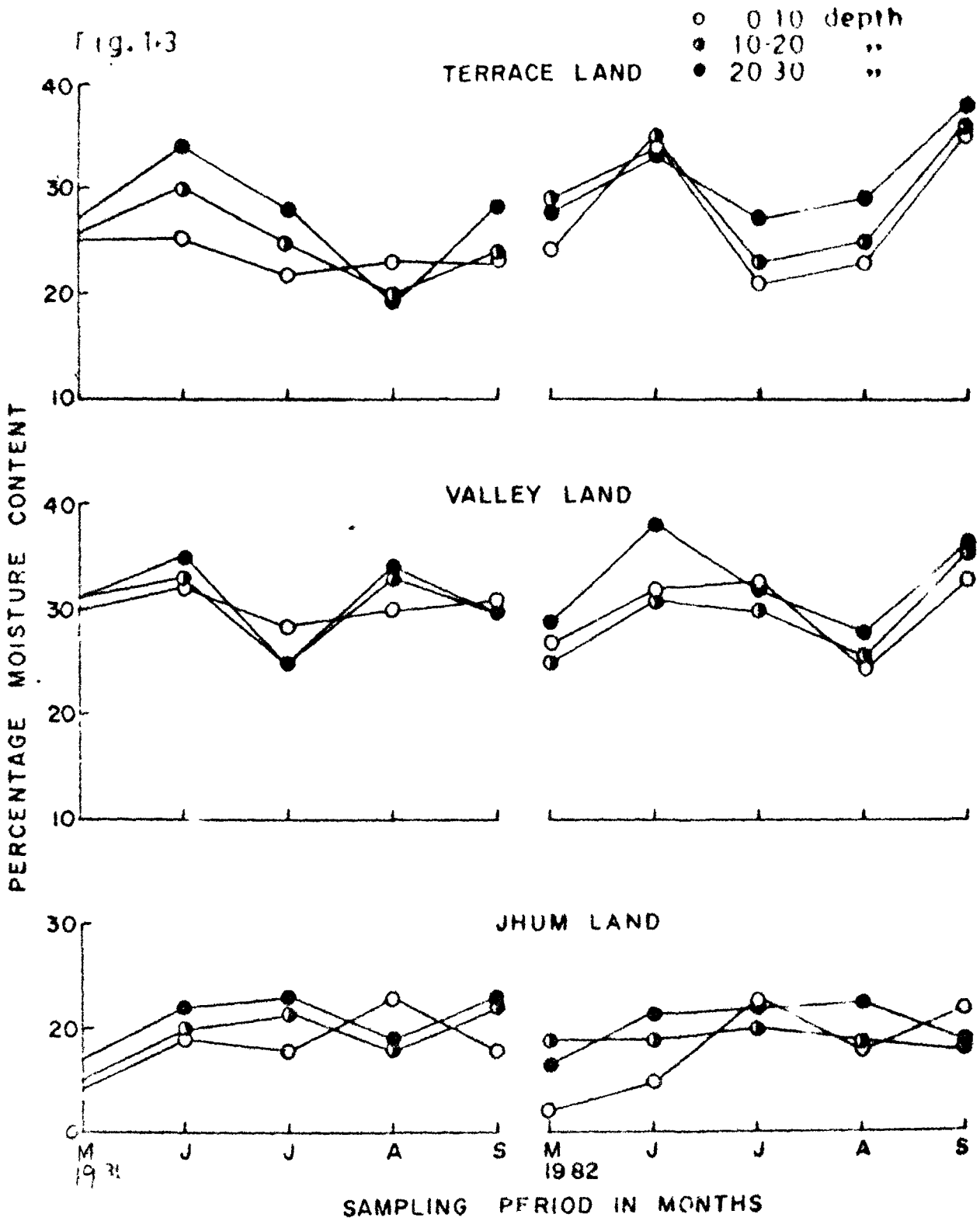
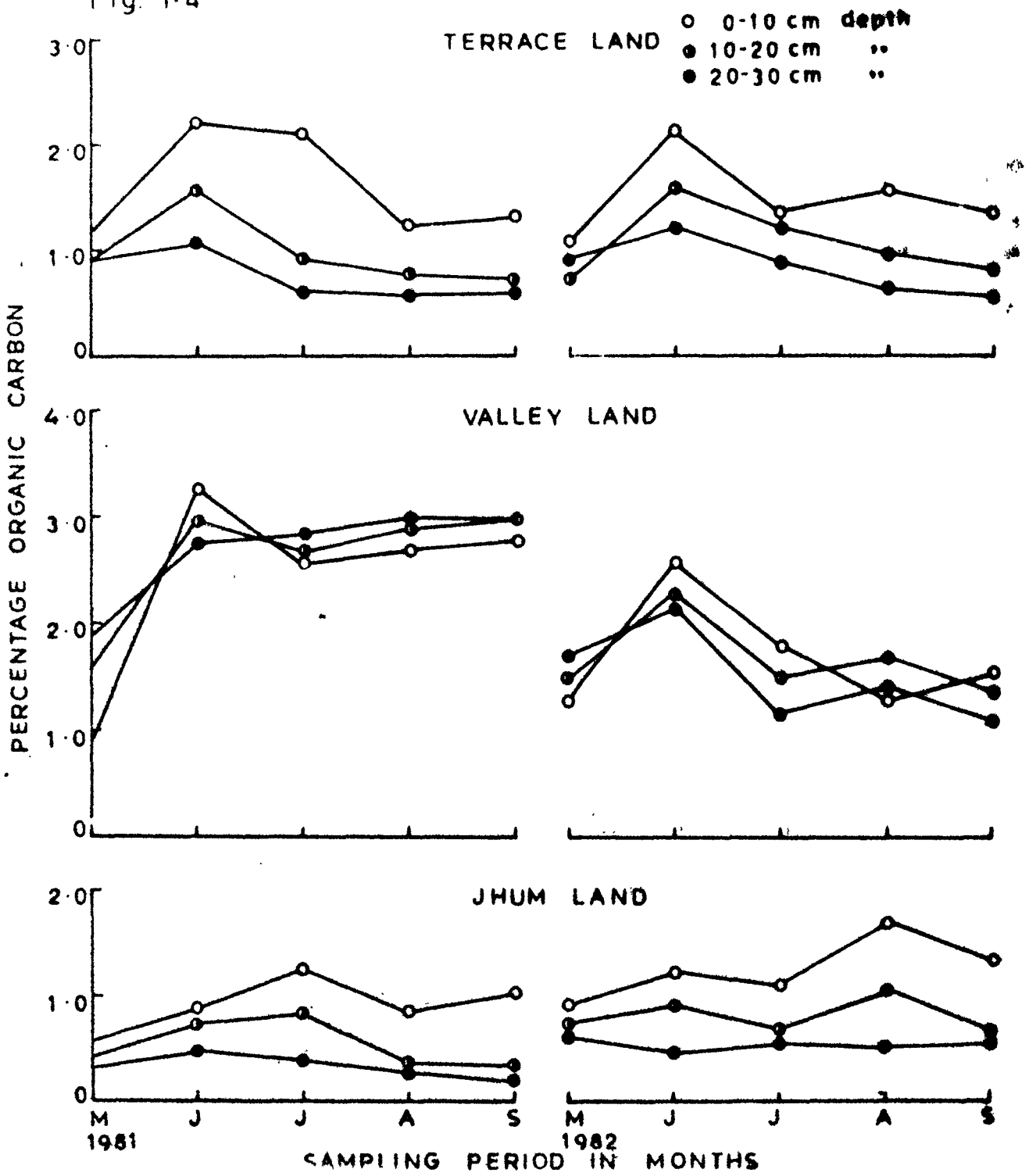

o 0 10 depth
10-20 ,,
20 30 ,,
TERRACE LAND
VALLEY LAND
JHUM LAND
PERCENTAGE MOISTURE CONTENT
40
30
20
10
0
M
J
J
A
S
1981
1982
SAMPLING PERIOD IN MONTHS

Fig. 1.4 Monthly variation in organic carbon of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·4


TERRACE LAND
○ 0-10 cm depth
● 10-20 cm ,,
● 20-30 cm ,,
VALLEY LAND
JHUM LAND
PERCENTAGE ORGANIC CARBON
3·0
2·0
1·0
0
4·0
M
J
J
A
S
1981
1982
SAMPLING PERIOD IN MONTHS

while in case of terrace land the maximum value recorded was in the month of August. In case of jhum land maximum was found in June (1981) and September (1982). Nitrogen content was always higher in the surface soil of terrace land and jhum land while in valley land soil it was slightly less in the upper and increased along with depth (Fig. 1.5).

Available Phosphorus:

Peak in available phosphorus was obtained in the month of July in all the three agricultural soils which decreased thereafter. The available phosphorus decreased along depth of the soil (Fig. 1.6).

Exchangeable Potassium:

Peak in exchangeable potassium was observed in the month of June, July and August in the soil of valley land, terrace land and jhum land respectively. Potassium content decreased with increase in depth in all the three different agricultural soils. Maximum exchangeable potassium was found in the soil of jhum land followed by valley land and minimum was observed in terrace land (Fig. 1.7).

DISCUSSION

Nutrient concentration was generally higher in the surface (0-10 cm) soil. In the soil of valley land the high

Fig. 1.5 Monthly variation in total nitrogen of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·5

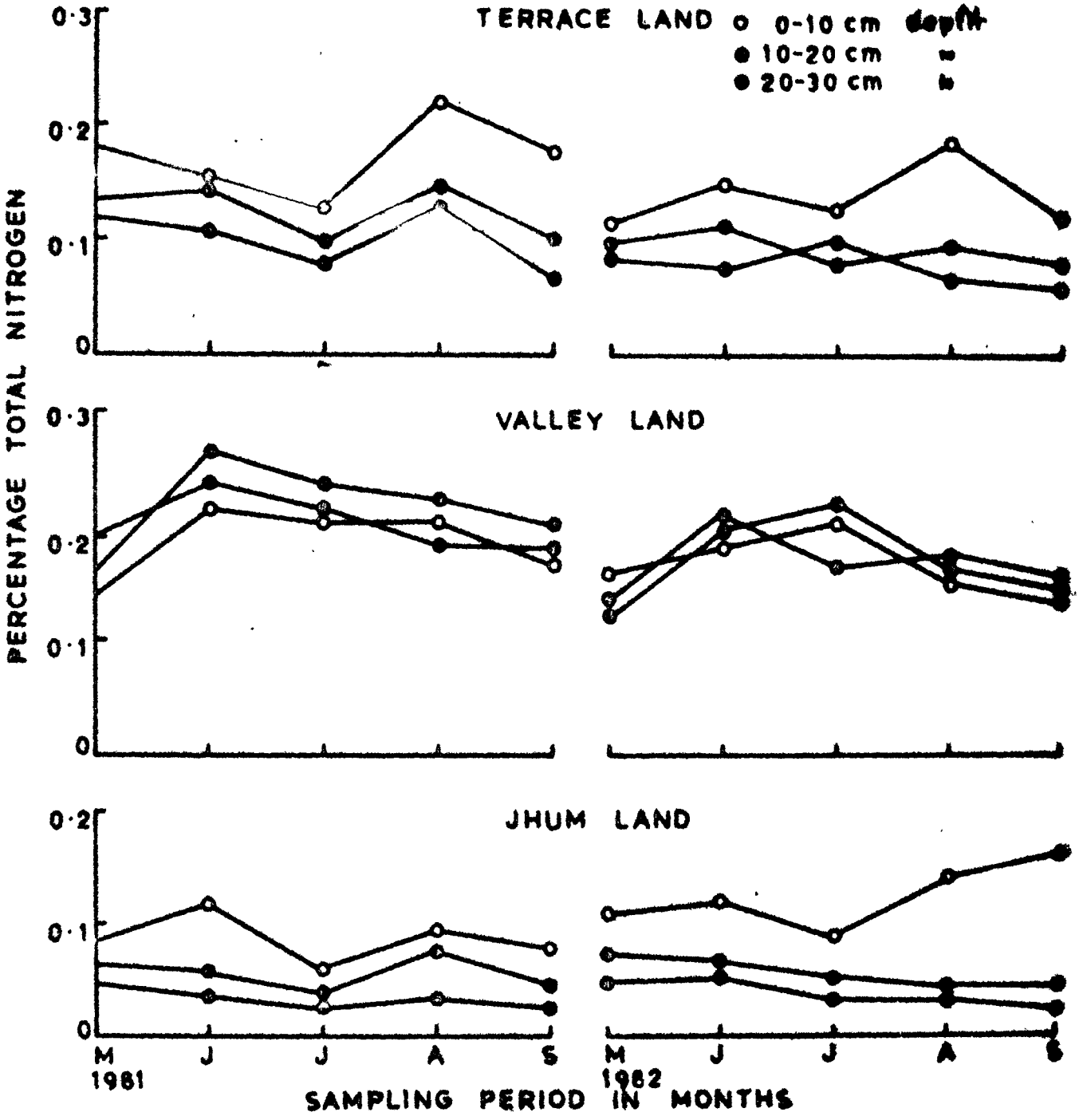

TERRACE LAND
o 0-10 cm depth
● 10-20 cm "
● 20-30 cm "
VALLEY LAND
JHUM LAND
PERCENTAGE TOTAL NITROGEN
0·3
0·2
0·1
0
M
J
J
A
S
1961
1962
SAMPLING PERIOD IN MONTHS

Fig. 1.6 Monthly variation in available phosphorus of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·6

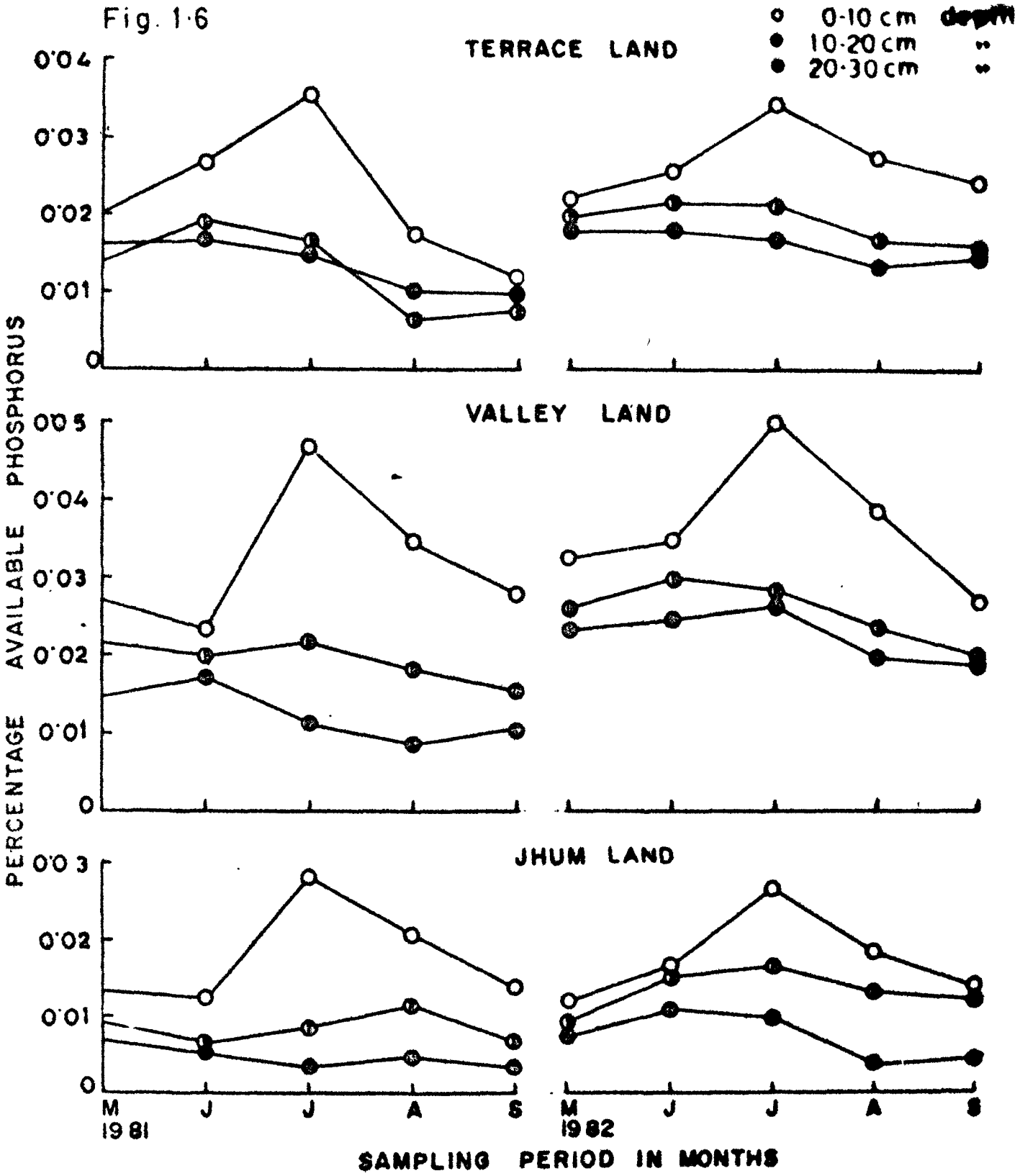
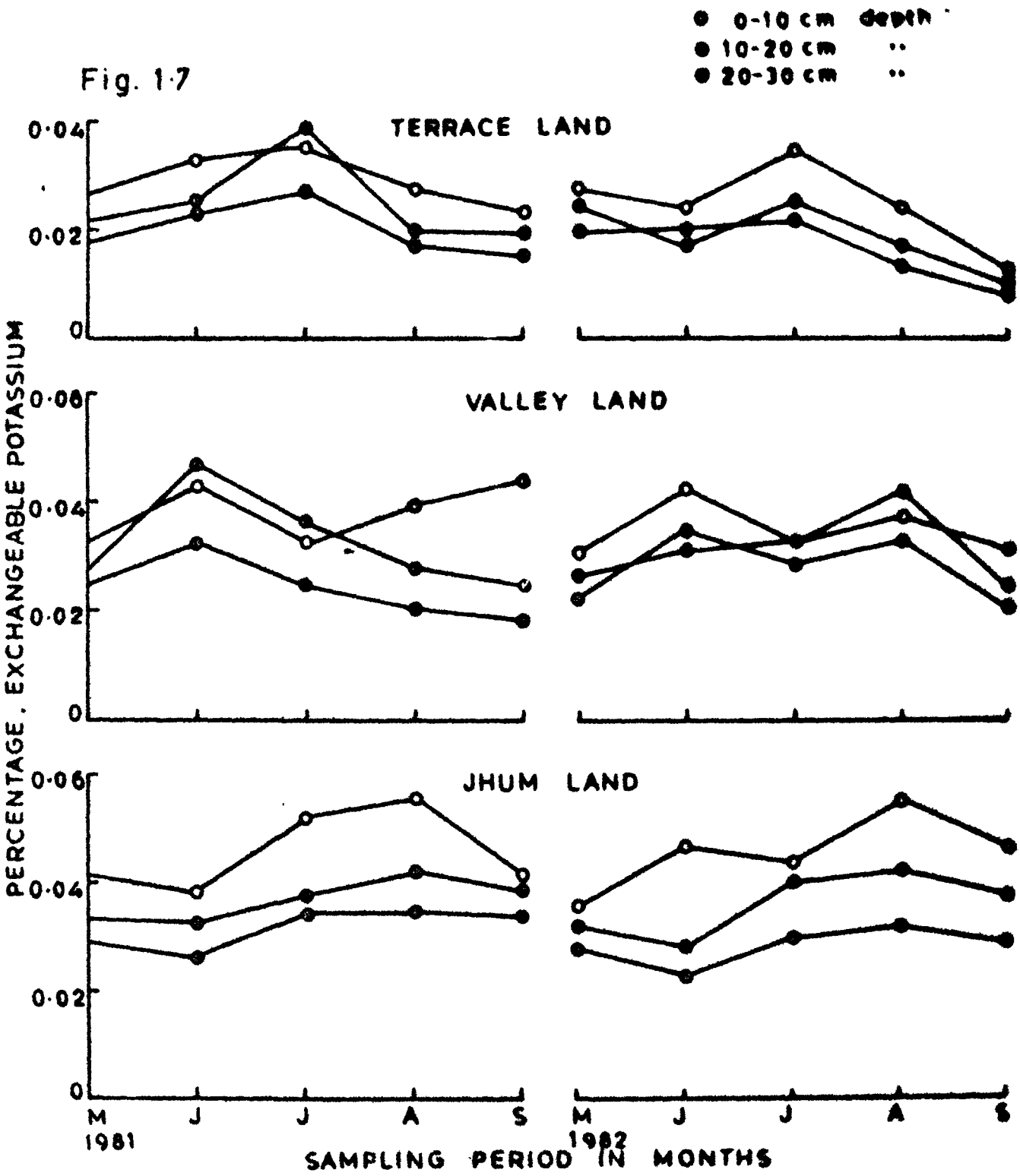

0-10 cm depth
10-20 cm "
20-30 cm "
TERRACE LAND
VALLEY LAND
JHUM LAND
PERCENTAGE AVAILABLE PHOSPHORUS
0·04
0·03
0·02
0·01
0
0·05
0·04
0·03
0·02
0·01
0
0·03
0·02
0·01
0
M
J
J
A
S
1981
M
J
J
A
S
1982
SAMPLING PERIOD IN MONTHS

Fig. 1.7 Monthly variation in exchangeable potassium of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 1·7


● 0-10 cm depth
● 10-20 cm ..
● 20-30 cm ..
TERRACE LAND
VALLEY LAND
JHUM LAND
PERCENTAGE, EXCHANGEABLE POTASSIUM
0·04
0·02
0
0·06
0·04
0·02
0
0·06
0·04
0·02
0
M
J
J
A
S
M
J
J
A
S
1981
1982
SAMPLING PERIOD IN MONTHS

organic carbon and nitrogen at all the depths was probably due to the continuous deposition of the eroded soil rich in organic matter brought by the surface run off of water from the adjacent hill areas. Similar reasons may be attributed to the generally high nutrient concentration at valley land soil than the other two agricultural soils. Topography and different agricultural systems play an important role in determining the nutrient status of the soil. In case of jhum land, high annual rainfall and steep cultivated slopes cause maximum loss of nutrients through soil erosion. This is more pronounced in the absence of crop cover particularly at the time of sowing and after the harvest (Toky and Ramakrishnan, 1981). This may be the reason for the minimum nutrient content in the soil of jhum land.

Soil physical factors also contribute to the low nutrient levels of the soil. Arnason et al. (1982) reported that the steep slope creates a favourable condition for the accelerated soil erosion particularly when the aggregating power of the soil is reduced considerably. The coarse texture of jhum land soil might be expected to have a lower water and nutrient retention capacity.

The low organic carbon and nitrogen observed in jhum land soil could be attributed to high temperature which accelerates the rate of decomposition. A substantial amount of nitrogen is lost through volatilization during burning of slash in jhum land soil (DeBell and Ralston, 1970; White et al., 1973; Sanchez, 1976).

The concentration of available phosphorus was found to be maximum in the month of July in all the three fields (Fig. 1.6) This may be due to the greater microbial activity (Ahlgren and Ahlgren, 1965) and realease of soluble phosphate because of the suitable temperature. The concentration of available phosphorus remained more or less constant at the lower depths at all the sites. The phosphate is relatively immobile (Bieleski, 1973) and thus its transport along the water flow from the upper layer is relatively less which may be accounted for nearly uniform distribution of available phosphorus.

The potassium concentration was found to be maximum in the jhum land soil (Fig. 1.7). Higher temperature conditions of this field perhaps has to play a significant role in high potassium accumulation (Woodruff, 1955; Golden, 1962). Haagsma and Miller (1963) reported that rate of release of non-exchangeable potassium to the exchangeable form has been found to be a temperature dependent phenomenon. A significent positive correlation was found between temperature and exchangeable potassium in jhum land soil ($r = 0.641$). Nye and Greenland (1960) and Zinke *et al.* (1978) also reported that the high temperature releases cations to the surface soil thereby increasing the potassium concentration of the soil. Significant positive correlation was found between temperature and pH in jhum land soil ($r = 0.893$). With increase in temperature, pH also increased which was found to be true in jhum land soil. Ahlgren

and Ahlgren (1965) also reported the similar finding.

The higher concentration of nutrients in the surface layer could be associated with high organic carbon concentration of the layer. Blakemore (1966), Gupta and Rorison (1975) also reported the similar findings. The surface layer of the soil is continuously enriched by the organic matter deposition and other input of nutrients from adjoining areas. The nutrient status of the below ground soil largely depends on the mineralization process at the top because the nutrients once released percolate down the profile along with the water.

CHAPTER II

STUDIES ON MICROBIAL POPULATIONS AND THEIR ACTIVITIES IN MAIZE FIELD SOILS

INTRODUCTION

The microbial communities constitute one of the most important component of the soil. The organisms in the soil live in a habitat of their own - an underworld which remains shut away from the pronounced biological manifestations with which we are so familiar around us. Soil is a favourable natural medium for the growth of microorganisms such as fungi, bacteria, actinomycetes, and soil fauna. These organisms are said to live in a state of dynamic equilibrium.

The occurrence of microorganisms in the soil is influenced by various factors such as type of vegetation, agricultural practices, quality and quantity of organic matter, pH, climate etc. The abundance and physiological activity of the fungal flora of different habitats vary considerably. Both the generic composition as well as size of the flora vary with type of soil and with its physico-chemical characteristics.

One of the features common to most soil microbiological studies is the counting of cells or the measurement of hyphal lengths present in a soil sample. Such counts and measures are intended to provide evidence on the relative importance of organisms in the soil, but they are of limited value in this respect, and must be converted to weights of living material (biomass) to allow true comparison. Soil microbial biomass in a

given weight of soil and the size of the soil component are influenced by many environmental factors. Length of time after sampling is one of the important factors for biomass estimation. Soil agar film method of Jones and Mollison (1948) as modified by Thomas **et al.** (1965) emerged as the most accurate assessment of hyphal-length in soil samples.

In the past few years several investigators have devoted considerable attention to the development of enzymatic tests for determining the microbial activity in soil. Most of the enzyme techniques involve the addition of known substrates to soil samples and then the activity is estimated by determining the end products formed. Several parameters have been used to measure the biological activities of soil microbes. Among them the important ones are: total microbial counts, rates of nitrification, ammonification, cellulose decomposition, CO_2 evolution and individual enzyme activities such as dehydrogenase, urease etc. A number of controversies has arisen out of such studies. Some authors have found a relationship between specific enzyme activity in soil and known indices of microbial activity. However, in most instances no relationship could be found between the enzyme tests and total number of microorganisms, carbon dioxide production, nitrification and ammonification in soils (Drobnik and Seifert, 1955).

Drobnik (1955) has pointed out that although enzyme tests are suitable for qualitative studies of various soil processes,

they are not applicable for the quantitative estimation of total activity. The failure of such tests to serve as reliable criteria of total microbial activity from the fact that most of the substrate used resulted in an adaptive response by the specific organisms or groups of organisms involved. The drawbacks led the workers to turn to the methods like carbon dioxide evolution, oxygen uptake and dehydrogenase activity. Studies on different enzyme activities in soil are important as they indicate the potentiality of soil to carry out the biochemical processes which are important to maintain the soil fertility.

Levels of dehydrogenase activity of soils are considered to give an estimate of the microbiological activity of soil. Dehydrogenase enzymes are considered to play an essential role in the initial stages of oxidation of soil organic matter. Dehydrogenase activity appears to be more dependent upon the metabolic state of the microbial population of soil than the activity of free enzyme available in the soil.

Another important enzyme in soil is urease. The increase use of urea as fertilizer has led many workers to study its fate in soil and also the relationship with other soil factors. Proper evaluation of the urea hydrolyzing power of any soil is essential to improve the efficiency of fertilizer use especially in the soils with low organic carbon and high pH.

Many ecological studies also involve estimation of

biological activity through measurements of oxygen uptake or carbon dioxide evolution (Seto & Tazaki, 1975). Respiratory measurements are widely used for estimating the general biological activity of soil (Wagner, 1975). They are of great significance in ecological studies because they provide some indication of the rate at which soil organic matter may be broken down. Most of the organic matter added to the soil in the form of litter and dead roots is processed through microbial activity resulting to the production of carbon dioxide and release of nutrients. A major part of the CO_2 evolved from the soil comes from the microbial respiration, which in certain cases may account for as high as 90% of the total CO_2 evolved. Thus, studies on soil respiration in a given ecosystem may lead to an assessment of microbial activity, organic matter input into the system, the flow of energy through microbial compartment and the rate of mineralization.

The present work was undertaken with a view to understand the distribution pattern of soil microflora (Fungi and bacteria) and their biological activities in maize field soils.

REVIEW OF LITERATURE

Soil Microflora:

The microorganisms in the soil have been subjected to intensive studies during the recent years. Studies on soil fungi have received much attention since the problem of soil mycologi-

cal investigation was probed in by Adametz (1886). A vast literature has accumulated on nature and activities of soil microflora. The impetus has come through the realization of the importance of microorganisms in the field of antibiotics, soil fertility and seed and root pathology (Waksman, 1952; Parkinson and Waid, 1960; Garrett, 1963). According to Waksman (1952) the abundance of microorganisms in soil is influenced by various factors such as organic matter, soil reaction, moisture, temperature, aeration and nature of crop grown. The distribution of microorganisms in the soil is, therefore, controlled by numerous ecological factors, comprising climatic, edaphic, and biotic.

Saksena (1955) and Warcup (1957) found a marked decrease in viable propagules of soil in summer. He further observed that moisture, as a rule, was favourable for the growth of fungi as long as there was no water logging.

Orput and Curtis (1957) reported that soil moisture was an important factor in controlling the distribution of fungi.

Roy and Dwivedi (1962) studied the fungal flora of different grass species situated in the same field and found variation in the population of fungal flora which they thought was due to variation in the vegetation.

Though several workers have reported that there is a decrease in microbial population with the increase in depth of the soil, Apinis (1963) observed a relatively uniform distribu-

tion in microfungal population throughout the soil horizons. Soil texture has been shown to affect oxygen and CO_2 relationships as well as moisture content and nutrient availability, all of which have been suggested to influence the composition of microfungal community (Griffin,1963).

Ishizawa and Toyoda (1964) studied the microflora of two groups of cultivated soils in Japan, usual field and submerged paddy field soil. They observed that the former soil is rich in fungi and actinomycetes, whereas bacteria are more abundant in the latter type of soil particularly anaerobic bacteria such as sulfate reducing ones.

Mishra (1966a) studied the seasonal variation in fungal flora of grasslands of Varanasi (India) and recorded a seasonal effect on the prevalence of different fungal species. A positive correlation between the fungal population and temperature and moisture content of soils was observed (Mishra, 1967)

Mishra and Kanaujia (1972) studied the distribution of soil fungi in relation to cover vegetation and physicochemical characters of the soil collected from different places of North India and observed that fungal population was generally higher in cultivated fields, and nature and duration of cropping affects the population. It is suggested that moisture content, organic matter content and pH of the soil affected fungal flora.

Hattori (1967) found in a garden soil of Sendai, a rather high correlation in fungal flora at various points in November and February, although the flora at the same study site varied widely in November and June. He concluded that fungal flora at various study sites in a field are not uniform and always vary widely.

Soil types of some mountain ranges in the Caucasus and Middle Asia were studied by Mishustin and Mirzoyeva (1968). They found that the effect of altitudinal zonality on the composition of soil microflora is the same as that of latitudinal zonality. At higher mountains, where the average annual soil temperature is low, both the number of bacteria and actinomycetes decreases. Some soil types have characteristic profiles and they possess specific pattern in distribution of organic matter. These differences affect the microbiological profile of different soils.

