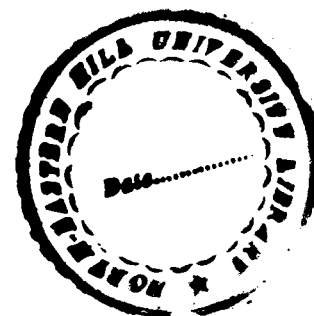


**ROLE OF EARTHWORMS IN LITTER DECOMPOSITION AND FUNGAL
DISPERSAL IN PINE FORESTS**

By

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

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For the present investigation, two forest stands of *Pinus kesiya* (Royle ex-Gordon) at different altitudes of Meghalaya were selected. The study sites chosen were Upper Shillong at a higher altitude situated at 1825m above sea level and the other site at Mawlai at a lower altitude situated at 1400m above sea level.

The soil and the earthworm casts collected from the two pine forest stands were analysed for various physico-chemical properties and fungal population. Generally soils at low altitude showed higher temperature than soils at high altitude. During winter months (October to January) temperature decreased and started to increase again after February at both the study sites. There was not much variation in the moisture content of the soil at both the study sites. Low soil moisture content was recorded during the winter months which increased with the onset of rains. When comparison was made between the moisture content of the earthworm casts with that of the surrounding soil, it was observed that the earthworm casts generally harboured higher moisture content except in few instances at both the study sites. The soil was found to be more acidic at high altitude than at low altitude. The casts had high pH values as compared to the surrounding soil. Generally, soil at high altitude showed higher organic carbon, nitrogen and phosphorus contents than soil at low altitude. The earthworm casts contained higher organic carbon, nitrogen and phosphorus contents than the surrounding soil at both the study sites.

Isolation of fungi from soil and casts was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. Fungal population of soils exhibited a more or less similar trend of monthly variation at both the study sites. Higher fungal population was recorded in the month of May in the soil at high altitude, whereas, at low altitude the fungal population was higher in the month of June. It was observed that the earthworm casts generally harboured maximum population as compared to that of the surrounding soil. A positiv

correlation was observed between the fungal population of soil and soil temperature at high altitude ($P < 0.05$). A significant positive correlation was also observed between soil organic carbon and fungal population at low altitude ($P < 0.10$). The pine forest soil at high altitude exhibited highest number of fungal isolates as compared to that at low altitude. Qualitatively, there was not much difference in the composition of microflora of the two pine forest soils. Most of the fungal species which were obtained from the soil at high altitude were also isolated from the soil at low altitude except for *Aspergillus terreus*, *Botrytis* sp., *Mucor plumbeus*, *M. racemosus*, *Trichoderma harzianum* and black sterile mycelia which were isolated from the soil at high altitude only, while *Absidia glauca*, *A. versicolor*, *Cladosporium herbarum*, *Fusarium merismoides* and *F. sporotrichioides* and *Oidodendron* sp. which were restricted to the soil at low altitude only.

The casts collected from the pine forest stand at low altitude exhibited a highest number of fungal isolates as compared to that collected from the pine forest stand at high altitude. Qualitatively, there was not much difference in the composition of the microflora of the casts collected from both the study sites except for *Alternaria tenuis*, *Mucor racemosus* and *M. circinelloides* which were isolated from the casts at high altitude only and *Aspergillus candidus*, *Cunninghamella elegans*, *Fusarium oxysporum*, *Mortierella parvispora* and *Penicillium janthinellum* which were isolated from the casts at low altitude only.

The earthworm species found at high altitude were identified as *Eutyphoeus* sp., *Amyntas corticis* (Kinberg), *Eutyphoeus festivus* Gates, *Drawida papillifer papillifer* Steph, *Lenoscolex strigosus* Gates and *Kanchuria sumerianus* Julka while those found at low altitude were identified as *Drawida papillifer papillifer* Stephensen, *Amyntas corticis* (Kinberg) and *Metaphire anomala* (Michaelson). However, *Drawida papillifer papillifer* Steph. and *Amyntas corticis* (Kinberg) were found to be common at both the study sites.

Drawida papillifer papillifer was chosen for studying the fungal population from the gut of the earthworm. The fungal population in the gut of earthworm *Drawida papillifer papillifer* at both the study sites was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. Results showed that the population was generally higher in the fore-gut as compared to that of the mid-gut and hind-gut regions. Qualitatively, there was not much difference in the composition of the fungal flora in the gut contents of the earthworm at both the study sites except for *Cladosporium macrocarpum*, *Fusarium moniliforme* and *Penicillium digitatum* which could be isolated from the gut regions of the earthworm collected from high altitude only and *Aspergillus japonicus*, *A. versicolor*, *Alternaria alternata*, *Cunninghamella elegans* and *Verticillium alboatrum* which could be isolated from the gut regions of earthworm collected from low altitude only.

A comparative study of the fungal flora present in the soil, gut contents of the earthworm and the casts collected from the pine forest stand, at both the altitudes showed that altogether a total of 28 fungal species could be isolated from the soil, 32 from the gut contents of the earthworm and 19 from the casts at high altitude. Of the 32 fungal species occurring in the different gut regions, 26 species could be isolated from the foregut, 23 from the midgut, and 25 from the hindgut regions. A total of 28 fungal species could be isolated from the soil, 32 from the gut contents and 21 from the casts at low altitude. Of the 32 fungal species occurring in the different gut regions, 29 species could be isolated from the foregut, 27 from the midgut and 26 from the hindgut regions. The earthworm gut contained higher number of fungal species as compared to that of the surrounding soil.

Moisture content and pH of both the undecomposed and the partially decomposed pine litter was also analysed. Moisture content of undecomposed and partially decomposed pine litter at high altitude was generally higher than that at low altitude. The moisture content of the

partially decomposed pine litter was more than that of the undecomposed litter at both the study sites. There was not much difference in the pH of the litter collected from the two study sites. However, pH of the partially decomposed litter at high altitude was more acidic as compared to that at low altitude. When comparison was made between the pH of the two types of litter it was found that pH of the undecomposed pine litter was more acidic as compared to that of the partially decomposed pine litter.

Similarly, the fungal population from the undecomposed as well as the partially decomposed pine litter was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. There was not much variation in the fungal population of the undecomposed litter at both the altitudes. However, the fungal population of the partially decomposed pine litter was found to be more at low altitude. There was not much variation in the fungal population between the two types of pine litter also at both the study sites. A positive significant correlation was observed between fungal population and moisture content of the litter at high altitude ($P < 0.5$). Undecomposed litter at high altitude exhibited highest number of fungal isolates as compared to the litter at low altitude. Qualitatively, there was not much difference in the fungal flora of the litter except for *Mucor racemosus*, *Aspergillus carneus* and *A. flavus* which were isolated from the litter at high altitude only, and *Mucor circinelloides* and *Alternaria alternata* which could be isolated from the litter at low altitude only. The partially decomposed pine litter at high altitude exhibited more or less similar number of fungal isolates at both the study sites. Qualitatively, there was not much difference in the composition of the fungal flora of the litter except for *Mucor racemosus*, *Absidia cylindrospora*, *Aspergillus terreus*, *A. carneus*, *A. candidus*, *A. flavus* and *Acremonium* sp. which could be isolated from the litter at high altitude only and *Aspergillus wentii*, *Penicillium brevicompactum*, *P. rubrum*,

Monilia sp. and yellow sterile mycelia which were associated with the litter at low altitude only.

There was not much difference in the composition of the fungal species associated with the undecomposed and partially decomposed pine litters at both the study sites. When comparison was made between the fungal species present in the undecomposed and partially decomposed litters, slight difference in the fungal species composition was observed in which *Alternaria alternata*, *A. tenuis*, *Aspergillus niger* and *Mucor circinelloides* were found to be present in undecomposed litter only, whereas *Absidia cylindrospora*, *Ambylosporium* sp., *Aspergillus wentii*, *Fusarium merismoides*, *Monilia* sp., *Penicillium rubrum* and *P. waksmanii* were found to be present in partially decomposed litter only.

Dehydrogenase activity of soil and the casts was determined by 2, 3, 5- triphenyl tetrazolium chloride (TTC) reduction technique modified by Casida *et. al.* (1977). The dehydrogenase activity was higher in the soil at high altitude as compared to that at low altitude except in the months of September, February, March, April and November where a lower activity was recorded. It was observed that the earthworm casts generally showed higher dehydrogenase activity as compared to that of the surrounding soil at both the study sites. Dehydrogenase activity of soil was found to vary significantly between the sampling periods at 5% level of significance.

Urease activity was measured by the modified McGarity and Myers' (1967) method. Higher urease activity was recorded in the soil at high altitude except in the months of October, April and May where lower activity was recorded. Urease activity of earthworm casts was also recorded to be higher than that of the surrounding soil at both the study sites. Statistically, a significant correlation was observed between urease activity of soil and fungal population at high altitude ($P < 0.10$). A significant correlation was observed between urease activity and

moisture content at both the study sites ($P < 0.05$ at high altitude and $P < 0.001$ at low altitude). A significant variation was observed for urease activity of the soil between the study sites as well as between the sampling periods at 5% and 1% level of significance.

Wilcke's (1955) method of handsorting was adopted for the estimation of earthworm population from the two study sites. The earthworm population was more at high altitude as compared to that at low altitude. Population was highest in June at high altitude and in July at low altitude. Minimum population was recorded in February at both the study sites. It was found that earthworm population was positively correlated with soil temperature ($P < 0.05$) and organic carbon ($P < 0.2$) at high altitude.

For determining the role of earthworms in litter decomposition under laboratory condition the method adopted by Haimi and Huhta (1990) was followed. Since *Drawida papillifer papillifer* appeared to be the dominant species, therefore, this particular species has been selected for the study. Two sets were maintained for this study with one set without earthworms being treated as a control, while another set treated with earthworms. Weight loss of pine litter was slightly more in the treated sets as compared to that of the control. Percentage weight remaining after 120 days was 83.4% in the control and 82.1% in the treated sets. The various components like cellulose, hemicellulose, lignin, total amino acids and sugars of the decomposing pine litter in the controls as well as in the sets treated with earthworms were maximum in the initial stages of decomposition which was observed to decrease in the latter stages as decomposition proceeded. pH from the leachates was found to be more acidic in the treated sets as compared to that of the control sets. Total Nitrogen and $\text{PO}_4^{3-} - \text{P}$ from the leachates also were observed to be more in the treated sets as compared to the controls. The organic carbon content of the mineral soil in the second and third samplings was higher in the sets treated with earthworms, while in the first and fourth samplings the organic carbon content

was higher in the control sets. Total nitrogen content and available phosphorus of the mineral soil and humus was found to be more in the treated sets as compared to that of the control sets. However, when comparison was made between the mineral soil, humus and earthworm casts, it was found that the casts had higher organic carbon, nitrogen and available phosphorus as compared to that of mineral soil and humus in both the sets. CO₂ evolution was higher in the sets treated with earthworms than in the control sets.

To study the role of earthworms in fungal dispersal, screening of fungi was done from soil collected from earthworm furrows and the soil adjacent to the furrows. Moisture content, pH and fungal population were determined from the two study sites (earthworm furrows and the adjacent soil). The fungi were isolated from the soil samples by soil plate method (Warcup, 1950) using rose bengal agar medium (Martin, 1950). pH of the adjacent soil was found to be more acidic as compared to that of the surrounding soil. The moisture content of the soil collected from earthworm furrows was slightly higher than that of the adjacent soil. The fungal population was also recorded to be more in the soil from earthworm furrows as compared to that of adjacent soil. Moisture content was found to be positively correlated with fungal population in adjacent soil ($P < 0.5$) as well as in soil from earthworm furrows ($P < 0.5$). There was not much variation in the fungal species collected from the two sites. However, *Humicola* sp. could be isolated from the soil from furrows only whereas, *Penicillium waksmanii* could be isolated from adjacent soil only. It was observed that the percentage relative abundance of the fungal species *P. intermedium* was high in the soil from earthworm furrows as compared to that in the adjacent soil.

Feeding habit of the earthworm species *Drawida papillifer papillifer* Steph. in relation to certain fungi was studied in conical flasks (250 ml) containing 150g of sterilized soil and 1g of sterilised partially decomposed pine litter. The test fungi used for this experiment were

Penicillium chrysogenum, *Pythium intermedium*, *Mucor hiemalis* and *Trichoderma koningii*. Results showed that there was an increase in the earthworm biomass at the initial stage, thereby, indicating that *D. papillifer papillifer* can utilise fungi as their source of food. There was a gain in the earthworm biomass initially with the different fungal species except for *P. intermedium* where the biomass showed a decreasing trend. It was also observed that the earthworms did not survive till the end of the experiment. The length of the earthworm fed with the mixed culture, *T. koningii* and *P. intermedium* showed a decreasing trend at the latter stages. The length of the earthworm fed with *P. chrysogenum* increased initially and thereafter decreased. It was also observed that the length of the earthworm remained unaffected for 35 days fed with *M. hiemalis*.

Litter bag technique (Bocock *et. al.*, 1960) was applied to study the rate of pine litter decomposition in the field. The percentage weight remaining of the litter placed in the field after 360 days was 53.9%. For the isolation of fungi from the decomposing litter bags was done following dilution plate technique (Waksman, 1922). The fungal population was observed to be more in the later stages of decomposition as compared to that in the initial stages. A positive correlation was observed between fungal population and pH of the decomposing litter ($P < 0.5$).

Four dominant fungi (*Penicillium chrysogenum*, *Pythium intermedium*, *Mucor hiemalis* and *Trichoderma koningii*) were selected to assess their efficacy in litter decomposition in the laboratory condition. In terms of different fungal inoculum, *Mucor hiemalis* proved to be more efficient in the initial stage of decomposition of the pine litter followed by *Penicillium chrysogenum*, *Trichoderma koningii* and *Pythium intermedium*. In the control (without fungi) the decomposition was recorded to be the least. A mixture of all the test fungi, however, was observed to be still more efficient as compared to the individual species. During the later stage

of decomposition *Pythium intermedium* proved to be more efficient as compared to the other fungal species.

Comparing the decomposition rates of the pine litters placed under field and laboratory conditions, it was observed that the rate of pine litter decomposition was more faster under field condition, which was observed to be approximately 80% as compared to that under laboratory condition which was observed to be approximately 90% in 105 days.

Cellulose, hemicellulose, lignin, total sugars and amino acids of the decomposing pine litters were estimated by methods described by Peach and Tracey (1955). The various organic components viz. cellulose, hemicellulose, lignin, total amino acids and sugars were observed to be maximum in the initial stages of decomposition both under field and laboratory conditions. The rate of decomposition of these various constituents was similar to that of weight loss of the decomposing litters at various sampling periods. Similar trend in the rate of decomposition was observed in the case of cellulose and lignin. The rate of degradation of the various constituents was more rapid in the field condition as compared to that in the laboratory condition. Total sugars, amino acids and hemicellulose decomposed faster than cellulose and lignin. The rate of decomposition of cellulose, hemicellulose, lignin and sugars under laboratory condition were recorded to be the highest in the mixed culture of all the test fungi and minimum in the control sets without the fungi. However, *M. hiemalis* was observed to be more efficient in the degradation of amino acids.

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CERTIFICATE

I, Darisuk Neolicia Dkhar, hereby declare that the subject matter of the thesis entitled "Role of earthworms in litter decomposition and fungal dispersal in pine forests" is the record done by me, that the contents of this thesis did not form basis of the award of any previous degree or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Botany.

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Darshan
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INTRODUCTION

The decomposition of dead plant material is an important process responsible for the release of nutrients from the litter. It plays a major role in the structure and dynamics of ecosystems. It also helps in the transfer of elements and energy and in control mechanisms and feedback processes. It is a key process in all terrestrial ecosystems because it controls nutrient availability and hence primary production. A large amount of plant litter is added annually to the soil through leaf fall and death of plants. Meentemeyer *et. al.* (1982) showed that above ground litter production is the dominant pathway joining the living biological component to the non-living soil of the ecosystem through organic matter decomposition. It acts as an input-output system on the soil surfaces and determines several other functions of the ecosystem. It forms an important nutrient pool and plays a significant role in regulating structure and function of forest ecosystem in a variety of ways.

In forest ecosystems, litter decomposition is considered as an indispensable factor influencing the growth and development of forest stand. This is relevant especially in nutrient deficient coniferous forests where decomposition is slow and availability of nutrients to plants is low (Das, 1980 ; Staaf and Berg, 1982). The slow rate of decomposition can result in the accumulation of amount of locked up nutrients on the soil surface thus limiting the nutrient supply for primary producers (Florence, 1965 ; Lamb, 1971).

The rate of litter decomposition is influenced by a number of factors, including moisture, temperature (Donnelly *et. al.*, 1990 ; Berg *et. al.*, 1993), substrate quality, particularly the chemical composition of the decomposing material (Waksman and Tenney, 1927 ; Singh and Gupta, 1977). The process is also influenced by lignin content (Meentemeyer , 1978 ; Melillo *et. al.*, 1982), nutrient status of the soil (Verhoeven and Toth, 1995) and the nature of

the microorganisms and soil fauna active in the decomposition process (Singh and Gupta, 1977).

The role of microorganisms in the decomposition of plant materials is of immense importance. As the plant materials fall on the ground, they are attacked immediately 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 tissue which in turn are used by other organisms. Fungi, being heterotrophic in their mode of nutrition along with bacteria are responsible for the decay process and thereby in the release of locked up nutrients from the dead materials of plants and animals. In the process of decomposition, fungi by the peculiarities of their physical organizations are especially effective in attacking hard and woody parts. The majority of the litter consists of structural components of plants such as cellulose, hemicellulose and lignin, all of which are broken down relatively slowly. Cellulose, a widely distributed polysaccharide is the vast replenishable resource and is recycled by the microbes. Cellulosic portion of the biomass can be converted to glucose by enzymatic hydrolysis. Hemicellulose occurs in small amounts than cellulose, making up, for instance, 12% of pine needles and 10-30% of wood. Besides these, lignin decomposition is difficult due to their heterogeneity. Lignin remains closely associated with cellulose and hemicellulose (Lundquist *et. al.*, 1980). Sometimes the lignin degradation products particularly oxidized phenolics react with nitrogeneous compounds and limit the availability of nitrogen to decomposers.

Estimates of enzyme activity are often used as indices of microbial activity and soil fertility. Dehydrogenase and urease are commonly used for this purpose. Dehydrogenase being a respiratory enzyme provides a measure of catabolic activity. It is believed to be linked intracellularly with microbial respiratory process. Dehydrogenase activities are thought to

reflect the total range of oxidative activities of the soil microflora. Urease is another important soil enzyme and is unique in that it greatly affects the fate and performance of an important fertilizer (urea). It is responsible for the breakdown of urea into carbon dioxide and ammonia. Owing to this property it has an applied importance in the N-economy of soil.

Earthworms also have a major role in the breakdown of organic matter and release and recycling of nutrients. They are the most obvious fauna in soils, comprising of more than fifty percent of the biomass. They are known to enhance decomposition by mechanical breakdown and stimulation of microbial activity. Their faecal material is in the form of casts which tends to be microbially active and contains more nutrients than the surrounding soil.

Earthworms remove partially decomposed plant litter and crop residues from the soil surface, ingest and fragment it and then transport it to the subsurface layers. The mixing and fragmentation of forest litter by earthworms have been identified as being of fundamental importance to renewal of spruce forest ecosystems in the French Alps (Bernier and Bonge, 1994). Earthworm species such as *Lumbricus terrestris* is responsible for a large proportion of the overall fragmentation and incorporation of litter in many woodlands of the temperate zone, and is primarily responsible for the formation of mulls, which are forest soils in which the surface litter and the organic layers are mixed thoroughly with the mineral soil (Muller, 1878 and Scheu and Wolters, 1991a).

The rate of breakdown of litter by earthworms depends upon the type of litter. Earthworms consume very large amount of litter and the amount they ingest seems to depend more on the total quantity of suitable organic matter than on any other factors. Some workers have calculated the amount of leaf litter of different plant species eaten by earthworms (Needham, 1957 ; Haimi and Huhta, 1990). It has also been reported that earthworms increase overall microbial respiration in soil thereby enhancing microbial degradation of organic matter

(Barley and Jennings, 1959 ; Haimi and Huhta, 1990).

Interaction between microflora and fauna also seems to play an important role in the decomposition process (Visser, 1985). Symbiotic interactions between earthworms and microorganisms help in breakdown and fragmentation of organic matter progressively, and finally incorporating it into waterstable aggregates. Earthworms depend upon microorganisms as their major source of nutrients. The best experimental evidence for the importance of microorganisms to the diet of earthworms comes from the studies of *Eisenia foetida*, a species common in decaying organic matter, however, it can also live in soils with a large amount of organic matter (Miles, 1963a). Feeding-preference studies have also shown that earthworms prefer to feed on materials inoculated with particular groups of microorganisms (Cooke and Luxton, 1980 ; Edwards and Fletcher, 1988). They can enhance dispersal of microorganisms by ingesting them or by transporting the microbes that adhere to their body surface. Most of the microorganisms transported by the earthworms are those involved in the decomposition of organic material, but they can also consume and transport beneficial as well as harmful microbial groups.

Since earthworms are widely distributed throughout the world, the importance of their accurate estimation is, therefore, necessary. A number of workers have followed Wilcke's (1955) method in selecting a sample unit area of 50x50 sq. cm. and a depth of 40 or 50 cm. Reports show that handsorting was much more efficient than using potassium permanganate (Svendsen, 1955) or formalin (Bouche, 1969).

For better understanding of the effect of earthworm activity on the soil fertility and microbe-mediated processes of soil ecosystem, knowledge on the microbial population and their activity in the earthworm casts is a prerequisite. Considering the importance of earthworms in the breakdown of litter and recycling of nutrients, it was thought desirable to

study their role in litter decomposition and fungal dispersal in pine forest soils at two altitudes of Meghalaya. To achieve the objectives set in, the following aspects were undertaken:

1. Estimation of physico-chemical properties of soil and the casts.
2. Estimation of fungal population of soil, casts, earthworm gut and litter (undecomposed and partially decomposed)
3. Enzymatic assay of soil and earthworm casts.
4. Estimation of earthworm population and identification of earthworms.
5. Role of earthworms in litter decomposition.
- 6 (a). Role of earthworms in fungal dispersal.
(b). Feeding habit of earthworms in relation to certain fungi.
- 7 (i). Efficacy of soil fungi in litter decomposition.
(a) Field condition.
(b) Laboratory condition.
(ii). Chemical analysis of the decomposing pine litters.

REVIEW OF LITERATURE

Physico-chemical properties of soil and earthworm casts

Physico-chemical properties viz. temperature, moisture, pH and nutrient contents of soil regulate the population and activity of microorganisms. Soil organic matter is a major pool of carbon and nutrients, and regulates to a large extent the physical, chemical and biological properties of the soil. Soil organic matter dynamics and the interaction between plants, microbes and physico-chemical processes influence nutrient and water availability to the plants. Nitrogen is the most limiting nutrient in many plant communities (Vitousek *et. al.*, 1982; Vitousek and Howarth, 1991). Phosphorus is another essential element of all living organisms. Wormcasts are known to have more favourable physico-chemical properties for crop growth than the parent soil (Lal and Akinremi, 1983; Mulongoy and Bedoret, 1989).

Mishra and Singh (1973) studied the leaching of applied nitrogen in the soils. They reported that the ammonium ions from N-fertilizers are retained in 0-4 cm depth, whereas only very little ammonium ions were able to move down to the bottom of the soil.

Yadav and Badolka (1973) found that soils of deodar forests of Uttar Pradesh have a high water holding capacity and cation exchange capacity. They also noted that the soils were deficient in phosphorus.

Aldag and Graff (1975) kept the earthworms *Lumbricus terrestris* in pots and reported that the content of available nitrogen in their casts was 40% greater than in the surrounding soil.

Sharpley and Syers (1976) observed that the rate of release of inorganic and organic phosphorus in the earthworm casts was about four times faster than that in the surface soil during 3 days of sequential extraction.

Fardeau (1981) showed that exchangeable and water extractable inorganic P in casts of

Pontoscolex corethrus was more abundant than in non-ingested control soils.

Waring and Schlesinger (1985) suggested that soil inorganic N is a small but dynamic pool that plays a central role in the control of production in N-limited forest ecosystems.

Lee (1985) reported that casts had higher quantities of carbon, phosphorus (5-10 times more) than the surrounding soil.

Shaw and Pawluk (1986a,b) reported a greater amount of clay-associated carbon in earthworm casts than in the surrounding soil.

Mulongoy and Bedoret (1989) noted higher pH values, organic carbon and total nitrogen in the casts as compared to the surrounding soil. They observed significant correlations between properties of the casts and of the corresponding soil for various chemical and biological parameters.

Tiwari *et. al.* (1989) observed a seasonal variation in the concentration of inorganic phosphorus in earthworm casts with highest levels relative to the surrounding soil occurring in the middle of July- August.

Rastin *et. al.* (1990) investigated a number of biological and biochemical factors in different horizons from the upper and lower slopes of a spruce forest. They reported that NH_4 - and NO_3 - N concentrations in the soil solution showed significant correlations with most of the biological and biochemical soil factors investigated.

Scheu (1991) reported that secretion of mucus in casts and from the body wall accounted for 63% of total carbon losses from a geophagus earthworm, *Octolasion lacteum*.

Lavelle *et. al.* (1992) observed that levels of inorganic N were often quite high in fresh casts.

Lopez-Hernandez *et. al.* (1993) reported that the contents of water soluble and exchangeable phosphorus were much greater in earthworm casts than in the surrounding tropical

soil.

Godde *et. al.* (1996) reported that sieving had increased the production of dissolved organic carbon from organic material collected under red spruce stands.

Schrader and Zhang (1997) reported that the organic carbon content of the earthworm casts was higher than the parent soil. They also reported that the organic C content in the casts of *Lumbricus terrestris* was more than that of *Aporrectodea caliginosa*.

Smith *et. al.* (1998) reported that the release of inorganic and organic forms of N and P were similar for forest floor and mineral soil samples collected from 3 disturbed black spruce forests. They also suggested that dissolved organic N and P is an important part of N and P cycling in boreal systems.

Fungal population of soil, casts, earthworm gut and litter

The microbial population and their activities may be regulated by the soil physico-chemical characters (Mishra, 1966; Tiwari *et. al.*, 1987). Due to their inseparable relationships, these parameters were widely investigated by various workers (Waksman, 1927; Thakur and Morris, 1928; Warcup, 1950).

Soil and casts

Waksman (1927) considered organic matter, soil pH, moisture, temperature, aeration and nature of the crop grown to be responsible for the distribution and abundance of microbes in soil.

Saksena (1955) suggested that high moisture content of soil was favourable for the growth of fungi as long as there was no water logging.

Schultz and Felber (1956) reported increase in microbial population in earthworm casts compared with the surrounding soil.

Ghilarov (1963) reported that increase in the number of microorganisms in the earthworm gut and casts was twice than that observed in the surrounding soil.

Went (1963) reported little or no difference between microfloral population of earthworm casts and soil.

Mishra (1966) suggested that factors like organic matter, pH, moisture content, aeration, temperature, season and the state of litter decomposition governed the distribution of microbes in the soil.

Mishra and Kanaujia (1972) analysed the ecological aspects of soil fungi in relation to climatic condition, vegetation and soil physico-chemical characteristics and they found that organic matter, pH, soil depth and season played a critical role in the distribution of mycoflora.

Dash *et. al.* (1979) reported that earthworm casts contained higher number of microorganisms than the surrounding soil.

Dkhar and Mishra (1987 & 1992) observed that the microbial population was higher in the soils of permanent agriculture as compared to that of 'slash and burn' type of shifting agriculture. They further reported that the soil of the valley land harboured maximum microbial populations followed by the terrace land agriculture and minimum in the soil of jhum land agriculture.

Tiwari *et. al.* (1987, 1989 & 1991) reported that soil moisture significantly alters the microbial population, its activity and relationships between parameters. They reported that earthworm casts contained higher microbial population as compared to the soil of pineapple plantations and also observed positive correlation between microfungi population and organic C, available P and exchangeable K in pineapple orchard soil.

Mulongoy and Bedoret (1989) reported higher microbial counts in the casts than in the surrounding soil.

Behera *et. al.* (1991) while conducting an ecological investigation of some microfungi in a tropical forest soil of Orissa could isolate 36 fungal species and one sterile mycelia from the soil. They observed seasonal variations of fungal population to be more pronounced in upper soil layers. They also observed positive correlation between fungal population, soil moisture and organic matter content.

Altemuller and Joschko (1992) by using fluorescent staining technique for thin microscopy observed that earthworm casts had higher microbial population as compared to the surrounding soil.

Tiwari and Mishra (1993) sampled earthworm casts and adjacent soil at 30 different sites in India and observed that the casts usually contained larger fungal population and a greater number of fungal species than the surrounding soil.

Shukla and Mishra (1996 &1997) reported that the fungal and bacterial populations dropped significantly following application of fungicides in potato field soil. They further reported that application of herbicides generally inhibited fungal populations.

Schmidt *et. al.* (1997) reported higher numbers of *Pseudomonas corrugata* in fresh casts of the earthworm species *Aporrectodea caliginosa*, *A. longa*, *Lumbricus rubellus* and *L. terrestris*.

Berg *et. al.* (1998) investigated the abundance and micro stratification of bacteria and fungi inhabiting the organic layers of a Scots pine forest. They suggested that the abundance of bacteria was influenced by water and that of fungi by water and temperature.

Tiwari and Sharma (1998) reported that the fungal and bacterial populations in highland soils increased with increase in altitude upto 1100 m, but thereafter, the populations declined sharply. They also observed positive correlation between fungal and bacterial populations with organic matter content of the soil.

Gut contents

Bassalik (1913) isolated more than 50 species of bacteria from the alimentary canal of *Lumbricus terrestris* and found none that differed from those in the soil from which the worms had been taken.

Stockli (1928) reported that there was an increase in the total number of bacteria and actinomycetes occurring in the earthworm gut as compared with those in the surrounding soil.

Dawson (1947) reported that the number of species of bacteria in soil that had passed through the gut of an earthworm decreased whereas those of fungi remained unaffected.

Parle (1959 & 1963a) showed that number of microorganisms increased exponentially from the anterior to the posterior portions of the earthworm gut.

Dash *et. al.* (1979) isolated 19 species of microfungi from the soil, 16 species from the anterior portion of the earthworm's gut and 8 species from the posterior portion of the gut.

Tiwari *et. al.* (1990) could isolate a total of 17 species of microfungi from the gut and casts, out of which 16 occurred in the anterior region, 12 in the middle region and 10 in the posterior region of the gut. They suggested that there existed a gradient with regard to the digestive capability of different regions of the gut of earthworms for utilization of microfungi as food.

Dkhar and Mishra (1991) observed that the fungal population was maximum in the foregut and minimum towards the hindgut of the earthworm *Amyntas diffringens*.

Kristufek *et. al.* (1992) reported that the number of fungi increased during passage through the gut of *Lumbricus rubellus*.

Moody *et. al.* (1996) studied the fate of fungal spores associated with the wheat straw decomposition on passage through the guts of two earthworms *L. terrestris* and *Aporrectodea longa*. They found that the effect of passage through the earthworm gut on the viability of spores

of saprophytic fungi was found to vary depending upon the fungal and earthworm species. They also found that *Fusarium lateritium*, *Agrocybe temulenta*, *Trichoderma* sp., *Mucor hiemalis* and *Chaetomium globosum* failed to germinate. They further reported that germination of *Trichoderma* sp. and *Mucor hiemalis* was significantly reduced in the case of *L. terrestris* whereas the reverse was observed in the case of *A. longa* in which there was a significant increase in the spore germination after gut transit.

Ranee and Dkhar (1998) observed that the fungal population in the gut of the earthworm was of the order : hindgut > foregut > midgut. They also observed that there was a gradual decrease in the number of fungal species from soil to hindgut.

Litter

Sharma and Dwivedi (1972) examined the fungal succession on decaying grass and observed differences in the total number of fungi at different time intervals on the different plant parts like the stem, sheath and the blade. They attributed this variation to the various factors like the moisture content of the substrate, temperature, humidity and the competition between the colonizers.

Eicker (1973) studied the microflora of *Eucalyptus maculata* and observed a total of 45 species representing 22 genera from various litter horizons.

Black and Dix (1977) studied the pattern of colonization of soil fungi on the Scots pine. They observed a difference in the colonizing ability from species to species. Furthermore, they reported that *Trichoderma* sp., *Penicillium* sp. and members of mucorales were the surface colonizers and that their colonizing ability was dependent on the internal tissues of the litter.

Das (1980) studied the fungal succession and microbial population of litter *Pinus kesiya* of different plantations and found that *Trichoderma viride* and *Penicillium chrysogenum* were the dominant mycoflora during decomposition.

Mishra and Dickinson (1981) obtained a marked seasonal variation in the mycoflora from the litter of *Ilex aquifolia*.

Kuter (1986) isolated different fungal species from both the green and senescent decomposing maple leaves and also observed a quantitative variation in the fungal population.

Mittal and Wang (1987) isolated 13 fungal species from seeds and cone scales of *Pinus sylvestris* and 17 species from *Picea glauca*.

Tiwari (1988) studied the fungal and bacterial populations of pineapple litter of different plantations. He recorded a low bacterial and fungal count in the beginning that increased as decomposition continued but later declined towards the final stage. He isolated a total of 19 fungal species from decomposing leaf and root litters.

Kshatriya (1990) reported minimum microbial population in the litter during winter months.

Shukla *et. al.* (1990) found highest microbial population in the leaf litter of potato followed by stem and the root litters.

Soil Enzymes

Studies of enzyme activities in soil are important as they indicate the potential of the soil to support biochemical processes which are essential for the maintenance of soil fertility (Dkhar and Mishra, 1983). Enzyme activities in soil catalyze various reactions necessary for decomposition of organic matter, cycling of nutrients, formation of organic matter and soil structure (Dick, 1994).

Dehydrogenase

Determination of dehydrogenase activity in soils is based on the use of soluble tetrazolium salts as artificial electron acceptors, which are reduced to red coloured formazans, extracted and then determined colorimetrically (Trevors, 1984; Von Mersi and Schinner, 1991).

Lenhard (1956) was the first to apply the method for the estimation of dehydrogenase activity in the soils, using anaerobic conditions and triphenyltetrazolium chloride (TTC).

Ross (1971) considered dehydrogenases to play an essential role in the initial stages of the oxidation of soil organic matter by transferring hydrogen and electrons from substrates to acceptors.

Skujins (1976) studied the seasonal variation in enzyme activity. He recorded that once the enzyme is stabilized in the soil, it develops resistance to humidity, temperature and other environmental changes.

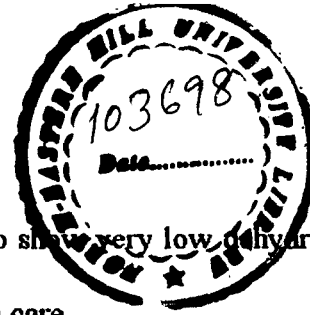
Das and Mishra (1986) estimated dehydrogenase activity and CO₂ evolution from pine forest soil. They did not find any correlation between dehydrogenase activity and microbial population and suggested that these two parameters are governed by different environmental factors.

Dkhar and Mishra (1986) observed that the dehydrogenase activity of soil was positively correlated with moisture content and the bacterial population.

Tiwari *et. al.* (1987a,b) studied the influence of moisture regime on dehydrogenase activity and reported that moisture plays a significant role in soils. They further reported maximum dehydrogenase activity in the summer season and also suggested that soil temperature, moisture and organic carbon were the most important factors regulating the dehydrogenase activity.

Von Mersi and Schinner (1991) found that dehydrogenase activity closely correlates with respiratory activity in soils.

Jha *et. al.* (1992) observed positive correlation between dehydrogenase activity and fungal population in forest stands at two altitudes.



Alef (1995) reported that acidic soil below pH 5 tend to show very low dehydrogenase activity and also pointed out that they should be interpreted with care.

Shukla and Mishra (1997&1998) reported that dehydrogenase activity tend to decrease after application with the herbicides thiobencarb and fluchloralin. However, they observed no marked variation in the dehydrogenase activity in the sets treated with 2,4-dichlorophenoxyacetic acid. They further reported a sharp decline in the dehydrogenase activity in the soil applied with P and K after 4 weeks of incubation.

Brzezinska *et. al.* (1998) suggested that soil water content and temperature influence the dehydrogenase activity indirectly by affecting the soil-reduction status. They also observed that soil dehydrogenase activity was highly correlated with redox potential and with oxygen diffusion.

Tiwari and Sharma (1998) showed that the organic matter content of the soil is an important factor that regulates enzyme and microbial activities in the Eastern Himalayan highland soils.

Camina *et. al.* (1998) while studying the dehydrogenase activity in acid soils rich in organic matter showed that Iodonitrotetrazolium formazan (INTF) was adsorbed by the soils with an intensity that closely relates with soil carbon content. They also suggested that a mixture of 1:1 Dimethyl-formamide-ethanol (DMF- ethanol) was more effective than methanol in extracting INTF, thereby improving estimates of dehydrogenase activity.

Urease

The presence of urea in large amounts in the soil allows its use as a nitrogen fertilizer worldwide. A few studies regarding relationships between urease activity and other soil properties have indicated that urease activity tends to increase with increase in 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; Skujins and McLaren, 1967; Myers and McGarity, 1968). Urease hydrolyses urea, yielding ammonia and carbonate (Andrews *et. al.*, 1989).

McGarity and Myers (1967) found that urease activity in 100 Australian surface soils was highly correlated with organic carbon and weakly correlated with pH.

Myers and McGarity (1968) also observed a highly significant relationship between urease activity and organic carbon in profile samples of five great soil groups, but did not detect a significant correlation between urease activity and pH.

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

Zantua *et. al.* (1977) reported that urease activity was significantly correlated with organic carbon, total nitrogen and cation-exchange capacity but was not correlated with pH, silt and CaCO_3 equivalent.

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

Nor (1982) studied the urease activity in several Malaysian soils and indicated that the soils have varying capacity to hydrolyse urea. He also observed positive correlation between urease activity and soil pH, but did not observe any significant relationship with neither organic carbon nor cation exchange capacity.

O'Toole *et. al.* (1985) suggested that urease activity was affected by temperature, soil factors, such as moisture content, pH, organic matter and number of microorganisms.

Tiwari *et. al.* (1987b) noted higher urease activity during April in the surface soil which was attributable to higher organic carbon, bacterial population, temperature and favourable moisture content.

Douglas and Hendrickson (1989) reported that because of the greater stability and resistance to degradation, thiophosphoric triamide provides longer sustained activity against soil urease.

Joshi *et. al.* (1991) reported that soil with higher microbial population harboured higher urease activity.

Pagliai and ^{De}Nobili (1993) showed that urease activity was positively correlated with soil porosity.

Rao *et. al.* (1995) observed higher urease activity during rainy season than in winter in subtropical pine forest soils collected from different age group stands.

Shukla and Mishra (1997 & 1998) reported that urease does not mediate the degradation pathway of herbicides but rather remained unaffected by the herbicides. They further did not observed any significant difference in the urease activity of fertilizer-treated potato field soils and the control.

Earthworm population

Many efforts have been made in recent decades to find an optimal method to determine earthworm population in soils. The use of an electrical method for the determination of earthworm population from the soil was suggested by various workers (Doeksen, 1950; Satchell, 1955d; Edwards and Lofty, 1975; Rushton and Luff, 1984). Generally handsorting and formalin extraction seem to be the most suitable methods for the determination of earthworm populations in the field (Nordstrom and Rundgren, 1972; Walther and Snider, 1984; Lee, 1985; Mukherji and Singh, 1986; Dunger and Fiedler, 1989). Handsorting and subsequent washing and

seiving can also improve the efficiency of separating earthworms from soil (Lavelle, 1978; Walther and Snider, 1984; Judas, 1988). Chloroacetophenone and mustard solutions have also been suggested for the estimation of *Lumbricus terrestris* and other earthworms as well (Daniel *et. al.*, 1992; Gunn, 1992).

Evans and Guild (1947c) suggested that earthworm activity was mostly affected by temperature and moisture.

Gates (1961) observed that earthworm activity in the tropics was limited to certain seasons only.

Gerard (1967) reported that in pasture soil in England, *Aporrectodea chlorotica*, *A. caliginosa* and *A. rosea* usually occurred within 10 cm of the soil surface. However, when the soil temperature fell below 5°C or when the soil became dry, individuals of these species move to deeper soil.

Edwards and Lofty (1977) suggested that earthworm population dynamics are relatively complex and that they depend principally on the availability of soil moisture and temperature for development and activity.

Lee (1985) suggested that the availability of organic matter was probably the most important factor determining the size of earthworm populations.

Reddy (1987) observed that earthworms were found in the upper (0-10cm) layer of the soil during the rainy season but penetrated downwards into the deeper soil as winter approached.

Mishra and Ramakrishnan (1988) reported a monthly fluctuation in the population of earthworms and attributed this fluctuation to be related to soil temperature, moisture and litterfall pattern.

Bhadoria and Ramakrishnan (1989) reported that earthworm population declined significantly after slashing and burning. They also suggested that population size was significantly correlated with soil moisture, temperature and organic matter.

Clare *et. al.* (1992) while determining the effect of liming in earthworm populations of two European forests found that liming increased the earthworm density. They further concluded that earthworms have a beneficial effect in limed coniferous soils by increasing nutrient turnover.

Baker *et. al.* (1993a,b) found that the populations of *Aporrectodea caliginosa* and *Octolasion cyaneum* in pasture soil increased from May to July and decreased from July to October. They further determined the abundance of *Aporrectodea rosea*, *A. trapezoides*, *Microscolex dubius* and *M. phosphoreus* in lucerne and cereal fields in Australia and recorded highest numbers of earthworms occurring during winter and spring.

Reddy and Pasha (1993) reported that earthworms migrated to deeper layers during winter and summer. They suggested that their seasonal population structure was significantly influenced by the seasonal patterns in rainfall, soil temperature, pH electrical conductivity, phosphorus, organic carbon, nitrogen and potassium. They further suggested that the physical factors of the soil were collectively more effective in causing the seasonal variation in their population size than the chemical factors.

Kaushal and Bisht (1994) while studying the population of the earthworms in Kumaon Himalayan pasture soil found that of the total 13310 individuals collected 99% were those of *Amyntas alexandri*. The other species found were *Amyntas diffringens* and *Eisenia foetida*. They also found that the maximum density and biomass were recorded during the wet season.

Cothrel *et. al.* (1997) reported that insitu recycling of leaf litter can increase earthworm populations in urban soil.

Decomposition by earthworms

Earthworms have a major role in the breakdown of organic matter and the release and recycling of nutrients. They remove the partially decomposed plant litter and crop residues from the soil surface, ingest it, fragment it and transport it to the subsurface layer.

Edwards and Heath (1963) who placed disks, cut from freshly fallen oak and beech leaves in nylon bags of four different mesh sizes observed that after one year 92% of the total oak leaf material and 70% of the beech litter had been removed by earthworms. They observed that earthworms ate not only the softer parts of the leaves but also the veins and the ribs.

Madge (1966) reported that in tropical forests of Nigeria the litterfall was three to four times greater than in the temperate forests and earthworms were the most important animals causing its fragmentation and incorporation.

Vimmerstedt and Finney (1973) reported large increases in deciduous litter disappearance on mine spoils following the introduction of *L. terrestris*.

Brattsen (1979) showed that *Quercus ilex* litter was unpalatable for *L. terrestris* and attributed this unpalatability to the relative toxicity of aromatic polyphenols in this litter.

Anderson *et. al.* (1983) measured nitrogen mineralization in forest soil incubated with oak litter and with or without the earthworm *Lumbricus rubellus*. They observed that *L. rubellus* increased the mobilization of nitrate nitrogen by 10 times and that of ammonium nitrogen by 80 times relative to soil without earthworms.

Ferriere and Bouche (1985) reported that the entire carbon content of the earthworm could turnover in 40 days and also suggested that a considerable portion of this turnover was due to mucus secretion.

MacKay and Kladivko (1985) reported that after 36 days, pots with no earthworms had retained 60% of the soybean residues and 85% of the maize residues, whereas, pots with earthworms had only 34% of the original soybean residues and 52% of the original maize residues.

Cortez *et. al.* (1989) reported relatively large increase in decomposition of plant litter on the soil by addition of earthworms.

Haimi and Huhta (1990) showed that *L. rubellus* increased the mass loss of coniferous forest humus by a factor of 1.4 in a 48 week laboratory incubation. They also observed that earthworm respiration accounted for 21-32% of the increase in CO₂ evolution. They further reported that the earthworm species *L. rubellus* and *Dendrobaena octaedra* significantly raised the pH of the leaching waters and humus and observed that both the worms increased N-mineralization but influenced the level of PO₄³⁻- P only slightly.

Haimi and Boucelham (1991) in a laboratory study reported that *L. rubellus* had a positive effect on CO₂ evolution during the period when the worms were present .

Robinson *et. al.* (1992) reported that *Aporrectodea caliginosa* were shown to increase nitrogen in leachates from field lysimeters in limed peat soil. They estimated a total of 60 kg nitrogen/ ha/ yr was collected from leachates with earthworms whereas, only 36 kg nitrogen/ ha/ yr was collected from leachates without earthworms.

Ruz-Jerez *et. al.* (1992) reported that mineral nitrogen concentrations were about 50% greater in soils with earthworms than in soils without earthworms. They also observed that earthworms increased CO₂ evolution after the addition of clover and grass by 1.35 and 1.25 fold respectively.

Haimi and Einbork (1992) reported that the earthworm *Aporrectodea caliginosa tuberculata* had mixed the organic matter into the mineral layer of the soil. They also observed

that pH values and N concentrations in the leaching water showed no consistent differences between soil with and without earthworms. They further reported that the worm increased the CO₂ production of the soil.

Scheu (1993a,b) reported that earthworms increased the mineralization of ¹⁴C- labelled lignin in limestone soils. He reported that during 253 days of laboratory incubations, *Octolasion lacteum* increased the mineralization of labelled lignin for the first 10 weeks, but decreased later.

Bohlen and Edwards (1995) reported that earthworms increased soil respiration rates during the first 15 days of incubation by 1.24 to 2.42 fold.

Wessells *et. al.* (1997) reported that earthworms had significant effects on soil respiration but their effects varied seasonally and were influenced by environmental conditions.

Cortez and Bouche (1998) reported that anecic earthworms make litters more palatable by a particular type of behaviour. They reported that during the first stage of decomposition the litters were ploughed in by earthworm casts involving both an increase of microbial activity and preliminary microbial litter decomposition.

Role of earthworms in dispersal

Earthworms can enhance the dispersal of microorganisms by ingesting them at one location from a particular food source and egesting them elsewhere or by transporting microbes that adhere to their body surface.

Baweja (1939) suggested that earthworms dispersed the pathogenic fungus *Pythium*.

Khambata and Bhatt (1957) suggested that earthworms dispersed spores of harmful fungus *Fusarium*.

Hutchinson and Kamel (1956) inoculated sterilized soil with several species of fungi and reported that the rate of spread of the fungi through the soil was much greater when worms were present than when they were absent.

Hoffman and Purdy (1964) reported that teleospores produced by *Telletia controversa*, a pathogen causing dwarf bunt could pass through the earthworm gut without harm.

Rao (1979) reported that the earthworm *Megascolex insignis* fed on decaying roots of *Carica papaya* and spread viable spores.

Madsen and Alexander (1982) reported that *L. rubellus* enhanced the translocation of rhizobium bacterium *Bradyrhizobium japonicum* to greater soil depths.

Huss (1989) fed *L. terrestris* with separate suspension of spores and myxamoebae of *Dictyostelium mucoroides* and found that the spores survived passage through the gut. He, therefore, concluded that earthworms may play an important role in the short- range dispersal of slime mould propagules.

Gange (1993) reported that earthworms had a significant impact on the distribution of VAM propagules in early (1 and 3 year) and later (5,8 and 11 year) successional plant communities.

Doube *et. al.* (1994c) showed that earthworms *A. trapezoides* and *A. rosea*, common species found in cereal soils of southern Australia could disperse *Pseudomonas corrugata* strain 2140R which was used as a biocontrol agent for the take all disease of wheat.

Stephens *et. al.* (1994a,b) reported that *A. trapezoides* enhanced the rates of dispersal of *Rhizoctonia meliloti* as well as the levels of root nodulation in infected alfalfa plants. They further showed that the earthworm *A. trapazoides* dispersed the bacterium *Pseudomonas corrugata* 2140R strain through the soil thereby resulting in bacterial colonization of the roots of wheat seedlings.

Toyota and Kimura (1994) found that the earthworm *Pheretima* sp. dispersed the soil borne plant pathogen *Fusarium oxysporum* in the top soil but decreased the total propagules of this pathogen.

Brown (1995) suggested that by feeding, burrowing and casting activity earthworms influenced the dispersal of microorganisms throughout the soil.

Feeding habit of earthworms

Microorganisms constitute an important component of earthworm diet. Reports suggest that fungal tissues are digested as the principle source of nourishment (Morgan, 1988). Suggestions that preferential consumption of fungal species by earthworms in field studies have also been made by several authors (Dash *et. al.*, 1986; Striganova *et. al.*, 1989) after examinations of the contents of the alimentary tracts of earthworms and comparison with the surrounding soil.

Miles (1963a) introduced *Elsinia fetida* into soils inoculated with fungi and bacteria and showed that the worms were unable to reach sexual maturity unless protozoans were added to the cultures.

Wright (1972) reported that when *Lumbricus terrestris* was allowed to feed on disks of apple leaves inoculated with *Pseudomonas aeruginosa*, they consumed about 35% more than when offered with uninoculated leaf disk.

Atlavinyte and Pociene (1973) found that earthworms grew best in soil with green and blue green algae, while Pearce (1978) found that fungi and algae were a significant component of the food of 6 lumbricid species.

Neuhauser *et. al.* (1980) reported that *E. fetida* increased in weight in presence of bacteria, protozoa and fungi.

Cooke and Luxton (1980) and Cooke (1983) showed that *L. terrestris* preferred to feed on paper disks that were inoculated with particular species of fungi such as *Fusarium oxysporum*, *Alternaria solani* and *Trichoderma viride* and rejected the disks inoculated with other species such as *Cladosporium cladosporioides*, *Poronia piliformis* and *Chaetomia globosum*.

Flack and Hartenstein (1984) showed that earthworms grew well with many species of protozoa and bacteria, although growth rates were 20% greater in the presence of bacteria alone.

Hand and Hays (1988) provided 18 individual species of bacteria and 22 different species of fungi to *E. fetida* and observed that earthworm growth was improved in the presence of some but remained unaffected by others.

Edwards and Fletcher (1988) suggested that bacteria were of minor importance in the diet, algae of moderate importance while protozoa and fungi were the major source of nutrients.

Moody *et. al.* (1995 & 1996) suggested that selective grazing by earthworms on some fungal species may reduce their competitive ability and allow slow growing fungal species to gain competitive advantage. They further studied the feeding preference of three species of earthworms (*L. terrestris*, *Aporrectodea longa* and *Allolobophora chlorotica*) on a choice of mixtures of soil and small wheat straw fragments inoculated with 6 fungal species and found that all the earthworm species showed preferences between the six fungal species offered.

Microbial decomposition of litter

Decomposition of plant material is an important factor in forest ecosystems because of its critical role in the cycling of essential plant materials particularly N (Hendersen *et. al.*, 1978 ; Van Cleve and Oliver, 1982). Rates of decomposition and nutrient cycling are determined by the rates of input of dead plant tissues, resource quality, abiotic factors and the

soil microorganisms (Singh and Gupta, 1977 ; Swift *et. al.*, 1979). Decomposition results in the release of substrate for the growth of other organisms and in the establishment of food webs.

Mikola (1955) and Minderman (1968) suggested that chemical composition of the decomposing materials has been considered a critical factor in determining the extent and rate of decay.

Cromack and Monk (1975) have shown that lignin concentration of the substrate is an excellent index to use to predict the rate of disappearance and weight loss by forest litter samples.

Antheunisse (1979) has made an investigation on the decomposition of coco fibres in soil. He used the unaffected and partly decayed fibres for his investigation. He found that around the partly decayed fibres nearly always fungi developed.

Nagy and Macauley (1982) described a method to determine the effect of relative humidity and substrate moisture content on the rates of decomposition of *Eucalyptus* leaf litter. Their results suggested that dry weight loss of the litter can occur at relative humidity and moisture content values of approximately 32% and 5% respectively.

Herlitzius (1983) used weight loss as a variable to monitor the decomposition of hazel leaves in different temperate forest, alluvial, beech and spruce. He concluded that the duration of exposure was important in the decomposition of hazel litter in both alluvial and beech forests. For the weight loss in the spruce forest, he concluded that the date of incubation was important than the duration of exposure.

DeCantazaro and Kimmins (1985) while studying the changes in weight and nutrient composition of litter fall in three forest ecosystem types found that confined broadleaf foliage decomposed faster at about the same rates on all sites, but unconfined samples decomposed significantly faster on the lower hygric sites, probably as a result of greater fragmentation

losses. They further reported that twig weight loss of conifers was extremely variable ranging from 10% to 28% after 12 months.

Bockheim and Leide (1986) studied the breakdown of dry matter from a 34 year old *Pinus resinosa* plantation using leaf-litter bags. They observed that after 1 year of decomposition, 77% of the original dry matter in the leaf-litter bags remained.

Edmonds (1987) reported that conifer twigs decomposed faster than cones and branches.

Fyles and McGill (1987) while studying the decomposition of boreal forest litters from jack pine, white spruce, balsam fir, green alder, feather mosses and lichen observed that relative differences in decomposition rates among litter types appeared to be reflected in forest floor properties suggesting that plant species play different roles in the control of decomposition-related ecosystem processes.

