STUDIES ON THE MICROBIOLOGY OF COAL MINE SPOILS OF JAINTIA HILLS, MEGHALAYA

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CERTIFICATE

I, Easter Meena Blah, hereby, declare that the subject matter of the thesis entitled "Studies on the microbiology of coal mine spoils of Jaintia Hills, Meghalaya" is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me 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.

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CHAPTER I

GENERAL INTRODUCTION

Mineral resources are essential for human existence. Though, the exploitation of mineral resources is important for developmental activities, it has led to degradation and destruction of ecosystems. During the last few decades, large scale coal mining activities have resulted in the degradation and destruction of natural vegetation in Meghalaya. Degradation of natural resources have lead to the loss of gene pool and biological diversity. For exploration of coal, heaps of debris consist of consolidated and unconsolidated overburden overlying the coal seams have to be removed and they form a waste material known as "overburden" of "coal mine spoil" (Wali, 1987). Spoils materials associated with mining often contain pyrite (FeS_2) when it is exposed to the atmosphere produces H_2SO_4 (Pitchtel and Dick, 1991). Acidic coal mine spoils contain toxic level of soluble elements such as Fe, Al, Mn and Cu (Lyngdoh, 1995). Soil, the most important natural resource harbours a variety of microorganisms and is considered to be the most dynamic site of interactions in nature. The degradation of soil has an effect on vegetation and soil, especially on the microbial populations (Sharma, 1981 and Deka and Mishra, 1982). The microorganisms are responsible for the breakdown of organic matter and release of nutrients. They are regarded as the principal agents of mineral cycling in soil due to their role in translocation of elements like carbon, nitrogen, phosphorus and potassium. Thus, the microflora influences the availability of nutrients in the system which consequently regulates the structure and function of ecosystem. Understanding of the variation in fungal population in time and space has paramount importance due to its relevance in biodiversity and role of fungi in regulating population of other organisms and ecosystem processes. The microorganisms are important in soil formation and revegetation through their activities as decomposers and in nutrient cycles. The microorganisms contribute not only to establishment of the biogeochemical cycles in developing soils but may also contribute to amelioration of adverse physical and chemical limitations of mine spoils e.g. heavy metal contents, acidic pH, poor structure (Tate, 1985 and Visser, 1985). These organisms also play a beneficial and primary role in the amelioration of the detrimental effect. Re-establishment of the necessary biogeochemical cycles may also contribute to a healthy belowground and aboveground ecosystems (Miller and Cameron, 1978).

Soil microbial biomass contribute to soil fertility and it represents an important living part of soil organic matter. It is an agent for the transformation of added and native organic matter and acts as a labile reservoir for plant available N, P and S (Jenkinson and Ladd, 1981). The physico-chemical characteristics of soil influence the level of biomass and the activity of microorganisms. Soil with a relatively high organic matter input usually develops a larger microbial biomass which accounts for 1-3% of soil organic C, but it acts as a medium through which all organic nutrients that enter the soil must pass (Jenkinson, 1977). Values of microbial biomass can provide one of the most satisfactory estimates of the restoration of soil microbial populations (Maithani, 1996).

Microbial biomass is defined as the living part of the soil organic matter excluding plant roots and soil animals larger than 5 x 10^3 cm size (Maithani, 1996). The active phase of soil organic matter is intimately related to the biomass serving as the main energy source for the biomass and recycling its organic output (Jensson and Persson, 1982). Recognition of the importance of soil microorganisms in the functioning of ecosystems has led to an increased interest in measuring the nutrients held in soil biomass (Brookes *et al.*, 1982).Numerous studies on the measurement of microbial C, N and P in different natural and disturbed ecosystems have shown that the soil microbial biomass contain important labile pools of C and mineral nutrients which are liberated after the death of microorganisms (Anderson and Domsch, 1980, Smith and Paul, 1990 and Diaz-Ravina *et al.*, 1993). Large annual fluctuations in the microbial biomass has been reported by Lynch and Panting (1980 a, b) and Ross *et al.* (1981), while others observed only small annual changes (Schnurer *et al.*, 1986 and Patra *et al.*, 1990). The turnover time of microbial biomass is estimated at 1-3 years (Paul and

Voroney, 1980 and Schnurer *et al.*, 1985). This rapid turnover rate makes the soil microbial biomass an important dynamic source of plant nutrients (Jenkinson and Ladd, 1981 and Lodge, 1993). Microbial biomass can also serve as a sensitive indicator of toxicity. Brookes and McGrath (1984) observed a decline in soil microbial biomass in a heavy metal contaminated sludge. Stress by heavy metal toxicity and low pH has been shown to result in a reduction in the size of soil microbial biomass (Chander and Brookes, 1991b, Wilter *et al.*, 1993, Bardgete *et al.*, 1994 and FlieBbach *et al.*, 1994). Soil microbial biomass is an active fraction of organic matter which constitutes a reservoir of nutrients and participates in nutrient cycling (Smith and Paul, 1990). The microbial biomass content of the soil depends on the quality and quantity of resource and its distribution of the C-input varies with time and depth (Kaiser and Heinemeyer, 1993). Generally, microbial biomass-C comprises about 2-4% of total organic C (Anderson and Domsch, 1989) but variation within this range is influenced by the quantity and quality of organic inputs to the soil (Wardle, 1992).

Soil microorganisms are of great importance for soil ecosystems because they affect plant available nutrients and soil structural stability (Paul and Clark, 1989). The size and activity of the microbial populations depend on quantity and quality of soil organic matter, soil texture, soil pH and other properties of soil (Insam *et al.*, 1989 and Kaiser *et al.*, 1992).

Major biological processes such as mineralization, immobilization, nitrification, nitrate reduction etc. are the result of microbial activities and are catalysed by enzymes (Rastin *et al.*, 1988). Chemical reaction in soil is highly influenced by enzymatic activity. A low level of biological activity is associated with soil deterioration (Gildon and Rimmer, 1993). The biological activity in soil provides better insight in the understanding of transformation of organic matter (Pietkainen and Fritze, 1995). Therefore, knowledge about enzymes activities and their temporal and seasonal variation in soil has considerable biological significance.

The general name "Phosphatase" has been widely used to describe a broad group of enzymes that catalyze and hydrolyse both esters and anhydrides of phosphoric acid (Schmidt and Laksowski, 1961). Labile organic phosphorus compounds, although a minor part of the phosphorus resources are mineralized rapidly in soils (Bowman and Cole, 1978a) by enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Eivazi and Tabatabai, 1977). The phosphatases are involved in transformation of organic phosphorus compounds in soil. Their activity may play a significant role in release of phosphorus compounds (Rastin *et al.* 1988). The phosphatase activity of soil has been shown to vary with the standing vegetation (Neal, 1973).Phosphatase has been found a good indicator of the organic matter in soil (Hattori, 1988).

Urease enzyme is responsible for the break-down of urea into carbon dioxide and ammonia. Due to this property it has got an applied importance in the N-economy of soil (Jha, 1990). Soil ureases are microbial products that can accumulate in cell free form in the soil because they are highly resistant to environmental degradation (McNaughton *et al.* 1997).

Dehydrogenase enzymes are considered to play an essential role in the initial stage of the oxidation of soil organic matter by transferring hydrogen or electron from substrates to acceptors (Ross, 1971). Dehydrogenase activity being a respiratory enzyme provides a measure of catabolic activity of soil (Skujins, 1976). Dehydrogenase activity is considered to be caused by a broad group of endocellular enzymes (Skujins, 1978). Dehydrogenase activity act as an indicator of the microbiological system in soils and can be considered a good measure of microbial oxidative activity.

An important component of ecosystem C exchange is soil CO_2 efflux commonly referred to as soil respiration (Russel and Varoney, 1988). Measurements of soil CO_2 efflux can be used to characterize soil biological activity and the response of soil biota to environmental variables (Nadelhoffer, 1990, Anderson and Domsch, 1993 and Howard and Howard, 1993). Total soil respiration is an important ecosystem attribute that provides an estimate of the turnover of soil organic matter (Parker *et al.*, 1983). Soil microbes are the major contributors to the CO_2 flux from soil. Soil microorganisms such as bacteria and fungi play a major role in releasing CO_2 by metabolizing organic debris. Temperature, soil aeration and nutrient availability are important factors regulating the rate of organic matter decomposition as a result of past sludge additions (Brookes and McGrath, 1984 and FlieBbach *et al.*, 1994). The condition of the soil influences plant growth and the activities of microorganisms. The conditions of coal mine spoils are extremely unfavourable for plant growth. Williams (1975) and Wittwer *et al.* (1981) reported that nitrogen and phosphorus are the two limiting nutrients on coal mine spoils. Metal toxicity is a problem encountered in coal mine spoils. The toxic level of Fe, Al, and Mn was found in coal mine spoils (Berg and Vogel, 1973). The extreme acidity caused due to the oxidation of pyrites (FeS_2) (Chadwick, 1973 and Caruccio, 1975).