Soil moisture content, seasonal weather fluctuations (Prakash and Khan, 1971) and depth (Tyagi, 1973) have been shown to be important factors governing the frequency and population of soil microflora.

Holding et al. (1974) noted that the viable counts of bacteria decreased with depth and was correlated with the decrease in moisture content, organic matter and inorganic nutrients. They further stated that the decrease in numbers with the increase in depth was less marked for the anaerobes

than the aerobes.

Wong (1975) studied the distribution of soil fungi as influenced by some edaphic factors and reported that the total number of species was related with the soil moisture and pH. Singh and Charaya (1975) studied the distribution of soil microflora of a sugarcane field. They reported that fungal population decreased with increase in depth.

Jones and Richards (1977) reported that the vegetation type has no effect on the total number of bacterial and actinomycetes populations.

Martinez and Ramirez (1979) studied the microfungal community of an andosol, under an acid beech forest and found that there was a marked decrease of fungal propagules and mycelial biomass with soil depth.

Soil Fungal Biomass:

Very few studies have been made on the live and active part of the soil fungal biomass. Biomass provided an indication of the overall variation in microbial population, whereas dehydrogenase activity reflected the respiratory rate of the microbial community (Casida, 1977; Skujins, 1976). Flanagan and Vancleve, (1977) stained the soil mycelium with a cytoplasmic and nuclear stain, and by measuring the nucleated hyphae they were able to estimate the live fungal biomass.

Soderstrom (1979) assessed the metabolically active fungal biomass in three horizons of a podzolized pine-forest soil. He found that biomass increased during winter with soil temperature below $0^{o}C$. Recurrent definite biomass peaks were observed in autumn and during early spring. Only a small fraction (2.4-4.3%) of the total fungal biomass was found to be active. The amount of FDA active biomass was correlated with soil moisture content.

Baath (1980) observed that there was a decrease in fungal biomass after felling of the forests trees throughout the soil profile. FDA-active hyphae decreased more in the mineral soil than in the organic soil.

Tate and Terry (1980) found that microbial biomass, dehydrogenase activity and aerobic bacterial population were correlated with moisture level of the soil.

Dehydrogenase Activity In Soil :

Level of dehydrogenase activity in soil is considered to provide some guide to the microbiological activity of soil. This aspect has been investigated intensively since the introduction by Lenhard (1956) of a method for estimation of the activity. Lenhard (1956) introduced the concept of determining the metabolic activity of micro-organisms in soil and other habitats by measuring dehydrogenase activity using 2, 3, 5 - triphenyl tetrazolium chloride (TTC) reduction. The method is based on

the assumption that in the absence of oxygen, TTC acts quantitatively as the terminal H - acceptor for dehydrogenase system with the formation of red triphenyl-tetrazolium formazan (TPF): $TTC + 2H^{+} + 2e^{-} = TPF + HCl$.

Stevenson (1959) found a close correlation between the oxygen uptake and dehydrogenase activity in the soil samples. According to him there is a close relationship between dehydrogenase activity and bacterial number.

Stevenson (1959) and Casida et al. (1964) used 2, 3, 5 - TTC in an attempt to equate the dehydrogenase activity with the biological activities of soil.

Dehydrogenase activities appear to be greater in the less acid samples and are strongly favoured by moisture. Gilot and Dommergues (1967) found that dehydrogenase activities /g soil organic C in some sub-alpine forest soils were greater in horizons of highest pH values.

Skujins and McLaren (1968) studied the dehydrogenase activity in some air dried soils that had been stored for a few years. Harris (1968) found high dehydrogenase activity in light sand, as well as in recently wetted soil.

Ross and Roberts (1970) investigated the enzyme activities and oxygen uptake of pasture soil in relation to temperature and rainfall. They observed that dehydrogenase activities

were highest in winter samples and were significantly related with both soil moisture content and pH.

Ross (1971) studied some factors influencing the estimation of dehydrogenase activity of pasture soils in New Zealand. He observed that dehydrogenase activities of samples of the same soil collected at different times differ. He suggested that enzyme dehydrogenase is considered to play an important role in the initial stages of the oxidation of soil organic matter by transferring hydrogen or electrons from substrate to acceptors.

Ross and McNeilly (1972) while studying the effects of storage on oxygen uptake and dehydrogenase activities of beech forest litter and soil observed that dehydrogenase activities of some samples maintained at $4^{o}C$ increased but those of all samples held at $-20^{o}C$ decreased. According to them fresh samples gave better dehydrogenase activity and if necessary storage at $4^{o}C$ may be preferrable for retaining dehydrogenase activities. According to Skujins (1973) the dehydrogenase activity did not correlate with microbial numbers, and an assay of dehydrogenase activity was used to predict the proteolytic, nitrifying and respiratory activities in soils.

Vishwanath et al.(1975) studied the behaviour of bacteria surviving in chloroform and toluene treated soils with regard to their dehydrogenase activity.

Tu (1982) while studying the effect of physical treatments on the numbers of microorganisms and soil enzyme activities indicated that none of the treatments inhibited dehydrogenase activity.

Urease Activity in Soil:

The increased use of urea as a fertilizer has led many workers to study its fate in soil and also its relationship with soil factors. Stojanovic (1959) found marked seasonal variation in urease activity in Mississippi soils. Seasonal variations in the enzymatic activities of soil reported in a number of studies are biologically important because they change the quantity and quality of substrates upon which they act and are also responsible for altering the rate of various soil processes influenced by seasonal changes (Freytag, 1965; Galstyan, 1965; Ramirez-Martinez and McLaren, 1966).

A few studies regarding relationships between urease activity and other soil properties have indicated that urease activity tends to increase with organic matter content and that sandy or calcareous soils tend to have a lower urease activity than heavy textured or non-calcareous soils (McGarity and Myers, 1967; Skujins,1967; Myers and McGarity, 1968; Skujins and McLaren 1969). McGarity and Myers (1967) found that urease activity in Australian surface soils was highly related with pH, but they

could not detect a significant relationship between pH and urease activity in a subsequent study of five profile samples (Myers and McGarity, 1968).

Gould et al. (1973) observed a significant relationship between urease activity and organic carbon in profile samples of an Alberta soil, but Pancholy and Rice (1973) found no such relationship in nine Oklahama soil. They concluded that the level of urease activity in these soils was determined by the type of vegetation.

Dalal (1975) observed a highly significant correlation between urease activity and organic carbon or cation-exchange capacity.

Zantua and Bremner (1976) concluded that addition of a small amount of urea to a Drummer silty clay loam induced urease activity i.e. promoted production of urease by soil microorganisms. They further observed that production of urease activity on addition of glucose and other organic materials promote microbial activity. They concluded that soil constituents protect urease against microbial degradation and other processes leading to inactivation of enzymes and that every soil has a stable level of urease activity determined by the ability of its constituents to provide this protection.

Zantua et al.(1977) studied surface samples of 21 diverse

Iowa soils representing a varied pH, texture, and organic matter content to determine the relationships between soil urease activity and other soil properties. They reported that the activity was correlated very significantly with organic carbon, total N and cation-exchange capacity, however, it was not significantly correlated with pH, silt or $CaCO_3$ equivalent.

Speir (1977) found that enzyme activities were correlated significantly with several soil chemical properties related to the amount of organic matter.

Verstraete and Voets (1976) reported that urease activities and soil respiration increased with increasing soil organic matter, clay and $CaCO_3$ content.

Tabatabai (1977) reported that cropping history, soil amendments and environmental factors have greater influence on the activity of urease and other enzymes in soils. According to Speir and Ross (1981) soil air drying generally increases urease activity in soil.

Dash *et al*. (1981) observed that total N and organic carbon were positively correlated with urease activity. Further they reported that high temperature also affects the activity in tropical soils.

Nor (1982) studied the urease activity in several Malaysian soil and indicated that the soils have varying capacity

to hydrolyze urea. Soil pH and urease activity correlated well, but neither organic carbon content nor cation-exchange capacity had any significant relationship.

Carbon Dioxide Evolution in Soil:

The estimation of soil respiration either as oxygen uptake or as carbon dioxide evolution is the most widely used measure to correlate with the biological activities of the soils. Katznelson and Stevenson (1956) studied the evolution of carbon dioxide from the forest floor and showed that a number of environmental factors has been found to affect the evolution of carbon dioxide from the soil such as temperature, moisture, pH and the substrates.

Stotzky (1960) established a correlation between carbon dioxide evolution and microbial biomass.

Dobson and Wilson (1963) studied the soil activities from a strip-mine spoil areas in West Virginia and observed a decreased rate of respiration along with the low bacterial numbers.

Witkamp (1966b) studied the evolution of carbon dioxide from forest floor of Pine and Maple stands and found a correlation between the bacterial numbers and carbon dioxide evolution. He further stated that the temperature, moisture level and substrates have been shown to affect the level of carbon dioxide

evolution from soil. Witkamp (1971) further reported that moisture had the greatest limiting effect on carbon dioxide evolution from the floor of an Oak-hicory forest in Missouri and that temperature was the most limiting factor in the spring and winter.

Wildung et al. (1975) suggested that temperature and moisture content were interdependent in their effects on soil respiration.

Ross et al. (1975) studied respiratory activities and their relationship with temperature, moisture and soil properties in tussock grasslands in New Zealand. They found significant correlation with contents of soil moisture, organic carbon, and total nitrogen.

Tesarova and Gloser (1976) studied total carbon dioxide output from alluvial soils with two types of grassland communities and found that soil moisture content was one of the most important parameter controlling carbon dioxide evolution from soil.

The importance of temperature and soil respiration has been well documented for various types of soils (Bunnell et al. 1977; Singh and Gupta, 1977). Temperature and moisture are positively correlated with the soil respiration (Singh and Gupta, 1977).

Ross and Cairns (1978) studied the influence of temperature on biochemical processes in some soils from tussock grasslands. They found that the patterns of carbon dioxide evolution at different temperatures and incubation periods varied with the different soils and that carbon dioxide evolution increased with increase in temperature. The soil activities have been found to depend upon a number of other factors. Kowalenko et al. (1978) stated that below the optimum water content for microbial activity, increasing moisture results in increasing carbon dioxide evolution .

Cleve et al. (1979) reported that the estimation of carbon dioxide evolution provides an insights into the rate of organic matter breakdown and mineralization.

Upadhyaya et al. (1981) reported that soil temperature and moisture were linearly related with carbon evolution.

MATERIALS AND METHODS

The investigation of the soil microflora and their various activities was carried out on monthly interval for two crop cycles of maize (Zea mays L.) starting from May 1981 to September 1981 and May 1982 to September 1982.

Soil Sampling Procedure:

Soil samples were collected randomly from five places at each study site from three depths (0-10 cm, 10-20 cm and 20-30 cm). Following the removal of the surface vegetation, a pit was dug down the profile and the soil samples were collected from each range of depths by inserting a clear sterile trowel vertically into the soil. Care was taken to avoid contamination by other horizons and the trowel was cleaned with alcohol between each sampling. Each set of five samples was thoroughly mixed in a sterile polythene bag to make a composite soil sample which was then secured with a rubber band. The samples were taken to the laboratory immediately. All aseptic precautions were taken to avoid contaminations. Samples were kept in the freeze at $4^{o}C$ until they were processed. Christensen (1969) reported that freezing had no significant effect on the prevalence of micro-fungi in mineralized soil samples. Additional bulk of the samples collected from each site provided material for pH and moisture determination. The same procedure of soil sampling was followed for the investigation of soil microflora throughout the sampling periods. With the samples collected the following studies were made

Isolation Of Soil Fungi:

The soil plate method (Warcup, 1950) using Rose-bengal agar medium (Martin, 1950) was followed for isolation of fungi. A small amount (0.015g) of soil was taken from the composite

sample with the help of a sterile nichrome spatula having a flattened tip. The soil aggregates were crushed and dispersed in a few drops of sterile distilled water in the bottom of the sterile Petri dishes. Approximately, 10-15 ml of melted and cooled (below 45°C) Martin's Rose-Bengal Agar medium supplemented with streptomycin sulphate, was poured and the soil particles were dispersed throughout the medium by gentle shaking and rotating the dishes. Three replicates were maintained for each sample. The plates were then incubated upside down at a temperature of 25 ± 1°C for 5-6 days in BOD incubator. The number of fungal colonies was counted and the total number of fungi per gram dry soil was calculated by taking into consideration of the moisture content. The isolation of fungi was carried out in a "Laminar Flow Chamber". The pure culture of fungi was maintained in culture tubes containing agar slants of Czapex-Dox Agar medium and preserved in the freeze at 4°C. The identification of fungi was done with the help of the books of Gilman (1956), Subramanian (1971), Barnett and Hunter (1972).

Estimation Of Fungal Biomass:

Jones and Mollison's (1948) agar film technique as modified by Thomas et al. (1965) was followed for the determination of fungal biomass which in turn was standardized during the preliminary investigation. The details of the method are as follows.

Soil sample (3.5 g) was grinded with mortar and pestle for 2 minutes in 20 ml of sterile distilled water. Supernatent was poured into a beaker and the process was repeated for the second time. A final grinding for 2 minutes in 10 ml of sterile distilled water was done and the supernatent was decanted off to the beaker. To the resulting 50 ml of the supernatent in the beaker, 50 ml of molten 2.5% water agar suspension was added to make the volume 100 ml. This 100 ml soil-water-agar suspension was mixed by rotating it by hand and was maintained at $45^{o}C$-$50^{o}C$.

Prior to the preparation of agar film, the soil-water-agar suspension was agitated by hand and then allowed to settle for 5-10 seconds. A small amount of the suspension was pipetted from approximately 4 cm below the surface of the suspension and was placed on the 0.1 mm deep platform on a haemocytometer slide. The platform was then covered with a coverslip and a 5.0g weight was put on the coverslip to ensure the correct thickness of the film. The film was allowed to solidify. After this the slide was immersed in distilled water and the coverslip was removed. The surplus agar on the slide was cut away with a sharpened razor blade and the agar film was floated off into a clean microscopic slide. The preparation was allowed to dry at room temperature and was stained with phenolic aniline blue stain for 1 hour, washed in 98% alcohol (3 to 4 changes) and mounted in the glycerine.

For each soil sample, ten films were prepared and ten observations were made from each film, using low power objective (X 10) of the microscope.

Calculations of Fungal Biomass From Hyphal Length:

Assuming that mycelium is a perfect cylinder, its volume can be calculated as $\pi r^2 L$ where

r = radius of mycelium.

L = length of mycelium.

Average density of mycelium = 1.1 (for the density of microbial protoplasm).

Therefore, Biomass (Wet weight) = Volume X Density

$= \pi r^2 L \times 1.1.$

Assuming 80% of the microbial protoplasm as water (Clark and Paul, 1970), biomass (dry weight) was calculated.

Isolation Of Bacteria:

Dilution plate method (Waksman, 1922) was followed for the isolation of bacteria from the soil samples using Nutrient agar medium (Johnson and Curl, 1972) throughout the sampling period.

1.0 g of soil from each sample was taken in a 250 ml conical flask containing 100 ml of sterilized distilled water to make 1:100 dilution. The soil suspension was shaken thoroughly for about 10-15 minutes for homozenizing the suspension. 10ml

of this mixture was transferred aseptically by means of a sterilized 10 ml pipette to another 250 ml sterilized conical flask containing 90 ml of sterilized distilled water to get a suspension of 1:1000 dilution. The process was repeated once again to get a suspension of 1:10,000 dilution which was considered to be suitable for isolation of bacteria. Throughout the investigation, the same dilution was used for various samples.0.5 ml of soil suspension from 1:10,000 dilution was transferred aseptically into each of several sterilized Petri dishes containing approximately 10-15 ml of cooled, solidified Nutrient agar medium for isolation of bacteria. The Petri dishes were rotated gently in a swirling motion so that the inoculum was dispersed uniformly over the surface of the agar medium. Three replicates were maintained for each sample. In each case, separate sterilized pipette was used for transferring the soil suspension. The isolation was carried out in a sterilized "Laminar Flow Chamber" throughout the investigation. Inoculated Petri dishes were incubated upside down at a temperature of $30^{\circ}C$ for 24 hours. Population of bacteria per gram dry soil was calculated by taking into consideration of the moisture content and dilution factor. In this study, only the bacterial population was considered and attempt was not made for their identification.

Composition Of Media Used For The Isolation Of Fungi And Bacteria:

For Fungi:

(a) Peptone-Dextrose-Rose Bengal Agar (Martin, 1950)

Agar	20.0 g.
kH_2PO_4	1.0 g.
$MgSO_4 7H_2O$	0.5 g.
Peptone	5.0 g.
Dextrose	10.0 g.
Rose Bengal (%)	3.3 ml.
Distilled water	1000.0 ml.
Streptomycin	30.0 mg.

(b) Czapek-Dox Agar (Raper and Thom, 1949)

Agar	15.0 g.
$NaNO_3$	3.0 g.
K_2HPO_4	1.0 g.
$MgSO_4 7H_2O$	0.5 g.
KCl	0.5 g.
$FeSO_4 7H_2O$	10.0 mg.
Sucrose	30.0 g.
Distilled water	1000.0 ml.

For Bacteria:

(c) Nutrient Agar (Johnson and Curl, 1972)

Agar	15.0 g.

Beef extract	3.0 g.
Peptone	5.0 g.
NaCl	8.0 g.
Distilled water	1000.0 ml.

Determination of Percentage Relative Abundance:

The determination of percentage relative abundance of a particular species of fungi was done by means of the following formula:

$$\frac{\text{Total number of individual species of fungus}}{\text{Total number of individuals of all species}} \times 100$$

Determination Of Dehydrogenase And Urease Activities And Carbon Dioxide Evolution From Soil:

Dehydrogenase activity:

Dehydrogenase activity of soil was determined by 2, 3, 5 triphenyl tetrazolium chloride (TTC) reduction technique modified by Casida *et al.*(1964). To 10 grams of soil taken in three sterile stoppered test tubes, 0.1 gram of $CaCO_3$ and 1.0 ml of 1.5% (W/V) 2, 3, 5-TTC were added. To each of these tubes, 3 ml of distilled water were added. The quantity of liquid was found to be enough to saturate the soil and to form a liquid layer on the soil sample. This ensures adequate anaerobiosis for TTC reduction. The content of each tube was mixed thoroughly and then incubated at $37^{o}C$ for 24 hours. The extraction of triphenyl tetrazolium formazan produced was carried out with

methanol. The soil from tube was quantitatively transferred to Whatman No.1 filter paper by shaking with methanol and washed down into a 50 ml volumetric flask. The soil was washed with methanol until no more colour could be extracted. The volume of the filtrate was made upto the mark with methanol. Three replicates were maintained for each sample. The optical density due to pink colour of the filtrate was determined at 485 mm in a spectrophotometer using methanol extract (without soil) from the control as the blank. Concentrations of the formazan in the extract are calculated by comparison with a standard curve of triphenylformazan (TPF) in methanol and expressed as formazan mg per gram dry soil.

Urease activity:

Urease activity was measured by the modified McGarity and Myers (1967) method. To 10 grams of soil, 1ml of toluene was added and left for about 15 minutes for penetration of toluene into the soil sample. 10 ml of buffer solution (pH 7.0) and 5 ml of urea (10%) were added and incubated at 37^{o}C for three hours. Then the volume was made upto 50 ml with distilled water, shaken and filtered through Whatman No.1 filter paper. The filtrate varied from colourless to brown depending on amount of organic matter in the soil.

Ammonia released as a result of urease activity was determined by indophenol blue method: 1 ml of filtrate was

placed in a 50 ml volumetric flask and made upto 10 ml with distilled water. 5 ml freshly prepared phenolate solution and 3 ml sodium hypochlorite solution were added. This mixture was thoroughly mixed and after 20 minutes made upto 50 ml by adding distilled water. Optical density was read at 630 nm in a spectrophotometer. Three replicates were maintained for each sample. The amount of ammonia-N formed are calculated by reference to a calibration curve and expressed as mg NH_4^+ -N per g dry soil.

Preparation Of Phenolate Solution:

20 ml of phenol solution + 20 ml of caustic soda solution diluted to 100 ml with distilled water.

Phenol Solution:

62.5 g of phenol was dissolved in minimum volume of methanol denatured alcohol, 18.5 ml of acetone was added and this mixture was made upto 100 ml with ethyl alcohol.

Caustic Soda Solution:

27 g of sodium hydroxide was dissolved in 100 ml of distilled water.

Both the solutions were kept in a freeze.

Carbon Dioxide Evolution:

The amount of CO_2 evolved from the soil was measured in the laboratory by the following procedure:

1 kg of soil from each sample was taken in a rectangular jar (12.5 cm x 10.3 cm x 20.8 cm) and one 100 ml glass beaker containing 20 ml of 0.1 N KOH solution was placed inside the jar. Then the jar was covered with a glass lid sealing nicely with grease and thus making air tight to prevent O_2 exchange with the atmosphere. Control set contained sterilized sand instead of soil. After 24 hours of exposure the amount of CO_2 fixed by the KOH solution was measured by back titration with 0.1 N HCl solution using phenolphthalein as an indicator. In each case three replicates were maintained. Evolution of CO_2 was calculated on mg per kg dry soil per day basis.

RESULTS

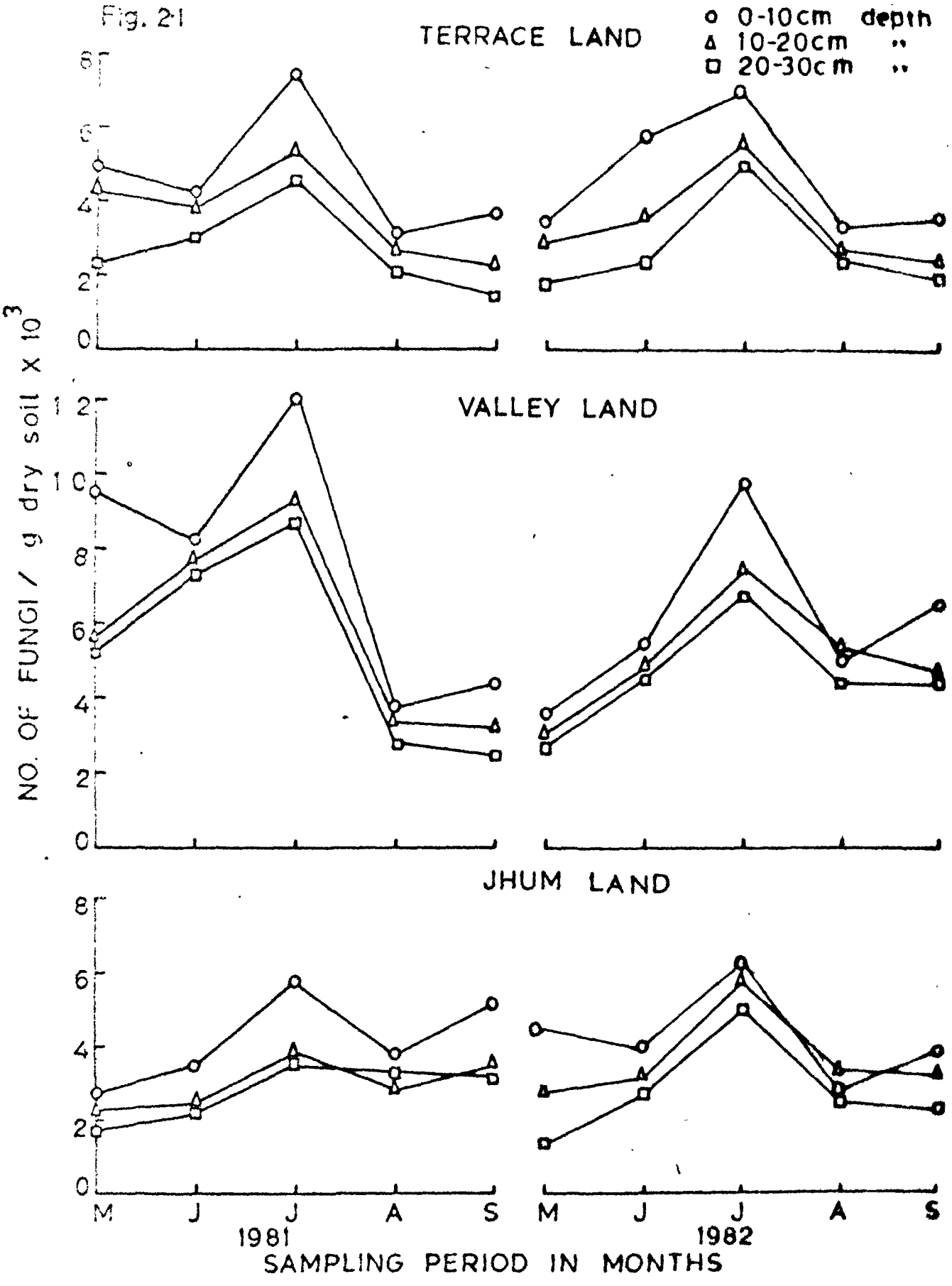
Quantitative Estimation of Soil Fungal Population, Biomass and Bacterial Population:

The fungal population exhibited almost a similar trend of monthly variation in the soils of three different agricultural systems during the study period of 1981 and 1982. Maximum population was recorded in the month of July and thereafter a fall in the population was noted (Fig. 2.1). In the month of September, there was a slight increase in the population of the surface soil (0-10cm).

Fungal biomass of the soil followed a similar trend as that of the fungal population in all the three study sites. The

Fig. 2.1 Monthly variation of fungal population of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 21


TERRACE LAND
o 0-10cm depth
Δ 10-20cm ..
□ 20-30cm ..
VALLEY LAND
JHUM LAND
NO. OF FUNGI / g dry soil X 10³
0
2
4
6
8
10
12
M
J
J
A
S
1981
1982
SAMPLING PERIOD IN MONTHS

biomass was maximum in the month of July and thereafter it showed a decreasing trend (Fig. 2.2).

Bacterial population also exhibited almost a similar trend of monthly variation in all the three agricultural systems. The population was maximum in the month of June and thereafter it decreased. A slight increase in the population of the surface soil (0-10cm) was noted in the month of September (Fig. 2.3).

Both fungal and bacterial populations and the fungal biomass decreased with increase in depth. From the Figures 2.1-2.3, it is clear that the soil of valley land agriculture always harboured maximum fungal and bacterial populations and fungal biomass followed by terrace land and minimum was observed in the soil of jhum land agriculture.

Distribution of Fungal Species and their Percentage Relative Abundance:

Altogether 33 fungal species were isolated from the soil of all the three agricultural systems studied during the period of 1981 and 1982. Of which 12 belonged to the class Phycomycetes, 18 to Fungi imperfecti and 3 were Mycelia sterilia. Most of the species were distributed to all the depths (Tables 1.1-1.18).

Qualitatively the composition of fungal species was almost similar in all the three agricultural fields. Majority of the species isolated were common to all the soils. The class

Fig. 2.2 Monthly variation of fungal biomass of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 2·2

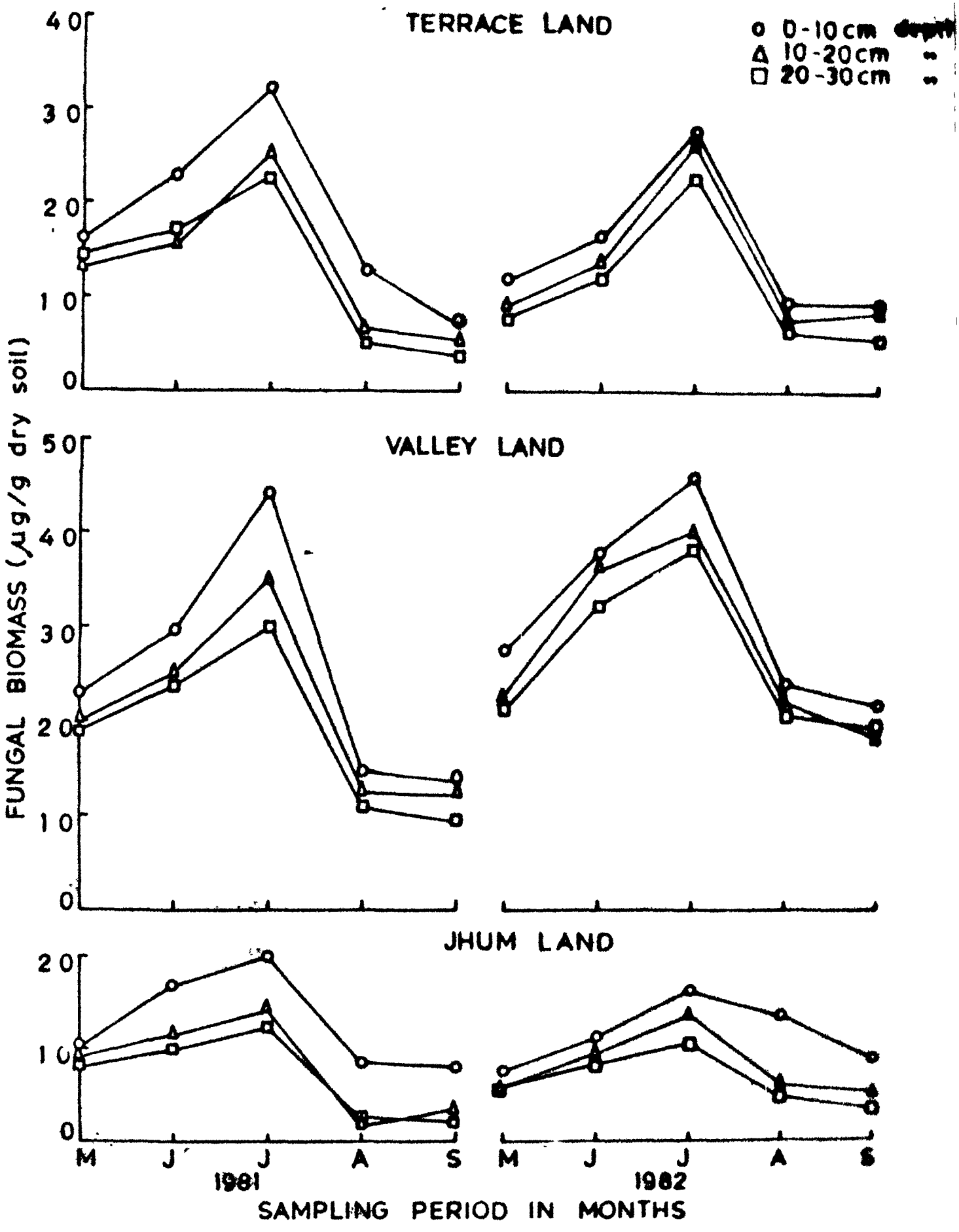

TERRACE LAND
o 0-10 cm depth
Δ 10-20 cm "
□ 20-30 cm "
VALLEY LAND
JHUM LAND
FUNGAL BIOMASS (µg/g dry soil)
0
10
20
30
40
50
M
J
J
A
S
1981
1982
SAMPLING PERIOD IN MONTHS

Fig. 2.3 Monthly variation of bacterial population of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 2·3