Jordan *et. al.* (1989) studied the decomposition of *Typha* litter in mesh bags placed in the plots containing litter. They found that in plots with increased litter placed on the top of the litter layer decomposed faster than litter placed on the sediment surface. They also reported that nutrient additions did not affect the rate of litter decomposition.

Shukla *et. al.* (1990) studied the decomposition of potato litter in relation to microbial population and plant nutrients under field conditions. They found that the rate of decomposition was dependent on the chemical composition of litter and associated microbial population. Their study also demonstrated that the turnover of nutrients will take less than one year.

Bowen (1990) studied the decomposition of wheat straw by cultures of *Fusarium culmorum*, *Trichoderma viride*, *Chaetomium globosum*, lignin degrading basidiomycetes and *Typhula* sp. isolated from arable soils. He reported that the rate of straw decay may be affected

by the species of the cellulose and lignin decomposers and by the relative timing of their inoculation on to the straw.

Berg and Ekbohm (1991) showed that the decomposition process in the later stages of decay of needle and leaf litter is closely related to lignin turnover.

Alban and Pastor (1993) while studying the decomposition of aspen, spruce and pine boles of two sites at Minnesota observed that decomposition constant (K) decreased in the order aspen > spruce > red pine > jack pine. Although the decomposition rate was strongly species dependent, it did not differ between the two sites. Nutrient concentration also increased during decomposition, particularly for nitrogen and phosphorus.

Virzo De Santo *et. al.* (1993) while studying the decomposition dynamics of needle litter of *Pinus pinea*, *P. laricio*, *P. sylvestris* and *Abies alba* suggested the importance of litter moisture as a rate regulating factor in the early stages of decomposition.

Martin *et. al.* (1994) while studying the leaf litter decomposition of *Quercus pyrenaica* from Spain and *Q. lanuginosa* from France for over three years reported that leaf mass loss amounted to 32-38%, 42-53% and 53-65% respectively after one, two or three years of decomposition. They also showed that decomposition progressed faster at the French site as a consequence of climatic factors. They also concluded that environmental factors, especially the moisture level in Mediterranean areas play an important role in leaf decomposition. They attributed this direct effect on the microbial and mesofaunal activity in the soil.

Santa-Regina and Gallardo (1995) while studying the litter decomposition of three forest ecosystems of oak, sweet-chestnut copice and scots pine found that higher decomposition rates were in the chestnut leaves than in the oak leaves. Under almost identical climatic condition, chestnut leaves decomposed faster than oak and scots pine. Litter accumulation also was recorded to be the highest in the pine forest followed by the oak and chestnut forests.

Berg *et al.* (1996) analysed various available data from temperate and boreal coniferous forests and observed that high N concentrations had a very significant and strong suppressive effect on the later stages of decomposition but by contrast, high concentrations of Mn enhanced decomposition at this stage.

Hasegawa and Takeda (1996) suggested that decomposition rates of pine needles decreased with the field exposed time and were significantly higher in the first year than in the rest of the experimental period.

Gunadi *et al.* (1998) while studying the decomposition of coniferous litter at two layers (L and F layers) in a pine forest plantation in two plots at two altitudes observed that after one year the weight loss in the upper plot was 32% in the L layer and 53% in the F layer. The weight loss in the lower plot, however, was 60% in the L layer and 42% in the F layer. They also suggested that temperature and moisture were not responsible for these differences in weight loss but the chemical composition of the litter seemed to be more important in determining the decay rate of the L layer.

Couteaux *et al.* (1998) while studying the chemical changes in the decomposing scots pine needles exposed for 22 months in litter bags reported that the concentrations of N, P, cellulose, lignin, lignin-C and lignin-N content were related to accumulated mass loss.

Chemical analysis of decomposing litters

The plant litters differ in their chemical composition depending upon the species. The chemical composition of litter changes as the process of decomposition proceeds. The soluble organic and inorganic components leach out of the material within a very short span of time which may range from a week to few months depending upon the physical structure of the material. Macromolecules such as lignin, cellulose, hemicellulose, sugar, amino acid etc. have been generally considered as a measure of degree of decomposition.

Sokolov and Karpova (1965) observed that during decomposition of starch, hemicellulose and amino acids in organic residues decomposed at a faster rate while lignin was the last to decompose.

Suberkropp *et. al.* (1976) observed increments in the percentage of nitrogen and lignin. They also found that the change in percentage of hemicellulose and cellulose concentration was quite high and generally remained near original values.

Antheunisse (1979) reported that coco fibres had no homogeneous composition relative to lignin and sugar percentage.

Mishra (1979) reported maximum cellulose, hemicellulose, lignin, soluble sugars and amino acids in the roots of *Pennisetum typhoides* than in the roots of *Triticum aestivum*, *Hordeum vulgare*, *Paspalum scrobiculatum* and *Echinochloa crusgalli*.

Mishra and Tiwari (1984) reported that sugars and amino acid concentration declined very fast during the initial periods of decomposition and became stabilized in the later periods.

Agrosin *et. al.* (1985) reported that the water soluble phenolic compounds are produced in large concentrations by basidiomycetes during straw decay.

Berg (1986) suggested that decomposition of litter may be divided into at least two phases. In the first phase soluble substances and non-lignified carbohydrates (cellulose and hemicellulose) are decomposed by saprophytic fungi. In the late decomposition phase, on the other hand, primarily lignin and lignified cellulose remained.

Summerbell and Burgess (1989) reported that the percentage of hot water- soluble components, hemicellulose and cellulose decreased, while the proportion of lignin increased as the wheat straw decomposed.

Bowen (1990) suggested that the rate of straw decay was affected by the species of the cellulose and lignin decomposition and by their relative timing of inoculation onto the straw.

Dkhar and Mishra (1992) reported that the amount of cellulose, hemicellulose and lignin contents of the decomposing maize litters varied with different litter types. They observed maximum amount of lignin in the root followed by the stem and minimum in the leaf. They also observed significant positive correlation between percentage weight remaining and various chemical compounds associated with different types of litter.

Entry and Backman (1995) observed that the concentration of C and N (together) in the soil correlated with cellulose and lignin degradation. They concluded that cellulose:lignin:N ratio may be a more accurate predictor of organic matter decomposition rates than C : N or lignin : N ratios.

Berg *et. al.* (1996) reported relatively low lignin concentrations in sugar maples as compared to scots pine needles, white pine, white oak and aspen leaves.

Cortez *et. al.* (1996) studied the decomposition of four Mediterranean species leaf litter of *Quercus petraea* L., *Q. ilex*, *Castanea sativa* and *Fagus sylvatica*. They observed that all the litters showed a reduction of lipid, hydrosoluble, hemicellulose and cellulose content and a relative increase of lignin content.

Couteaux *et. al.* (1998) reported that at 50% mass loss of scots pine needles, 70% of the absolute amount of cellulose decomposed where after it was observed to have approached an asymptotic value of about 18% afterwhich it decomposed at the same rate as lignin. They also reported that during the active initial phase of decomposition the concentration of lignin increased from about 24% to about 35%.

Plate No. 1 Study site at high altitude.

Plate No. 2 Study site at low altitude.



Plate No. 1



Plate No. 2

STUDY AREA, CLIMATE AND VEGETATION

Meghalaya also known as the “abode of clouds” is situated in the north eastern part of our country. It is the 21st state of the Indian Union that was declared a full fledged state on January 21st, 1972. Meghalaya lies between 25°1' N and 26°5' N latitude and 89°49' E and 92°52' E longitude. Its capital, *Shillong* is situated at an altitude of 1496m above sea level.

The hills of Meghalaya are geologically of an older formation and are more stable than the Himalayas. The rock formations of Meghalaya are of the earlier pre-Cambrian era - composed of archaean gneiss. The higher ridges of the state lie in the coniferous belt, gradually sloping down to sub-tropical and tropical zones. The wet hill mixed coniferous forest stands of Meghalaya are dominated by pine. *Pinus kesiya* (Royle ex-Gordon) commonly known as Khasi pine is distributed in the sub tropics between an altitude of 800m to 2000m of North East India. It is an important conifer in the higher altitude of North East India that dominates the forest plants of Meghalaya consisting of about 30% of the total forest cover. It has a high potential value as producer of resin, wood and wood products.

For the present investigation, two forest stands of *Pinus kesiya* (Royle ex-Gordon) at different altitudes of Meghalaya were selected. The study sites chosen were Upper Shillong at a higher altitude and Mawlai at a lower altitude. The site at a higher altitude was situated at 1825m above sea level and lies at 25°34' N latitude and 91°56' E longitude. The other site at a lower altitude was situated at 1400m above sea level and lies at 25°36' N latitude and 91°53' E longitude. Both the study sites were 20 km apart from each other.

Meghalaya is directly influenced by south-west monsoon and the north east winter winds. There is fairly evenly distributed precipitation for over six months in the year and two

Fig. 1 (a&b). Monthly variation in rainfall (histogram) and ambient temperature of two pine forest stands (o-o) =Max. temperature, (•-•) =Min. temperature.

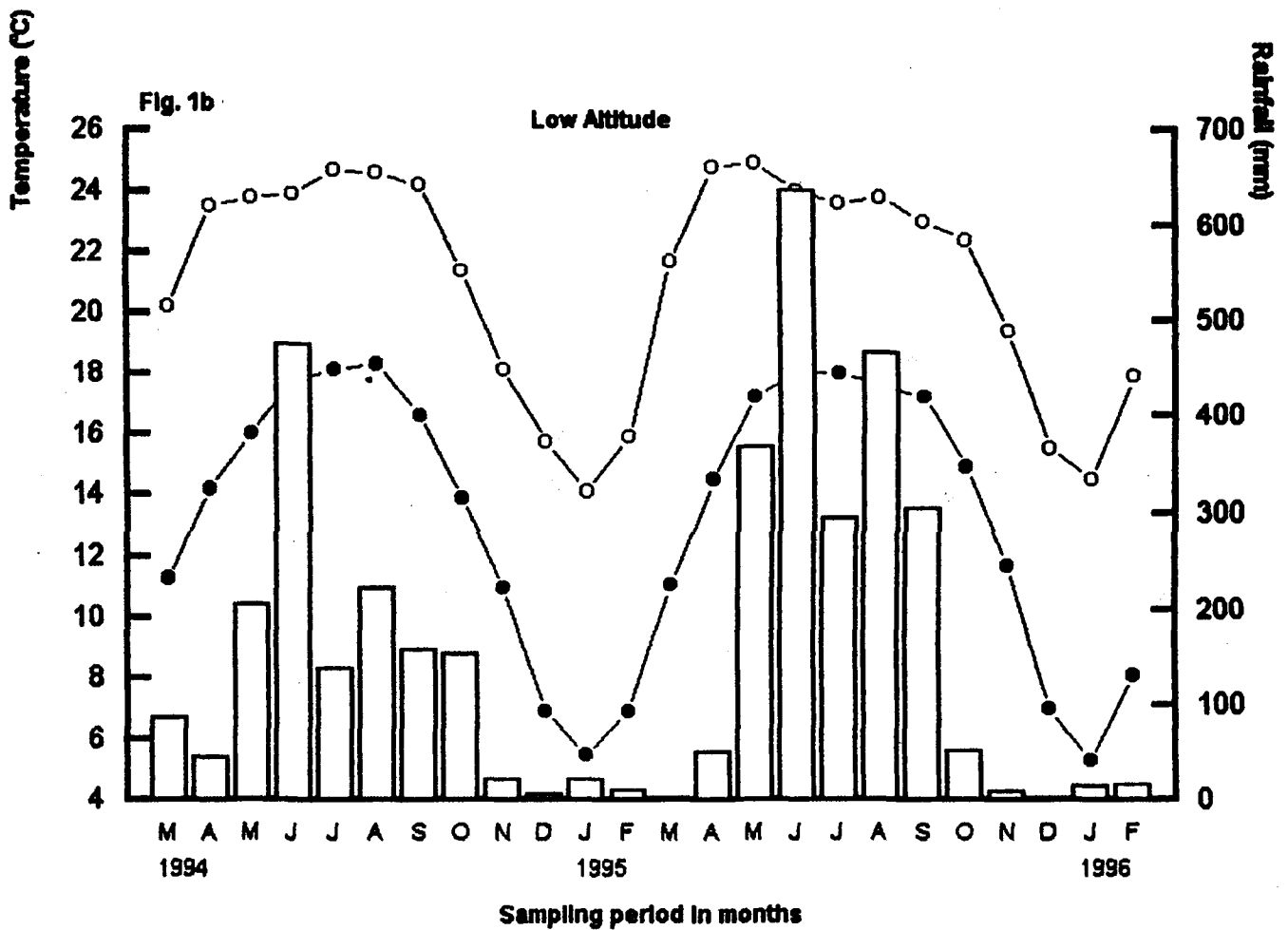
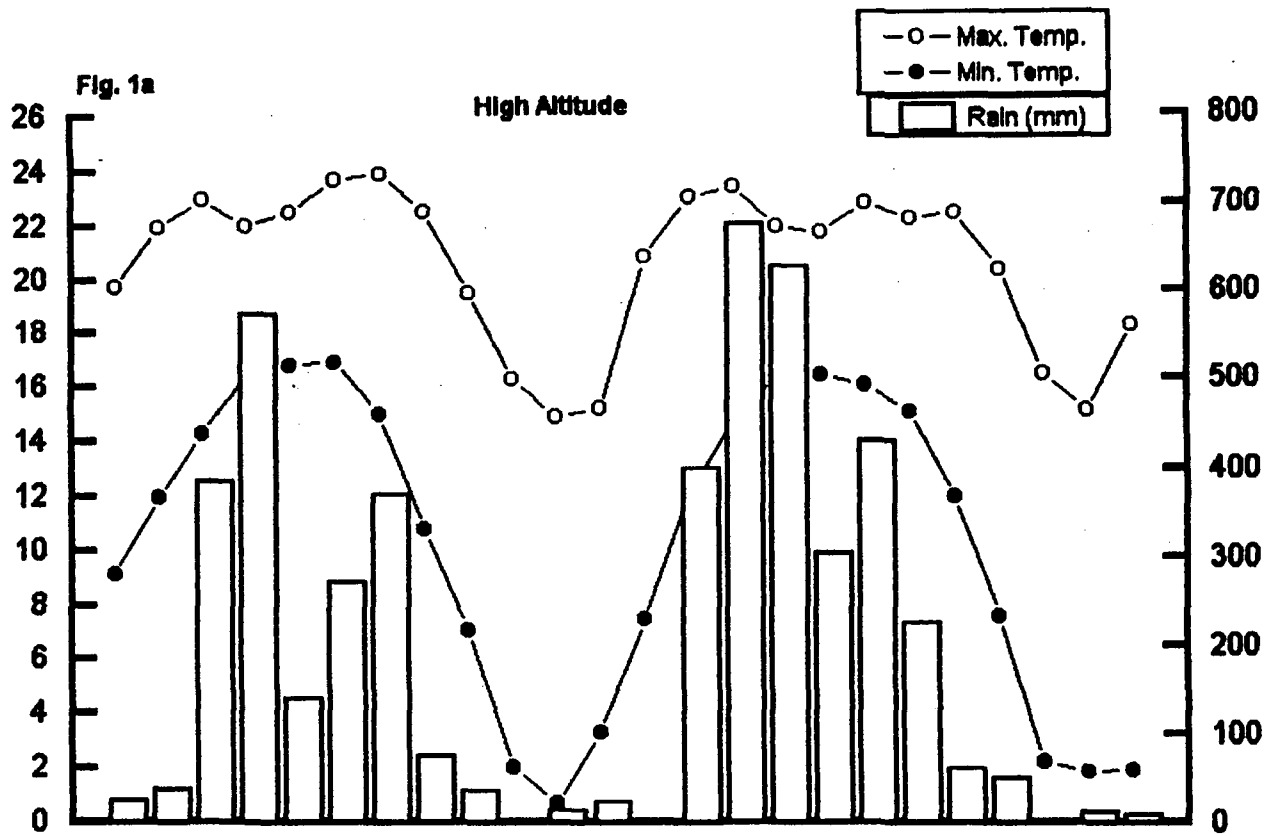
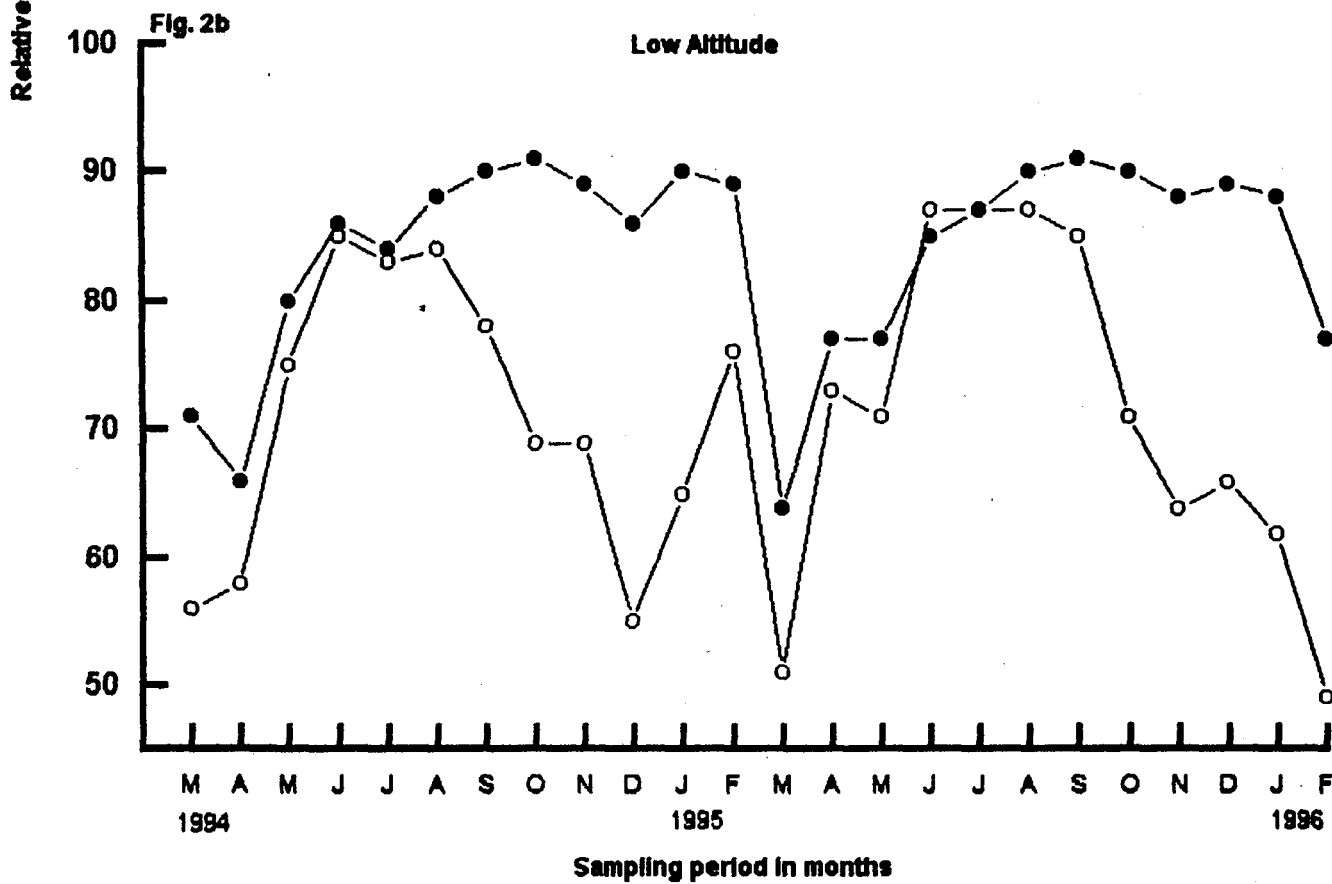
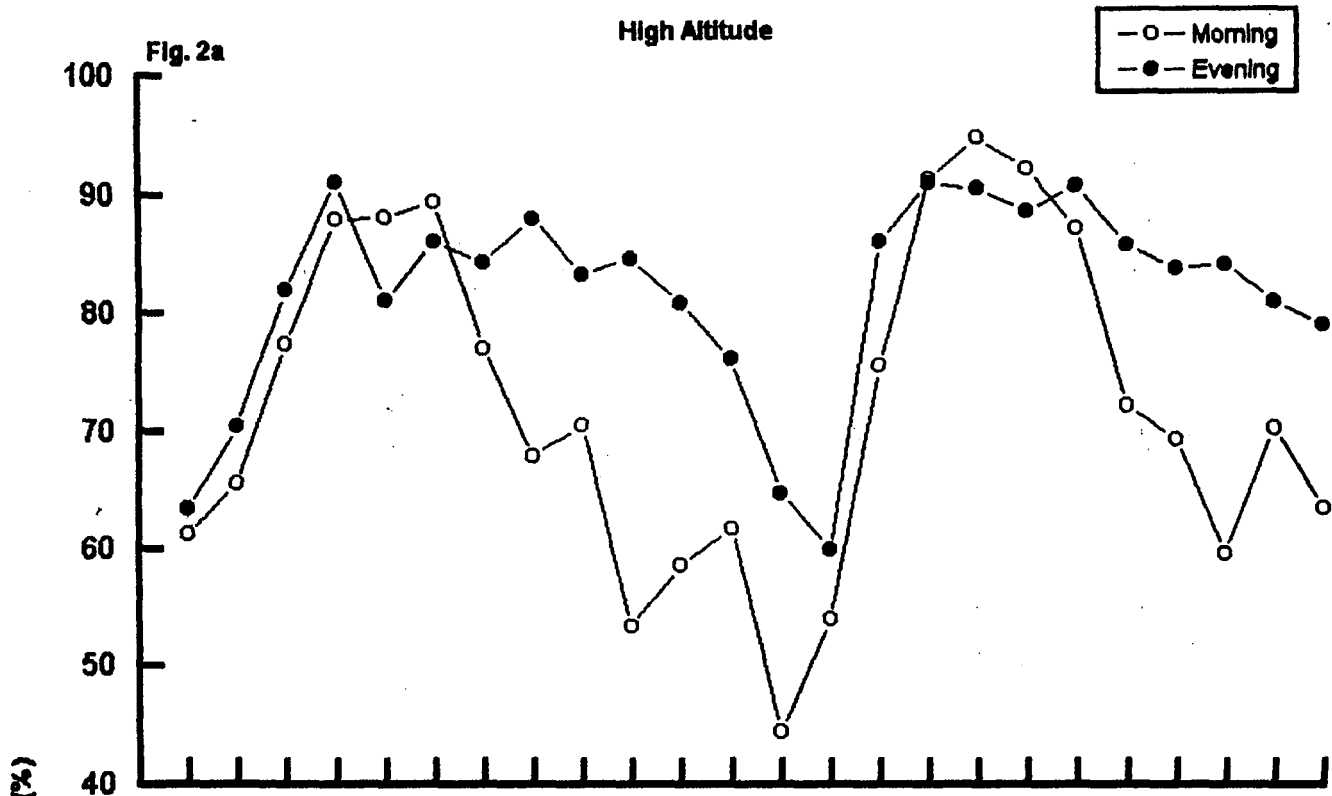


Fig. 2 (a&b). Monthly variation in percentage relative humidity of two pine forest stands. (o-o) =Morning, (e-e) = Evening.



places in the state, that is, Cherrapunjee and Mawsynram have been vying with each other for the distinction of being the rainiest place on earth.

Based on the meteorological conditions the year may be broadly classified into four seasons: spring, summer, autumn and winter. Spring season includes March and April. Spring is characterized by high velocity of wind, less humidity and moderate temperature. Summer or rainy season includes May to September and maximum rainfall occurs during June to July. Autumn season includes October and November and is the shortest season of the year. Winter season includes December to February. This period is characterized by low temperature and less rainfall.

The annual rainfall at high altitude ranged from 0 mm to 676 mm from March 1994 to February 1996 (fig.1a). The annual rainfall at low altitude ranged from 1.2mm to 635.8mm during March 1994 to February 1996 (fig. 1b). The average maximum and minimum temperature at high altitude varied from 14.9°C to 23.9°C and 0.67°C to 16.9°C (fig 1a), while the average maximum and minimum temperature at low altitude varied from 14.1°C to 24.9°C and 5.3°C to 18°C respectively during March 1994 to February 1996 (Fig. b). The percentage relative humidity at high altitude in the morning ranged between 44.39% and 94.68%, while that in the evening ranged between 59.9% and 91.13% (fig 2a). The percentage relative humidity at low altitude in the morning ranged between 49% and 87%, while that in the evening ranged between 64% and 91% (fig. 2b).

Soil

The soils of the study areas have originated from the hard rocks representing gneisses, schists and granite. The texture of the soil at high altitude is sandy while that at low altitude is sandy loam.

Vegetation

Both the forest stands are dominated by *Pinus kesiya* (Royle ex-Gordon). The other species inhabiting the study site at high altitude includes *Alnus nepalensis*, *Myrica esculenta*, *Rubus ellipticus*, *Lantana camara*, *Osbeckia crinata*, *Eupatorium adenophorum*, *Oxalis corniculata*, *Centella asiatica*, *Hypocharis radicata*, *Plantago major*, *Paspalum dilatatum*, *Poa annua* etc. The other species inhabiting the study site at low altitude includes *E. adenophorum*, *C. asiatica*, *L. camara*, *O. corniculata*, *H. radicata*, *P. major* etc.

MATERIALS AND METHODS

Collection of the samples:

Freshly fallen undecomposed, partially decomposed pine litter, surface soil (0-10 cm), earthworms and earthworm casts were collected in sterilized polythene bags at monthly intervals for a period of two years. The following investigations were carried out:-

1. Estimation of the physico-chemical properties of soil and the earthworm casts:

Soil temperature :

Temperature of the soil (0-10cm) was measured with the help of thermometer.

pH and moisture content :

5g of the sample (soil and casts) separately was diluted in 25 ml distilled water and stirred for 15 minutes with a magnetic stirrer. The pH was read in an electric digital pH meter. For determination of moisture content, 10g of the sample (soil and casts) taken separately 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:-

$$\% \text{ Moisture content} = \frac{\text{loss in weight (g)}}{\text{initial weight (g)}} \times 100$$

Three replicates were maintained for each sample.

Organic Carbon :

Walkley and Black's (1934) rapid titration method was followed for the determination of organic carbon of the soil and the casts. To 0.5g of sieved soil (through 0.2mm sieve) and 0.25g of sieved casts (through 0.2mm sieve) taken separately in a 500 ml conical flask, 10 ml of K₂Cr₂O₇ (1N) and 20 ml of conc. H₂SO₄ were added and left for 30 minutes. The mixture was then diluted with 200 ml of distilled water and then 10 ml of Orthophosphoric acid (85%) was added. Finally, it was titrated with ammonium ferrous sulphate (1N) using diphenyl amine as an

indicator. The percentage organic carbon was calculated according to the formula given below:-

$$\% \text{ Organic carbon} = \frac{V1-V2}{W} \times 0.003 \times 100$$

where, V1 = volume of blank titrated.

V2 = volume of ammonium ferrous sulphate in the sample titrated.

W = weight of the sample (g).

Total Nitrogen :

Total Nitrogen in the soil and the casts were estimated by micro-kjeldahl method (Allen, 1974). 1 g of finely ground sample (sieved through 0.2mm) was taken in a micro-kjeldahl flask with one kjeltab. To it 6 ml of conc. H₂SO₄ was added slowly down the neck while the flask was rotated. The digestion flask was heated on a digestion rack. The digestion was stopped after the colour turned green. After digestion the flask was allowed to cool. The digested material was diluted with distilled water and the volume was made upto 50 ml in a volumetric flask and then filtered through whatman No. 1 filter paper. The blank digestion was also prepared only with the mixture (without the sample). Through digestion all the organic nitrogen converts into ammonia that was determined by distillation method.

Distillation was done in a kjeldahl distillation set with 10 ml of the sample solution and 10 ml of 40% of NaOH. The distillate was collected in a beaker containing 5 ml of boric acid indicator [100g boric acid in 10 litres of distilled water + 100 ml bromocresol green (100mg in 100 ml methanol) + 70 ml of methyl red (100mg in 100 ml methanol)]. The distillation was stopped when the pink colour of the boric acid indicator turned greenish. The distillate collected in the beaker was titrated against N/140 N HCl. The titration was stopped when the colour turned to pink. The nitrogen was calculated by using the following formula:-

$$\text{Total N (\%)} = \frac{\text{T- blank} \times \text{solution volume (ml)}}{10^2 \times \text{aliquot (ml)} \times \text{sample weight (g)}}$$

Available phosphorus :

For the determination of available phosphorus Anderson and Ingram's method (1993) was followed. 2 g each of air dried soil and the casts (through 0.2 mm sieve) were taken separately in 20 ml of sodium bicarbonate (0.5 M). The mixture was shaken in a mechanical shaker and filtered through whatman No. 44 filter paper. In cases where muddy precipitation appeared after filtering, a pinch of activated charcoal was added and then filtered again through whatman No. 1 filter paper. Then 5 ml of the aliquot was taken in a 50 ml volumetric flask and to it 10 ml of doubled distilled water was added. About 2 ml of ammonium molybdate solution and 1 ml of stannous chloride were added subsequently. The volume was finally made upto 50 ml with doubled distilled water and the optical density was read at 700 nm within 30 minutes. A calibration curve from the standards was used to determine phosphorus in the aliquot. Percentage available phosphorus was calculated as follows:

$$\text{Available Phosphorus (\%)} = \frac{\text{C (mg)} \times \text{solution volume (ml)}}{10 \times \text{Aliquot (ml)} \times \text{sample weight (g)}}$$

Preparation of ammonium molybdate solution:

25g of $(\text{NH}_4)_6 \text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ was dissolved in 200 ml double distilled water in a beaker. The mixture was warmed slightly and 280 ml of conc. H_2SO_4 was added slowly to about 400 ml of double distilled water in a 1 litre volumetric flask. The mixture was then mixed thoroughly and allowed to cool.

Preparation of stannous chloride:

Stock solution: 10g of stannous chloride was added to 25 ml of conc. HCl. 1 ml of the stock solution was taken and 330 ml of double distilled water was added to it.

2. Isolation of fungi from soil , casts, earthworm gut and litter

Isolation of fungi from soil, casts, earthworm gut and litter (undecomposed and partially decomposed) was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. A small amount (0.01g) of soil and the casts was taken separately from both the composite samples with the help of sterile nichrome spatula having a flattened tip and then inoculated into plates containing approximately 10 -15 ml of rose bengal agar medium . For the isolation of fungi from the earthworm gut, the earthworm species *Drawida papillifer papillifer* Steph. which appeared to be dominant was chosen for this study. The earthworms were brought to the laboratory and washed thoroughly with sterilized distilled water and 70% alcohol and each worm was cut into three parts— anterior, middle and posterior using sterilized blade. The gut content of the different regions was inoculated in sterilized Petri-dishes containing the medium. For the isolation of fungi from the pine litter (undecomposed and partially decomposed), the litter was first cut into 10mm wide strips using sterilized blades and then inoculated into plates containing the same medium. Three replicates were maintained in each case. The plates were then incubated upside down at a temperature of $25\pm 1^{\circ}\text{C}$ for 5-6 days in a BOD incubator. The number of fungal colonies was counted. The isolation was carried out in a Laminar Flow Chamber. The pure cultures of some dominant fungi were maintained in culture tubes containing agar slants of Czapek-Dox agar medium (Raper and Thom, 1949) and preserved in the freeze at 4°C . The identification of fungi was done with the help of manuals of Gilman (1965), Subramanian (1971) Barnett and Hunter (1972), Domsch (1980) and with the help of the International Mycological Institute, Surrey, U.K.

Composition of Media used for the isolation of Fungi:

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

Agar : 20.0g

KH ₂ PO ₄	: 1.0g
MgSO ₄	: 0.5g
Peptone	: 5.0g
Dextrose	: 10.0g
Rose Bengal (%)	: 3.3 ml
Distilled water	: 1000.0 ml
Streptomycin	: 30 mg

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

Agar	: 15.0g
NaNO ₃	: 3.0g
K ₂ HPO ₄	: 1.0g
MgSO ₄ 7H ₂ O	: 0.5g
KCl	: 0.5g
FeSO ₄	: 10.0 mg
Sucrose	: 30.0g
Distilled water	: 1000.0 ml

Determination of percentage relative abundance:

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

$$\% \text{ Relative Abundance} = \frac{\text{Total number of colonies of the particular species}}{\text{Total number of colonies of all the species}} \times 100$$

pH and moisture content of pine litter :

5g of litter (undecomposed and partially decomposed) taken separately was diluted in 25 ml distilled water and stirred for 15 minutes with a magnetic stirrer. The pH was read in an electric digital pH meter. For determination of moisture content, 10g of the litter was dried in a

hot air oven at 60°C for 24 hours and the weight was taken. The percentage moisture content was calculated as follows:-

$$\% \text{ Moisture content} = \frac{\text{Loss in dry weight (g)}}{\text{Initial sample weight}} \times 100$$

Three replicates were maintained for each sample.

3. Estimation of Enzyme activity :

Dehydrogenase activity

Dehydrogenase activity of soil and the casts was determined by 2, 3, 5- triphenyl tetrazolium chloride (TTC) reduction technique modified by Casida *et. al.* (1977). To 10g of soil and the casts taken separately in sterile stoppered test tube, 0.1g of CaCO₃ and 1 ml of 1.5 % (w/v) 2, 3, 5- triphenyl tetrazolium chloride were added. To each of these tubes, 3 ml of distilled water was added. Three replicates were maintained in each case. The quantity of liquid was found to be enough to saturate the sample and to form a liquid layer on the sample. This ensures adequate anaerobiosis for TTC reduction. The content of each tube was mixed thoroughly and then incubated at 37°C for 24h. The extraction of triphenyl tetrazolium formazan produced was carried out with methanol. The sample from each tube was transferred to whatman No. 1 by shaking with methanol. Similarly, a blank (without sample) was also prepared. The optical density, the orange colour of the filtrate was determined at 485nm in a spectrophotometer. Concentrations of the formazan in the extract were calculated by comparing with a standard curve of triphenyl formazan (TPF) in methanol and expressed as mg Formazan g⁻¹ dry sample.

Urease Activity

Urease activity was measured by the modified McGarity and Myers' (1967) method. To 10g each of soil and the casts, 1 ml of toluene was added and left for 15 minutes for penetration

of toluene into the sample. 10 ml of buffer solution (pH 7.0) and 5 ml of urea (10%) were added and incubated at 37°C for 3h. Then the volume was made upto 50 ml with distilled water, shaken and filtered through whatman No. 1. The filtrate varied from colourless to brown depending on the amount of organic matter present in the sample.

Ammonia released as a result of urease activity was determined by indophenol blue method. 1 ml of the filtrate was placed in a 50 ml volumetric flask and made upto 10 ml with distilled water. 5 ml of freshly prepared phenolate solution and 3 ml of sodium hypochlorite solution were added. The mixture was thoroughly mixed and after 20 minutes the volume was made upto 50 ml by adding distilled water. Optical density was read at 630nm in a spectrophotometer. Three replicates were maintained in each case. The amount of ammonia-N formed was calculated by reference to a calibration curve and expressed as mg per $\text{NH}_4\text{-N g}^{-1}$ dry sample.

Preparation of phenolate solution :

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

Phenol solution :

62.5g 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

27g of sodium hydroxide was dissolved in 100 ml of distilled water. Both the solutions were kept in a freeze.

4. Estimation of earthworm population and identification of earthworms

Wilcke's (1955) method of handsorting was adopted for the estimation of earthworm population from the two study sites for a period of one year. A quadrat of 50x50 sq. cm. and

a depth of 30 cm was taken and the number of earthworms present was counted and noted down. Five replicates were maintained in each case. For the identification of the earthworms in both the study sites, the earthworms were collected in the every sampling period from the beginning of the present investigation and were then sent to Dr. J.M. Julka in Solan, Himachal Pradesh for identification.

5. Role of earthworms in litter decomposition :

For determining the role of earthworms in litter decomposition under laboratory condition the method adopted by Haimi and Huhta (1990) was followed. The experiment was conducted in sixteen plastic containers for four months and the environment similar to the coniferous forest floor was created in these containers by including mineral soil, humus and litter horizons. Since *Drawida papillifer papillifer* appeared to be the dominant species, therefore, this particular species has been selected for this study.

2 kg of air dried, sieved and sterilized mineral soil (about 3 cm layer) was spread on the bottom of each container (29 cm x 23 cm diameter) covered with a nylon net (3mm mesh) and watered with 450 ml of distilled water. Then 300g of humus (sieved and sterilized) was added and covered with a similar net. Finally 10g of freshly fallen pine litter (oven dried) was spread on the upper net. Ten adult specimens of *Drawida papillifer papillifer* were introduced into eight of the containers. The vessels were then incubated and sampling was done after every 30 days interval. The vessels were irrigated with 450 ml after every 30 days interval and the water that leached out through the soil profile was used for determining pH and nutrient contents (total nitrogen and PO_4^{3-} -P). Total nitrogen from the leachates was estimated by Sulphuric acid- hydrogen peroxide procedure (Allen, 1974). The water sample was first reduced to small volume (20 ml) after which 0.9 ml of the digestion mixture was added. It was then boiled to white fumes with care being taken to avoid dryness. The entire digest was retained for

distillation (details given on page 36). The nitrogen was calculated by using the following formula:-

$$\text{Total N (mg l}^{-1}\text{)} = \frac{\text{T- blank (ml)} \times 10^2}{\text{aliquot (ml)}}$$

PO_4^{3-} - P from the leachates was determined by Molybdenum Blue Method (Anderson and Ingram, 1993). 5 ml of leachate sample was taken directly without extracting in 0.5 M sodium bicarbonate solution. The rest of the procedure was as given on page 37. Phosphate-phosphorus was calculated as follows:

$$\text{PO}_4^{3-}\text{- P (mg l}^{-1}\text{)} = \frac{\text{C (mg)} \times 10^3}{\text{aliquot (ml)}}$$

The amount of casts obtained from each sampling period was also collected. The mineral soil, and humus were collected from the treated (with earthworms) and the untreated (without earthworms) containers. The mineral soil, humus and the casts were then used for determining the organic carbon (Walkley and Black, 1934), total nitrogen (Allen, 1974) and available phosphorus (Anderson and Ingram, 1993).

The pine litter was collected at every 30 days interval, washed thoroughly with distilled water to remove soil particles and dried in a hot air oven at 60°C for 48 hours. The final dry weight of the litter was taken and the percentage weight was calculated on the basis of oven dry weight of the sample. The decay constant (K) was calculated using Olson's (1963) decay model (details given on page 46). For the analysis of total cellulose, hemicellulose, lignin, amino acid and sugar of the litter, the methods described by Peach and Tracey (1955) was followed (details given on page 47).

Carbon dioxide evolution was determined by rapid titration method (Macfadyen, 1970) to assess the role of earthworms in decomposition activity. 1 kg of soil from each sampling period was taken in a rectangular jar (12.5 cm x 10.3 cm x 20.8 cm) and 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 sealed nicely with grease and thus making air tight to prevent O₂ exchange with the atmosphere. Control set containing sterilized sand instead of soil was also maintained. After 24 hours of incubation the amount of CO₂ fixed by the KOH solution was measured by back titration with 0.1 (N) HCl solution using phenolphthalein as an indicator.

Evolution of carbon dioxide was calculated on mg per kg fresh soil per 24 hour basis.

$$\text{CO}_2 \text{ (mg)} = (\text{B}-\text{V}) \text{ N} \times \text{E}$$

where, B= volume of acid used to titrate for blank

V= volume of acid used to titrate the alkali in soil filled jar

N= Normality of acid

E= Equivalent of CO₂

6 (a) Study on the role of earthworms in fungal dispersal :

Screening of fungi was done from earthworm furrows and the soil adjacent to the furrows. Soil from the earthworm furrows and adjacent to furrows were collected from the pine forest stand in separate sterilized polythene bags with sterile spatula and brought to the laboratory for isolation of fungi. Moisture content and pH were determined by the methods described earlier. The fungi were isolated from the soil samples by soil plate method (Warcup, 1950) using rose bengal agar medium (Martin, 1950). The identification of fungi was done with the help of manuals of Gilman (1965), Subramaniam (1971), Barnett and Hunter (1972) and Domsch (1980).

6 (b) Feeding habit of earthworm in relation to certain fungi:

Feeding habit of the earthworm species *Drawida papillifer papillifer* Steph. in relation to certain fungi was studied in conical flasks (250 ml) containing 150g of sterilized soil and 1g of sterilized partially decomposed pine litter. The test fungi used for this experiment were

Penicillium chrysogenum, *Pythium intermedium*, *Mucor hiemalis* and *Trichoderma koningii*. The pure cultures of the test fungi were maintained on Czapek-dox agar medium (Raper and Thom, 1949).

16 blocks of 5mm diameter were cut out with the help of sterilized cork borer from the periphery of 7 day old cultures of the test fungi and were transferred to the flasks containing sterilized soil and litter. In case of mixed inoculum, 4 blocks of each fungal species were introduced into the flasks. A control set was also maintained where no test fungi were added. Three replicates were maintained for each set. One adult species of *Drawida papillifer papillifer* was added to each flask. The harvesting was done at 7 days interval for 56 days. After every 7 days interval, the weight of the earthworm and its length were recorded.

7 (i) Efficacy of soil fungi in litter decomposition:

(a) Field condition:

Litter bag technique (Bocock *et. al.*, 1960) was applied to study the rate of pine litter decomposition in the field. Freshly fallen pine needles were collected from the pine forest stands, cleaned thoroughly and oven dried at 60°C till the constant weight was recorded. Nylon bags were prepared into 20 cm size having a mesh size of 1.0 mm. Each bag was filled with 10g of oven dried litter and was closed by folding over the open and stitched. The bags containing the pine litter were placed randomly in the field. The litter in the bags was then allowed to decompose. The sampling was done at 30 days interval for 360 days (one year). On each sampling date five bags were collected aseptically in sterilized polythene bags and were brought to the laboratory. Of the five litter bags, one was used for the estimation of fungi and two each for pH and moisture content (details given on page 40). The loss in weight of the litter was measured by drying in a hot air oven at 60°C until a constant weight was recorded. The decomposed litters were kept for analysis of cellulose, hemicellulose, lignin, amino acid and

sugar contents. The decay constant (K) was calculated using Olson's (1963) decay model, using the following formula:

$$\frac{X_t}{X_0} = e^{-kt}, \text{ where, } X_0 = \text{Initial weight}$$

$$X_t = \text{weight after time 't'}$$

$$K = \text{the annual exponential (base e) decay coefficient.}$$

For the isolation of fungi from the decomposing litter bags dilution plate technique (Waksman, 1922) was followed. 1g litter was cut into 1 cm. small pieces using sterilized scissors and transferred into a 250 ml 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 to homogenize 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. Enumeration of fungal population was done using rose bengal agar medium (Martin, 1950).

0.5 ml of the litter suspension was transferred aseptically from 1:1000 dilution into each of the sterilized petridishes containing 15 ml of the cooled solidified rose bengal agar medium. The petridishes were rotated gently with hand so that the inoculum was dispersed uniformly over the surface of the agar medium. Three replicates were maintained in each case. The plates were then incubated at a temperature of $25 \pm 1^\circ\text{C}$ for 5-6 days in a BOD incubator. The number of fungal colonies was counted. The isolation was carried out in a Laminar Flow Chamber. The identification of fungi was done with the help of manuals of Gilman (1965), Subramaniam (1971) Barnett and Hunter (1972), Domsch (1980) and with the help of the International Mycological Institute, Surrey, U.K.

(b) Laboratory condition:

Four dominant fungi (*Penicillium chrysogenum*, *Pythium intermedium*, *Mucor hiemalis* and *Trichoderma koningii*) were selected to assess their efficacy in litter decomposition. 1g of oven dried undecomposed pine litter was introduced into 250 ml conical flask plugged properly with cotton. The litter was moistened with 15 ml distilled water and sterilized by autoclaving at 15lbs and 121°C for 15 minutes. Sixteen blocks of 5mm diameter were cut out with the help of sterilized cork borer from the periphery of 7 day old cultures of the test fungi and were transferred to the flasks containing sterilized pine litter. In case of mixed inoculum, 4 blocks of each fungus were introduced into the flasks. In the control sets same numbers of agar blocks (without fungi) were given. The flasks were then incubated at 25±1°C. Three replicates were maintained for each set of fungi and the harvesting was done at 15 days time interval for 105 days. Whenever necessary, required amount of sterilized water was poured to maintain the moisture level. After harvesting, the litter was taken out from the flask and the fungal mycelium was removed by brushing and rubbing the litter gently. The litter was then dried in a hot air oven at 60°C until 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 analysis of cellulose, hemicellulose, lignin, amino acid and sugar contents.

7 (ii). Chemical analysis of the decomposing pine litters :

Cellulose, hemicellulose, lignin, amino acid and sugar content of pine litter were estimated by methods described by Peach and Tracey (1955). Decomposing pine litter collected on different sampling periods were washed with distilled water to remove soil particles and dried in a hot air oven at 60°C for 48 hours. The samples were cooled to room temperature over anhydrous calcium chloride in a dessicator and were ground to fine powder by electric grinder and processed for cellulose, hemicellulose and lignin.

0.25g of the powder was treated with 10 ml of 25% aqueous KOH (w/v). In each case, the mixture was centrifuged at 3000 rpm. The decant obtained in each case was used for the detection of hemicellulose. The residue left at the end of the digestion was washed several times in distilled water till the trace of KOH was removed. It was dried in a hot air oven at 105°C for 24 hours, cooled to room temperature in a dessicator and weighed. The amount thus obtained was designated as total cellulose.

The decant obtained was neutralized with equal amount of glacial acetic acid and ethanol. The precipitate was filtered, washed, dried and weighed as above for determination of total hemicellulose.

For the estimation of lignin, 0.25g of oven dried litter powder was taken in a test tube with 10 ml of 72% H₂SO₄ and kept in deep freeze for 24 hours. It was then centrifuged and the residue was calculated and washed thoroughly to remove the traces of H₂SO₄ present. It was then oven dried and weighed. The amount so obtained gave the total lignin content in the litter.

Three replicates were maintained in each case. The cellulose, hemicellulose and lignin content were estimated on initial dry weight of the litters.

For the estimation of total sugars and amino acids of the decomposing pine litter 100mg of the powdered sample was taken in a test tube and treated with 80% ethanol. Occasionally, when any colour develop, it was treated with activated charcoal and centrifuged at 6000rpm. This solution was filtered through whatman No.1 filter paper. The clear filtrate was boiled in a hot water bath to remove traces of alcohol. To it, distilled water was added to make the volume to 5 ml.

To 3 ml of the above solution 6 ml of anthrone reagent (0.4% in H₂SO₄) was added slowly by the side of the test tube and shaken gently. The solution was then kept for 3 minutes in the boiling water bath and cooled at room temperature. The optical density was read in a

spectrophotometer at 610nm. The standard curve from transmittance of varying concentration of glucose solution were treated exactly as the samples. From the standard curve the values of the total sugars were expressed as mg/ g dry weight of the sample.

To the rest of the 2 ml , 2.5 ml of ninhydrin citrate buffer solution (pH 5.0) was added. The mixture was kept on the boiling water bath for 30 minutes and then cooled at room temperature. A light purple colour developed in the solution . The optical density was read at 540 nm in the spectrophotometer. The total amino acid was calculated from the standard curve obtained from transmittance of different concentration of leucine solution treated as the samples. It was expressed as mg /g dry weight of the samples.

RESULTS:

1. Physico-chemical properties of soil and earthworm casts

Soil Temperature

Generally soil at low altitude showed higher temperature than soil at high altitude (Fig. 3). During winter months (October to January) temperature decreased and started to increase again after February at both the study sites.

Moisture content

Moisture content of the soil at high altitude varied from 18% to 43.33% while that of the soil at low altitude varied from 13.3% to 40% (Fig. 4a & b). There was not much variation in the moisture content of the soil at both the study sites. Low soil moisture content was recorded during the winter months which increased with the onset of rains. However, when comparison was made between the moisture content of the earthworm casts with that of the surrounding soil, it was observed that the earthworm casts generally harboured higher moisture content except in few instances at both the study sites (Figs. 4a & b). Moisture content of the casts at high altitude varied between 6.67% to 56.67% while that at low altitude varied between 6.67% to 43.33%.

pH :

The soil was found to be more acidic at high altitude than at low altitude. pH of soil at high altitude varied from 4.74 to 5.90, while that at low altitude varied from 4.78 to 6.28 (Figs. 5a & b). However, when comparison was made between pH of casts and that of the surrounding soil at both the study sites, it was found that the casts had high pH values as compared to that of the surrounding soil (Figs. 5a & b).

Fig. 3 Monthly variation in soil temperature of two pine forest stands. HA (High Altitude), LA (Low Altitude).

Fig. 3

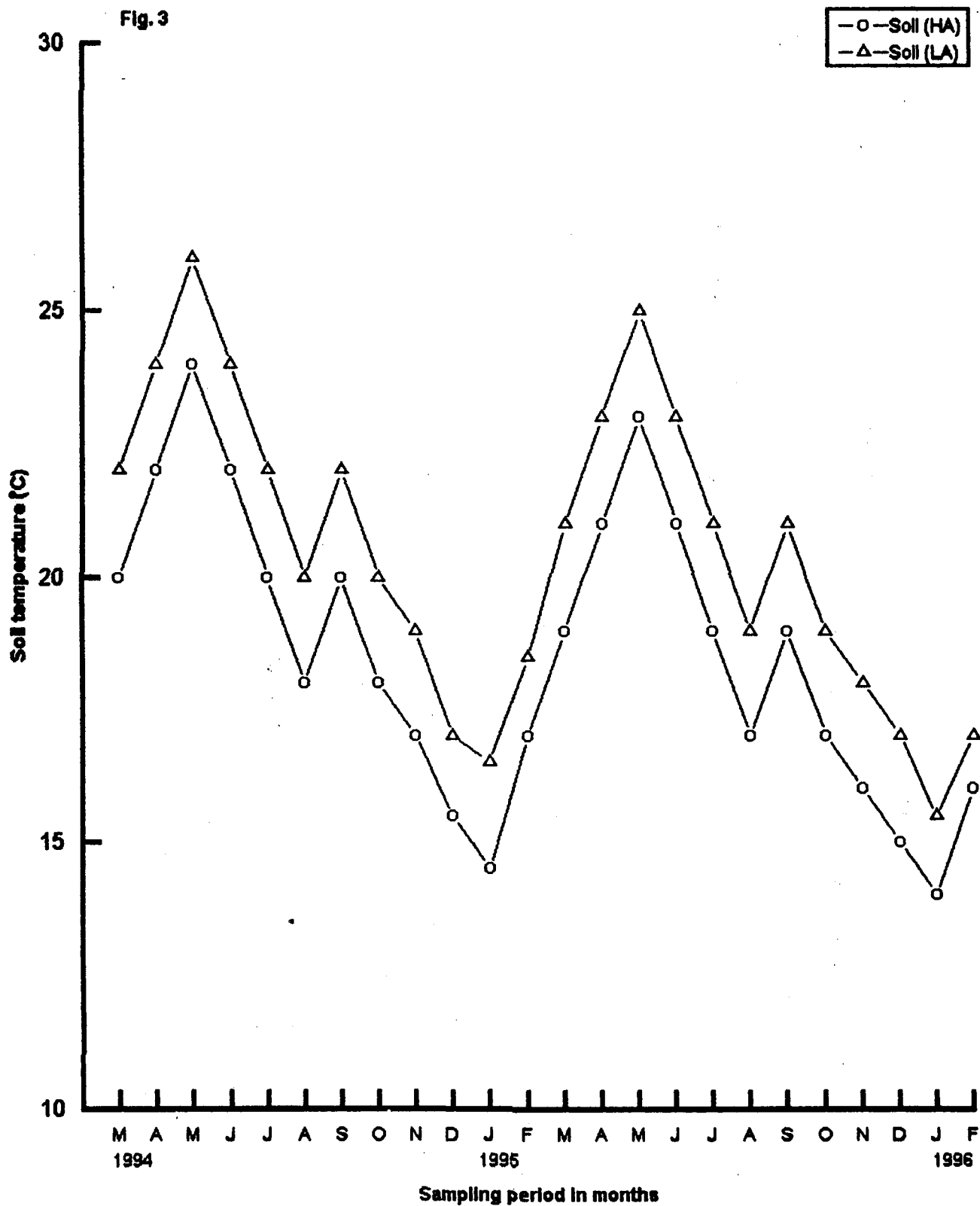


Fig. 4 (a&b) Monthly variation in moisture content of soil and earthworm casts of two pine forest stands.