Meghalaya is rich in mineral resources, particularly coal, limestone, sillimanite and clay. Coal production in this region was started way back in the 19th century. Surface mining (Locally known as rat hole method) of coal increased dramatically during the past few decades in Jaintia Hills of Meghalaya. As a result, the primary broadleaved forest has been destroyed . The prevailing unscientific and unplanned mining operations in these areas have led to large number of hazards to the local people. Past and present mining operations have resulted in the removal of large quantities of waste material known as overburden or coal mine spoil and its subsequent dumping on adjacent areas, resulting in large areas that are devoid of vegetation and soil (Plate 1.1A). Revegetation of these disturbed areas is normally very difficult due to the extreme physical and chemical properties of the substrate which limit seedling establishment and plant growth (Bradshaw and Chadwick, 1980 and Bradshaw, 1983). Several workers have identified salinity, poor water holding capacity, inadequate supplies of plant nutrients, accelerated rate of soil erosion and soil texture as the major problems in coal mine spoils that affect revegetation process (Wali and Freeman, 1973, Down, 1975 and Archibold, 1980). Coal mine spoils are usually susceptible to drought and the nutrient deficiency and coal spoils can lead to poor root development and ultimate death due to drought (Fitter and Bradshaw, 1974).

Lowering of ground water-table, pollution of rivers and streams in the coal fields due to discharge of pumped mine water, serious air pollution in the form of generation of dust, release of noxious gases affecting the health of mine workers, habitat degradation of forest and agricultural land and decline in biodiversity are some of the adverse effects of coal mining in the Jaintia Hills district of Meghalaya.

Mining and the resultant dumping of spoils have adverse effects in the soil microbial and biochemical processes due to inhibition of enzymes by metals (Tyler, 1974, Jordan and Lechevalier, 1975 and Freedman and Hutchinson, 1980).

India is one of the largest coal producers in the world. The natural energy policy clearly stresses the need for increased production and utilization of India's vast resources with total environmental protection through use of demonstrated pollution control and reclamation technology. This can be attained through the combined effort of multidisciplinary team work of experts in mining and reclamation.

In Meghalaya, coal mining is carried out by private operators and is done manually by a very crude method known as "Rat-Hole Method" (Plate 1.1B). In this method, pits ranging from 5 to 10 m^2 are excavated into the soil till the seam of coal is reached (Plate 1.2A). Prior to mining, however, the land is cleared by felling of trees, shrubs and by the removal of ground vegetation. The consolidated and unconsolidated materials overlying the coal seam are brought out manually from the tunnels and dumped on adjacent unmined land. These materials are called as mine spoils or overburden. Coal is then removed from this pit using sharp iron rod. Tunnels are then made into the seam side-ways. Coal is normally brought out of the tunnel using basket or by wheel barrow and then dumped on the nearby area and later on to the roadside from where it is transported to different parts of the country (Plate 1.2 B).

The objective of the present research was to study the microbiology of coal mine spoils of Jaintia Hills and to evaluate the role of microbes in improving the physico-chemical properties of spoils. The study covered the following major aspects :

(i) Bimonthly analysis of microbial community and their biomass in coal mine strip soil and unmined forest soil.

(ii) Isolation and maintenance of soil microbes from coal mine spoil and forest soil.

(iii) Screening of acid tolerant microbes capable of removal of coal toxicity (Sulphates) and the efficiency of such microbes to break down of sulfate.

(iv) Bimonthly study of microbial activity (dehydrogenase, urease and phosphatase) and carbon dioxide evolution from coal mine spoil and forest soil.

(v) Analysis of physico-chemical properties (organic C, N, P, K, Fe, S, pH and moisture content) of coal mine spoil and forest soil.

Plate 1.1 A. Heaps of debris consisting of haphazardly mixed material.

Plate 1.1B . Pit formed as a result of " rat hole" method of mining on the hill slope.

Plate 1.2 A. Presence of coal seam on the hill slope.

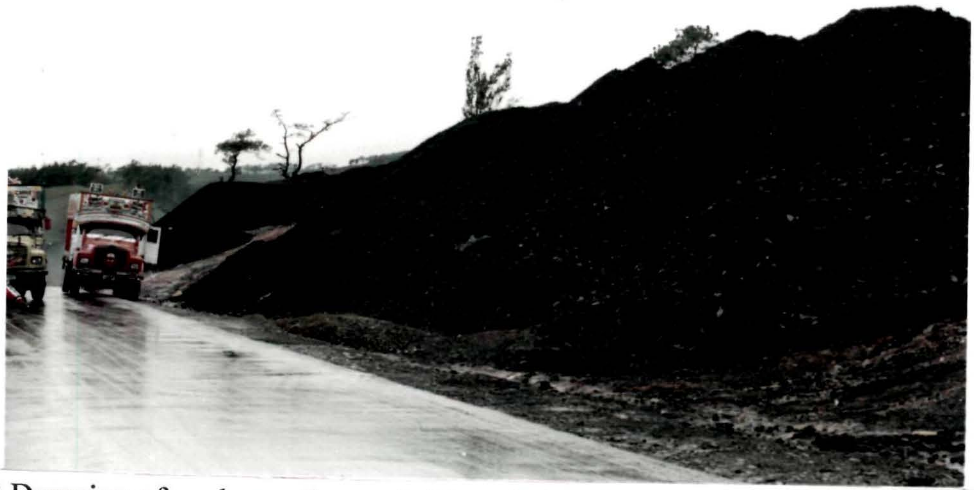

Plate 1.2 B.Dumping of coal near the road side and its subsequent transportation to different parts of the country.

CHAPTER II

REVIEW OF LITERATURE

Mining operation results in severe terrain disturbance and in the development of substrate which differ in biological activity from that in the undisturbed locations. Excavation of minerals and dumping of the waste material thrown on the adjacent site form the spoil. The conditions of the spoil are extremely unfavourable for plant growth and establishment. The soil physico-chemical characteristics regulate the microbial community, an important component of the soil. Microbial population conserves and regulates the plant nutrients in forest and agriculture ecosystems.

Soil microbes : Microbiological analysis of soil might provide sensitive index of fertility and that the relative numbers of bacteria, actinomycetes and fungi also indicate the chemical composition of the soil (Waksman, 1927). Seasonal variation in bacterial numbers in several European forest soil was studied by Feher (1933). Considering their importance, various workers have studied the microbial population in soil (Waksman, 1927 and Warcup, 1951). Under natural conditions, the soil microflora is ready to make use of any available substrate (Stotzky and Norman, 1964). Fungi may be more efficient in their use of organic substrate in forest soil and therefore have competitive advantage over bacteria (Witkamp, 1964).

Mishra (1966) studied the ecological factors responsible for the distribution of soil microflora. He concluded that factors like organic matter, pH, moisture content, aeration, temperature, soil depth, season and state of litter decomposition governed the distribution of microbes in soil. The number of fungal species is highest in the soil surface (Warcup, 1967). From the ecological point of view, bio-chemical and physiological capacities of the populations are of more interest.

Soil microorganisms exhibit synergistic decomposition of soil substrates, but are capable of much less activity when acting individually (Salonius *et al.*, 1970 and Ivarson, 1974). It is likely that fungi are the most important primary colonizer of plant litter (Dickinson and Pugh, 1974). They concentrate materials into their hyphae (Cromack *et al.*, 1975), which are then degraded by bacteria (Mitchell and Alexander, 1963 and Cromack *et al.* 1975).

The survival of a microbe in the soil is greatly influenced by the inoculum potential and saprophytic colonization of substrate in a mixed population (Johri *et al.*, 1975). Acidity significantly influences microbial abundance and it has been observed that high acidity (pH<4.2) contains the lowest population of algae, cellulolytic microbes, *Nitrosomonas, Nitrobacter* and denitrifiers (Ayanaba and Omayuli, 1975).

Some bacteria colonize only forest soil in the presence of fungal hyphae

(Gray, 1973, Siala and Gray, 1974 and Malajczuk *et al.*, 1977). Much work has been directed towards understanding the complexity of pesticide microbial interactions in soil (Hill and Wright, 1978). Herbicides generally appear to have no adverse effects on the population of total bacteria in soil except at concentrations exceeding recommended rates (Anderson, 1978).

Changes in the soil bacterial numbers over the years in coniferous forests soil have been demonstrated by Laudelout *et al.* (1978) . Seasonal fluctuations in microbial population have been found in pine forest by Clarholm and Rosswall (1980). The activity of many common bacteria is inhibited or suppressed by strong acidic conditions in soils but the relative abundance of fungi rises at lower pH because of their greater tolerance to acidity and through reduced competition from other microorganisms (Alexander, 1977, 1980). Soil acidity is generally linked with decreased rates of organic matter decomposition although the extent of the decrease varies with the nature of the materials (Abrahamsen *et al.*, 1977, 1980, Baath *et al.*, 1980, Alexander, 1980 and Jenkinson, 1981). Studies have focussed on comparing the generic composition of the bacterial populations in the root-free soil with that of rhizosphere (Jager and Velvis, 1981). Abiotic factors greatly alter the survival of a bacterial species either promoting its decline or favouring its persistence (Osa-Afiana and Alexander, 1982). Biological factors also determine the fate of bacterial species introduced into soil.

Studies on microorganisms in manipulated system are of interest not only in general ecology and forestry but also in soil microbiology, since investigations of populations in disturbed situations help to understand and interpret results from the natural undisturbed system (Lundgren, 1982). Lundgren and Soderstrom (1983) reported that availability of nutrients, soil water regime and temperature are the major factors which regulate the growth and survival of bacteria in the soil.