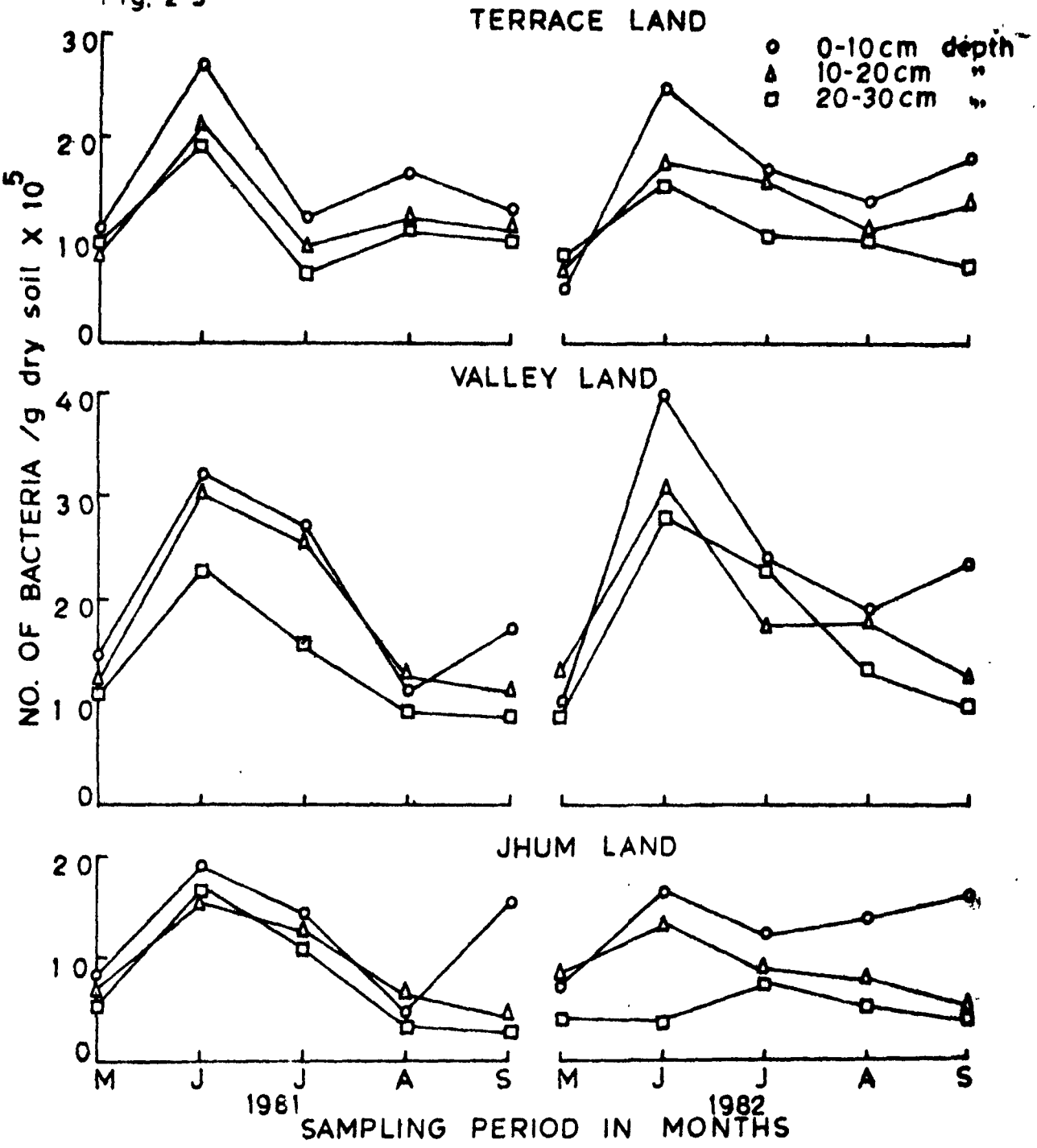

TERRACE LAND
0-10cm depth
10-20cm "
20-30cm "
NO. OF BACTERIA /g dry soil X 10^5
30
20
10
0
VALLEY LAND
40
30
20
10
0
JHUM LAND
20
10
0
M
J
J
A
S
1981
M
J
J
A
S
1982
SAMPLING PERIOD IN MONTHS

Table 1.1 Monthly variation of fungal population (per gram dry soil) in the soil of terrace land agriculture at 0-10 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981)				
	May	June	July	Aug.	Sept.
Mucor hiemalis	0.042 (10.00)	0.418 (26.40)	0.187 (35.40)	0.019 (4.76)	0.071 (15.93)
M. plumbeus	-	-	0.020 (3.80)	0.096 (14.29)	-
M. racemosus	0.006 (3.30)	-	0.040 (7.60)	-	0.058 (14.49)
Pythium sp.	-	0.069 (4.40)	0.020 (3.80)	0.099 (19.05)	0.019 (4.34)
Rhizopus nigricans	0.022 (5.20)	-	-	-	0.065 (15.94)
Aspergillus niger	0.025 (5.55)	0.013 (0.80)	-	-	-
Fusarium moniliforme	-	0.069 (4.40)	-	-	-
Geotrichum candidum	0.006 (3.30)	-	-	0.019 (4.76)	-
Penicillium chrysogenum	0.100 (27.40)	0.095 (6.00)	0.073 (13.93)	0.135 (33.30)	0.195 (42.47)
P. humicola	0.075 (14.90)	-	0.153 (29.10)	-	-
Penicillium sp.	0.045 (8.33)	0.443 (28.00)	-	-	-
Trichoderma viride	0.055 (10.50)	0.360 (22.80)	0.033 (6.33)	0.100 (23.81)	0.033 (7.25)
Trichoderma sp.	0.030 (6.30)	0.095 (6.00)	-	-	-
Verticillium sp.	0.023 (5.22)	-	-	-	-
Yellow sterile mycelia	-	0.019 (1.20)	-	-	-

Table 1.2 Monthly variation of fungal population (per gram dry soil) in the soil of terrace land agriculture at 0-10 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982) May	June	July	Aug.	Sept.
Mucor hiemalis	0.007 (1.17)	0.047 (2.96)	0.142 (29.8)	0.032 (6.70)	0.085 (15.70)
M. plumbeus	-	0.008 (0.59)	0.009 (0.59)	0.055 (9.70)	0.069 (12.86)
M. racemosus	-	-	0.072 (6.90)	-	0.054 (9.99)
Pythium sp.	0.007 (1.17)	-	0.052 (4.80)	-	0.023 (4.29)
Rhizopus nigricans	-	0.016 (1.19)	0.045 (2.40)	0.023 (4.70)	-
Aspergillus niger	0.063 (10.59)	-	-	-	-
Fusarium moniliforme	-	-	-	0.009 (2.30)	-
Geotrichum candidum	-	0.039 (2.52)	0.022 (1.20)	0.003 (0.50)	-
Penicillium chrysogenum	0.345 (57.66)	1.08 (82.84)	0.245 (46.70)	0.220 (33.80)	0.162 (30.00)
P. humicola	0.134 (22.35)	0.054 (4.14)	0.040 (2.30)	0.095 (19.90)	-
Trichoderma viride	0.042 (7.06)	0.062 (4.74)	0.065 (5.31)	0.100 (20.90)	0.085 (15.70)
Verticillium sp.	-	-	-	0.006 (1.50)	0.054 (9.99)

Table 1.3 Monthly variation of fungal population (per gram dry soil) in the soil of terrace land agriculture at 10-20 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981) May	June	July	Aug.	Sept.
Mortierella sp.	-	0.047 (5.42)	-	-	0.039 (9.38)
Mucor hiemalis	0.005 (1.20)	0.392 (29.45)	0.068 (12.82)	0.049 (18.61)	0.019 (4.69)
M. plumbeus	-	-	-	0.106 (39.54)	-
Pythium sp.	-	0.061 (6.97)	0.020 (3.85)	0.038 (13.96)	-
Rhizopus nigricans	-	-	-	-	0.007 (1.57)
Aspergillus niger	0.005 (1.20)	0.027 (4.30)	0.007 (1.28)	-	-
Cladosporium herbarum	0.072 (16.20)	-	-	-	-
Fusarium moniliforme	0.055 (10.20)	-	-	-	-
Penicillium chrysogenum	0.070 (15.30)	0.143 (14.72)	0.108 (20.50)	0.056 (20.94)	0.342 (76.26)
P. humicola	0.099 (20.5)	0.283 (23.25)	0.034 (6.41)	-	-
Trichoderma viride	0.110 (22.40)	0.167 (17.82)	0.291 (55.10)	-	0.033 (7.81)
Verticillium sp.	0.065 (12.60)	-	-	-	-
White sterile mycelia	-	-	-	0.019 (5.98)	-

Table 1.4 Monthly variation of fungal population (per gram dry soil) in the soil of terrace land agriculture at 10-20 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982) May	June	July	Aug.	Sept.
Mucor hiemalis	0.007 (2.11)	0.009 (2.70)	0.023 (2.68)	0.065 (4.00)	0.032 (5.79)
M. plumbeus	-	-	-	-	0.087 (15.96)
Pythium sp.	-	0.065 (6.40)	0.008 (0.88)	0.072 5.70)	0.060 (10.05)
Rhizopus nigricans	-	-	-	0.006 (0.50)	0.008 (1.44)
Aspergillus niger	0.007 (2.11)	0.003 (0.90)	0.015 (1.79)	-	0.008 (1.44)
Cladosporium herbarum	0.007 (2.11)	-	-	-	-
Cephalosporium sp.	-	0.085 (7.50)	-	0.090 (10.20)	-
Fusarium moniliforme	-	0.095 (8.10)	-	0.123 (15.00)	-
Penicillium chrysogenum	0.109 (31.93)	0.185 (39.30)	0.508 (59.83)	0.206 (30.80)	0.167 (30.45)
P. humicola	0.022 (6.39)	0.024 (4.90)	0.030 (3.56)	0.099 (11.00)	-
Trichoderma viride	0.123 (36.21)	0.100 (30.20)	0.227 (26.72)	0.200 (20.50)	0.119 (21.75)
Verticillium sp.	0.036 (10.66)	-	0.030 (3.56)	0.026 (2.30)	0.071 (13.05)
Yellow sterile mycelia	0.029 (8.46)	-	0.008 (0.88)	-	-

Table 1.5 Monthly variation of fungal population (per gram dry soil) in the soil of terrace land agriculture at 20-30 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981)				
	May	June	July	Aug.	Sept.
Mucor hiemalis	0.005 (0.70)	0.205 (20.14)	0.063 (11.50)	0.035 (14.64)	-
M. plumbeus	-	0.099 (9.70)	0.014 (2.56)	0.094 (39.04)	-
Pythium sp.	-	-	-	0.006 (2.24)	0.056 (36.88)
Rhizopus nigricans	-	-	-	0.010 (4.88)	0.021 (13.83)
Zygorhynchus sp.	0.065 (2.90)	-	-	-	0.007 (4.61)
Aspergillus niger	-	0.258 (25.37)	-	-	0.007 (4.61)
Cladosporium herbarum	0.123 (12.50)	-	-	-	0.007 (4.61)
Fusarium moniliforme	0.100 (12.37)	0.076 (7.46)	-	-	-
Paecilomyces sp.	-	-	-	-	0.021 (13.83)
Penicillium chrysogenum	0.060 (2.60)	0.219 (21.64)	0.438 (80.80)	0.065 (26.84)	0.028 (20.44)
P. humicola	0.150 (15.80)	0.083 (8.20)	-	-	-
Trichoderma viride	0.123 (12.50)	0.076 (7.46)	0.028 (5.10)	0.019 (7.32)	-
Verticillium sp.	0.290 (30.10)	-	-	-	-
White sterile mycelia	0.099 (8.33)	-	-	0.010 (4.88)	-

Table 1.6 Monthly variation of fungal population (per gram dry soil) in the soil of terrace land agriculture at 20-30 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982) May	June	July	Aug.	Sept.
Mucor hiemalis	-	-	-	0.003 (2.30)	0.032 (9.05)
M. plumbeus	-	0.007 (2.75)	0.012 (2.80)	-	-
Pythium sp.	0.008 (3.30)	-	-	0.025 (10.30)	0.040 (11.36)
Rhizopus nigricans	0.008 (3.30)	0.015 (5.58)	0.030 (5.60)	-	0.032 (9.05)
Zygorhynchus sp.	-	0.002 (1.23)	-	-	-
Acremonium acremonella	-	-	-	-	0.024 (6.80)
Aspergillus flavus	-	0.007 (2.75)	0.012 (2.80)	-	-
A. niger	-	-	-	0.029 (11.81)	-
Cephalosporium sp.	-	0.015 (5.58)	0.030 (5.60)	0.010 (5.16)	0.024 (6.80)
Cladosporium herbarum	0.038 (16.70)	-	-	0.006 (4.20)	-
Fusarium moniliforme	-	-	0.090 (16.54)	0.040 (20.54)	-
Penicillium chrysogenum	0.129 (56.70)	0.179 (66.67)	0.145 (37.46)	0.075 (35.00)	0.105 (29.46)
P. humicola	0.023 (10.00)	0.019 (6.70)	0.075 (12.50)	0.015 (5.30)	-
Trichoderma viride	0.015 (6.70)	0.037 (13.92)	0.080 (13.90)	0.006 (4.20)	0.081 (22.65)
Verticillium sp.	0.008 (3.30)	-	-	-	0.008 (2.25)
White sterile mycelia	-	0.007 (2.70)	-	-	-
Yellow sterile mycelia	-	-	0.012 (2.80)	-	0.008 (2.25)

Table 1.7 Monthly variation of fungal population (per gram dry soil) in the soil of valley land agriculture at 0-10 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981)				
	May	June	July	Aug.	Sept.
Absidia sp.	0.015 (8.40)	-	-	-	0.022 (2.63)
Mortierella sp.	0.052 (15.50)	0.053 (20.00)	-	0.021 (6.45)	-
Mucor hiemalis	0.035 (10.00)	0.088 (26.00)	0.134 (21.70)	0.043 (12.77)	0.087 (10.53)
M. plumbeus	-	-	0.082 (13.25)	0.057 (17.05)	0.065 (7.89)
M. racemosus	-	-	0.017 (1.19)	-	-
Pythium sp.	0.010 (4.00)	0.010 (9.00)	0.017 (1.19)	0.057 (17.05)	0.043 (5.26)
Rhizopus nigricans	0.039 (10.20)	-	-	0.007 (2.11)	0.109 (13.16)
Syncephalastrum sp.	-	-	-	0.029 (8.49)	-
Aspergillus niger	-	-	-	0.007 (2.11)	-
Cephalosporium sp.	-	0.009 (5.8)	-	-	-
Endomyces sp.	-	-	-	-	0.043 (5.26)
Fusarium moniliforme	0.006 (2.00)	-	-	-	0.087 (10.53)
Geotrichum candidum	-	-	0.099 (14.47)	-	-
Monilia sp.	-	-	0.022 (1.21)	-	-
Penicillium chrysogenum	0.035 (10.00)	0.038 (12.00)	0.142 (22.89)	0.071 (21.26)	0.043 (5.26)
P. humicola	0.006 (2.00)	-	-	-	0.087 (10.53)
Penicillium sp.	0.049 (10.50)	-	-	-	-
Trichoderma viride	0.075 (25.60)	0.065 (22.20)	0.040 (12.04)	-	0.239 (28.95)
Trichoderma sp.	-	-	-	0.043 (12.77)	-
White sterile mycelia	-	-	0.017 (1.19)	-	-
Yellow sterile mycelia	-	0.007 (4.00)	-	-	-

Table 1.8 Monthly variation of fungal population (per gram dry soil) in the soil of valley land agriculture at 0-10 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982)				
	May	June	July	Aug.	Sept.
Mucor hiemalis	-	0.075 (15.10)	0.096 (17.48)	-	0.099 (20.69)
M. plumbeus	-	0.010 (3.00)	0.015 (3.00)	0.033 (9.45)	-
Pythium sp.	0.021 (5.66)	-	-	0.100 (28.30)	-
Rhizopus nigricans	0.048 (13.15)	-	-	0.013 (3.79)	-
Syncephalastrum sp.	-	0.075 (15.10)	-	-	0.027 (6.42)
Aspergillus niger	0.027 (7.53)	0.005 (1.20)	0.015 (3.00)	-	-
Cephalosporium sp.	-	0.033 (7.50)	-	-	0.012 (3.85)
Endomyces sp.	-	0.040 (8.50)	-	-	-
Fusarium moniliforme	0.014 (3.79)	-	-	-	0.032 (8.96)
Geotrichum candidum	-	0.033 (7.50)	0.037 (7.48)	-	-
Penicillium chrysogenum	0.095 (20.77)	0.100 (22.00)	0.287 (58.19)	-	0.157 (26.92)
Trichoderma viride	0.178 (49.07)	0.099 (20.00)	0.007 (1.48)	0.207 (58.46)	0.150 (24.62)
Verticillium sp.	-	-	-	-	0.029 (7.69)
White sterile mycelia	-	-	0.007 (1.48)	-	-
Yellow sterile mycelia	-	-	0.037 (7.48)	-	-

Table 1.9 Monthly variation of fungal population (per gram dry soil) in the soil of valley land agriculture at 10-20 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981)				
	May	June	July	Aug.	Sept.
Absidia sp.	-	0.015 (4.44)	0.033 (6.19)	-	-
Mucor hiemalis	0.052 (10.50)	0.075 (22.22)	0.033 (6.19)	0.067 (18.00)	0.015 (5.43)
M. plumbeus	-	-	0.040 (7.49)	0.007 (1.90)	0.015 (5.43)
Pythium sp.	0.055 (13.30)	-	-	0.015 (4.02)	0.010 (4.20)
Rhizopus nigricans	0.003 (1.20)	-	-	-	0.015 (5.43)
Syncephalastrum sp.	-	-	0.007 (1.22)	-	-
Aspergillus candidus	-	-	-	-	0.015 (5.43)
A. niger	-	0.029 (8.88)	-	-	0.022 (8.10)
Cephalosporium sp.	0.035 (7.50)	-	-	-	-
Fusarium moniliforme	0.055 (13.30)	0.045 (13.33)	0.040 (7.49)	0.082 (22.02)	0.044 (16.19)
Geotrichum candidum	0.007 (2.20)	-	0.027 (4.93)	0.015 (4.02)	0.015 (5.43)
Penicillium chrysogenum	0.001 (1.00)	0.015 (4.44)	1.32 (51.26)	0.075 (19.98)	0.059 (21.62)
Penicillium sp.	0.040 (8.60)	-	-	-	-
Phoma humicola	-	-	0.033 (6.19)	-	-
Trichoderma viride	0.115 (27.10)	0.104 (31.11)	0.047 (8.63)	0.112 (29.99)	0.074 (26.96)
Trichoderma sp.	-	0.052 (15.55)	-	-	-
Verticillium sp.	0.088 (15.50)	-	-	-	-

Table 1.10 Monthly variation of fungal population (per gram dry soil) in the soil of valley land agriculture at 10-20 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982)				
	May	June	July	Aug.	Sept.
Mucor hiemalis	-	0.065 (12.08)	0.022 (6.25)	0.007 (2.61)	0.008 (1.45)
Pythium sp.	-	0.012 (4.02)	0.007 (2.06)	-	-
Rhizopus nigricans	0.033 (11.65)	0.035 (7.10)	-	-	-
Acremonium acremonella	-	-	-	-	0.047 (8.81)
Aspergillus niger	0.033 (11.65)	-	-	-	0.148 (27.99)
Cephalosporium sp.	-	-	-	0.007 (2.61)	0.039 (7.36)
Fusarium moniliforme	0.027 (9.27)	0.070 (12.80)	0.015 (4.19)	-	0.094 (17.62)
Geotrichum candidum	-	-	0.015 (4.19)	-	-
Penicillium chrysogenum	0.020 (6.97)	0.025 (5.00)	0.072 (20.81)	0.081 (31.60)	0.055 (10.26)
P. humicola	-	0.060 (10.50)	-	-	-
Trichoderma viride	0.140 (48.81)	0.950 (33.80)	0.196 (56.25)	0.162 (63.10)	0.101 (19.08)
Verticillium sp.	0.033 (11.65)	0.030 (5.50)	-	-	0.039 (7.36)
White sterile mycelia	-	0.045 (9.10)	0.022 (6.25)	-	-

Table 1.11 Monthly variation of fungal population (per gram dry soil) in the soil of valley land agriculture at 20-30 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981) May	June	July	Aug.	Sept.
Mucor hiemalis	0.009 (8.70)	0.015 (6.25)	0.015 (3.19)	0.023 (17.70)	0.036 (12.22)
M. plumbeus	-	-	0.015 (3.19)	0.008 (5.84)	0.020 (7.32)
M. racemosus	-	-	-	0.008 (5.84)	0.007 (2.41)
Pythium sp.	-	0.023 (9.48)	-	-	0.007 (2.41)
Rhizopus nigricans	-	-	-	0.008 (5.84)	-
Aspergillus niger	0.003 (2.00)	-	-	-	-
Cephalosporium sp.	0.055 (10.50)	-	-	-	-
Fusarium moniliforme	0.055 (10.50)	0.084 (21.88)	0.130 (27.69)	0.008 (5.84)	0.036 (12.22)
Geotrichum candidum	0.001 (1.75)	-	-	-	-
Penicillium chrysogenum	0.168 (24.70)	0.010 (5.83)	0.065 (13.84)	0.008 (5.84)	0.071 (24.36)
P. humicola	0.055 (10.50)	-	0.138 (29.21)	-	0.049 (17.04)
Trichoderma viride	0.062 (15.50)	0.154 (56.50)	0.036 (7.25)	0.111 (47.26)	0.057 (19.53)
Trichoderma sp.	-	-	0.072 (15.37)	-	-
Verticillium sp.	0.060 (12.80)	-	-	-	-
White sterile mycelia	0.004 (3.05)	-	-	-	-

Table 1.12 Monthly variation of fungal population (per gram dry soil) in the soil of valley land agriculture at 20-30 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982)				
	May	June	July	Aug.	Sept.
Mucor hiemalis	0.007 (3.67)	0.006 (2.80)	0.022 (5.08)	-	0.008 (1.68)
Pythium sp.	0.004 (2.25)	-	0.007 (1.68)	0.014 (5.58)	-
Rhizopus nigricans	-	0.007 (3.20)	-	-	0.071 (15.23)
Aspergillus niger	-	0.007 (3.20)	-	-	0.024 (5.08)
Cephalosporium sp.	-	-	-	-	0.032 (6.75)
Fusarium moniliforme	0.028 (14.79)	0.076 (23.20)	0.029 (6.76)	-	-
Geotrichum candidum	-	0.052 (11.07)	-	-	0.103 (21.98)
Penicillium chrysogenum	0.007 (3.67)	0.009 (5.20)	0.059 (13.57)	0.125 (50.00)	0.095 (20.30)
Penicillium sp.	-	0.066 (15.58)	-	-	-
Trichoderma viride	0.113 (53.30)	0.105 (35.70)	0.284 (64.41)	0.100 (38.80)	0.048 (10.15)
Verticillium sp	0.042 (22.25)	-	0.037 (8.49)	-	0.087 (18.63)
White sterile mycelia	-	-	-	0.014 (5.58)	-

Table 1.13 Monthly variation of fungal population (per gram dry soil) in the soil of jhum land agriculture at 0-10 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981)				
	May	June	July	Aug.	Sept.
Blakeslea sp.	0.022 (1.64)	0.004 (1.71)	-	0.012 (2.11)	-
Cunninghamella echinulata	-	0.008 (4.08)	0.002 (1.22)	0.018 (2.22)	-
Mucor hiemalis	0.043 (4.13)	0.006 (3.20)	0.009 (5.00)	0.035 (4.90)	0.116 (32.75)
Pythium sp.	0.024 (1.67)	-	-	-	0.006 (1.73)
Rhizopus nigricans	0.029 (1.80)	-	0.004 (3.90)	0.001 (1.00)	0.012 (3.45)
Aspergillus candidus	0.150 (21.35)	0.240 (38.59)	0.220 (30.00)	0.199 (26.80)	0.058 (12.50)
A. flavus	-	-	0.020 (8.60)	0.059 (7.08)	-
A. niger	0.055 (6.37)	-	0.100 (16.75)	0.070 (12.50)	0.104 (29.32)
Fusarium moniliforme	-	0.012 (5.69)	0.015 (7.40)	0.002 (1.90)	-
Geotrichum candidum	0.099 (8.60)	0.008 (4.08)	-	-	0.006 (1.73)
Penicillium chrysogenum	0.175 (36.10)	0.100 (20.90)	0.055 (11.50)	0.095 (20.09)	0.061 (17.24)
Trichoderma viride	0.110 (17.80)	0.110 (21.50)	0.095 (15.55)	0.085 (18.50)	-

Table 1.14 Monthly variation of fungal population (per gram dry soil) in the soil of jhum land agriculture at 0-10 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982) May	June	July	Aug.	Sept.
Cunninghamella echinulata	0.034 (9.50)	-	0.006 (1.25)	-	-
Mucor hiemalis	-	0.006 (1.80)	-	0.032 (3.25)	0.015 (2.20)
Rhizopus nigricans	-	0.012 (3.70)	-	-	-
Aspergillus flavus	-	-	-	0.032 (3.25)	-
A. niger	0.091 (25.40)	0.006 (1.80)	0.829 (70.91)	0.468 (41.39)	1.25 (82.88)
Fusarium moniliforme	-	-	-	0.019 (1.95)	-
Geotrichum candidum	0.034 (9.50)	-	-	-	0.028 (3.90)
Penicillium chrysogenum	0.085 (23.80	0.199 (61.81)	0.006 (1.25)	0.148 (14.90)	0.020 (2.80)
Penicillium sp.	-	-	0.024 (5.04)	-	-
Trichoderma viride	0.091 (25.40)	0.100 (30.80)	0.053 (11.39)	0.199 (20.10)	0.072 (7.70)
Verticillium sp.	0.012 (5.40)	-	0.041 (8.85)	0.122 (12.30)	-

Table 1.15 Monthly variation of fungal population (per gram dry soil) in the soil of jhum land agriculture at 10-20 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981)				
	May	June	July	Aug.	Sept.
Mucor hiemalis	-	0.004 (0.70)	0.015 (3.00)	0.095 (2.70)	0.008 (4.50)
Rhizopus nigricans	0.095 (8.00)	0.072 (6.90)	0.082 (9.10)	0.120 (6.20)	-
Aspergillus candidus	-	-	-	0.250 (12.20)	0.006 (3.00)
A. niger	0.352 (20.00)	0.220 (26.90)	0.163 (17.20)	0.485 (30.20)	0.096 (45.45)
Cephalosporium sp.	0.082 (6.60)	-	0.012 (2.10)	0.200 (11.50)	-
Cladosporium herbarum	-	0.019 (1.50)	-	-	0.019 (9.09)
Fusarium moniliforme	0.110 (15.20)	0.100 (10.80)	0.159 (17.00)	0.095 (2.70)	-
Geotrichum candidum	-	0.055 (3.20)	0.025 (4.50)	-	-
Monilia sp.	-	0.050 (2.20)	-	-	-
Penicillium chrysogenum	0.625 (40.00)	0.243 (29.00)	0.205 (24.80)	0.390 (21.40)	0.039 (18.18)
Trichoderma viride	0.085 (10.20)	0.123 (15.30)	0.199 (20.50)	0.190 (11.10)	0.039 (18.18)

Table 1.16 Monthly variation of fungal population (per gram dry soil) in the soil of jhum land agriculture at 10-20 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982) May	June	July	Aug.	Sept.
Mucor hiemalis	-	-	-	0.068 (9.90)	-
Rhizopus nigricans	0.025 (13.80)	0.019 (7.70)	0.019 (4.10)	-	0.021 (6.38)
Aspergillus flavus	0.031 (17.27)	-	0.006 (1.35)	-	-
A. niger	0.019 (10.30)	0.012 (5.20)	0.025 (5.50)	0.148 (21.60)	0.363 (45.80)
Cladosporium herbarum	-	0.006 (2.60)	-	-	0.015 (4.30)
Fusarium moniliforme	0.025 (13.80)	0.019 (7.70)	-	0.068 (9.90)	0.006 (1.05)
Geotrichum candidum	-	0.025 (10.20)	-	0.068 (9.90)	0.056 (9.60)
Monilia sp.	-	-	-	0.019 (2.80)	-
Penicillium chrysogenum	0.049 (27.60)	0.110 (46.20)	0.134 (32.93)	0.105 (15.30)	0.060 (10.00)
Trichoderma viride	0.012 (6.90)	0.043 (17.90)	0.191 (42.50)	0.148 (21.60)	0.099 (22.30)
Verticillium sp.	0.012 (6.90)	0.006 (2.40)	0.049 (10.97)	0.006 (0.90)	-
White sterile mycelia	0.006 (3.40)	-	-	-	-

Table 1.17 Monthly variation of fungal population (per gram dry soil) in the soil of jhum land agriculture at 20-30 cm depth during the sampling period of 1981. Values in the parentheses are percentage relative abundance.

Fungi	Months (1981) May	June	July	Aug.	Sept.
Gongronella sp.	0.010 (9.20)	-	0.008 (6.00)	0.085 (9.30)	-
Mucor hiemalis	0.005 (2.10)	0.006 (5.80)	-	0.100 (10.70)	0.026 (21.01)
Rhizopus nigricans	-	0.019 (12.70)	-	-	0.006 (5.21)
Acremonium acremonella	-	-	0.005 (4.20)	0.002 (1.20)	0.013 (10.60)
Aspergillus niger	0.085 (30.30)	0.048 (32.60)	0.075 (32.70)	0.150 (14.50)	0.013 (10.60)
Fusarium moniliforme	0.011 (10.20)	0.003 (3.47)	-	0.169 (18.30)	0.006 (5.21)
Penicillium chrysogenum	0.038 (19.20)	0.009 (9.10)	0.022 (20.80)	0.280 (24.40)	0.013 (10.60)
Trichoderma viride	0.022 (15.90)	0.032 (19.90)	0.052 (28.10)	0.075 (9.10)	0.099 (30.50)
Verticillium sp.	0.009 (7.70)	0.007 (7.90)	0.010 (8.20)	0.095 (9.90)	-
Black sterile mycelia	0.005 (2.10)	-	-	-	0.006 (5.21)
White sterile mycelia	0.006 (2.30)	0.006 (5.80)	-	-	-

Table 1.18 Monthly variation of fungal population (per gram dry soil) in the soil of jhum land agriculture at 20-30 cm depth during the sampling period of 1982. Values in the parentheses are percentage relative abundance.

Fungi	Months (1982) May	June	July	Aug.	Sept.
Mucor hiemalis	-	-	0.013 (2.20)	0.037 (6.60)	0.012 (1.20)
Rhizopus nigricans	0.030 (15.20)	0.032 (15.70)	-	0.013 (2.20)	0.012 (1.20)
Aspergillus flavus	-	0.013 (6.30)	-	-	0.012 (1.20)
A. niger	0.054 (27.30)	0.026 (12.50)	0.013 (2.20)	0.088 (15.20)	0.220 (21.40)
Cladosporium herbarum	-	-	-	-	0.167 (19.00)
Fusarium moniliforme	-	0.032 (15.70)	0.220 (20.10)	0.062 (10.90)	0.029 (3.60)
Penicillium chrysogenum	0.042 (21.10)	0.039 (18.70)	0.301 (35.94)	0.212 (36.90)	0.120 (17.86)
Penicillium sp.	-	-	0.013 (2.20)	-	0.095 (14.30)
Trichoderma viride	0.054 (27.30)	0.064 (31.20)	0.135 (24.99)	0.062 (10.96)	0.095 (14.30)
Verticillium sp.	-	-	0.058 (10.70)	0.075 (13.10)	0.045 (5.90)
White sterile mycelia	0.018 (9.00)	-	0.006 (1.20)	0.025 (4.10)	-

Phycomycetes was represented by 11 genera and 12 species. Mucor spp. and Rhizopus nigricans were common to all the three fields. Pythium sp. was rarely obtained from the soil of jhum land agriculture whereas this species was commonly isolated from the soils of valley land and terrace land agricultures.