Fig. 4a

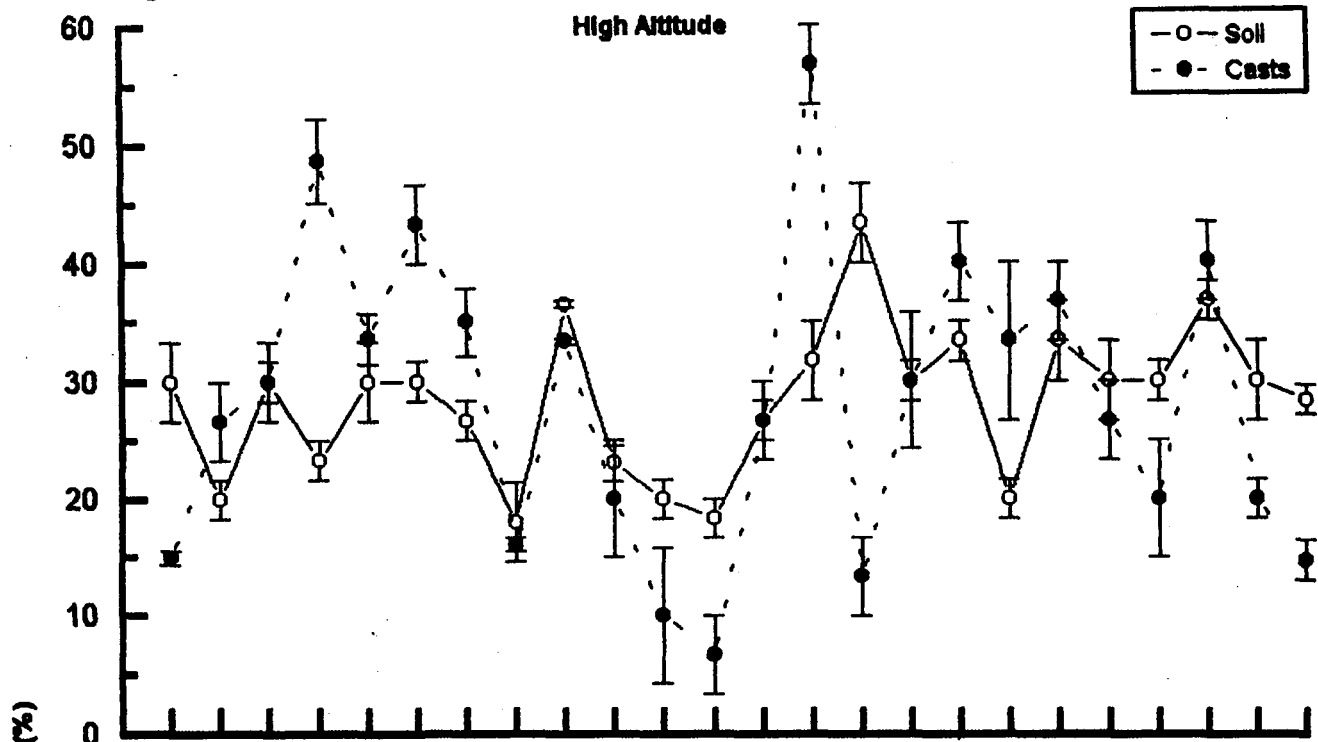


Fig. 4b

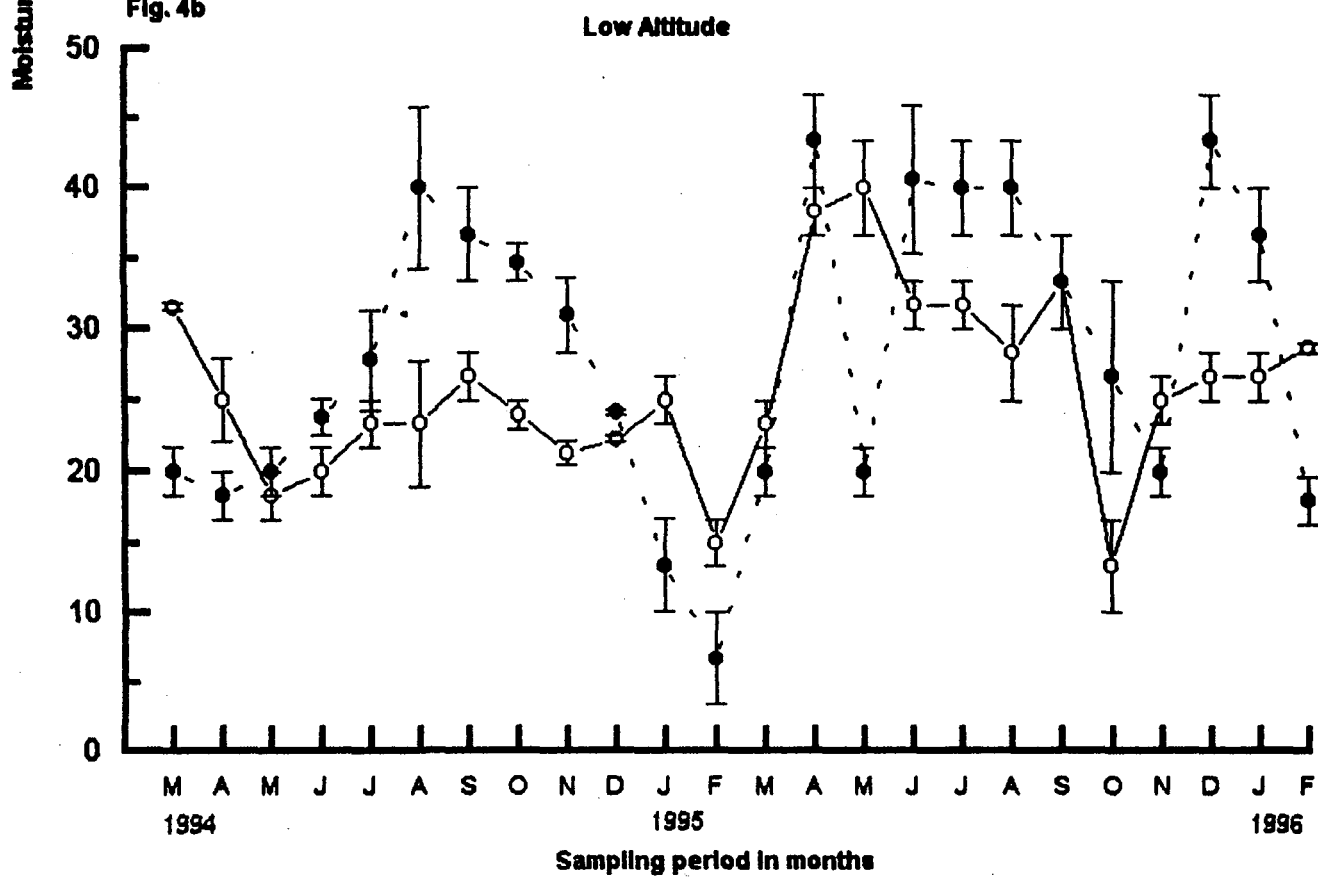
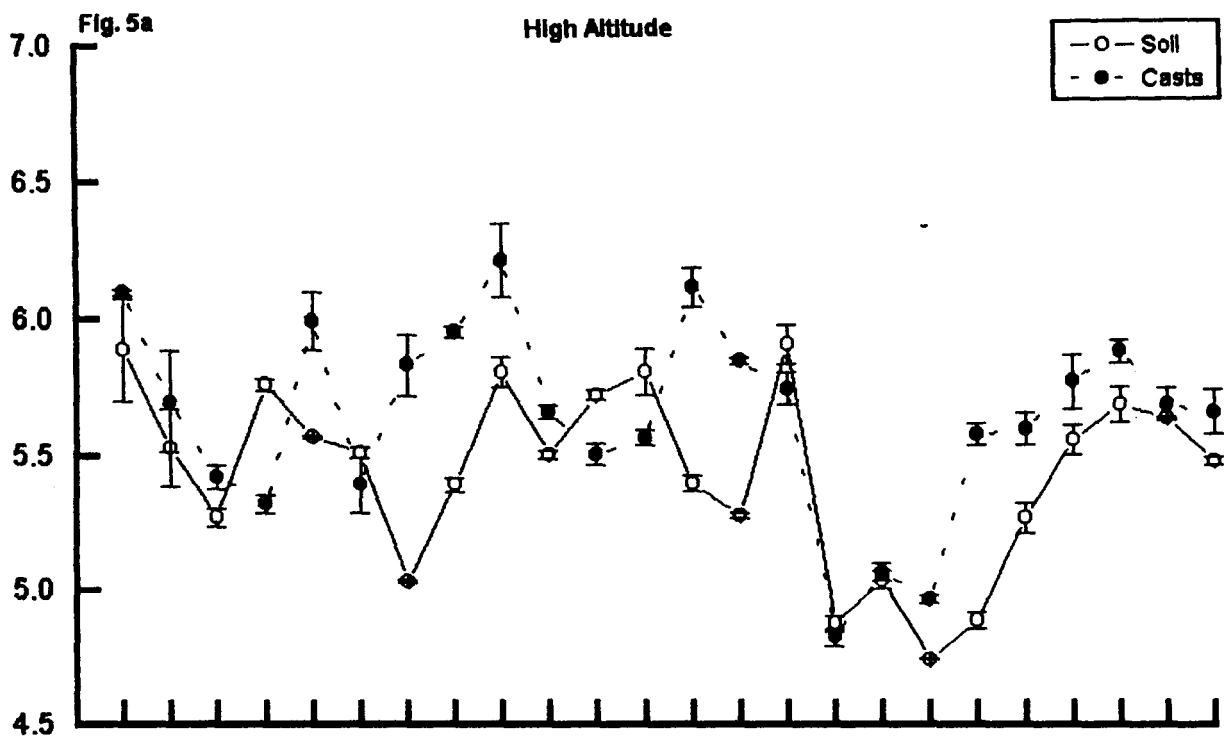


Fig. 5 (a&b) Monthly variation in pH of soil and earthworm casts of two pine forest stands.



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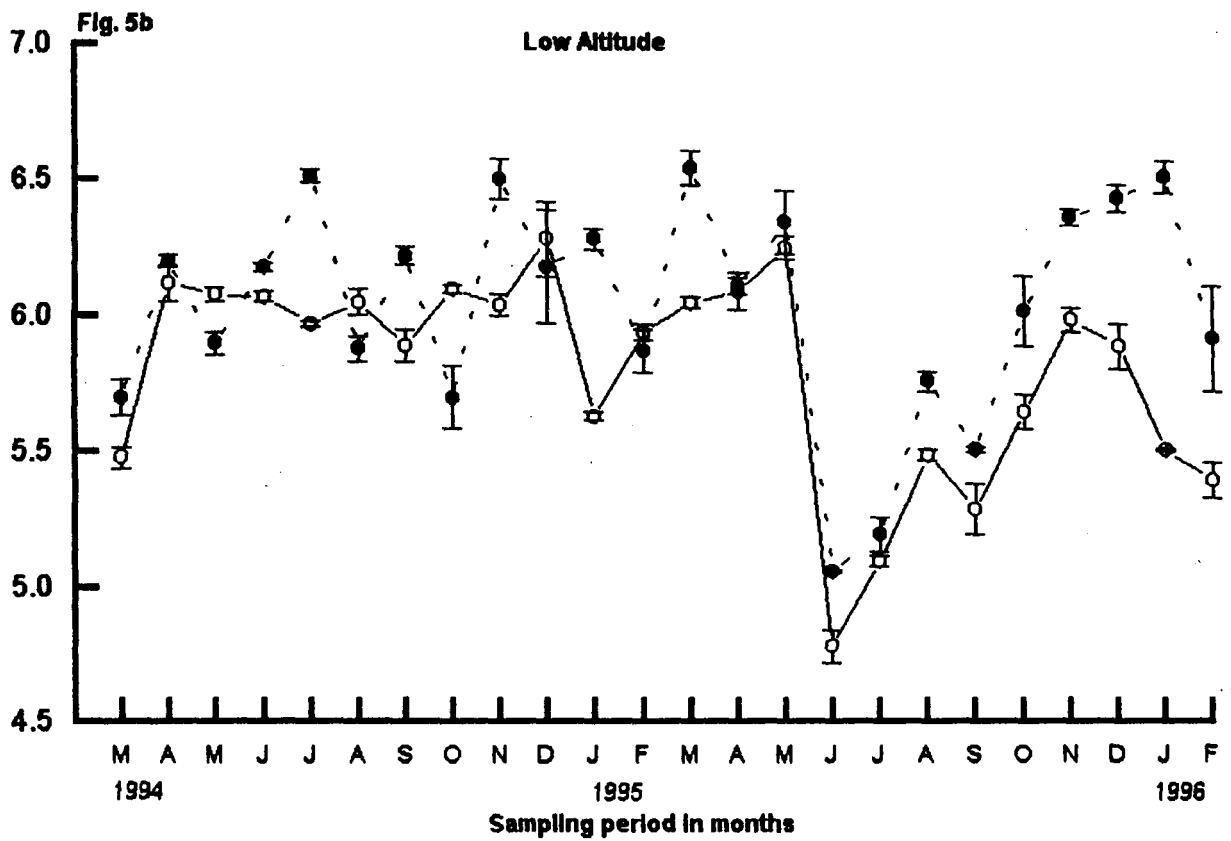


Fig. 6 (a&b) Monthly variation in organic carbon of soil and earthworm casts of two pine forest stands.

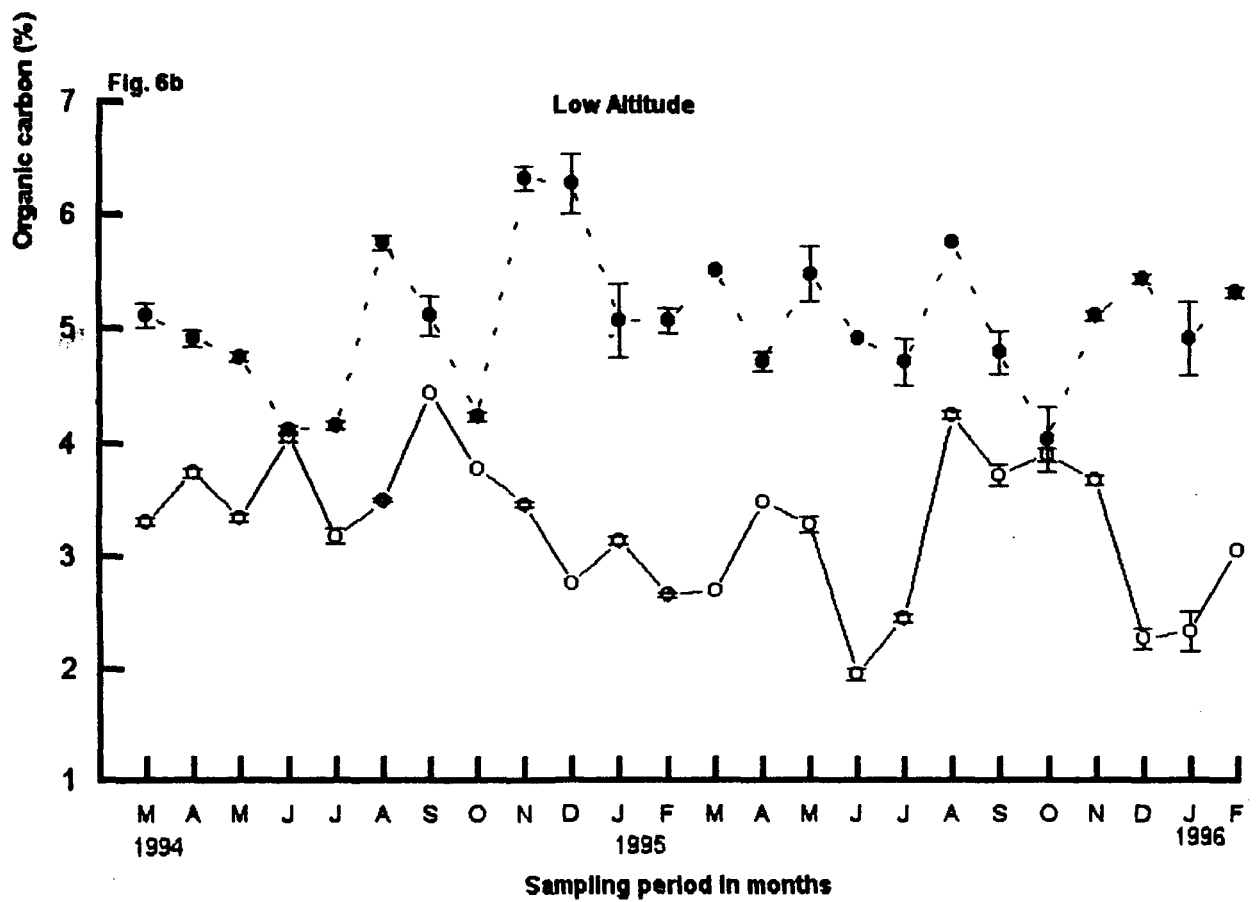
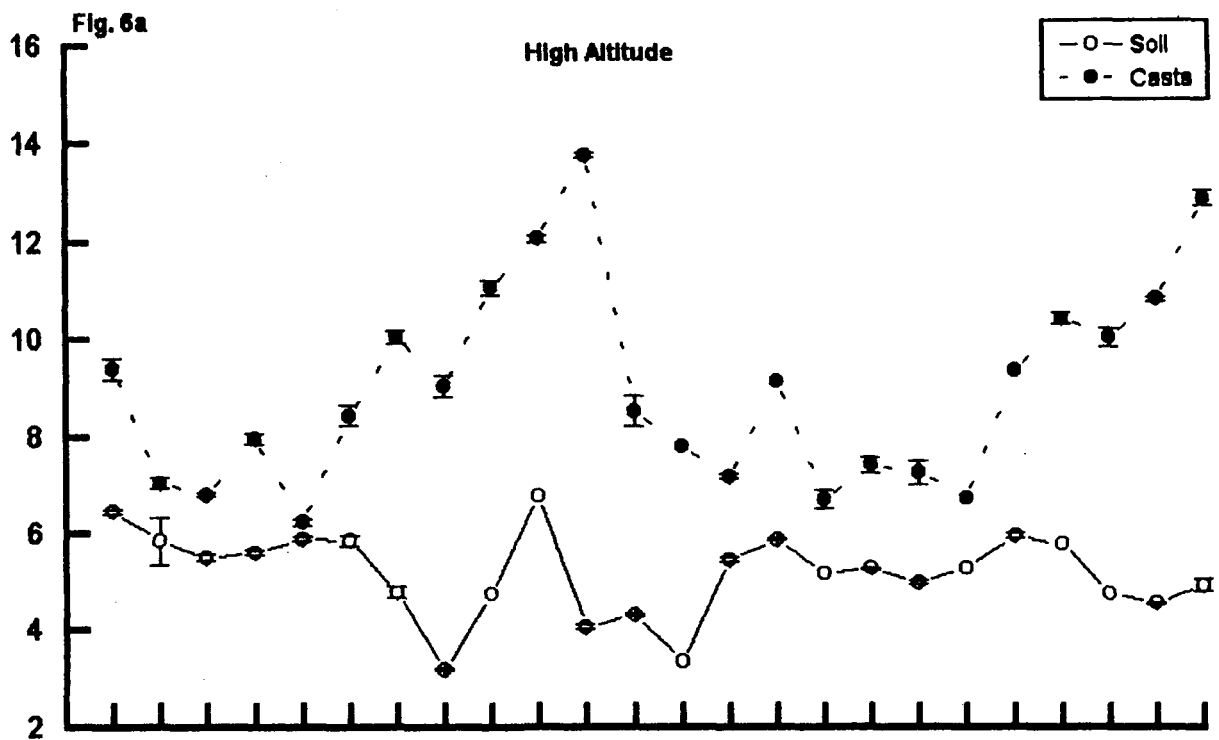


Fig. 7(a&b) Monthly variation in total nitrogen content of soil and earthworm casts of two pine forest stands.

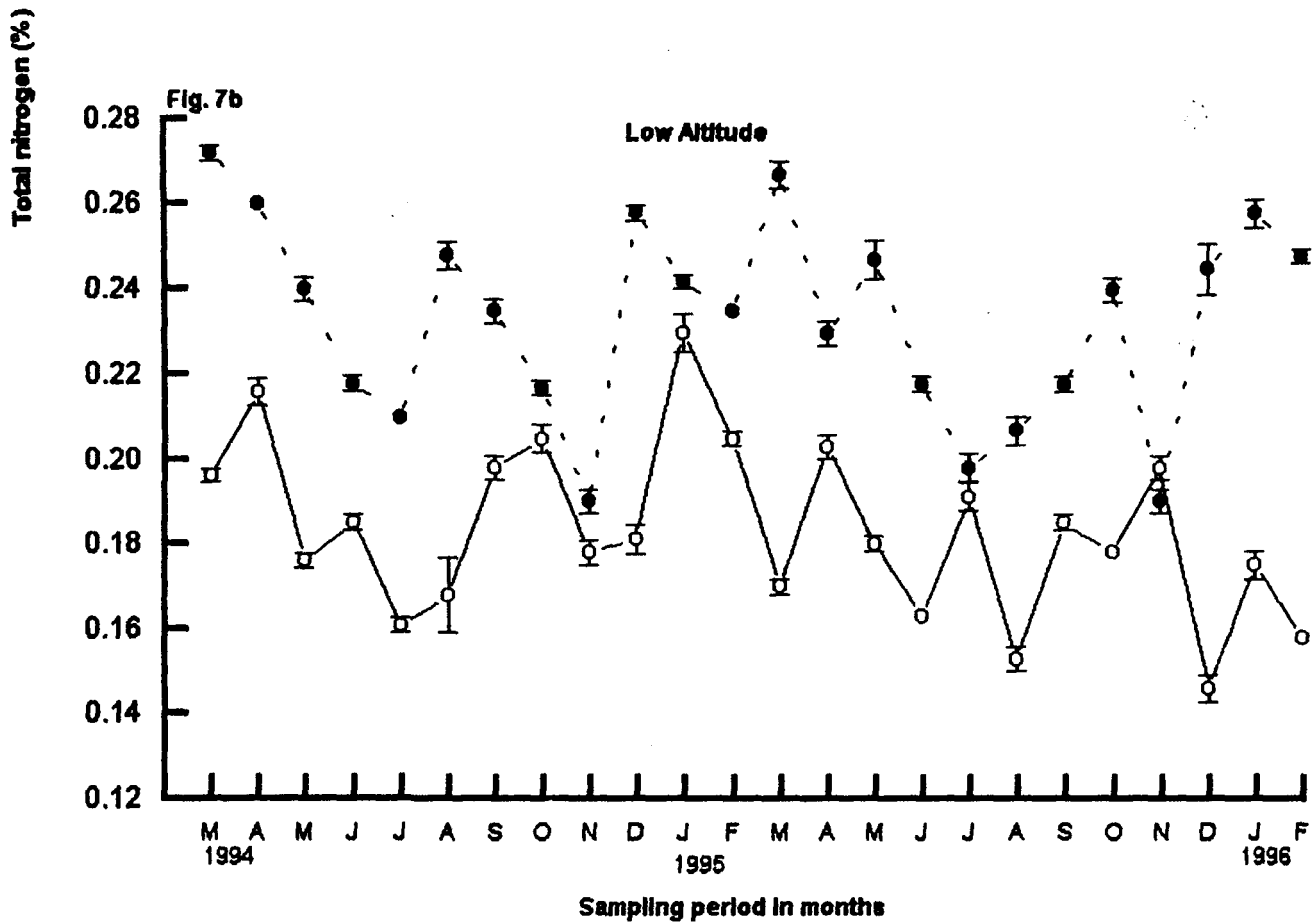
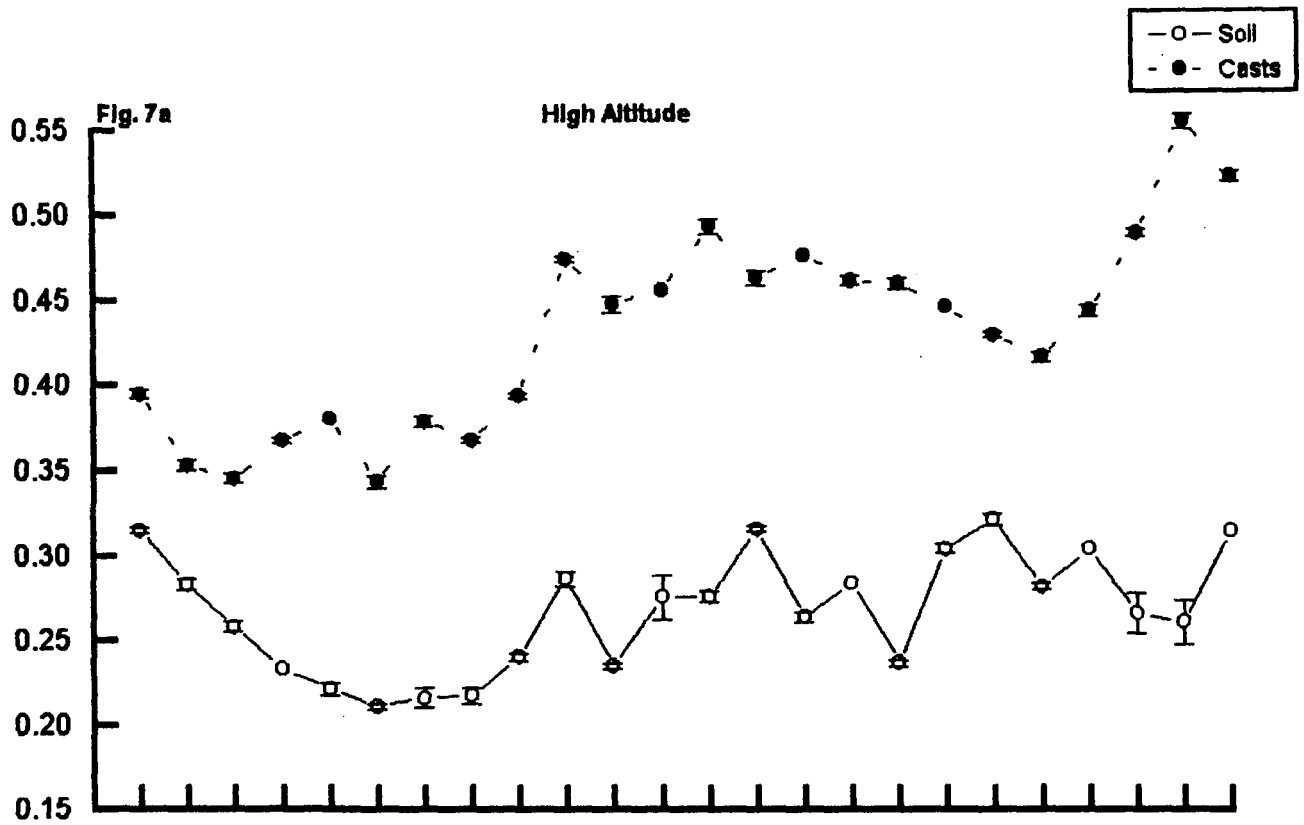
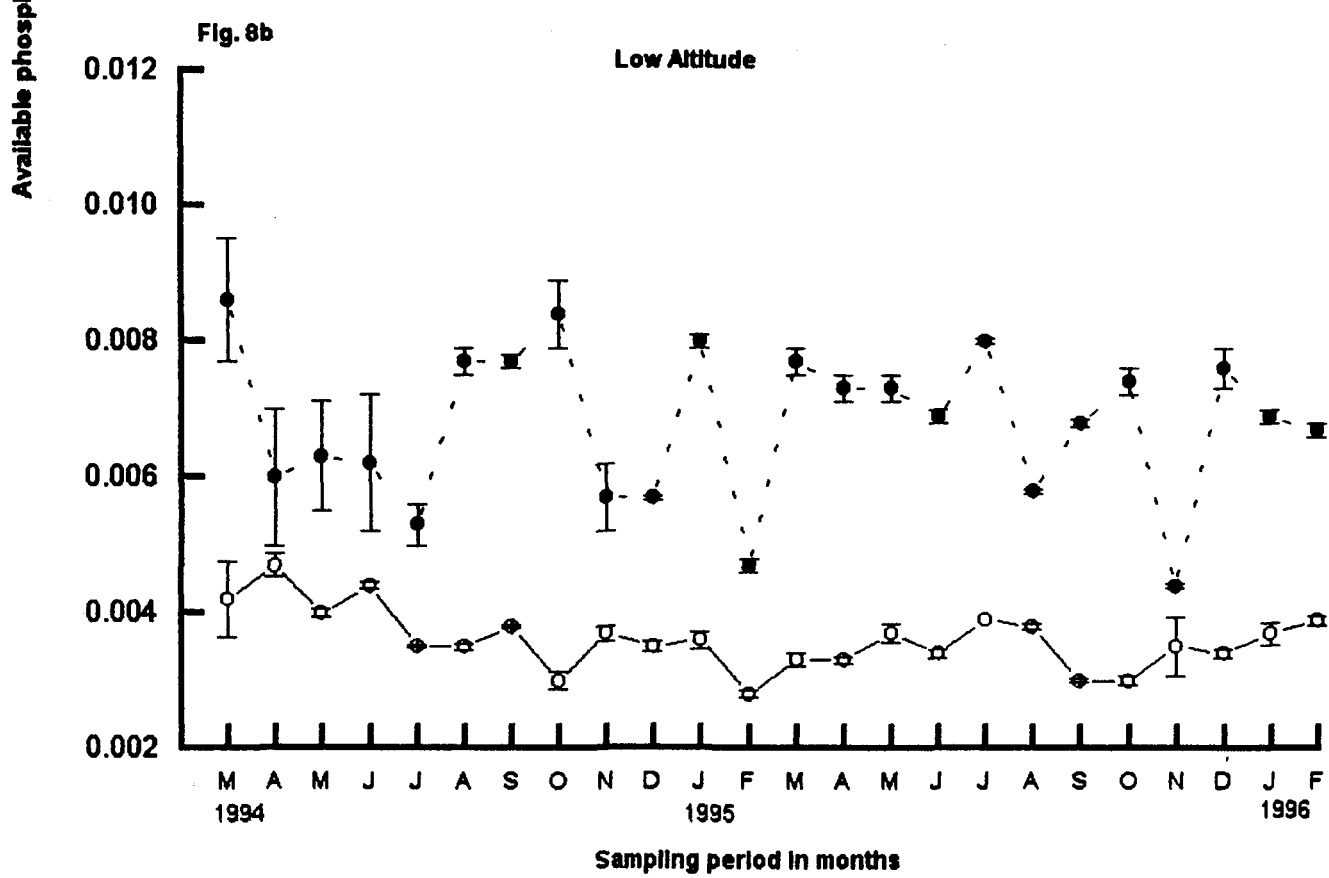
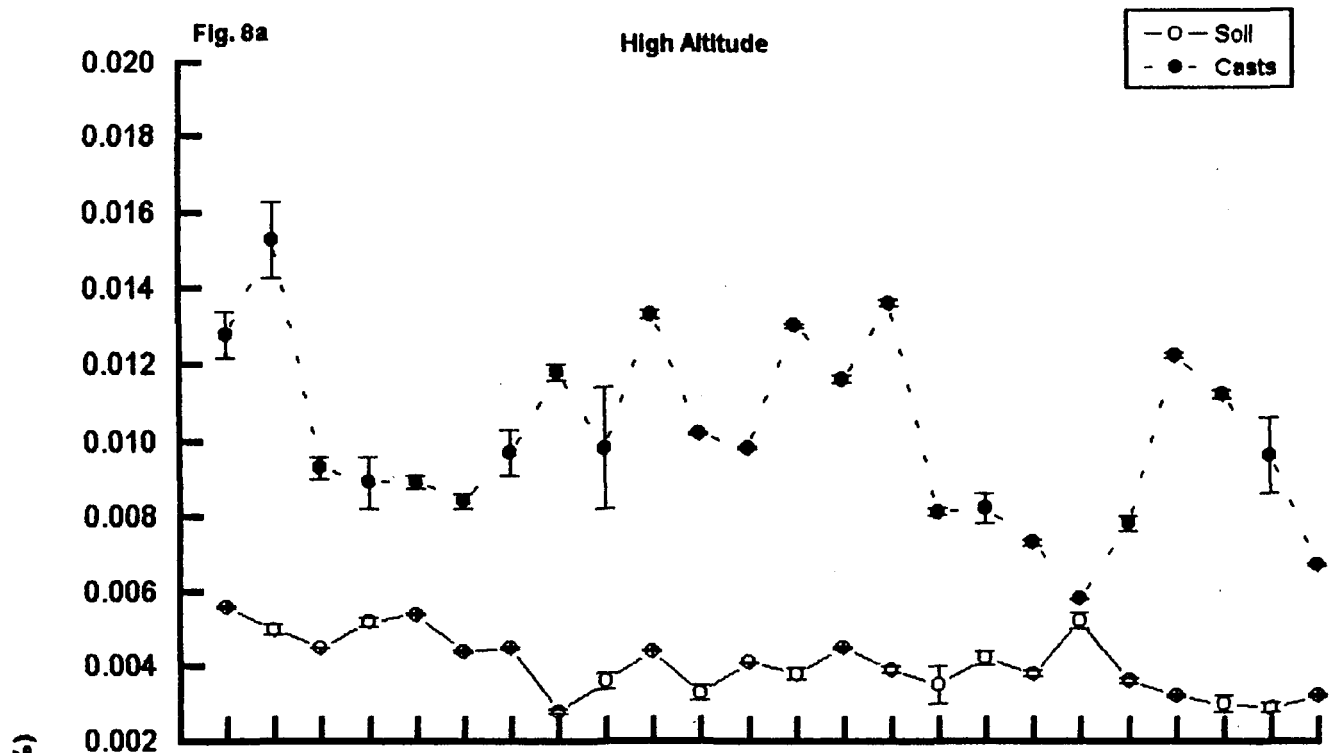


Fig. 8 (a&b) Monthly variation in available phosphorus of soil and earthworm casts of two pine forest stands.



Organic Carbon :

Generally, soil at high altitude showed higher organic carbon content than soil at low altitude (Figs. 6a & b). Organic carbon content of soil at high altitude ranged between 3.20% to 6.78%, while that at low altitude ranged between 1.96% to 4.44%. Figs. 6a & b depict the organic carbon content of earthworm casts and the surrounding soil at both the study sites. From both the figures, it can be predicted that earthworm casts contained higher organic carbon than the surrounding soil. Organic carbon content of earthworm casts at high altitude ranged between 6.24% to 13.76% while that of casts at low altitude ranged between 4.04% to 6.32%.

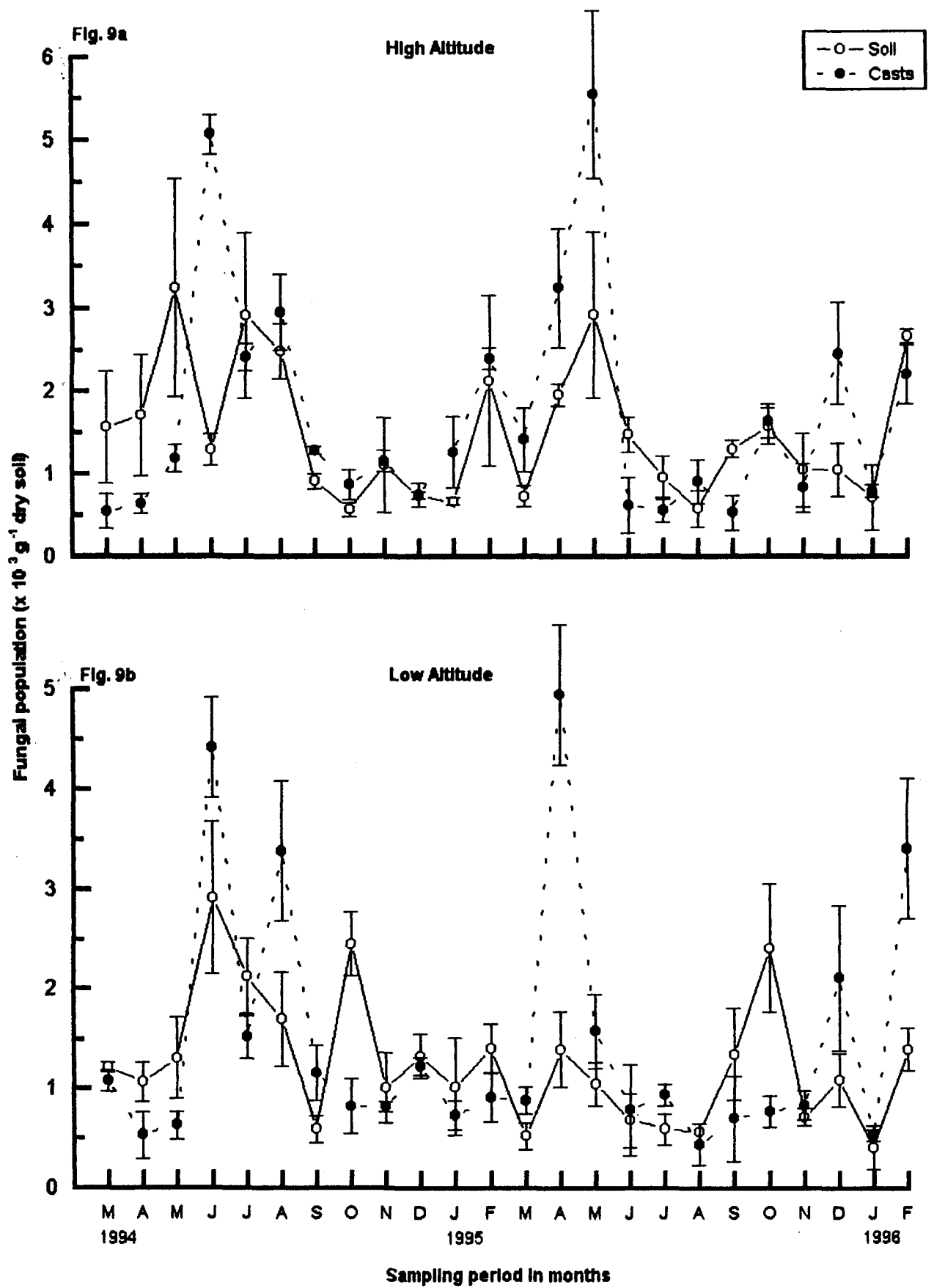
Nitrogen :

Generally, soil at high altitude showed higher nitrogen content as compared to soil at low altitude (Figs. 7a & b). Nitrogen content of soil at high altitude ranged from 0.211% to 0.315%, while that of soil at low altitude ranged between 0.146% to 0.230%. It was also recorded that earthworm casts had higher contents of nitrogen compared to the surrounding soil (Figs. 7a & b). Nitrogen content of earthworm casts at high altitude varied between 0.343% and 0.552%, while that of casts at low altitude varied between 0.190% and 0.272%. The nitrogen content of the earthworm casts at both the study sites was observed to be more during the winter months.

Available Phosphorus :

Available phosphorus of the soil was generally higher at high altitude than that of the soil at low altitude (Figs. 8a & b). A fluctuation in the monthly variation of available phosphorus was observed in the soil at high altitude as well as low altitude. However, when comparison was made between available phosphorus of earthworm casts with that of the surrounding soil at both the study sites it was found that the earthworm casts showed higher contents of available phosphorus (Figs. 8a & b).

Fig. 9 (a&b) Monthly variation in fungal population of soil and earthworm casts of two pine forest stands.



2. Fungal population of soil, casts, gut contents of the earthworm and pine litter (undecomposed and decomposed)

Fungal population of soils exhibited a more or less similar trend of monthly variation at both the study sites. Higher fungal population was recorded in the month of May in the soil at high altitude, whereas, at low altitude the fungal population was higher in the month of June (Figs. 9a & b). However, when comparison was made between the fungal population of the soil and the casts, it was observed that the earthworm casts generally harboured maximum population as compared to that of the surrounding soil (Figs. 9a & b).

Table 7 shows the correlation between fungal population with different soil parameters at both the study sites. A positive correlation was observed between the fungal population of soil and soil temperature at high altitude ($r = 0.491$, $P < 0.05$). Statistically, a positive correlation was observed between soil moisture and fungal population of soil at both the study sites but was not significant. A significant positive correlation was also observed between soil organic carbon and fungal population at low altitude ($r = 0.353$, $P < 0.1$).

Surface soil (0-10 cm) of the pine forest stand at high altitude harboured a total of 26 fungal species during the first year (table 1.1). The species were *Absidia cylindrospora*, *Aspergillus candidus*, *Aspergillus flavus*, *A. terreus*, *A. niger*, *A. japonicus*, *Botrytis* sp., *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Fusarium solani*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. racemosus*, *M. plumbeus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. rubrum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, and white sterile mycelia. During the second year of study the same soil harboured a total of 21 fungal species (table 1.2). The species isolated were *A. cylindrospora*, *Aspergillus flavus*, *A. japonicus*, *C. cladosporioides*, *Cunninghamella elegans*, *F. solani*, *Humicola* sp., *Mortierella parvispora*, *M. hiemalis*, *M. plumbeus*, *M. racemosus*, *P. brevicompactum*, *P. chrysogenum*,

Table 1.1 Fungal population ($\times 10^3 \text{ g}^{-1}$ dry soil) of soil at high altitude of pine forest stand during the different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspidia cylindrospora</i>	-	-	-	-	-	-	-	0.040 (7.14)	-	0.190 (17.65)	-	-
2. <i>Aspergillus candidus</i>	-	-	-	-	-	0.048 (1.92)	-	-	-	-	-	-
3. <i>A. farus</i>	-	-	-	0.174 (13.33)	-	-	-	0.040 (7.14)	-	-	-	0.326 (15.38)
4. <i>A. japonicus</i>	-	-	-	0.348 (26.67)	-	-	-	-	-	-	-	-
5. <i>A. niger</i>	-	-	-	-	0.047 (1.84)	-	-	-	-	-	-	-
6. <i>A. terreus</i>	-	-	0.048 (1.47)	-	-	-	-	-	-	-	-	-
7. <i>Botrytis</i> sp.	-	-	-	-	-	0.095 (3.85)	-	-	-	-	-	-
8. <i>Cladosporium cladosporioides</i>	-	-	-	-	0.047 (1.84)	-	-	-	0.152 (4.76)	-	-	-
9. <i>Fusarium oxysporum</i>	-	-	0.619 (19.12)	-	-	-	-	-	-	-	-	-
10. <i>F. solani</i>	-	-	-	-	0.667 (22.95)	-	-	-	-	-	-	0.734 (34.62)
11. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.125 (18.75)	-
12. <i>Mortierella parvispora</i>	-	-	-	-	-	0.048 (1.92)	-	-	-	-	-	-
13. <i>Mucor hiemalis</i>	-	-	0.190 (5.88)	-	-	0.333 (13.46)	-	-	-	0.130 (17.65)	-	-
14. <i>M. plumbeus</i>	-	-	-	-	-	-	-	-	0.210 (19.05)	-	-	-
15. <i>M. racemosus</i>	-	-	-	-	-	-	-	0.487 (85.71)	-	-	-	-
16. <i>Penicillium brevicompactum</i>	-	-	-	0.087 (6.67)	-	-	-	-	-	-	0.042 (6.25)	-
17. <i>P. chrysogenum</i>	0.476 (30.30)	1.208 (70.73)	0.048 (1.47)	0.347 (26.67)	1.666 (57.38)	0.048 (1.92)	-	-	0.420 (38.09)	-	-	0.857 (40.38)
18. <i>P. frequentans</i>	0.190 (12.12)	-	-	-	0.333 (11.48)	0.142 (5.77)	-	-	0.157 (14.29)	-	-	-
19. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.157 (14.29)	-	-	-
20. <i>P. rubrum</i>	-	-	0.095 (2.94)	-	-	-	-	-	-	-	-	0.163 (7.69)
21. <i>P. wakamatsii</i>	-	-	0.429 (13.24)	-	-	-	-	-	-	-	-	0.841 (1.92)
22. <i>Pythium intermedium</i>	0.905 (57.58)	-	0.190 (5.88)	0.260 (20.00)	0.095 (3.27)	1.667 (67.31)	-	-	-	-	-	-
23. <i>Fusicladium stolonifer</i>	-	1.125 (7.32)	0.238 (7.35)	-	0.047 (1.64)	-	0.136 (15.00)	-	-	0.346 (47.85)	-	-
24. <i>Trichoderma harzianum</i>	-	-	1.381 (42.65)	-	-	-	-	-	-	-	-	-
25. <i>T. konigii</i>	-	0.292 (17.07)	-	-	-	-	0.546 (60.00)	-	0.105 (9.52)	0.130 (17.65)	0.417 (62.50)	-
26. White sterile mycelia	-	0.083 (4.88)	-	0.087 (6.67)	-	0.095 (3.85)	0.227 (25.00)	-	-	-	0.083 (12.50)	-

Table 1.2 Fungal population ($\times 10^3$ g⁻¹ dry soil) of soil at high altitude of pine forest stand during the different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspidia cylindrospora</i>	-	-	-	-	-	-	-	0.143 (25.00)	-	0.211 (20.00)	-	-
2. <i>Aspergillus flavus</i>	-	-	-	0.048 (1.64)	0.049 (5.26)	-	-	0.048 (8.33)	-	-	-	-
3. <i>A. japonicus</i>	-	-	-	0.190 (6.56)	-	-	-	-	-	-	-	-
4. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	-	0.048 (6.67)	-
5. <i>Clostridium elegans</i>	-	-	-	-	-	0.042 (7.14)	-	-	-	-	-	-
6. <i>Fusarium solani</i>	-	-	-	-	0.449 (47.38)	-	-	-	-	-	-	-
7. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.571 (80.00)	2.649 (100.00)
8. <i>Mortierella parvispora</i>	-	-	-	-	-	0.041 (7.14)	-	-	-	-	-	-
9. <i>Mucor hiemalis</i>	0.227 (31.25)	-	0.059 (4.00)	-	-	-	-	-	0.238 (22.73)	0.105 (10.00)	-	-
10. <i>M. plumbeus</i>	-	-	-	-	-	-	-	-	0.095 (9.89)	-	-	-
11. <i>M. racemosus</i>	-	-	-	-	-	-	-	0.381 (66.67)	-	-	-	-
12. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	-	-	-	-	0.095 (13.33)	-
13. <i>P. chrysogenum</i>	-	0.244 (12.50)	-	0.048 (1.64)	-	-	-	-	0.095 (9.89)	-	-	-
14. <i>P. frequentans</i>	-	0.439 (22.50)	-	-	-	-	-	-	-	-	-	-
15. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.286 (27.27)	-	-	-
16. <i>P. wakamanii</i>	-	-	0.470 (32.00)	-	-	-	-	-	-	-	-	-
17. <i>Pythium intermedium</i>	0.227 (31.25)	-	0.941 (64.00)	2.619 (90.16)	0.049 (5.26)	0.500 (85.71)	-	-	-	-	-	-
18. <i>Rhizopus stolonifer</i>	-	-	-	-	-	-	0.250 (19.23)	-	-	0.632 (60.00)	-	-
19. <i>Trichoderma korringii</i>	0.273 (37.50)	1.025 (52.50)	-	-	0.049 (5.26)	-	0.900 (69.23)	-	0.333 (31.82)	0.105 (10.00)	-	-
20. White sterile mycelia	-	0.244 (12.50)	-	-	-	-	0.149 (11.54)	-	-	-	-	-
21. Black sterile mycelia	-	-	-	-	0.349 (36.49)	-	-	-	-	-	-	-

Table 1.3 Fungal population ($\times 10^3 \text{ g}^{-1}$ dry soil) of soil at low altitude of pine forest stand during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus carneus</i>	-	-	-	0.167 (5.71)	-	-	-	-	-	-	-	-
2. <i>A. flavus</i>	-	-	-	0.083 (2.86)	-	-	-	-	-	-	-	-
3. <i>A. niger</i>	-	0.044 (4.16)	-	0.042 (1.43)	-	-	-	-	-	-	-	-
4. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	-	0.089 (8.70)	0.392 (27.77)
5. <i>Cunninghamella elegans</i>	-	-	-	-	-	-	-	0.614 (25.00)	-	0.857 (64.52)	-	-
6. <i>Fusarium oxysporum</i>	-	-	-	0.333 (11.43)	-	-	-	-	-	-	-	-
7. <i>F. solani</i>	-	0.578 (54.17)	0.204 (15.62)	-	0.174 (8.16)	-	-	-	-	-	-	0.235 (16.67)
8. <i>F. sporotrichioides</i>	-	-	-	-	-	-	0.045 (7.69)	-	-	-	-	-
9. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.311 (30.43)	-
10. <i>Mucor hiemalis</i>	-	-	-	-	0.087 (4.08)	-	-	1.053 (42.86)	0.842 (3.85)	-	-	-
11. <i>Oidodendron</i> sp.	0.097 (8.00)	-	-	-	-	-	-	-	-	-	-	-
12. <i>Penicillium brevicompactum</i>	-	-	-	0.125 (4.29)	-	-	-	0.789 (32.14)	-	-	0.044 (4.35)	-
13. <i>Penicillium chrysogenum</i>	0.487 (40.00)	0.444 (41.67)	0.041 (3.21)	1.708 (58.57)	1.217 (57.14)	0.348 (20.51)	0.091 (15.38)	-	0.593 (53.85)	0.472 (35.48)	-	0.706 (50.00)
14. <i>P. frequentans</i>	-	-	-	-	-	0.304 (17.95)	0.045 (7.69)	-	0.127 (11.54)	-	-	-
15. <i>P. javanicum</i>	0.049 (4.00)	-	-	-	-	-	-	-	-	-	-	-
16. <i>P. rubrum</i>	-	-	-	-	0.087 (4.08)	0.130 (7.69)	-	-	-	-	-	-
17. <i>P. wakamarii</i>	0.146 (12.00)	-	-	-	-	-	-	-	-	-	-	-
18. <i>Pythium intermedium</i>	0.097 (8.00)	-	0.367 (28.13)	0.208 (7.14)	0.391 (18.37)	0.826 (48.72)	-	-	-	-	-	-
19. <i>Rhizopus stolonifer</i>	-	-	0.490 (37.50)	0.167 (5.71)	0.130 (6.12)	-	0.318 (53.85)	-	0.085 (7.69)	-	-	0.039 (2.78)
20. <i>Trichoderma korringii</i>	-	-	0.082 (6.25)	0.083 (2.86)	0.043 (2.04)	0.087 (5.13)	0.091 (15.38)	-	0.169 (15.38)	-	0.578 (56.52)	-
21. White sterile mycelia	0.341 (28.00)	-	0.122 (9.38)	-	-	-	-	-	-	-	-	0.039 (2.78)

Table 1.4 Fungal population ($\times 10^3 \text{ g}^{-1}$ dry soil) of soil at low altitude of pine forest stand during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Absidia cylindrospora</i>	-	-	-	-	-	-	-	-	-	-	0.091 (22.22)	-
2. <i>A. glauca</i>	-	-	-	-	0.049 (8.33)	-	-	-	-	-	-	-
3. <i>Aspergillus japonicus</i>	-	0.054 (3.85)	-	-	-	-	-	-	-	-	-	-
4. <i>Aspergillus versicolor</i>	0.043 (8.33)	-	-	-	-	-	-	-	-	-	-	-
5. <i>Cladosporium herbarum</i>	-	-	-	-	-	-	0.050 (3.70)	-	-	-	-	-
6. <i>C. cladosporioides</i>	-	-	-	-	-	-	-	-	-	0.045 (4.17)	-	-
7. <i>Cunninghamella elegans</i>	-	-	-	-	-	-	0.150 (11.11)	-	-	0.091 (8.33)	-	-
8. <i>Fusicarum oxysporum</i>	-	-	-	-	-	-	-	-	-	0.364 (33.33)	-	-
9. <i>F. solani</i>	0.043 (8.33)	0.918 (85.38)	-	-	-	-	-	-	-	-	-	-
10. <i>F. marismoides</i>	-	-	-	-	-	0.046 (8.33)	-	-	-	-	-	-
11. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.227 (55.56)	-
12. <i>Mucor circinelloides</i>	-	-	-	-	-	-	-	-	-	-	-	1.002 (100.00)
13. <i>M. hiemalis</i>	0.130 (25.00)	0.054 (3.85)	-	-	-	-	0.050 (3.70)	0.654 (26.98)	0.311 (43.75)	0.409 (37.50)	0.045 (11.11)	-
14. <i>Penicillium brevicompactum</i>	-	-	0.056 (5.26)	-	-	-	-	0.654 (26.98)	-	0.045 (4.17)	-	-
15. <i>P. chrysogenum</i>	-	0.108 (7.69)	0.056 (5.26)	0.244 (35.71)	0.098 (16.67)	-	0.050 (3.70)	0.308 (12.70)	-	0.091 (8.33)	-	-
16. <i>P. frequentans</i>	-	-	-	-	-	-	-	-	0.044 (6.25)	-	-	-
17. <i>Pythium intermedium</i>	0.043 (8.33)	-	0.888 (84.21)	0.195 (28.57)	0.195 (33.33)	0.464 (83.33)	0.050 (3.70)	-	-	-	-	-
18. <i>Rhizopus stolonifer</i>	0.130 (25.00)	-	-	-	-	-	0.150 (11.11)	-	-	-	-	-
19. <i>Trichoderma korringii</i>	0.043 (8.33)	0.054 (3.85)	0.056 (5.26)	0.244 (35.71)	0.244 (41.67)	-	0.849 (62.96)	-	0.356 (50.00)	0.045 (4.17)	-	-
20. White sterile mycelia	0.086 (16.67)	0.216 (15.38)	-	-	-	0.046 (8.33)	-	0.038 (1.59)	-	-	0.045 (11.11)	-