Among the different factors which affect the formation, stabilization and degradation of soil crumbs, microorganisms play an important role by producing binding agents to improve soil aggregation (Tisdall and Oades, 1982). The activities of microorganisms and soil fauna serve to promote soil aggregation (Oades, 1984). The more complex relationship which take place in the soil between the binding agents and the variable climatic conditions lead to seasonal changes in soil structure (Ojeniyi and Dexter, 1983 and Imerson and Vis, 1984).

The tall wet tropical forests have long vertical gradation of temperature and relative humidity that can accommodate fungal species adapted to different strata (Hedger, 1985). Observations on microbial interactions in the field are difficult to interpret due to variation in climate, soil fauna and vegetation, therefore microcosms have been used for their study (Habte and Alexander, 1977 and Griffiths, 1986).

Microbes constitute a significant part of natural and cultivated ecosystems and play a major role in the establishment of early colonization of plant species. The potential importance of microorganisms in soil formation and revegetation through their activities as decomposers and nutrient cyclers has been reported (Srivastava *et al.*, 1989). The size and activity of microorganisms depends on the quality and the distribution of soil organic matter and have been related to soil texture (Kaiser *et al.*, 1992), pH, soil climatic conditions (Insam et al., 1989).

Hammond (1992) hypothesise that overall forest architecture may be a better predictor of the number of fungi and small animals present in the given area than plant species richness. In addition to providing more resources and surfaces, forests with greater stature and structural complexity can create more microhabitats and micro-climates for insects and fungi. In tropical forests, temporal variation in fungal and microbial biomass can potentially alter the fate of nutrients in the ecosystem (Lodge, 1993, Lodge *et a*l., 1994, Zimmerman *et al.*, 1995). Microbial communities have great potential for temporal or spatial change and thus represent power tool for understanding community dynamic variation in microbial community structure which may have effects on ecosystem process (Garland, 1997).

MICROBIAL BIOMASS

The microbial biomass plays an important role in soil as an agent for nutrient transformation. The activity of much of the soil biomass is severely limited by nutrient availability and many soil organisms have very low metabolic rates or spend most of their life time in dormant or resting phases (Gray and William, 1971 and Gray, 1976).Relatively constant relationships between biomass C and mineral N were generally established for some African soil (Ayanaba *et al.*, 1976) and between biomass C and ATP for some Australian soil by Oades and Jenkinson (1979). The soil microbial biomass is a comparatively labile fraction of soil organic matter (Jenkinson and Ladd, 1981) and is a major nutrient sink during immobilization and a source of nutrients during mineralization. The soil microbial biomass is the agent of break down of organic materials in the sludges and in general, increases in response of inputs of decomposable material such as crop residues or animal manures (Jenkinson and Ladd, 1981). The active phase of soil organic matter is intimately related to the biomass serving as the main energy source for the biomass and receiving its organic output and dying cells (Jensson and Persson, 1982). Recognition of the importance of soil microorganisms in the functioning of ecosystems has led to an increased interest in measuring the nutrients held in soil biomass (Jenkinson and Poowlson, 1976, Ayanaba *et al.*, 1976, Anderson and Domsch, 1978 and Brookes *et al.*, 1982).

In soil of Saskatchewan, a close relationship between the microbial biomass and the amount of mineralized N was observed (Paul and Voroney, 1980). Microbial biomass can also serve as a sensitive indicator of toxicity.

Heavy metals from contaminated sewage sludge cause a decline in soil microbial biomass (Brookes and McGrath, 1984). Soil microbial biomass acts as a pool of biologically active C,N, P and S (Jenkinson and Powlson, 1976, Jenkinson and Ladd, 1981 and Brookes *et al.*, 1985) and has been widely used in investigations of nutrient dynamics and transformation in soil. It is widely accepted that soil microbial nutrients used by plants and that is the main mediator of carbon turnover (Paul and Juma, 1981, Marumato *et al.*, 1982, and McGill *et al.*, 1986). Microbial biomass carbon reflects the long term amount of C input into a soil (McGill *et a*l., 1986 and Anderson and Domsch, 1986).

Many soil microorganisms are known to be intolerant to low soil moisture contents (Harris, 1981 and Paul and Clark, 1989) and changes in soil moisture status can result in rapid shifts in the magnitude of the soil microbial biomass (Bottner 1985 and Schnurer *et al.*, 1986). Biomass measurements can reveal changes brought about by soil management long before such changes can be detected in total organic carbon or N (Powlson and Jenkinson, 1981 and Powlson *et al.*, 1987). Microbial biomass and enzyme activities can be used as early indicators of changes in soil properties induced by tillage (Carter, 1986 and Powlson *et al.*, 1987). Soil microbial biomass, the most active fraction of soil organic matter, responds rapidly to changes in the soil environment (Powlson *et al.,* 1987).

Values of biomass C were closely related to other biomass indices, viz., mineral N (min-N) flush (Ayanaba *et al.*, 1976), ATP content (Jenkinson and Oades, 1979), substrate induced respiration (Anderson and Domsch, 1978 and West and Sparling, 1986) in some of the soils (Ross *et al.*, 1980 and Sarathchandra *et al.*, 1984) . Soil microbial biomass (SMB) is a small but labile component of the soil organic matter. It is thought to exert a key controlling influence on the rate at which N, C and other nutrients cycle through agricultural and other ecosystems (Jenkinson, 1988). The size of the microbial biomass in soil is related to successional state of the ecosystem (Insam and Domsch, 1989). The importance of soil microbial biomass in the cycling of C, N and P is well documented (Van Veen *et al.*, 1989).

Microorganisms are the main mediators of C turnover in soil. By definition they are also part of the organic C and nutrient pool, as such they may be called microbial biomass. Microbial biomass and respiration are important variables in the C cycle (Insam 1990). Insam *et al.* (1989) and Insam (1990) found a close relationship between microbial biomass and organic C ratio with several climatic variables. Estimations of soil microbial biomass are now frequently made because of the importance of soil organisms in nutrient cycling and their role as a source and sink of plant nutrients (Jenkinson, 1988 and Smith and Paul, 1990). Recognition of the importance of soil microorganisms has led to increased interest in measuring the nutrients held in their biomass (Jenkinson and Powlson, 1976, Martikainer and Palojarvi, 1990 and Singh *et al.*, 1991).

Most of this biomass consists of bacteria and fungi, with the balance consisting of soil microflora and algae (Wardle, 1992). Low biomass C to soil organic C ratio observed in an acid soil and in a metal contaminated soil (Witter *et al.*, 1993). Changes in microbial biomass often take place before any change in the total organic matter can be measured (Angers *et al.*, 1993).

Microbial biomass has been assessed by many procedures, such as fumigation extraction (Vance *et al.,* 1987), measurement of specific microbial components (Miller and Casida, 1970), Phospholipid-linked ester fatty acid (White *et al.*, 1979), ATP (Oades and Jenkinson, 1979), ergosterol (Newell, 1992), physiological and biochemical (Alef, 1993), chloroform fumigation incubation (Jenkinson and Powlson, 1976), substrate induced respiration (Anderson and Domsch, 1978). Out of these, choloroform fumigation incubation method of Jenkinson and Powlson (1976) bas been widely accepted.

Amount of microbial biomass is influenced by soil texture and quality of soil organic matter (Wardle, 1992, Ross and Tate, 1993b, Bosatta and Agren, 1994 and Hassink, 1994). Stress by heavy metal toxicity and low pH has been shown to result in a reduction in the size of soil microbial biomass (Brookes and McGrath, 1984, Chander and Brookes, 1991b, Witter *et al.,* 1993, Bardgett *et al.*, 1994 and

FlieBbach *et al.*, 1994).

The quality and composition of microbial biomass are sensitive to changes in the soil physical and chemical environment (Wolters and Joergenson, 1991, Wardle, 1992). The size of the microbial biomass has been used to evaluate detrimental effects of heavy metals.

Soil enzymes

Knowledge about enzyme activity and their seasonal variation in soil had considerable biological significance. Most of the biological processes are carried out by enzymes. Ramirez-Martinez and McLaren (1966b) and Paulson and Kurtz (1969) reported that soil enzyme activity is independent of the microbial population. Soil is a living system where all biochemical activities proceed through enzymatic process. Enzymes accumulated in soil are present as free enzymes, such as exoenzymes released from living cells, endoenzymes released from proliferating cells (Kiss *et al.*, 1975). Most of the enzymes are added to soils by decaying microbial tissues and by plant and animal residues. Each of the organic and microbial fractions in soil has special influence on enzyme activity (McLaren, 1975).

The enzymatic activity of a soil depends both on the abiotic factors (Skujins, 1976) such as extracellular enzymes, active enzymes within dead and the living microbial cells.

Pollution of soil with heavy metal is widespread. Mining and other industrial waste contamination is expected to have an adverse effect on the soil microbial and biochemical processes due to inhibition of enzymes by metals (Tyler, 1974,Jordan and Lechevalier, 1975, Freedman and Hutchinson, 1980 and Mathur, 1981).