The class Fungi imperfecti was represented by 13 genera and 18 species Trichoderma viride and Penicillium chrysogenum were isolated frequently from all the three fields. Besides, Aspergillus niger was found to be more abundant in jhum land soil. Fusarium moniliforme, Geotrichum sp., Cephalosporium sp. and Verticillium sp. could be isolated occassionally from all the three fields. Fusarium moniliforme was mostly isolated from the deeper layer of the soil. Zygorhynchus sp. and Paecilomyces sp. could be isolated only from the soil of terrace land agriculture. Phoma humicola, Endomyces sp. and Syncephalastrum sp. were restricted to valley land soil. Blakeslea sp., Cunninghamella echinulata and Gongronella sp. were restricted to the jhum land soil (Tables 1.1-1.18).

Dehydrogenase and Urease Activities and Carbon Dioxide Evolution of the Soil of three Agricultural Systems:

Dehydrogenase and urease activities followed almost a similar trend of monthly variations. The activities of both the enzymes were maximum in the month of June and thereafter it decreased (Figs. 2.4, 2.5). Both the activities decreased with increasd in depth.

Fig. 2.4 Monthly variation of dehydrogenase enzyme activity of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 2·4

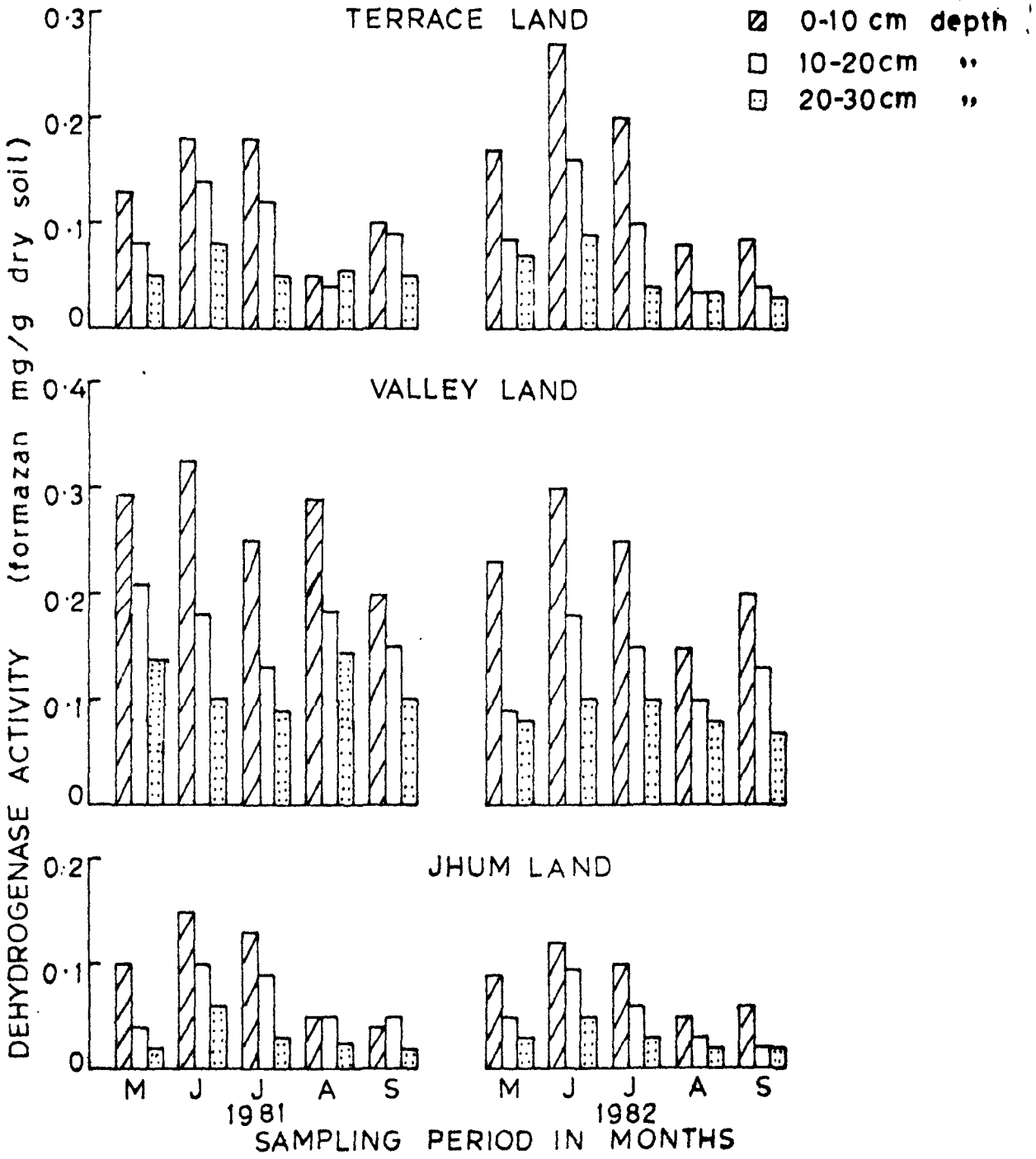
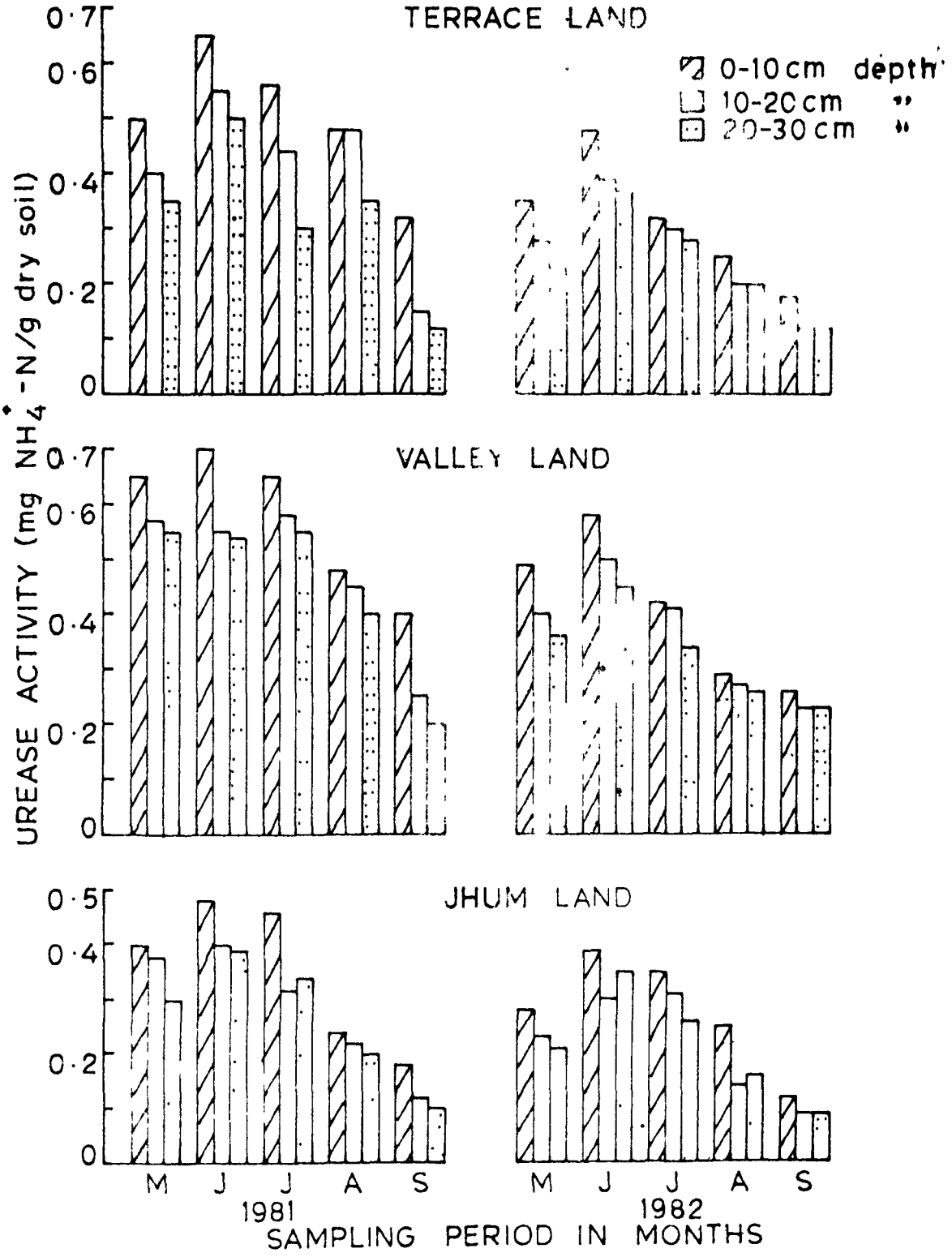

TERRACE LAND
0-10 cm depth
10-20cm ,,
20-30cm ,,
0·3
0·2
0·1
0
VALLEY LAND
0·4
0·3
0·2
0·1
0
JHUM LAND
0·2
0·1
0
DEHYDROGENASE ACTIVITY (formazan mg/g dry soil)
M J J A S
1981
M J J A S
1982
SAMPLING PERIOD IN MONTHS

Fig. 2.5 Monthly variation of urease enzyme activity of soil in three different agricultural systems during the study periods of 1981 and 1982.

Fig. 2·5


TERRACE LAND
0-10 cm depth
10-20 cm "
20-30 cm "
VALLEY LAND
JHUM LAND
UREASE ACTIVITY (mg NH_4^+-N/g dry soil)
0·7
0·6
0·4
0·2
0·5
0
M J J A S
1981
1982
SAMPLING PERIOD IN MONTHS

When correlation co-efficients between the enzyme activities, fungal and bacterial populations and soil physico-chemical characters were calculated, it was observed that there was no significant correlation between dehydrogenase activity and fungal and bacterial populations. A positive correlation was found between dehydrogenase activity and soil moisture content in all the three agricultural soils. A positive significant correlation was found between urease activity and bacterial population, organic carbon, total nitrogen and moisture content in the soil of valley land and terrace land agriculture, whereas it was not significant in jhum land soil (Tables 1.20-1.22).

Carbon dioxide evolution was maximum in the month of July and minimum was observed in the month of September in all the three agricultural soils (Fig. 2.6). Carbon dioxide evolution decreased with increase in soil depths. A significant positive correlation between carbon dioxide evolution, microbial population and fungal biomass was found in all the three agricultural soils (Tables 1.20-1.22). Moisture content was found to be positively related with carbon dioxide evolution in the valley land soil (r=0.866).

DISCUSSION

Quantitative Distribution of Soil Fungal and Bacterial Populations:

Maximum fungal population was observed in the month of

Table 1.19 Analysis of variance among various parameters.

Sources of variation	Fungi	Fungal biomass	Bacteria	Dehydrogenase	Urease	Carbon dioxide
Soil depth	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Site	< 0.05	< 0.05	NS	NS	NS	< 0.05
Observation date	NS	< 0.05	NS	NS	NS	NS
Soil depth X site	< 0.05	< 0.05	< 0.05	< 0.05	NS	NS
Soil depth X observation date	NS	< 0.05	NS	NS	NS	NS

NS = Not significant.

Table 1.20 Correlation coefficient values (r) for various parameters in terrace land soil.

Sources of variation	pH	M.C	O.C.	N	P	K	F	FB	B	D	U	CO_2
Temp	0.893**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
pH		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M.C			NS	NS	NS	NS	NS	NS	0.904**	0.697*	0.540*	NS
O.C.				NS	NS	NS	NS	NS	NS	NS	0.551*	NS
N					NS	NS	NS	NS	NS	NS	0.738**	NS
P						NS	NS	NS	NS	NS	NS	NS
K							NS	NS	NS	NS	NS	NS
F								0.925**	NS	NS	NS	0.937**
FB									NS	NS	NS	0.996**
B										NS	0.441*	NS
D											NS	NS
U												NS

Temp = Temperature, M.C.=Moisture content, O.C. = Organic carbon, N = Nitrogen, P = Phosphorus, K = Potassium, F = Fungi, FB = Fungal biomass, B = Bacteria, D = Dehydrogenase, U = Urease, NS = Not significant, * = Significant at 5% level, ** = Significant at 1% level.

Table 1.21 Correlation coefficient values (r) for various parameters in valley land soil.

Sources of Variation	pH	M.C.	O.C.	N	P	K	F	FB	B	D	U	CO_2
Temp	NS	NS	0.432*	0.502*	NS	NS	NS	NS	NS	NS	NS	NS
pH		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M.C.			0.620*	NS	NS	NS	NS	NS	NS	0.697*	0.771**	0.866**
O.C.				NS	NS	NS	NS	NS	NS	NS	0.755**	NS
N					NS	NS	NS	NS	0.592*	NS	0.738**	NS
P						NS	NS	NS	NS	NS	NS	NS
K							NS	NS	NS	NS	NS	NS
F								0.801**	NS	NS	NS	0.999**
FB									NS	NS	NS	0.888**
B										NS	0.444*	NS
D											NS	NS
U												NS

Temp = Temperature, M.C. = Moisture content, O.C. = Organic carbon, N = Nitrogen, P = Phosphorus, K = Potassium, F = Fungi, FB = Fungal biomass, B = Bacteria, D = Dehydrogenase, U = Urease, CO_2 = Carbon dioxide, NS = Not significant,

* Significant at 5% level, ** Significant at 1% level.

Table 1.22 Correlation coefficient values (r) for various parameters in jhum land soil.

Sources of variation	pH	M.C.	O.C.	N	P	K	F	FB	B	D	U	CO_2
Temp	0.893**	NS	NS	0.499*	NS	0.641*	NS	NS	NS	NS	NS	0.924**
pH		NS	0.852**	NS	NS	NS	NS	NS	NS	NS	NS	NS
M.C.			NS	NS	NS	NS	NS	NS	NS	0.639*	NS	NS
O.C.				NS	NS	NS	NS	NS	NS	NS	NS	NS
N					NS	NS	NS	NS	NS	NS	NS	NS
P						NS	NS	NS	NS	NS	NS	NS
K							NS	NS	NS	NS	NS	NS
F								0.987**	NS	NS	NS	0.837**
FB									NS	NS	NS	0.661*
B										NS	NS	NS
D											NS	NS
U												NS

Temp = Temperature, M.C. = Moisture content, O.C. = Organic carbon, N = Nitrogen, P = Phosphorus, K = Potassium, F = Fungi, FB = Fungal biomass, B = Bacteria, D = Dehydrogenase, U = Urease, NS = Not significant, * Significant at 5% level, ** Significant at 1% level.

Fig. 2.6 Monthly variation of carbon dioxide evolution of soil in three different agricultural systems during the study periods of 1981 and 1982.

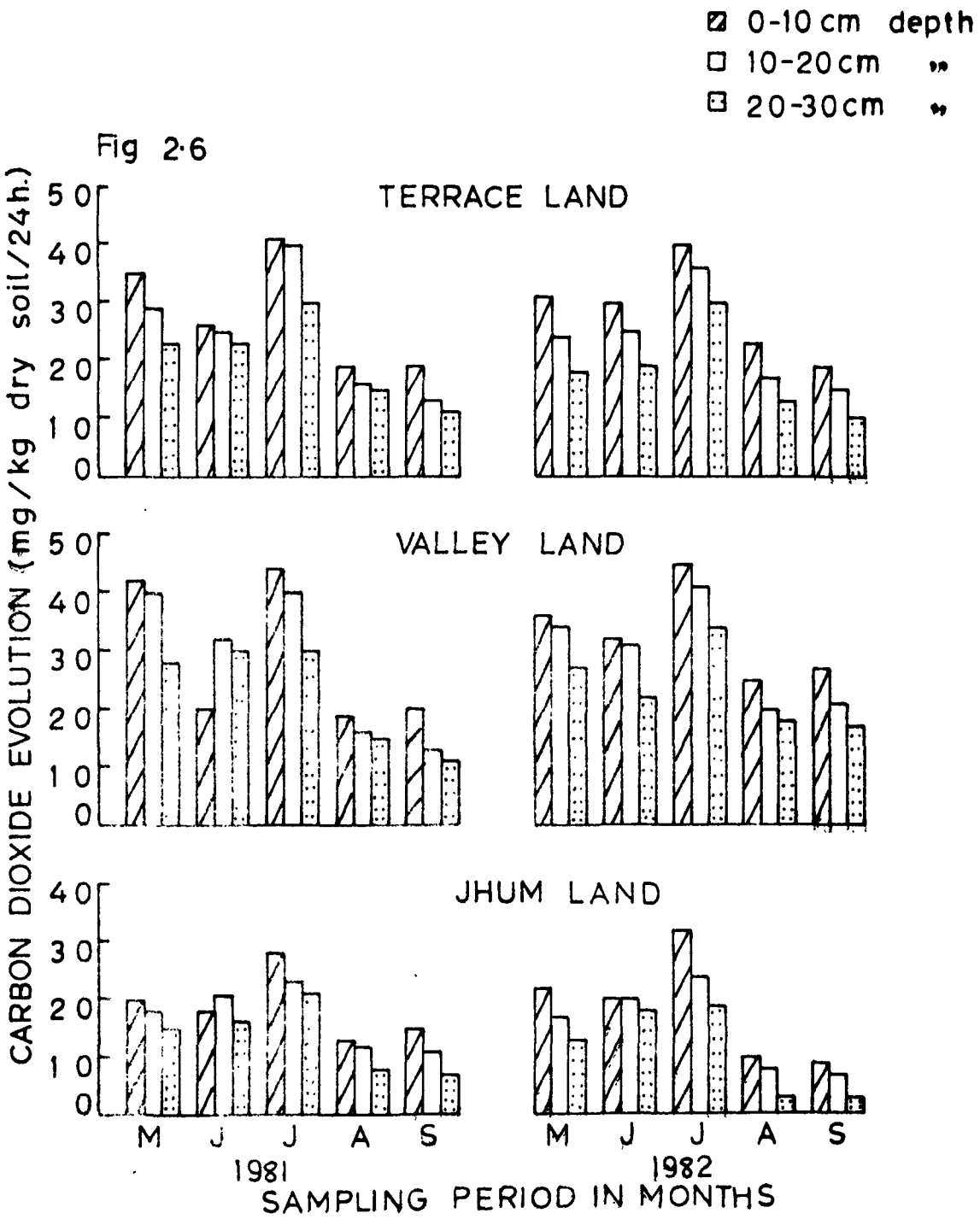

0-10 cm depth
10-20 cm ,,
20-30 cm ,,
TERRACE LAND
VALLEY LAND
JHUM LAND
CARBON DIOXIDE EVOLUTION (mg/kg dry soil/24h.)
50
40
30
20
10
0
M J J A S
1981
M J J A S
1982
SAMPLING PERIOD IN MONTHS

Fig 2·6

July when the crop exhibited maximum growth. The climatic conditions and availability of substrates at this period seem to influence favourably the growth and development of the soil microbes (Wright and Bollen, 1961; Christensen, 1969). The distribution of soil microbial population is not determined by a single environmental factor but by a set of factors like moisture, temperature, pH and organic carbon (Eiker, 1970a,b; Nazarov, 1970; Dadalauri, 1975; Singh and Charaya, 1975). Widden and Abitbol (1980) in their studies concluded that inspite of the tremendous spatial variation, significant seasonal variations occurred and they attributed this due to the changes in temperature. Parkinson and Balasoorya (1969) in their study of seasonal and spatial variation in the fungal population concluded that fungal population is more related to the soil moisture content than any other environmental factors. Saksena (1955), Dwivedi (1966a), Mishra and Kanaujia (1972) also observed a positive correlation between moisture content of soil and fungal population. Ghose and Dutta (1960) could not find such correlation between moisture content and fungal population. Jones and Richards (1977) explained the seasonal variation in bacterial population in terms of temperature and soil water regime.

However, during the present investigation statistically no significant correlation was observed between the soil moisture and soil microbial population and fungal biomass. Increased availability of substrates due to senescence and death of crop

plants may be responsible for the post harvest increase in the microbial population (Alexander, 1978).

Vertical Distribution of Soil Fungal and Bacterial Populations:

Depth-wise decrease in the fungal and bacterial populations observed in the present study may be attributed to the various soil factors such as variations in nutrient status at different depths and also the nature of the crop grown (Balasubramaniam et al., 1972). Selvaraj and Ramaswami (1978) opined that moisture regime and soil depth are important factors affecting microbial population and their activity. Similar pattern of vertical distribution of soil microbes has also been reported by several workers (Warcup, 1951; Saksena, 1955; Dwivedi, 1966b; Nicholas and Parkinson, 1967; Mishra and Kanaujia, 1972; Tyagi, 1973; Deka, 1981).

Fungal Species Distribution in Different Agricultural Systems:

Throughout the study period, mostly similar spectrum of fungal species were isolated and only a few species were restricted to each of the study sites. The similar type of fungal species composition may be due to the similar type of crop grown. Several workers have also observed that the composition of microfungal communities was correlated with the species composition in the above ground vegetation (Christensen et al., 1962; Christensen and Whittingham, 1965; Moral and Vanterpool, 1968;

Christensen, 1969). Slight variations in the fungal flora of the three agricultural systems at some of the samplings could be ascribed to the corresponding variations in the soil temperature, moisture and pH (Mishra and Kanaujia, 1972).

Only a few species were found to be dominant and could be isolated with high percentage relative abundance from each of the study sites. This finding is in conformity with the observation of Bissett and Parkinson (1979) who pointed out that for a given community only a few species were quite dominant which may strongly affect the environmental conditions for other species.

Martinez and Ramirez (1979), Deka (1981) observed that no species were in any instance confined to a particular horizon. They stated that the profile grouping were partly the result of the activity of soil fauna bringing about homogenization of the species composition along the whole profile. In the present study too, all the species were found to be distributed to all the depths and no definite pattern in distribution of fungal species was observed.

Among the fungi, members of Basidiomycetes and Ascomycetes could not be isolated at all. This may be attributed due to the fast growing nature of the forms like Trichoderma viride, Penicillium spp. and Mucor spp. which may probably inhibited the growth of such fungi. Another reason may be that the selec-

-tion of media and the method used were probably not conductive for their isolation.

Trichoderma viride was reported by Warcup (1951) to be common in acidic soils which is in agreement with the present studies. Mishra (1965) also isolated this genus repeatedly from waterlogged acidic soils. Baruah et al. (1980) also reported the similar finding. Several workers (Chesters, 1949; Warcup, 1951) have recorded that Pythium sp. show a seasonal distribution, being more regularly isolated when the soil moisture is relatively high. The similar observation was also made in the present study. The low moisture content may be accounted for the rare isolation of Pythium sp. from the jhum land soil. Trichoderma viride and Penicillium chrysogenum were repeatedly isolated for the soils of all the three agricultural systems with high percentage relative abundance. The dominance of these species in all the three agricultural soils may be due to their wide tolerance to the different extremes of environmental conditions. Aspergillus, Penicillium and Trichoderma are known to thermotolerant species and these species are reported to dominate fungal flora of the burnt soils (Tiwari and Rai, 1977; Jalaluddin, 1969; Herman and Kucera, 1979). Jensen (1931) and Garrett (1956) reported that Aspergillus are more abundant in warm regions of the world which is in conformities with the present study. The low number of fungal species and their population in jhum land soil may be due to direct effect of heat killing most of the propagules and

allowing only thermotolerant species to survive therein and also due to reduced nutrient status and moisture condition.

Dehydrogenase and Urease Activities and Carbon Dioxide Evolution:

Soil rich in microbial population harboured maximum enzyme activities which clearly depicts the relationship between the two. However, the monthly variation in the microbial population due to season, crop age and changes in the physico-chemical characters of the soil are not simultaneously reflected in the changed enzymatic activities. There may be a lag between the physico-chemical changes and their corresponding changes in microbial populations and their activities. Statistically no correlation between dehydrogenase activity and total microbial population of the soil was observed. It could be suggested that two parameters are governed by different environmental factors. Several workers (Stevenson, 1959,1962; Hirte, 1963; Gilot and Dommergues, 1967; Ross, 1973; Das, 1980; Deka, 1981) also could not correlate dehydrogenase activity and bacterial numbers. As dehydrogenase activity depends partly upon the metabolic activity of the soil microorganisms (Stevenson, 1962; Peterson, 1967), it is not surprising that its value in different soils does not always reflect the total numbers of viable organisms isolated on a particular medium. On the other hand a significent positive correlation was observed between dehydrogenase activity and moisture content. Ross and Roberts (1970) also found significant

positive correlation between dehydrogenase activity and soil moisture content. It seems that the increased moisture stimulated directly the microbial activity.

Both dehydrogenase and urease enzyme activities in the soils were higher during the growing period of the crop plants (Figs. 2.4, 2.5). This may be attributed to the continual addition of enzyme released from the plant roots (Skujins, 1967) and also due to associated microorganisms (Paulson and Kurtz, 1969; Speir and Ross, 1978).

With increase in organic carbon content enzyme activities increased. Speir (1977), Beri et al., (1978) and Ojeniyi (1980) reported that soil urease activity was largely controlled by the organic carbon status of the various soils. Dkhar and Mishra (1983) found positive correlation between urease activity and organic carbon and suggested that the organic carbon content may be accounted for most of the variations in soil urease activity. Dalal (1975) and Tabatabai (1977) also showed that urease activity was significantly correlated with organic carbon.

The microbial activities of the soil decreased with increase in depth [illegible] Tate (1979). The decrease in enzyme activities suggested that microbial activity was inhibited by unfavourable environmental conditions prevailing in the deeper region of the soil (Duxbury and Tate, 1981). Low microbial

population in the deeper soils may also be responsible for lesser microbial activities in deeper layer.

A significant positive correlation between carbon dioxide evolution, microbial population and fungal biomass was found in the soils of all the agricultural systems (Tables 1.20-1.22). It appears that the microorganisms are the most important contributor towards the CO_2 evolution. CO_2 evolution was maximum in valley land soil (Fig. 2.6), the higher rate reflects the favourable effect of soil moisture and moderate temperature on microbial activity (Gupta and Singh, 1981). Tesarova and Gloser (1976) have shown that soil moisture content was of paramount importance in controlling the CO_2 evolution from soil. Witkamp (1966) and and Anderson (1973) have indicated that temperature exerts a decisive influence on CO_2 metabolism of the soil. Similar result was observed in the soil of jhum land while same was not true in the other two agricultural lands.

Results of the present study demonstrate that enzyme activity is nutrient and moisture dependent. Soil moisture and temperature are important factors in governing the respiratory activity of maize field soils. The valley land agricultural system always harboured higher rates of CO_2 evolution, microbial population and fungal biomass, most possibly due to higher concentration of nutrients and sufficient availability of soil moisture. Considering enzyme activities, CO_2 evolution and

heterotrophic microbial population estimates as a nutrient release and decomposer activity function, it appears that the valley land agriculture is more stable and self sustainable. On the other hand, the terrace land and the jhum land agricultural systems seem to be fragile.

CHAPTER III

MICROBIAL DECOMPOSITION OF MAIZE LEAF, STEM AND ROOT LITTERS UNDER FIELD AND LABORATORY CONDITIONS

INTRODUCTION

In most terrestrial ecosystems, the amount of organic matter in the soil remains at a relatively constant level from one year to another. This results from the counterbalance of two converse actions: one is annual litter fall and annual death of roots, and the other is microbial decomposition of these litters. Satchell (1971) defined decomposition as the enzymatic oxidation of chemically complex woody plant materials by microorganisms, to more simple compounds and nutrients that become part of the soil. During decomposition many complex interactions, transformations and syntheses occur. Thus, at any one time the soil environment could contain a vast variety of chemical compounds, many of which could affect plants in many different ways. Plant residues and their decomposition products are involved in virtually all biochemical processes taking place in the soil, and most of these affect plant either directly or indirectly.

The role of microorganisms in the decomposition of plant material is of immense importance. As the plant materials fall on the ground, they are immediately attacked by various groups of soil microorganisms and soon after, the process of decomposition is initiated. The microbes are primarily responsible for the release of various nutrients locked up in the plant tissues which in turn are utilised by other organisms. Fungi, being

heterotrophic in their mode of nutrition, along with bacteria are responsible for the decay processes and thereby releasing locked up nutrients from the dead bodies of plants and animals. In the process of decomposition, fungi by the peculiarities of their physical organizations are specially effective in attacking hard and woody parts.

In any ecosystem, the microfungal population associated with the decomposition process is controlled by the environmental factors in which the plant is growing and also by the nature of plant parts involved (Garrett, 1963; Witkamp, 1963; Bakshi and Fokkema, 1976,1977; Alexander, 1978; MacLean and Wain, 1978; Edmonds, 1979). It has been suggested that the residues of the young materials decompose more readily due to their high carbohydrate and low lignin content, however, the residues formed from the old-growth timber are relatively resistant to decay. During the process of decomposition various compounds are released and become involved in biological interactions. These compounds may either have toxic or stimulating effects on microorganisms or there could also be no apparent effect at all.

Litter is also attacked by mites, other microfauna and by various microflora at first and readily utilizable materials are decomposed into carbon dioxide, water and minerals. Intermediate organic compound metabolites (sugar, amino acids etc.) are partly assimilated by microbes. The role of microorganisms in the conversion of cellulose into simple carbohydrates has

received sufficient attention. There is, however, a growing concern over the residues which result from agricultural wastes. The microbiological decomposition of cellulose and formation of humus is also one of the major processes maintaining the soil fertility. The rate of decomposition and the substances released may be affected by change in the nature of the soil environment by agricultural practices. A point which generally strikes to the biologist is whether the process of decaying may be accelerated. As microorganisms play an important role in decomposition of agricultural wastes, the selection and use of proper organisms after experimentation, is being taken up. In a consideration of the decomposition of crop residues, however, the underground parts of the plant should not be forgotten since they provide energy and nutrients to support the growth of organisms such as earthworms, fungi and bacteria, that can themselves improve soil structure or strengthen and thus preserve desirable soil aggregates.

The increased interest in the role of microorganisms in nutrient and energy flow relationships in natural as well as manmanipulated environments has emphasized the need of the studies related to litter decomposition. In the process of decomposition microbial attack is the most important and the process becomes further complicated since large variety of micro-organisms are involved in the process. To understand this complex phenomenon it is logically important to evaluate the role of the most important groups of organisms, their succession and

natural influence.

Addition of organic materials to the agricultural fields in the form of crop residues contributes significantly towards maintenance of the soil fertility. Maize is one of the most important agricultural crop of North-Eastern India. A large amount of maize litter is left in the fields after harvest. Detailed study on the rate of decomposition, microbial populations and chemistry changes during decomposition of maize litter in field and laboratory condition is lacking. Keeping this in view, the present investigation was undertaken to better understand the subject.

Present chapter deals with the studies on rate of decomposition, chemistry change and microorganisms associated with decomposing maize litters under field condition. Role of certain dominant fungal species in the process of maize litter decomposition has been studied under laboratory condition.

REVIEW OF LITERATURE

Studies of decomposition of plant leaf litter on the forest floor and crop debris in the agricultural fields are important for an understanding of soil fertility affecting both the physical structure of the soil and its nutrient status. In addition, the study of crop residues and their decomposition has been vigorously pursued with a view to the biological control

of plant pathogenic organisms found on the crop debris and in the soil. Last (1955) put forth his view that perhaps the litter is an admixture of propagules persisting from the phylloplane flora when the leaves were functional units and these species were capable of colonizing and decomposing the litter.