Table 2.1 Fungal population ($\times 10^3 \text{ g}^{-1}$ dry soil) of earthworm casts collected from the pine forest stand at high altitude during the different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	-	0.074 (5.88)	-
2. <i>Fusarium solani</i>	-	-	-	0.974 (19.23)	-	-	-	-	-	-	-	-
3. <i>Humicola</i> sp.	0.117 (21.43)	-	-	-	-	-	-	-	-	-	-	-
4. <i>Mucor hiemalis</i>	-	-	-	-	0.200 (8.33)	0.059 (2.00)	-	-	-	-	-	-
5. <i>M. circinalloides</i>	-	-	0.190 (16.00)	-	-	-	-	-	-	-	-	-
6. <i>M. racemosus</i>	-	-	-	-	-	-	0.525 (34.48)	0.833 (95.45)	-	-	-	-
7. <i>Periclitium brevicompactum</i>	-	-	-	-	0.050 (2.08)	-	-	-	0.050 (4.35)	-	-	-
8. <i>P. chrysogenum</i>	0.078 (14.29)	0.228 (35.71)	-	1.494 (29.49)	0.150 (6.25)	0.353 (12.00)	-	-	0.300 (26.08)	0.291 (38.89)	0.185 (14.71)	1.642 (68.22)
9. <i>P. frequentans</i>	-	-	-	-	0.150 (6.25)	-	-	-	-	-	0.037 (2.94)	-
10. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.751 (65.22)	-	0.037 (2.94)	-
11. <i>Pythium intermedium</i>	0.352 (64.29)	0.091 (14.29)	0.142 (12.00)	1.642 (32.05)	1.857 (77.08)	2.179 (74.00)	-	-	-	0.167 (22.22)	0.259 (20.59)	0.249 (10.45)
12. <i>Phizopus stolonifer</i>	-	-	-	-	-	0.059 (2.00)	0.420 (27.59)	-	0.050 (4.35)	-	-	-
13. <i>Trichoderma korringii</i>	-	0.273 (42.86)	0.619 (52.00)	0.974 (19.23)	-	-	0.578 (37.93)	-	-	0.292 (38.89)	0.667 (52.94)	-
14. White sterile mycelia	-	0.045 (7.14)	0.238 (20.00)	-	-	0.177 (6.00)	-	-	-	-	-	-
15. Black sterile mycelia	-	-	-	-	-	-	-	-	-	-	-	0.500 (20.89)
16. Yellow sterile mycelia	-	-	-	-	-	0.118 (4.00)	-	0.040 (4.55)	-	-	-	-

Table 2.2 Fungal population ($\times 10^3 \text{ g}^{-1}$ dry soil) of the earthworm casts collected from the pine forest stand at high altitude during the different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Acremonium</i> sp.	-	-	-	-	-	-	-	0.045 (2.78)	-	-	-	-
2. <i>Alternaria tenuis</i>	-	-	2.925 (52.78)	-	-	-	-	-	-	-	-	-
3. <i>Cladosporium cladosporioides</i>	0.045 (3.23)	-	-	-	-	-	-	-	-	-	-	-
4. <i>Hemicelia</i> sp.	-	-	-	-	-	-	-	-	-	-	-	2.069 (94.64)
5. <i>Mucor hiemalis</i>	-	-	-	0.809 (51.52)	-	0.050 (5.56)	-	-	-	-	-	-
6. <i>Mucor circinalloides</i>	-	-	0.115 (2.08)	-	-	-	-	-	-	-	-	-
7. <i>M. racemosus</i>	-	-	-	-	-	-	0.210 (40.00)	1.544 (94.44)	-	-	-	-
8. <i>Penicillium chrysogenum</i>	-	1.536 (47.62)	0.308 (5.56)	-	-	0.050 (5.56)	-	-	-	0.855 (2.27)	-	0.078 (3.57)
9. <i>P. frequentans</i>	-	0.077 (2.38)	-	0.047 (3.03)	-	0.050 (5.56)	-	-	-	-	-	-
10. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.041 (5.00)	-	-	0.039 (1.79)
11. <i>P. wakamatsii</i>	-	-	1.655 (29.86)	-	-	-	-	-	-	-	-	-
12. <i>Pythium intermedium</i>	0.363 (25.80)	-	0.539 (9.72)	0.714 (45.45)	0.555 (40.00)	0.650 (72.22)	-	-	-	2.056 (94.09)	0.792 (100.00)	-
13. <i>Phizopus stolonifer</i>	-	-	-	-	-	-	0.150 (20.00)	-	-	-	-	-
14. <i>Trichoderma korningii</i>	0.999 (70.97)	1.459 (45.24)	-	-	0.833 (60.00)	-	0.210 (40.00)	-	0.667 (80.00)	0.278 (11.36)	-	-
15. White sterile mycelia	-	0.154 (4.76)	-	-	-	-	-	0.045 (2.78)	0.125 (15.00)	0.055 (2.27)	-	-
16. Yellow sterile mycelia	-	-	-	-	-	0.100 (11.11)	-	-	-	-	-	-

Table 2.3. Fungal population ($\times 10^3 \text{ g}^{-1}$ dry soil) of the earthworm casts collected from the pine forest stand at low altitude during the different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Acromonium</i> sp.	-	-	-	-	-	-	-	0.305 (24.00)	-	-	-	-
2. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	0.055 (1.64)	-	-	-	-	0.038 (5.26)	0.218 (24.00)
3. <i>Fusarium oxysporum</i>	0.166 (15.38)	-	-	-	-	-	-	-	-	-	-	-
4. <i>F. solani</i>	-	-	-	3.849 (87.12)	-	-	-	-	-	-	-	-
5. <i>Humicola</i> sp.	0.416 (38.46)	-	-	-	-	-	0.525 (28.57)	-	-	-	-	-
6. <i>Mucor hiemalis</i>	0.375 (34.61)	-	0.208 (33.33)	-	0.507 (33.33)	0.056 (1.64)	-	0.509 (40.00)	0.338 (30.43)	0.923 (67.74)	-	-
7. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	-	0.305 (24.00)	-	-	-	-
8. <i>P. chrysogenum</i>	-	0.122 (23.08)	-	-	0.092 (6.06)	0.889 (26.23)	-	-	0.193 (17.39)	0.175 (12.90)	0.038 (5.26)	0.109 (12.00)
9. <i>P. frequentans</i>	-	-	-	-	-	0.167 (4.92)	-	0.101 (8.00)	-	-	0.038 (5.26)	-
10. <i>P. javanicum</i>	-	-	-	-	-	0.055 (1.64)	-	-	-	-	-	-
11. <i>P. wakamanii</i>	-	-	-	-	0.923 (60.61)	-	-	-	-	-	-	-
12. <i>Pythium intermedium</i>	-	-	-	0.349 (7.92)	-	-	0.683 (37.14)	-	-	0.263 (19.35)	-	0.255 (28.00)
13. <i>Trichoderma koningii</i>	0.125 (11.54)	0.326 (61.54)	0.416 (66.67)	0.218 (4.95)	-	0.111 (3.28)	0.630 (34.29)	-	0.289 (26.09)	-	0.577 (78.95)	-
14. White sterile mycelia	-	0.081 (15.38)	-	-	-	2.000 (59.01)	-	0.050 (4.00)	0.241 (21.74)	-	0.038 (5.26)	-
15. Black sterile mycelia	-	-	-	-	-	0.055 (1.64)	-	-	-	-	-	0.036 (4.00)
16. Yellow sterile mycelia	-	-	-	-	-	-	-	-	0.048 (4.34)	-	-	0.291 (32.00)

Table 2.4. Fungal population ($\times 10^3$ g⁻¹ dry soil) of earthworm casts collected from the pine forest stand at low altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentages relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Acromonium</i> sp.	-	-	-	-	-	-	-	0.182 (23.53)	-	-	-	-
2. <i>Aspergillus candidus</i>	0.333 (19.05)	-	-	-	-	-	-	-	-	-	-	-
3. <i>Ctenophtharrella elegans</i>	-	-	-	0.056 (7.14)	-	-	-	-	-	-	-	-
4. <i>Fusarium oxysporum</i>	-	-	-	0.505 (64.28)	0.388 (41.18)	-	-	-	-	-	-	-
5. <i>Hemicela</i> sp.	-	-	-	-	-	-	0.150 (10.34)	-	-	-	0.158 (30.00)	1.179 (34.52)
6. <i>Mortierella parvispora</i>	-	-	-	-	-	-	0.450 (31.03)	-	-	-	-	-
7. <i>Miscor hiemalis</i>	-	-	-	-	0.278 (29.41)	-	-	0.182 (23.53)	0.416 (35.71)	0.177 (8.33)	0.368 (70.00)	2.154 (63.10)
8. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	-	0.272 (35.29)	-	0.353 (16.67)	-	0.041 (1.19)
9. <i>P. chrysogenum</i>	0.083 (9.52)	2.061 (41.67)	0.083 (5.26)	0.112 (14.29)	-	-	-	-	-	0.294 (13.89)	-	-
10. <i>P. frequentans</i>	-	-	-	-	-	-	-	-	-	0.177 (8.33)	-	-
11. <i>P. janthinellum</i>	-	1.237 (25.00)	-	-	-	-	-	-	-	-	-	-
12. <i>P. wakamatsii</i>	-	-	-	-	0.278 (29.41)	-	-	-	-	-	-	-
13. <i>Pythium intermedium</i>	0.250 (28.57)	0.589 (11.90)	1.375 (86.84)	-	-	-	0.300 (20.69)	-	-	0.177 (8.33)	-	-
14. <i>Rhizopus stolonifer</i>	-	-	-	-	-	-	-	-	-	0.177 (8.33)	-	-
15. <i>Trichoderma koribgii</i>	0.875 (38.10)	0.353 (7.14)	-	0.112 (14.29)	-	1.667 (100.00)	0.550 (37.93)	-	0.750 (64.29)	0.765 (36.11)	-	-
16. White sterile mycelia	0.042 (4.76)	0.707 (14.29)	0.042 (2.63)	-	-	-	-	0.135 (17.65)	-	-	-	-
17. Black sterile mycelia	-	-	0.083 (5.26)	-	-	-	-	-	-	-	-	-
18. Yellow sterile mycelia	-	-	-	-	-	-	-	-	-	-	-	0.041 (1.19)

P. frequentans, *P. javanicum*, *P. waksmanii*, *P. intermedium*, *R. stolonifer*, *T. koningii*, white sterile mycelia and black sterile mycelia.

Surface soil (0-10 cm) of the pine forest stand at low altitude harboured a total of 21 fungal species during the first year (table 1.3). The fungal species isolated were *Aspergillus carneus*, *A. flavus*, *A. niger*, *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Fusarium oxysporum*, *F. solani*, *F. sporotrichioides*, *Humicola sp.*, *Mucor hiemalis*, *Oldodendron sp.*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. rubrum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia. During the second year of study the same soil harboured a total of 20 fungal species (table 1.4). The fungal species isolated were *A. cylindrospora*, *A. glauca*, *Aspergillus japonicus*, *A. versicolor*, *C. cladosporioides*, *C. herbarum*, *Cunninghamella elegans*, *F. oxysporum*, *F. merismoides*, *F. solani*, *Humicola sp.*, *M. hiemalis*, *M. circinelloides*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *Pythium intermedium*, *R. stolonifer*, *T. koningii* and white sterile mycelia.

From tables 1.1 to 1.4, it is clear that the pine forest soil at high altitude exhibited highest number of fungal isolates as compared to that at low altitude. Qualitatively, there was not much difference in the composition of microflora of the two pine forest soils. Most of the fungal species which were obtained from the soil at high altitude were also isolated from the soil at low altitude except for *Aspergillus terreus*, *Botrytis sp.*, *Mucor plumbeus*, *M. racemosus*, *Trichoderma harzianum* and black sterile mycelia which were isolated from the soil at high altitude only, while *Abstidia glauca*, *A. versicolor*, *Cladosporium herbarum*, *Fusarium merismoides* and *F. sporotrichioides* and *Oldodendron sp.* which were restricted to the soil at low altitude only.

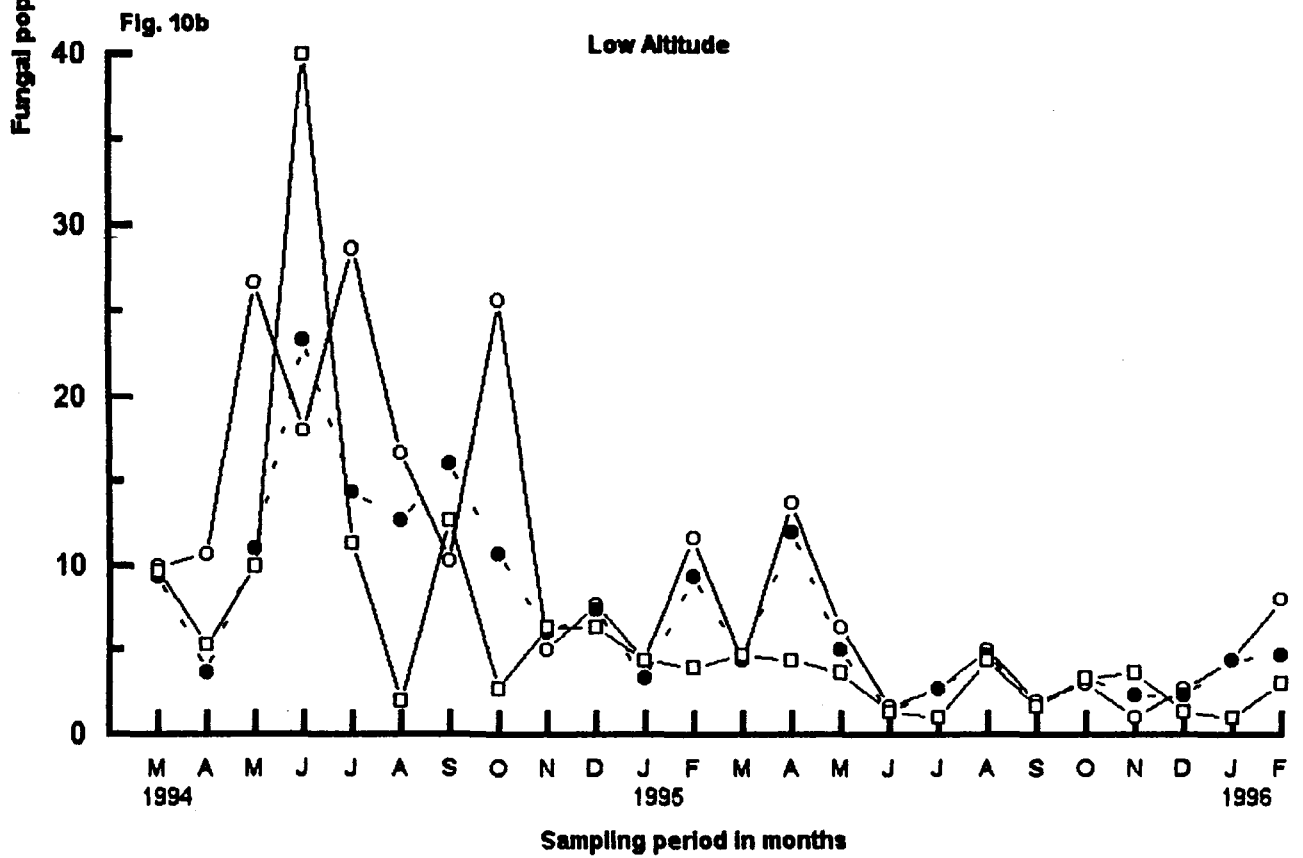
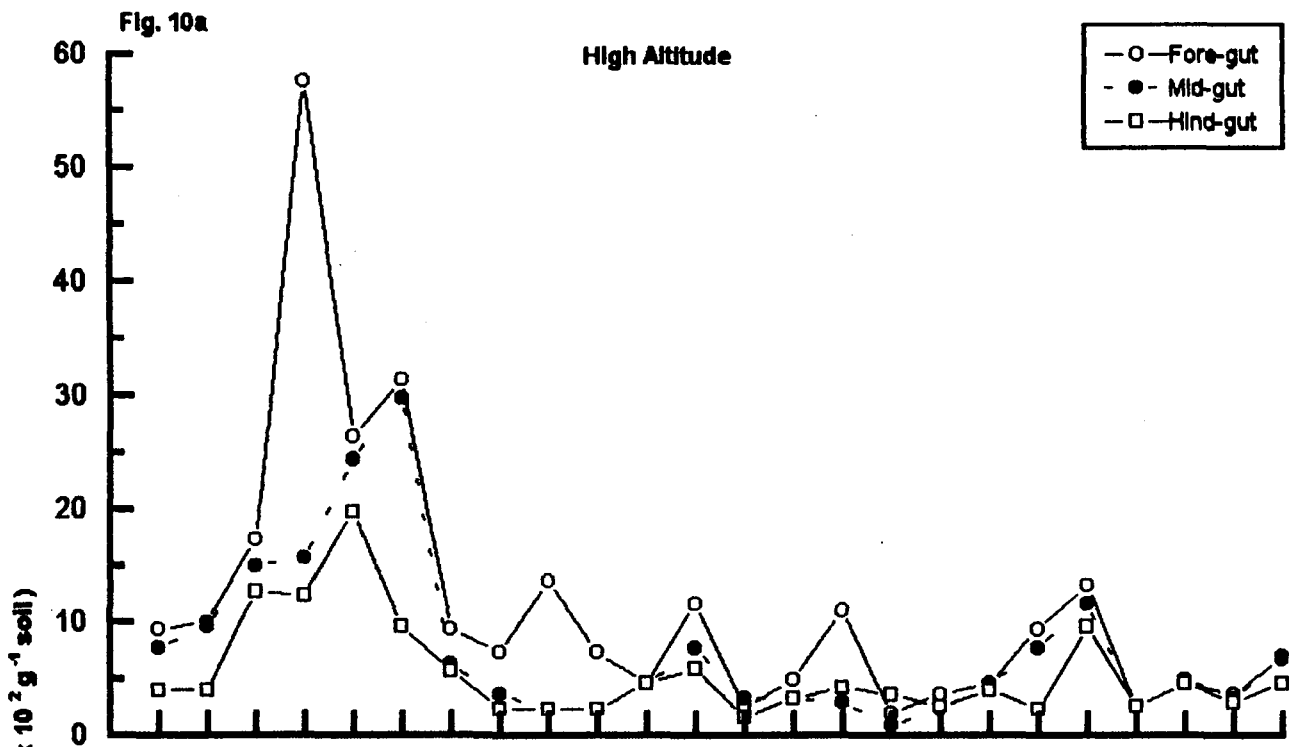
List of fungal species isolated from the earthworm casts collected from the two pine forest stands are given in tables 2.1 to 2.4. Casts collected from the pine forest stand at high

altitude harboured a total of 16 fungal species during the first year (Table 2.1). The species isolated were *Cladosporium cladosporioides*, *Fusarium solani*, *Humicola* sp., *Mucor hiemalis*, *M. racemosus*, *M. circinelloides*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, white sterile mycelia, black sterile mycelia and yellow sterile mycelia. During the second year of study the same soil harboured a total of 16 fungal species (table 2.2). The species isolated were *Acremonium* sp., *Alternaria tenuis*, *C. cladosporioides*, *Humicola* sp., *M. hiemalis*, *M. racemosus*, *M. circinelloides*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *P. intermedium*, *R. stolonifer*, *T. koningii*, white sterile mycelia and yellow sterile mycelia.

Earthworm casts collected from the pine forest stand at low altitude harboured a total of 16 fungal species during the first year (Table 2.3). The species isolated were *Acremonium* sp., *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *Humicola* sp., *Mucor hiemalis*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Trichoderma koningii*, white sterile mycelia, black sterile mycelia and yellow sterile mycelia. During the second year of study the same soil harboured a total of 18 fungal species (Table 2.4). The species isolated were *Acremonium* sp., *Aspergillus carneus*, *Cunninghamella elegans*, *F. oxysporum*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *P. brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. janthinellum*, *P. waksmanii*, *Pythium intermedium*, *R. stolonifer*, *Trichoderma koningii*, white sterile mycelia, black sterile mycelia and yellow sterile mycelia.

Tables 2.1 and 2.4 showed that the casts collected from the pine forest stand at low altitude exhibited a highest number of fungal isolates as compared to that collected from the pine forest stand at high altitude. Qualitatively, there was not much difference in the composition of the microflora of the casts collected from both the study sites except for *Alternaria tenuis*,

Fig. 10 (a&b) Monthly variation in fungal population of foregut, midgut and hindgut of the earthworm collected from two pine forest stands.



Mucor racemosus and *M. circinelloides* which were isolated from the casts at high altitude only and *Aspergillus candidus*, *Cunninghamella elegans*, *Fusarium oxysporum*, *Mortierella parvispora* and *Penicillium janthinellum* which were isolated from the casts at low altitude only.

Figs. 10 a & b depict the monthly variation of fungal population in the gut of earthworm *Drawida papillifer papillifer* Steph. at both the study sites. The fungal population was generally higher in the fore-gut as compared to that of the mid-gut and hind-gut regions. In other words, the fungal population exhibited a decreasing trend down the canal of the earthworm. Fungal population in the different gut regions was recorded to be more during the first year at both the study sites.

Altogether a total of 22, 19 and 21 fungal species were isolated from the fore-, mid- and hind- gut regions respectively during the first year and a total of 22, 19, 18 fungal species were isolated from the same regions during the second year at high altitude (Tables 3.1 - 3.6). The fungal species isolated from the fore-gut at high altitude during the study period were *Aspergillus carneus*, *A. flavus*, *A. terreus*, *A. wentii*, *Cladosporium cladosporioides*, *C. herbarum*, *C. macrocarpum*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *F. sporotrichioides*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. daleae*, *P. digitatum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, and white sterile mycelia. The fungal species isolated from the mid-gut region were *Aspergillus carneus*, *A. flavus*, *A. wentii*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. daleae*, *P. digitatum*, *P. frequentans*, *P. janthinellum*,

Table 3.1 Fungal population ($\times 10^3 \text{ g}^{-1}$ soil) in the fore-gut of the earthworm *Dravida papillifer papillifer* Steph. collected from the pine forest stand at high altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus carneus</i>	-	-	6.67 (38.48)	-	7.33 (27.88)	-	-	-	-	-	-	-
2. <i>A. flavus</i>	-	-	0.67 (3.86)	-	1.33 (5.06)	2.00 (6.38)	-	-	-	-	0.33 (7.14)	0.33 (2.86)
3. <i>A. wentii</i>	-	-	-	-	0.67 (2.53)	-	-	-	-	-	-	-
4. <i>A. terreus</i>	-	-	-	10.67 (18.50)	-	-	-	-	-	-	-	-
5. <i>Cladosporium cladosporioides</i>	-	-	0.33 (1.90)	0.67 (1.16)	1.67 (6.33)	-	-	2.00 (27.27)	0.67 (5.56)	-	-	-
6. <i>Fusarium moniliforme</i>	-	-	-	-	-	0.33 (1.06)	-	-	-	-	-	-
7. <i>F. solani</i>	-	3.33 (36.60)	2.00 (11.54)	0.33 (0.57)	-	-	-	0.33 (4.55)	-	-	-	-
8. <i>F. sporotrichioides</i>	-	-	-	-	-	9.33 (29.79)	-	-	2.33 (19.44)	-	-	-
9. <i>Hemicola</i> sp.	-	-	-	-	-	-	5.33 (60.74)	-	-	-	-	-
10. <i>Mortierella parvispora</i>	-	-	-	-	-	-	-	-	-	7.33 (100.00)	-	-
11. <i>Mucor hiemalis</i>	0.33 (3.33)	-	-	-	-	-	-	1.67 (22.73)	0.67 (5.56)	-	-	7.00 (60.00)
12. <i>M. racemosus</i>	-	-	-	-	-	-	-	-	1.00 (8.33)	-	-	-
13. <i>Penicillium brevicompactum</i>	-	-	-	18.00 (31.21)	2.67 (10.13)	2.67 (8.51)	-	-	-	-	-	2.67 (22.85)
14. <i>P. chrysogenum</i>	2.67 (26.67)	-	0.67 (3.86)	26.33 (45.66)	12.00 (45.57)	16.33 (52.13)	3.33 (35.73)	1.33 (18.18)	6.33 (52.78)	-	1.67 (35.72)	0.67 (5.71)
15. <i>P. frequentans</i>	-	-	-	0.67 (1.16)	1.00 (3.80)	-	-	0.33 (4.55)	-	-	-	-
16. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	-	-	-	0.67 (5.72)
17. <i>P. digitatum</i>	-	-	-	-	-	-	-	1.66 (22.73)	-	-	-	-
18. <i>Pythium intermedium</i>	-	-	-	-	-	-	-	-	-	-	2.67 (57.14)	0.33 (2.86)
19. <i>Fusicoccus stolonifer</i>	-	-	0.33 (1.90)	1.00 (1.74)	-	0.33 (1.06)	0.33 (3.57)	-	-	-	-	-
20. <i>Trichoderma harzianum</i>	-	-	6.67 (38.48)	-	-	-	-	-	-	-	-	-
21. <i>T. koningi</i>	7.00 (70.00)	-	-	-	-	-	-	-	0.67 (5.56)	-	-	-
22. White sterile mycelia	-	6.33 (63.4)	-	-	-	0.33 (1.06)	-	-	0.33 (2.78)	-	-	-

Table 3.2 Fungal population ($\times 10^2$ g⁻¹ soil) in the fore-gut of the earthworm *Dravidia papillifer papillifer* Steph. collected from the pine forest stand at high altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus carneus</i>	-	-	-	0.333 (16.67)	-	1.333 (28.57)	-	-	-	-	-	-
2. <i>A. flavus</i>	0.333 (12.50)	-	0.333 (9.23)	-	0.667 (18.18)	-	-	-	-	-	-	-
3. <i>Cladosporium cladosporioides</i>	-	-	1.333 (12.91)	-	-	-	-	-	-	-	0.333 (9.09)	-
4. <i>C. herbarum</i>	0.330 (12.50)	-	-	-	-	-	-	-	-	-	-	-
5. <i>C. macrocarpum</i>	-	-	0.667 (6.45)	-	-	-	-	-	-	-	-	-
6. <i>Fusicarium oxysporum</i>	-	-	4.000 (38.72)	-	-	0.667 (14.29)	-	-	-	-	-	-
7. <i>F. solani</i>	-	-	-	0.667 (33.33)	0.333 (9.09)	0.667 (14.29)	-	-	-	-	-	-
8. <i>F. sporotrichioides</i>	-	-	-	-	-	0.333 (7.14)	-	-	-	-	-	-
9. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.333 (9.09)	7.000 (100.00)
10. <i>Mortierella parvispora</i>	-	-	-	-	1.333 (36.36)	-	-	-	-	1.667 (35.72)	-	-
11. <i>Mucor hiemalis</i>	1.00 (37.50)	-	0.667 (6.45)	-	-	0.333 (7.14)	-	-	0.667 (25.00)	-	-	-
12. <i>M. racemosus</i>	-	-	-	-	-	0.333 (7.14)	-	-	0.667 (25.00)	-	-	-
13. <i>Penicillium brevicompactum</i>	-	0.333 (6.67)	-	0.333 (16.67)	-	-	-	-	1.000 (37.50)	0.333 (7.14)	0.333 (9.09)	-
14. <i>P. chrysogenum</i>	0.333 (12.50)	1.000 (20.00)	0.667 (6.45)	-	-	-	-	-	-	-	-	-
15. <i>P. frequentans</i>	-	-	0.333 (3.23)	-	0.333 (9.09)	-	-	-	-	-	-	-
16. <i>P. javanicum</i>	0.333 (12.50)	-	1.000 (9.68)	-	-	-	-	-	0.333 (12.50)	-	-	-
17. <i>P. daleae</i>	0.333 (12.50)	0.667 (13.33)	1.333 (12.91)	-	-	-	-	-	-	-	-	-
18. <i>P. digitatum</i>	-	-	-	-	-	-	-	7.000 (52.50)	-	-	-	-
19. <i>Pythium intermedium</i>	-	-	-	-	1.000 (27.27)	0.333 (7.14)	-	-	-	-	2.667 (72.73)	-
20. <i>Rhizopus stolonifer</i>	-	0.300 (60.00)	-	-	-	-	-	-	-	-	-	-
21. <i>Trichoderma koningii</i>	-	-	-	0.667 (33.33)	-	0.667 (14.29)	8.333 (89.29)	-	-	-	-	-
22. White sterile mycelia	-	-	-	-	-	-	1.000 (10.71)	6.333 (47.50)	-	2.667 (57.14)	-	-

Table 3.3 Fungal population ($\times 10^2 \text{ g}^{-1}$ soil) in the mid-gut of the earthworm *Dravida papillifer papillifer* Steph. collected from the pine forest stand at high altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus carneus</i>	-	0.33 (3.40)	-	-	0.33 (1.37)	1.00 (3.37)	-	-	-	-	-	-
2. <i>A. flavus</i>	-	-	1.00 (6.67)	-	4.67 (19.18)	-	-	-	-	-	-	-
3. <i>A. wentii</i>	-	-	-	-	2.33 (9.59)	-	-	-	-	-	-	-
4. <i>Cladosporium cladosporioides</i>	1.00 (4.41)	-	0.33 (2.20)	-	1.00 (4.11)	-	-	-	-	-	-	-
5. <i>Fusarium solani</i>	-	-	3.00 (20.00)	1.33 (8.48)	-	-	-	-	-	-	-	-
6. <i>Hemicola</i> sp.	-	-	-	-	-	-	0.67 (10.53)	0.33 (9.09)	-	-	-	-
7. <i>Mortierella parvispora</i>	-	-	-	-	-	-	-	-	-	1.33 (57.14)	-	-
8. <i>Mucor hiemalis</i>	2.33 (10.29)	-	-	0.67 (4.28)	-	-	-	2.33 (63.64)	-	-	3.67 (78.57)	7.67 (100.00)
9. <i>M. racemosus</i>	-	-	-	-	-	-	-	-	2.33 (100.00)	-	-	-
10. <i>Penicillium brevicompactum</i>	-	-	0.67 (4.47)	-	-	12.33 (41.57)	-	-	-	-	-	-
11. <i>P. chrysogenum</i>	9.33 (41.17)	5.00 (51.80)	3.00 (20.00)	12.33 (78.68)	6.00 (24.64)	13.33 (44.94)	5.67 (89.47)	-	-	-	-	-
12. <i>P. frequentans</i>	-	-	-	0.67 (4.28)	3.33 (13.70)	-	-	-	-	-	-	-
13. <i>P. javanicum</i>	-	-	-	-	-	1.00 (3.37)	-	-	-	-	-	-
14. <i>P. wakuzaii</i>	-	-	-	-	0.33 (1.37)	-	-	-	-	-	-	-
15. <i>Pythium intermedium</i>	6.33 (27.94)	-	-	-	-	2.00 (6.74)	-	-	-	-	1.00 (21.43)	-
16. <i>Rhizopus stolonifer</i>	-	0.33 (3.40)	-	0.67 (4.28)	-	-	-	-	-	-	-	-
17. <i>Trichoderma harzianum</i>	-	-	5.67 (37.80)	-	-	-	-	-	-	-	-	-
18. <i>T. koningii</i>	3.67 (16.17)	-	-	-	6.33 (26.03)	-	-	1.00 (27.27)	-	-	-	-
19. White sterile mycelia	-	4.00 (41.40)	1.33 (8.86)	-	-	-	-	-	-	1.00 (42.86)	-	-

Table 3.4 Fungal population ($\times 10^2 \text{ g}^{-1}$ soil) in the mid-gut of the earthworm *Drawida papillifer papillifer* Steph. collected from the pine forest stand at high altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus flavus</i>	0.667 (20.00)	-	-	-	0.333 (14.29)	-	-	0.333 (2.86)	-	-	-	-
2. <i>Cladosporium cladosporioides</i>	-	-	-	-	0.333 (14.29)	-	-	-	-	-	-	-
3. <i>Fusarium oxysporum</i>	-	-	-	-	-	1.000 (21.43)	-	-	-	-	-	-
4. <i>F. solani</i>	-	1.667 (50.00)	0.667 (22.22)	1.000 (100.00)	-	-	-	-	-	-	-	-
5. <i>Humicola</i> sp.	-	-	-	-	-	-	0.667 (8.69)	-	-	-	-	6.667 (100.00)
6. <i>Mortierella parvispora</i>	-	-	-	-	-	-	-	-	-	4.667 (93.33)	-	-
7. <i>Mucor hiemalis</i>	0.333 (10.00)	-	-	-	1.667 (71.42)	-	-	-	-	-	-	-
8. <i>M. racemosus</i>	-	-	-	-	-	-	0.333 (4.35)	-	2.000 (75.00)	-	-	-
9. <i>Penicillium brevicompactum</i>	-	0.333 (10.00)	1.333 (44.84)	-	-	-	-	-	0.333 (12.50)	-	-	-
10. <i>P. chrysogenum</i>	-	-	-	-	-	0.667 (14.29)	-	-	-	-	-	-
11. <i>P. frequentans</i>	-	-	0.333 (11.12)	-	-	-	-	0.333 (2.86)	-	-	-	-
12. <i>P. javanicum</i>	-	-	0.667 (22.22)	-	-	-	-	0.667 (5.71)	-	-	-	-
13. <i>P. janthinellum</i>	0.667 (20.00)	-	-	-	-	-	-	-	-	-	-	-
14. <i>P. daleae</i>	-	0.333 (10.00)	-	-	-	-	-	-	-	-	-	-
15. <i>P. digitatum</i>	-	-	-	-	-	-	-	-	0.333 (12.50)	-	-	-
16. <i>Pythium intermedium</i>	1.667 (50.00)	-	-	-	-	0.667 (14.29)	-	7.33 (62.86)	-	-	3.333 (100.00)	-
17. <i>Fusicladium stolonifer</i>	-	1.000 (30.00)	-	-	-	-	-	-	-	-	-	-
18. <i>Trichoderma korringii</i>	-	-	-	-	-	2.333 (50.00)	6.667 (86.96)	-	-	-	-	-
19. White sterile mycelia	-	-	-	-	-	-	-	3.000 (25.71)	-	0.333 (6.67)	-	-

Table 3.5 Fungal population ($\times 10^2$ g⁻¹ soil) in the hind-gut of the earthworm *Drawida papillifer papillifer* Steph. collected from pine forest stand at high altitude during different sampling periods from March 1994 to February 1995. The values in parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus candidus</i>	-	-	0.67 (5.30)	2.67 (21.62)	-	-	-	-	-	-	-	-
2. <i>A. flavus</i>	-	-	0.67 (5.30)	3.33 (27.03)	3.67 (18.64)	-	-	-	-	-	-	-
3. <i>A. niger</i>	-	-	-	-	0.33 (1.69)	-	-	-	-	-	-	-
4. <i>A. wentii</i>	-	-	1.00 (7.90)	-	0.33 (1.69)	-	-	-	-	-	-	-
5. <i>Cladosporium cladosporioides</i>	2.00 (50.00)	-	-	-	2.33 (11.86)	0.33 (3.44)	-	-	0.33 (14.29)	0.33 (14.29)	-	-
6. <i>Fusarium moniliforme</i>	-	-	-	-	-	2.67 (27.59)	-	-	-	-	-	-
7. <i>F. solani</i>	-	-	4.00 (31.50)	-	-	-	-	0.33 (14.29)	-	-	-	-
8. <i>Humicola</i> sp.	-	-	-	-	-	-	3.33 (58.82)	-	-	-	-	-
9. <i>Mucor hiemalis</i>	0.33 (8.30)	-	-	-	-	0.67 (6.90)	-	1.00 (42.85)	-	-	1.67 (35.71)	3.00 (50.00)
10. <i>M. circinalloides</i>	-	0.33 (8.30)	-	-	-	-	-	-	-	-	-	-
11. <i>M. racemosus</i>	-	-	-	-	-	-	-	-	1.33 (57.14)	-	-	-
12. <i>Penicillium brevicompactum</i>	-	-	-	3.00 (24.32)	-	3.33 (34.48)	-	-	0.67 (28.57)	-	-	-
13. <i>P. chrysogenum</i>	1.33 (33.40)	1.33 (33.40)	1.00 (7.90)	1.67 (13.51)	7.33 (37.29)	2.67 (27.59)	1.67 (29.41)	-	-	0.67 (28.57)	-	-
14. <i>P. frequentans</i>	-	-	-	0.67 (5.41)	1.33 (6.78)	-	-	-	-	-	-	-
15. <i>P. javanicum</i>	-	-	-	0.67 (5.41)	-	-	-	-	-	-	-	2.67 (44.44)
16. <i>P. wakamatsii</i>	-	-	-	-	1.67 (8.47)	-	0.67 (11.67)	-	-	-	-	-
17. <i>Pythium intermedium</i>	-	-	0.67 (5.30)	-	-	-	-	-	-	0.67 (28.57)	3.00 (64.29)	0.33 (5.56)
18. <i>Rhizopus stolonifer</i>	0.33 (8.30)	0.33 (8.30)	-	0.33 (2.70)	2.00 (10.17)	-	-	0.33 (14.29)	-	-	-	-
19. <i>Trichoderma harzianum</i>	-	-	3.33 (26.30)	-	-	-	-	-	-	-	-	-
20. <i>T. koningii</i>	-	-	0.67 (5.30)	-	0.67 (3.39)	-	-	0.67 (28.57)	-	0.33 (14.29)	-	-
21. White sterile mycelia	-	2.00 (50.00)	0.67 (5.30)	-	-	-	-	-	-	0.33 (14.29)	-	-

Table 3.6 Fungal population ($\times 10^2 \text{ g}^{-1}$ soil) in the hind-gut of the earthworm *Dravida papillifer papillifer* Steph. collected from the pine forest stand at high altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus niger</i>	-	-	-	-	-	0.333 (8.33)	-	-	-	-	-	-
2. <i>Cladosporium cladosporioides</i>	-	0.333 (10.00)	0.333 (7.69)	-	-	-	0.333 (14.29)	-	-	-	-	-
3. <i>Fusarium oxysporum</i>	-	-	-	-	1.000 (37.50)	-	-	-	-	-	-	-
4. <i>F. solani</i>	-	-	-	1.000 (27.27)	1.000 (37.50)	0.333 (8.33)	-	-	-	-	-	-
5. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	-	4.667 (100.00)
6. <i>Mortierella parvispora</i>	-	-	-	-	-	-	-	-	-	2.667 (57.14)	-	-
7. <i>Mucor hiemalis</i>	-	-	-	-	-	0.333 (8.33)	-	0.333 (3.45)	-	1.000 (21.43)	-	-
8. <i>M. racemosus</i>	-	-	-	-	-	-	-	0.333 (3.45)	1.000 (37.50)	-	-	-
9. <i>Penicillium brevicompactum</i>	-	2.000 (60.00)	-	1.333 (36.36)	-	-	-	-	1.667 (62.50)	-	-	-
10. <i>P. chrysogenum</i>	-	0.333 (10.00)	2.667 (61.54)	-	-	0.667 (16.67)	-	-	-	-	-	-
11. <i>P. frequentans</i>	-	-	-	-	-	-	-	2.000 (20.69)	-	-	-	-
12. <i>P. javanicum</i>	-	-	-	-	-	-	-	0.333 (3.45)	-	-	0.333 (11.11)	-
13. <i>P. daleae</i>	-	0.333 (10.00)	0.667 (15.38)	-	-	-	-	-	-	-	-	-
14. <i>P. digitatum</i>	-	-	-	-	-	-	-	6.667 (68.96)	-	-	-	-
15. <i>P. wakamatsii</i>	-	-	-	-	-	-	1.333 (57.14)	-	-	-	-	-
16. <i>Pythium intermedium</i>	1.667 (100.00)	-	-	0.333 (9.09)	0.667 (25.00)	1.000 (25.00)	-	-	-	-	2.667 (88.89)	-
17. <i>Trichoderma koningi</i>	-	0.333 (10.00)	-	0.333 (9.09)	-	1.333 (33.33)	-	-	-	-	-	-
18. White sterile mycelia	-	-	0.667 (15.38)	0.667 (18.18)	-	-	0.667 (28.57)	-	-	1.000 (21.43)	-	-

Table 3.7 Fungal population ($\times 10^2 \text{ g}^{-1}$ soil) in the fore-gut of the earthworm *Drawida papillifer papillifer* Steph. collected from the pine forest stand at low altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Alternaria alternata</i>	-	-	-	-	-	-	-	-	-	-	1.000 (23.08)	2.00 (17.14)
2. <i>Aspergillus carneus</i>	-	-	-	0.330 (6.00)	-	-	-	-	-	-	-	-
3. <i>A. candidus</i>	-	-	0.33 (1.25)	0.330 (6.00)	-	-	-	-	-	-	-	-
4. <i>A. flavus</i>	-	-	-	-	1.330 (4.65)	-	1.000 (9.68)	-	-	-	-	-
5. <i>A. japonicus</i>	-	0.067 (6.67)	-	-	-	-	-	-	-	-	-	-
6. <i>A. versicolor</i>	-	-	-	-	-	1.000 (6.00)	-	-	-	-	-	-
7. <i>Cladosporium cladosporioides</i>	-	-	-	2.000 (11.11)	-	-	-	-	-	-	-	0.330 (2.86)
8. <i>Fusarium sporotrichioides</i>	-	-	6.000 (22.50)	-	-	-	-	-	0.660 (13.33)	-	-	-
9. <i>Mucor hiemalis</i>	0.133 (12.90)	-	-	0.330 (6.00)	-	-	-	0.670 (2.60)	-	2.000 (26.09)	-	5.33 (45.72)
10. <i>M. circinalloides</i>	-	0.067 (6.67)	-	-	-	-	-	-	-	-	-	-
11. <i>M. racemosus</i>	-	-	-	-	-	-	7.320 (70.97)	-	-	-	-	-
12. <i>Penicillium brevicompactum</i>	-	-	-	-	16.700 (58.44)	10.030 (62.00)	-	-	-	-	0.660 (20.00)	0.660 (5.71)
13. <i>P. chrysogenum</i>	0.067 (16.13)	7.000 (70.00)	-	11.60 (64.81)	9.670 (33.72)	2.000 (12.00)	1.330 (12.90)	25.000 (97.40)	3.330 (66.67)	3.330 (43.47)	2.330 (53.84)	2.660 (22.86)
14. <i>P. javanicum</i>	-	-	-	0.330 (6.00)	-	-	-	-	-	-	-	0.660 (5.71)
15. <i>P. frequentans</i>	-	-	-	0.330 (6.00)	-	2.330 (14.00)	-	-	-	-	-	-
16. <i>Pythium intermedium</i>	-	-	1.330 (5.00)	1.330 (7.41)	-	-	-	-	-	-	-	-
17. <i>Phytosopus stolonifer</i>	-	-	-	-	0.670 (2.33)	1.000 (6.00)	-	-	-	-	-	-
18. <i>Trichoderma harzianum</i>	-	-	19.00 (71.25)	-	-	-	-	-	-	-	-	-
19. <i>T. koningi</i>	7.32 (70.97)	1.660 (16.67)	-	-	0.330 (1.16)	-	-	-	0.660 (13.33)	2.000 (26.09)	-	-
20. <i>Verticillium albo-atrum</i>	-	-	-	-	-	-	-	-	0.330 (6.67)	-	-	-
21. White sterile mycelia	-	-	-	1.330 (7.41)	-	-	0.670 (6.45)	-	-	0.330 (4.35)	-	-

Table 3.9 Fungal population ($\times 10^2$ g⁻¹ soil) in the mid-gut of the earthworm *Druidia papillifer papillifer* Steph. collected from the pine forest stand at low altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus candidus</i>	-	-	-	-	0.670 (4.56)	-	-	-	-	-	-	0.330 (3.57)
2. <i>A. carneus</i>	0.330 (6.67)	-	-	-	-	-	-	-	-	-	-	-
3. <i>A. flavus</i>	-	0.330 (9.09)	-	5.000 (21.43)	0.670 (4.65)	-	-	-	-	-	-	-
4. <i>A. terreus</i>	-	-	-	0.670 (2.86)	-	-	-	-	-	-	-	-
5. <i>A. waziti</i>	-	-	0.330 (3.03)	-	1.000 (6.98)	-	-	-	-	-	-	-
6. <i>Clavibotryella elegans</i>	-	-	-	-	1.330 (9.30)	-	-	-	-	-	-	-
7. <i>Cladosporium cladosporioides</i>	-	-	0.330 (3.03)	-	-	0.033 (2.63)	-	-	0.066 (11.11)	-	-	-
8. <i>C. herbarum</i>	0.330 (6.67)	-	-	-	-	-	-	-	0.033 (5.55)	-	-	0.330 (3.57)
9. <i>Fusarium oxysporum</i>	-	-	-	-	-	-	-	-	-	0.330 (4.55)	-	-
10. <i>F. sporotrichioides</i>	-	-	0.330 (3.03)	-	-	-	-	-	-	-	-	-
11. <i>Mortierella parvispora</i>	-	-	-	-	-	-	-	3.000 (28.12)	-	-	-	-
12. <i>Mucor hiemalis</i>	-	0.330 (9.09)	-	-	1.330 (9.30)	0.033 (2.63)	-	-	-	1.660 (22.72)	1.330 (40.00)	7.330 (78.58)
13. <i>Penicillium brevicompactum</i>	0.330 (6.67)	1.661 (45.46)	-	-	5.000 (34.88)	-	-	-	-	-	0.660 (20.00)	0.330 (3.57)
14. <i>P. chrysogenum</i>	0.330 (6.67)	-	0.330 (3.03)	12.33 (52.86)	2.330 (16.28)	-	13.00 (81.25)	2.330 (21.88)	2.330 (38.89)	3.000 (40.91)	1.000 (30.00)	0.330 (3.57)
15. <i>P. frequentans</i>	-	-	-	-	-	-	-	-	2.330 (38.89)	-	-	-
16. <i>P. javanicum</i>	-	-	-	2.330 (10.00)	1.000 (6.98)	5.670 (44.74)	-	-	-	-	-	0.660 (7.14)
17. <i>P. janthinellum</i>	2.000 (40.00)	-	-	-	0.330 (2.33)	4.330 (34.21)	-	-	-	-	-	-
18. <i>P. wakamatsii</i>	-	-	-	-	-	1.330 (10.53)	-	-	-	-	0.330 (10.00)	-
19. <i>Pythium tetramedum</i>	1.000 (20.00)	1.000 (27.27)	2.330 (21.21)	0.330 (1.42)	-	-	-	-	-	-	-	-
20. <i>Trichoderma koningii</i>	-	0.330 (9.09)	1.330 (12.12)	1.000 (4.28)	0.670 (4.65)	-	3.000 (18.75)	2.000 (18.75)	-	-	-	-
21. <i>T. harzianum</i>	-	-	4.67 (42.43)	0.670 (2.86)	-	-	-	-	-	-	-	-
22. <i>Rhizopus stolonifer</i>	-	-	-	-	-	0.067 (5.26)	-	3.330 (31.25)	-	-	-	-
23. <i>Verticillium albo-atrum</i>	-	-	-	-	-	-	-	-	0.033 (5.25)	-	-	-
24. White sterile mycelia	0.660 (13.33)	-	1.330 (12.12)	1.000 (4.28)	-	-	-	-	-	2.330 (5.26)	-	-

Table 4.0 Fungal population (per g soil x 10³) in the mid-gut of the earthworm collected from the pine forest stand at lower altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus carneus</i>	0.330 (6.67)	-	-	-	-	-	-	-	-	-	-	-
2. <i>A. flavus</i>	-	0.330 (2.77)	-	-	-	-	-	-	-	-	-	-
3. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	-	0.330 (7.69)	-
4. <i>C. herbarum</i>	0.330 (6.67)	-	-	-	-	-	-	-	-	-	-	-
5. <i>Claustrophiala elegans</i>	-	-	-	-	0.330 (12.50)	-	-	-	-	-	-	-
6. <i>Fusarium solani</i>	-	-	1.660 (33.33)	0.660 (50.00)	0.330 (12.50)	-	-	-	-	-	-	-
7. <i>F. sporotrichioides</i>	-	-	-	-	-	-	-	-	1.660 (71.43)	-	-	-
8. <i>Hemicolia sp.</i>	-	-	-	-	-	-	-	-	-	-	0.330 (7.69)	4.660 (100.00)
9. <i>Mortierella parvispora</i>	-	-	-	-	-	0.330 (7.14)	-	0.330 (10.00)	-	1.330 (57.14)	3.330 (76.92)	-
10. <i>Mucor hiemalis</i>	-	2.330 (19.44)	-	0.660 (50.00)	1.000 (37.50)	1.660 (35.71)	-	1.000 (30.00)	-	1.000 (42.86)	-	-
11. <i>M. circinaloides</i>	-	0.660 (5.56)	-	-	-	2.000 (42.86)	-	-	-	-	-	-
12. <i>M. racemosus</i>	-	-	-	-	-	-	1.670 (100.00)	-	-	-	-	-
13. <i>Penicillium brevicompactum</i>	0.330 (6.67)	0.660 (5.56)	0.066 (13.33)	-	-	-	-	1.330 (40.00)	0.660 (28.57)	-	-	-
14. <i>P. chrysogenum</i>	0.330 (6.67)	0.660 (5.56)	1.330 (26.67)	-	0.330 (12.50)	-	-	-	-	-	-	-
15. <i>P. javanicum</i>	-	-	1.330 (26.67)	-	-	-	-	-	-	-	-	-
16. <i>P. janthinellum</i>	2.000 (40.00)	-	-	-	-	-	-	-	-	-	-	-
17. <i>Pythium intermedium</i>	1.000 (20.00)	0.330 (2.77)	-	-	-	-	-	0.660 (20.00)	-	-	-	-
18. <i>Trichoderma kovingii</i>	-	-	-	-	0.660 (25.00)	0.660 (14.29)	-	-	-	-	-	-
19. White sterile mycelia	0.660 (13.33)	7.000 (58.33)	-	-	-	-	-	-	-	-	0.330 (7.69)	-

Table 4.1 Fungal population ($\times 10^2$ g⁻¹ soil) in the hind-gut of the earthworm *Dravidia papillifer papillifer* Steph. collected from the pine forest stand at low altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus flavus</i>	-	-	1.000 (10.00)	9.000 (22.50)	-	-	-	-	-	-	-	-
2. <i>A. niger</i>	-	-	-	-	-	-	-	-	-	-	-	0.330 (8.33)
3. <i>A. terreus</i>	-	-	-	3.670 (9.17)	-	-	-	-	-	-	-	-
4. <i>Claosporium elegans</i>	-	-	-	3.000 (7.50)	-	-	-	-	-	-	-	-
5. <i>Cladosporium cladosporioides</i>	-	-	0.330 (3.33)	0.670 (1.67)	-	-	0.330 (2.63)	-	3.660 (57.89)	-	-	-
6. <i>C. herbarum</i>	-	-	-	-	-	-	-	-	-	3.660 (57.89)	-	-
7. <i>Fusarium oxysporum</i>	-	-	1.670 (16.67)	-	-	-	-	-	-	-	-	-
8. <i>F. solani</i>	-	0.330 (6.25)	-	-	-	-	-	-	-	-	-	-
9. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	-	1.330 (33.34)
10. <i>Mortierella parvispora</i>	-	-	-	-	-	-	-	0.670 (25.00)	-	-	-	-
11. <i>M. hiemalis</i>	5.00 (51.18)	-	-	-	0.330 (2.94)	0.330 (16.67)	-	-	-	-	-	-
12. <i>M. circinalloides</i>	-	0.330 (6.25)	-	-	-	-	-	-	-	-	-	-
13. <i>Penicillium brevicompactum</i>	-	-	-	-	2.000 (17.65)	-	-	-	-	-	1.660 (38.46)	-
14. <i>P. chrysogenum</i>	4.00 (41.40)	3.000 (56.25)	1.000 (10.00)	22.00 (55.00)	6.000 (52.94)	0.670 (33.33)	6.330 (50.00)	0.670 (25.00)	1.000 (15.79)	1.000 (15.79)	0.330 (7.89)	1.660 (41.66)
15. <i>P. frequentans</i>	-	-	0.330 (3.33)	-	0.670 (5.88)	-	-	-	-	-	-	-
16. <i>P. javanicum</i>	-	-	0.670 (6.67)	-	2.000 (17.65)	-	-	-	-	-	-	-
17. <i>P. janthinellum</i>	0.330 (3.40)	-	-	-	-	-	-	-	-	-	-	0.660 (16.67)
18. <i>P. wakamarii</i>	-	-	-	-	-	0.330 (16.67)	-	-	-	-	1.660 (38.46)	-
19. <i>Pythium intermedium</i>	0.330 (3.40)	-	-	-	-	-	-	-	-	-	-	-
20. <i>Rhizopus stolonifer</i>	-	0.670 (12.50)	-	0.033 (0.01)	0.330 (2.94)	0.670 (33.33)	3.660 (28.95)	-	0.660 (10.53)	0.660 (10.53)	-	-
21. <i>Trichoderma harzianum</i>	-	-	4.330 (43.34)	1.330 (3.33)	-	-	-	-	-	-	-	-
22. <i>T. korringii</i>	-	1.000 (8.75)	0.330 (3.33)	-	-	-	-	0.670 (25.00)	0.660 (10.53)	0.660 (10.53)	-	-
23. <i>Verticillium albo-atrum</i>	-	-	-	-	-	-	-	-	0.330 (5.26)	-	-	-
24. White sterile mycelia	-	-	0.330 (3.33)	-	-	-	2.330 (18.42)	0.670 (25.00)	-	0.330 (5.26)	0.660 (15.39)	-

Table 4.2 Fungal population ($\times 10^2$ g⁻¹ soil) in the hind-gut of the earthworm *Dravida papillifer papillifer* Steph. collected from the pine forest stand at low altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus flavus</i>	0.330 (6.67)	0.330 (7.69)	-	-	-	-	-	-	-	-	-	-
2. <i>Claosporium elegans</i>	-	-	-	0.670 (50.00)	-	-	-	-	-	-	-	-
3. <i>Cladosporium cladosporioides</i>	0.330 (6.67)	-	-	-	-	-	-	-	-	-	-	-
4. <i>C. herbarum</i>	-	0.330 (7.69)	-	-	-	-	-	-	-	-	-	-
5. <i>Fusarium solari</i>	-	-	-	-	-	-	-	-	2.000 (54.55)	-	-	-
6. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	-	3.000 (100.00)
7. <i>Mortierella parvispora</i>	-	-	-	-	-	0.330 (7.69)	-	0.670 (20.00)	-	-	1.000 (100.00)	-
8. <i>Mucor hiemalis</i>	0.660 (13.33)	-	-	0.330 (25.00)	1.000 (100.00)	2.660 (61.54)	-	2.000 (60.00)	-	1.000 (75.00)	-	-
9. <i>M. circinaloides</i>	-	-	-	-	-	0.330 (7.69)	-	-	-	-	-	-
10. <i>M. racemosus</i>	-	-	-	-	-	-	0.670 (40.00)	-	-	-	-	-
11. <i>Paricbium brevicompactum</i>	-	-	-	-	-	0.330 (7.69)	-	-	0.670 (18.18)	-	-	-
12. <i>P. chrysoerum</i>	-	1.330 (30.77)	-	-	-	-	0.330 (20.00)	-	-	-	-	-
13. <i>P. frequentans</i>	-	0.330 (7.69)	-	-	-	-	-	-	-	-	-	-
14. <i>P. javanicum</i>	-	-	0.330 (9.09)	-	-	-	-	-	-	-	-	-
15. <i>P. jarthirallum</i>	1.660 (33.33)	-	-	-	-	-	-	-	-	-	-	-
16. <i>P. daleae</i>	-	1.330 (30.77)	-	-	-	-	-	-	-	-	-	-
17. <i>Pythium intermedium</i>	0.330 (6.67)	-	-	-	-	-	-	0.670 (20.00)	-	-	-	-
18. <i>Trichoderma kovringii</i>	-	-	3.000 (81.82)	0.330 (25.00)	-	0.670 (15.38)	0.670 (40.00)	-	1.000 (27.27)	-	-	-
19. White sterile zygoclia	1.660 (33.33)	0.660 (15.38)	0.330 (9.09)	-	-	-	-	-	-	3.000 (25.00)	-	-

P. javanicum, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum* and white sterile mycelia. The fungal species isolated from the hind-gut region were *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. wentii*, *Cladosporium cladosporioides*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. circinelloides*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. daleae*, *P. digitatum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, and white sterile mycelia.