Tabatabai (1982) reported that cropping history, soil amendments and environmental factors have a special influence in affecting the enzyme activity in soil. Studies on the extracellular enzyme activities in ecosystem have shown that vegetation, agricultural chemicals and industrial pollutants have marked influence on soil enzymes (Tabatabai, 1982). Like other biochemical reactions in soils, however, enzymes activities are associated with organic matter distribution profile and generally decrease with depth (Tabatabai, 1982).

Soil enzymes play an important role in soil mineralization processes (Tate, 1977) and have been related to other soil biological properties (Frankenberger and Dick, 1983). Soil as a system of humus and minerals consists of both immobilized enzymes, stabilized by a three dimensional network of macromolecules and occluded microbial cells. Few reports are available on the enzyme activity in forest soil (Harrison , 1983, Stott and Hagedorn, 1980 and Rastin *et al.,* 1984).

Numerous reports are available on the activity of enzymes in agricultural soils (Burns, 1978, Ross and Cairns, 1982, Frankenberger and Dick, 1983, Sarathchandra *et al.*, 1984, Nannipieri, 1984, Speir *et al.*, 1984 and Stott *et al.*, 1985). These studies have clearly shown that enzyme activities in soils are influenced by numerous factors. Soil microbial activity contributes to the regulation of soil carbon storage, soil respiration and ecosystem productivity (Bauhus *et al.*, 1998).

Dehydrogenase

Lenhard (1956) was the first to use Triphenyl Tetrazolium Chloride (TTC) for the determination of dehydrogenase activity. Ross (1970) reported that dehydrogenase activity depends more upon the metabolic state of the microbial population of the soil than the activity of specific free enzymes acting on particular substrates. The activity of dehydrogenase may act as an indicator of the microbiological redox system in soils and can be considered a good measure of microbial oxidative activity (Casida, 1977 and Tabatabai, 1982). Seasonal variation in dehydrogenase activity in sludge amended soil was lower due to the heavy metals (Reddy and Faza, 1989). Chander and Brookes (1991) examined the dehydrogenase activity in copper contaminated soils .

Phosphatase

Phosphatase activity may play a significant role in P availability to plants from native soil organic P compounds. A number of phosphatase esters occur in soils (Anderson, 1967). Khan (1970) reported that addition of fertilizer P increases phosphatase activity in soil. Rolstone *et al.* (1975) have shown that addition of inorganic P inhibits the production and secretion of phosphatase by plants. Spires McGill (1979) observed that phosphatase activity increases with increasing organic matter content. Phosphatases are involved in transformation of organic and inorganic phosphorus compounds in soil (Rastin *et al.*, 1988). Phosphatase activity might be an indicator of microbial action and evolution of organic matter in the composting process (Garcia *et al.*, 1993).

Urease

This enzyme plays an important role in maintaining the nitrogen economy of soil. Spier (1977) extracted urease activity and related it with soil pH. Burns (1978 and 1982) reported that urease activity would be influenced by the type and density of vegetational cover, climate and soil type. O'toole and Morgan (1984) reported that urease was resistant to thermal degradation. Soil ureases are microbial products that can accumulate in cell free form in the soil because they are highly resistant to environmental degradation (McNaughton *et al.*, 1997).

Carbon dioxide evolution

CO_2 evolution from soils used as an index of microbial activity because most of it comes form microbial source. Soil respiration may be considered as the sum total of all soil metabolic functions in which CO_2 is produced (Lundegardh, 1927).

Macfadyen (1970) reported that 50% of the total soil respiration is contributed by the roots. Coleman (1973) reported that root contributed 17% of the total CO_2 evolved from a grassland soil. Total soil respiration is an important ecosystem attribute that provides an estimate of the turnover of soil organic matter (Parker *et al.,* 1983). Linn and Doran (1984) observed a greater soil respiration in 0-15 cm soil layer due to surface accumulation of organic matter. CO_2 serve as an index of biological activity of cultivated soil. Soil respiration includes microbial decomposition of litter, root exudation and dead roots as well as respiration by root symbionts (Kursar, 1989). The biological activity in soil provides better insight in understanding the transformation of organic matter (Pietkainen and Fritze, 1995). Cool and humid conditions favour carbon accumulation in soil (Silvola *et al.*, 1996).

Nutrients

The major elements like carbon, nitrogen and oxygen are important for life which are brought about by different types of microorganisms. In coal mine spoils there is a great loss of nutrients in the system because of the disruption of the ecosystem (O'Niell *et al.*, 1977). The continued existence of any group of organic matter largely originate from plant litter and root residue (Swift *et al.*, 1979). Nutrients mineralized from soil organic matter are the primary source of energy for plant growth in many ecosystems (Berendse *et al.*, 1987b). Soil water content, temperature, microbial activity and substrate quality are the primary factors affecting the rate of organic matter decomposition (Clymo, 1984 andHogg, 1993). In coal mine spoils litter layer which is an exchange site of nutrient is lost from the system due to soil and wind erosion. Thus, nutrient holding capacity of coal mine spoils is drastically reduced (Lyngdoh, 1995).

Finer texture leads to increased C contents. Organic matter held in tree canopies in forest is highly microbically active (Brandy, 1974 and Jenny, 1980). The importance of microorganisms residing in the canopy organic matter lies in their role in transforming C and nutrient inputs (Vance and Nadkarni, 1990). There is close relationship of climate with carbon contents and pH (Insam, 1990).

Plants and animals as well as most microorganism acquired nitrogen for nutrition and growth. Nitrogen deficiency in coal mine spoils could be due to its great susceptibility to leaching losses (Richardson and Dicker , 1972 and Gemmell, 1973). Nitrogen is considered the most limiting nutrient in forest ecosystems.Nitrogen is the nutrient which increases production in fertilizer trail. Nitrogen deficiency is a major factor limiting the growth of plants on spoils (Davidson and Jefferies, 1966, Fitter and Bradshaw, 1974, Handley *et al.*, 1978 and Bradshaw and Chadwick, 1980). Restored soils at opencast site are characterised by low N-status (McRae, 1989).

Phosphorus is deficient in U.S. mine spoils (Bauer *et al.*, 1977 and Power *et al.*, 1978a).Iverson and Wali, 1982 also reported that phosphorus is the limiting nutrient during colonization and early successional process on surface-mined land in North-Dakota.Phosphorus and nitrogen are the two limiting nutrients on coal mine spoils (William, 1975, Witter *et al.*, 1981).

There are various factors which limit plant growth in coal mine spoils. The toxic level of Fe, Al and Mn (Berg and Vogel, 1973) was found in coal mine spoils. The extreme acidity is caused due to the oxidation of iron pyrite (FeS_2) (Chadwick, 1973 and Caruccio, 1975). Metal toxicity is a problem encountered on coal-mine spoils. Coal mine spoils of Scotland present a number of metals at levels which could be toxic to plants (Kimber *et al.*, 1978). Acidic coal-mine spoils contain toxic level of soluble element such as Fe, Al, Mn and Cu (Lyngdoh, 1995).

CHAPTER III

STUDY SITE AND CLIMATE

The present investigation was carried out in Bapung (Latitude 25⁰24'35"N, Longitude 92⁰18'30"E, Altitude 1300 m asl) in Jaintia Hills district which is about 85 km south east of Shillong the capital of Meghalaya, India (Fig.3.1). Coal mine spoil of about three years old (Plate 3.1) and a nearby forest as control were selected for the present investigation (Plate 3.2 A & B).

GEOLOGY AND SOIL

The Shillong plateau which covers the Jaintia Hills district is situated at an average elevation of 1500 m above the Brahmaputra valley (Gansser, 1964). Geologial and chrono-stratigraphic studies have revealed that rock formation ranging from pre-Cambrian to the Quaternary have gone into the making of the geological sub-strata of the plateau. The plateau region is composed of thick series of quartzites and schists with intrusions of granites, dolemites and predites and thin embedded bands of argillites (Lyngdoh, 1995).

The soils of the plateau are derived from the underlying quartzites, schites and granites. They have been grouped under latosol (oxisol) type (Pascoe, 1950). The soil of Meghalaya has been broadly divided into four categories viz., (1) red loamy soil (2) lateritic soil and (3) red and yellow soil and (4) alluvial soil. The soil are acidic in nature. The nutrient level varies form place to place depending upon the prevailing vegetation cover and topography.

CLIMATE

The climate of Meghalaya is monsoonic with an average annual rain fall of 2500 mm distributed over seven months of the year. Based on the atmospheric condition, the year can be divided into four seasons viz. spring (March-mid May), rainy (mid May-September), autumn (October-November) and winter (December-February). The spring season is characterised by occasional rain associated with high wind velocity. The rainy season commences with the onset of south-west monsoon in mid-May and lasts till September. This is followed by a short autumn (October-November). During this period, rainfall is considerably low and atmospheric temperature drops. The winter season extends from December to February. During this period, rainfall is scanty, the days are sunny and nights are frosty. Monthly rainfall pattern and mean maximum and minimum temperature during the study period is shown in Fig.3.2. The annual rainfall was 788.5 and 4331.4 mm and 5497.6 mm during 1995,1996 and 1997 respectively. The mean relative humidity was maximum (87.5%) during (rainy season|) and minimum (37.5%) during (winter season) (Fig.3.3).The mean maximum and minimum temperatures were 24.78^{0}C and 11.6^{0}C respectively.