There has been a number of studies concerned with the decomposition of above-ground parts of various plant species. However, the underground parts of the plant should not be ignored since they influence the composition and activity of the soil microflora and fauna and thus affect the rate of decomposition. Waid (1957) while studying the distribution of rye grass roots, pointed out that the most active population originated from the surface of intact roots and the primary colonisers were fungi with a low degree of competitive saprophytic ability whereas secondary colonisers possessed a high degree of competitive saprophytic ability.

The rate of litter decomposition is regulated by different environmental factors. Pugh (1958) studied the leaf litter decomposition of Carex paniculata L. and recorded the changes in the fungal flora as decomposition proceeded. He suggested that the temperature and moisture content of leaf litter were the main factors that influence the seasonal activities of microbes during the decomposition process. Douglas and Tedrow (1959) investigated the decomposition of organic matter in the field in the arctic soils using an evolved CO_2 technique. They found that

temperature, moisture potential and soil type influenced the rate of decomposition. Mikola (1960) noted that temperature was the most important factor regulating the litter decomposition rate, since rainfall was evenly distributed throughout the year.

Garrett (1963) stated that succession of fungi is regulated mainly by the nature of carbon substrate available to the microorganisms during the decay. He further putforth his view that the amount of nitrogen content is probably the most important factor limiting decomposition.

Caldwell (1963) has provided an account of the fungal flora associated with decomposing beach leaf litter in soil. He observed some of the commonest fungi from the earliest to the latest stages of decomposition with a little change in their frequency.

Das (1963) while studying the successional pattern of fungi on roots of Oryza sativa distinguished three groups of fungi colonizing one after the other depending on the different growth stages. In the early stages of growth, species of Aspergillus and Penicillium are common isolates but specialized root inhabitants like Thielavia angulata and Humicola sp. occur dominantly on mature roots. Trichoderma viride and Cephalosporium sp. become prominent only on dead and decomposing roots left in the field after harvesting.

Witkamp (1963) observed that fungal and bacterial popula-

tions are positively correlated with the rate of litter breakdown. He also noted that temperature, moisture and stage of litter decay effectively controlled the microbial population and rate of litter breakdown.

Little is known about the distribution of bacteria on decomposing roots. Stenina (1964) sampled the bacterial populations that developed on a mixture of Phleum and Trifolium roots enclosed within thin glass bags buried in soil. He observed that the bacterial population increased in number to reach a maximum between the 30th and 60th day of burial and declined thereafter. He reported that there is a correlation between number of bacteria present and the rate at which the roots were decomposed.

Sokolov and Karpova (1965) studied decomposition, humification and mineralization in a mixed pine forest podsol. They found that the first substances to be decomposed were the water-soluble alcohol and benzene extractives such as starch, hemicelluloses and amino acids in organic residues and lignin was the last to be decomposed.

Macauley and Thrower (1966) while studying the succession of fungi on the leaf litter of Eucalyptus regnans recognized five groups of fungi. They observed that species of coleomycetes and some moniliales were the important initial colonizers on the litter whereas the species of Penicillium and Mucorales appeared

at later stages of decomposition. They further indicated that the fungi capable of utilizing cellulose or pectin are the important early colonizers.

Smith (1966) while studying the decomposition of three plants, maize (Zea mays L.), wheat (Triticum aestivum L.) and soyabean (Glycine max L Merr.) observed that root decomposed more slowly than leaf or stalk tissue. His results indicated the relatively greater resistance of roots to decomposition even when they have been ground to very small fragments. He showed that this resistance was not due to a lack of nitrogen in the root tissue. Perhaps the relative distribution and proportion of resistant carbonaceons materials in roots confered upon them this resistance to biological breakdown.

Heal et al. (1967) studied the litter decomposition in antarctic soil and reported that the slow decomposition rate is due to low temperatures and a restricted growing season.

Rai (1973) studied the mycofloral succession on decaying leaves of Saccharum munja and recognised three groups on the basis of sporing periods of fungi. He reported considerable variation in the quality and quantity of fungi situated at various nodes of the plants. Further, he concluded that the succession of fungi on decaying leaves seems to be governed by their competitive saprophytic as well as cellulolytic ability, moisture content of the substrate, temperature and relative

humidity of the atmosphere, inter-competition between fungi and nutritional variation in substrates.

The removal of above ground vegetation and soil fauna also seems to have some effect on the leaf litter decomposition. Study of Likens *et al.* (1970) has shown that the removal of forest vegetation increased the decomposition which in turn enhanced the nutrient availability to the soil mycoflora. Edwards *et al.* (1970) showed ample evidence of the role of soil fauna and their involvement in litter decomposition in terrestial habitats.

Ivarson (1973) observed that the rate of decomposition increased with the increase in temperature. Malone and Reichle (1973) showed that shoot litter decomposed more rapidly than the roots suggesting that the latter are tougher than shoots. He found that fragmentation by soil animals accelerates the decomposition by microorganisms.

Recently much attention has been paid to the techniques devised for studying the rate of decomposition of different types of plant litter in varying climatic conditions (Howard and Howard, 1974; Jensen, 1974). Williams and Gray (1974) reported that litter decomposition may be regulated by a combination of factors including the physico-chemical properties of the substrate and the external environment.

Heal and French (1974) suggested that high decomposition

rates in the first year after litter accumulation are mainly due to leaching effects and not due to biological activity.

Temperature, moisture, soil reaction and the amount and availability of nutritional components affect the biological activity and consequently, the rate and direction of transformation of the organic substances in the soil (Hoflich, 1975). Parnas (1975) concluded that the rate of decomposition of any substrate is proportional to the growth-rate of its decomposers.

Martyniuk and Myskow (1976) found the highest number of bacteria and actinomycetes in the first month of decomposition of plant material and fungi developed more intensively in the later period.

Brinson (1977) has suggested that both temperature and moisture were important factors controlling the decomposition rate. Lawrey (1977) stated that an important factor which may influence early stages of decomposition in the bare area is the degree of exposure. He observed that an exposed soil surface affords less favourable conditions for colonization and growth of microorganisms than shaded. He further stated that a bare soil surface also supplies less energy for the support of microbial populations.

Alexander (1978) stated that as the decomposition proceeds, hemicellulose and cellulose disappear whereas lignin like materials remain. He observed that during decomposition

hemicellulose disappeared at a more rapid rate than the cellulose. He further stated that as the plant materials are decomposed by fungi, a variety of organic constituents are released which provide substrate for the succession of ecological groups of fungi, each organism or group of organisms altering the organic constituents until complete decomposition occurs. Subsequently, he suggested that such organic constituents serve as the basis for categorising the fungi into different ecological groups. He further reported that decomposition of plant materials is influenced by soil pH.

Mishra (1979) reported that the distribution pattern of the fungal species on and within the decomposing roots, besides a number of other factors, is regulated by the enzymatic activities of the fungi.

Kanazawa and Yoneyama (1980) investigated the decay of rice residues under both flooded and upland soil conditions. They reported that carbon loss from the soils amended with rice residues and decrease in the weight of total plant debris proceeded at a rapid speed in the early periods and then at a slow speed in the subsequent periods under both flooded and upland soil conditions.

Antheunisse (1981) studied the influence of pH, temperature and organic matter on the degradation of the lignin-cellulose complex of coconut fibres. He found that the quick and

total decay of the coir wrapper around drain pipes seems to be caused by Basidiomycetes only, however, many fungi belonging to Deuteromycetes and Ascomycetes and bacteria were isolated from partly decayed coir.

Harper and Lynch (1981) while studying the chemical components and decomposition of wheat straw observed that internodes have more cellulose and less water soluble fractions than the leaves and the nodes. Lignin and hemicellulose contents are almost similar. The leaves and nodes of wheat straw lose weight more rapidly than the internodes during the early stages of aerobic decomposition.

Moustafa and Sharkas (1982) investigated the fungi associated with cellulose decomposition in the tidal mud-flats of Kuwait. They found no coincidence between the frequency of occurrence and cellulytic ability of fungal species. They suggested that the high frequency of any fungus do not imply that the fungus is an active cellulose decomposer in the soil and similarly low frequency fungi are not necessarily weak decomposers.

Knapp et al. (1983) while studying the microbial respiration and growth during the decomposition of wheat straw observed that increasing rates of nitrogen did not appreciably increase the overall decomposition rates of crop residues and the stimulatory effect of nitrogen on the mineralization of

straw residues is shown to be nonuniform over time. Finally, they postulated that the turnover of substrate and of microbially associated organic nitrogen results in significant decomposition of crop residues even under presumably nitrogen deficient condition.

MATERIALS AND METHODS

The study regarding decomposition of litters (leaf, stem, and root) of maize (Zea mays L.) plants was carried out under field and laboratory conditions.

In the field decomposition study, the following parameters were taken into account.

1. Rate of litter decomposition,
2. Population dynamics of litter microflora (Fungi and bacteria), pH and moisture contents of the litters,
3. Changes in cellulose, hemicellulose, lignin, sugar and amino acid contents of the litters.

1. Rate of Litter Decomposition:

Litter bag technique (Bocock et al., 1960) was applied to study the rate of litter decomposition of maize in the field. Different parts i.e. leaf, stem, and root of maize plant were collected from the field and cleaned and oven-dried at 60°C till the constant weight was recorded. Plastic bags were prepared into 20 cm size having a mesh size of 1.0 mm. Each bag was filled

with 10.0 grams of oven-dried litter and was closed by folding over the open end and stitched. The bags, containing leaf and stem litters separately were placed randomly in the field while those containing root litter were buried under the soil (5.0 cm deep) in the same field on November 15, 1981. The litter in the bags was then allowed to decompose. The samplings were done at monthly interval for a period of seven months. On each sampling date five replicate bags of each type of litter were collected aseptically in sterilized polythene bags and were brought to the laboratory to measure the loss in dry weight and for the analysis of microbial population of the litter. Of the five litter bags, one was used for microbial analysis and one for the pH and moisture content of decomposing litter. The remaining three litter bags were made open and from each bag, litter was carefully separated, cleaned from any adhering soil particles and was allowed to dry in a hot air oven at a temperature of 60^{o}C until a constant dry weight was obtained. The percentage weight loss of litter was calculated on the basis of oven dry weight of the sample.

2. Population Dynamics of Litter Decomposition:

The aim of this study was to evaluate the succession of microflora during the litter decomposition. The assessment of litter microflora was done mostly on the same day of collection. Occasionally, the bags were stored overnight in the freeze

at $4^{o}C$ when the processing could not be completed on the same day (Ruscoe, 1971).

Isolation of Microflora:

Dilution plate method (Waksman, 1922) was followed for the estimation of fungal and bacterial populations associated with the decomposing litters. One gram of litter was cut into 1.0 cm wide strips using sterilized scissors and introduced into a 250 ml sterilized conical flask containing 100 ml of sterilized distilled water. Litter suspension of 1:100 dilution was prepared. The flask was thoroughly shaken for fifteen minutes for homogenizing the suspension. 10 ml of the suspension was then transferred aseptically by means of a sterilized 10 ml pipette to another 250 ml sterilized conical flask containing 90 ml of sterilized distilled water to get a suspension of 1:1000 dilution. The process was repeated once more to get a suspension of 1:10000 dilution. 1:1000 and 1:10000 dilutions were found suitable for isolation of fungi and bacteria respectively. Isolation of fungi and bacteria was done using Rose Bengal Agar (Martin, 1950) and Nutrient Agar media (Johnson and Curl,1972) respectively.

Litter suspension of 0.5 ml was transferred aseptically from 1:1000 and 1:10000 dilutions into each of sterilised Petri dishes containing 10-15 ml of cooled solidified Rose Bengal Agar and Nutrient Agar media for the isolation of fungi and

bacteria respectively. The Petri dishes were rotated gently with hand so that the inoculum was dispersed uniformly over the surface of the agar media. Three replicates were maintained for each sample. In each case separate sterile pipettes were used for transferring the litter suspension. The isolation was carried out in a "Laminar Flow Chamber". Inoculated Petri dishes of fungi and bacteria were incubated upside down at temperatures of 25°C for five days in BOD incubator and 30°C for 24 hours in a bacteriological incubator respectively. The total numbers of fungi and bacteria per gram dry litter were calculated by taking into consideration of moisture content and dilution of the litter. Identification of microfungi was done by following the manuals of Gilman (1956), Subramanian (1971) and Barnett and Hunter (1972). Only the quantitative estimation was done in case of bacteria and no attempt was made to identify them. Composition of different media used for the isolation of litter microflora is given in Chapter II.

pH and Moisture Content of Litter:

pH of the decomposing litters was determined with the help of a digital pH meter and moisture content was estimated by drying the litters in oven at 60°C for 24 hours.

3. Quantitative Estimation of Cellulose, Hemicellulose and Lignin:

Cellulose, hemicellulose and lignin contents of the

decomposed litters were estimated by the method described by Peach and Tracey (1955).

A litter powder of 0.3 g from each harvest was separately treated with 20 ml of 25% aqueous potassium hydroxide (KOH) solution (W/V) and kept for twelve hours at room temperature. In each case, the samples were centrifuged at 3000 rpm for ten minutes. The decant obtained from each sample was used for detection of hemicellulose. The residue left at the end of the digestion was washed several times in distilled water till the last trace of KOH was removed. It was then dried in a hot air oven at $60^{o}C$ for 24 hours, cooled to room temperature over anhydrous calcium chloride in a desiccator and was weighed. The amount so obtained was designated as total cellulose.

The decant of each sample was collected and neutralized with equal amount of glacial acetic acid and ethanol. The precipitate developed was filtered, washed, dried and weighed. The amount so obtained was accounted for total hemicellulose.

For the estimation of lignin, 0.3 g of dried powdered litter was taken in a test tube and treated with 72% sulphuric acid (AR) (H_2SO_4), and kept in a deep freeze for 24 hours, this mixture was centrifuged and decanted out. The clear solution obtained was discarded, whereas, the residue was washed many times to remove the traces of H_2SO_4 present. It was then dried and weighed. The amount so obtained was accounted as lignin

component of the litter.

Quantitative Estimation of Total Sugar and Amino Acid:

Methods described by Mahadevan and Sridhar (1982) were followed for the estimation of sugar and amino acid. 0.3 g of dried powdered litter was weighed out into a test tube and treated with 15 ml of 80% ethyl alcohol. Occasionally, when any colour developed, it was treated with activated charcoal and centrifuged at 6000 rpm. The solution was then filtered through a Whatman No. 1 filter paper. The clear filtrate was boiled on a hot water bath to remove the last trace of alcohol and then distilled water was added to the test tube to make the volume upto 5 ml. To 3 ml of this solution, 6 ml of anthrone reagent (0.4% in H_2SO_4) was added gently to the test tube from the side while the test tube was kept in a icecold water. The test tubes were gently shaken and warmed on a hot water bath for few minutes till the green colour developed. The optical density of the filtrate was read in a Spectro photo meter at 610 nm. Standard curve was prepared from transmittance of varying concentration of glucose solution treated with anthrone reagent as described for the samples. From the standard curve the values of the total sugar of the samples were calculated and expressed in mg/g dry weight of the samples.

To the rest of the 2 ml solution, 2.5 ml of ninhydrin citrate buffer solution was added. The mixture was then placed

on a hot water bath for half an hour. A light purple colour developed and optical density of the solution was read at 540 nm in a spectro photo meter. Standard curve was prepared from the transmittance of the varying concentration of leucine solution treated with ninhydrin citrate buffer solution. From the standard curve the values of the total aminoacid of the samples were calculated and expressed in mg/g dry weight of the sample. The estimation in each case was done in triplicates.

Decomposition Under Laboratory Condition:

This experiment was carried out by selecting a few known fungi to assess their roles in litter decomposition under laboratory condition.

Two grams of oven dried maize litter of leaf, stem and root were introduced into 500 ml of conical flasks which were plugged properly with cotton. The litter was moistened with 20 ml of distilled water and sterilized by autoclaving at 15 lb pressure for 15 minutes.

Pure cultures of few selected, dominant litter fungi were maintained on Czapek Dox Agar medium (Raper and Thom, 1949). The composition of the medium was as below:

Agar	.....	15.0 g
$NaNO_3$	.....	3.0 g
K_2HPO_4	.....	1.0 g

$MgSO_4$ $7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4$	10.0 mg
Sucrose	30.0 g
Distilled water	1000.0 ml

Trichoderma viride and Absidia glauca were selected as test fungi for determining their efficiency for the decomposition. 20 blocks of 5 mm diameter were cut out with the help of a sterilized cork-borer from the periphery of 7 days old cultures of the fungi which were transferred to the flasks containing sterilized litter. In case of mixed inoculum, 10 blocks of each fungi were introduced into the flasks. In the control sets only the agar blocks were put (without fungi). The flasks were shaken nicely to mix the agar blocks with the litter. All aseptic measures were taken during inoculation. The flasks were then incubated in a BOD incubator at $25\pm1^{o}C$. Three replicates were maintained for each set of fungi and the harvesting was done at monthly interval for the first two months and subsequently at bimonthly intervals. Whenever necessary, required amount of sterilized distilled water was poured to maintain the moisture level. After harvesting, the litters were dried in a hot air oven at a temperature of $60^{o}C$ untill a constant weight was recorded. The percentage weight loss of litter was calculated on the basis of oven dry weight of the samples.

The decomposed litters were kept for the analyses of cellulose, hemicellulose, lignin, sugar and amino acid.

RESULTS

Decomposition Under Field Condition

Rate of Decomposition of Maize Residues:

The rate of breakdown of leaf, stem and root litters was slow during the early stages of decomposition (during winter) and increased slowly thereafter. Maximum rate was observed in leaf followed by stem and root (Fig. 3.1a). During the present study when the exponential decay model (Olson, 1963) was applied it was found that while in leaf the 95% life was 1578.9 days, in stem it was 1764.7 days and in root it may prolong upto 2000.0 days (Table 2.15)

Quantitative Estimation of Fungi and Bacteria in Decomposing Maize Residues:

The fungal population associated with the decomposing leaf, stem and root litters was minimum on first sampling date. It attained the maximum peak in the month of April in case of leaf and root and exhibited a decreasing trend thereafter, whereas in case of stem the maximum fungal population was observed in the month of May after which it declined to a low level (Fig. 3.2a).

Fig. 3.1 (a) Percentage weight remaining of maize leaf, stem and root after various periods of decomposition under field condition.

(b) Percentage weight remaining of maize leaf after various periods of decomposition by Trichoderma viride, Absidia glauca and mixed culture of T. viride and A. glauca under laboratory condition.

(c) Percentage weight remaining of maize stem after various periods of decomposition by Trichoderma viride, Absidia glauca and mixed culture of T. viride and A. glauca under laboratory condition.

(d) Percentage weight remaining of maize root after various periods of decomposition by Trichoderma viride, Absidia glauca and mixed culture of T. viride and A. glauca under laboratory condition.

Fig. 3·1

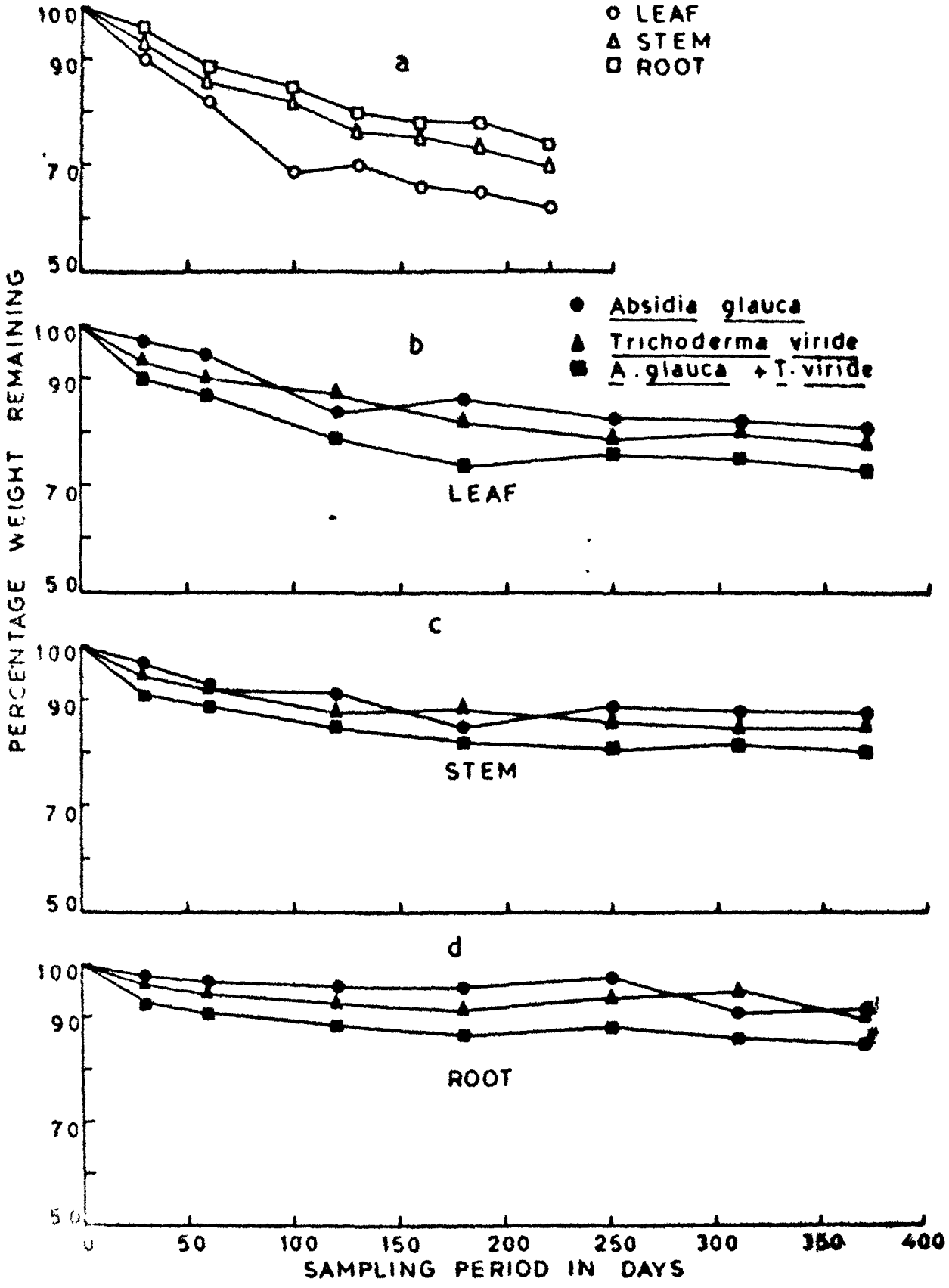
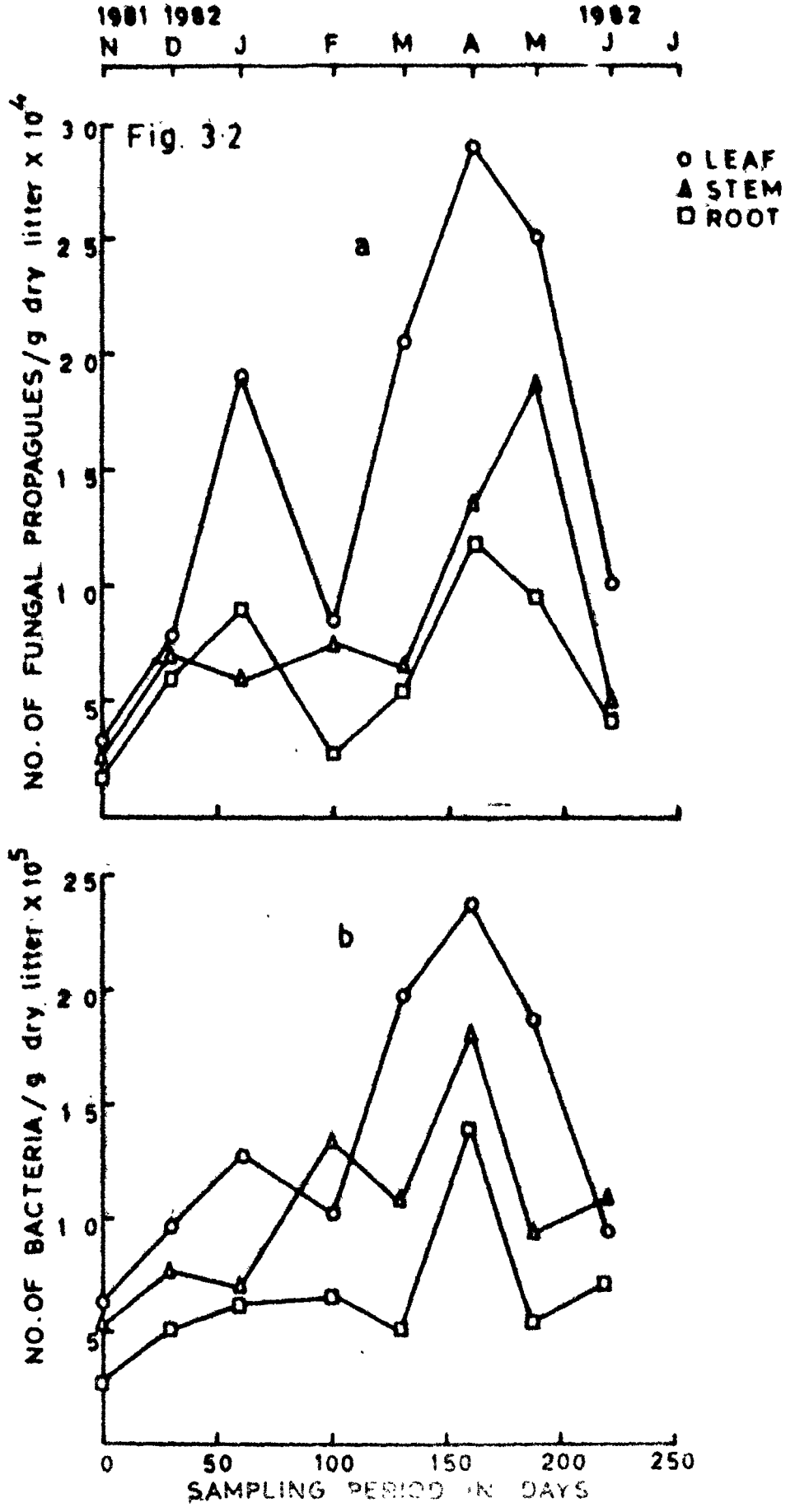

o LEAF
Δ STEM
□ ROOT
a
b
c
d
● Absidia glauca
▲ Trichoderma viride
■ A. glauca + T. viride
LEAF
STEM
ROOT
PERCENTAGE WEIGHT REMAINING
100
90
70
50
0
50
100
150
200
250
300
350
400
SAMPLING PERIOD IN DAYS

Fig. 3.2 (a) Fungal population of decomposing maize leaf, stem and root under field condition.

(b) Bacterial population of decomposing maize leaf, stem and root under field condition.


1981 1982
1982
N D J F M A M J J
Fig. 3·2
LEAF
STEM
ROOT
a
NO. OF FUNGAL PROPAGULES/g dry litter X 10⁴
30
25
20
15
10
5
b
NO. OF BACTERIA/g dry litter X 10⁵
25
20
15
10
5
0 50 100 150 200 250
SAMPLING PERIOD IN DAYS

Bacterial population of the decomposing litters also exhibited more or less similar pattern as that of fungal population. It was minimum during the early stages of decomposition. The population reached to its maximum in the month of April in all the litters and thereafter dropped to quite a low level in case of leaf, whereas in case of stem and root a slight increase was observed at the last sampling period (Fig. 3.2b).

Qualitative Estimation of Fungi:

Qualitatively there was not much difference in the fungal species isolated from leaf, stem and root litters. A total of 17 fungal species was isolated from leaf, stem and root litters of which 6 belonged to the class Phycomycetes, 9 to Fungi imperfecti and 2 belonged to Mycelia sterilia. Higher number of fungal species was isolated from the leaf litter as compared to the stem and root. Among Phycomycetous fungi, Mucor spp., Rhizopus nigricans and Absidia glauca were common to all the three litters (Tables 2.1-2.3)

In case of leaf, Penicillium chrysogenum, Aspergillus niger, Cladosporium herbarum, and Trichoderma viride were found to be present on most of the sampling periods (Table 2.1). Other species, however, occurred sporadically. In case of stem, Alternaria alternata, Penicillium chrysogenum and Trichoderma viride were isolated [illegible] more frequently (Table 2.2).

Table 2.1 Population of fungi (per gram dry litter X 10^3) associated with the decomposing leaf litter at various sampling periods. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING PERIOD IN DAYS							
	0	30	60	100	130	160	190	220
PHYCOMYCETES								
Absidia glauca	0.045 (12.20)	0.050 (14.31)	-	0.023 (6.76)	-	-	0.022 (2.31)	-
Mucor hiemalis	0.003 (1.25)	-	0.087 (7.92)	-	-	-	-	0.015 (2.30)
M. plumbeus	-	-	-	0.035 (10.17)	-	0.007 (2.68)	-	-
Pythium sp.	0.022 (10.20)	0.030 (8.57)	-	-	-	-	-	-
Rhizopus nigricans	-	0.030 (8.57)	-	-	0.004 (5.00)	-	-	-
FUNGI IMPERFECTI								
Alternaria alternata	-	-	-	-	-	0.007 (2.68)	0.006 (0.57)	0.002 (0.35)
Aspergillus flavus	-	0.049 (11.39)	-	-	-	-	-	-
A. niger	-	-	-	0.188 (54.25)	0.006 (7.62)	-	0.022 (2.31)	0.098 (20.20)
Cladosporium herbarum	-	0.090 (25.71)	-	-	0.006 (7.62)	-	0.483 (49.72)	0.290 (35.50)
Fusarium moniliforme	-	0.030 (8.57)	-	-	-	0.036 (13.54)	-	-
Penicillium chrysogenum	0.190 (45.57)	0.030 (8.57)	0.290 (25.90)	0.100 (28.83)	0.029 (38.57)	0.221 (65.53)	0.089 (9.24)	0.038 (5.42)
Penicillium sp.	-	0.050 (14.31)	-	-	0.012	-	-	-
Trichoderma viride	0.142 (30.50)	-	0.590 (53.96)	-	0.020 (25.72)	0.045 (15.25)	0.348 (35.85)	0.290 (35.50)
MYCELIA STERILIA								
White sterile mycelia	-	-	0.007 (0.71)	-	-	-	-	-
Yellow sterile mycelia	-	-	0.130 (11.50)	-	-	-	-	-

Table 2.2 Population of fungi (/g dry litter X 10^3) associated with the decomposing stem litter at various sampling periods. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING PERIOD IN DAYS							
	0	30	60	100	130	160	190	220
PHYCOMYCETES								
Absidia glauca	0.150 (15.20)	0.065 (10.50)	0.025 (2.34)	-	-	0.010 (0.58)	-	0.020 (1.68)
Cunninghamella echinulata	-	0.010 (2.30)	-	0.027 (9.82)	-	-	-	-
Mucor hiemalis	-	-	0.025 (2.34)	-	0.021 (16.65)	-	0.085 (10.25)	-
Rhizopus nigricans	0.003 (1.25)	-	0.049 (4.64)	-	-	-	-	0.030 (2.50)
FUNGI IMPERFECTI								
Alternaria alternata	0.120 (10.55)	0.980 (43.55)	-	-	0.064 (41.68)	0.033 (4.71)	0.029 (4.28)	0.090 (8.33)
Aspergillus niger	-	-	-	-	-	0.010 (0.58)	0.012 (1.72)	-
Cladosporium herbarum	-	0.020 (4.68)	-	-	**0.021** (16.65)	**0.121** (16.65)	**0.070** (9.00)	-
Fusarium moniliforme	-	-	-	-	0.005 (4.13)	-	0.029 (4.28)	-
Penicillium chrysogenum	0.300 (27.20)	0.420 (27.91)	0.146 (13.95)	0.196 (70.59)	0.029 (20.90)	-	0.099 (13.00)	1.05 (87.50)
Penicillium sp.	-	-	0.354 (33.72)	-	-	0.976 (54.12)	-	-
Trichoderma viride	0.520 (45.50)	-	0.427 (40.69)	0.054 (19.59)	-	-	0.550 (57.25)	-
MYCELIA STERILIA								
White sterile mycelia	-	0.020 (11.01)	0.025 (2.34)	-	-	0.155 (23.10)	-	-

Table 2.3 Population of fungi (per gram dry litter X 10^3) associated with the decomposing root litter at various sampling periods. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING PERIOD IN DAYS							
	0	30	60	100	130	160	190	220
PHYCOMYCETES								
Absidia glauca	0.075 (20.00)	-	0.038 (3.76)	-	-	-	-	0.017 (2.84)
Mucor hiemalis	-	0.054 (9.24)	-	0.017 (1.77)	0.022 (9.25)	-	0.015 (5.28)	-
Pythium sp.	-	-	-	0.020 (2.25)	-	-	-	-
Rhizopus nigricans	-	0.540 (58.80)	-	-	-	0.220 (10.28)	0.015 (5.28)	0.065 (17.24)
FUNGI IMPERFECTI								
Alternaria alternata	-	-	-	-	-	0.022 (1.14)	-	-
Aspergillus candidus	-	0.035 (11.42)	-	-	-	-	-	-
A. niger	0.009 (5.88)	-	0.038 (3.76)	0.078 (8.29)	-	-	0.014 (4.94)	-
Cladosporium herbarum	-	-	-	-	0.056 (25.00)	-	-	0.033 (8.62)
Curvularia pallescens	-	-	-	-	-	-	0.035 (12.52)	0.009 (2.59)
Fusarium moniliforme	0.120 (42.50)	-	0.644 (63.22)	0.148 (18.50)	0.099 (45.00)	0.440 (22.59)	0.112 (32.50)	0.097 (25.86)
Penicillium chrysogenum	-	0.030 (11.29)	0.029 (2.83)	0.549 (58.57)	0.044 (20.00)	-	0.070 (24.44)	0.087 (23.02)
Penicillium sp.	-	-	-	0.070 (6.75)	-	-	-	0.065 (17.24)
Trichoderma viride	0.099 (30.60)	0.025 (9.24)	0.269 (26.42)	-	-	1.390 (60.35)	0.043 (15.02)	0.009 (2.59)
MYCELIA STERILIA								
White sterile mycelia	-	-	-	0.038 (3.77)	-	0.110 (5.64)	-	-

In case of root, Penicillium chrysogenum, Trichoderma viride and Fusarium moniliforme were dominant fungi (Table 2.3). From the tables 2.1-2.3 it is evident that different members of Fungi imperfecti were found to be dominant at various stages of decomposition.