Altogether a total of 21, 25 and 24 fungal species were isolated from the fore-, mid- and hind- gut regions during the first year and a total of 20, 19 and 19 fungal species were isolated from the same regions during the second year of study at low altitude (Tables 3.7- 4.2). The fungal species isolated from the fore-gut region during the study period were *Alternaria alternata*, *Aspergillus candidus*, *A. carneus*, *A. flavus*, *A. japonicus*, *A. terreus*, *A. versicolor*, *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Fusarium solani*, *F. sporotrichioides*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. circinelloides*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. daleae*, *P. frequentans*, *P. janthinellum*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, *Verticillium alboatrum* and white sterile mycelia. The fungal species isolated from the mid-gut region were *Aspergillus candidus*, *A. carneus*, *A. flavus*, *A. terreus*, *A. wentii*, *C. cladosporioides*, *C. herbarum*, *Cunninghamella elegans*, *Fusarium oxysporum*, *F. solani*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. circinelloides*, *M. racemosus*, *P. brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. janthinellum*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *T. koningii*, *T. harzianum*, *Verticillium alboatrum* and white sterile mycelia. The fungal species isolated from

Table 4.3 List of fungi isolated from soil, gut contents of earthworm *Dravidia papillifer papillifer* Steph. and earthworm casts collected from the pine forest stands at high altitude and at low altitude during the study period.

Fungi	Soil	High Altitude			Casts	Soil	Low Altitude			Casts
		Gut contents					Gut contents			
		Fore-gut	Mid-gut	Hind-gut			Fore-gut	Mid-gut	Hind-gut	
1. <i>Absidia cyindrospora</i>	+	-	-	-	-	+	-	-	-	-
2. <i>A. glauca</i>	-	-	-	-	-	+	-	-	-	-
3. <i>Acromonium</i> sp.	-	-	-	-	+	-	-	-	-	+
4. <i>Alternaria alternata</i>	-	-	-	-	-	-	+	-	-	-
5. <i>A. terreus</i>	-	-	-	-	+	-	-	-	-	-
6. <i>Aspergillus candidus</i>	+	-	-	+	-	+	+	+	-	+
7. <i>A. carneus</i>	-	+	+	-	-	-	+	+	-	-
8. <i>A. flavus</i>	+	+	+	+	-	+	+	+	+	-
9. <i>A. japonicus</i>	+	-	-	-	-	+	+	-	-	-
10. <i>A. niger</i>	+	-	-	+	-	+	-	-	-	-
11. <i>A. terreus</i>	+	+	-	-	-	-	+	+	+	-
12. <i>A. wentii</i>	-	+	+	+	-	-	-	+	-	-
13. <i>A. versicolor</i>	-	-	-	-	-	+	-	-	-	-
14. <i>Botrytis</i> sp.	+	-	-	-	-	-	-	-	-	-
15. <i>Cladosporium herbarium</i>	-	+	-	-	-	+	-	+	-	-
16. <i>C. macrocarpum</i>	-	+	-	-	-	-	-	-	-	-
17. <i>C. cladosporioides</i>	+	+	+	+	+	+	+	+	+	+
18. <i>Cucurbitulicium elegans</i>	+	-	-	-	-	+	+	+	+	+
19. <i>Fusicium moniliforme</i>	-	+	-	+	-	-	-	-	-	-
20. <i>F. oxysporum</i>	+	+	+	+	-	+	-	+	+	+
21. <i>F. solani</i>	+	+	+	+	+	+	+	+	+	+
22. <i>F. varicosoides</i>	-	-	-	-	-	+	-	-	-	-
23. <i>F. sporotrichioides</i>	-	+	-	-	-	+	-	-	-	-
24. <i>Hemicola</i> sp.	+	+	+	+	+	+	+	+	+	+
25. <i>Mortierella parvispora</i>	+	+	+	+	-	-	+	+	+	+
26. <i>Mucor circinalioides</i>	-	-	-	+	+	+	+	+	+	-
27. <i>M. hiemalis</i>	+	+	+	+	+	+	+	+	+	+
28. <i>M. plumbeus</i>	+	-	-	-	-	-	-	-	-	-
29. <i>M. racemosus</i>	+	+	+	+	+	-	+	+	+	-
30. <i>Oidodendron</i> sp.	-	-	-	-	-	-	-	-	-	-
31. <i>Penicillium brevicompactum</i>	+	+	+	+	+	+	+	+	+	+
32. <i>P. chrysogenum</i>	+	+	+	+	+	+	+	+	+	+
33. <i>P. doleae</i>	-	+	+	+	-	-	+	-	-	-
34. <i>P. digitatum</i>	-	+	+	+	-	-	-	-	-	-
35. <i>P. frequentans</i>	+	+	+	+	+	+	+	+	+	+
36. <i>P. javanicum</i>	-	-	+	-	-	-	+	+	+	+
37. <i>P. javanicum</i>	+	+	+	+	+	+	+	+	+	+
38. <i>P. rubrum</i>	+	-	-	-	-	+	-	-	-	-
39. <i>P. wakamatsii</i>	+	-	+	+	+	+	+	+	+	+
40. <i>Pythium intermedium</i>	+	+	+	+	+	+	+	+	+	+
41. <i>Phaeoascus stolonifer</i>	+	+	+	+	+	+	+	+	+	+
42. <i>Trichoderma koningii</i>	+	+	+	+	+	+	+	+	+	+
43. <i>T. harzianum</i>	+	+	-	+	-	-	+	+	+	-
44. <i>Verticillium albo-atrum</i>	-	-	-	-	-	-	+	+	+	-
45. White sterile mycelia	+	+	-	+	+	+	+	+	+	+
46. Black sterile mycelia	+	-	-	-	+	-	-	-	-	+
47. Yellow sterile mycelia	-	-	-	-	+	-	-	-	-	+

Present = +, Absent = -

the hind-gut region were *Aspergillus flavus*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *C. herbarum*, *Cunninghamella elegans*, *Fusarium oxysporum*, *F. solani*, *F. sporotrichioides*, *Mortierella parvispora*, *Mucor hiemalis*, *M. circinelloides*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. janthinellum*, *P. javanicum*, *P. daleae*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, *Verticillium alboatrum* and white sterile mycelia. Tables 3.1- 4.2 indicate that qualitatively, there was not much difference in the composition of the fungal flora in the gut contents of the earthworm at both the study sites except for *Cladosporium macrocarpum*, *Fusarium moniliforme* and *Penicillium digitatum* which could be isolated from the gut regions of the earthworm collected from high altitude only and *Aspergillus japonicus*, *A. versicolor*, *Alternaria alternata*, *Cunninghamella elegans* and *Verticillium alboatrum* which could be isolated from the gut regions of earthworm collected from low altitude only.

Table 4.3 shows a comparative study of the fungal flora present in the soil, gut contents of the earthworm and the casts collected from the pine forest stands at both the altitudes. Altogether a total of 28 fungal species could be isolated from the soil, 32 from the gut contents of the earthworm and 19 from the casts at high altitude. Of the 32 fungal species occurring in the different gut regions, 26 species could be isolated from the foregut, 23 from the midgut, and 25 from the hindgut regions. A total of 28 fungal species could be isolated from the soil, 32 from the gut contents and 21 from the casts at low altitude. Of the 32 fungal species occurring in the different gut regions, 29 species could be isolated from the foregut, 27 from the midgut and 26 from the hindgut regions. From the table, it is evident that the earthworm gut contained higher number of fungal species as compared to that of the surrounding soil. The fungal species found to be common in the soil, earthworm gut and the casts at high altitude include *Cladosporium cladosporioides*, *Fusarium solani*, *Humicola sp.*, *Mucor hiemalis*, *M. racemosus*, *Penicillium*

brevicompactum, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia. *Aspergillus carneus*, *A. wentii*, *Cladosporium herbarum*, *C. macrocarpum*, *Fusarium sporotrichioides*, *F. moniliforme*, *Penicillium daleae*, *P. digitatum* and *P. janthinellum* were found to be present in the gut only, while, *Absidia cylindrospora*, *Aspergillus japonicus*, *Botrytis* sp. and *Mucor plumbeus* were restricted to the soil only. The species found throughout the digestive tract were *Aspergillus flavus*, *A. wentii*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. daleae*, *P. digitatum*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia. Comparison in the different gut regions of the earthworm showed that *Aspergillus carneus* was found to be present in the foregut and midgut regions. *Aspergillus terreus*, *Cladosporium herbarum*, *C. macrocarpum* and *Fusarium sporotrichioides* were found to be present in the foregut only, *Penicillium janthinellum* was restricted to the midgut region only while *Aspergillus niger*, *A. niger* and *Mucor circinelloides* were found to be present in the hindgut region only. *P. waksmanii* was found to be present in the midgut and hindgut only while *Acremonium* sp., *Alternaria tenuis* and yellow sterile mycelia could be isolated from the casts only.

The fungal species found to be common in the soil, earthworm gut and the casts at low altitude include *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Fusarium solani*, *Humicola* sp., *Mortierella parvispora*, *Mucor hiemalis*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia. *Aspergillus terreus*, *A. wentii*, *Mucor racemosus*, *Penicillium daleae*, *Trichoderma harzianum* and *Verticillium albo-atrum*

Fig. 11 (a&b) Monthly variation in the moisture content of undecomposed (UL) and partially decomposed (UL) pine litters of two pine forest stands.

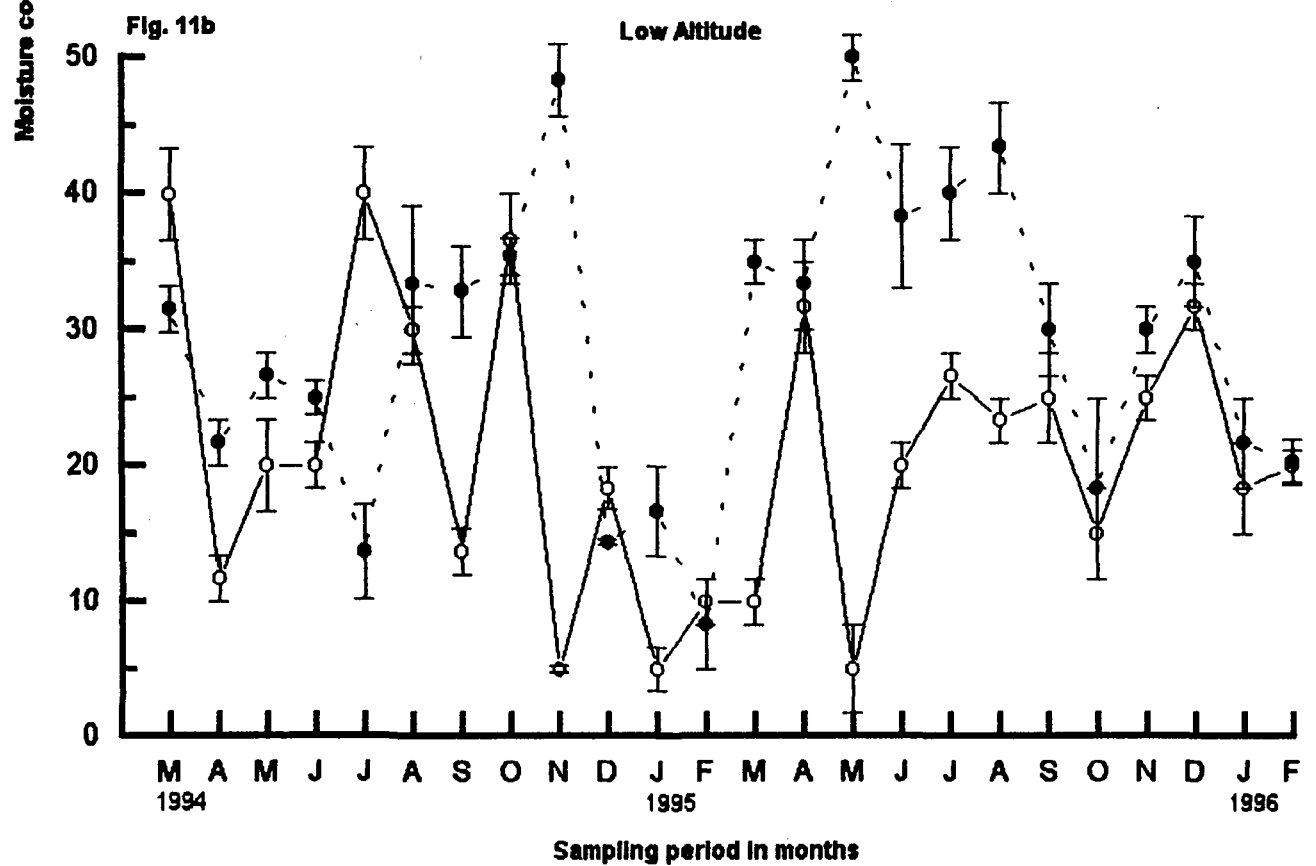
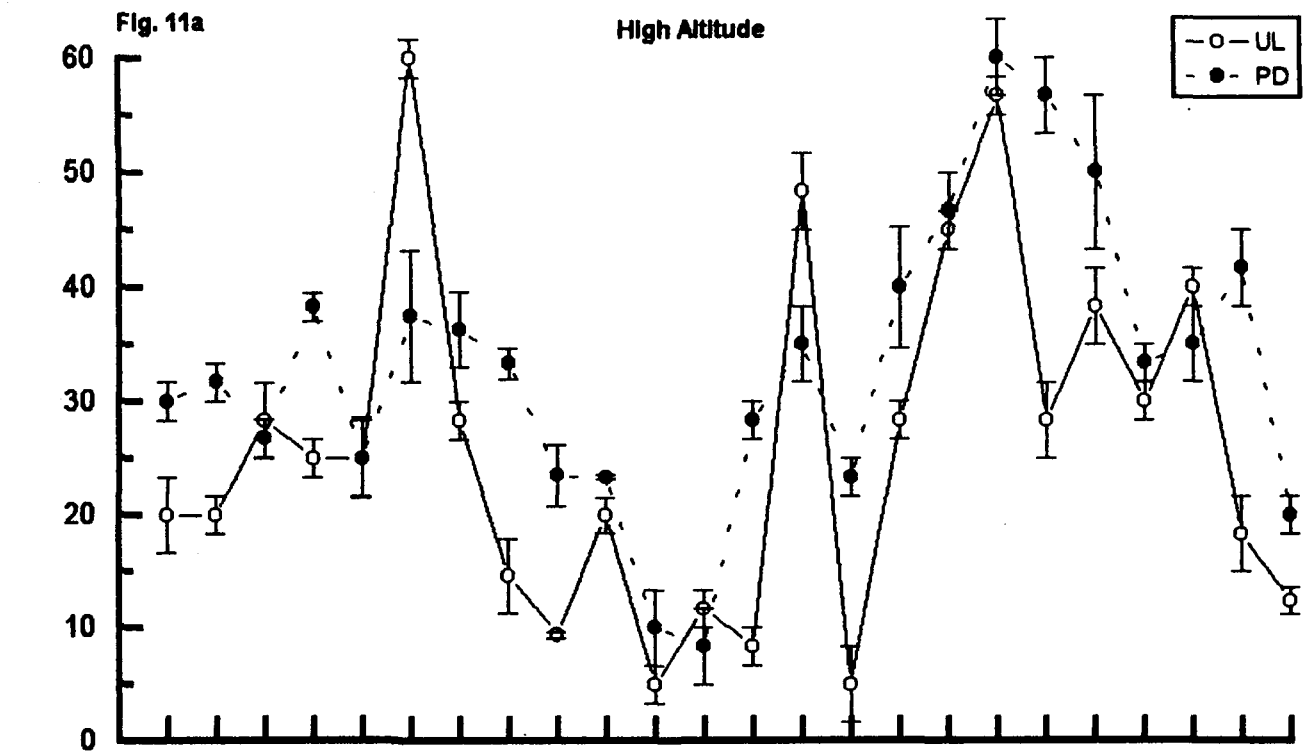
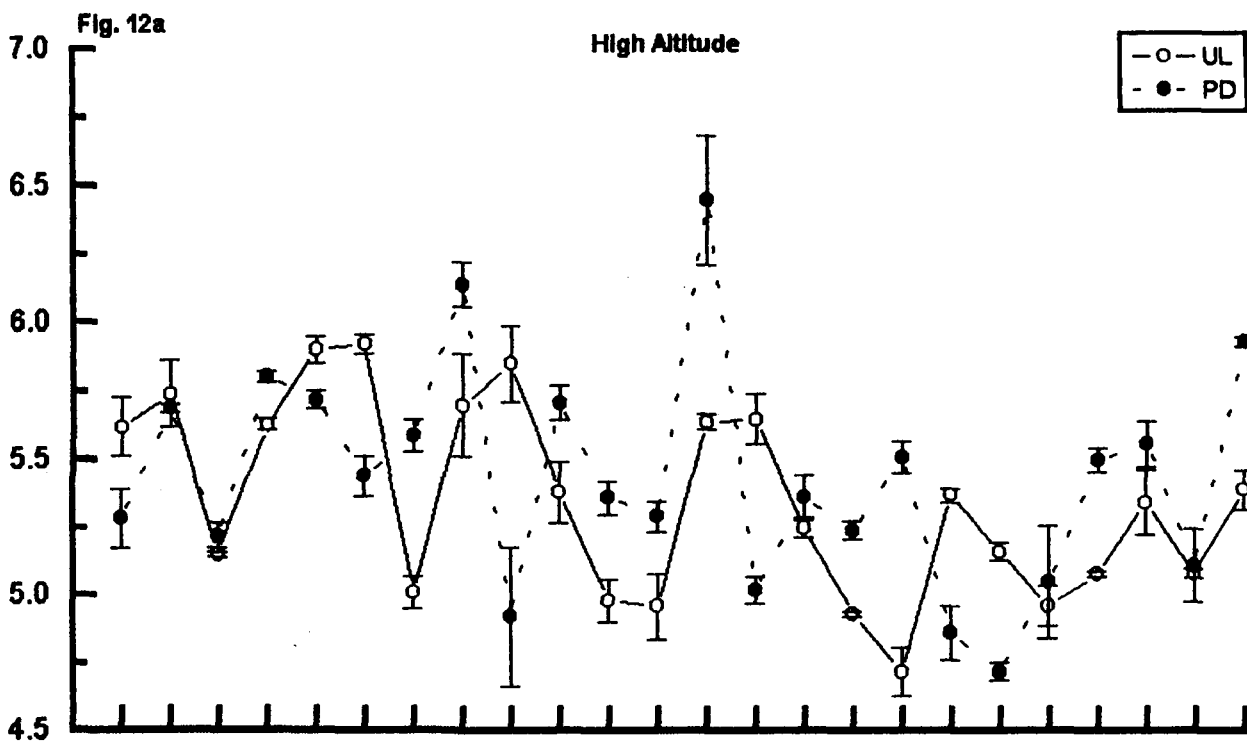


Fig. 12 (a&b) Monthly variation in pH of undecomposed (UL) and partially decomposed (PD) pine litters of two pine forest stands.



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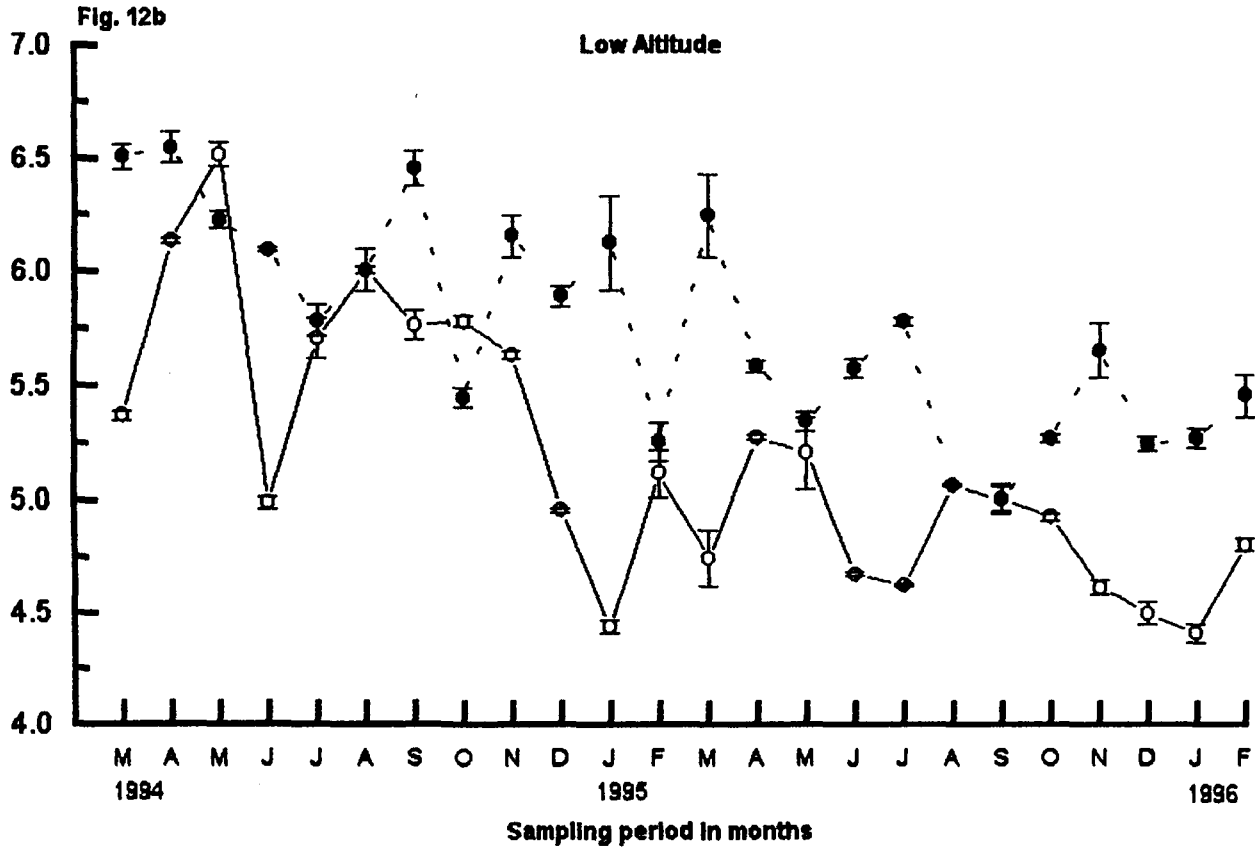
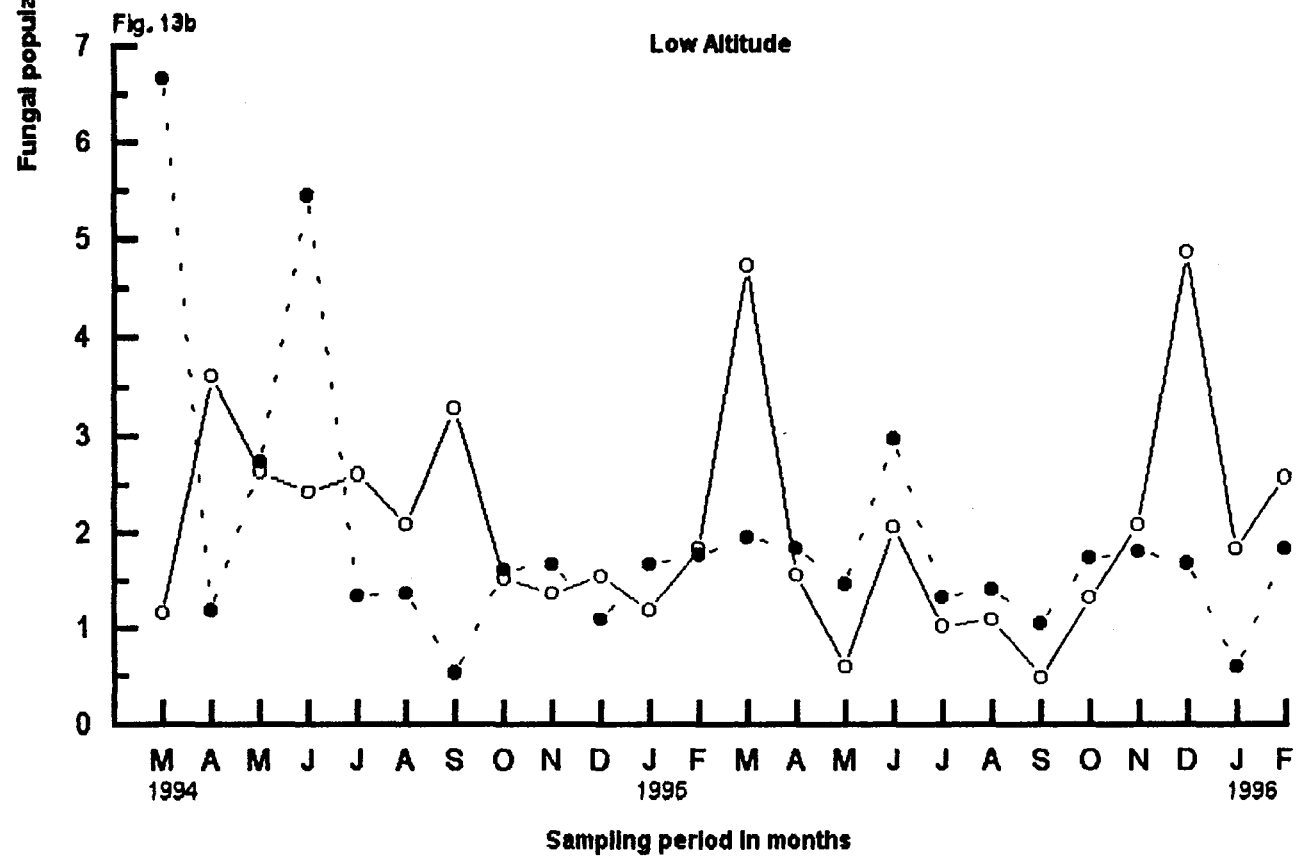
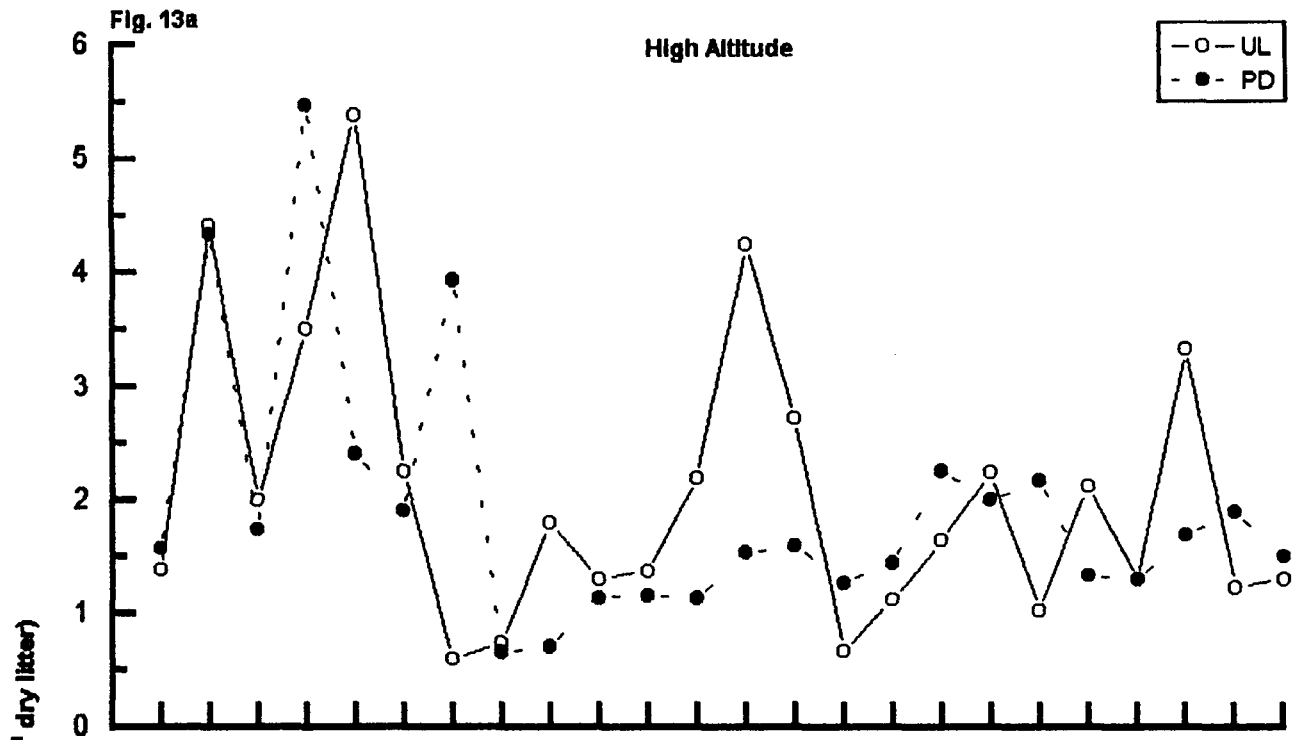


Fig. 13 (a&b) Monthly variation in fungal population of undecomposed (UL) and partially decomposed (PD) pine litters of two pine forest stands.



were found to be present in the gut only while *Absidia cylindrospora*, *A. glauca*, *Fusarium poae*, *F. merismoides*, *Oldodendron* sp. *Penicillium rubrum* were found to be present in the soil only. The fungal species found throughout the digestive tract were *Aspergillus flavus*, *A. terreus*, *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Fusarium solani*, *F. sporotrichioides*, *Humicola* sp., *Mucor hiemalis*, *M. circinelloides*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, *Verticillium alboatrum* and white sterile mycelia. Comparison in the different gut regions of the earthworm showed that *Aspergillus carneus* and *A. candidus* were found to be present in the foregut and the midgut only while *Cladosporium herbarum*, *Fusarium oxysporum* were found in the midgut and the hindgut only. *Alternaria alternata*, *Aspergillus japonicus*, *A. versicolor* and *Fusarium sporotrichioides* were found to be present in the foregut, *Aspergillus wentii* in the midgut and *Aspergillus niger* was found to be present in the hindgut only. *Acremonium* sp., black sterile mycelia and yellow sterile mycelia were found to be present in the casts only.

Moisture content and pH of undecomposed litter and partially decomposed litter

Percentage moisture content of undecomposed and partially decomposed pine litters of both the study sites are depicted in figs. 11a & b. Moisture content of undecomposed and partially decomposed pine litter at high altitude was generally higher than that at low altitude. When comparison was made between the moisture content of both the litter types, it was found that the moisture content of the partially decomposed pine litter was more than that of the undecomposed litter at both the study sites. pH of the undecomposed litter was more acidic at both the study sites (figs. 12 a & b). There was not much difference in the pH of the litter collected from the two study sites. However, pH of the partially decomposed litter at high altitude was more acidic as compared to that at low altitude (figs. 12a & b). When comparison

Table 5.1. Fungal population ($10^3 \times \text{g}^{-1}$ dry litter) associated with the undecomposed pine litter collected from the pine forest stand at high altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Acromonium</i> sp.	-	-	-	0.266 (7.60)	-	-	-	-	-	-	-	-
2. <i>Attermaria tenuis</i>	-	-	-	-	-	-	-	-	-	-	-	0.716 (32.76)
3. <i>Aspergillus carneus</i>	-	-	-	0.088 (2.53)	-	-	-	-	-	-	-	-
4. <i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-	0.280 (20.51)	-
5. <i>A. terreus</i>	-	-	-	0.088 (2.53)	-	-	-	-	-	-	-	-
6. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	0.110 (6.12)	-	0.035 (2.54)	-
7. <i>Fusarium oxysporum</i>	-	-	1.255 (62.79)	0.088 (2.53)	0.257 (4.76)	-	-	0.078 (10.53)	-	-	-	-
8. <i>F. solani</i>	-	-	0.093 (4.65)	-	-	-	-	-	-	0.041 (3.23)	-	-
9. <i>F. poae</i>	-	-	-	-	1.796 (33.33)	-	-	-	-	-	-	-
10. <i>F. sporotrichioides</i>	-	-	-	-	-	-	-	0.390 (52.63)	-	-	-	-
11. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.105 (7.69)	-
12. <i>Mucor hiemalis</i>	-	0.375 (8.50)	-	-	-	-	-	-	0.515 (28.57)	0.041 (3.23)	-	-
13. <i>M. racemosus</i>	-	-	-	-	-	-	0.093 (15.39)	-	-	-	-	-
14. <i>Penicillium brevicompactum</i>	0.166 (12.12)	-	-	-	-	-	0.046 (7.69)	0.078 (10.53)	-	-	-	0.150 (6.90)
15. <i>P. chrysogenum</i>	0.291 (21.21)	3.125 (70.75)	-	2.222 (63.29)	2.180 (40.48)	-	-	-	0.735 (40.82)	0.209 (16.12)	0.350 (25.64)	0.876 (3.45)
16. <i>P. frequentans</i>	-	-	-	-	-	0.167 (7.41)	-	-	-	0.084 (6.45)	-	-
17. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.183 (10.20)	0.041 (3.23)	-	-
18. <i>Pythium intermedium</i>	0.791 (57.58)	-	-	0.355 (10.13)	-	1.583 (70.37)	-	-	0.257 (14.29)	0.834 (64.51)	-	1.018 (46.55)
19. <i>Rhizopus stolonifer</i>	-	0.916 (20.75)	0.511 (25.58)	0.088 (2.53)	1.153 (21.43)	-	0.046 (7.69)	0.196 (26.31)	-	-	-	0.837 (1.72)
20. <i>Trichoderma harzianum</i>	-	-	0.046 (2.33)	-	-	-	-	-	-	-	-	-
21. <i>T. korringii</i>	0.125 (9.09)	-	-	0.311 (8.86)	-	0.083 (3.70)	0.046 (7.69)	-	-	0.041 (3.23)	0.385 (28.21)	0.076 (3.45)
22. White sterile mycelia	-	-	0.093 (4.65)	-	-	0.417 (18.52)	0.372 (61.54)	-	-	-	-	-
23. Yellow sterile mycelia	-	-	-	-	-	-	-	-	-	-	0.210 (15.38)	-

Table 5.2 Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the undecomposed pine litter collected from the pine forest stand at high altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Acromorium</i> sp.	0.204 (4.81)	-	-	0.046 (4.16)	-	-	-	-	-	-	-	-
2. <i>Attervaria tenuis</i>	1.305 (30.77)	-	-	-	-	-	-	-	-	-	-	-
3. <i>Aspergillus carneus</i>	-	-	-	-	-	-	-	-	-	-	0.040 (3.33)	-
4. <i>A. flavus</i>	-	-	-	0.046 (4.16)	-	-	-	-	-	-	0.040 (3.33)	-
5. <i>Cladosporium cladosporioides</i>	-	0.238 (30.95)	-	-	-	-	-	0.162 (7.69)	0.095 (7.41)	0.667 (20.00)	0.204 (16.67)	0.342 (26.67)
6. <i>Fusarium oxysporum</i>	-	-	0.182 (27.27)	-	-	-	-	-	-	-	-	-
7. <i>F. solani</i>	-	-	0.060 (9.09)	-	-	-	-	-	-	-	-	-
8. <i>F. sporotrichioides</i>	-	-	-	-	-	-	-	0.918 (43.59)	-	-	-	-
9. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.857 (70.00)	0.950 (73.53)
10. <i>Mucor hiemalis</i>	0.408 (9.61)	0.967 (35.71)	-	-	-	-	0.418 (40.91)	0.432 (20.51)	0.476 (37.04)	0.500 (15.00)	-	-
11. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	0.186 (18.18)	0.162 (7.69)	-	-	-	-
12. <i>P. chrysogenum</i>	0.694 (16.35)	0.387 (14.29)	-	-	-	-	-	-	-	0.055 (1.67)	0.082 (6.67)	-
13. <i>P. frequentans</i>	0.204 (4.81)	-	-	-	-	0.154 (6.90)	-	-	-	-	-	-
14. <i>P. javanicum</i>	-	-	-	-	-	-	-	0.270 (12.84)	0.238 (18.51)	-	-	-
15. <i>Pythium intermedium</i>	0.204 (4.81)	-	-	1.023 (91.67)	1.636 (100.00)	2.001 (89.66)	-	-	-	-	1.833 (55.00)	-
16. <i>Rhizopus stolonifer</i>	-	0.064 (2.38)	0.242 (36.36)	-	-	-	0.418 (40.91)	-	-	0.500 (15.00)	-	-
17. <i>Trichoderma korringii</i>	1.184 (27.88)	-	0.182 (27.27)	-	-	-	0.279 (27.27)	-	-	0.11 (3.38)	-	-
18. White sterile mycelia	0.040 (0.96)	0.451 (16.67)	-	-	-	-	0.093 (9.09)	0.162 (7.69)	0.476 (37.04)	-	-	-
19. Yellow sterile mycelia	-	-	-	-	-	0.077 (3.44)	-	-	-	-	-	-

Table 5.3 Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the undecomposed pine litter collected from the pine forest stand at low altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Alternaria alternata</i>	0.111 (14.29)	-	-	-	-	-	-	-	-	-	-	-
2. <i>A. tenuis</i>	-	0.453 (12.50)	-	-	-	-	-	-	-	-	-	-
3. <i>Aspergillus candidus</i>	-	-	-	-	-	0.047 (3.22)	-	-	-	-	-	-
4. <i>A. niger</i>	-	-	-	-	-	-	-	-	-	0.040 (2.63)	-	-
5. <i>A. terreus</i>	-	-	0.041 (1.59)	-	-	-	-	-	-	-	-	-
6. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	-	-	0.074 (4.00)
7. <i>Fusarium oxysporum</i>	-	-	-	0.041 (1.72)	0.173 (6.67)	-	-	-	-	-	-	-
8. <i>F. solani</i>	0.333 (42.86)	-	-	-	0.809 (31.11)	-	-	-	-	-	-	1.837 (56.00)
9. <i>F. poae</i>	-	-	-	0.084 (3.45)	0.462 (17.78)	-	-	-	-	-	-	-
10. <i>F. sporotrichioides</i>	-	-	-	-	-	0.191 (12.90)	-	-	-	-	-	-
11. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	-	0.597 (30.00)	-
12. <i>Mucor hiemalis</i>	-	-	-	-	-	-	0.078 (2.35)	0.422 (27.59)	0.982 (71.79)	0.257 (55.26)	-	-
13. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	-	0.158 (10.34)	-	-	-	-
14. <i>P. chrysogenum</i>	0.055 (7.14)	1.019 (28.13)	0.209 (7.94)	1.125 (46.55)	1.039 (40.00)	0.286 (19.35)	2.703 (82.35)	-	0.176 (12.82)	0.612 (39.47)	0.315 (26.47)	0.418 (26.00)
15. <i>P. frequentans</i>	0.111 (14.29)	-	-	-	-	-	-	-	0.210 (15.38)	-	0.140 (11.76)	-
16. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	-	0.040 (2.63)	-	-
17. <i>Pythium intermedium</i>	-	-	1.041 (39.68)	1.084 (44.83)	-	0.761 (51.61)	-	0.947 (62.07)	-	-	-	-
18. <i>Rhizopus stolonifer</i>	-	0.150 (4.17)	0.584 (22.22)	0.041 (1.72)	0.116 (4.44)	-	0.463 (14.12)	-	-	-	-	0.259 (14.00)
19. <i>Trichoderma harzianum</i>	0.055 (7.14)	-	0.375 (14.29)	-	-	-	-	-	-	-	-	-
20. <i>T. korangii</i>	-	-	0.291 (11.11)	0.041 (1.72)	-	0.191 (12.90)	-	-	-	-	0.140 (11.76)	-
21. White sterile mycelia	0.111 (14.29)	2.001 (55.21)	0.084 (3.17)	-	-	-	0.038 (1.18)	-	-	-	-	-

was made between the pH of the two types of litter it was found that pH of the undecomposed pine litter was more acidic as compared to that of the partially decomposed pine litter at both the study sites.

Figs. 13 a & b depict the monthly variation in the fungal population of pine litter (undecomposed & partially decomposed) at both the study sites. There was not much variation in the fungal population of the undecomposed litter at both the altitudes. However, the fungal population of the partially decomposed pine litter was found to be more at low altitude. When a comparison was made between the fungal population associated with the type of litters, it was found that there was not much variation in the fungal population between the two types of pine litter at both the study sites. There was a positive significant correlation between pH and fungal population of undecomposed litter at high altitude only ($r = 0.504$, $P < 0.02$). A positive correlation exist between pH and fungal population of the partially decomposed litter at both the study sites. However, it was observed to be significant at low altitude only ($r = 0.347$, $P < 0.1$). A positive significant correlation was also observed between fungal population and moisture content of the litter at high altitude ($r = 0.265$, $P < 0.5$).

Undecomposed litter at high altitude harboured a total of 23 fungal species during the first year of study (Table 5.1). The species isolated were *Acremonium* sp., *Alternaria tenuis*, *Aspergillus carneus*, *A. flavus*, *A. terreus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. poae*, *F. sporotrichioides*, *Humicola* sp., *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, white sterile mycelia and yellow sterile mycelia. During the second year of study the same litter harboured a total of 19 fungal species (Table 5.2). The fungal species isolated were *Acremonium* sp., *Alternaria tenuis*, *Aspergillus carneus*, *A. flavus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. sporotrichioides*, *Humicola* sp., *Mucor hiemalis*, *Penicillium*

Table 6.1 Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the partially decomposed pine litter collected from the pine forest stand at high altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Acremonium</i> sp.	-	-	-	-	-	-	-	0.250 (41.67)	-	-	-	-
2. <i>Aspergillus candidus</i>	0.047 (3.03)	0.098 (2.25)	-	-	-	-	-	-	-	-	-	-
3. <i>A. carneus</i>	-	-	-	0.324 (5.94)	-	-	-	-	-	-	-	-
4. <i>A. terreus</i>	-	-	-	0.432 (7.92)	-	-	-	-	-	-	-	-
5. <i>Cladosporium cladosporioides</i>	-	-	-	-	0.178 (7.41)	-	-	-	-	-	-	-
6. <i>Fusarium oxysporum</i>	-	0.098 (2.25)	0.136 (7.89)	0.756 (13.86)	0.044 (1.85)	-	-	-	-	-	-	-
7. <i>F. solani</i>	-	-	0.546 (31.58)	0.162 (2.97)	0.400 (16.67)	-	-	-	0.392 (56.25)	0.478 (42.31)	-	-
8. <i>F. sporotrichioides</i>	-	-	-	-	-	-	-	0.050 (8.33)	-	-	-	-
9. <i>Mucor hiemalis</i>	-	0.732 (16.85)	0.091 (5.26)	-	0.133 (5.56)	0.158 (8.33)	-	0.150 (25.00)	-	-	0.111 (9.68)	0.473 (41.94)
10. <i>M. racemosus</i>	-	-	-	-	-	-	-	0.050 (8.33)	-	-	-	-
11. <i>Penicillium chrysogenum</i>	0.667 (42.42)	2.635 (60.67)	0.091 (5.26)	3.350 (61.39)	0.800 (33.33)	0.211 (11.11)	-	0.100 (16.67)	0.044 (6.25)	-	0.222 (19.35)	-
12. <i>P. frequentans</i>	0.190 (12.13)	-	-	-	-	-	-	-	-	-	-	-
13. <i>P. javanicum</i>	-	-	-	0.324 (5.94)	-	-	-	-	0.087 (12.50)	0.043 (3.85)	-	-
14. <i>Pythium intermedium</i>	0.667 (42.42)	-	0.091 (5.26)	0.108 (1.98)	0.533 (22.22)	0.895 (47.22)	-	-	-	0.435 (38.46)	-	0.654 (58.06)
15. <i>Fibropus stolonifer</i>	-	-	0.045 (2.63)	-	0.311 (12.96)	-	-	-	-	-	-	-
16. <i>Trichoderma harzianum</i>	-	-	0.455 (26.32)	-	-	-	-	-	-	-	-	-
17. <i>T. koningi</i>	-	0.781 (17.98)	0.273 (15.79)	-	-	-	3.925 (100.00)	-	-	0.174 (15.38)	0.215 (70.97)	-
18. White sterile mycelia	-	-	-	-	-	0.632 (33.33)	-	-	0.174 (25.00)	-	-	-

Table 6.2 Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the partially decomposed pine litter collected from the pine forest stand at high altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Abidita cylindrospora</i>	-	-	-	0.167 (11.54)	-	-	-	-	-	-	-	-
2. <i>Acremonium</i> sp.	0.046 (3.03)	-	-	0.333 (23.08)	-	-	-	0.600 (45.00)	-	-	-	-
3. <i>Aspergillus flavus</i>	-	-	-	-	-	-	-	-	-	0.103 (6.06)	-	-
4. <i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	0.099 (7.69)	-	-	0.083 (5.56)
5. <i>Fusarium oxysporum</i>	-	-	0.043 (3.44)	-	-	-	-	-	-	-	-	1.167 (77.77)
6. <i>F. solani</i>	0.046 (3.03)	-	0.391 (31.03)	0.444 (30.77)	-	-	-	-	0.700 (53.85)	-	-	-
7. <i>F. sporotrichioides</i>	-	-	-	-	-	0.167 (8.33)	-	0.133 (10.00)	-	-	0.172 (9.09)	-
8. <i>Hemicola</i> sp.	-	-	-	-	-	-	-	-	-	0.769 (45.45)	0.200 (42.42)	-
9. <i>Mucor hiemalis</i>	-	1.538 (96.77)	0.130 (10.34)	-	-	-	0.231 (10.71)	0.266 (20.00)	-	0.410 (24.24)	-	-
10. <i>M. racemosus</i>	-	-	-	-	-	-	-	0.200 (15.00)	-	-	-	-
11. <i>Penicillium chrysogenum</i>	0.093 (6.06)	0.051 (3.23)	0.043 (3.44)	-	-	-	-	0.067 (5.00)	-	-	-	-
12. <i>P. frequentans</i>	0.093 (6.06)	-	-	-	-	-	-	-	-	-	-	0.083 (5.56)
13. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.300 (23.08)	-	-	0.125 (8.33)
14. <i>P. wakamarii</i>	-	-	-	-	-	-	-	-	-	-	-	0.042 (2.78)
15. <i>Pythium intermedium</i>	0.883 (57.58)	-	0.217 (17.24)	0.500 (34.62)	2.251 (100.00)	1.833 (91.67)	-	-	-	-	-	-
16. <i>Trichoderma korningii</i>	0.372 (24.24)	-	0.435 (34.48)	-	-	-	1.925 (89.29)	-	-	0.410 (24.24)	0.915 (48.48)	-
17. White sterile mycelia	-	-	-	-	-	-	-	0.067 (5.00)	0.200 (15.38)	-	-	-

Table 6.3 Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the partially decomposed pine litter collected from the pine forest stand at low altitude during different sampling periods from March 1994 to February 1995. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Ambylosporium</i> sp.	2.725 (40.88)	-	-	-	-	-	-	-	-	-	-	-
2. <i>Aspergillus wentii</i>	-	-	0.227 (8.33)	-	-	-	-	-	-	-	-	-
3. <i>Cladosporium cladosporioides</i>	-	-	-	0.044 (1.79)	-	-	-	-	-	-	0.200 (11.90)	0.582 (32.65)
4. <i>Fusarium oxysporum</i>	-	0.383 (32.14)	0.227 (8.33)	0.400 (16.07)	0.039 (2.86)	-	-	-	-	0.117 (10.71)	-	0.763 (42.86)
5. <i>F. solani</i>	-	0.170 (14.29)	-	-	0.734 (54.29)	-	-	-	-	-	-	-
6. <i>F. poae</i>	-	-	-	-	0.232 (17.14)	-	-	-	-	-	-	-
7. <i>Mucor hiemalis</i>	0.049 (1.00)	0.255 (21.43)	-	0.044 (1.79)	-	0.105 (7.69)	0.189 (36.36)	0.673 (41.94)	0.774 (46.15)	0.467 (42.86)	-	-
8. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	-	0.569 (35.48)	-	0.117 (10.71)	-	-
9. <i>P. chrysogenum</i>	2.822 (42.34)	-	0.045 (1.67)	0.488 (19.64)	0.116 (8.57)	-	-	-	-	0.155 (14.29)	0.640 (39.10)	0.400 (22.45)
10. <i>P. frequentans</i>	-	-	-	-	-	-	-	-	-	-	0.080 (4.76)	-
11. <i>P. rubrum</i>	0.049 (1.00)	-	-	-	-	-	-	-	-	-	-	-
12. <i>P. wakamatsii</i>	-	-	-	-	-	-	-	-	-	-	-	0.036 (2.04)
13. <i>Pythium intermedium</i>	0.535 (8.03)	0.128 (10.71)	0.364 (13.33)	0.266 (10.71)	-	0.737 (53.85)	-	0.362 (22.58)	-	-	-	-
14. <i>Rhizopus stolonifer</i>	-	0.255 (21.43)	1.410 (51.67)	0.088 (3.57)	0.116 (8.57)	-	-	-	-	-	-	-
15. <i>Trichoderma harzianum</i>	0.486 (7.30)	-	0.409 (15.00)	1.155 (46.43)	0.116 (8.57)	-	-	-	-	-	-	-
16. <i>Trichoderma koningii</i>	-	-	-	-	-	0.527 (38.46)	0.320 (63.63)	-	0.193 (11.54)	0.233 (21.43)	0.280 (16.67)	-
17. White sterile mycelia	-	-	0.045 (1.67)	-	-	-	-	-	0.709 (42.31)	-	0.480 (28.57)	-

Table 6.4 Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the partially decomposed pine litter collected from the pine forest stand at low altitude during different sampling periods from March 1995 to February 1996. The values in the parentheses are percentage relative abundance.

Fungi	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
1. <i>Aspergillus wentii</i>	-	-	0.266 (18.18)	-	-	-	-	-	-	-	-	-
2. <i>Cladosporium cladosporioides</i>	0.369 (18.92)	-	-	-	-	-	-	-	-	-	-	0.125 (6.81)
3. <i>Fusarium oxysporum</i>	-	-	0.066 (4.55)	-	0.111 (8.33)	-	-	-	-	0.103 (6.06)	-	-
4. <i>F. solani</i>	0.053 (2.70)	-	-	1.566 (52.94)	0.278 (20.83)	-	-	-	-	-	-	-
5. <i>F. marismoides</i>	-	-	-	1.392 (47.06)	-	-	-	-	-	-	-	-
6. <i>F. poae</i>	-	-	-	-	-	-	0.190 (18.18)	-	-	-	-	-
7. <i>Hemicola sp.</i>	-	-	-	-	-	-	-	-	-	-	-	2.000 (4.55)
8. <i>Moravia sp.</i>	0.263 (13.53)	-	-	-	-	-	-	-	-	-	-	-
9. <i>Mucor hiemalis</i>	0.631 (32.43)	1.149 (62.16)	-	-	-	-	0.524 (50.00)	0.571 (32.56)	0.952 (52.63)	0.667 (39.39)	0.426 (71.43)	1.630 (88.64)
10. <i>Penicillium brevicompactum</i>	-	-	-	-	-	-	-	0.571 (32.56)	0.571 (31.59)	0.154 (9.09)	-	-
11. <i>P. chrysogenum</i>	0.211 (10.81)	0.650 (35.14)	0.200 (13.64)	-	-	0.059 (4.17)	-	-	-	0.256 (15.15)	-	-
12. <i>P. frequentans</i>	0.105 (5.41)	-	-	-	-	-	-	-	0.095 (5.62)	-	-	-
13. <i>P. javanicum</i>	-	-	-	-	-	-	-	-	0.047 (2.63)	0.051 (3.83)	-	-
14. <i>Pythium intermedium</i>	0.316 (16.22)	0.050 (2.70)	0.200 (13.64)	-	0.944 (70.83)	0.470 (33.33)	0.143 (13.64)	0.612 (34.88)	-	-	-	-
15. <i>Rhizopus stolonifer</i>	-	-	0.533 (36.36)	-	-	-	0.048 (4.55)	-	-	-	-	-
16. <i>Trichoderma kovringii</i>	-	-	0.133 (9.09)	-	-	0.882 (62.50)	0.143 (13.64)	-	-	0.461 (27.27)	0.170 (28.57)	-
17. <i>White sterile stycelia</i>	-	-	0.066 (4.55)	-	-	-	-	-	0.142 (7.89)	-	-	-

brevicompactum, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, white sterile mycelia and yellow sterile mycelia.