Vegetation

The vegetation of Meghalaya has been broadly divided into three types of forests viz., tropical forests, subtropical forests and temperate forests (Chauhan and Singh, 1992). According to the forest survey report (1995), Meghalaya has 4045 sq. km closed forest and 11669 sq. km open forest, the total forest cover being 15714 sq. km, which is 70.06 % of the total geographical area of the state. Age-old practice of shifting agriculture, locally known as jhum cultivation, mining and other developmental activities during the last few decades have resulted in the degradation of most of the natural forests in the state, particularly in Jaintia Hills. At present most of the primary forests in the state are represented by sacred groves which are preserved by the local people due to some religious belief and few national parks.

The forest stand where the present investigation was conducted is dominated by trees such as *Schima wallichii*, *Schima khasiana*, *Alnus nepalensis*, *Myrica esculenta*, *Castanopsis* sp., *Engelhardia spicata*, *Ficus elastica*, *Prunus nepalensis*, *Rhodopdendron arborium*, *Pinus kesiya*. The under story vegetation comprised of shrubs, weeds and grasses. This layer was composed of the species such as *Osbekia* sp., *Rubus ellipticus*, *Viburnum foetidum*, *Rhus semiadata*, *Arisaema* sp., *Litsea* sp., *Phyllanthus glaucus*, *Sacandra glabra*, *Mussaenda glabra*, *Urena lobata*,*Lantana camara*, *Eupatorium adenophorum*, *Ageratum conyzoides*, *Hypocharis* sp., *Polygonum* sp., *Viola sp.*, *Fagophyrum esculentum*, *Hautonia cordata, Solanum* sp., *Plantago major, Asparagus* sp., *Pteridium* sp. Climbers like *Smilax* and *Ipomea* sp were also present in the forest stand.

As a result of mining activities, the forest area has been converted into grassland. The coal mine spoils of Bapung has a high acidity and the soils have a very low nutrients. The young (0-3 year old) mine spoils which have been selected for the present investigation were devoid of any vegetal cover. However, grasses such as *Axonopus compressus, Cyanotis vaga, Cynodon dactylon, Cyperus sp., Kayllinga brevifolia, Arunidinella sp.,* and herbs such as *Osbekia* sp., *Lycopodium* sp., *Borreria hispida, Centella asiatica , Lantana camara,* and *Dicranopteris linearis*. were seen to colonise the mine spoils.

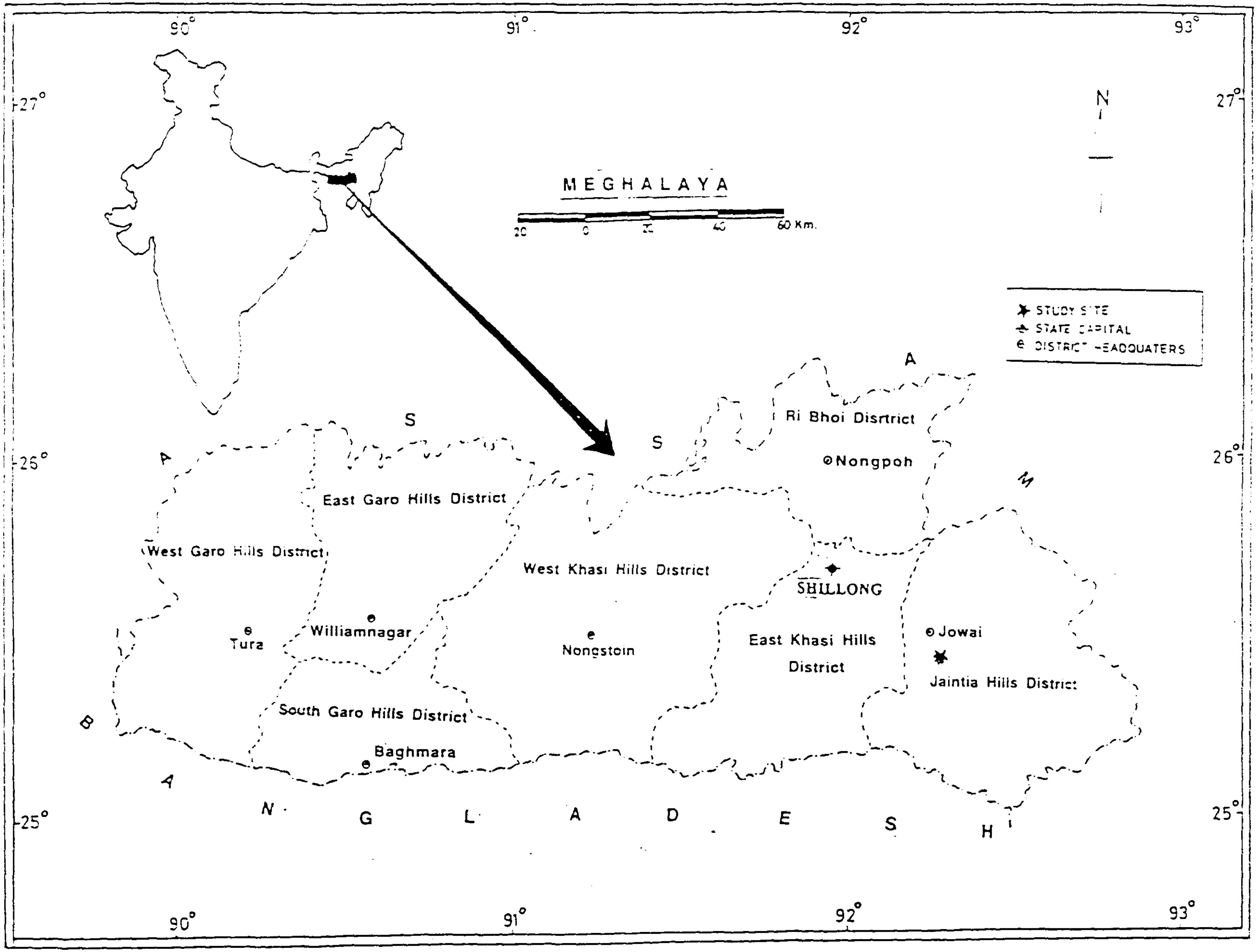

Fig. 3.1. Map showing geographical location of the study site.

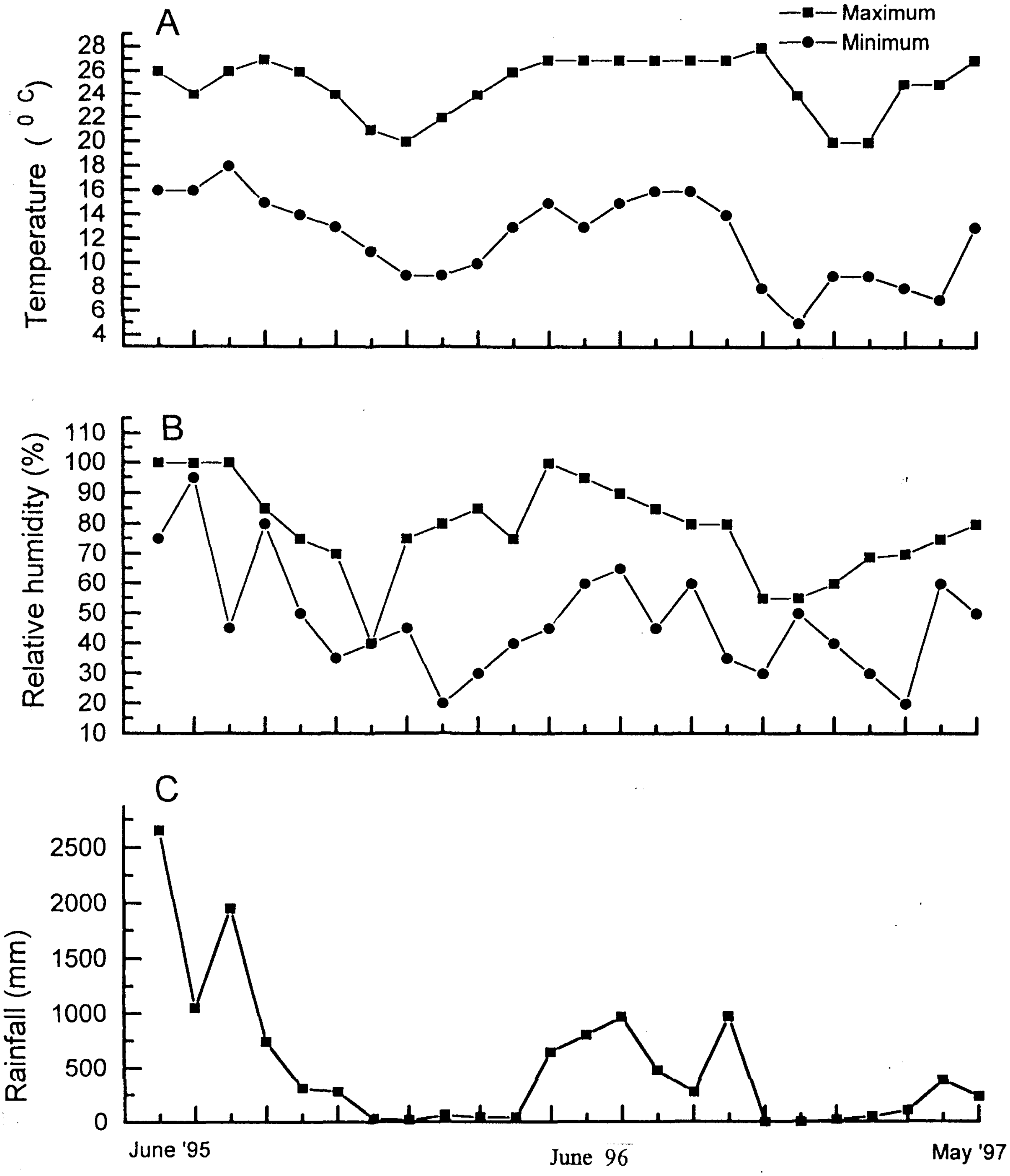

Fig.3.2 . Maximum and minimum temperature (A), maximum and minimum relative humidity (B) and total rainfall (C) during the study period.