Changes in pH and Moisture Content of the Decomposing Maize Residues:

pH of the decomposing leaf, stem and root litters was higher at early stages of decomposition. As the decomposition proceeded pH of all the three typesof litters decreased (Table 2.4).

Moisture content of the decomposing leaf, stem and root varied with time. At the early stages of decomposition (in winter) the moisture content was very low in all the litters studied and the values increased and reached to their maximum at the end of the experiment (Table 2.4).

Changes in Cellulose, Hemicellulose and Lignin Contents of the Decomposing Maize Residues:

The amount of cellulose, hemicellulose and lignin contents was maximum on the first observation date and minimum at the end of the experiment in all the cases. As the decomposition preceeded the amount of these components decreased. The loss was less at the early stages of decomposition. In case of

Table 2.4 pH and moisture content (%) of decomposing leaf, stem and root litters under field condition.

Period (days)	Leaf		Stem		Root	
	pH	Moisture content (%)	pH	Moisture content (%)	pH	Moisture content (%)
Initial	6.55	0	6.20	0	6.32	0
30	6.49	11	5.95	8	5.20	10
60	6.84	30	6.25	15	6.43	24
100	6.78	13	6.64	7	6.64	13
130	5.75	30	6.36	32	6.13	43
160	4.44	37	6.72	39	6.44	40
190	5.50	50	6.80	59	6.46	48
220	4.65	60	5.25	50	5.30	54

cellulose and hemicellulose, after a slight increment, the value decreased gradually (Table 2.5). Maximum amount of lignin was detected in root followed by stem and minimum was found in case of leaf (Table 2.5). The rate of disappearance of this component was much slower than that of cellulose and hemicellulose. After a slight increment in lignin content at the early stages of decomposition, the value decreased gradually (Table 2.5). The amount of all the three components declined at a faster rate at the later stages of decomposition.

Changes in Amount of Total Sugar and Amino-Acid of Decomposing Maize Residues:

The amount of sugar and amino acid was maximum on the first observation date and minimum at the end of the experiment in case of leaf, stem and root litters. A slight increment in sugar and amino-acid was observed as the decomposition proceeded and thereafter decreased gradually upto the end of the experiment (Table 2.6).

Changes in Total Nitrogen of the Decomposing Maize Residues:

The nitrogen content (%) of leaf, stem and root varied with time. Maximum values were recorded after 130 days of processing in case of leaf and stem and minimum was recorded at the end of the experiment, whereas, in the case of root the maximum was found after 100 days of processing and minimum

Table 2.5 Percentage cellulose, hemicellulose and lignin in decomposing maize leaf, stem and root litters under field condition.

Period (days)	Leaf			Stem			Root		
	C	H	L	C	H	L	C	H	L
Initial	40	30	26	44	22	36	30	16	51
30	33	28	30	37	20	40	26	15	52
60	28	29	33	34	18	37	22	10	47
100	29	23	31	36	13	38	23	13	47
130	26	19	22	30	15	35	24	12	42
160	20	16	18	23	19	36	22	8	37
190	18	17	17	23	10	33	20	7	38
220	15	12	15	20	9	32	18	6	35

C = Cellulose, H = Hemicellulose, L = Lignin.

Table 2.6 Amount of total sugar and amino acid (mg/g) in decomposing maize leaf, stem and root litters under field condition.

Period (days)	Leaf		Stem		Root	
	Sugar	Amino acid	Sugar	Amino acid	Sugar	Amino acid
Initial	25	60	28	50	20	49
30	15	39	17	38	14	39
60	12	33	18	35	15	35
100	9	35	15	30	13	35
130	10	25	19	34	10	40
160	7	31	13	32	11	38
190	6	26	10	31	9	33
220	5	20	7	24	8	25

was at the end of the experiment (Table 2.7). The amount of nitrogen (%) initially was found to be maximum in leaf followed by stem and minimum in case of root (Table 2.7).

Decomposition Under Laboratory Condition

Rate of Decomposition of Maize Residues:

The growth rate of both the test fungi on the decomposing leaf, stem and root was vigorous during the early stages of decomposition and was very feable at the later stages of decomposition. The rate of decomposition of leaf, stem and root coincided with the growth rate of test fungi. It was faster during the first phase of decomposition and thereafter the rate of decomposition became slower (Figs. 3.1b,c, d). However, when the Olson's (1963) exponential decay model was applied it was observed that in leaf the 95% life value ranged from 2500.0 days for mixed culture of Trichoderma viride and Absidia glauca to 4285.7 days for A. glauca. In case of stem the value ranged from 5000.0 days for mixed culture of T. viride and A. glauca to 7500.0 days for A. glauca, and in case of root, the values ranged from 6000.0 days in mixed culture of T. viride and A. glauca to 15000.0 days for A. glauca (Table 2.16).

Changes in Cellulose, Hemicellulose and Lignin Content of the Decomposing Maize Residues:

The amount of cellulose, hemicellulose and lignin of

Table 2.7 Total nitrogen (%) in decomposing maize leaf, stem and root litter under field condition.

Period (days)	Leaf	Stem	Root
Initial	0.44	0.33	0.29
30	0.42	0.39	0.36
60	0.38	0.36	0.49
100	0.49	0.33	0.53
130	0.59	0.43	0.33
160	0.56	0.38	0.36
190	0.53	0.36	0.30
220	0.23	0.20	0.23

decomposing leaf, stem and root amended with pure and the mixed cultures varied in a regular fashion. In all the cases, the amount of these components was maximum on first sampling date and minimum at the end of the experiment (Tables 2.8-2.10). In case of leaf, stem and root, there was a slight increment in cellulose, hemicellulose and lignin contents at the early stages of decomposition after which it decreased gradually. However, in all the cases, the disappearance of these three components was rapid in the set amended with mixed culture of I. viride and A. glauca and minimum in A. glauca.

Changes in Total Sugar and Amino Acid of Decomposing Maize Residues:

The disappearance of the amount of sugar and amino acid was faster at the early stages of decomposition and slowed down at the later stages in all the three types of litters (Tables 2.11-2.13). The maximum loss in sugar was observed in the sets amended with pure culture of [illegible] A. glauca and minimum loss was observed in I. viride set in case of leaf, stem and root (Tables 2.11-2.13). The maximum loss in amino acid was observed in the sets amended with mixed culture of I. viride and A. glauca and minimum loss was observed in the set of A. glauca in leaf, stem and root (Tables 2.11-2.13).

Table 2.8 Percentage cellulose, hemicellulose and lignin in decomposing maize leaf by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*			*Absidia glauca*			*T. viride* + *A. glauca*		
	C	H	L	C	H	L	C	H	L
Initial	40	30	26	40	30	26	40	30	26
30	38	28	29	39	26	27	35	23	23
60	35	26	24	32	23	25	32	22	20
120	37	27	25	37	24	26	33	17	22
190	32	23	21	35	22	24	28	14	23
250	28	25	19	29	23	24	26	15	19
310	26	21	19	28	22	23	25	14	18
370	24	19	18	26	20	21	23	13	17

C = Cellulose, H = Hemicellulose, L = Lignin.

Table 2.9 Percentage cellulose, hemicellulose and lignin in decomposing maize stem by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*			*Absidia glauca*			*T. viride* + *A. glauca*		
	C	H	L	C	H	L	C	H	L
Initial	44	22	36	44	22	36	44	22	36
30	37	17	34	42	19	34	35	18	33
60	35	15	31	40	16	33	33	17	30
120	36	18	33	42	17	35	31	13	32
190	33	18	30	39	16	33	32	15	28
250	26	16	29	40	15	32	25	12	29
310	27	15	30	38	15	31	26	11	27
370	25	15	29	36	15	30	24	11	26

C = Cellulose, H = Hemicellulose, L = Lignin.

Table 2.10 Percentage cellulose, hemicellulose and lignin in decomposing maize root by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*			*Absidia glauca*			*T. viride* + *A. glauca*		
	C	H	L	C	H	L	C	H	L
Initial	30	16	51	30	16	51	30	16	51
30	33	15	46	34	15	46	28	11	44
60	30	11	49	31	12	50	26	10	39
120	29	14	45	29	13	40	25	12	40
190	27	9	43	30	9	42	27	11	37
250	24	9	39	27	8	40	25	7	36
310	24	8	38	25	9	39	24	6	35
370	21	7	36	23	8	38	20	6	35

C = Cellulose, H = Hemicellulose, L = Lignin.

Table 2.11 Amount of total sugar and amino acid (mg/g) in decomposing maize leaf by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*		*Absidia glauca*		*T. viride* + *A. glauca*	
	Sugar	Amino acid	Sugar	Amino acid	Sugar	Amino acid
Initial	25	60	25	60	25	60
30	15	43	14	41	13	43
60	17	39	16	40	11	38
120	8	35	8	32	5	30
190	10	34	9	30	10	27
250	9	25	7	32	9	20
310	8	24	6	28	6	25
370	7	23	5	24	6	22

Table 2.12 Amount of total sugar and amino acid (mg/g) in decomposing stem by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*		*Absidia glauca*		*T. viride* + *A. glauca*	
	Sugar	Amino acid	Sugar	Amino acid	Sugar	Amino acid
Initial	28	50	28	50	28	50
30	19	44	15	48	16	39
60	17	42	14	46	14	40
120	15	40	12	43	10	30
190	12	43	11	44	11	32
250	11	38	10	39	10	30
310	10	32	9	35	9	29
370	11	30	8	33	9	28

Table 2.13 Amount of total sugar and amino acid (mg/g) in decomposing root by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*		*Absidia glauca*		*T. viride* + *A. glauca*	
	Sugar	Amino acid	Sugar	Amino acid	Sugar	Amino acid
Initial	20	49	20	49	20	49
30	19	42	18	42	16	40
60	15	45	14	43	13	41
120	11	35	16	40	14	36
190	13	36	11	35	13	35
250	12	35	11	38	11	36
310	11	31	13	33	10	33
370	12	30	9	33	10	29

Changes in Total Nitrogen of Decomposing Maize Residues:

The total nitrogen content of the litters varied significantly in all the sets amended with different fungi. In all the three types of litters the total nitrogen content increased at the early stages of decomposition and decreased at the end of the experiment (Table 2.14).

DISCUSSION

The slower rate of decomposition at the early stages of decomposition (in winter) coincided with the low microbial population, low moisture content of the litters and low temperature (Fig. 3.2a,b, Table 2.4, Fig I). It appears that the environmental factors and season played considerable role on the rate of decomposition and the growth of microbes. Williams and Gray, (1974) and Witkamp (1966a) also observed similar results. Besides, the accumulation of refractory materials like lignin and the subsequent slower breakdown of the same also slows the rate of decomposition. The higher rate of decomposition at the later stages coincided with the increase in the populations of fungi and bacteria (Figs. 3.2a,b). This clearly depicts the relationship between the two. The rate of decomposition was fastest in leaf which may be, at least in part, due to rapid losses of polysaccharides from the leaf. On the other hand, the higher amount of lignin in root and stem may contribute to their slow

Table 2.14 Total nitrogen (%) in decomposing leaf, stem and root litters by *Trichoderma viride*, *Absidia glauca* and mixed culture of *T. viride* and *A. glauca* under laboratory condition.

Period (days)	*Trichoderma viride*			*Absidia glauca*			*T. viride* + *A. glauca*		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Initial	0.44	0.33	0.29	0.44	0.33	0.29	0.44	0.33	0.29
30	0.73	0.29	0.39	0.64	0.26	0.26	0.74	0.66	0.33
60	0.59	0.22	0.66	0.43	0.66	0.54	0.56	0.56	0.53
120	0.59	0.30	0.43	0.43	0.32	0.49	1.1	0.75	0.36
190	0.77	0.49	0.63	0.42	0.35	0.53	0.89	0.24	0.23
250	0.63	0.43	0.33	0.36	0.37	0.59	0.39	0.39	0.23
310	0.46	0.34	0.29	0.33	0.28	0.37	0.59	0.39	0.53
370	0.33	0.28	0.26	0.29	0.28	0.26	0.53	0.22	0.43

Table 2.15 Decay constant (K), Half life, and 95% life (percentage weight remaining vs. time in days) values for maize leaf, stem and root litters decomposition under field condition.

Litter type	K (per day	Half life (days)	95% life (days)
Leaf	0.0019	364.7	1578.9
Stem	0.0017	407.6	1764.7
Root	0.0015	462.0	2000.0

Table 2.16 Decay constant (K), Half life and 95% life (percentage weight remaining vs. time in days) values for maize leaf, stem and root decomposition by certain selected fungal species under laboratory condition.

Litter type	Fungal species	K (per day)	Half life (days)	95% life (days)
Leaf	*Trichoderma viride*	0.0010	693.0	3000.0
	Absidia glauca	0.0007	990.0	4285.7
	T. viride + *A. glauca*	0.0012	577.5	2500.0
Stem	*Trichoderma viride*	0.0005	1386.0	6000.0
	Absidia glauca	0.0004	1732.5	7500.0
	T. viride + *A. glauca*	0.0006	1155.0	5000.0
Root	*Trichoderma viride*	0.0003	2310.0	10000.0
	Absidia glauca	0.0002	3465.0	15000.0
	T. viride + *A. glauca*	0.0005	1386.0	6000.0

rate of decomposition indicating their relatively greater resistance to decomposition than the leaf (Sinha, 1972). Satchell (1974) also reported that the high lignin content acts as a barrier to microorganisms thereby affecting the rate of decomposition. The faster rate of leaf litter breakdown may be attributed to the low lignin content (Table 2.5).

Low fungal and bacterial populations observed during early stages of decomposition in leaf, stem and root may be, because of the resistant nature of the maize litters towards the microbial attack and also due to unfavourable moisture and temperature condition prevailing at that time. Further, low moisture content at the time perhaps did not favour leaching of soluble nutrients to support the microbial growth. Both the populations increased after an initial lag phase reaching to their maximum levels in the month of April when the moisture content was fairly high thereby helping in the rapid growth of the microbes. Another reason may be the availability of more soluble sugars and amino acid as these two components were lost at a faster rate at that stage of decomposition. It seems that release of soluble nutrients from the litter coupled with favourable moisture and temperature conditions played an important role in the growth of microflora during the process of decomposition.

From the Figs. 3.2a,b it is evident that both fungal and

bacterial populations after reaching their maximum, declined sharply to a quite low level. This may be accounted to the exhaustion of soluble nutrients in the form of soluble sugars and amino acids and also to very high moisture observed during that period which probably led to a decrease in microbial population of the decomposing litters. Excess moisture results in reduced aeration which affects the microbial activity adversely (Mikola, 1954; Witkamp, 1966a; Das, 1980). Higher fungal population associated with the leaf litter may also be ascribed to the higher surface area available for the microbial colonization.

Very little difference in the species composition of fungi associated with leaf, stem and root suggests that most of the fungi are non-selective and they can utilise a wide variety of substrates. Considerable monthly variation in the fungal population noticed throughout the decomposition process may be attributed to the changes in weather conditions (Holm and Jensen, 1972). The gradual increase in the microflora observed may be due to the favourable moisture condition and temperature and intensive activity of fauna which exposed the litter surface for microbial colonization (Das, 1980). The higher rate of available nitrogen source might have favoured the faster multiplication of the microflora during that time. Myskow (1961, 1975) also reported the similar findings.

pH of the decomposing litters invariably affected the

growth of fungi during decomposition. Acidic nature of litters perhaps favoured the growth of more and more fungi on decomposing litters at the later stages of decomposition (Das, 1980).

The occurrence of a good number of Phycomycetous fungi at the early stages of decomposition may be accounted to their greater affinity towards the simple carbohydrates (Miller, 1974). Decrease in the Phycomycetous fungi at the later stages could be attributed to the changed chemistry of the substrate. The discontinuous occurrence of litter fungi in varying frequencies may be attributed to certain antagonistic factors, nutrient status of the decomposing litters and the release of different substances (Garrett, 1963; Lockwood, 1964). The chemical composition and the environmental condition of the microhabitats exercise a broad selective effect upon the kind of microorganisms that colonize the litters.

A major part of the mycoflora was represented by the members of Fungi imperfecti which played an important role in the maize litters decomposition (Tables 2.1-2.3). The dominance of this group of fungi is believed to be due to their better capacity to decompose cellulose (Singh and Charaya, 1975). Trichoderma viride, Aspergillus spp. and Penicillium spp. appeared abundantly during the process of decomposition. Such observation may be explained on the basis of faster growth of these fungi in addition to their better intrinsic capacity to

utilise the litter (Garrett, 1950). Other fungal species exhibited lower percentage of occurrence. Almost similar set of fungal species were found to be associated with all the litter types which may be due to similar environmental conditions and very little difference in the chemistry of the litters. Also, it appears that most of the fungi were not substrate specific.

The lignin content increased during the course of study. This clearly indicates the slow rate of decay of the component. Similar increase in lignin content has been reported by Waksman and Tenney (1927), King and Heath (1967). The accumulation and slow rate of lignin decomposition has been accounted to the complex chemistry of this component (Tiwari and Mishra, 1983). It is generally assumed that decomposition of lignin under natural condition is carried out exclusively by Basidiomycetes (Lindeberg, 1947; Mikola, 1958; Garrett, 1963; Hering, 1967). Basidiomycetes are rather slow growing organisms and they donot develop in the loose litter layer. This may be one of the reasons for slow loss in lignin content.

Increase in the amount of total sugar and amino acid during the decomposition study suggests the possibility of the incorporation of fungal mycelia and small animals with litter due to the increased activity of the microbes during the period.

The increase in the nitrogen content during the later stages of decomposition is in accordance with the findings of

Gilbert and Bocock (1960), Saito (1957), Suberkropp et al. (1976) and Das (1980). This has been attributed to the immobilization of nitrogen to the microbial biomass (Iverson, 1973). The low nitrogen content at the initial stage in all the litters (Table 2.7) may be due to rapid breakdown of nitrogenous compounds thereby releasing nitrogen from the litter (Burges, 1967).

Difference in the rate of litter breakdown by the fungal species under laboratory condition indicates that the different fungal species have varying decomposing ability even under the identical environmental situations (Figs.3.1 b,c,d). The differences in the loss of various plant components by different fungal species indicate that the rate of decomposition of an organic material is closely linked with the growth rate of the associated decomposers. The rate of decomposition of any substrate is generally proportional to the growth rate of its decomposers (Parnas, 1975).

The rate of decomposition under laboratory condition was relatively slower than that under field condition (Figs.3.1a,b,c, d). This may be primarily due to the total exclusion of decomposer fauna from the litter due to sterilization, limited number of fungal species and chenged physical conditions. The importance of micro-fauna in decomposing litter has been well documented by Heath and King (1964) and Anderson (1975).

As regards the loss of various plant components, different

fungal species exhibited varied ability to decompose these components. The maximum loss of sugar in the set amended with A. glauca may be accounted for its ability of utilising the readily available sugars, pentasans etc. by the fungus (Burges, 1939). In the present study, the active degradation of cellulose and hemicellulose by the test fungi probably led to the production of toxins which perhaps adversely affected the further growth of the fungi at the later stages of decomposition (Patrick et al., 1964; Patrick, 1971).

With the progress of decomposition, more toxic compounds were perhaps produced due to which the growth of the inoculated fungi was inhibited. This may be attributed for the slow rate of decomposition at later stages of decomposition under laboratory condition (Figs. 3.1b,c,d).

CHAPTER IV

STUDIES ON MICROBIAL POPULATIONS AND THEIR ACTIVITIES OF GUT CONTENTS AND THE CASTS OF EARTHWORM IN MAIZE FIELD

INTRODUCTION

Earthworms exert a beneficial influence on the soil. They improve aeration, water- retaining capacity, and nutrient status and enhance decomposition by mechanical breakdown and stimulation of microbial activity. They are the chief agents responsible for the crumb structure and mull formation, typical of fertile soils. (Satchell, 1967; Edwards and Lofty, 1972). Knowledge of the food requirements and digestive capability of members of soil fauna is essential to understand the decomposition process of the terrestial ecosystem. In many ecosystems, such as pastures, cropfields and mull forest soils, the earthworms constitute a large percentage of soil faunal biomass.

The role of earthworms in the soil has been studied for at least a hundred years, and the physical and chemical transformations brought about by earthworms has been thoroughly understood. There are also several references in the literature stating or assuming a belief that the earthworms influence the soil microflora. Usually, these references have reported only observations on the microflora of soils in which earthworms were present or absent, without establishing any causal relationship. On agricultural land, earthworms are often the most conspicuous group of soil animals and they constitute from half to three-

quarters of the total weight of the fauna. The earthworms incorporate and help to decompose plant litter and dung and their tunnel making habit helps to keep soils open and porous. Fungal mycelium and other microbial tissue in the decaying material ingested, may be an important constituent of the diet for earthworms.

The rich chemical composition of casts as well as the burrowing and ploughing action of worms are well known, but there are very few studies on the microflora in the intestinal tract of earthworms. The castings of earthworms, which consist largely of digested soil and particles of organic matter, is more chemically neutral than the surrounding soil. So by consuming soil, processing it, and excreting the remainder as castings, the earthworms help to keep a field closer to the neutral pH range. Chemical analyses of earthworm castings show that they can contain up to two times as much available magnesium, five times as much available nitrogen, seven times as much available phosphorus, and eleven times as much available potassium as the surrounding soil. The passage of soil through the earthworm's gullet also greatly promotes bacterial growth. In particular, actinomycetes and bacteria that create humus, thrive in the presence of earthworms. The content of actinomycetes in castings is six to seven times greater than in the original soil.

The present work was undertaken with a view to study

the interaction between soil microflora and earthworm in a maize field.

REVIEW OF LITERATURE

The influence of earthworms on soil formation and in changing the structure of agricultural soil has received considerable attention, but little has been published on the effects of worms on the soil microflora. The pioneer work of Darwin (1881) established the importance of earthworms as a major influence on the fertility of the soil.

Bassalik (1913) isolated more than 50 species of bacteria from the alimentary canal of the earthworm, Lumbricus terrestris. He found no difference between the type of bacteria isolated from the gizzard, intestine and casts and those present in the soil from which they were collected.

Aichberger (1914) observed that no diatoms, blue-green algae, desmids, yeasts or rhizopods were found alive in the alimentary canal of the worm. Taylor (1917) and Rathbun (1918) considered earthworms as disseminating agents for spores of soil fungi.

Heymons (1923) described the intestine of the worm as a breeding place for bacteria. Duggeli (1927) reported that earthworms play a part in spreading bacteria in deeper levels of the soil. According to Delaunay (1927), bacteria are respon-

sible for the degradation of urea in the intestinal tract of earthworms.

Stockli (1928) found a larger number of microorganisms present in worm casts than in soil. The flora of the canal and of the surrounding soil was qualitatively similar, but that there was a great increase in the total number of organisms present in the canal and an increase in the relative proportions of pectin-splitting fungi.

Thomson (1934) thought that bacteria in the soil are brought up to the surface in casts and visualised the possibility of the spread of the tetanus bacillus through this agency.

Gianferrari and Cantoni (1936) found a microorganism resembling the mucous capsulated bacteria and the semi-colour group, to be regularly present in Lumbricus terrestris.

Dawson (1947) stated that the number of bacteria was reduced by passage through the canal while the number of fungi remained unaffected.

Information on the microorganisms associated with the Indian earthworm is rare. Kelkar (1949) seems to have been the only person who did some work in this connection at that time. He observed that the number of bacteria, as seen on nutrient agar plates was generally less in casts but that the nitrifying power of the casts was higher than that of the corresponding

soils. Swaby (1949) stated that the intestinal bacteria of earthworms produce gums which cement the casts into waterstable aggregates.

Day (1950) found that while the total number of bacteria, actinomycetes, and fungi was not consistently greater or lesser in fresh casts of Lumbricus terrestris than in the surrounding soil, there was a difference in the proportions of species present.

Russell (1950) pointed out that the microflora present in the earthworm gut may bring about the breakdown of organic matter and thus make the casts richer in plant nutrients. He further stated that the earthworm intestine may be the site for lignin oxidation and humus formation. He observed that,whilst passing through the earthworm intestine, nitrifying bacteria did not undergo any considerable increase or decrease in numbers, Bacillus cereus var. mycoides, however, underwent slight reduction in number, while Serrata marcescens was completely killed.

Tracey (1951) reported the existence of cellulase and chitinase in British earthworms and suggested the possibility of these enzymes being produced by microorganisms living in their intestines.

Ruschmann (1953) and later Schultz and Felber (1956) were the first to observe the now generally known phenomenon

that actinomycetes are capable of rapid growth in the digestive canal of earthworm, forming thereby an important co-dominant fraction in the gut microbiota of these animals. Further, as implied already by Ruschmann (1953), the large actinomycetes population density in the earthworm's digestive canal may also considerably influence the species composition of the total gut bacterial community, above all through the antibiotic activities of certain Streptomyces and Nocardia species capable of colonizing many micro-habitats of the gut milieu.

The work of Hutchinson and Kamel (1956) has shown that at least some fungal spores may survive during their passage through the alimentary canal, and that there may be some differential killing of the species of the original population. It is also probable that the earthworms bear a surface contamination of spores and mycelial fragments, and that such combined characters may make an important factor in fungal dispersal. They studied the living fungal flora of some typical alimentary canal of earthworms and the effect of the presence of latter on the rate of spread of some selected fungi through a sterilized soil. Their results showed that a substantial number of viable fungal spores may be isolated from the intestine, including species of both thick-walled and thin-walled spore types. They further observed that rate of spread of fungi through a sterile soil was much higher when earthworms were present. There is also report that the comminution of soil aggregates in the gut may

expose fresh surfaces to microbial attack (Rovira and Greacen, 1957).

Parle (1963 a,b) found more organisms in the gut or casts of European earthworms than in the surrounding soils.

The enrichment of earthworm casts in essential plant nutrients such as N,P,K Ca, and Mg has been demonstrated by several workers (Lunt and Jacobson, 1944; Parle, 1963 b; Graff, 1970). Studies have also indicated that the amount of available P in earthworm casts is greater than that in underlying soil (Barley, 1961; Gupta and Sakal 1967; Graff, 1970; Vimmerstedt and Finney, 1973).

Dash and Cragg (1972) working on Enchytraeidae, Mitchell and Parkinson (1976) and McMillan (1976) working on micro arthropods have shown that they graze over soil microfungi and show preference for some species of fungi as their food. Earthworms are also utilized as decomposers for some industrial wastes in Japan (Watanabe and Tsukamoto, 1976).

In a recent study, Dash and Patra (1977) have shown that earthworms utilise about 14% of the total energy input into the decomposer system of a tropical grassland and this further emphasises the importance of earthworms in the ecosystem functions. Dash (1978) recommended the use of earthworms along with organic manures as a substitute for costly inorganic

fertilizers. Grazing of the earthworms over the soil microflora enhances the growth of microflora in soil (Dash et al.,1979). Informations on the fungi causing disease and decomposition of earthworm tissues are lacking and the role of parasite fungi on the regulation of earthworm population is not understood. Dash et al. (1979) studied the fungal feeding by a tropical earthworm. They reported that earthworms are unable to utilise antibiotic producing microorganisms or having spores with thick layered spore coats.

Sharpley et al. (1979) reported that the total loss of P and N was substantially increased when the production of earthworm casts was eliminated. They also indicated the importance of earthworms in improving the drainage characteristics of surface soils.

Abbott and Parker (1981) studied the effect of food quantity and quality on the biomass of earthworms, and the influence of earthworms on plant growth and infiltration of water into soil. They observed that earthworms with more food gained weight faster than those with little or no supplementary food.

Edwards and Lofty (1982) while studying the effect of nitrogenous fertilizers on earthworm population in agricultural soils found positive correlation between amounts of inorganic nitrogen applied and population of earthworms. Mackay et al.

(1983) suggested that burrowing and casting of the earthworms increase the availability of phosphorus to the plants.

MATERIALS AND METHODS

The present study was carried out in a maize field. The common earthworm found in the field was Amynthas diffringens (Baird) belonging to Family Megascolecidae. Collection of earthworms was done from the first week of May when the maize seedlings were coming out and continued upto the post harvest period i.e. upto the month of September. Samplings were done at 10 days intervals.

Estimation of Fungi, Bacteria and Actinomycetes Populations of Earthworm Gut Contents and the Casts:

Five large worms (12.0cm approximately) were brought to the laboratory, cleaned thoroughly with sterilized distilled water and each worm was cut into three parts- anterior (upto 1.0-4.0cm), middle (from 4.0-8.0cm) and posterior (from 8.0-12.0cm) using sterilized scissors. The gut contents of different regions were inoculated in sterilized Petri dishes by soil plate method (Warcup, 1950) using Martin's Rose Bengal Agar medium (Martin, 1950) for isolation of fungi. For isolation of bacteria and actinomycetes, dilution plate method (Waksman, 1922) was followed using Nutrient Agar medium (Johnson and Curl, 1972) and Starch casein Agar medium (Kuster and Williams, 1964)

respectively. The details of the methods and chemical composition of media (Martin's Rose Bengal Agar and Nutrient Agar media) followed are given in chapter 2.