Undecomposed pine litter at low altitude harboured a total of 21 fungal species during the first year of study (Table 5.3). The species isolated were *Alternaria alternata*, *A. tenuis*, *Aspergillus candidus*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. poae*, *F. sporotrichioides*, *Humicola* sp., *Mucor hiemalis*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum* and white sterile mycelia. During the second year of study the same litter harboured a total of 18 fungal species (Table 5.4). The fungal species isolated were *Acremonium* sp., *Alternaria tenuis*, *Aspergillus terreus*, *Cladosporium cladosporioides*, *Fusarium solani*, *F. poae*, *F. sporotrichioides*, *Humicola* sp., *Mucor hiemalis*, *M. circinelloides*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, white sterile mycelia and yellow sterile mycelia.

Tables 5.1- 5.4 show that undecomposed litter at high altitude exhibited highest number of fungal isolates as compared to the litter at low altitude. Qualitatively, there was not much difference in the fungal flora of the litter except for *Mucor racemosus*, *Aspergillus carneus* and *A. flavus* which were isolated from the litter at high altitude only, and *Alternaria alternata* and *Mucor circinelloides* which could be isolated from the litter at low altitude only.

Partially decomposed litter at high altitude harboured a total of 18 fungal species during the first year and 17 during the second year of study (Tables 6.1 & 6.2). The species isolated during the first year of study were *Acremonium* sp., *Aspergillus candidus*, *A. carneus*, *A. terreus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. sporotrichioides*, *Mucor hiemalis*, *M. racemosus*, *Penicillium chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum* and white

Table 6.5 List of fungi isolated from the undecomposed and partially decomposed pine litter collected from the pine forest stands at high altitude and at low altitude during the study period.

Fungi	High Altitude		Low Altitude	
	Undecomposed	Partially decomposed	Undecomposed	Partially decomposed
1. <i>Absidia cylindrospora</i>	-	+	-	+
2. <i>Acremonium</i> sp.	+	+	+	-
3. <i>Atornata albarnata</i>	-	-	+	-
4. <i>A. tenuis</i>	+	-	+	-
5. <i>Amblyosporium</i> sp.	-	-	-	+
6. <i>Aspergillus candidus</i>	-	+	+	-
7. <i>A. carneus</i>	+	+	-	-
8. <i>A. flavus</i>	+	+	-	-
9. <i>A. niger</i>	-	-	+	-
10. <i>A. terreus</i>	+	+	+	-
11. <i>A. versii</i>	-	-	-	+
12. <i>C. cladosporioides</i>	+	+	+	+
13. <i>F. oxysporum</i>	+	+	+	+
14. <i>F. solani</i>	+	+	+	+
15. <i>F. merimoides</i>	-	-	-	+
16. <i>F. poae</i>	+	-	+	+
17. <i>F. sporotrichioides</i>	+	+	+	-
18. <i>Hemicola</i> sp.	+	+	+	+
19. <i>Mucor circinelloides</i>	-	-	+	-
20. <i>M. himmalis</i>	+	+	+	+
21. <i>M. racemosus</i>	+	+	-	-
22. <i>Morilla</i> sp.	-	-	-	+
23. <i>Penicillium brevicompactum</i>	+	-	+	+
24. <i>P. chrysogenum</i>	+	+	+	+
25. <i>P. frequentans</i>	+	+	+	+
26. <i>P. javanicum</i>	+	+	+	+
27. <i>P. rubrum</i>	-	-	-	+
28. <i>P. wakamarii</i>	-	+	-	+
29. <i>Pythium intermedium</i>	+	+	+	+
30. <i>Fuizopus stolonifer</i>	+	+	+	+
31. <i>Trichoderma koningii</i>	+	+	+	+
32. <i>T. harzianum</i>	+	+	+	+
33. White sterile mycelia	+	+	+	+
34. Yellow sterile mycelia	+	-	+	-

Present = +; Absent = -

sterile mycelia. The species isolated during the second year of study were *Absidia cylindrospora*, *Acremonium* sp., *A. flavus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. sporotrichioides*, *Hemicola* sp., *Mucor hiemalis*, *M. racemosus*, *Penicillium chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Trichoderma koningii* and white sterile mycelia.

Partially decomposed litter at low altitude harboured a total of 17 fungal species during the first year and 18 during the second year of study (Tables 6.3 & 6.4). The species isolated during the first year were *Amblyosporium* sp., *Aspergillus wentii*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. poae*, *Mucor hiemalis*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. rubrum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum* and white sterile mycelia. The species isolated during the second year were *Aspergillus wentii*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*, *F. poae*, *F. merismoides*, *Hemicola* sp., *Monilia* sp., *Mucor hiemalis*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, white sterile mycelia and yellow sterile mycelia.

Tables 6.1- 6.4 show that the partially decomposed pine litter exhibited a more or less similar number of fungal isolates at both the study sites. Qualitatively, there was not much difference in the composition of the fungal flora of the litter except for *Mucor racemosus*, *Absidia cylindrospora*, *Aspergillus terreus*, *A. carneus*, *A. candidus*, *A. flavus* and *Acremonium* sp. which could be isolated from the litter at high altitude only and *Amblyosporium* sp., *Aspergillus wentii*, *Penicillium brevicompactum*, *P. rubrum*, *Monilia* sp. and yellow sterile mycelia which were associated with the litter at low altitude only.

Fig. 14 (a&b) Monthly variation in the dehydrogenase activity of soil and earthworm casts of two pine forest stands.

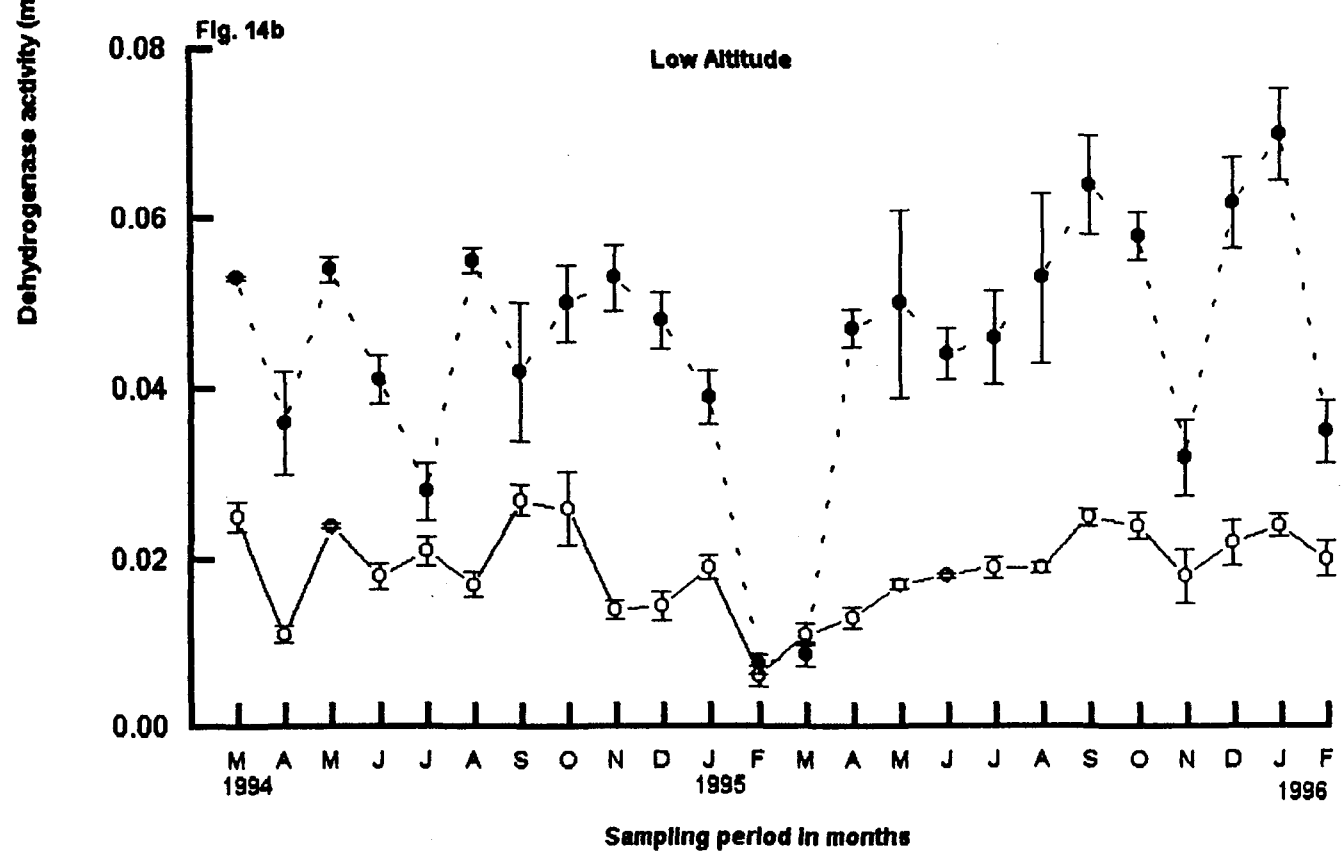
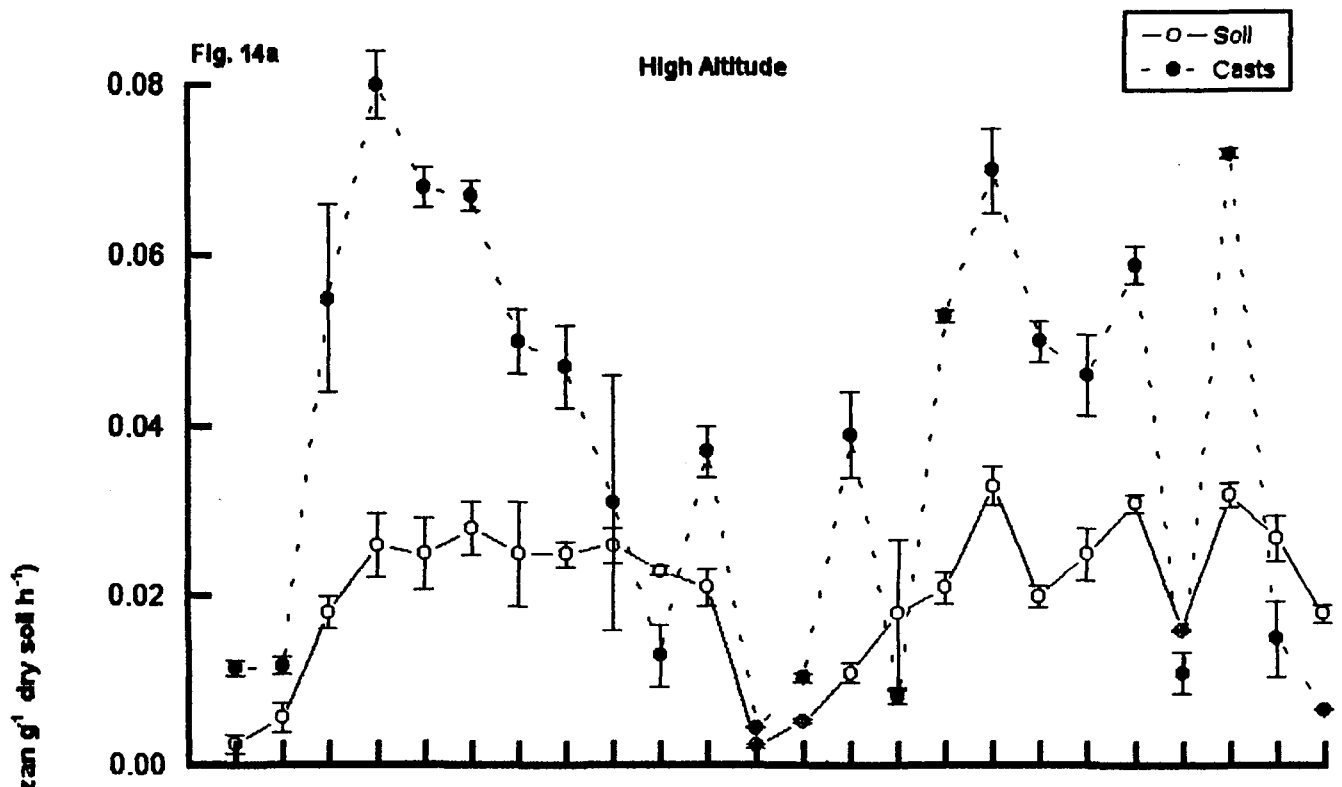


Fig. 15 (a&b) Monthly variation in the urease activity of soil and earthworm casts of two pine forest stands.

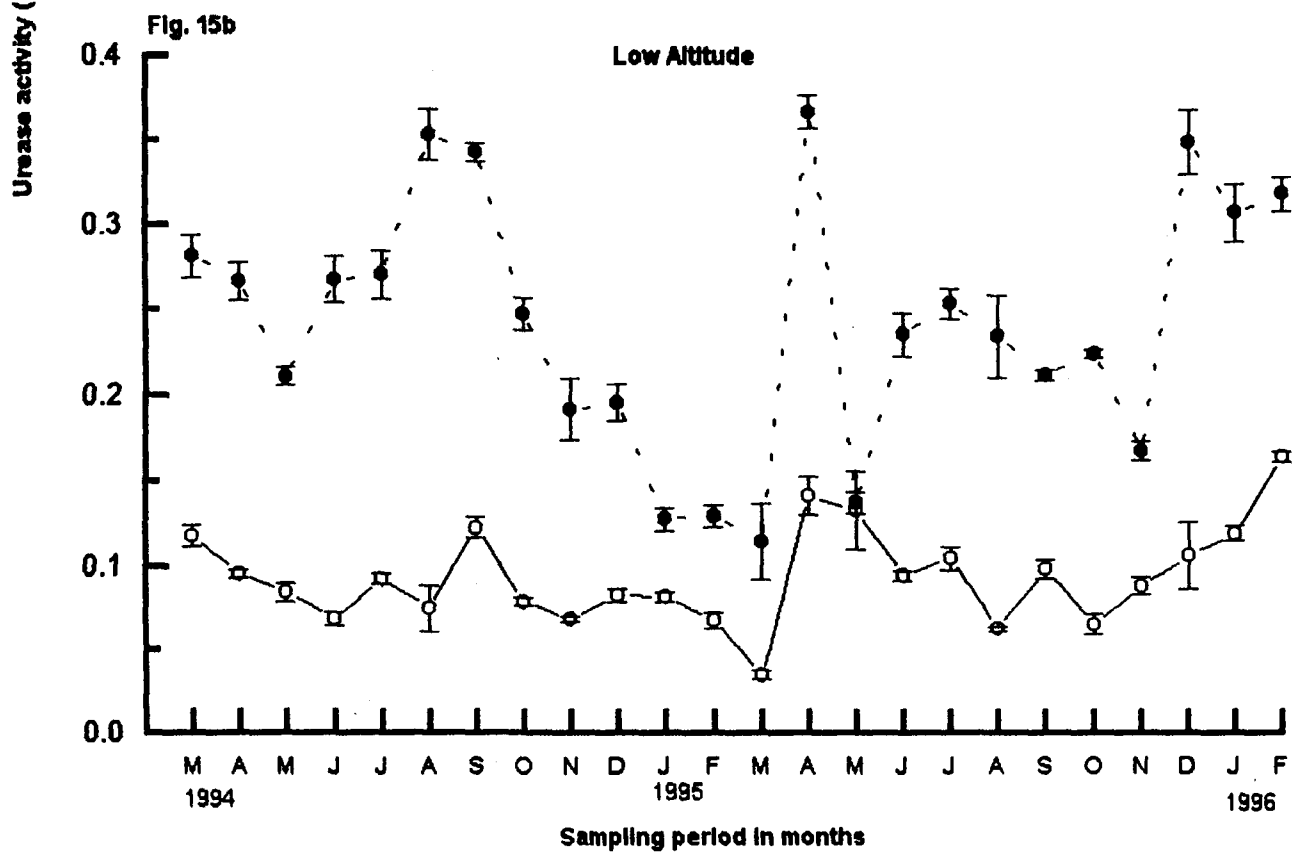
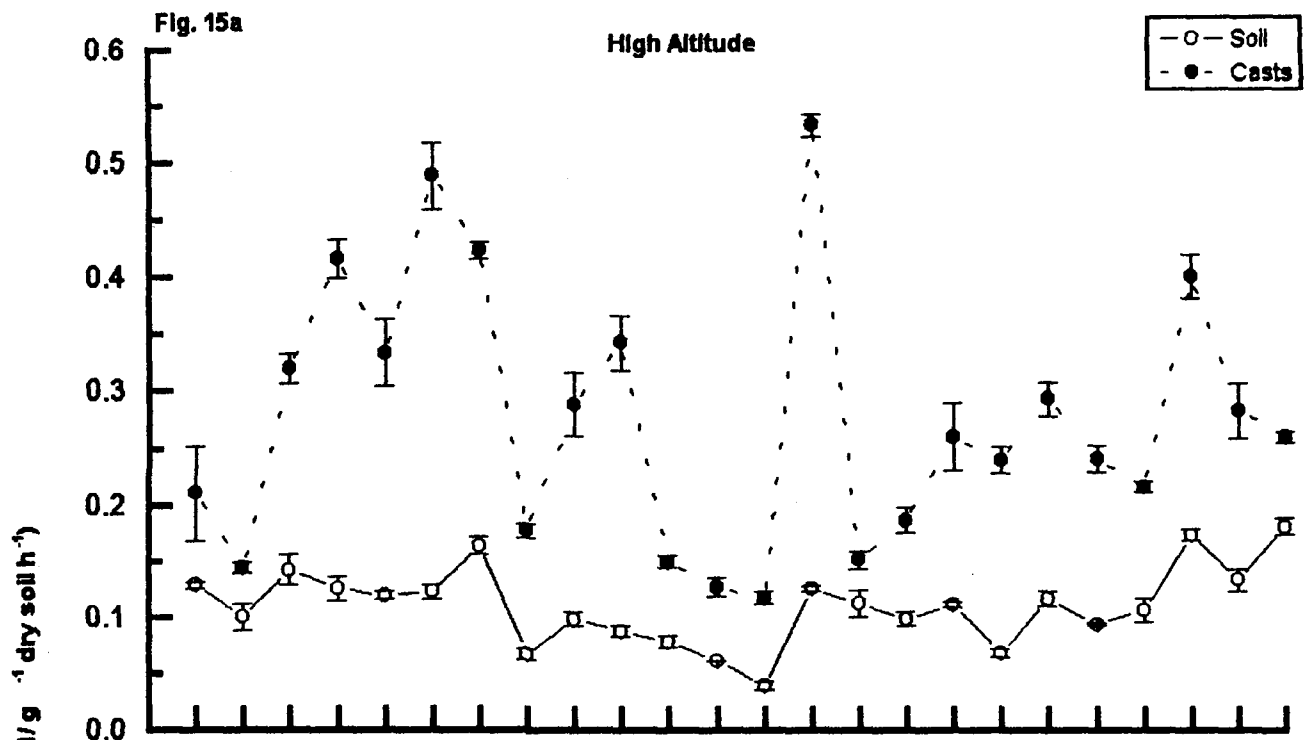


Table 7. Correlation coefficient values (r) for various soil parameters in two pine forest stand.

Sources of Variation	D.F	High Altitude			Low Altitude		
		Fungal population	Dehydrogenase activity	Urease activity	Fungal population	Dehydrogenase activity	Urease activity
Soil Temperature	22	0.491**	NS	NS	NS	NS	NS
%Moisture content	22	NS	NS	0.468**	NS	NS	0.655***
Soil pH	22	NS	NS	NS	NS	-0.317*	NS
% Organic carbon	22	NS	NS	0.325*	0.353*	NS	NS
Total nitrogen	22	NS	-0.490**	NS	NS	NS	NS
Available phosphorus	22	NS	NS	NS	NS	NS	NS
Fungal population	22		NS	0.354*		NS	NS

*, **, ***, P < 0.1, 0.05 and 0.001 respectively.
 NS = Not significant

Table 8. Analysis of variance of enzyme activities (dehydrogenase and urease) of the soils collected from the two pine forest stands.

Source of variation	D.F.	S.S.	M.S.S.	Variance ratio calculated F	Table F Value		Remark
					5 %	1 %	
Dehydrogenase :							
Between the sites	1	2.2 E- 05	2.2 E-05	0.737	4.279	7.881	Not Significant
Between sampling periods	23	0.0019	8.05 E- 05	2.697	2.014	2.719	Significant at 5 % level of probability
Error	47	0.0026	2.98 E- 05				
Total	47	0.0026					
Urease :							
Between the sites	1	0.0035	0.0035	12.574	4.279	7.881	Significant at 5 % and 1 % level of probability
Between sampling periods	23	0.0406	0.0018	8.367	2.014	2.719	Significant at 5 % and 1 % level of probability
Error	47	0.0064	0.0003				
Total	47	0.0504					

Table 6.5 shows a comparative study of the fungal flora associated with the undecomposed and partially decomposed pine litters. It was also observed that there was not much difference in the composition of the fungal species associated with the undecomposed and partially decomposed pine litters at both the study sites. When comparison was made between the fungal species present in the undecomposed and partially decomposed litters, slight difference in the fungal species composition was observed in which *Alternaria alternata*, *A. tenuis*, *Aspergillus niger* and *Mucor circinelloides* were found to be present in undecomposed litter only, whereas *Absidia cylindrospora*, *Ambylosporium* sp., *Aspergillus wentii*, *Fusarium merismoides*, *Monilia* sp., *Penicillium rubrum* and *P. waksmanii* were found to be present in partially decomposed litter only.

3. Estimation of enzyme activities from the soil and the earthworm casts

Dehydrogenase activity

Monthly variation in dehydrogenase activity of soils as well as earthworm casts at both the study sites is depicted in Figs. 14a & b. The activity was generally higher in the soil at high altitude as compared to that at low altitude except in the months of September, February, March, April and November where a lower activity was recorded. It was observed that the earthworm casts generally showed higher dehydrogenase activity as compared to that of the surrounding soil at both the study sites. No significant correlation could be observed between soil temperature and dehydrogenase activity at both the study sites. A significant negative correlation was observed between dehydrogenase activity of soil and soil pH at low altitude [$r = -0.317$, $P < 0.1$] Table 7]. Dehydrogenase was negatively correlated with nitrogen content of the soil at both the study sites but was significant only at high altitude ($r = -0.490$, $P < 0.05$). Dehydrogenase activity of soil was found to vary significantly between the sampling periods at 5% level of significance (Table 8).

Fig. 16 Monthly variation in earthworm population of two pine forest stands. HA (High Altitude), LA (Low Altitude).

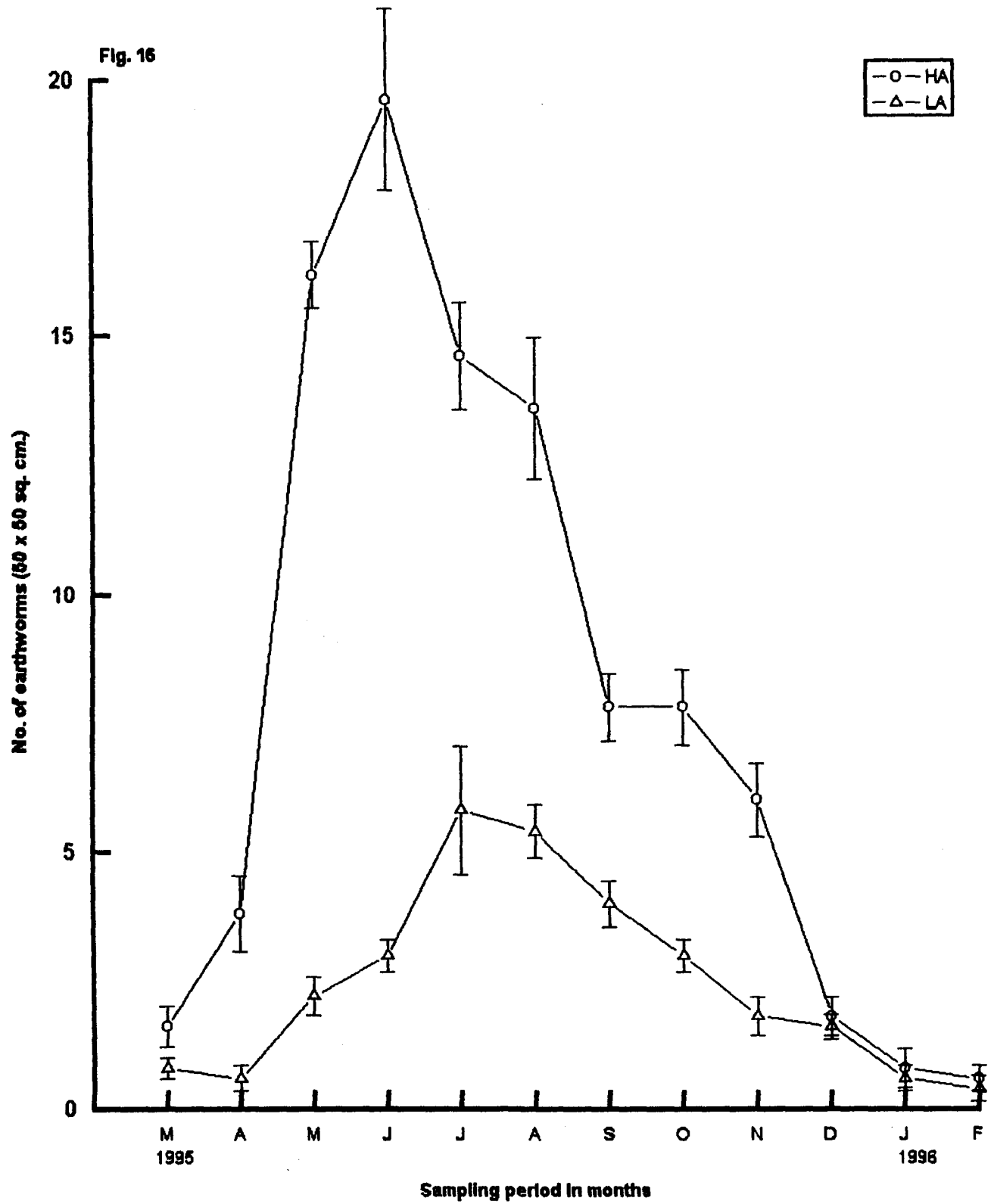


Table 9. Correlation coefficient (r) for various soil parameters with earthworm population in two pine stands.

Sources of variation	D.F	Earthworm population	Earthworm population
Soil temperature	10	0.627***	NS
% moisture content	10	NS	NS
soil pH	10	-0.444*	-0.508**
% Organic carbon	10	0.451*	NS
Total nitrogen	10	NS	NS
Available phosphorus	10	NS	NS

*, ** and *** , P < 0.2, 0.1 and 0.05 respectively.
 NS = Not significant

Urease activity :

Figs. 15a & b depict the monthly variation of urease activity in the soils and casts of the two study sites. Generally, higher urease activity was recorded in the soil at high altitude except in the months of October, April and May where lower activity was recorded. Urease activity of earthworm casts was also recorded to be higher than that of the surrounding soil at both the study sites. Statistically, a significant correlation was observed between urease activity of soil and fungal population at high altitude ($r = 0.354$, $P < 0.10$). A significant correlation was observed between urease activity and moisture content at both the study sites ($r = 0.468$, $P < 0.05$ at high altitude and $r = 0.655$, $P < 0.001$ at low altitude). A significant variation was observed for urease activity of soil between the study sites as well as between the sampling periods at 5% and 1% level of significance (Table 8).

4. Earthworm population and identification of earthworms :

Soil at the pine forest stand at high altitude was sandy while that at low altitude was sandy loam. Soil temperature, soil moisture and pH of both the study sites are depicted in figs. 3,4 (a & b) and 5 (a & b) respectively. Soil temperature at high altitude varied from 14°C to 23°C while that of the soil at low altitude varied from 15.5°C to 25°C during the study period. Moisture content of the soil at high altitude varied from 26.67% to 43.33% while that of the soil at low altitude varied from 13.3% to 40%. The soil was found to be more acidic at high altitude than at low altitude. pH of the soil at high altitude varied from 4.74 to 5.90 while at low altitude varied from 4.78 to 6.25.

The earthworm species found at high altitude were identified as *Eutyphoeus* sp., *Amyntas corticis* (Kinberg), *Eutyphoeus festivus* Gates, *Drawida papillifer papillifer* Steph., *Lenoscolex strigosus* Gates and *Kanchuria sumerianus* Julka while those found at low altitude were identified as *Drawida papillifer papillifer* Stephensen, *Amyntas corticis*

**Fig. 17 Percentage of weight remaining of pine litter after different time intervals (o-o)
= control (without earthworms), (●-●) = treated (with earthworms).**

Fig. 17

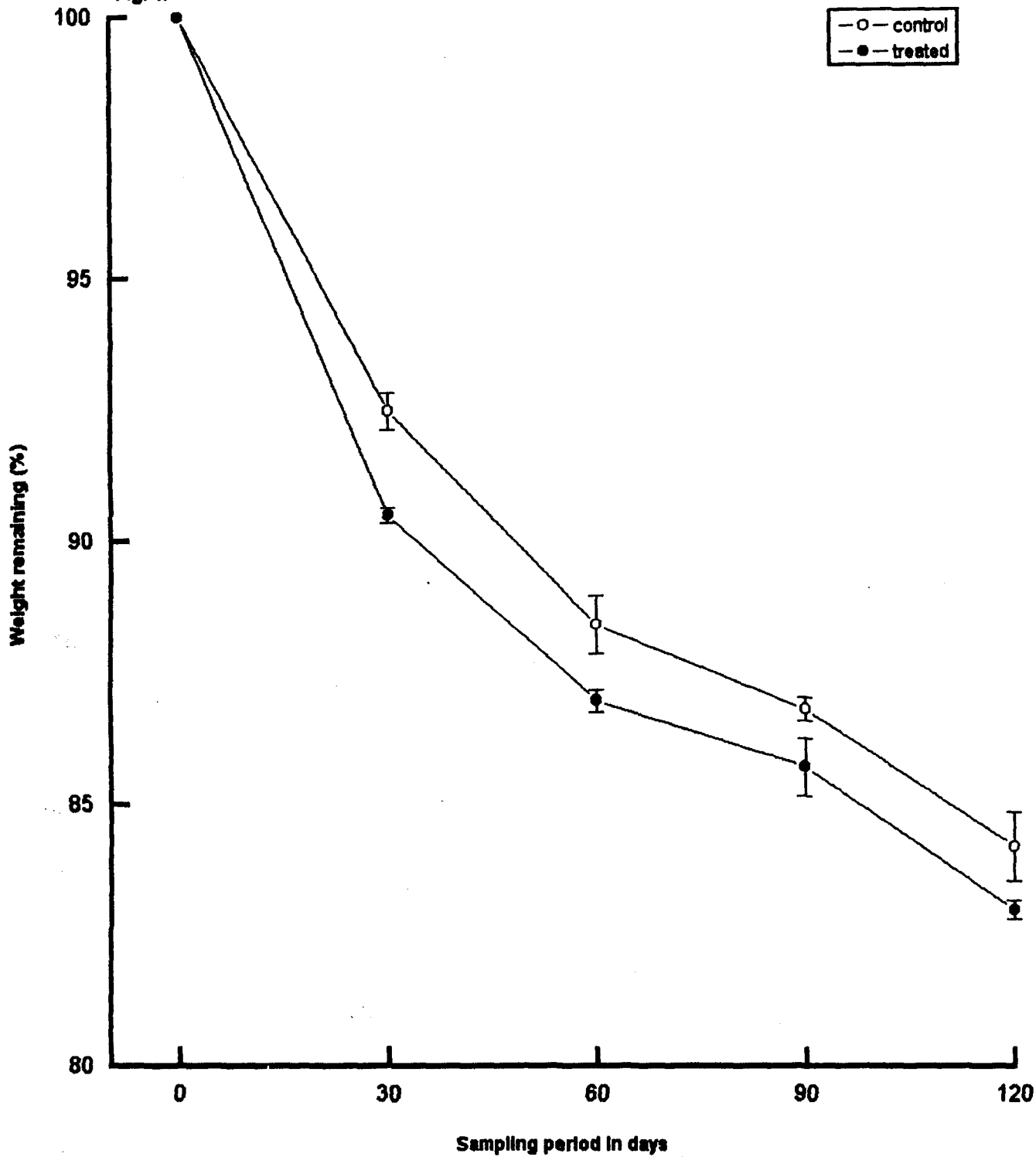


Table 10. Decay constant (K), Half life and 95% life (percentage weight remaining versus time in year) values for pine litter decomposition under laboratory condition.

Treatment	K (per year)	Half life (year)	95% life (year)
Control (without earthworms)	0.638	1.09	4.70
Treated (with earthworms)	0.730	0.949	4.11

Fig. 18 (a,b,c&d) Variation of cellulose, hemicellulose, lignin, total sugars and amino acids in the control (without earthworms) and treated (with earthworms) at different time intervals.

Fig. 18

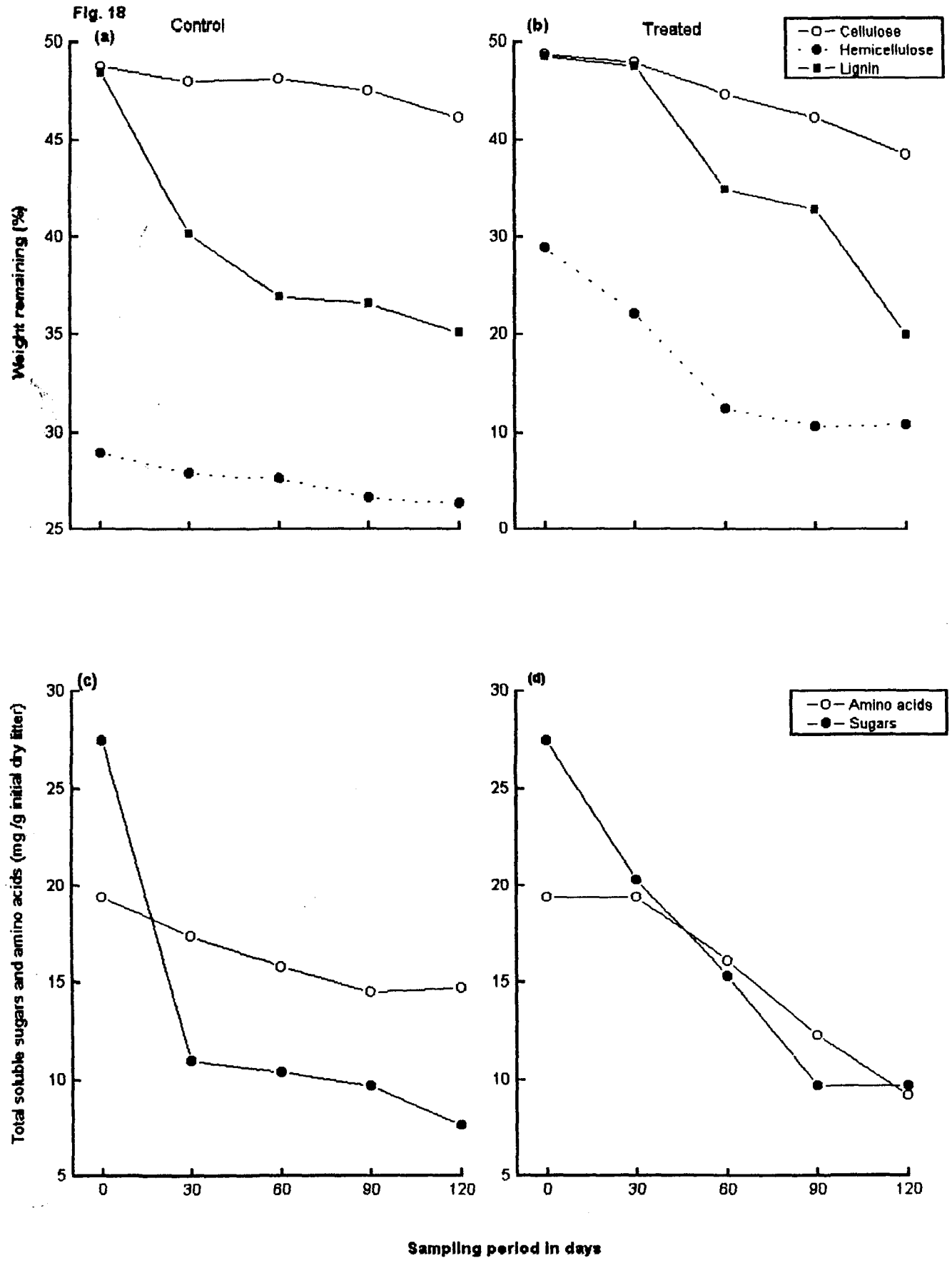
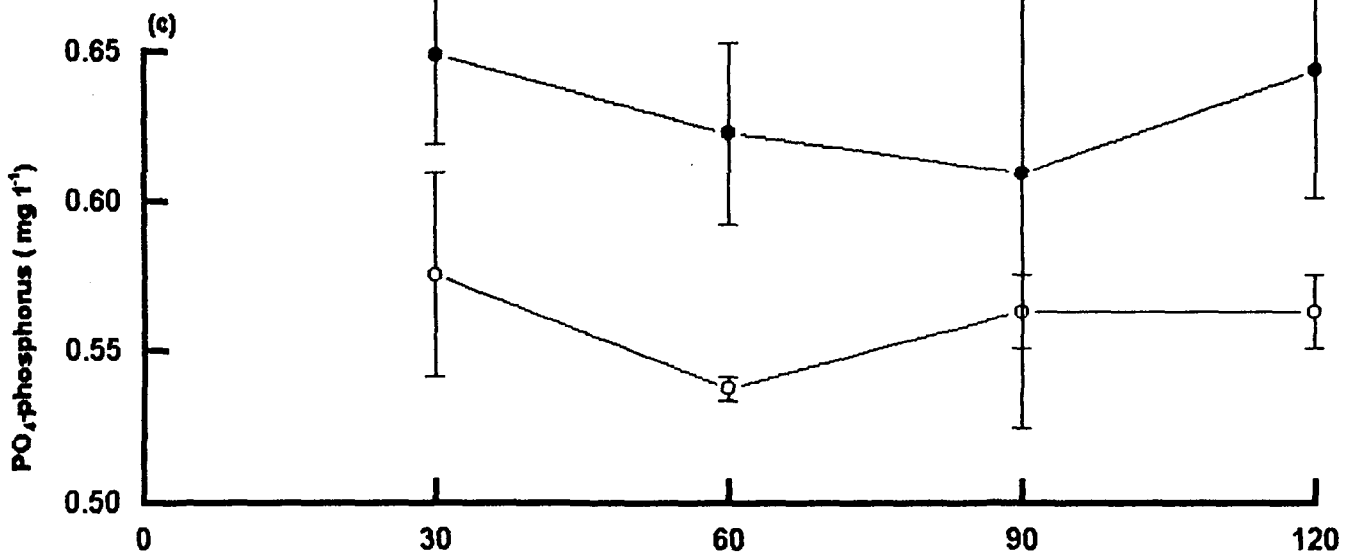
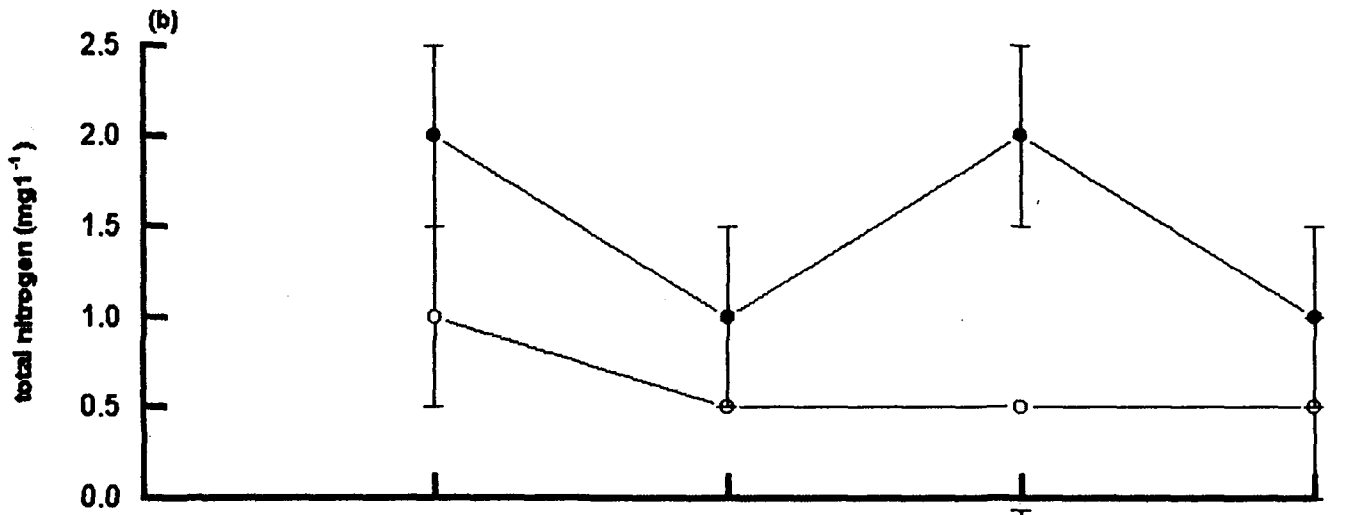
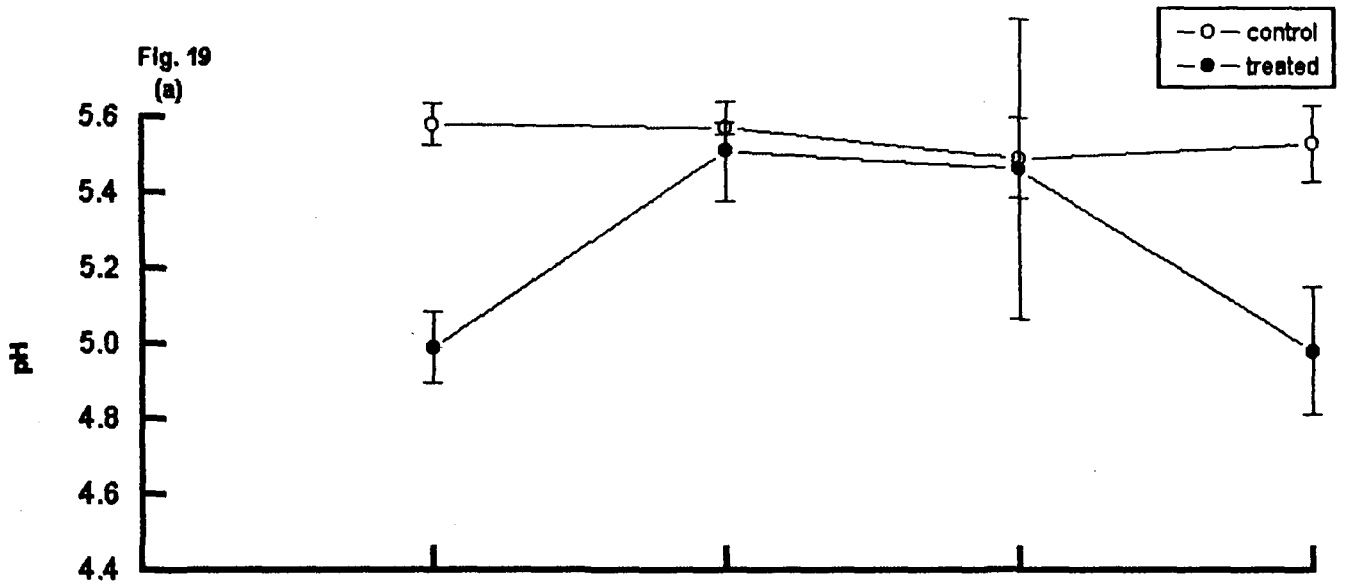


Fig. 19 (a,b&c) pH, amounts of nitrogen and phosphorus in leaching waters. (o-o) = control (without earthworms), (●-●) = treated (with earthworms).

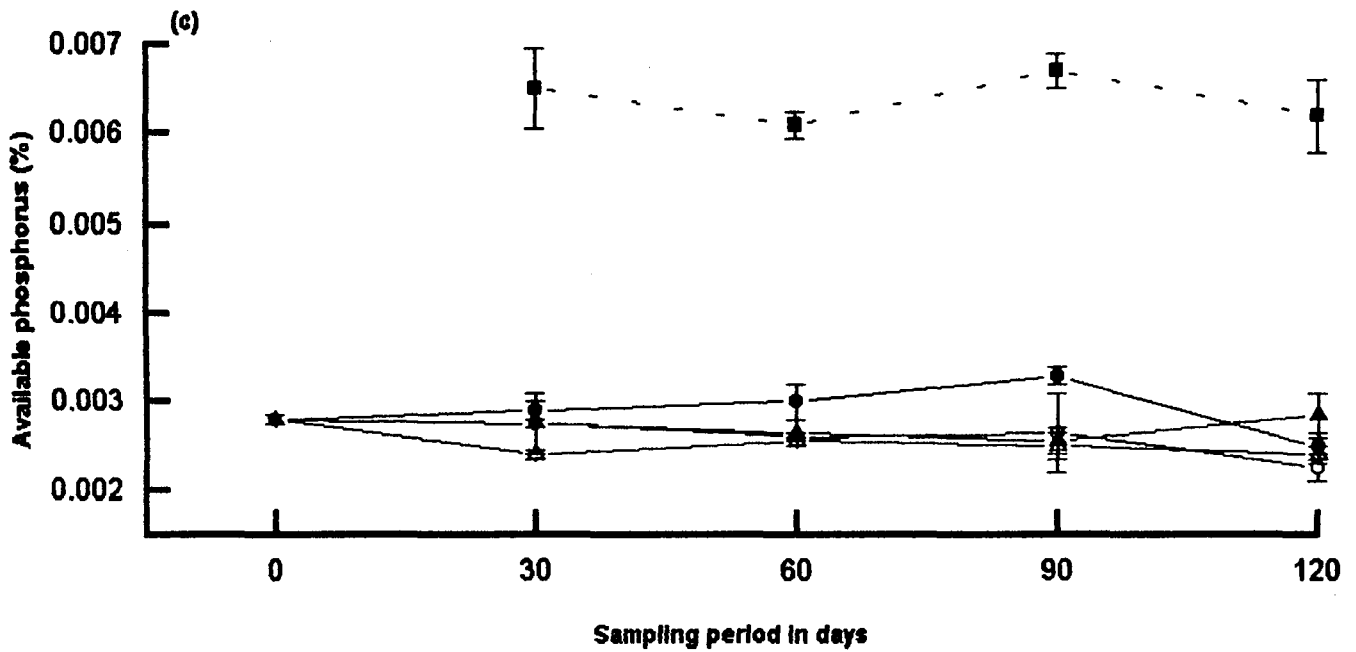
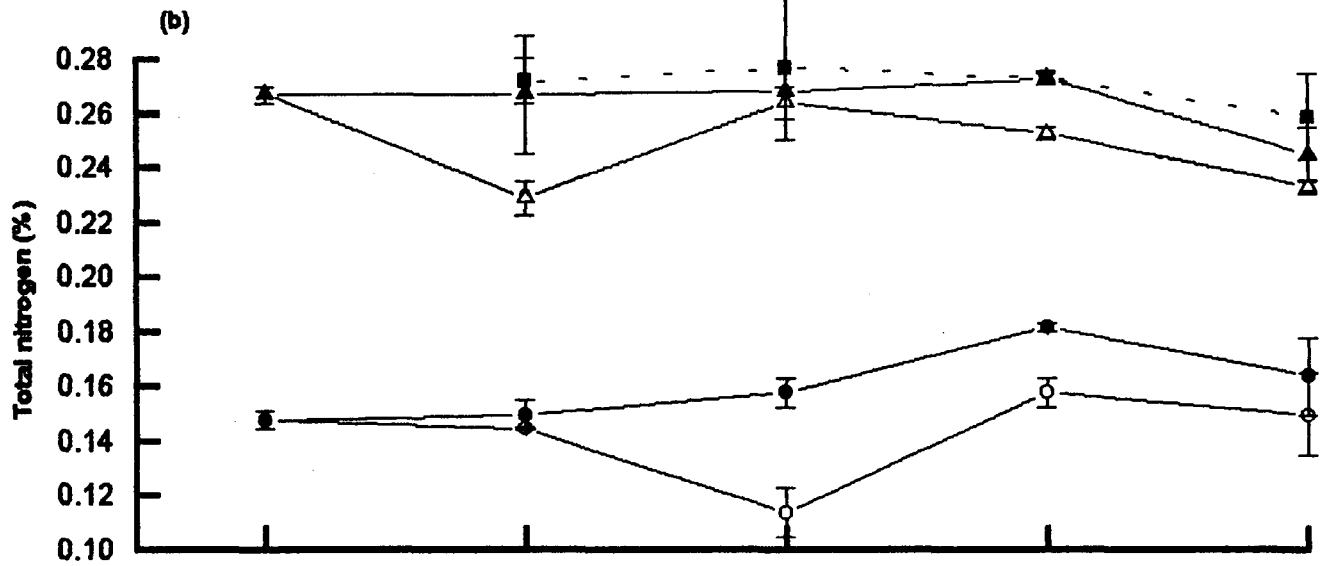
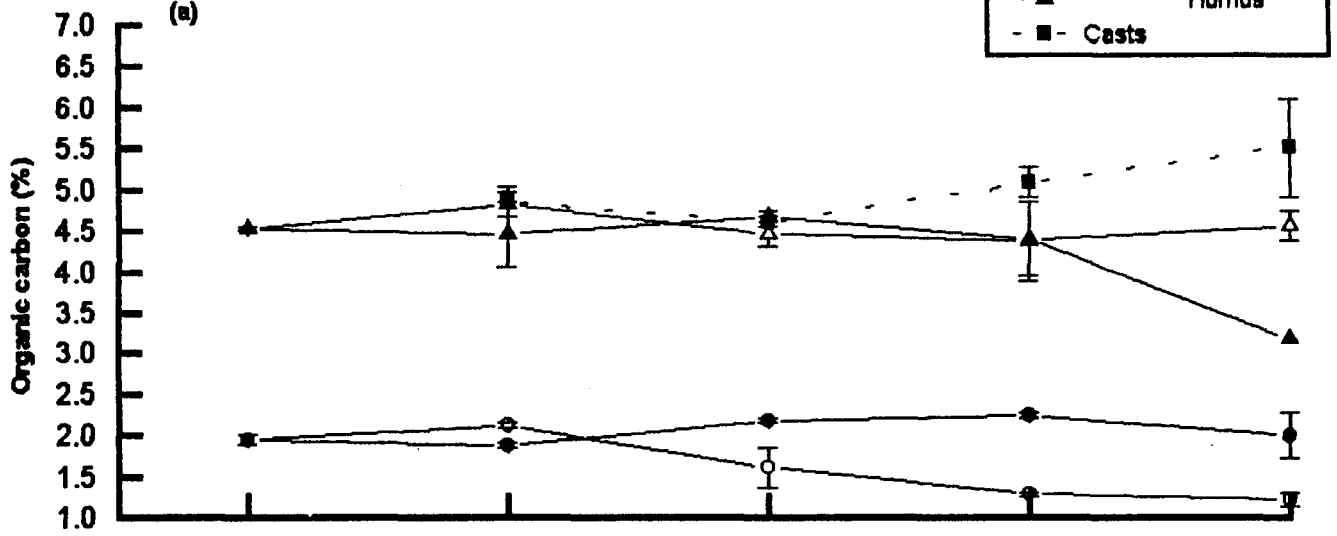
Fig. 19
(a)



Sampling period in days

Fig. 20 (a,b&c) Organic carbon, total nitrogen and available phosphorus of mineral soil, humus and casts.

Fig. 20
(a)



(Kinberg) and *Metaphire anomala* (Michaelsen). However, *Drawida papillifer papillifer* Steph. and *Amyntas corticis* (Kinberg) were found to be common at both the study sites.

Fig. 16 depicts the monthly variation of the earthworm population at the two study sites. It was observed that the earthworm population was more at high altitude as compared to that at low altitude. Population was highest in June at high altitude and in July at low altitude. Minimum population was recorded in February at both the study sites. Table 9 shows the correlation co-efficients calculated between earthworm population with various soil physico-chemical properties at both the study sites. It was found that earthworm population was positively correlated with soil temperature ($r = 0.627$, $P < 0.05$) and organic carbon ($r = 0.451$, $P < 0.2$) at high altitude. However, a significant negative correlation was observed between earthworm population and pH at both the study sites ($r = -0.444$, $P < 0.2$ and $r = -0.508$, $P < 0.1$).

5. Role of earthworms in litter decomposition :

Fig. 17 depicts the percentage weight remaining of litter in the control sets (without earthworms) and the treated sets (with earthworms). From the figure, it was observed that weight loss was slightly more in the treated sets as compared to that of the control. Percentage weight remaining after 120 days was 83.4% in the control and 82.1% in the treated sets. It was observed that the rate of decay of pine litter was more faster in the sets treated with earthworms than in the controls as calculated from the decay rate constant (Table 10).