Plate 3.2 A. Forest stand (overview).


Plate 3.2 B. Forest stand (closer view).

CHAPTER IV

MICROBIAL COMMUNITY AND BIOMASS IN COAL MINE SPOIL AND FOREST SOIL

Introduction

Extensive coal mining in the hill state of Meghalaya has resulted into soil degradation. There is always extreme acidity in the coal mining area due to the oxidation of iron pyrites (FeS_2) (Chadwick, 1973 and Caruccio, 1975). The microorganisms play a beneficial role in the amelioration of the detrimental effect of toxic substances. Soil is inhabited by diverse groups of microorganisms which are of great importance for soil ecosystem because they affect available nutrient, soil structure and its stability (Paul and Clark, 1989). Soil bacteria, fungi and fauna are essential components of soil ecosystem due to their major role in the regulation and decomposition of soil organic matter and recycling of nutrients (Coleman *et al.*, 1983, Wolters, 1991 and Beare *et al.*, 1992). Organic matter is decomposed mainly by bacteria and fungi and part of the decomposed material is used for the production of microbial biomass (Bloem et al., 1994). The survival of the microbes in the soil is greatly influenced by the inoculum potential and saprophytic colonization of a substrate in a mixed population (Johri *et al.*, 1975).

The size and activity of the microbial population depend on quality and quantity of soil organic matter which are related to soil texture (Kaiser *et al.*, 1992), soil pH and other properties of soil (Insam *et al.*, 1989). The microbial population in soil is greatly influenced by physico-chemical properties of soil and vegetal cover (Mishra and Sharma, 1977). Influence of canopy cover on microbial population dynamics and composition of microbial communities in forest ecosystems (Henrot and Robertson,1994). The microbial population in soil is greatly influenced by environmental factors such as temperature and rainfall. Acidity and soil temperature have a determining effect on the distribution of fungi (Warcup, 1951). Organic matter influences the growth of microbes (Lynch, 1981 and Guidi *et al.*, 1988). Microbial populations can immobilize significant quantities of a variety of nutrients as well as organic pesticides and pollutants (Smith and Paul, 1990). Microbial population can also be important degraders of organic pollutants (Alexander, 1994).

Microbial biomass is a key component of soils since it defines the functional component of the microbiota primarily responsible for decomposition, soil organic matter turnover and nutrient transformations (Dalal and Meyer, 1987, Smith and Paul, 1990 and Witter, 1996). The soil microbial biomass is the agent of break-down of the organic materials in the sludges and in general increases in response to inputs of decomposable materials such as crop residues or animal manures (Jenkinson and Ladd, 1981). Soil microbial biomass is affected by many factors such as temperature, moisture content, clay content and pH (Carter, 1986, Kaiser *et al.*, 1992 and Gestel *et al.*, 1993). Microbial biomass can serve as a sensitive indicator of toxicity particularly of heavy metals from contaminated sewage sludge (Brookes and McGrath, 1984). Stress by heavy metal toxicity and low pH have been shown to result in reduction in the size of soil microbial biomass (Brookes and McGrath, 1984, Chander and Brookes, 1991 b, Witter *et al.*, 1993, Bardgett *et al.*, 1994 and FlieBbach, *et al.*, 1994). The microbial biomass in stressed degraded systems play a significant role in turn-over of organic matter and establishment of pioneer plants on coal mine wastes in Scotland and Pennsylvania (Daft *et al.*, 1975). The microbial biomass content of soil depends on the quantity, quality and distribution of the C-input, factors that vary with time and depth (Kaiser and Heinemeyer, 1993).

Recognition of the importance of soil microorganisms has led to increased interest in measuring the nutrients held in their biomass (Jenkinson and Powlson, 1976, Martikainen and Palojurvi, 1990 and Singh *et al.*, 1991).

Study of microbial communities and biomass in soil of disturbed site may give insights into the role of microbes in restoring soil fertility. The present chapter deals with the seasonal and spatial variation in bacterial, fungal population and microbial biomass- C and their role in the restoration of degraded coal mine area.

Materials and methods

Soil samples were collected on bimonthly basis from June'95 to April'97 with the help of a soil corer (6.5 cm diameter). In each sampling, ten soil cores were collected randomly from two soil depths (0-10 cm and 10-20 cm). The soil samples of each depth were mixed thoroughly to obtain a composite sample and were kept in sterilized polythene bags. The samples were brought to the laboratory on the same day and kept at 4^0C.

Isolation of soil fungi

Fungal population was estimated by dilution plate method (DPM) (Waksman, 1922) using rose bengal agar (RBA) medium (Martin, 1950). 1 g of soil was taken in a 250 ml conical flask containing 100 ml sterilized distilled water to give 1:100 dilution. To prepare homogenous solution flasks were swirled for 15 minutes. 1 ml of this mixture was transferred aseptically with the help of sterilized pipette to another conical flask containing 100 ml of sterilized distilled water to get a dilution of 1:1000. 1 ml of this dilution was inoculated in Petri dishes containing RBA medium and were gently rotated to disperse the suspension. Three replicate were maintained for each sample. The Petri dishes were incubated upside down at 25 ± 1^0C for 5 days in a B.O.D. incubator. Total number of fungi in soil was calculated on dry weight basis. The fungi were identified following the keys of Gilman (1957), Subramanium (1971) and Barnett and Hunter (1972).

The following formulae were used for the determination of fungal population and relative abundance of a fungal species .

$$\text{Fungal population per gram} : \frac{\text{No. of fungal colonies X dilution factor X inoculum}}{\text{Dry weight of one gram soil}}$$

$$\text{Relative abundance \% of fungi} : \frac{\text{Total number of colonies of individual species}}{\text{Total number of colonies of all species}} \text{ X } 100$$

Bacterial population

Dilution plate method (Waksman, 1922) was followed for the enumeration of the bacteria using nutrient agar medium (Difco manual, 1953). Dilution of soil was made similar as in case of fungi except 1:10000 dilution.0.5 ml of final soil suspension was inoculated in Petri dishes containing the medium. Three replicates were maintained for each sample. The Petri dishes were incubated at $30\pm1^{0}C$ for 24 h in a bacteriological incubator. Bacterial population was calculated by taking moisture content and dilution factor into consideration.

Microbial biomass-C

The soil sieved through 2 mm mesh screen and was used in field moist condition for the determination of microbial biomass C. It was estimated by chloroform fumigation incubation (FI) method of Anderson and Ingram (1993). 10 g of soil was taken in a beaker and was placed in a vacucum desiccator containing 30 ml of alcohol free chloroform in a shallow dish. The lid was closed and sealed and the vacuum was used until the chloroform clearly evaporates and thereafter the desiccator was stored in the dark for 5 days at $25^{0}C$. After 5 days, the soil was transferred to watertight extraction bottle (125 ml) and to it 50 ml of

0.5 M K_2SO_4 was added and shaken for 30 minutes. Extract was filtered through whatman filter paper (No.42). The filtrate was used for analysis. To 4 ml of filtrate, 4 ml of 0.0667 M Potassium dichromate and 5 ml of concentrated sulphuric acid were added. Two blanks were prepared i.e. one preheated at 150^0C for 30 minutes and the other was without heating. The digested sample was transferred to 100 ml conical flask and to it 0.3 ml of indicator solution (Ophenantholine monohydrate) was added. The sample was then titrated with acidified ferrous ammonium sulphate solution. The end point was a colour change from green/violet to red.