Composition Of Starch-Casein Agar (Kuster and Williams, 1964): For Actinomycetes:

Agar	.....	18.00 g
Starch	.....	10.00 g
Casein (vitaminfree)	...	0.30 g
KNO_3	.....	2.00 g
NaCl	.....	2.00 g
K_2HPO_4	.....	2.00 g
$MgSO_4 7H_2O$	.....	0.50 g
$CaCO_3$	.....	0.20 g
$FeSO_4 7H_2O$	.....	0.10 g
Distilled water	.....	1000.00 ml
Nystatin	.....	mg/ml

The casts and the surrounding soil were collected using a sterilized spatula and forcep from the same study site where earthworms were collected. Soil samples were collected from the area close to the casts. For isolation of fungi, bacteria and actinomycetes, the methods mentioned above were followed.

Determination Of Physico-Chemical Characters of Earthworm Casts and the Soil:

For determination of pH, moisture content, organic

carbon, total nitrogen and available phosphorus of casts and the surrounding soil, methods described in chapter 1 were followed.

Determination of Dehydrogenase and Urease Enzyme Activities in Earthworm Casts and the Soil:

Methods followed for the determination of dehydrogenase and urease activities in casts and the soil are discribed in chapter 2.

RESULTS

Quantitative Assessment of Microflora of Gut Contents and Casts of Earthworm:

The populations of bacteria and actinomycetes were found to be maximum in the hindgut whereas, the population of fungi was found to be maximum in the foregut and exhibited a decreasing trend down the gut canal of the earthworm (Figs. 4.1a,b,c). In the casts, microbial population(Fungi, bacteria and actinomycetes) was found to be higher than the soil (Figs. 4.2 a,b,c). Much fluctuation in the microbial population was observed in gut contents and in the casts throughout the sampling period.

Fig. 4.1 (a) Fungal population in the gut contents of earthworm.

(b) Bacterial population in the gut contents of earthworm.

(c) Actinomycetes population in the gut contents of earthworm.

Fig. 4·1

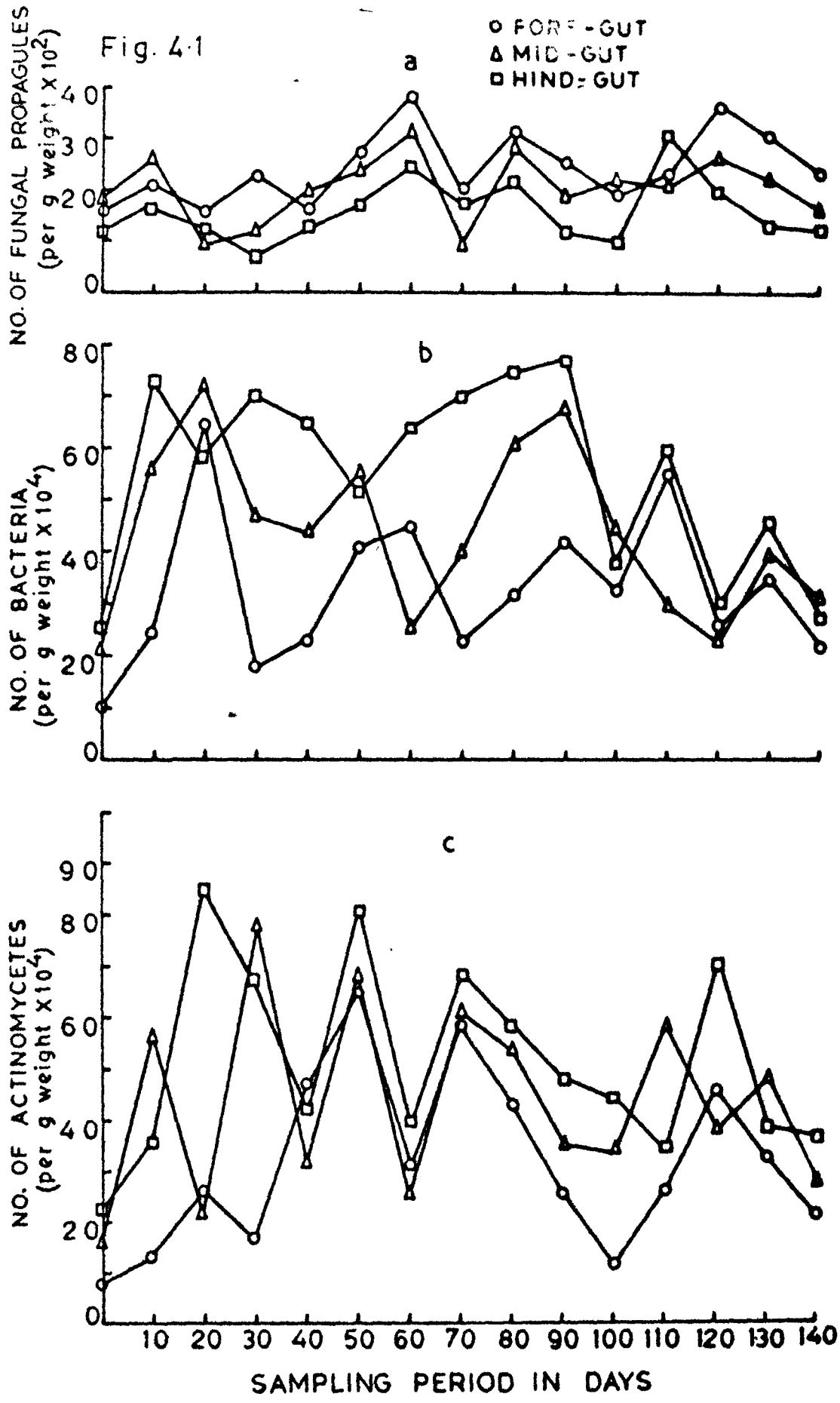
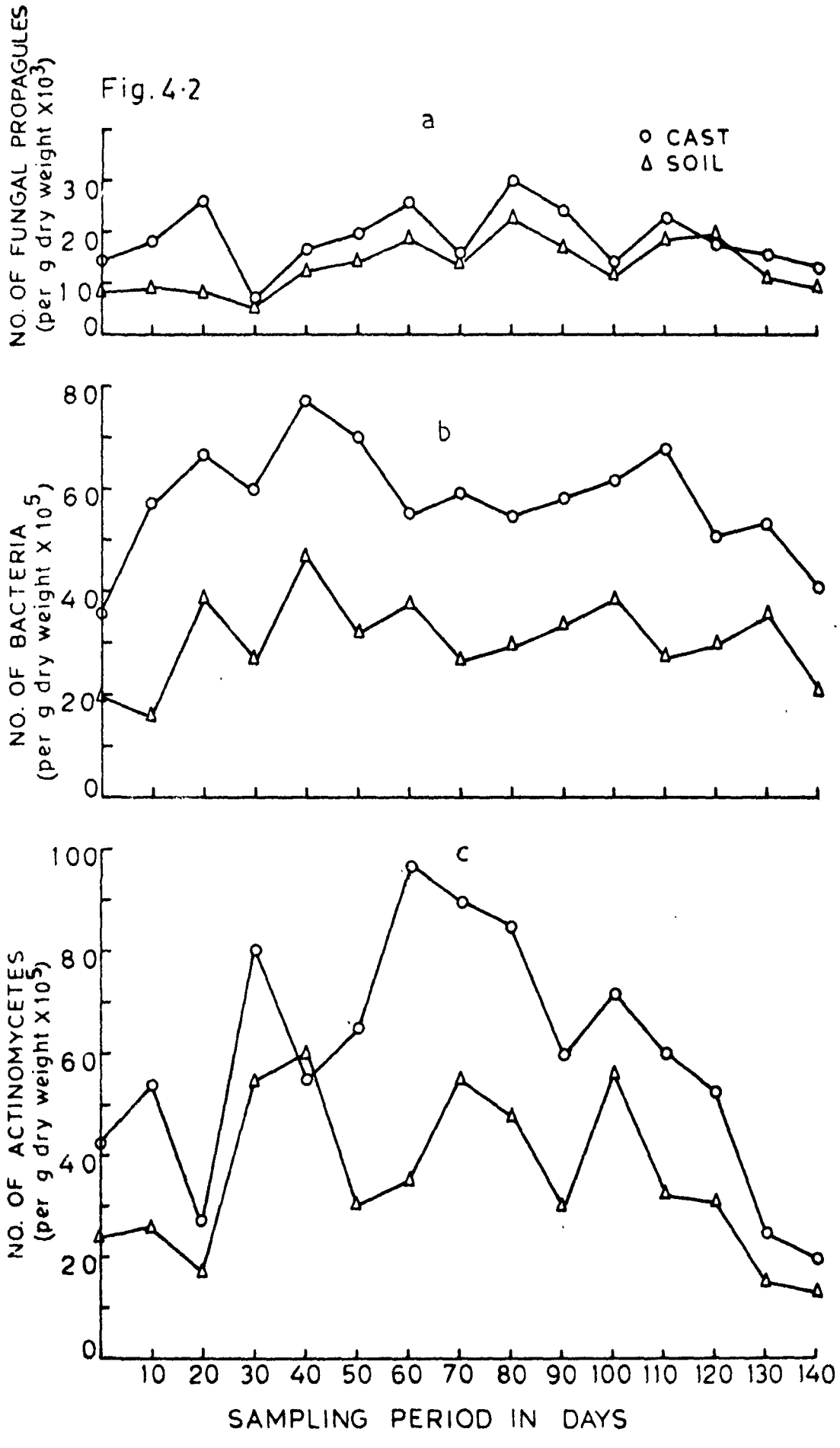

o FORE-GUT
Δ MID-GUT
□ HIND-GUT
a
NO. OF FUNGAL PROPAGULES (per g weight X 10²)
0
20
30
40
b
NO. OF BACTERIA (per g weight X 10⁴)
0
20
40
60
80
c
NO. OF ACTINOMYCETES (per g weight X 10⁴)
0
20
40
60
80
90
10 20 30 40 50 60 70 80 90 100 110 120 130 140
SAMPLING PERIOD IN DAYS

Fig. 4.2 (a) Fungal population of the earthworm casts and the soil.

(b) Bacterial population of the earthworm casts and the soil.

(c) Actinomycetes population of the earthworm casts and the soil.

Fig. 4·2


a
o CAST
Δ SOIL
NO. OF FUNGAL PROPAGULES (per g dry weight X10³)
0
10
20
30
b
NO. OF BACTERIA (per g dry weight X 10⁵)
0
20
40
60
80
c
NO. OF ACTINOMYCETES (per g dry weight X10⁵)
0
20
40
60
80
100
10 20 30 40 50 60 70 80 90 100 110 120 130 140
SAMPLING PERIOD IN DAYS

Percentage Relative Abundance of Fungal Species Isolated from Gut contents and Casts of Earthworm:

The list of fungi isolated from the surrounding soil, gut contents and the casts of the earthworm is given in table 3.1. Qualitatively there was no difference in the microflora of soil, gut contents and the casts. Most of the fungal species which were obtained from the casts were also isolated from all the regions of earthworm gut and the soil (Table 3.1). From the table, it is evident that soil and casts contained higher number of fungal species than the gut of the earthworm. The genus Didiodendron sp. and Alternaria alternata were restricted to casts and the soil respectively. Mucor hiemalis, Penicillium chrysogenum, Trichoderma viride and Geotrichum sp. were of frequent occurrence in the soil and the casts (Tables 3.2, 3.6). On the other hand Aspergillus niger, Fusarium moniliforme, Geotrichum sp., Mucor hiemalis and Penicillium chrysogenum were found to be present throughout the gut contents of the earthworm (Tables 3.3-3.5).

Physico-Chemical Characters of Earthworm Casts and the Soil:

pH:

pH of the earthworm casts and the soil varied within a narrow range. There was no significant difference between the pH of casts and that of the surrounding soil. The range being 6.24-7.52 and 6.05-7.35 respectively (Table 3.7).

Table 3.1 List of fungi isolated from soil, gut contents and the casts of earthworm *Amynthas diffringens* (Baird).

Fungi	Soil	Gut contents			Casts
		Fore-gut	Mid-gut	Hind-gut	
PHYCOMYCETES					
Absidia sp.	+	-	-	-	-
Mucor hiemalis	+	+	+	+	+
M. plumbeus	+	+	+	+	+
M. racemosus	+	+	+	-	+
Pythiopsis sp.	-	-	-	-	+
Pythium sp.	+	+	-	-	+
Rhizopus nigricans	+	+	+	-	+
FUNGI IMPERFECTI					
Alternaria alternata	+	-	-	-	+
Aspergillus candidus	-	+	+	+	-
A. flavus	+	+	+	+	+
A. niger	+	+	+	+	+
Cephalosporium sp.	+	+	+	-	+
Cladosporium cladosporioides	+	+	+	+	+
Fusarium moniliforme	+	+	+	+	+
Geotrichum candidum	+	+	+	+	+
Oidiodendron sp.	-	-	-	-	+
Penicillium chrysogenum	+	+	+	+	+
P. humicola	+	+	+	+	+
Penicillium sp.	+	+	+	-	-
Trichoderma viride	+	+	+	-	+
Trichoderma sp.	+	+	-	-	+
Verticillium sp.	+	+	-	-	+
MYCELIA STERILIA					
Black sterile mycelia	+	-	-	-	-
White sterile mycelia	+	+	-	+	+
Yellow sterile mycelia	+	+	+	-	+

+ = Present, - = Absent.

Table 3.2 Fungal population* of soil surrounding the earthworm casts during different periods of sampling. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING DATES - 1982														
	13/5	23/5	3/6	13/6	23/6	3/7	13/7	21/7	31/7	10/8	20/8	30/8	9/9	19/9	29/9
Absidia sp.	0.032 (5.70)	-	-	-	-	-	0.030 (5.60)	-	-	-	-	-	0.054 (10.50)	0.030 (3.70)	-
Mucor hiemalis	0.080 (14.30)	0.077 (18.20)	0.064 (44.90)	0.106 (20.90)	-	-	0.088 (11.10)	0.092 (13.00)	0.057 (11.40)	0.099 (40.50)		0.014 (1.90)	0.032 (5.50)	-	-
M. plumbeus	-	0.054 (12.70)	-	-	0.030 (4.00)	0.029 (4.80)	-	-	-	0.008 (5.10)	0.008 (15.50)	-	-	0.099 (14.80)	0.250 (5.10)
Mucor racemosus	-	-	-	-	0.096 (13.10)	0.110 (19.10)	-	-	-	-	-	0.210 (27.90)	-	-	0.200 (40.50)
Pythium sp.	-	-	-	-	-	-	-	0.088 (11.10)	-	-	-	-	-	-	-
Rhizopus nigricans	0.088 (15.70)	-	-	0.030 (5.90)	-	-	0.009 (1.80)	-	0.014 (2.90)	-	0.045 (9.30)	0.036 (4.80)	-	-	-
Alternaria alternata	-	0.023 (5.50)	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	0.007 (1.20)	-	-	-	-	-	0.029 (3.80)	-	-	-
Aspergillus niger	0.032 (5.70)	0.008 (1.80)	-	0.053 (10.40)	0.023 (3.00)	-	-	-	0.007 (1.40)	-	0.072 (10.20)	0.014 (1.90)	0.035 (5.60)	-	-
Cephalosporium sp.	-	-	-	-	-	-	0.030 (5.68)	-	-	-	-	-	0.031 (5.40)	0.089 (13.80)	-
Cladosporium cladosporioides	-	-	-	-	-	-	0.088 (11.10)	0.009 (1.80)	-	0.010 (6.90)	0.022 (8.60)	-	0.055 (11.10)	0.104 (27.00)	0.050 (8.90)
Fusarium moniliforme	0.016 (8.90)	-	-	0.167 (32.80)	0.008 (1.00)	-	0.039 (6.10)	0.075 (10.40)	0.079 (15.70)	0.045 (22.80)	0.009 (1.20)	0.014 (1.90)	-	-	0.120 (22.80)
Geotrichum candidum	-	-	-	-	0.083 (11.10)	0.029 (4.80)	-	0.045 (5.40)	-	0.072 (12.70)	-	0.700 (13.50)	0.092 (14.60)	-	0.060 (12.70)
Penicillium chrysogenum	0.144 (25.70)	-	0.079 (55.00)	0.129 (25.40)	0.455 (60.60)	-	0.163 (46.30)	0.123 (32.20)	0.136 (27.10)	-	0.152 (34.20)	0.029 (3.80)	-	0.085 (11.50)	-
P. humicola	-	0.162 (38.20)	-	-	-	0.164 (27.40)	-	-	-	-	-	0.199 (26.90)	0.111 (26.90)	-	-
Penicillium sp.	-	-	-	-	-	-	-	-	-	0.010 (6.90)	-	-	-	-	0.050 (8.90)
Trichoderma viride	0.160 (28.60)	0.069 (16.40)	-	-	0.030 (4.00)	0.093 (15.50)	-	0.100 (20.00)	0.193 (39.60)	-	-	0.029 (3.80)	-	0.112 (28.60)	-
Trichoderma sp.	-	-	-	-	-	-	0.004 (1.00)	-	-	-	0.022 (5.60)	-	-	-	-
Verticillium sp.	-	0.031 (7.30)	-	-	-	0.157 (26.20)	0.042 (7.40)	-	0.014 (2.90)	-	0.086 (12.50)	0.071 (9.60)	-	-	-
Black sterile mycelia	0.018 (1.41)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
White sterile mycelia	-	-	-	-	-	0.017 (1.28)	0.022 (4.60)	0.015 (3.90)	-	-	-	-	0.100 (17.20)	-	-
Yellow sterile mycelia	-	-	-	0.023 (4.50)	0.023 (3.00)	-	-	-	-	0.003 (1.10)	0.023 (5.80)	-	-	-	0.010 (1.30)

* Fungal population was calculated on per gm dry weight basis.

Table 3.3 Fungal population* in fore-gut of earthworm *Eisynthes diffringens* (Baird) during different periods of sampling. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING DATES - 1982														
	13/5	23/5	3/6	13/6	23/6	3/7	13/7	21/7	31/7	10/8	20/8	30/8	9/9	19/9	29/9
Mucor hiemalis	0.009 (2.00)	-	-	-	0.115 (13.20)	-	0.090 (20.00)	-	-	0.082 (15.50)	0.075 (15.50)	0.019 (0.90)	0.055 (9.90)	-	0.040 (6.70)
M. plumbeus	-	-	0.005 (2.50)	0.065 (16.90)	0.019 (2.30)	-	0.022 (5.40)	0.024 (5.60)	-	0.036 (7.60)	-	-	-	0.120 (20.50)	0.035 (7.60)
M. racemosus	-	-	-	0.019 (5.20)	-	0.026 (5.50)	-	-	-	0.038 (8.70)	0.020 (5.40)	-	-	-	-
Pythium sp.	-	0.005 (2.40)	-	-	-	-	0.005 (2.10)	-	-	-	0.006 (3.00)	-	-	-	-
Rhizopus nigricans	-	-	0.055 (28.30)	0.005 (1.30)	0.005 (0.60)	-	0.049 (25.50)	0.040 (9.30)	0.019 (4.50)	0.009 (3.00)	-	-	0.032 (6.00)	-	-
Aspergillus candidus	0.008 (2.00)	-	-	-	-	-	0.002 (1.10)	-	-	-	-	-	0.050 (9.20)	-	-
A. flavus	-	-	-	-	-	-	0.007 (2.00)	-	-	0.040 (10.90)	0.002 (1.20)	0.049 (9.90)	0.023 (2.30)	-	0.049 (10.90)
A. niger	-	0.010 (4.00)	0.005 (2.50)	0.010 (2.60)	0.015 (1.70)	0.005 (1.08)	-	-	0.025 (5.70)	0.007 (2.20)	0.019 (5.30)	0.049 (9.00)	-	0.060 (7.90)	0.010 (2.20)
Cephalosporium sp.	-	-	-	-	0.009 (1.10)	-	-	0.008 (3.20)	-	-	0.023 (8.90)	0.049 (9.90)	-	-	-
Cladosporium cladosporioides	-	0.015 (7.20)	-	0.025 (6.50)	-	-	0.020 (4.60)	-	-	-	-	-	0.059 (10.90)	0.075 (8.90)	-
Fusarium moniliforme	-	0.005 (2.40)	0.035 (17.90)	-	-	-	0.001 (0.90)	0.030 (8.60)	-	0.008 (3.00)	0.075 (15.50)	-	0.040 (8.50)	-	0.015 (3.30)
Geotrichum candidus	0.105 (28.00)	0.030 (14.30)	-	0.040 (10.40)	0.040 (4.60)	0.095 (20.70)	-	0.210 (37.00)	0.065 (14.80)	0.201 (40.50)	-	0.270 (53.50)	0.123 (40.30)	0.230 (32.50)	0.250 (55.60)
Penicillium chrysogenum	0.135 (56.00)	-	-	-	0.545 (62.30)	0.049 (10.90)	0.030 (22.50)	0.099 (17.80)	0.199 (45.50)	-	-	-	-	-	-
P. humicola	-	0.066 (30.90)	0.095 (48.70)	0.169 (44.20)	0.009 (1.10)	-	-	-	-	0.038 (7.60)	-	0.019 (3.95)	0.043 (7.40)	0.106 (16.70)	-
Penicillium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.035 (7.60)
Trichoderma viride	-	0.049 (23.80)	-	0.045 (11.70)	0.115 (13.2)	0.250 (54.40)	0.012 (15.20)	0.065 (11.50)	0.125 (28.40)	0.002 (0.80)	0.220 (44.30)	-	0.031 (5.20)	0.089 (11.10)	0.015 (4.30)
Trichoderma sp.	0.113 (30.00)	-	-	-	-	-	-	-	-	-	-	0.055 (10.90)	-	-	-
Verticillium sp.	-	0.025 (11.50)	-	0.005 (1.30)	-	0.035 (7.60)	-	-	-	-	-	-	-	-	-
White sterile mycelia	-	0.005 (2.40)	-	-	-	-	-	0.018 (6.00)	-	-	-	-	-	-	-
Yellow sterile mycelia	0.005 (2.00)	-	-	-	-	-	-	-	0.005 (1.10)	-	-	-	-	-	-

* Fungal population was calculated on per gram dry weight basis

Table 3-4 Fungal population* in mid-gut of earthworm Amynthas diffringens (Baird) during different periods of sampling. Values in the parenthesis are percentage relative abundance.

Fungal species isolated	SAMPLING DATES - 1982														
	12/5	23/5	3/6	13/6	23/6	3/7	13/7	21/7	31/7	10/8	20/8	30/8	9/9	19/9	29/9
Fusarium bipolaris	0.055 (8.80)	-	-	-	0.070 (8.90)	-	0.040 (10.50)	-	0.040 (8.20)	0.014 (2.40)	0.094 (15.20)	0.035 (7.10)	0.102 (12.90)	-	0.035 (11.60)
F. oxysporum	-	-	0.005 (1.90)	0.075 (12.40)	0.055 (7.80)	-	0.014 (3.90)	0.006 (2.30)	-	0.010 (10.80)	-	-	-	0.040 (10.50)	0.070 (10.60)
F. roseopicum	-	-	-	0.030 (3.70)	-	0.025 (8.20)	-	-	-	0.002 (2.30)	0.012 (5.40)	-	-	-	-
Rhizopus nigricans	-	-	-	-	-	-	0.006 (2.10)	-	-	-	-	-	-	-	-
Aspergillus candidus	0.020 (2.50)	-	-	-	-	-	0.190 (96.90)	-	-	-	-	-	-	-	-
A. flavus	-	-	-	-	-	-	0.010 (4.80)	-	-	0.010 ([illegible])	0.200 (51.30)	0.070 (14.20)	0.011 (1.20)	0.011 (2.30)	0.060 (13.50)
A. niger	-	0.010 (7.40)	0.005 (1.90)	0.025 (4.10)	0.035 (6.80)	0.015 (4.90)	-	-	0.015 (5.80)	0.003 (3.00)	0.025 (8.10)	0.045 (6.10)	-	0.013 (3.40)	0.009 (0.52)
Cephalosporium sp.	-	-	-	-	0.010 (2.80)	-	-	0.030 (7.80)	-	-	0.005 (2.80)	0.084 (15.10)	-	-	-
Cladosporium cladosporioides	*	0.002 (1.20)	-	0.030 (5.80)	-	-	0.030 (9.70)	-	-	-	-	-	0.120 (11.30)	0.04 (10.50)	-
Fusarium moniliforme	-	0.015 (11.10)	0.049 (61.60)	-	-	-	0.003 (1.10)	0.009 (15.50)	-	0.014 (10.50)	0.139 (25.50)	-	0.110 (15.50)	-	0.045 (6.80)
Gantrachae candidum	0.225 (37.50)	0.029 (21.80)	-	0.022 (4.70)	0.450 (58.40)	0.015 (4.90)	-	0.163 (40.00)	0.125 (25.50)	0.053 (37.20)	-	0.100 (22.20)	0.220 (35.20)	0.231 (46.58)	0.240 (36.40)
Penicillium chrysogenum	0.308 (31.30)	-	-	-	0.010 (2.80)	0.210 (80.80)	0.200 (34.20)	0.120 (22.20)	0.139 (28.70)	-	-	-	-	0.019 (2.60)	-
P. funiculo	-	0.015 (11.10)	0.146 (34.70)	0.350 (87.80)	-	-	-	-	-	0.015 (10.60)	-	0.020 (14.20)	0.125 (13.90)	0.010 (10.20)	-
Trichoderma viride	-	0.050 (40.40)	-	0.070 (13.20)	0.094 (13.40)	0.097 (15.10)	0.046 (11.10)	0.065 (12.30)	0.169 (34.70)	0.010 (9.90)	0.040 (10.70)	-	-	0.026 (5.90)	-
Trichoderma sp.	-	-	-	-	-	-	-	-	-	-	-	0.030 (10.20)	-	-	-
Yellow sterile mycelia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.032 (4.60)

* Fungal population was estimated on per gram dry weight basis

Table 3.5 Fungal population* in hind-gut of earthworm Aeynthes diffringens (Baird) during different periods of sampling. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING DATES - 1982														
	13/5	25/5	3/6	13/6	23/6	3/7	13/7	21/7	31/7	10/8	20/8	30/8	9/9	19/9	29/9
Mucor hiemalis	0.031 (7.10)	-	-	-	0.180 (27.90)	-	0.022 (12.50)	-	0.019 (9.10)	0.170 (19.90)	0.095 (17.90)	0.105 (22.20)	-	-	0.170 (19.90)
M. plumbeus	-	-	0.075 (14.30)	0.085 (17.40)	0.050 (6.80)	0.005 (1.86)	0.008 (5.20)	0.007 (0.90)	-	0.016 (2.33)	-	-	-	0.072 (9.10)	0.070 (8.40)
Aspergillus candidus	0.150 (37.60)	-	-	-	-	-	0.105 (30.80)	-	-	-	-	-	-	-	-
A. flavus	-	-	-	-	-	-	0.003 (2.90)	-	-	0.049 (5.70)	0.103 (24.70)	0.134 (20.60)	0.005 (1.00)	-	0.024 (3.00)
A. niger	-	0.199 (23.40)	0.175 (34.20)	0.109 (24.20)	0.070 (10.90)	0.049 (10.90)	-	-	0.044 (20.50)	0.009 (1.20)	0.160 (33.10)	0.023 (5.60)	-	0.086 (11.40)	0.005 (0.60)
Cladosporium cladosporioides	-	0.099 (12.20)	-	0.029 (16.20)	-	-	0.032 (20.70)	-	-	-	-	-	0.100 (38.70)	0.072 (9.90)	0.029 (4.30)
Fusarium moniliforme	-	0.100 (15.20)	0.040 (7.60)	-	-	-	-	0.100 (28.90)	-	-	0.108 (24.10)	-	0.020 (12.30)	-	0.010 (1.20)
Geotrichum candidum	0.080 (24.60)	0.420 (40.60)	-	0.090 (16.10)	0.190 (29.60)	0.145 (31.50)	-	0.120 (21.80)	0.070 (31.80)	0.400 (58.50)	-	0.155 (29.50)	0.091 (31.50)	0.290 (31.80)	0.520 (62.70)
Penicillium chrysogenum	0.075 (23.20)	-	-	-	0.155 (24.00)	0.255 (56.50)	0.095 (25.90)	0.231 (1.70)	0.085 (38.70)	-	-	-	0.069 (17.60)	-	-
P. humicola	-	0.099 (12.20)	0.135 (45.80)	0.109 (22.20)	-	-	-	-	-	0.070 (11.80)	-	0.105 (22.20)	0.009 (5.20)	0.350 (38.70)	-
White sterile mycelia	0.015 (3.60)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Fungal population was calculated on per gram weight basis.

Table 3.6 Fungal population* of earthworm casts during different periods of sampling. Values in the parentheses are percentage relative abundance.

Fungal species isolated	SAMPLING DATES - 1982														
	19/5	23/5	3/6	13/6	23/6	3/7	13/7	21/7	31/7	10/8	20/8	30/8	6/9	16/9	26/9
Mucor hiemalis	0.012 (2.56)	0.201 (21.20)	0.083 (25.89)	0.227 (31.18)	0.031 (2.49)	-	0.100 (11.90)	0.042 (13.00)	0.051 (8.63)	0.009 (1.90)	-	0.088 (11.44)	0.044 (5.50)	-	0.132 (34.62)
M. plumbeus	-	0.059 (6.17)	-	-	0.039 (3.13)	0.044 (6.45)	-	-	-	-	0.086 (15.50)	0.008 (0.81)	-	-	-
Mucor racemosus	-	-	-	-	-	0.044 (6.45)	0.045 (5.68)	-	-	-	-	-	0.100 (10.50)	0.016 (3.70)	-
Pythiopsis sp.	0.012 (2.56)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pythium sp.	-	-	-	-	-	0.007 (1.09)	-	-	-	-	-	-	-	-	-
Rhizopus nigricans	0.146 (32.03)	0.029 (3.02)	0.023 (11.11)	-	0.016 (1.26)	0.080 (12.91)	0.009 (1.80)	-	0.022 (3.71)	0.015 (4.80)	0.079 (9.30)	0.228 (23.76)	0.005 (1.26)	-	-
Alternaria alternata	-	0.036 (3.79)	-	-	-	-	-	0.065 (8.80)	-	-	-	-	-	0.056 (10.80)	0.054 (10.27)
Aspergillus flavus	-	-	-	-	0.020 (1.80)	-	-	-	-	0.010 (3.80)	-	-	-	-	-
Aspergillus niger	0.175 (3.85)	-	0.045 (22.22)	0.023 (3.22)	0.031 (2.49)	0.007 (1.07)	-	-	0.029 (4.93)	0.009 (1.90)	0.080 (10.20)	-	0.045 (5.60)	-	-
Cephalosporium sp.	-	-	-	-	0.031 (2.49)	0.022 (3.23)	0.045 (5.60)	-	-	0.042 (14.80)	-	0.008 (0.81)	0.043 (5.40)	0.067 (13.80)	0.028 (5.30)
Cladosporium cladosporioides	-	-	-	-	-	-	0.100 (11.10)	0.003 (1.80)	0.014 (2.81)	-	0.048 (5.60)	-	0.012 (11.10)	0.068 (18.90)	0.026 (3.85)
Fusarium moniliforme	0.012 (2.58)	0.144 (15.16)	-	-	0.031 (2.49)	-	0.055 (6.10)	0.005 (2.40)	-	0.009 (1.80)	0.005 (1.20)	-	-	-	-
Geotrichum candidum	-	0.094 (9.84)	-	0.227 (31.18)	0.039 (3.13)	0.066 (9.68)	-	0.010 (5.40)	-	0.048 (13.50)	-	0.110 (11.48)	0.032 (14.68)	-	0.115 (21.91)
Gliocladium sp.	0.012 (2.58)	-	-	-	-	-	-	0.010 (5.40)	-	0.010 (3.80)	-	-	-	-	-
Penicillium chrysogenum	-	0.072 (7.57)	-	0.211 (29.02)	0.867 (69.38)	0 -	0.123 (46.30)	0.105 (34.20)	-	0.010 (3.90)	0.130 (34.28)	0.303 (32.70)	-	0.123 (21.50)	0.054 (10.27)
P. humicola	0.141 (30.76)	0.180 (18.43)	-	-	-	0.338 (49.47)	-	-	0.162 (27.16)	0.099 (28.90)	-	0.140 (14.75)	0.920 (28.90)	-	-
Trichoderma viride	0.087 (19.22)	0.036 (3.79)	-	0.039 (5.39)	0.141 (11.28)	0.044 (6.45)	0.005 (1.40)	0.110 (20.00)	0.330 (52.60)	0.010 (3.80)	0.040 (5.60)	-	-	0.244 (28.60)	0.007 (1.27)
Trichoderma sp.	-	-	0.063 (40.78)	-	-	-	-	-	-	0.030 (8.60)	-	-	0.065 (7.00)	-	-
Verticillium sp.	-	0.001 (10.61)	-	-	-	-	0.087 (2.80)	0.007 (3.90)	-	0.030 (0.80)	0.080 (60.80)	0.038 (4.11)	-	-	0.001 (11.54)
White sterile mycelia	0.006 (1.27)	-	-	-	-	-	0.035 (4.00)	0.007 (3.90)	-	-	-	-	0.011 (10.20)	-	-
Yellow sterile mycelia	0.012 (2.56)	-	-	-	-	0.022 (3.23)	-	-	-	-	0.005 (5.80)	-	-	-	-

*Fungal population was calculated on per gram dry weight basis.