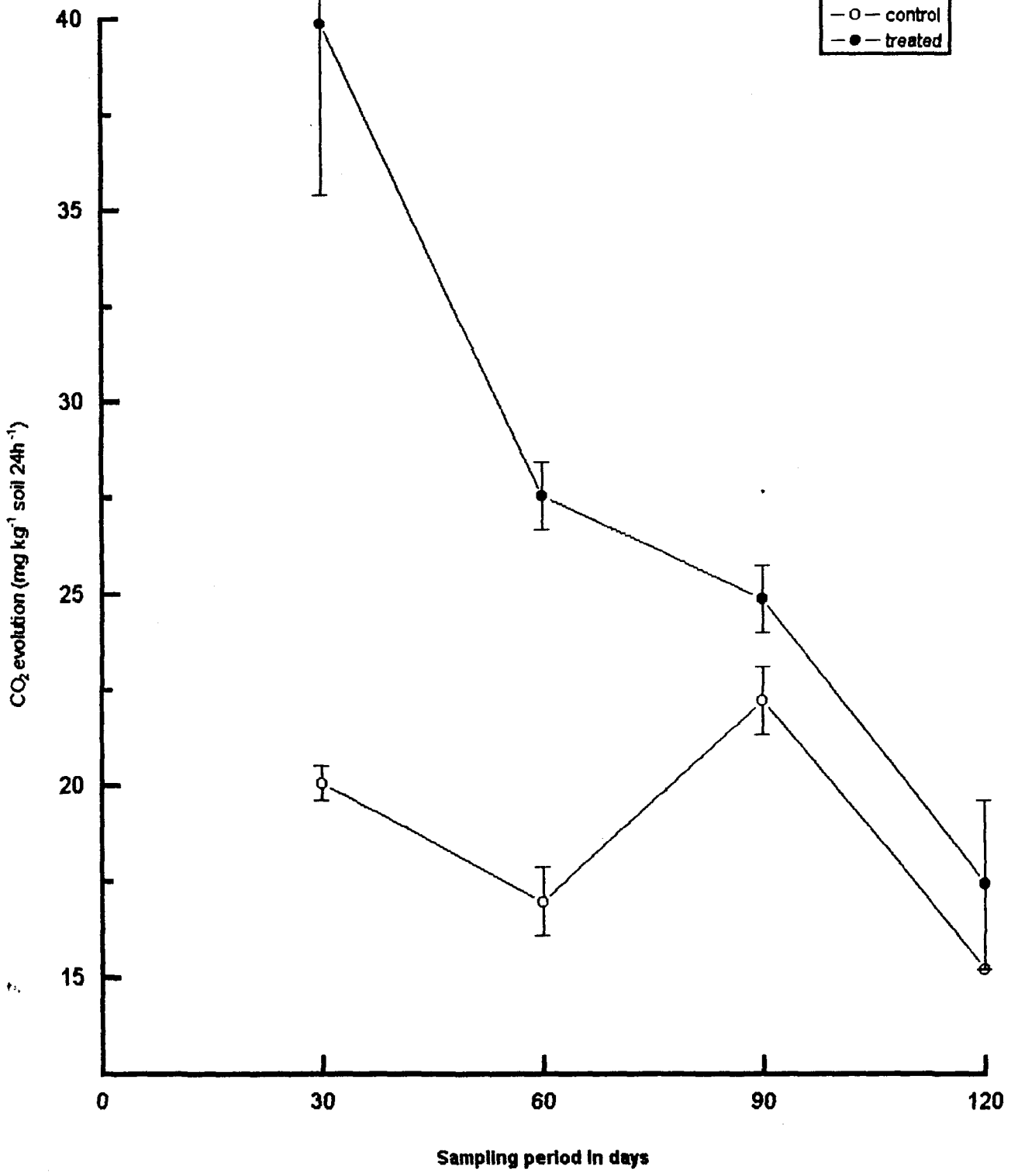
The various components like cellulose, hemicellulose, lignin, total amino acids and sugars of the decomposing pine litter in the controls as well as in the sets treated with earthworms were maximum in the initial stages of decomposition which was observed to decrease in the latter stages as decomposition proceeded (fig. 18a,b,c&d). The weight loss of cellulose, hemicellulose, lignin and amino acids was observed to be more in the sets treated

with earthworms whereas amount of sugars was more in the controls as compared to the sets treated with earthworms. Positive significant correlation was observed between weight loss of litter with the different components in both the treatments. In the control set, weight loss of litter was positively correlated at different levels with the different components [cellulose ($r = 0.836$, $P < 0.10$), hemicellulose ($r = 0.962$, $P < 0.01$), lignin ($r = 0.985$, $P < 0.005$), total amino acids ($r = 0.978$, $P < 0.005$), sugars ($r = 0.932$, $P < 0.05$)]. Similarly, in the sets treated with earthworms, weight loss was positively correlated at different levels of significance [cellulose ($r = 0.853$, $P < 0.10$), hemicellulose ($r = 0.955$, $P < 0.02$), lignin ($r = 0.844$, $P < 0.10$), total amino acids ($r = 0.811$, $P < 0.10$) and total sugars ($r = 0.962$, $P < 0.01$)].

Figs. 19 a, b & c depict the pH, nitrogen content and $\text{PO}_4^{3-}\text{-P}$ content from the leachates. pH from the leachates was found to be more acidic in the treated sets as compared to that of the control sets (fig. 19a). Total Nitrogen and $\text{PO}_4^{3-}\text{-P}$ from the leachates also were observed to be more in the treated sets as compared to the controls (figs. 19 b & c). A positive significant correlation existed for $\text{PO}_4^{3-}\text{-P}$ of the leachates between the two treatments [$\text{PO}_4^{3-}\text{-P}$ ($r = 0.492$, $P < 0.5$), whereas, a negative correlation exists for total nitrogen of the leachate between the treatments ($r = -0.577$, $P < 0.5$). The organic carbon content of mineral soil in the second and third samplings was higher in the sets treated with earthworms, while in the first and fourth samplings the organic carbon content was higher in the control sets. The organic carbon content of the mineral soil was negatively correlated between the treatments ($r = -0.610$ $P < 0.5$). Total nitrogen content and available phosphorus of the mineral soil and humus was found to be more in the treated sets as compared to that of the control (figs. 20a,b&c). However, when comparison was made between the mineral soil, humus and earthworm casts, it was found that the casts had higher organic carbon, nitrogen and available phosphorus as compared to that of mineral soil and humus in both the sets. The nitrogen content of mineral soil and humus was positively correlated between the treatments for nitrogen ($r = 0.526$ & $r = 0.529$, $P < 0.5$).

**Fig. 21 CO₂ evolution at different time intervals. (o-o) = control (without earthworms),
(•-•) = treated (with earthworms).**

Fig. 21



Phosphorus content of the mineral soil between the treatments was positively correlated ($r = 0.760, P < 0.2$) while that of humus was negatively correlated ($r = -0.775, P < 0.2$).

Fig. 21 depicts the CO₂ evolution in the control and the treated sets. From the figure, it was observed that the CO₂ evolution was higher in the sets treated with earthworms than in the control sets. When the number of earthworms were counted after every destructive sampling, it was found that the number of earthworms decreased from 10, 9, 7, 4 to 2 at the end of the experiment (Initial to the fourth sampling period).

6 (a) Role of earthworms in fungal dispersal :

Fig. 22 (a, b & c) shows the comparison between the pH, moisture content and fungal population in the soil from earthworm furrows and adjacent soil to study the role of earthworms in fungal dispersal. pH of the adjacent soil was found to be more acidic as compared to that of the surrounding soil. Similarly, the moisture content of the soil collected from earthworm furrows was slightly higher than that of the adjacent soil. The fungal population was also recorded to be more in the soil collected from earthworm furrows as compared to that of adjacent soil. Moisture content was found to be positively correlated with fungal population in adjacent soil ($r = 0.608, P < 0.5$) as well as in soil from earthworm furrows ($r = 0.627, P < 0.5$).

A total of 8 fungal species could be isolated from both the adjacent soil and soil from earthworm furrows (Table 11). *Fusarium poae*, *Mucor hiemalis*, *Penicillium chrysogenum*, *P. frequentans*, *Pythium intermedium* and *Trichoderma koningii* could be isolated from adjacent soil as well as from the soil from furrows. There was not much variation in the fungal species collected from the two sites. However, *Humicola* sp. could be isolated from the soil from furrows only whereas, *Penicillium waksmanii* could be isolated from adjacent soil only. From the table, it was also observed that the percentage relative abundance of the fungal

Fig. 22 (a,b&c) pH, moisture content and fungal population of soil from earthworm furrows and adjacent soil at different time intervals.

Fig. 22

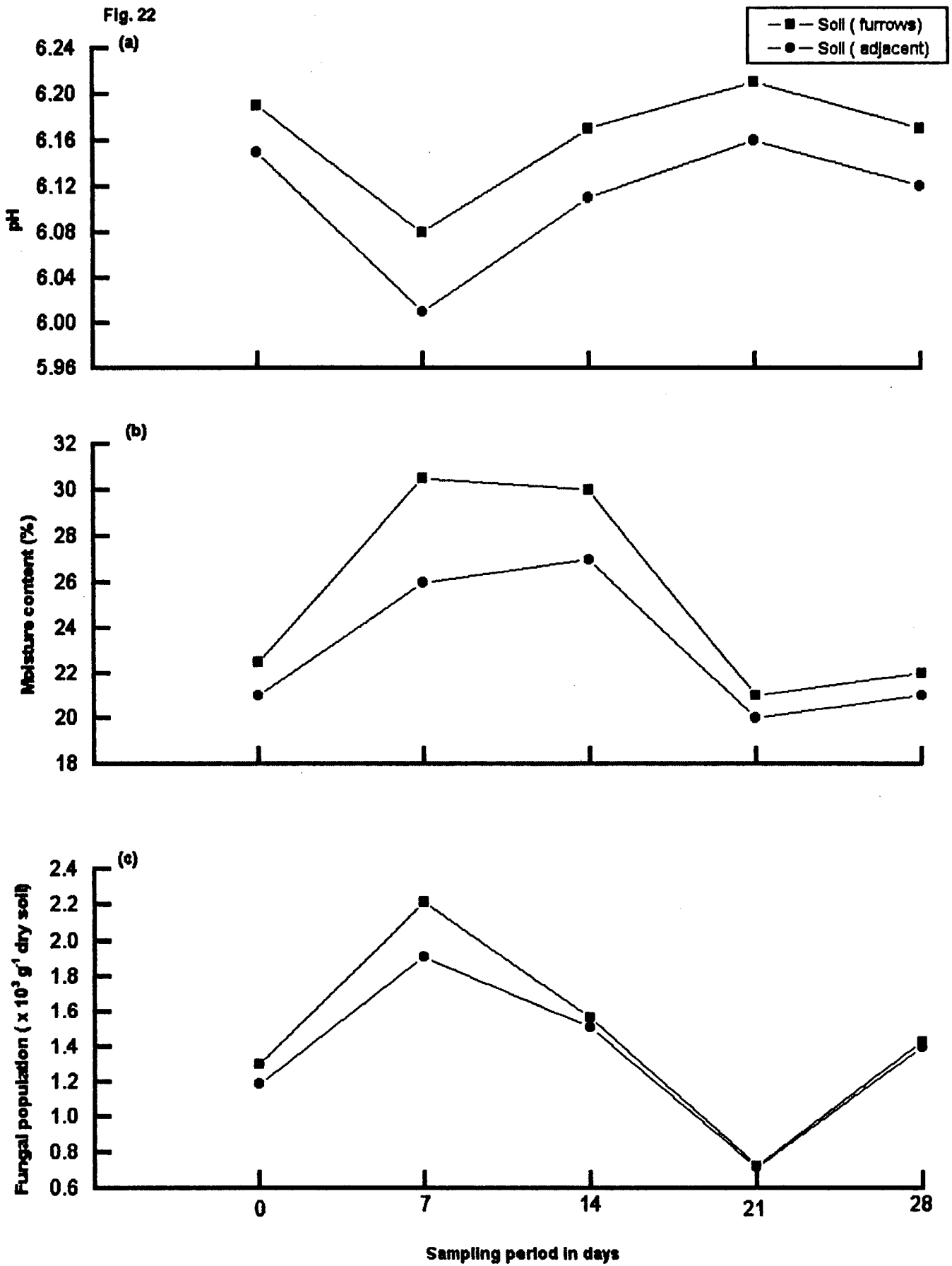


Table 11. Fungal population ($10^3 \times g^{-1}$ dry soil) in the soil collected from earthworm furrows and soil adjacent to furrows during the different sampling days. The values in the parentheses are percentage relative abundance.

Fungi	Sampling period (days)									
	0		7		14		21		28	
	Soil (furrow)	Soil (adjacent)	Soil (furrow)	Soil (adjacent)	Soil (furrow)	Soil (adjacent)	Soil (furrow)	Soil (adjacent)	Soil (furrow)	Soil (adjacent)
1. <i>Fusarium poae</i>	-	-	-	-	0.619 (39.39)	1.280 (84.85)	-	0.252 (35.29)	-	-
2. <i>Humicola</i> sp.	-	-	-	-	-	-	0.638 (88.24)	-	-	-
3. <i>Mucor hiemalis</i>	0.734 (56.67)	0.424 (35.71)	0.627 (28.26)	1.000 (52.38)	0.714 (45.45)	0.137 (9.09)	-	-	-	0.382 (27.27)
4. <i>Penicillium chrysogenum</i>	-	0.084 (7.14)	-	0.090 (4.76)	-	-	-	0.126 (17.65)	0.863 (60.61)	0.339 (24.24)
5. <i>P. frequentans</i>	-	-	-	-	0.142 (9.09)	0.045 (3.03)	-	-	-	-
6. <i>P. waksarii</i>	-	-	-	0.045 (2.38)	-	-	-	-	-	-
7. <i>Pythium intermedium</i>	0.518 (40.00)	0.382 (32.14)	1.543 (69.57)	0.773 (40.48)	-	-	-	-	0.474 (33.33)	0.382 (27.27)
8. <i>Trichoderma koningi</i>	0.043 (3.33)	0.297 (25.00)	0.048 (2.17)	-	0.095 (6.06)	0.045 (3.03)	0.085 (11.76)	0.336 (47.06)	0.086 (6.06)	0.297 (21.22)

species *P. intermedium* was high in the soil from earthworm furrows as compared to that in the adjacent soil.

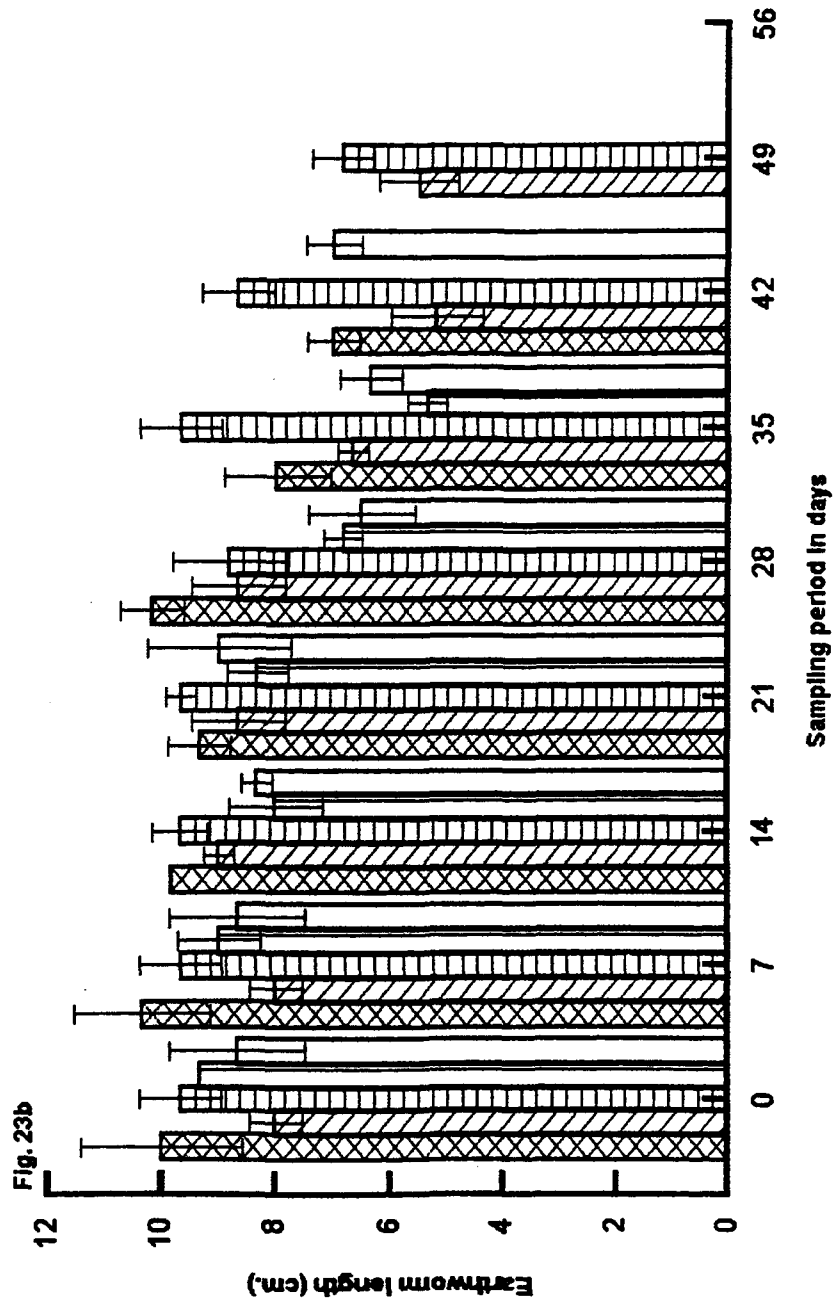
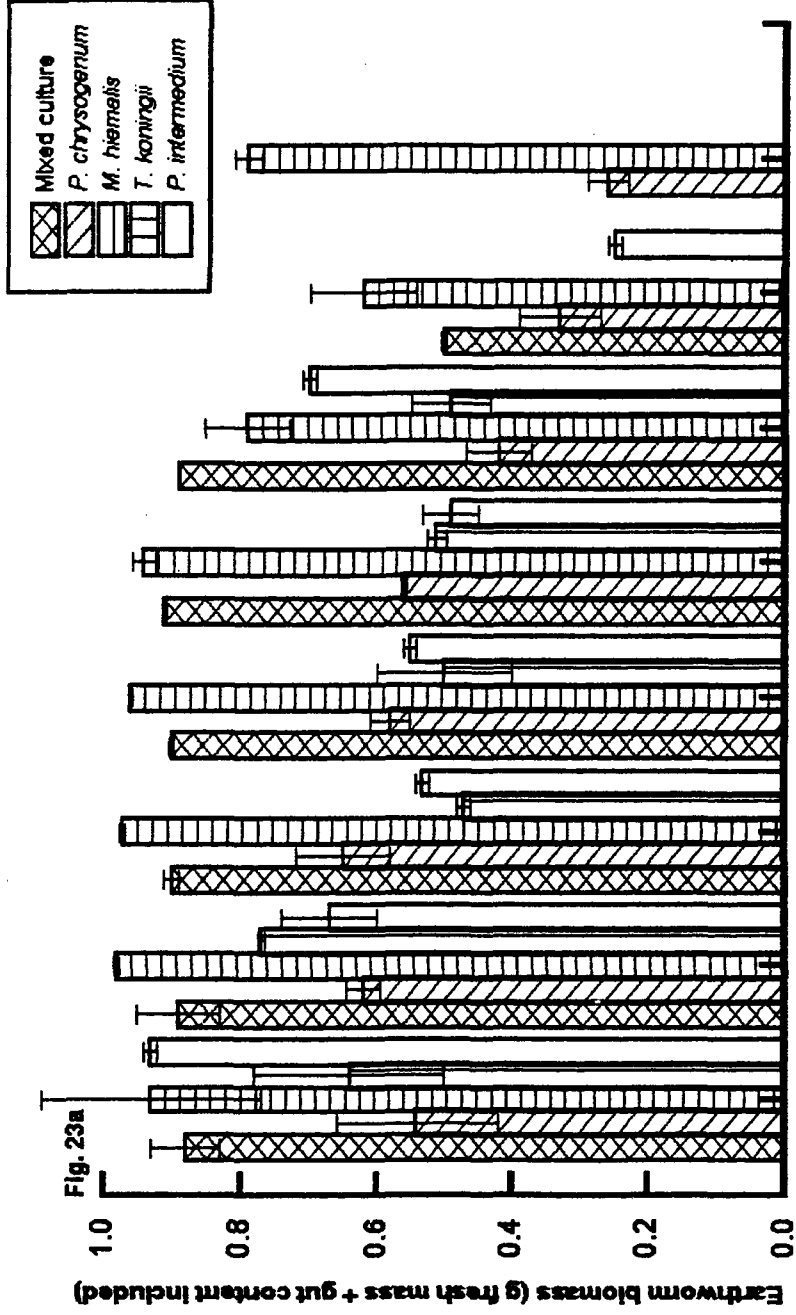
6 (b). Feeding habit of the earthworm species *Drawida papillifer papillifer* in relation to certain fungi :

Fig. 23a shows that there was an increase in the earthworm biomass at the initial stage indicating that *D. papillifer papillifer* can utilise fungi as their source of food. From the figure, it was evident that there was a gain in the earthworm biomass initially with the different fungal species except for *P. intermedium* where the biomass showed a decreasing trend. It was observed that there was an increase in the biomass of earthworm when fed with the mixed culture of all the test fungi for 35 days, 28 days when fed with *P. chrysogenum* and *M. hiemalis* but only 7 days when fed with *T. koningii*. It was also observed that the earthworms did not survive till the end of the experiment.

Fig. 23b shows the length of *D. papillifer papillifer* fed with the test fungi. It was observed that the length of the earthworm fed with the mixed culture showed a decreasing trend at the latter stages. The length of the earthworm fed with *P. chrysogenum* increased initially and thereafter decreased. It was also observed that the length of the earthworm remained unaffected for 35 days when fed with *M. hiemalis*. However, the length of the worm fed with *T. koningii* showed a decreasing trend. Similarly, when it was fed with *P. intermedium*, its length showed a decreasing trend at the latter stage.

A negative significant correlation was observed between the earthworm biomass and the period of sampling in the case of *P. chrysogenum*, *M. hiemalis* and *P. intermedium* ($P < 0.01, 0.05$ and 0.10) respectively. A negative significant correlation also exists between the length of the earthworm and period of sampling in all the treatments ($P < 0.05$). However,

Fig. 23 (a&b) Biomass and length of the earthworm *Drawida papillifer papillifer* Steph. fed with different test fungi at different time intervals.



when correlation coefficient was calculated between the biomass and length of the earthworm there exists a negative significant relationship in the case of *P. intermedium* ($P < 0.20$).

7 (I). Efficacy of certain fungi in decomposition :

(a) Field condition :

Fig. 24 depicts the microbial decomposition of pine litter under field condition. Decomposition was interpreted in terms of percentage weight remaining of the litter at different sampling periods. From the figure, the percentage weight remaining of the litter after 360 days was 53.9% . Moisture content of the decomposing litters exhibited fluctuation at different time intervals (fig. 25a). The pH of the decomposing litter was acidic (fig. 25b). The fungal population (fig. 26) was observed to be more in the later stages of decomposition as compared to that in the initial stages. A positive correlation was observed between fungal population and pH of the decomposing litter ($r = 0.229$, $P < 0.5$).

Table 12 shows the fungal species which were isolated from the decomposing litter placed under field condition. A total of 16 fungal species could be isolated from the litter bags during the different sampling periods. The fungal species isolated were *Alternaria alternata*, *Aspergillus japonicus*, *Cladosporium cladosporioides*, *C. herbarum*, *Fusarium sporotrichioides*, *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Trichoderma koningii*, white sterile mycelia , yellow sterile mycelia and black sterile mycelia.

(b) Laboratory condition :

Fig. 27 depicts the efficacy of the different fungal species to decompose the pine litter under laboratory condition. In terms of different fungal inoculum, *Mucor hiemalis* proved to be more efficient in the initial stage of decomposition of the pine litter followed by *Penicillium chrysogenum*, *Trichoderma koningii* and *Pythium intermedium*. In the control (without fungi) the decomposition was recorded to be the least. A mixture of all the

Fig. 24 Percentage of weight remaining of the decomposing pine litter placed in the field after different time intervals.

Fig. 24

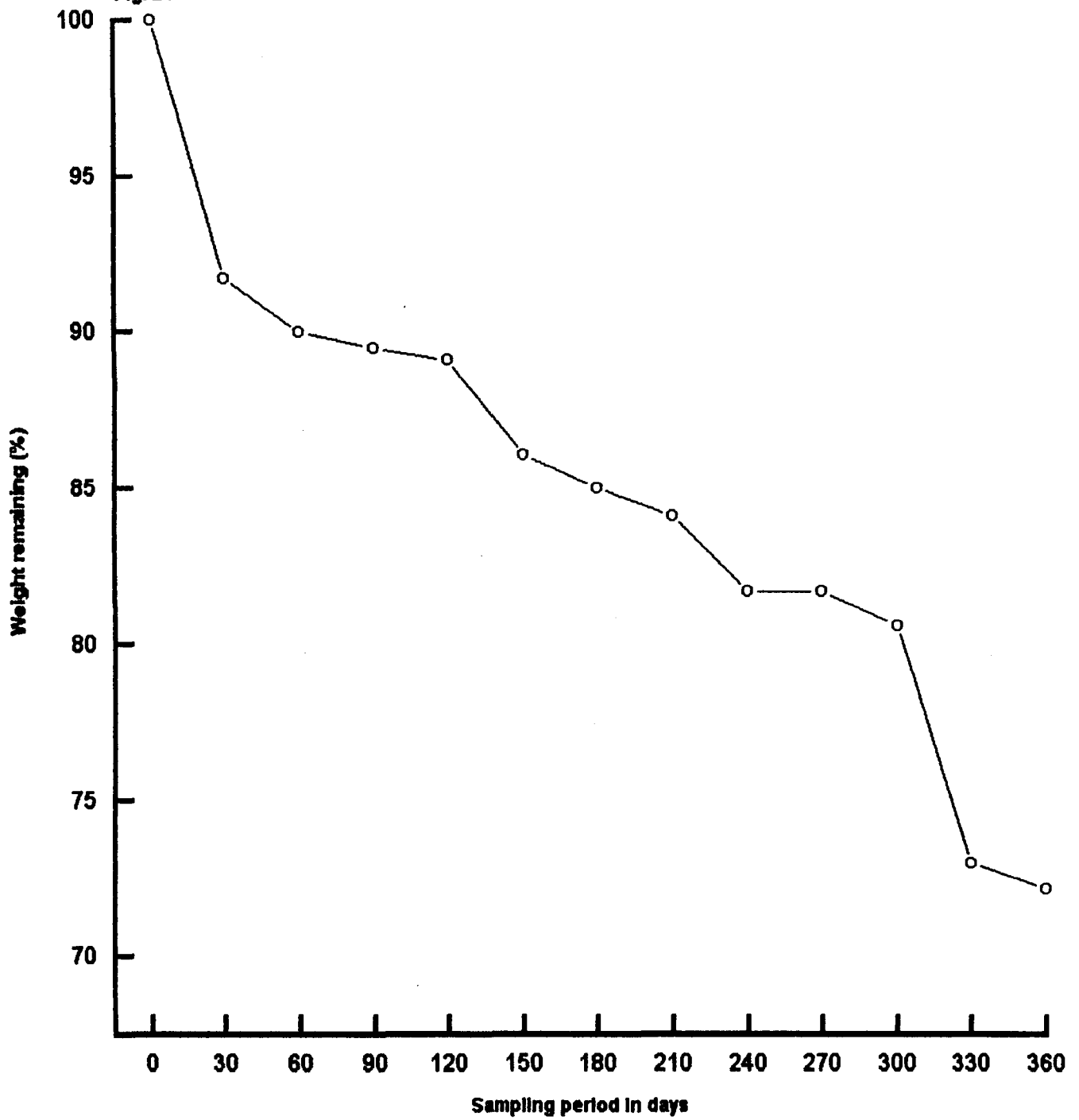


Fig. 25 (a&b) Moisture content and pH of the decomposing pine litter at different time intervals.

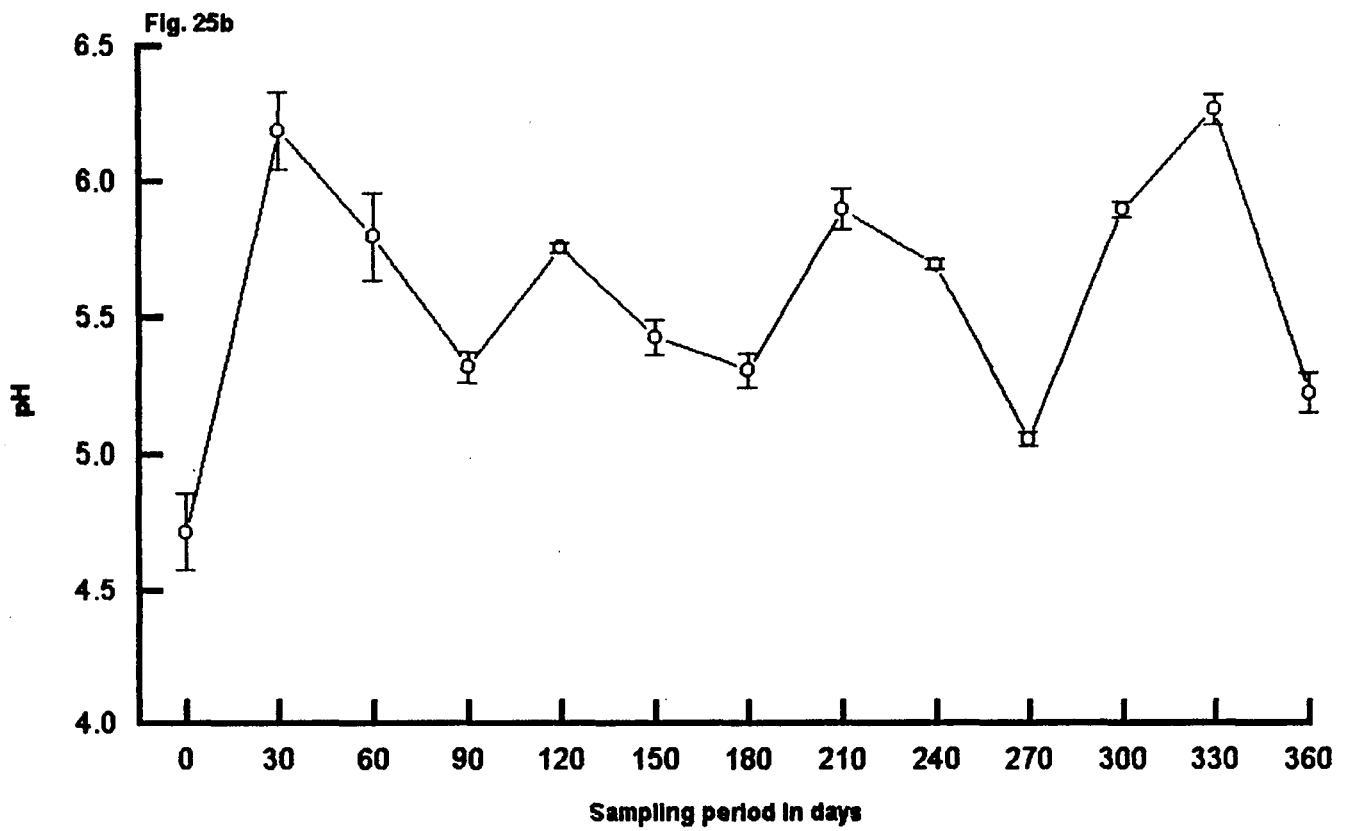
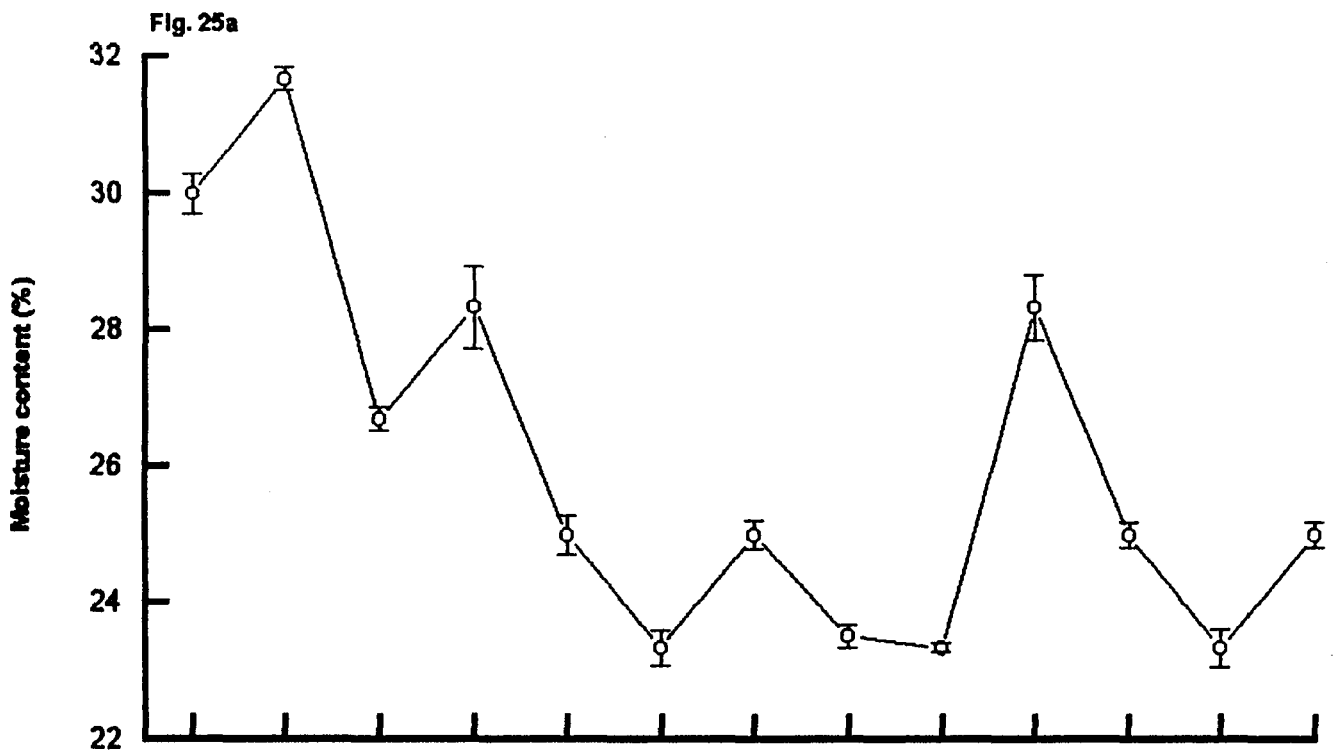


Fig. 26 Fungal population of the decomposing pine litter placed under field condition at different time intervals.

Fig. 26

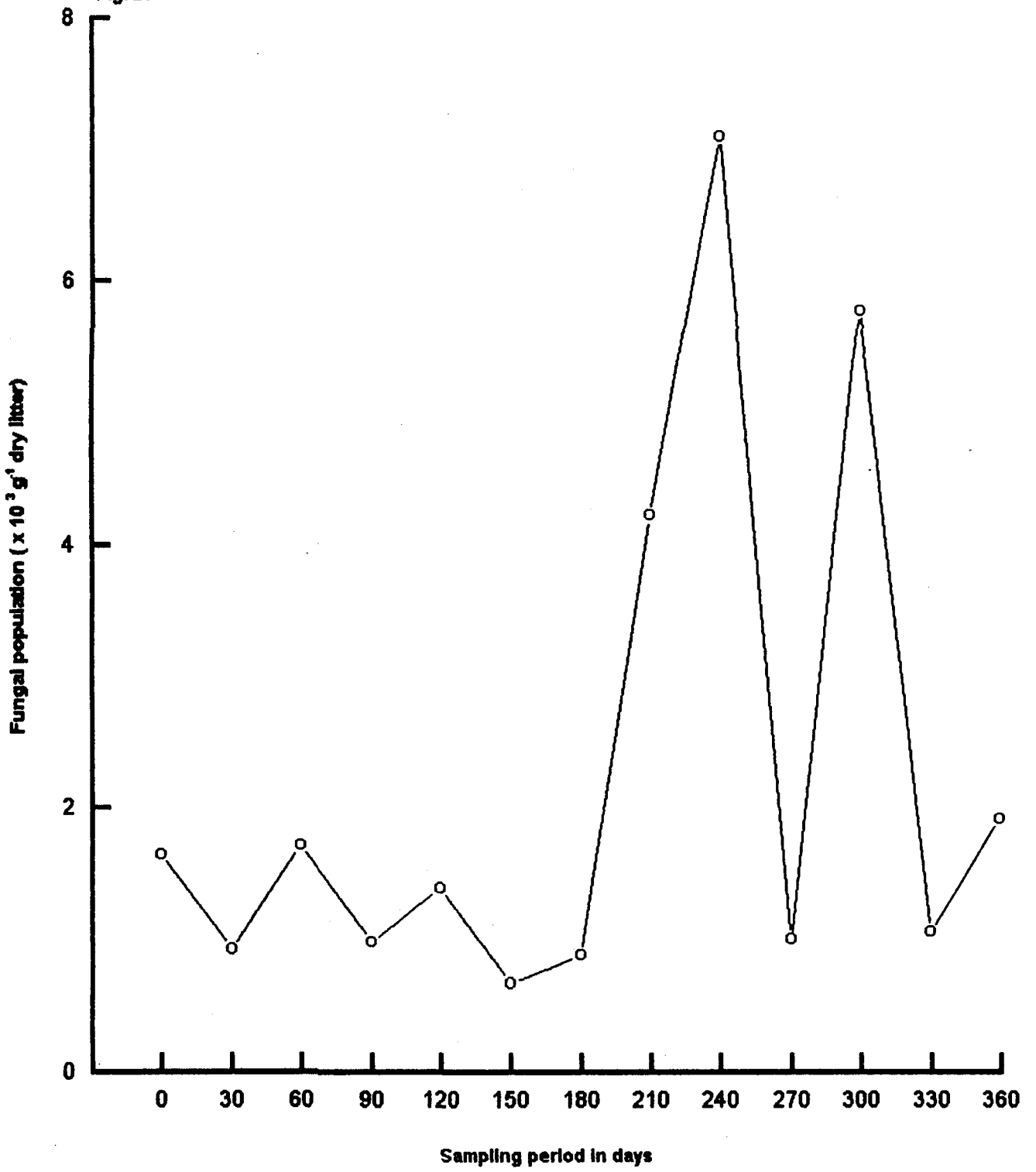


Table 12. Fungal population ($10^3 \times g^{-1}$ dry litter) associated with the decomposing pine litters placed in the field at various sampling periods. The values in the parentheses are percentage relative abundance.

Fungi	Sampling period in days												
	Initial	30	60	90	120	150	180	210	240	270	300	330	360
1. <i>Alternaria alternata</i>	-	-	-	-	-	-	-	-	0.041 (0.52)	-	-	-	-
2. <i>Aspergillus japonicus</i>	-	-	-	-	-	0.079 (11.76)	-	-	-	-	-	-	-
3. <i>Cladosporium cladosporioides</i>	-	-	-	-	0.447 (35.29)	-	-	0.356 (8.41)	-	-	-	-	-
4. <i>C. herbarum</i>	-	-	-	0.150 (16.00)	-	-	-	0.079 (1.87)	-	-	-	-	-
5. <i>Fusarium sporotrichioides</i>	-	-	-	0.935 (64.00)	0.112 (8.82)	-	-	-	-	-	-	-	-
6. <i>Mucor hiemalis</i>	0.673 (41.94)	-	0.036 (2.13)	-	-	0.079 (11.76)	-	-	-	0.082 (7.41)	-	-	0.320 (16.28)
7. <i>M. racemosus</i>	-	-	-	-	-	-	-	-	-	-	-	-	1.142 (58.14)
8. <i>Penicillium brevicompactum</i>	0.569 (35.48)	-	-	-	0.037 (2.94)	-	-	-	0.163 (2.09)	0.571 (51.85)	-	-	-
9. <i>P. chrysogenum</i>	-	-	-	0.112 (12.00)	-	-	-	0.119 (2.80)	0.857 (10.99)	0.082 (7.41)	-	0.092 (8.70)	0.046 (2.32)
10. <i>P. frequentans</i>	-	-	1.490 (87.23)	0.075 (8.00)	0.223 (17.65)	-	-	-	-	0.082 (7.41)	0.165 (2.86)	0.139 (13.04)	-
11. <i>P. javanicum</i>	-	-	-	-	-	0.079 (11.76)	-	-	-	0.041 (3.70)	-	-	-
12. <i>Pythium intermedium</i>	0.362 (22.58)	0.925 (100.00)	-	-	-	-	0.852 (95.65)	1.582 (37.38)	1.714 (21.99)	-	5.045 (87.14)	0.831 (78.26)	0.457 (23.26)
13. <i>Trichoderma korringii</i>	-	-	0.145 (8.51)	-	-	0.196 (29.42)	-	-	-	-	-	-	-
14. White sterile mycelia	-	-	-	-	0.447 (35.29)	0.235 (35.30)	0.039 (4.35)	-	-	0.245 (22.22)	0.579 (10.00)	-	-
15. Black sterile mycelia	-	-	-	-	-	-	-	2.096 (49.53)	4.937 (63.35)	-	-	-	-
16. Yellow sterile mycelia	-	-	0.036 (2.13)	-	-	-	-	-	0.082 (1.05)	-	-	-	-

Fig. 27 Percentage of weight remaining of the pine litter after different periods of decomposition by certain dominant fungi in laboratory condition.

Fig. 27

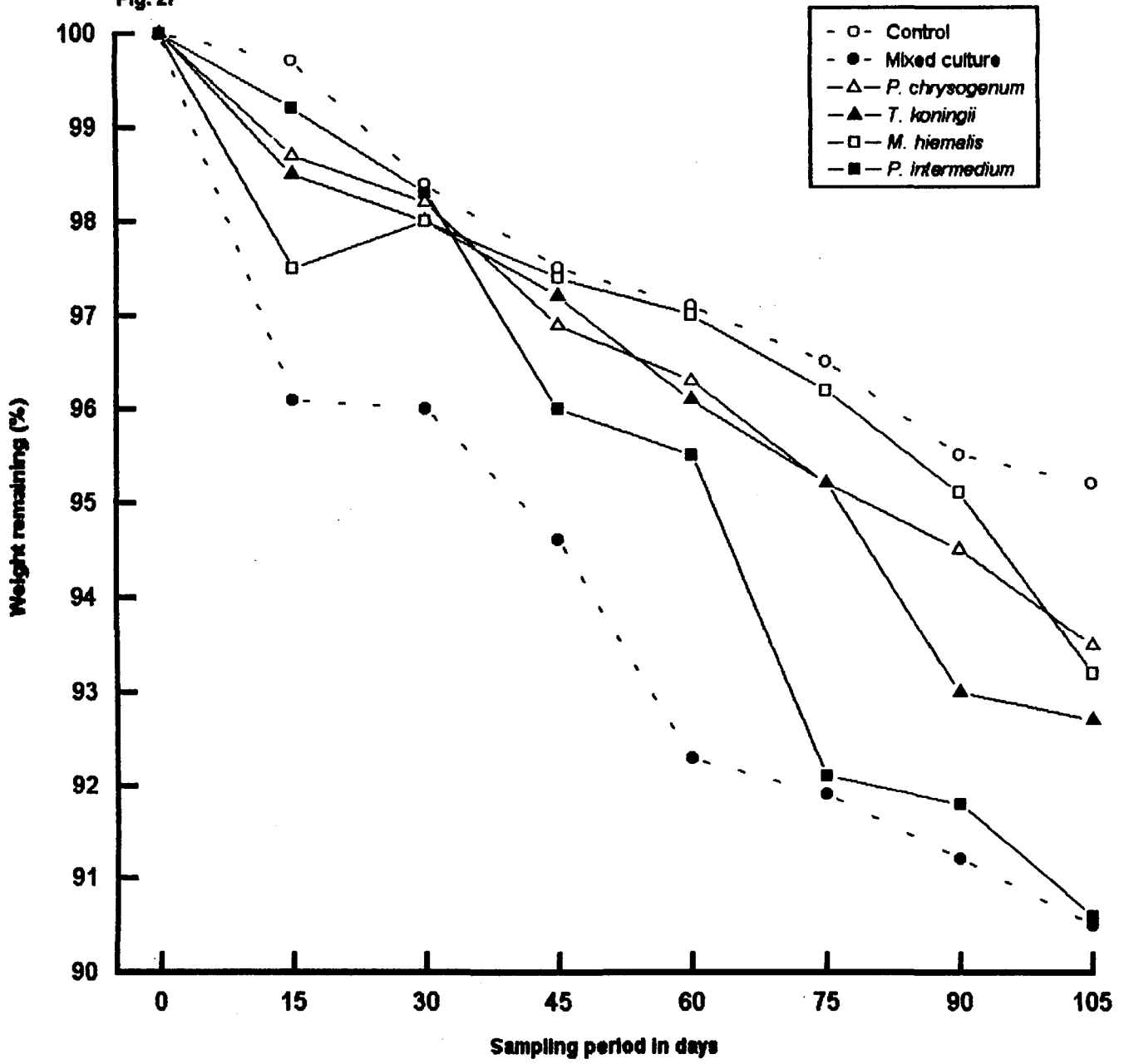


Fig. 28 (a&b) Variation of cellulose, hemicellulose, lignin, total sugars and amino acids of the decomposing pine litter at different periods of decomposition in field condition.

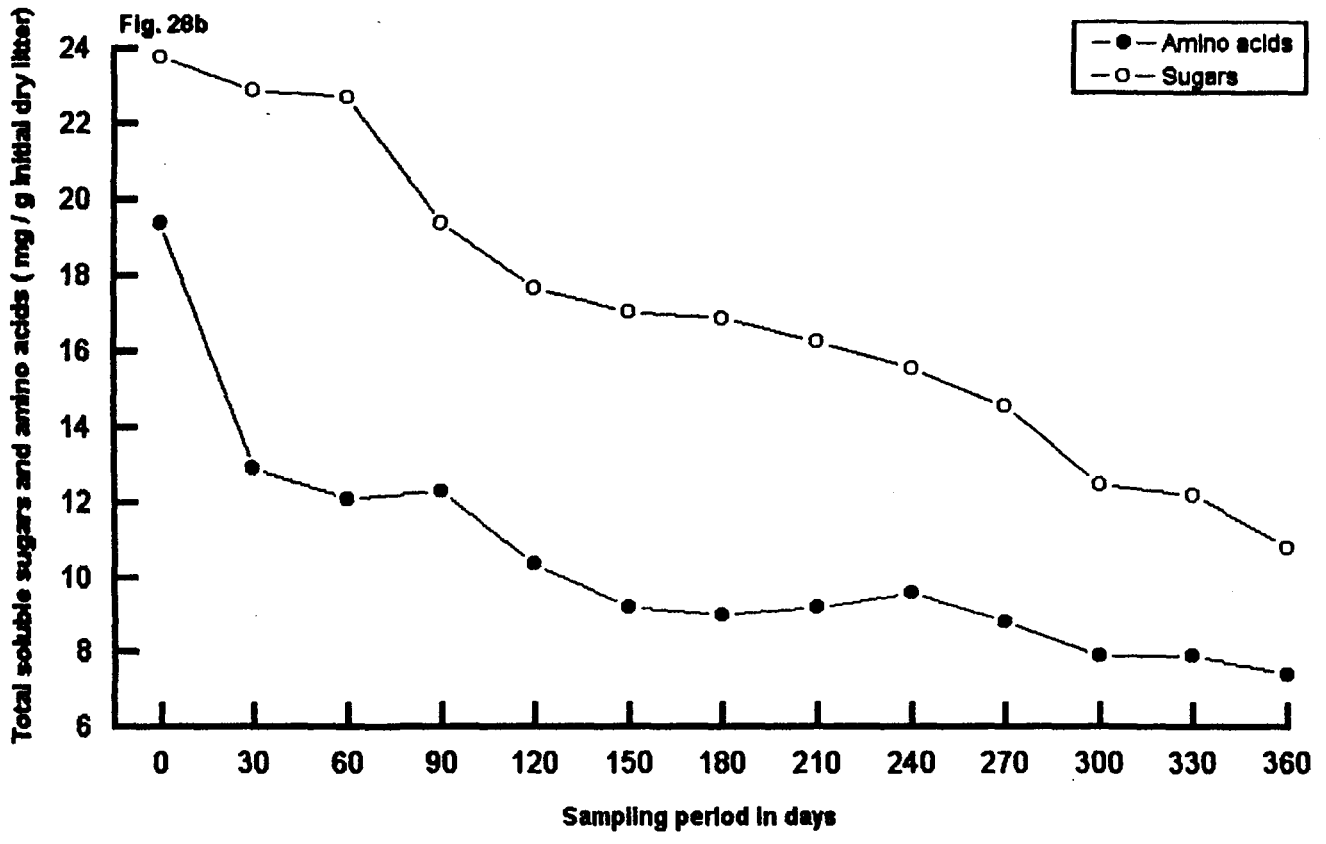
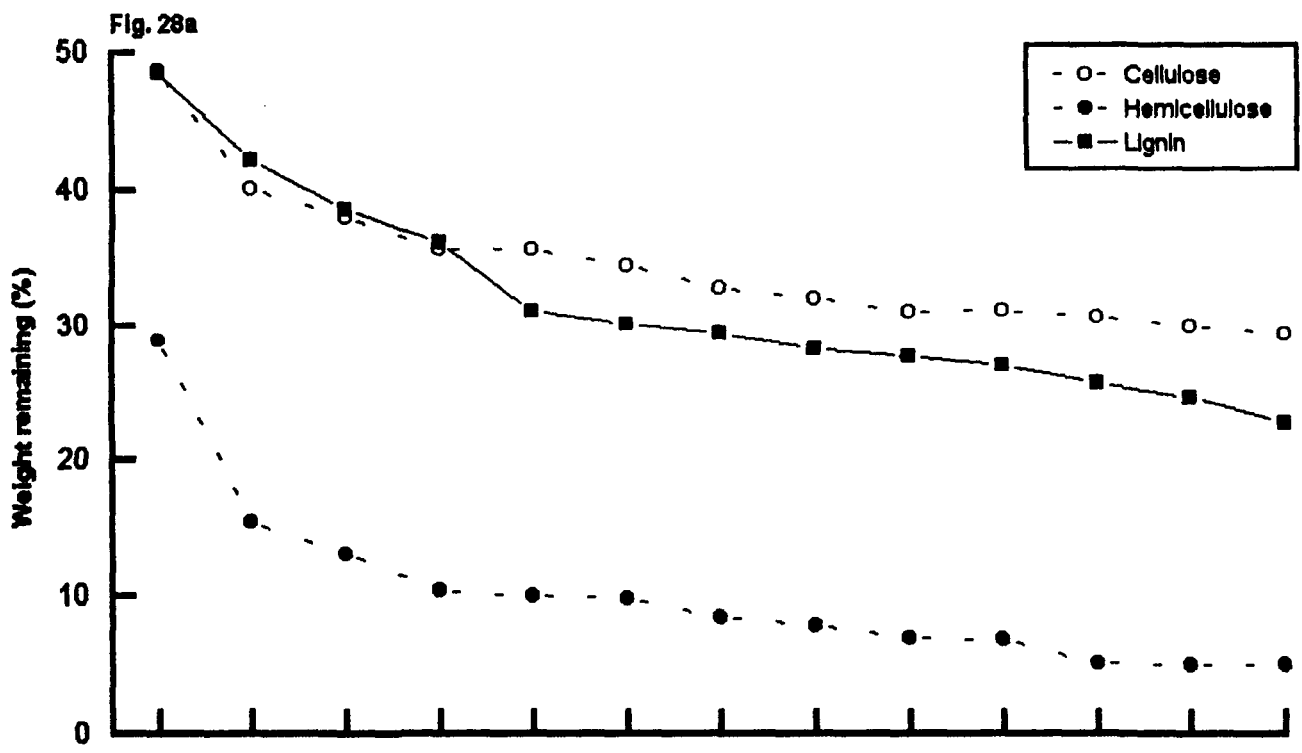
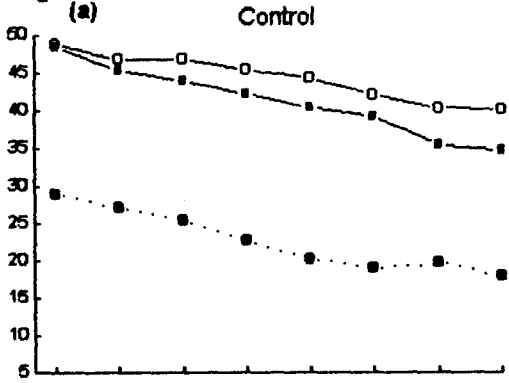
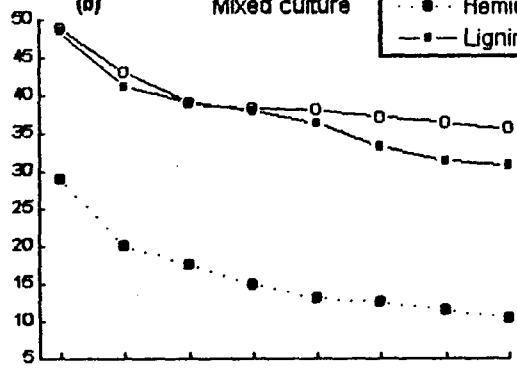


Fig. 29 (a,b,c,d,e&f) Variation of cellulose, hemicellulose and lignin content of the decomposed pine litter at different periods of decomposition by the test fungi in laboratory condition.