The biomass C was calculated as follows :

Organic (%) = {A x M x 0.003)/g} x (E/S) x 100

Where, M = Molarity of ferrous ammonium sulphate (0.033)

$A = (ml_{HB}-ml_{Sample}) \text{ x } \{ml_{UB}-ml_{HB})ml_{HB}\} +(ml_{HB}-ml_{Sample})$

g = Dry soil mass (g)

E = Extraction volume (ml)

S = Digest sample volume (ml)

Microbial biomass-C = (Extracted ct_1-Extracted ct_2) x 2.46

Results

Fungal population

The fungal population varied significantly ($P<0.01$) between the sampling period in both the coal mine spoil and the forest stand (Fig.4.1). It showed high value in the forest stand. In both coal mine spoil and the forest stand, the number of fungi was high during April and low during June, August and December. The fungal population in both the sites was greater in the surface layer than the sub-surface layer. Twenty four species of fungi were isolated from coal mine spoil and nineteen species from forest stand. In coal mine spoil *Pythium intermedium* and *Penicillium* sp. were dominant in both the depths (Table 4.1). However, in the sub-surface layer, in addition to *P. intermedium* and *Penicillium* sp., and *Trichoderma viride* was also dominant (Table 4.2). On the other hand, in forest *P. intermedium* was the dominant species at both 0-10 cm and 10-20 cm soil depths (Table 4.3 & 4.4). The fungus which has the highest relative abundance was *P. intermedium.*

There was a difference in the composition of the fungal flora at both the sites. *Mamaria* sp., *Monocillium* sp., *Paecilomyces carneus* and *Scopulariopsis brevicaulis* could be found in coal mine spoil whereas *Acremonium murorum, Cunninghamella elegans, Gliomastix murorum, Torulomyces* sp., and *Verticillium tennerum* could be found in forest soil.

Bacterial population

Bacterial population also varied significantly (P<0.01) between the sampling periods and it declined with soil depth in both the coal mine spoil and the forest stand (Fig. 4.2). It showed high value in forest stand. The bacterial population was more than the fungal population in both forest and coal mine spoil. In both coal mine spoil and forest stand, the bacterial population was maximum during June thereafter it declined gradually. In forest soil, *Pseudomonas* sp. and *Bacillus* sp. were dominant and *Thiobacillus* sp. was the dominant species in coal mine spoil (Plate 4.1, 4.2 & 4.3.)

Microbial biomass-C

Microbial biomass-C showed significant (P<0.01) variation between the sampling periods in coal mine spoil and the forest stand (Fig. 4.3). It declined significantly ($P<0.01$) from the surface (0-10 cm) to sub-surface (10-20 cm) soil layer in both the cases. Microbial biomass-C was significantly ($P<0.05$) higher in the forest stand. In both forest soil and the coal mine spoils, microbial biomass was high during April and August and minimum during December in the Ist year of study and in the 2nd year of study it was high during April, June and August and minimum during October.

Discussion

The seasonal changes in microbial population observed in both the coal mine spoil and the forest stand could be due to the variation in environmental conditions and soil nutrients. The high population of fungi during spring season was due to the favourable soil moisture, relatively higher temperature and better availability of organic matter and mineral nutrients. Whereas, the low population during the rainy season linked to run-off losses of fungal propagules along with plant materials from the hill slope due to heavy rainfall in the region (Kshattriya *et al.*, 1992 and Maithani, 1996) resulting in increase moisture level which is harmful to fungi. When water logging occurs the diffusion of oxygen necessary for aerobic metabolism is in adequate and in such conditions fungi suffer. Rainfall, temperature, and soil moisture thus had some effect on changes in size of soil microbial population. At low temperature, the composition of the fungal community seems to differ from that of the warmer period. The high number of fungal species in coal mine spoil may be attributed to the low pH. Alexander (1980) reported that the relative abundance of fungi rises at lower pH due to a greater tolerance of acidity and through reduced competition from other

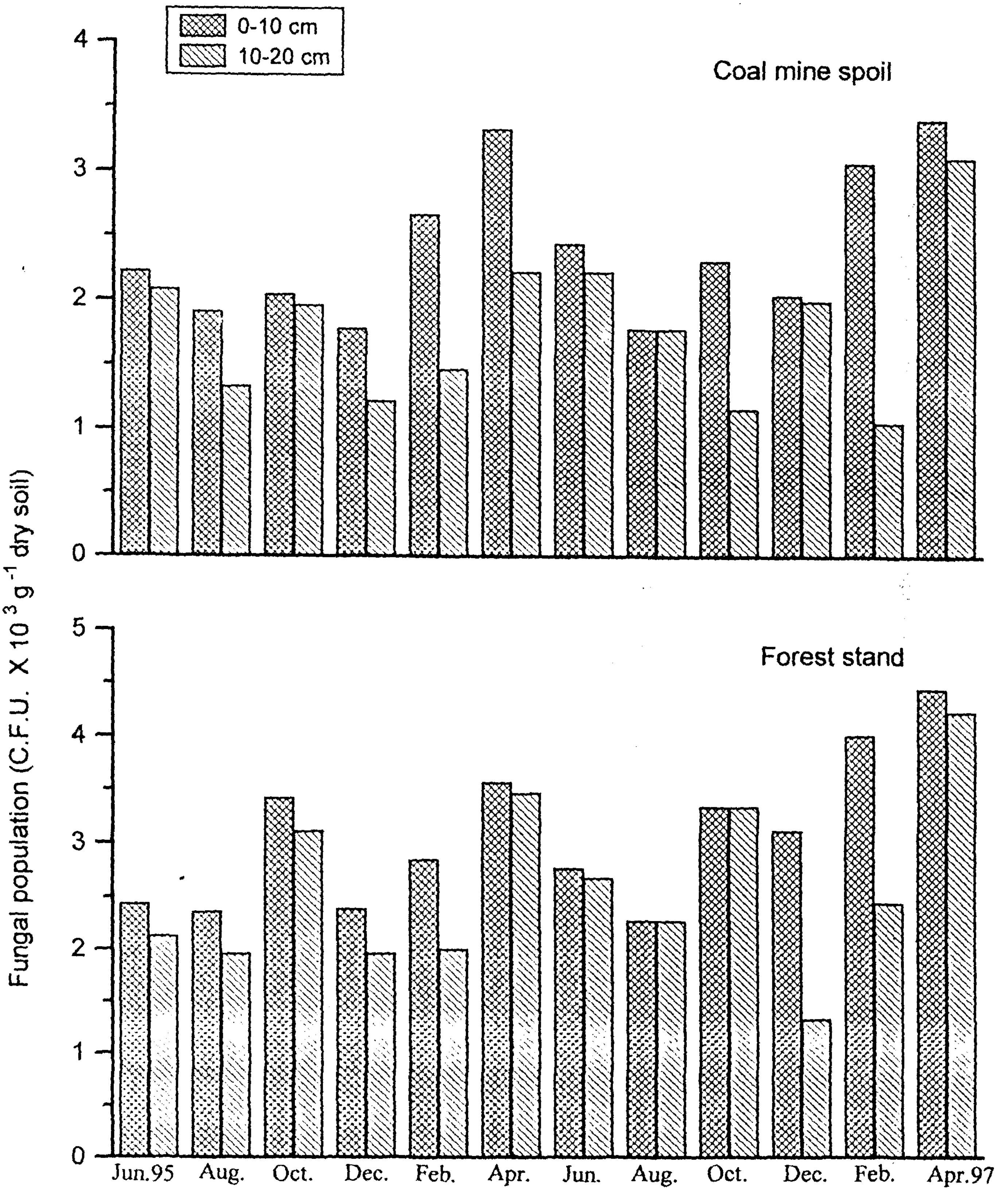

Fig.4.1. Bimonthly variation of fungal population in coal mine spoil and the forest stand.

Table 4.1. Temporal variation in relative abundance of fungal species in the 0-10 cm soil layer in the coal mine spoil

Species	Sampling period											
	Jun. 95	Aug. 95	Oct. 95	Dec. 95	Feb. 96	Apr. 96	Jun. 96	Aug. 96	Oct. 96	Dec. 96	Feb. 97	April. 97
Absidia glauca	-	-	-	-	-	-	-	-	-	-	-	3.33
Aspergillus flavus	10.34	-	-	-	-	5.26	-	-	-	-	-	-
Aspergillus sp.	-	-	-	-	-	-	-	-	-	-	-	3.33
Humicola grisea	-	-	-	-	-	-	-	50.00	30.00	-	-	-
Mortierella remanniana	-	-	-	-	12.9	-	-	-	-	-	-	-
Mucor circinelloides	-	-	5.12	-	-	-	-	-	10.00	-	-	-
M. hiemalis	-	-	-	-	7.69	15.78	-	-	-	4.34	-	-
M. racemosus	-	-	-	-	15.38	-	-	-	-	-	-	-
Mucor sp.	-	17.64	-	-	15.38	36.84	-	-	-	-	-	-
Penicillium brevicompactum	-	-	-	-	-	-	-	-	-	-	-	13.33
P. canescens	31.57	11.76	2.60	15.38	26.00	20.83	-	-	47.82	19.20	-	-
P. chrysogenum	-	-	50.00	-	-	4.50	-	-	-	-	-	-
P. frequentans	5.26	-	5.88	-	12.82	-	-	-	10.00	-	-	23.33
Phoma sp.	-	-	5.88	-	-	-	-	-	-	13.04	40.90	36.66
Pythium irregulare	-	-	77.00	-	-	-	-	-	-	-	-	-
P. intermedium	21.05	76.47	-	10	-	26.31	58.40	-	50.00	17.39	60.00	13.33
Pythium sp.	-	-	-	-	21.05	-	-	-	-	-	-	-
Scopulariopsis brevicaulis	-	-	-	20.00	-	-	-	-	-	4.34	-	-
Trichoderma koningii	-	-	-	-	-	9.09	-	-	-	-	-	-
T. viride	42.10	5.88	-	20.00	-	-	20.83	50.08	-	-	-	6.66

Table 4.2. Temporal variation in relative abundance of fungal species in the 10-20 cm soil layer in coal mine spoil.