Table 3.7 Physico-chemical characters of soil and the casts

Sampling dates	pH		M.C.		O.C. (%)		Total N (%)		Available P (%)	
	I	II	I	II	I	II	I	II	I	II
13.5.82	6.20	6.90	18.2	13.5	2.81	4.33	0.10	0.36	0.07	0.09
23.5.82	7.34	6.94	15.5	14.6	2.91	4.22	0.31	0.63	0.05	0.07
3.6.82	6.62	6.48	35.0	30.6	2.95	3.64	0.55	0.70	0.06	0.07
13.6.82	6.77	6.40	30.0	34.0	2.25	4.50	0.40	0.52	0.07	0.06
23.6.82	6.59	6.71	34.0	36.0	3.50	4.69	0.73	0.04	0.08	0.09
3.7.82	6.50	7.04	33.0	33.0	2.15	3.97	0.70	1.0	0.08	0.09
13.7.82	6.05	6.24	34.0	36.0	1.89	3.93	0.24	0.46	0.04	0.06
21.7.82	6.90	7.52	30.0	32.0	1.97	3.46	0.31	0.62	0.04	0.05
31.7.82	7.35	7.44	32.0	33.0	2.05	3.20	0.42	0.73	0.05	0.07
10.8.82	6.48	6.92	30.0	37.6	2.50	3.08	0.39	0.42	0.06	0.09
20.8.82	6.40	6.95	30.0	32.0	2.22	2.92	0.51	0.53	0.04	0.08
30.8.82	6.54	6.40	21.0	34.0	2.18	2.80	0.42	0.56	0.08	0.07
9.9.82	6.09	6.32	17.0	23.0	1.83	2.62	0.27	0.43	0.06	0.07
19.9.82	6.60	6.70	16.0	19.0	1.85	2.50	0.22	0.38	0.06	0.06
29.9.82	6.70	6.73	18.5	16.0	1.82	2.00	0.15	0.03	0.05	0.06

I = Soil, II = Casts, M.C. = Moisture content, O.C. = Organic carbon, N = Nitrogen, P = Phosphorus.

Moisture Content:

The moisture content was higher in the casts than the surrounding soil. The moisture content remained relatively higher at most of the sampling periods and decreased at the end (Table 3.7).

Organic Carbon, Total Nitrogen and Available Phosphorus:

It was found that the casts were richer in nutrients than the surrounding soil. At the beginning, organic carbon, total nitrogen and available phosphorus exhibited an increasing trend upto the 5th sampling periods and gradually decreased thereafter (Table 3.7).

Dehydrogenase and Urease Enzyme Activities in Casts and the Soil:

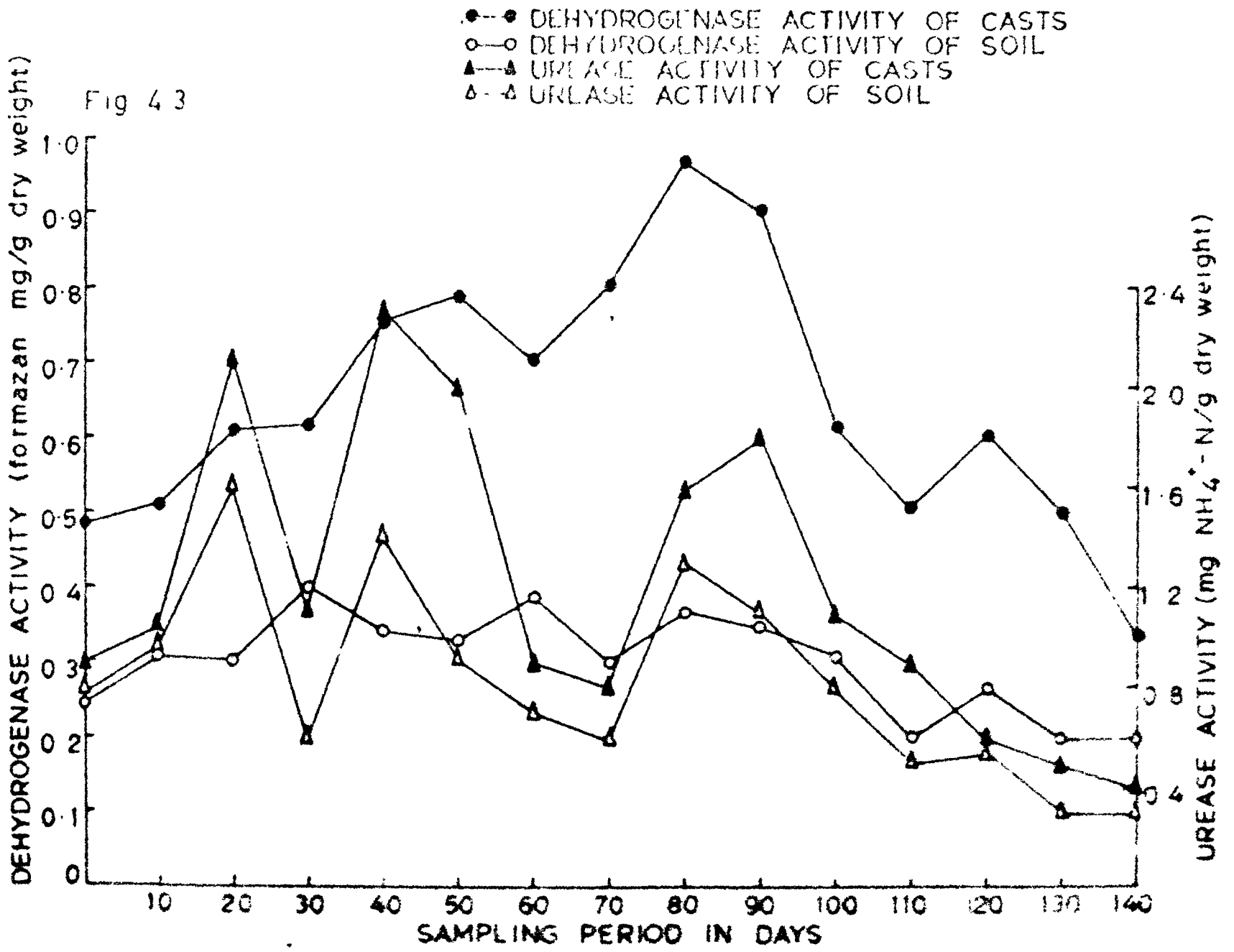
Enzyme activities were found to be more in the casts than the soil (Fig. 4.3). A positive correlation was observed between urease activity and total nitrogen and bacterial population (r=0.624, 0.721 respectively). Dehydrogenase activity was positively correlated with moisture content and bacterial population (r=0.616, 0.677 respectively).

DISCUSSION

Bacteria and actinomycetes in the gut flora increased greatly in number. Conditions were less suitable for fungi which usually did not increase in the gut. These results suggest

Fig. 4.3 Dehydrogenase and urease enzyme activities of the earthworm casts and the soil.

Fig 4.3


DEHYDROGENASE ACTIVITY OF CASTS
DEHYDROGENASE ACTIVITY OF SOIL
UREASE ACTIVITY OF CASTS
UREASE ACTIVITY OF SOIL
DEHYDROGENASE ACTIVITY (formazan mg/g dry weight)
UREASE ACTIVITY (mg NH4+-N/g dry weight)
SAMPLING PERIOD IN DAYS
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
0·4
0·8
1·2
1·6
2·0
2·4
10
20
30
40
50
60
70
80
90
100
110
120
130
140

that increases were by bacterial growth and not by the worm selecting food material with a high bacterial count. Similar findings have also been reported by Parle (1963a). Ruschmann (1953) and Schultz and Felber (1956) reported that actinomycetes are capable of rapid growth in the digestive canal of earthworm forming thereby an important co-dominant fraction in the gut microflora of the animals. However, these results are contrary to Dawson (1947) who observed that the number of bacteria was reduced by passage through the canal while the number of fungi was unaffected. The limited number of fungal species isolated from the gut contents may be on account of selective effect of the passage through the canal on the viability of different spores ingested. The small size of the sample and selective effect of the culture medium may also be responsible for lesser fungal species in the gut contents.

Many of the fungal species were common to all the three regions of the gut of earthworm (Tables 3.3-3.5). This indicates that there was no complete killing of such species as they pass down the canal, however, this does not give any information regarding the possibility of quantitative selective killing which may have occurred.

Most of the gut fungi were also found in the soil from where the worms were collected. Parle (1963a), Satchell (1967) and Lofty (1974) also reported the similar findings and they

concluded that the earthworms are unlikely to possess an indigenous microfloral population in their guts.

Earthworms are not capable of digesting all the fungal species they ingest (Nielson, 1962). There is evidence that some of the microorganisms which are taken with the soil by the earthworm are digested during their passage through the earthworm gut (Dash *et al.*, 1979). From the tables 3.3-3.5, it is observed that *Pythium* sp. and *Verticillium* sp. were found to be present in the fore-gut and did not occur in the mid-gut and hind-gut of the earthworm, thereby indicating that these species are digested in the mid-gut of the earthworm. *Rhizopus nigricans*, *Trichoderma* spp. and *Cephalosporium* sp. could be isolated from fore-gut and mid-gut which indicates that they might be digested in the hind-gut of the earthworm. *Fusarium moniliforme*, *Geotrichum candidum*, *Cladosporium cladosporioides*, *Aspergillus* spp. and *Penicillium* spp. were found to be present throughout the gut canal suggesting that these species were not digested by the the earthworm. Earthworm *Amynthas diffringens* is not capable of digesting those fungi having thich-walled spores or antibiotic producing properties.

Koylovskaya and Zhdannikova (1961) reported that the casts contained proportionately more fungi, actinomycetes and bacteria than the surrounding soil. Similar findings also observed in the present study. Ponomareva (1962) found 13 times more

bacteria in earthworm casts than in the surrounding soil. Ghilarov (1963) and Went (1963) have also reported larger microfloral population in casts than in surrounding soil. The worm may ingest soil at one place and lay casts at another place. The casts are therefore foci from which soil microorganisms can spread into surrounding soil (Lofty, 1974; Dash *et al.*, 1979). Differences in microbial population in earthworm casts and surrounding soil are either due to environmental changes within the earthworm gut or due to the food material ingested which provides a rich substrate, thereby increasing microbial activity. The type and quality of ingested material helps to determine the size of microfloral populations in casts. Parle (1963a) showed that number of bacteria and actinomycetes in ingested material increased by a factor of 1000 while passing through the earthworm's intestine and microbial activity in the casts was still much greater than in the surrounding soil.

The genus *Didiodendron* which occurs in association with decaying wood litter (Dennis and Wakefield, 1946; Smith, 1946; Tribe, 1957) but is not common in soil was isolated from the casts only. This suggests that no particular group of fungi is restricted to the casts. The composition of fungi possibly depends on the flora of the plant material recently eaten by the worm. Further, the occurrence of same fungal species in the casts as well as in the gut of the earthworm indicates that majority of them are not digested in the gut.

The higher urease activity in the casts may be due to higher nutrient contents of the casts and presumably the microbial activity (McGarity and Myers, 1967). Dehydrogenase activity increased with increase in moisture content of the casts. It seems that increased moisture stimulated directly the microbial activity (Ross and Roberts, 1970). The higher values of organic carbon and total nitrogen in the casts than the surrounding soil may be due to higher comsumption or slower decomposition rate of organic materials during the period of maximum earthworm activity or both (Table 3.7). There are also reports by several workers that earthworm casts contain more nitrogen than the soil (Lunt and Jacobson, 1944; Shrikhande and Pathak, 1951; Wittich, 1953; Parle, 1963b; Syers et al., 1979). Patra (1975) observed that casts contained some 0.47% of nitrogen compared to 0.35% in the surrounding soil. Several studies have shown that earthworm casts are rich in available phosphorus than the soil (Barley, 1961; Ponomareva, 1962; Gupta and Sakal, 1967; Graff, 1970; Vimmersted and Finney, 1973; Sharpley and Syers, 1976). The increase in pH of earthworm casts might be responsible for the higher phosphorus content in casts (Barley, 1961).

From the present study it has been observed that there exists an interaction between earthworm and the soil micro-organisms which form a source of food for the earthworm. Earthworm may affect the soil microbial community by consuming the latter as their food at one place and laying casts at

another place. The activity of earthworm may have a very significant effect on the distribution of the soil fungi. Further, earthworm casts are much richer in number of microflora than the soil. Casts also provide a good substrate for the growth of microorganisms. No evidence was obtained that the worms had a specialized gut flora, qualitatively different from that of the soil they feed on.

GENERAL DISCUSSION

The study of physico-chemical characteristics of the soil reveals that the soil of valley land agriculture contained maximum organic carbon, nitrogen, phosphorus and moisture content followed by terrace land agriculture and minimum was observed in the soil of jhum land (Figs 1.3-1.6). Topography and physical characteristics of soil play important role in determining the nutrient status of the soil. The maximum nutrient level in the soil of valley land agriculture was probably due to the deposition of eroded top soil rich in organic matter and other nutrients from the adjacent hill areas brought by runoff water. In case of jhum land agriculture, steep cultivated slopes cause maximum loss of nutrients through soil erosion. This is more pronounced in absence of crop cover particularly at the time of sowing and after the harvest. In case of terrace land agriculture, the nutrient losses are not severe as compared to that of jhum land agriculture because of the terracing. Arnason et al. (1982) reported that erosion is the most important single factor which determines the fertility of soil. The high annual rainfall also creates a favourable condition for accelerated soil erosion particularly when the aggregating power of the soil is reduced (Sanchez, 1976; Toky and Ramakrishnan, 1981).

Depth-wise distribution of nutrients exhibited a decreasing trend along with increase in soil depth in all the three

agricultural systems (Figs. 1.4-1.7). The higher concentration of nutrients in the surface layer could be associated with higher organic carbon concentration of the layer. Blakemore (1966), and Gupta and Rorison (1975) also reported the similar results. The surface layer of the soil is continuously enriched by the organic matter deposition and other input of nutrients from adjoining areas. The nutrient status of the below ground soil largely depends on the mineralization process at the top because the nutrients once released percolate down the profile along with the water.

From the results of the population dynamics of fungi and bacteria (Figs. 2.1, 2.3), it could be noted that the valley land agricultural soil harboured highest population of fungi and bacteria followed by terrace land and lowest was noted in the soil of jhum land agriculture. Almost a similar trend of distribution of fungal population was observed in the soils of all the three study sites. In general, the fungal population peaked in the month of July (Fig. 2.1). The favourable climatic condition and other factors like soil moisture and nutrient status, at this period, seem to influence favourably the growth and development of the microbes. The fall in the population thereafter may be ascribed to the decrease in nutrient content (Figs. 1.4-1.7) and non-availability of substrates (Christensen, 1969). Similarly, population of bacteria also exhibited a similar trend of distribution in all the three different agricultural soil. The peak

was generally noted in the month of June (Fig. 2.3). The population reached its peak not corresponding with the fungal population. This may probably be due to the higher counts of bacterial population, which might have inhibited the growth of fungi. Wright and Bollen (1961) also reported the similar finding. The distribution of soil microbial population may or may not be governed by a single environmental factor. It has been suggested that a number of soil factors such as pH, moisture, temperature and organic carbon regulate the distribution of microorganisms (Dadalauri, 1975; Jones and Richards, 1977). Widden and Abitbol (1980) in their studies concluded that inspite of the tremendous spatial variation, significant seasonal effects occurred and they attributed this to be due to the changes in temperature. It was noted that the microbial population decreased with increase in soil depths and the maximum population was isolated from the surface soils (Figs. 2.1-2.3). This may be attributed to the corresponding changes in nutrient status and rhizosphere effect. The nature of the crop also determines the depth-wise distribution of microbial population in the soil (Balasubramaniam et al., 1972). Selvaraj and Ramaswami (1978) opined that moisture regime and soil depth are the most important factors affecting microbial population and their activity. The maximum population of microbes in the surface layer of the soil was also reported by Warcup (1951), Saksena (1955), Dwivedi (1966b), Nicholas and Parkinson (1967), Mishra and Kanaujia (1972), Tyagi (1973) and Deka (1981).

Depth-wise distribution of microbes and their population are also influenced by soil pH and moisture content. In the present study, pH and moisture content increased with increase in depth (Figs. 1.2, 1.3). Generally the upper horizon had slightly lower pH value than the lower one. The data obtained during the present study depicts that slight depth-wise variation in soil pH does not exert any remarkable influence on the microbial population. Jensen (1931), Warcup (1951), Saksena (1955), Dwivedi (1956a) and Mishra (1966b) have also reported similar findings. Hall (1933) and Howker (1950) reported that most of the fungi have optimum growth at neutral or slightly acidic side. Similar observations were also made in the present study. Menon and Williams (1957) also reported that the soil pH was apparently not a factor contributing to mycofloral changes.

Throughout the study period, almost a similar set of fungal species was isolated, and only a few species were restricted to a particular study site. Pythium sp. could be isolated more frequently in the soil of valley land and terrace land agricultures whereas this species was rarely obtained from the soil of jhum land agriculture (Tables 1.1-1.18). Several workers (Chesters, 1949; Warcup, 1951) have reported that Pythium sp. shows a seasonal distribution being more regularly isolated when the soil moisture is relatively high. No distinct variation in the species composition was noticed from month to month (Tables 1.1-1.18). Christensen et al. (1962) and Morral and

Vanterpool (1968) also found that the composition of microfungal species does not change appreciably with time and the composition of microfungal communities was correlated with the species composition of the above ground vegetation.

Only a few species were found to be dominant and could be isolated with high percentage relative abundance. The above finding is in conformity with the observation of Bissett and Parkinson (1979), who pointed out that for a given community only a few species were quite dominant which may strongly affect the occurrence of other species.

The less fungal flora in jhum land soil may be due to direct effect of heat which might have allowed only thermotolerant species to survive therein (Tiwari and Rai, 1977). Suboptimal moisture and burning of organic materials during fire may be other causes for minimum population and species number in the soil of jhum land agriculture.

Soil rich in microbial population and nutrients exhibited maximum enzyme activities which clearly depicts the positive relationship between the two. It is apparent that the nutrient level and moisture play important role towards the enzyme activities in the soil. The enzyme activities were higher during the growing period of the plants (Figs. 2.4, 2.5). This could be attributed to the continual addition of enzyme released from the plant roots (Skujins, 1967) and also due to associated micro-

organisms (Paulson and Kurtz, 1969; Speir and Ross, 1978).

With increase in organic carbon content, urease activity increased. Speir (1977), Beri et al. (1978) and Ojeniyi (1980) reported that the enzyme activity is a direct function of organic carbon content of the soil. Tabatabai (1977), Zantua et al. (1977) and Dash et al. (1981) also reported significant positive correlation between urease activity and soil organic carbon. Dkhar and Mishra (1983) suggested that the organic carbon content may be accounted for most of the variations in soil urease activity. Dehydrogenase activity was positively correlated with the soil moisture (Tables 1.20-1.22). Ross and Roberts (1970) and Tate and Terry (1980) also reported the similar findings. The high moisture content results in the increased anaerobic and facultative bacteria which might be responsible for the increased enzymatic activity.

A positive correlation between carbon dioxide evolution, microbial population and fungal biomass was observed in the soil of all the three agricultural systems (Tables 1.20-1.22). It appears that the microorganisms were the most important contributors towards the carbon dioxide evolution. The carbon dioxide evolution was maximum in the valley land soil (Fig. 2.6), the higher rate reflects the favourable effect of soil moisture and moderate temperature on microbial activity (Gupta and Singh, 1981). Witkamp (1966a) and Anderson (1973) have indicated that temperature

also exerts a decisive influence on carbon dioxide metabolism of the soil. Similar result was observed in the soil of jhum land agriculture, while same was not true in the terrace land and valley land agricultural soils. It may be concluded that soil moisture and temperature are important factors in governing the respiratory activity of the maize field soil.

Depth-wise decline in the microbial populations and their activities (Figs. 2.1-2.6) suggests that microbial activities were inhibited by unfavourable environmental conditions prevailing in the deeper region of the soil (Duxbury and Tate, 1981). Low microbial population in the deeper soil may also be responsible for lesser microbial activities.

In the decomposition study of maize leaf, stem and root litters, it has been noted that during early stages of decomposition (during winter) the rate of decomposition was slow and the same increased thereafter (Fig. 3.1a). It may be accounted to the low temperature in winter which did not favour the growth of decomposer microorganisms (Waid, 1960; Witkamp, 1966). The low nitrogen content at the initial stages may also be responsible for the slow rate of decomposition (Mishra, 1979). Low nitrogen content at the initial stages may be due to rapid breakdown of nitrogenous compounds thereby releasing nitrogen from the litter (Burges, 1967). The rate of decomposition was maximum in leaf than stem and root. This may be, in part, as a

consequence of more rapid losses of polysaccharides from the leaf. The higher lignin content in stem and root (Table 2.4) may contribute to their slow rate of decomposition (Sinha, 1972).

The increase in microbial population at the later stages of decomposition (Figs.3.2a,b) could be attributed to the favourable moisture condition, temperature and release of nutrients from the litter (Das, 1980). Microbial population was more in leaf than stem and root which may be ascribed to the higher surface area available for the microbial colonization on the broad surfaces of the leaf.

The mycroflora of the decomposing leaf, stem and root exhibited almost a similar spectrum of fungal species (Tables 2.1-2.3). This indicates that the fungi are non-selective and may utilise a wide variety of substrates.

Majority of the fungal species was represented by the members of Fungi imperfecti. The frequent occurrence of this group is believed to be due to their better capacity to decompose cellulose (Singh and Charaya, 1975). Trichoderma viride, Penicillium chrysogenum, Aspergillus niger, Cladosporium herbarum, Fusarium moniliforme and Absidia glauca were the most common fungal species isolated from the decomposed leaf, stem and root litters (Tables 2.1-2.3). The presence of these fungi on decomposing litters suggests their importance in decomposition of litters.

The rate of decomposition under laboratory condition was much slower than that under field condition. This may be attributed to the total exclusion of the decomposer fauna from the litter studied in laboratory coupled with other unnatural conditions. However, the maximum loss in weight was noted in the set where it was amended with mixed inoculum of Trichoderma viride and Absidia glauca and minimum was observed in the set of A. glauca (Figs. 3.1b,c,d). It is believed that during the degradation of cellulose and hemicellulose by the test fungi, toxic substances might be released (Miller, 1955; Miller et al., 1958; Patrick and Toussoun, 1965). These toxic compounds probably inhibited further growth of fungi in litters. Therefore, a slower rate of decomposition was noted at the later stages of decomposition under laboratory condition.

It may be suggested that the process of decomposition is largely governed by the chemical composition of the substrate. The breakdown of various chemical components is affected as a sequence of specific reactions with enzyme systems of the organisms. The variation in the loss of different components (Tables 2.5-2.14) suggested that the rate of decomposition is closely linked with the activity of the decomposer organisms. Parnas (1975) stated that the rate of decomposition of any substrate is generally proportional to the growth rate of its decomposers.

In the study of microflora of gut contents and casts of earthworm, it is evident from the Table 3.1 that the species composition of the gut and the casts was almost similar to that of the soil from where the earthworms have been collected. It has been suggested that the earthworms are unlikely to possess an indigenous microbial population in their guts and the casts (Parle, 1963a,b; Satchell, 1967). The limited number of fungal species isolated from the gut contents of earthworm (Tables 3.3-3.5) may be on account of the selective effect of the passage through the canal and on the viability of different spores ingested. The small size of the sample and selective effect of the method of isolation may also be responsible for lesser number of fungal species in the gut contents of the earthworm. Fungal population exhibited a decreasing trend towards the mid-gut and hind-gut (Fig. 4.1a). This indicates that most of the fungal propagules were digested in the mid-gut and hind-gut of the earthworm. The gradual increase in the populations of bacteria and actinomycetes along the gut canal (Figs. 4.1b,c) may be accounted for the favourable condition for their rapid growth. Ruschmann (1953) and Schultz and Felber (1956) also reported similar findings, whereas Dawson (1947) observed that the number of bacteria was reduced by passage through the canal while the number of fungi was unaffected.

From the Figs. 4.2a,b,c it is noted that the casts contained higher microbial population than the soil. Similar

observations were also made by Parle (1963a), Ponomareva (1962) and Lofty (1974). Differences in fungal population in earthworm casts and the soil are either due to environmental changes within the earthworm gut or due to the food material ingested which provides rich substrate thereby increasing microbial activity in the casts. The type and quality of ingested material help to determine the size of microbial population in casts. The microflora present in the earthworm gut may bring about the breakdown of organic matter and thus make the casts richer in microbial population and its activity than the soil (Russell, 1950). The genus Didiodendron which occurs in wood litter (Dennis and Wakefield, 1964; Smith, 1946; Tribe, 1957), but is not common in soil was isolated from the casts only (Table 3.1). This indicates that the composition of fungi possibly depends on the flora of the plant material eaten by the earthworm. The occurrence of similar types of fungal species in the casts and the gut of earthworm may be accounted for the incapability of the earthworms to digest the same in their guts.

Many of the fungal species were found to be present throughout the entire gut canal with variation in their percentage relative abundance (Tables 3.3-3.5). This indicates that there was no complete killing of such species as they pass down the canal, however, this does not give any information regarding the possibility of quantitative selective killing which may have occurred.

The higher organic carbon and total nitrogen content found in the casts than the soil (Table 3.7) during the present study may be due to higher consumption and/or slower decomposition rate of organic materials during the period of maximum earthworm activity (Syers et al., 1979). The higher nutrient contents and microbial activity could be attributed to the higher enzyme activities in the casts than the soil (McGarity and Myers, 1967). No evidence was obtained whether the worms had a specialized gut flora qualitatively different from that of the soil they feed on.

A comparison of the three agricultural systems reveals that the soil of valley land agricultural system always harboured maximum microbial populations and their activities followed by terrace land agriculture and minimum in the soil of jhum land agriculture. Considering enzyme activities and heterotrophic microbial population estimates as a nutrient release and decomposer activity function, it appears that the valley land agriculture is the most stable and self sustainable. On the other hand, the terrace land and the jhum land agricultural systems seem to be fragile. Jhum land agriculture can be regarded as the most degraded system.

SUMMARY

Three maize fields differing in agricultural systems were selected for the present study. They were terrace land, valley land and jhum land agricultural systems. Analyses of various physico-chemical characters of the soils of all the three agricultural fields reveal that in general the nutrients followed a trend valley land > terrace land > jhum land. Both quantitative and qualitative assessment of soil microflora and their various activities viz., dehydrogenase and urease enzyme activities and carbon dioxide evolution measurement were carried out at monthly intervals for two crop cycles of maize (Zea mays L.). Results demonstrate that the microbial populations and their various activities also followed the trend valley land > terrace land > jhum land. Qualitatively, the composition of mycoflora was found to be almost similar in all the three agricultural fields. Penicillium chrysogenum, Trichoderma viride, Aspergillus niger and Mucor hiemalis were found to be of frequent occurrences in all the study sites. Pythium sp. was rarely obtained from the soil of jhum land agriculture whereas, this species was commonly isolated from the soil of valley land and terrace land agricultures. Zygorhynchus sp. and Paecilomyces sp. could be isolated only from the soil of terrace land agriculture. Phoma humicola and Endomyces sp. were restricted to valley land soil. Blakeslea sp., Cunninghamella echinulata and Gongronella sp. could be

isolated from the jhum land soil only.

Relationships between dehydrogenase and urease activities, carbon dioxide evolution, microbial population and physico-chemical characters of the soil were worked out. Statistically no significant correlation was obtained between dehydrogenase activity and microbial populations. However, a positive correlation was obtained between carbon dioxide evolution and fungal population. Urease activity was positively correlated with moisture content, bacterial population, organic carbon and total nitrogen.

Decomposition of maize litters in field condition was studied using litter bag method for a period of seven months. It was found that the rate of decomposition was largely influenced by the types of litter. The rate of decomposition of leaf was more than that of stem and root. The weight loss of all the litters followed Olson's (1963) negative exponental model. Both fungal and bacterial populations were minimum at the early stages of decomposition coinciding with the slow rate of decomposition at the time and attained maximum at the later stages of decomposition. Qualitatively, composition of mycoflora was almost similar in case of all the three types of litters. In decomposed litters, Trichoderma viride, Penicillium chrysogenum, Fusarium moniliforme, Absidia glauca and Mucor hiemalis were the most common fungal species. The pattern of change in cellulose, hemicellulose,

lignin, sugar and amino acid was almost similar for all the litters. Loss in sugar and amino acid was more rapid and the lignin content of the litters degraded at the slowest rate. Increment in the total nitrogen was noted in all the litters which declined towards the end of the experiment.

Under laboratory condition, the rate of litter decomposition by Trichoderma viride and Absidia glauca was very slow as compared to that under field condition. Fastest rate of decomposition was observed in the set amended with mixed culture of Trichoderma viride and Absidia glauca and slowest rate was recorded in case of Absidia glauca alone.

The study of the mycoflora of gut contents and casts of earthworm did not show difference in species composition. It was found that fungal numbers was maximum in fore-gut and exhibited a decreasing trend towards the distal region of the gut canal while the number of bacteria and actinomycetes increased towards the distal end of the gut canal. Penicillium chrysogenum, Fusarium moniliforme, Geotrichum candidum and Aspergillus niger were found to be present throughout the gut canal. Casts of the earthworm contained always higher number of microorganisms (fungi, bacteria and actinomycetes) than the surrounding soil.

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*Original not seen.