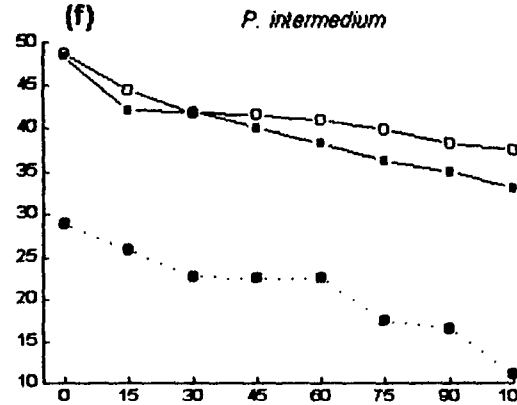
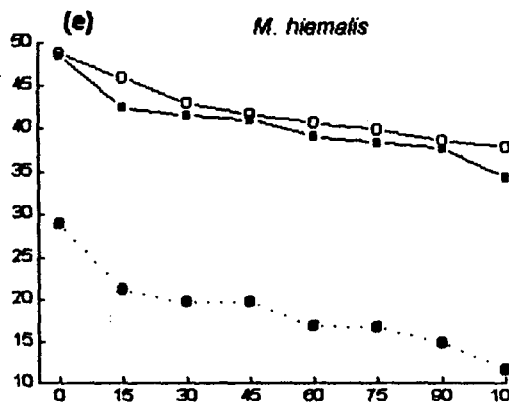
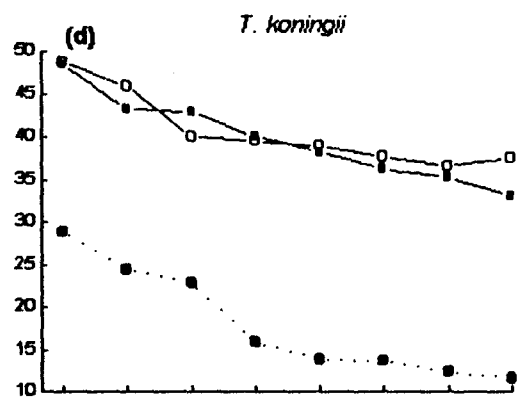
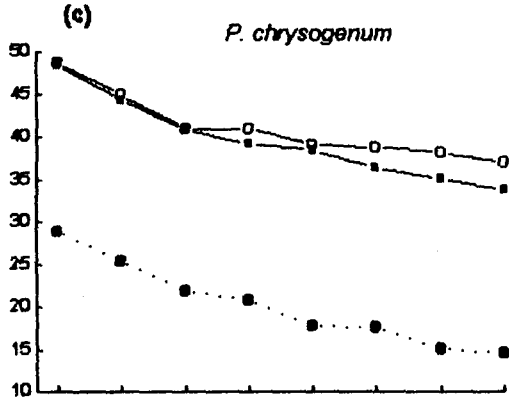
Fig. 29
(a)



(b) Mixed culture



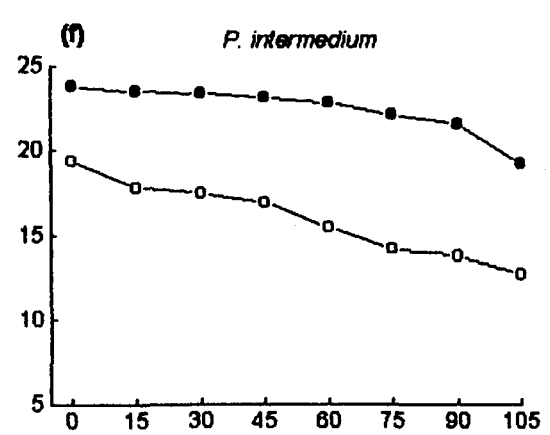
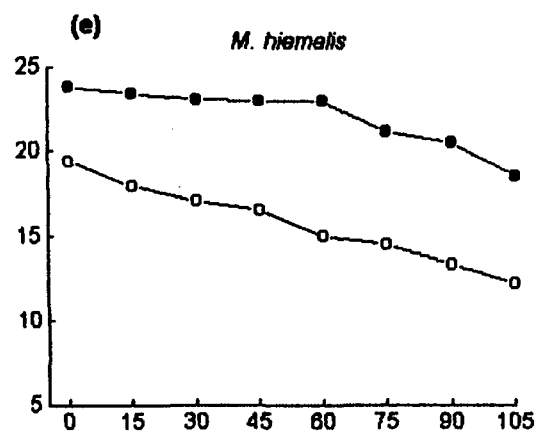
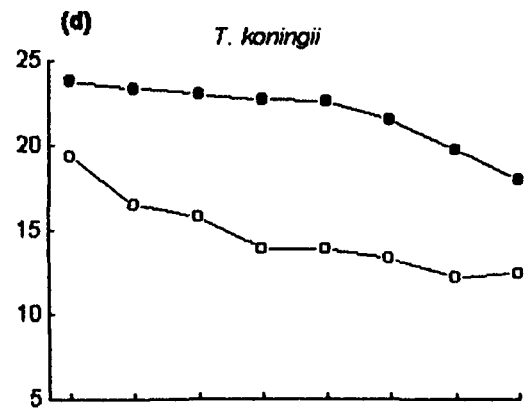
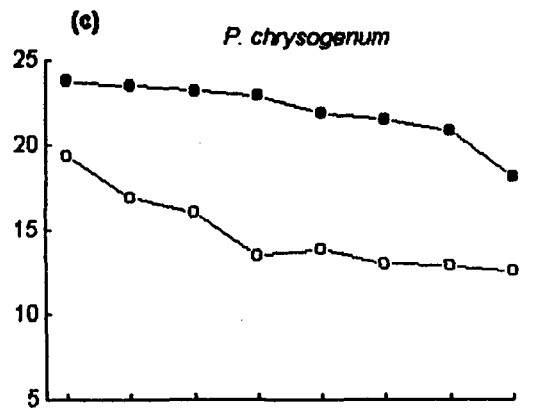
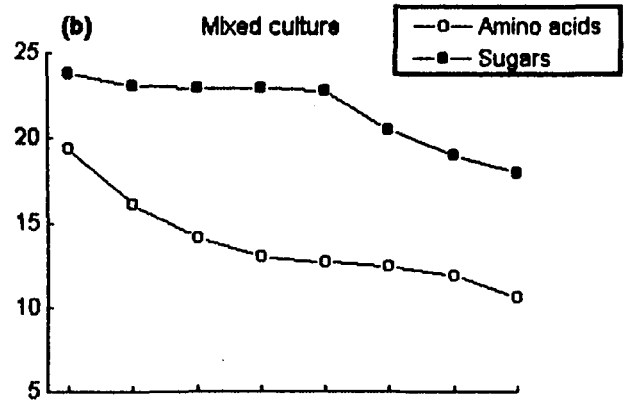
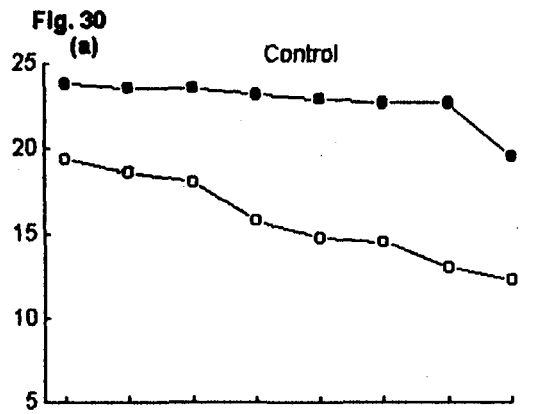
Weight Remaining (%)



Sampling period in days

Fig. 30 (a,b,c,d,e&f) Variation of total sugars and amino acid contents of the decomposed pine litter at different periods of decomposition by the test fungi in laboratory condition.

Total soluble sugars and amino acids (mg / g initial dry litter)



Sampling period in days

Table 13. Decay constant (K), Half life and 95% life (percentage weight remaining versus time in year) values for pine litter decomposition under field and by some test fungi under laboratory condition.

	K (per year)	Half life (year)	95% life (year)
Field	0.271	2.56	11.07
Laboratory :			
Control	0.069	10.04	43.48
Mixed culture	0.171	4.05	17.54
<i>Mucor hiemalis</i>	0.103	6.73	29.13
<i>Penicillium chrysogenum</i>	0.103	6.73	29.13
<i>Pythium intermedium</i>	0.137	5.06	21.80
<i>Trichoderma koningii</i>	0.103	6.73	29.13

Table 14. Correlation coefficients (r) for cellulose, hemicellulose, lignin, total sugars and amino acids with weight loss of pine litter with the different treatments under laboratory condition.

Sources of variation	D.F	Control	Mixed culture	<i>Penicellium chrysogenum</i>	<i>Mucor hiemalis</i>	<i>Trichoderma koningii</i>	<i>Pythium intermedium</i>
Cellulose	5	0.984***	0.933 ***	0.925 ***	0.885 ***	0.866**	0.329
Hemicellulose	5	0.977***	0.971***	0.971***	0.931 ***	0.917**	0.359
Lignin	5	0.965***	0.982 ***	0.972 ***	0.929***	0.970 ***	0.241
Total amino acids	5	0.988***	0.947 ***	0.938 ***	0.955 ***	0.929 ***	0.329
Total sugars	5	0.743 *	0.818 *	0.918 **	0.939 ***	0.919 ***	0.310

*, ** & *** (P < 0.05, 0.01 & 0.001 respectively).

test fungi, however, was observed to be still more efficient compared to the individual species. During the later stage of decomposition *Pythium intermedium* proved to be more efficient as compared to the other fungal species.

Comparing the decomposition rates of the pine litters placed under field and laboratory conditions, it was observed that the rate of pine litter decomposition was more faster under field condition ^{(Table 13),} which was approximately 80% as compared to that under laboratory condition which ^{was} approximately 90% in 105 days.

7(ii). Determination of cellulose, hemicellulose, lignin, total amino acids and sugars of the decomposing pine litter:

The various organic components viz. cellulose, hemicellulose, lignin, total amino acids and sugars were observed to be maximum in the initial stages of decomposition both under field and laboratory conditions (figs. 28a&b, 29a,b,c,d,e&f & 30a,b,c,d,e&f). The rate of decomposition of these various constituents was similar to that of weight loss of the decomposing litters at various sampling periods. Similar trend in the rate of decomposition was observed in the case of cellulose and lignin. The rate of degradation of the various constituents was more rapid in the field condition as compared to that in the laboratory condition.

Total sugars, amino acids and hemicellulose decomposed faster than cellulose and lignin. The rate of decomposition of cellulose, hemicellulose, lignin and sugars under laboratory condition was recorded to be highest in the mixed culture of all the test fungi and minimum in the control sets without the fungi. However, *M. hiemalis* was observed to be more efficient in the degradation of amino acids. High significant correlation was observed between weight loss of the litters and the various constituents [(P < 0.05, 0.01 and 0.001 respectively) Table 14].

DISCUSSIONS

1. Physico-chemical properties of soil and earthworm casts

Soil Temperature

Lowering of soil temperature at both the study sites in the winter months corresponds to the low atmospheric temperature during the period (fig. 3).

Moisture content

There was not much variation in the moisture content of the soil at both the study sites (figs. 4a&b), since variation in rainfall at both the study sites was not much (figs. 1a&b).

pH

The soil was found to be more acidic at high altitude than at low altitude (figs.5a&b) . The high acidic nature of the soil at high altitude is related to the dominance of pine litter. Higher pH values in the casts (figs. 5a&b) is in conformity with the reports of earlier workers (Lee, 1985; Mulongoy and Bedoret, 1989; Dkhar and Mishra, 1992). Higher pH values in the casts is due to ammonia excretion into the worm intestine and also due to the action of the calciferous glands in the worm pharynx when the soil is being ingested (Lee, 1985). Prento (1979) also suggested that the increase in pH during gut transit is related to mucus production and to the activity of the morren glands which secretes large amount of CaCO_3 .

Organic carbon

Soil at high altitude showed higher organic carbon content than the soil at low altitude (figs. 6a&b). Casts also showed higher organic carbon as compared to that of the surrounding soil. The high organic carbon content of the soil at high altitude is due to the continuous deposition of eroded soil rich in organic matter brought about by the surface run off of water from the adjacent hill areas. Lee (1985) suggested that the enrichment of organic carbon in casts

as compared to the surrounding soil was due to ingestion of plant and microbial residues and large quantities of mucopolysaccharides produced by the worm during digestion. The higher organic carbon content in the casts is ascribed to the fact that earthworms may select soil fractions enriched in organic compounds (Blair *et. al.*, 1994).

Total nitrogen

The increased nitrogen content in the soil at high altitude (figs. 7a&b) is also probably due to the continuous deposition of the eroded soil rich in organic matter brought about by the surface run off of water from the adjacent hill areas. The increase in total nitrogen content in casts as compared to the surrounding soil (figs. 7a&b) is a result of excretion of ammonia into the soil as it passes through the gut. Results of the present study are in conformity with the results of other workers (Graff, 1971; Lavelle *et. al.*, 1992) who also reported higher nitrogen content in the casts than that of the surrounding soil. Needham (1957), on the other hand, reported that very little nitrogen is excreted in the faeces of earthworms.

Available phosphorus

Higher concentration of available phosphorus in the soil at high altitude (figs. 8a & b) could be related to higher microbial activity (figs. 14a,b & 15a,b). Increase in pH in the casts (figs. 5 a & b) is responsible for the higher phosphorus content in the casts as was suggested by Barley (1961). Another possible reason could be that the gut passage may have altered the P-sorption capacities of the soil (Lopez-Hernandez *et.al.*, 1993).

2. Fungal population in the soil, casts , the earthworm gut and litter

Higher fungal population in the soil recorded in the month of May at high altitude and in June at low altitude (figs.9a&b) could be due to climatic conditions and availability of substrates during these months which appear to favour the growth and development of the soil microbes (Wright and Bohlen, 1961:). Lower fungal population during winter

at both the study sites may be ascribed to low moisture content during this period (figs. 4a&b).
^{Low}
 temperature during this period may not be conducive for fungal growth and multiplication. Low fungal population recorded in August at high altitude and June, July and August at low altitude is due to the adverse effect of excess moisture on fungal population (Kamal and Bhargava, 1973). *Penicillium chrysogenum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia were found to be dominant and with high percentage relative abundance at both the study sites during the study periods (Tables 1.1 to 1.4). Except for *R. stolonifer* the same species appeared to be dominant during the second year of study also (Tables 1.2 & 1.4). This result is in accordance with the findings of Bissett and Parkinson (1979) who pointed out that for a given community only a few species were dominant which may strongly affect the growth of other species. The dominance of these fungal species is due to their fast growing nature which inhibit the growth of slow growing species or also due to selection of media and method and which probably were not conducive for the isolation of other fungi which require specific media for their isolation. Another possible reason could be due to their wide tolerance to different extreme environmental conditions. The dominance of a few members of Fungi Imperfecti for example *P. chrysogenum* and *T. koningii* in the present study is in agreement with other workers (Kamal and Bhargava, 1973; Mishra and Kanaujia, 1972). It is believed that due to their wide tolerance and capability to decompose cellulose, Fungi Imperfecti dominate the soil fungal flora (Singh and Charaya, 1975). Qualitatively, there was difference in the fungal flora isolated from the soil at both the study sites. *Aspergillus terreus*, *Botrytis*, *Mucor plumbeus*, *M. racemosus*, *Trichoderma harzianum* and black sterile mycelia could be isolated from the soil at high altitude only, whereas, *Absidia glauca*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Fusarium merismoides*, *F. sporotrichioides* and *Oidodendron* sp. could be isolated from the soil at low altitude only. This variation in the

fungal flora may be due to the conditions which were conducive for the isolation of these fungal species from the respective study sites.

Statistically, a positive correlation was observed between fungal population and soil temperature at high altitude ($P < 0.05$). There was a significant positive correlation

between soil organic carbon and fungal population at low altitude ($P < 0.10$). These results are in agreement with the findings of other workers (Tiwari *et. al.*, 1987a; Tiwari *et. al.*, 1987b; Behera *et. al.* 1991) who suggested that organic matter, pH, moisture content and temperature influenced the abundance and distribution of micro-organisms in soil. A number of studies have also indicated that the vegetational cover had significant influence on the microbial population (Dwivedi, 1966; Mishra and Kanaujia, 1972). However, Jones and Richard (1972) did not find any such type of effect of vegetation on the microbial population.

Higher fungal population in the casts (figs.9a&b) is due to the fact that casts are usually rich in ammonia and partially digested organic matter and act as a good substrate for the growth of microorganisms. The intestinal mucus secreted during passage through the earthworm gut is egested with the casts and this secretion continues to stimulate microbial activity and growth (Barois and Lavelle, 1986; Scheu, 1991). Many other workers have also reported that microbial population of the earthworm casts is much larger than that of the surrounding soil (Dkhar and Mishra, 1986; Tiwari *et.al.*, 1989; Tiwari and Mishra, 1993).

Fungal population was observed to be higher in the fore-gut as compared to that of the mid-gut and hind-gut regions at both the study sites (fig. 10a&b). This result is in agreement with the results of earlier workers (Domsch and Banse, 1972; Dash *et.al.*, 1979) who reported destruction and digestion of various species of microfungi during passage through the earthworm gut. The earthworm gut contained higher number of fungal species as compared to that of the surrounding soil at both the study sites (Table 4.3). This result is in accordance with

the findings of Ghilarov (1963) and Atlavinyte *et. al.* (1971). Increase in the number of fungal species in the gut may be due to increased organic content of the digested soil due to the digestive activity of the earthworm. The fungal species found to be common in the soil, earthworm gut and the casts at high altitude include *Cladosporium cladosporioides*, *Fusarium solani*, *Humicola sp.*, *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia, while those found to be common in the soil, earthworm gut and the casts at low altitude include *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Fusarium solani*, *Humicola sp.*, *Mortierella parvispora*, *Mucor hiemalis*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia. These fungal species were found to be common in the soil, earthworm gut and casts because of the fact that they may be either having thick walled spores, or having antibiotic producing capacity and are not readily killed but are excreted with the casts whereby they germinate and grow fast due to increased nutrient level and act as foci for their dissemination in soil (Rabatin and Stinner, 1988). *Aspergillus carneus*, *A. wentii*, *Cladosporium herbarum*, *C. macrocarpum*, *Fusarium sporotrichioides*, *F. moniliforme*, *Penicillium daleae*, *P. digitatum* and *P. janthinellum* were found to be present in the gut of the earthworm collected from high altitude while, *Aspergillus terreus*, *A. wentii*, *Mucor racemosus*, *Penicillium daleae*, *Trichoderma harzianum* and *Verticillium alboatrum* were found to be present in the gut only from low altitude. The reason why these fungal species were restricted to the gut only could be that they may be suppressed in the soil due to the fast growth of other fungal species. The species found throughout the digestive tract of earthworms collected from high altitude were *Aspergillus flavus*, *A. wentii*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *F. solani*,

Humicola sp., *Mortierella parvispora*, *Mucor hiemalis*, *M. racemosus*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. daleae*, *P. digitatum*, *P. javanicum*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii* and white sterile mycelia. *Aspergillus flavus*, *A. terreus*, *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Fusarium solani*, *F. sporotrichioides*, *Humicola* sp., *Mucor hiemalis*, *M. circinnelloides*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. frequentans*, *P. javanicum*, *P. waksmanii*, *Pythium intermedium*, *Rhizopus stolonifer*, *Trichoderma koningii*, *T. harzianum*, *Verticillium alboatrum* and white sterile mycelia were found to be present throughout the digestive tract of the earthworms collected from low altitude. Presence of these fungal species throughout the canal of the earthworm could be due to the fact that various fungal spores have thick walled or wrinkled coats (Dash *et al.*, 1979) or are resistant to breakdown by intestinal enzymes of earthworm (Striganova *et al.*, 1989) thus leading to their survival during passage through the alimentary canal (Reddell and Spain, 1991a and Harinikumar and Bagyaraj, 1994). However, the presence of *Aspergillus terreus*, *Cladosporium herbarum*, *C. macrocarpum* and *Fusarium sporotrichioides* in the foregut region and *Aspergillus carneus* in the foregut and midgut regions of the earthworm collected from the stand at high altitude and *Alternaria alternata*, *Aspergillus japonicus*, *A. versicolor* and *Fusarium sporotrichioides* in the foregut and *Aspergillus carneus* and *A. candidus* in the fore-gut and mid-gut of the earthworm collected from the forest stand at low altitude indicated that they might have been digested in the midgut and hindgut regions of the earthworm. Although certain fungal species occurred throughout the digestive tract yet there was still some variation in the fungal flora of the different regions of the gut. Similar observations were also made in the variation of the mycoflora in different regions of the gut by various workers (Bhattacharya and Chakrabarty, 1986; Ghosh *et al.*, 1989). This variation in the mycoflora of the different regions of the gut suggests that some fungi which are in the

dormant stage gets broken down in course of their passage through the gut. The presence of the fungal species *Acremonium* sp., *Alternaria tenuis* and yellow sterile mycelia in the casts at high altitude and *Acremonium* sp., black sterile mycelia and yellow sterile mycelia in the casts at low altitude could be attributed to the nutrient enrichment in the casts which makes it ideal for the growth of these fungal species.

The monthly fluctuation in the fungal population of undecomposed and partially decomposed pine litter at both the study sites (figs. 13a&b) is due to the changes in weather conditions during the study period (Holm and Jensen, 1972).

The fungal population of undecomposed litter was recorded to be more at high altitude as compared to that at low altitude. This is due to high moisture content encountered in the litter at high altitude (figs. 11a&b). The maximum peak in the fungal population during the month of August is ascribed to high moisture content in this month (figs. 11a&b). The drop in fungal population during winter is due to low moisture content in the litter as well as low atmospheric temperature. This result is in accordance with the findings of various workers (Edmonds, 1979 and Rai and Srivastava, 1982) who also attributed low microbial counts to moisture stress and low temperature.

Although the moisture content of partially decomposed litter was observed to more at high altitude (figs. 11a&b), yet the fungal population was recorded to be less as compared to that at low altitude. Low fungal population may probably be due to excess moisture content in the litter. This result is in accordance with the findings of earlier workers (Witkamp, 1966 and Das, 1980) who also suggested that low fungal population during excess of moisture content in the litters is due to lack of aeration.

From table 6.5, it was observed that there was slight difference in the composition of the fungal species associated with the undecomposed and partially decomposed pine litters at

both the study sites suggesting that most of the fungi are non-selective and that they can utilize a wide variety of substrates.

3. Enzyme activities in soil and earthworm casts

Dehydrogenase and urease activities were recorded to be higher at high altitude as compared to that at low altitude (figs. 14a,b & 15a,b). The increase in enzyme activities at high altitude could be related to higher levels of organic carbon content in the soil (O'Toole *et. al.*, 1985 ; Tiwari *et. al.*, 1987; Tiwari and Sharma, 1998).

Dehydrogenase activity was observed to be low during winter at both the study sites. This result is in conformity with the results of Ross and Roberts (1970). Lower activity during winter months may be on account of lower moisture in the soil during these months. In the present study, dehydrogenase activity was recorded to be higher in the earthworm casts as compared to that of the surrounding soil. Higher dehydrogenase activity in the casts could be related to the higher moisture content in the casts (figs. 4a&b). An increase in moisture content enhanced the availability of organic carbon to heterotrophs responsible for the increased microbial activities in the casts. Brzezinska *et. al.* (1998) also suggested that the activities of soil dehydrogenases increased with increase in soil water content. In the present study, no significant correlation could be observed between dehydrogenase and fungal population and moisture content of the soil at both the study sites. Lack of correlation between these parameters indicated that some other biotic components may also regulate the enzyme activities. As dehydrogenase depends partly on the metabolic activity of the soil microorganisms (Paterson, 1967) it is not surprising that its value does not always reflect the total number of viable organisms isolated on a particular medium. Skujins (1973) also did not find any correlation between dehydrogenase activity and microbial numbers.

4. Earthworm population

Earthworm population was observed to be more at high altitude as compared to that at low altitude (fig. 16). The reason for this could be due to favourable conditions available in the soil at high altitude. The soil at high altitude had higher nutrient contents (organic carbon, total nitrogen and available phosphorus) as compared to that of the soil at low altitude. Higher nutrient contents in the soil at high altitude favoured the growth of a large number of earthworms. The seasonal variation in the earthworm population observed at both the study sites could be attributed to the earthworm species, soil type and altitude (Guild, 1952 and Murchie, 1958). Maximum earthworm populations were recorded in the month of June at high altitude and in the month of July at low altitude. This result is in accordance with the findings of Lavelle and Fragoso (1992) who also observed that earthworm activity in the humid tropics was recorded to be maximum during the hot wet seasons. Gates (1961) also suggested that earthworm activity was limited to certain seasons. Maximum populations during the summer months (June- July) could be attributed to favourable moisture and temperature in the soil as moisture and temperature are regarded as important factors affecting earthworms (Dash and Senapati, 1980 and Sahu and Senapati, 1986). Evans and Guild (1947c) also suggested that the soil conditions affecting earthworm activity were temperature and moisture besides another important factor which is the obligatory diapause or the period of quiescence during adverse conditions. Minimum earthworm population during the winter months is due to migration of earthworms away from the soil due to unsuitable temperatures (Dowdy, 1944). During the winter months the soil also was observed to be low in moisture content as a result of which the earthworms may have constructed cells lined with mucus fairly deep in the soil, and remained in a coiled position during the dry period (Edwards and Lofty, 1977). The low population could also be due to low availability of food supply. The positive significant correlation between

earthworm population, soil temperature ($P < 0.05$) and organic carbon ($P < 0.20$) at high altitude indicated that these two parameters are responsible for the maximum earthworm population at this site. Tiwari *et al.* (1992) also found significant correlation between earthworm populations and temperature and moisture in a pineapple field.

5. Role of earthworms in litter decomposition

From the present study, the percentage weight remaining of the litter in the sets treated with the earthworm species *Drawida papillifer papillifer*^{was} slightly less as compared to that remaining in the control. It appears that this earthworm species consumed less pine litter (17% after 120 days) as compared to the study made by MacKay *et al.* (1983) who observed that 50% of the wheat baits was consumed by *Lumbricus rubellus* within 10 days and 45% by *Aporrectodea caliginosa* within 12 days. The reason could be due to high polyphenolic contents in litter which reduced the palatability for earthworms (Slapokas and Granhall, 1991).

pH from the leachates was found to be more acidic in the sets treated with earthworms as compared to that in the controls (fig. 19a). This result is in accordance with the findings of MacKay *et al.* (1983) who suggested that earthworms remained active even at relatively low pH. However, the reverse was observed by Haimi and Einbork (1992) who observed that earthworm species *L. rubellus* raised the pH of the leaching waters. Total nitrogen and PO_4^{3-} phosphorus from the leachates in the sets treated with earthworms was observed to be more than that of the controls (figs. 19b&c). The difference in the total nitrogen and phosphate-phosphorus might have been caused indirectly by the stimulation of microbial activity or directly through mucus secretions. Theenhaus and Scheu (1996) also reported that leaching of N and P from the beech leaf litter was significantly increased in treatments with mucus and faecal material. It was also observed that Total -N and PO_4^{3-} -P decreased as the incubation period was increased. Similarly, the number of earthworms which was initially 10 in each container

was reduced to 2 after 120 days. Since only matured specimens were introduced into the containers, the specimens must have died since they are considered to have a certain lifespan. This reduction in the number of earthworms could also be attributed to the organic status of the soil in the containers for not being able to maintain the population of the earthworms (Boyer *et. al.*, 1999). The decreasing trend in Total-N and PO_4^{3-}P during different periods of sampling could be due to the reduction in the number of earthworms.

Total nitrogen and available phosphorus in the mineral soil and humus was recorded to be higher in the sets treated with earthworms than in the controls (figs. 20b&c). This increase demonstrates that the presence of earthworms can improve and enhance decomposition process and nutrient release. Ruz-Jerez *et. al.* (1992) also reported higher $\text{NO}_3\text{-N}$ concentration in the sets treated with earthworms *L. terrestris* and *Eisenia fetida* than in the control treatments (without earthworms). This increase could be due to mucus secretions and also due to the decaying tissues of the worms themselves. However, the main influence may be due to the indirect effects of their burrowing and feeding activity.

Higher CO_2 evolution in the sets treated with earthworms (fig. 21) may be caused by the direct contribution of earthworm respiration or by the stimulation of microbial activity. Similar observations were also made by different workers (Ross and Cairns, 1982; Haimi and Huhta, 1990 and Ruz-Jerez *et. al.*, 1992). Theenhaus and Scheu (1996) attributed the increase in basal respiration and microbial biomass to mucus secretion.

6 (a) Role of earthworms in fungal dispersal

Higher pH values in the soil collected from earthworm furrows (fig. 22a) may be due to secretion of mucous compounds on the body surface of the earthworms which tends to increase the pH value to neutral or even slightly basic reaction (Schrader, 1991). Earthworm burrows enhance percolation and improve vertical movement of water (Ehlers, 1975; Carter *et.al.*, 1982

and Roth and Joschko, 1991). This enhancement of percolation and vertical movement of water may have caused the high moisture content in the soil from furrows than from the adjacent soil (fig. 22b).

Higher fungal population in the soil collected from furrows (fig. 22c) may be due to the presence of mucous compounds secreted by the earthworms which may stimulate microbial activity and act as energy substrates for microorganisms (Scheu, 1991 and Edwards and Bohlen, 1996). From table 11, it is evident that there was slight variation in the the composition of of the fungal flora corresponding to the variation in the environmental factors like pH and moisture content (Dkhar and Mishra, 1986 and Ranee and Dkhar, 1998). The higher percentage relative abundance of *Pythium intermedium* in the soil from furrows (Table 11) indicates that the earthworms help in the dispersal of this pathogenic fungus. Similar observations were also made in the case of pathogenic fungi, *Fusarium* and *Pythium* that they can be transmitted through soil by earthworms (Baweja, 1939; Khambata and Bhatt, 1957 and Edwards and Fletcher, 1988). However, Toyota and Kimura (1994) made a reverse observation in which the earthworm *Pheretima* sp. disseminate the soil borne plant pathogen *Fusarium oxysporum* in the top soil but decreased the total propagules of this pathogen.

6 (b) Feeding habit of earthworms in relation to certain fungal species

Fig. 23 a shows an increase in the earthworm biomass at the initial stage indicating that *D. papillifer papillifer* can utilise fungi for its nutritional requirement (Pearce, 1978; Flack and Harstenstein, 1984; Dash *et.al.*, 1986). However, in case of *P. intermedium* the biomass showed a decreasing trend. This result is in accordance with the findings of Michon (1954) who suggested that earthworms gained weight until the disappearance of the clitellum that indicated the onset of senescence and that during this period the earthworms slowly declined in weight until their eventual death. There was an increase in the biomass of the earthworm when

fed with the mixed culture of all the four test fungi for 35 days, 28 days when fed with *P. chrysogenum* and *M. hiemalis* separately but 7 days only when fed with *T. koningii*. In other words, we can say that *P. chrysogenum* and *M. hiemalis* were taken most frequently as compared to the other fungal species (*T. koningii* and *P. intermedium*). This preference of *P. chrysogenum* and *M. hiemalis* over *T. koningii* and *P. intermedium* could possibly be due to higher nutritional value of the former fungi than the latter ones. Suggestions regarding preferential consumption of fungal species by earthworms occurring in the field has also been made by several workers (Dash *et.al.*, 1986; Striganova *et.al.* 1989). The preference in the mode of feeding of earthworm may also exist because fungi produce a number of volatile compounds that may be attractive or repellent to the earthworm as was noted for collembolans (Bengtsson *et. al.* 1988). It was also observed that the earthworms did not survive till the end of the experiment that could be due to exhaustion of nutrients or that the fungi may have produced certain toxins that may not be conducive for the growth of earthworms. That earthworms have certain lifespan cannot be ruled out also.

Fig. 23 b shows the length of *D. papillifer papillifer* fed with the test fungi. It was observed that the length of the earthworms fed with the mixed culture showed a decreasing trend at the latter stages. The length of the earthworms fed with *P. chrysogenum* increased initially and thereafter decreased. It was also observed that the length of the earthworms remained unaffected for 35 days fed with *M. hiemalis*. However, the length of the worms fed with *T. koningii* showed a decreasing trend. Similarly, when they were fed with *P. intermedium*, their lengths showed a decreasing trend at the latter stage. This difference in the length of the earthworms may be due to the quiescent behaviour of the worms themselves thus enabling them to exist for a longer period on their own reserves especially when there was limited supply of nutrients (Gerard, 1967). Since the food supplies in the later periods of incubation have become

exhausted the earthworms may have entered into a diapause resulting in the difference in length. Doeksen and van Wingerden (1964) considered that this diapause was induced by substances excreted by the worms themselves. Selective consumption of fungi by earthworms could have considerable implication for nutrient cycling and organic matter decomposition (Newell, 1984b). With sufficient knowledge of the earthworm-microbial interactions it is also possible to manipulate soil faunal and microbial communities to enhance soil fertility (Doube *et. al.*, 1994) and earthworm population could also be managed to provide soils in which disease severity to crops can be reduced as was suggested by Doube *et. al.* (1994).

7 (i) Efficacy of fungi in litter decomposition

(a) Field condition

The percentage weight remaining of pine litter placed under field condition decreased as decomposition proceeded (fig. 24). The rate of decomposition of the pine litter was comparatively slow with 53.9% weight remaining after 360 days as compared to that observed by Shukla *et. al.* (1990) in potato field with 41.4% only remaining after 30 days. This is perhaps due to a combination of a low base status substrate of the pine litter and containing resistant substances like waxes, resins and lignin and also due to cold climate (Das, 1980). The rate of litter decomposition is governed by environmental factors as well (Williams and Gray, 1974).

The pH and moisture content of the decomposing litter showed fluctuation during different sampling periods (figs. 25a&b). The fungal population also showed fluctuation during different sampling periods with the minimum population during the initial stage of decomposition which increased at latter stage (fig. 26). The reduced fungal population during the initial stages of decomposition may be due to unavailability of soluble nutrients or specific substrates which are still in complex forms. The progressive rate of decomposition must have

caused the mobilisation of the nutrients from the litter for microbial growth (Das, 1980). The gradual increase in the fungal population may also be due to favourable moisture condition, temperature and intensive activity of fauna which exposed the litter surface for microbial attack (Das, 1980). Fungi belonging to the class Fungi Imperfecti, Phycomycetes and the sterile forms belonging to the class Basidiomycetes were observed to be dominant in the decomposing litter. Among the Fungi Imperfecti, *Penicillium chrysogenum* and *Trichoderma koningii* appeared to be dominant indicating the free spore nature of these species. Similar result was observed by Bangar *et al.* (1979) who also reported that *T. viride* and *Penicillium sp.* were the most important cellulose degrading fungi. The presence of *Mucor hiemalis*, *P. chrysogenum*, *P. frequentans*, *Pythium intermedium* and *T. koningii* (Table 12) as dominant fungi may be explained on the basis of their faster growth in addition to their better intrinsic capacity to utilise the litter (Garrett, 1950). The frequent occurrence of *M. hiemalis* and *Pythium intermedium* may also be because of their greater affinity to utilise simple carbohydrates. The presence of sterile forms in high frequencies during the decomposition period may be due to their high saprophytic ability which helps in their survival even under adverse environmental conditions. These sterile forms may belong to Basidiomycetes which do not produce fruiting bodies on the agar medium (Mishra and Tiwari, 1984) and are responsible for the decomposition of lignin in the last stage of decomposition (Garrett, 1963).

(b) Laboratory condition

The differences in the weight loss of pine litter (fig. 27) treated with the different test fungi (*Penicillium chrysogenum*, *Pythium intermedium*, *Trichoderma koningii* and *Mucor hiemalis*) under laboratory condition suggests that these fungal species vary in their decomposing ability even under identical environmental conditions. In terms of individual fungal inoculum, *M. hiemalis* proved to be efficient in the initial stages of decomposition of the

litter followed by *P. chrysogenum*, *T. koningii* and *P. intermedium*. This result is in conformity with the results of several workers (Sadasivan, 1939 and Sinha and Dayal, 1983) who reported that some members of Phycomycetes were involved at the initial stage of decomposition.

(ii) Chemical analysis of the decomposing pine litter

The various organic components viz. cellulose, hemicellulose, lignin, total amino acids and sugars were observed to be maximum at the initial stages of decomposition both under field and laboratory conditions (figs. 28a&b, 29a,b,c,d,e&f and 30a,b,c,d,e&f). The decrease in these constituents may be due to their utilization by the microbes (Harper and Lynch, 1981). The rate of decomposition of these various constituents was similar to that of weight loss of the decomposing litters at various sampling periods. Similar trend in the rate of decomposition was observed in the case of cellulose and lignin. The rate of degradation of the various constituents was more rapid in the field condition as compared to that in the laboratory condition. This is primarily due to the total exclusion of decomposer fauna from the litter due to partial sterilization and also due to several biotic and abiotic factors, which influence the rate of litter decomposition under field condition.

Total sugars, amino acids and hemicellulose decomposed faster than cellulose and lignin. This result is in accordance with the findings of Das (1980). Autolytic process in the decomposing litters led to the decomposition of sugars very rapidly. The slow decomposition of cellulose and lignin in the decomposing litters may be due the slow exposure of these constituents to microbial attack through removal of the other constituents. The rate of decomposition of cellulose, hemicellulose, lignin and sugars under laboratory condition were recorded to be the highest in the mixed culture of all the test fungi and minimum in the control sets without the fungi. However, *M. hiemalis* was observed to be more efficient in the degradation of amino acids. The efficiency of *M. hiemalis* in the degradation of amino acids has

also been suggested by Das (1980) since this species is known to have greater affinity towards amino acids. The high significant correlation observed in table 14 between weight loss of the litters and the various constituents ($P < 0.05$, 0.01 and 0.001 respectively) indicate that these components or constituents need to be considered in predicting the decomposition rates.

From the present investigation, we can conclude that earthworm casts are important microsites with high microbial abundance, activity and nutrient availability as compared to the surrounding soil. However, further research quantifying the production of earthworm casts by earthworms with different ecological characteristics and by determining the distribution of casts in the soil profile would help us to assess the overall importance of earthworms on soil structure and fertility. Further research is still needed for better understanding on the distribution and abundance of most species of earthworms and their activities in forest ecosystems of the north-eastern region. The increase in total nitrogen, phosphate phosphorus in the leachates and in total nitrogen, available phosphorus and CO_2 evolution in the sets treated with the earthworm species *Drawida papillifer papillifer*^{Steph.} clearly demonstrates that the presence of earthworms can improve and enhance decomposition process and nutrient release. Further, they help in the dispersal of beneficial as well as harmful fungi. Studies of different earthworm species on vermicomposting will be of significant importance in vermitechnology since the vermicompost so produced will provide a highly needed organic manure, an alternative replacement for chemical fertilizers.

SUMMARY

For the present investigation, two forest stands of *Pinus kesiya* (Royle ex-Gordon) at different altitudes of Meghalaya were selected. The study sites chosen were Upper Shillong at a higher altitude situated at 1825m above sea level and the other site at Mawlai at a lower altitude situated at 1400m above sea level.

The soil and the earthworm casts collected from the two pine forest stands were analysed for various physico-chemical properties and fungal population. Generally soils at low altitude showed higher temperature than soils at high altitude. During winter months (October to January) temperature decreased and started to increase again after February at both the study sites. There was not much variation in the moisture content of the soil at both the study sites. Low soil moisture content was recorded during the winter months which increased with the onset of rains. When comparison was made between the moisture content of the earthworm casts with that of the surrounding soil, it was observed that the earthworm casts generally harboured higher moisture content except in few instances at both the study sites. The soil was found to be more acidic at high altitude than at low altitude. The casts had high pH values as compared to the surrounding soil. Generally, soil at high altitude showed higher organic carbon, nitrogen and phosphorus contents than soil at low altitude. The earthworm casts contained higher organic carbon, nitrogen and phosphorus contents than the surrounding soil at both the study sites.

Isolation of fungi from soil and casts was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. Fungal population of soils exhibited a more or less similar trend of monthly variation at both the study sites. Higher fungal population was recorded in the month of May in the soil at high altitude, whereas, at low altitude the fungal

population was higher in the month of June. It was observed that the earthworm casts generally harboured maximum population as compared to that of the surrounding soil. A positive correlation was observed between the fungal population of soil and soil temperature at high altitude ($P < 0.05$). A significant positive correlation was also observed between soil organic carbon and fungal population at low altitude ($P < 0.10$). The pine forest soil at high altitude exhibited highest number of fungal isolates as compared to that at low altitude. Qualitatively there was not much difference in the composition of microflora of the two pine forest soils. Most of the fungal species which were obtained from the soil at high altitude were also isolated from the soil at low altitude except for *Aspergillus terreus*, *Botrytis* sp., *Mucor plumbeus*, *M. racemosus*, *Trichoderma harzianum* and black sterile mycelia which were isolated from the soil at high altitude only, while *Absidia glauca*, *A. versicolor*, *Cladosporium herbarum*, *Fusarium merismoides* and *F. sporotrichioides* and *Oidodendron* sp. which were restricted to the soil at low altitude only.

The casts collected from the pine forest stand at low altitude exhibited a highest number of fungal isolates as compared to that collected from the pine forest stand at high altitude. Qualitatively, there was not much difference in the composition of the microflora of the casts collected from both the study sites except for *Alternaria tenuis*, *Mucor racemosus* and *M. circinelloides* which were isolated from the casts at high altitude only and *Aspergillus candidus*, *Cunninghamella elegans*, *Fusarium oxysporum*, *Mortierella parvispora* and *Penicillium janthinellum* which were isolated from the casts at low altitude only.

The earthworm species found at high altitude were identified as *Eutyphoeus* sp., *Amyntas corticis* (Kinberg), *Eutyphoeus festivus* Gates, *Drawida papillifer papillifer* Steph., *Lenoscolex strigosus* Gates and *Kanchuria sumerianus* Julka while those found at low altitude were identified as *Drawida papillifer papillifer* Stephensen, *Amyntas corticis*

(Kinberg) and *Metaphire anomala* (Michaelsen). However, *Drawida papillifer papillifer*

Steph. and *Amyntas corticis* (Kinberg) were found to be common at both the study sites.

✓ *Drawida papillifer papillifer* was chosen for studying the fungal population from the gut of the earthworm. The fungal population in the gut of earthworm *Drawida papillifer papillifer* at both the study sites was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. Results showed that the population was generally higher in the fore-gut as compared to that of the mid-gut and hind-gut regions. Qualitatively, there was not much difference in the composition of the fungal flora in the gut contents of the earthworm at both the study sites except for *Cladosporium macrocarpum*, *Fusarium moniliforme* and *Penicillium digitatum* which could be isolated from the gut regions of the earthworm collected from high altitude only and *Aspergillus japonicus*, *A. versicolor*, *Alternaria alternata*, *Cunninghamella elegans* and *Verticillium albo-atrum* which could be isolated from the gut regions of earthworm collected from low altitude only.

A comparative study of the fungal flora present in the soil, gut contents of the earthworm and the casts collected from the pine forest stands at both the altitudes showed that altogether a total of 28 fungal species could be isolated from the soil, 32 from the gut contents of the earthworm and 19 from the casts at high altitude. Of the 32 fungal species occurring in the different gut regions, 26 species could be isolated from the foregut, 23 from the midgut, and 25 from the hindgut regions. A total of 28 fungal species could be isolated from the soil, 32 from the gut contents and 21 from the casts at low altitude. Of the 32 fungal species occurring in the different gut regions, 29 species could be isolated from the foregut, 27 from the midgut and 26 from the hindgut regions. The earthworm gut contained higher number of fungal species as compared to that of the surrounding soil.

Moisture content and pH of both the undecomposed and the partially decomposed pine litter was also analysed. Moisture content of undecomposed and partially decomposed pine litter at high altitude was generally higher than that at low altitude. The moisture content of the partially decomposed pine litter was more than that of the undecomposed litter at both the study sites. There was not much difference in the pH of the litter collected from the two study sites. However, pH of the partially decomposed litter at high altitude was more acidic as compared to that at low altitude. When comparison was made between the pH of the two types of litter it was found that pH of the undecomposed pine litter was more acidic as compared to that of the partially decomposed pine litter.

Similarly, the fungal population from the undecomposed as well as the partially decomposed pine litter was done following Warcup's (1950) method using rose bengal agar (Martin, 1950) medium. There was not much variation in the fungal population of the undecomposed litter at both the altitudes. However, the fungal population of the partially decomposed pine litter was found to be more at low altitude. There was not much variation in the fungal population between the two types of pine litter also at both the study sites. A positive significant correlation was observed between fungal population and moisture content of the litter at high altitude ($P < 0.5$). Undecomposed litter at high altitude exhibited highest number of fungal isolates as compared to the litter at low altitude. Qualitatively, there was not much difference in the fungal flora of the litter except for *Mucor racemosus*, *Aspergillus carneus* and *A. flavus* which were isolated from the litter at high altitude only, and *Mucor circinelloides* and *Alternaria alternata* which could be isolated from the litter at low altitude only. The partially decomposed pine litter at high altitude exhibited more or less similar number of fungal isolates at both the study sites. Qualitatively, there was not much difference in the composition of the fungal flora of the litter except for *Mucor racemosus*, *Absidia cylindrospora*, *Aspergillus*

terreus, *A. carneus*, *A. candidus*, *A. flavus* and *Acremonium* sp. which could be isolated from the litter at high altitude only and *Aspergillus wentii*, *Penicillium brevicompactum*, *P. rubrum*, *Monilia* sp. and yellow sterile mycelia which were associated with the litter at low altitude only.

There was not much difference in the composition of the fungal species associated with the undecomposed and partially decomposed pine litters at both the study sites. When comparison was made between the fungal species present in the undecomposed and partially decomposed litters, slight difference in the fungal species composition was observed in which *Alternaria alternata*, *A. tenuis*, *Aspergillus niger* and *Mucor circinelloides* were found to be present in undecomposed litter only, whereas *Absidia cylindrospora*, *Ambylosporium* sp., *Aspergillus wentii*, *Fusarium merismoides*, *Monilia* sp., *Penicillium rubrum* and *P. waksmanii* were found to be present in partially decomposed litter only.

Dehydrogenase activity of soil and the casts was determined by 2, 3, 5- triphenyl tetrazolium chloride (TTC) reduction technique modified by Casida *et. al.* (1977). The dehydrogenase activity was higher in the soil at high altitude as compared to that at low altitude except in the months of September, February, March, April and November where a lower activity was recorded. It was observed that the earthworm casts generally showed higher dehydrogenase activity as compared to that of the surrounding soil at both the study sites. Dehydrogenase activity of soil was found to vary significantly between the sampling periods at 5% level of significance.

Urease activity was measured by the modified McGarity and Myers' (1967) method. Higher urease activity was recorded in the soil at high altitude except in the months of October, April and May where lower activity was recorded. Urease activity of earthworm casts was also recorded to be higher than that of the surrounding soil at both the study sites. Statistically, a

significant correlation was observed between urease activity of soil and fungal population at high altitude ($P < 0.10$). A significant correlation was observed between urease activity and moisture content at both the study sites ($P < 0.05$ at high altitude and $P < 0.001$ at low altitude). A significant variation was observed for urease activity of the soil between the study sites as well as between the sampling periods at 5% and 1% level of significance.

Wilcke's (1955) method of handsorting was adopted for the estimation of earthworm population from the two study sites. The earthworm population was more at high altitude as compared to that at low altitude. Population was highest in June at high altitude and in July at low altitude. Minimum population was recorded in February at both the study sites. It was found that earthworm population was positively correlated with soil temperature ($P < 0.05$) and organic carbon ($P < 0.2$) at high altitude.

For determining the role of earthworms in litter decomposition under laboratory condition the method adopted by Haimi and Huhta (1990) was followed. Since *Drawida papillifer papillifer* appeared to be the dominant species, therefore, this particular species has been selected for the study. Two sets were maintained for this study with one set without earthworms being treated as a control, while another set treated with earthworms. Weight loss of pine litter was slightly more in the treated sets as compared to that of the control. Percentage weight remaining after 120 days was 83.4% in the control and 82.1% in the treated sets. The various components like cellulose, hemicellulose, lignin, total amino acids and sugars of the decomposing pine litter in the controls as well as in the sets treated with earthworms were maximum in the initial stages of decomposition which was observed to decrease in the latter stages as decomposition proceeded. pH from the leachates was found to be more acidic in the treated sets as compared to that of the control sets. Total Nitrogen and $\text{PO}_4^{3-} - \text{P}$ from the leachates also were observed to be more in the treated sets as compared to the controls. The

organic carbon content of mineral soil in the second and third samplings was higher in the sets treated with earthworms, while in the first and fourth samplings the organic carbon content was higher in the control sets. Total nitrogen content and available phosphorus of the mineral soil and humus was found to be more in the treated sets as compared to that of the control sets. However, when comparison was made between the mineral soil, humus and earthworm casts, it was found that the casts had higher organic carbon, nitrogen and available phosphorus as compared to that of mineral soil and humus in both the sets. CO₂ evolution was higher in the sets treated with earthworms than in the control sets.

To study the role of earthworms in fungal dispersal, screening of fungi was done from soil collected from earthworm furrows and the soil adjacent to the furrows. Moisture content, pH and fungal population were determined from the two study sites (earthworm furrows and the adjacent soil). The fungi were isolated from the soil samples by soil plate method (Warcup, 1950) using rose bengal agar medium (Martin, 1950). pH of the adjacent soil was found to be more acidic as compared to that of the surrounding soil. The moisture content of the soil collected from earthworm furrows was slightly higher than that of the adjacent soil. The fungal population was also recorded to be more in the soil from earthworm furrows as compared to that of adjacent soil. Moisture content was found to be positively correlated with fungal population in adjacent soil ($P < 0.5$) as well as in soil from earthworm furrows ($P < 0.5$). There was not much variation in the fungal species collected from the two sites. However, *Humicola* sp. could be isolated from the soil from furrows only whereas, *Penicillium waksmanii* could be isolated from adjacent soil only. It was observed that the percentage relative abundance of the fungal species *P. intermedium* was high in the soil from earthworm furrows as compared to that in the adjacent soil.

Feeding habit of the earthworm species *Drawida papillifer papillifer* Steph. in relation to certain fungi was studied in conical flasks (250 ml) containing 150g of sterilized soil and 1g of sterilised partially decomposed pine litter. The test fungi used for this experiment were *Penicillium chrysogenum*, *Pythium intermedium*, *Mucor hiemalis* and *Trichoderma koningii*. Results showed that there was an increase in the earthworm biomass at the initial stage, thereby, indicating that *D. papillifer papillifer* can utilise fungi as their source of food. There was a gain in the earthworm biomass initially with the different fungal species except for *P. intermedium* where the biomass showed a decreasing trend. It was also observed that the earthworms did not survive till the end of the experiment. The length of the earthworm fed with the mixed culture, *T. koningii* and *P. intermedium* showed a decreasing trend at the latter stages. The length of the earthworm fed with *P. chrysogenum* increased initially and thereafter decreased. It was also observed that the length of the earthworm remained unaffected for 35 days fed with *M. hiemalis*.

Litter bag technique (Bocock *et. al.*, 1960) was applied to study the rate of pine litter decomposition in the field. The percentage weight remaining of the litter placed in the field after 360 days was 53.9%. For the isolation of fungi from the decomposing litter bags was done following dilution plate technique (Waksman, 1922). The fungal population was observed to be more in the later stages of decomposition as compared to that in the initial stages. A positive correlation was observed between fungal population and pH of the decomposing litter ($P < 0.5$).

Four dominant fungi (*Penicillium chrysogenum*, *Pythium intermedium*, *Mucor hiemalis* and *Trichoderma koningii*) were selected to assess their efficacy in litter decomposition in the laboratory condition. In terms of different fungal inoculum, *Mucor hiemalis* proved to be more efficient in the initial stage of decomposition of the pine litter followed by *Penicillium*

chrysogenum, *Trichoderma koningii* and *Pythium intermedium*. In the control (without fungi) the decomposition was recorded to be the least. A mixture of all the test fungi, however, was observed to be still more efficient as compared to the individual species. During the later stage of decomposition *Pythium intermedium* proved to be more efficient as compared to the other fungal species.

Comparing the decomposition rates of the pine litters placed under field and laboratory conditions, it was observed that the rate of pine litter decomposition was more faster under field condition, which was observed to be approximately 80% as compared to that under laboratory condition which was observed to be approximately 90% in 105 days.

Cellulose, hemicellulose, lignin, total sugars and amino acids of the decomposing pine litters were estimated by methods described by Peach and Tracey (1955). The various organic components viz. cellulose, hemicellulose, lignin, total amino acids and sugars were observed to be maximum in the initial stages of decomposition both under field and laboratory conditions. The rate of decomposition of these various constituents was similar to that of weight loss of the decomposing litters at various sampling periods. Similar trend in the rate of decomposition was observed in the case of cellulose and lignin. The rate of degradation of the various constituents was more rapid in the field condition as compared to that in the laboratory condition. Total sugars, amino acids and hemicellulose decomposed faster than cellulose and lignin. The rate of decomposition of cellulose, hemicellulose, lignin and sugars under laboratory condition were recorded to be the highest in the mixed culture of all the test fungi and minimum in the control sets without the fungi. However, *M. hiemalis* was observed to be more efficient in the degradation of amino acids.

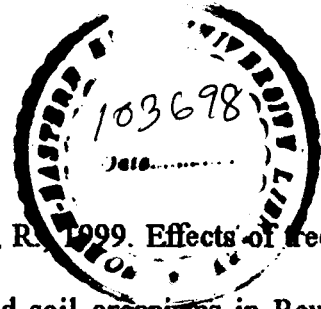
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