Species	Sampling period											
	Jun. 95	Aug. 95	Oct. 95	Dec. 95	Feb. 96	Apr. 96	Jun. 96	Aug. 96	Oct. 96	Dec. 96	Feb. 97	Apr. 97
Absidia glauca	-	-	-	-	-	-	-	-	-	-	-	16.2
A. niger	-	-	-	-	-	-	-	-	-	-	-	23.2
Humicola grisea	-	-	2.56	-	-	-	-	42.85	51.42	-	-	-
Mamaria sp.	-	-	-	-	-	-	-	-	-	42.30	-	-
Monocillium	-	-	-	20.00	-	-	-	-	-	-	-	-
Mortierella remanniana	-	-	-	-	-	-	-	-	-	42.30	-	-
Mortierella sp	-	-	-	-	-	-	-	-	-	13.04	-	-
Mucor circinalloides	-	-	-	-	-	-	-	-	5.71	-	-	-
M. hiemalis	-	-	-	-	-	-	-	-	-	3.84	-	-
Paecilomyces carneus	-	-	-	-	40.00	-	-	-	-	-	-	-
Penicillium canescens	62.06	41.66	-	-	-	-	-	-	-	-	-	-
Pythium intermedium	10.34	25.00	25.60	-	18.50	68.18	75.00	-	-	-	6.45	4.65
Scopulariopsis brevicaulis	-	-	-	-	-	-	-	-	-	7.60	-	-
T. viride	17.24	16.66	5.12	30.00	7.40	-	-	57.14	-	-	11.11	-

Table 4.3.Temporal variation in relative abundance of fungal species in the 0-10 cm soil layer in the forest stand.

Species	Sampling period											
	Jun. 95	Aug. 95	Oct. 95	Dec. 95	Feb. 96	Apr. 96	Jun. 96	Aug 96	Oct. 96	Dec. 96	Feb. 97	Apr. 97
Absidia cylindrosprora	-	-	-	-	-	-	-	-	-	22.20	-	-
Actinomucor elegans	-	-	-	11.76	-	-	-	-	-	-	-	-
Cunninghamella elegans	-	45.45	-	-	-	-	-	-	-	-	-	-
Gliomastix muroxum	-	-	-	-	11.76	-	-	-	-	-	-	-
Humicola gvisea	-	-	-	-	-	-	-	100	29.62	-	-	-
Mucor circinelloides	-	-	-	-	2.94	-	-	-	-	-	-	-
M. hiemalis	-	-	-	-	-	14.28	-	-	-	-	-	-
Penicillium canescens	-	12.12	-	-	-	-	-	-	-	-	-	-
P. chrysogenum	-	-	6.89	-	-	-	-	-	-	-	-	53.12
P. frequentans	18.18	-	-	88.20	-	-	5.55	-	11.11	44.44	-	-
Phoma sp.	-	-	1.73	-	-	-	-	-	37.03	-	20.00	-
Pythium intermedium	18.18	57.75	31.03	11.76	41.17	85.70	88.88	-	22.22	24.22	40.00	46.87
Torulomyces sp.	-	-	-	-	17.60	-	-	-	-	-	-	-
Trichoderma koningii	-	-	-	-	14.70	-	-	-	-	-	-	-
T. viride	-	-	60.34	-	-	-	5.55	-	-	11.11	40.08	-
Verticillium tennerum	63.63	-	-	-	-	-	-	-	-	-	-	-

Table 4.4..Temporal variation in relative abundance of fungal species in the 10-20 cm soil layer in the forest stand.

Species	Sampling period											
	Jun. 95	Aug. 95	Oct. 95	Dec. 95	Feb. 96	Apr. 96	Jun. 96	Aug. 96	Oct. 96	Dec. 96	Feb. 97	Apr. 97
Acremonium murorum	-	-	-	-	-	-	-	-	-	-	-	16.66
Cunninghamella elegans	-	42.85	52.77	-	-	-	-	-	-	-	-	-
Gliomastix muroxum	-	-	-	11.11	5.26	-	-	-	-	-	-	-
Humicola gisea	-	-	-	-	-	-	-	48.71	33.33	-	-	-
Mortierella horticola	17.64	-	-	-	-	-	-	-	-	-	-	-
Mucor circinelloides	-	-	5.55	5.55	21.05	-	-	7.69	8.33	-	-	-
M. hiemalis	-	-	-	-	-	14.76	-	-	-	-	-	-
Penicillium canescens	-	11.42	-	-	36.84	-	-	-	-	-	-	13.88
P. chrysogenum	-	-	13.88	-	-	-	-	-	-	-	-	-
P. jensenii	-	-	-	-	-	-	-	-	-	-	13.88	-
Phoma sp.	-	-	-	-	-	32.86	-	-	-	-	-	-
Pythium intermedium	11.76	45.71	-	38.88	36.64	52.38	86.95	10.25	16.66	21.92	16.60	33.33
Trichoderma koningii	-	-	-	-	-	-	-	-	-	-	27.77	-
T. viride	-	-	27.27	-	-	-	-	-	-	50.00	13.88	16.66
Verticillium tennerum	70.58	-	-	-	-	-	-	-	-	-	-	-

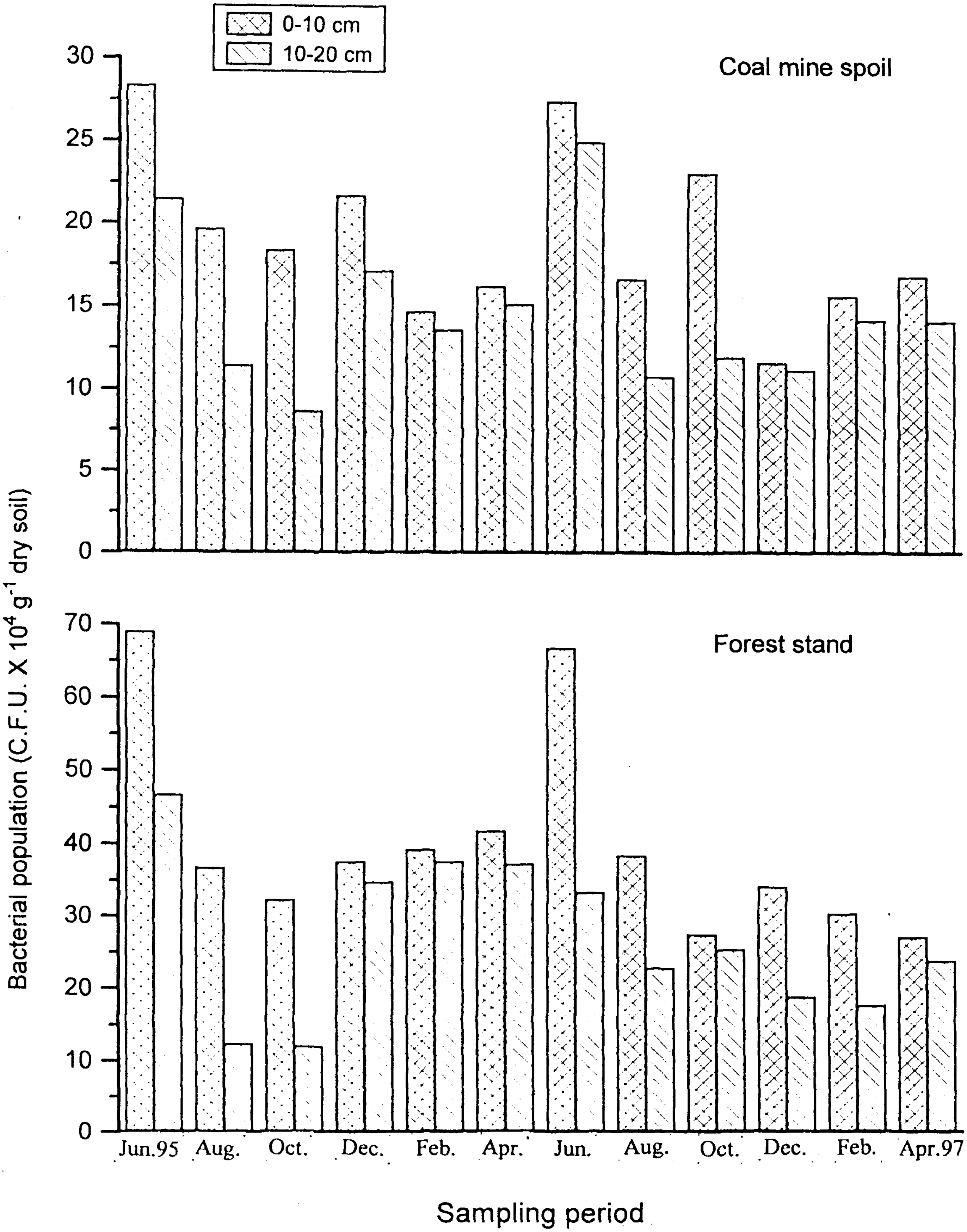

Fig. 4.2. Bimonthly variation in bacterial population coal mine spoil and the forest stand.

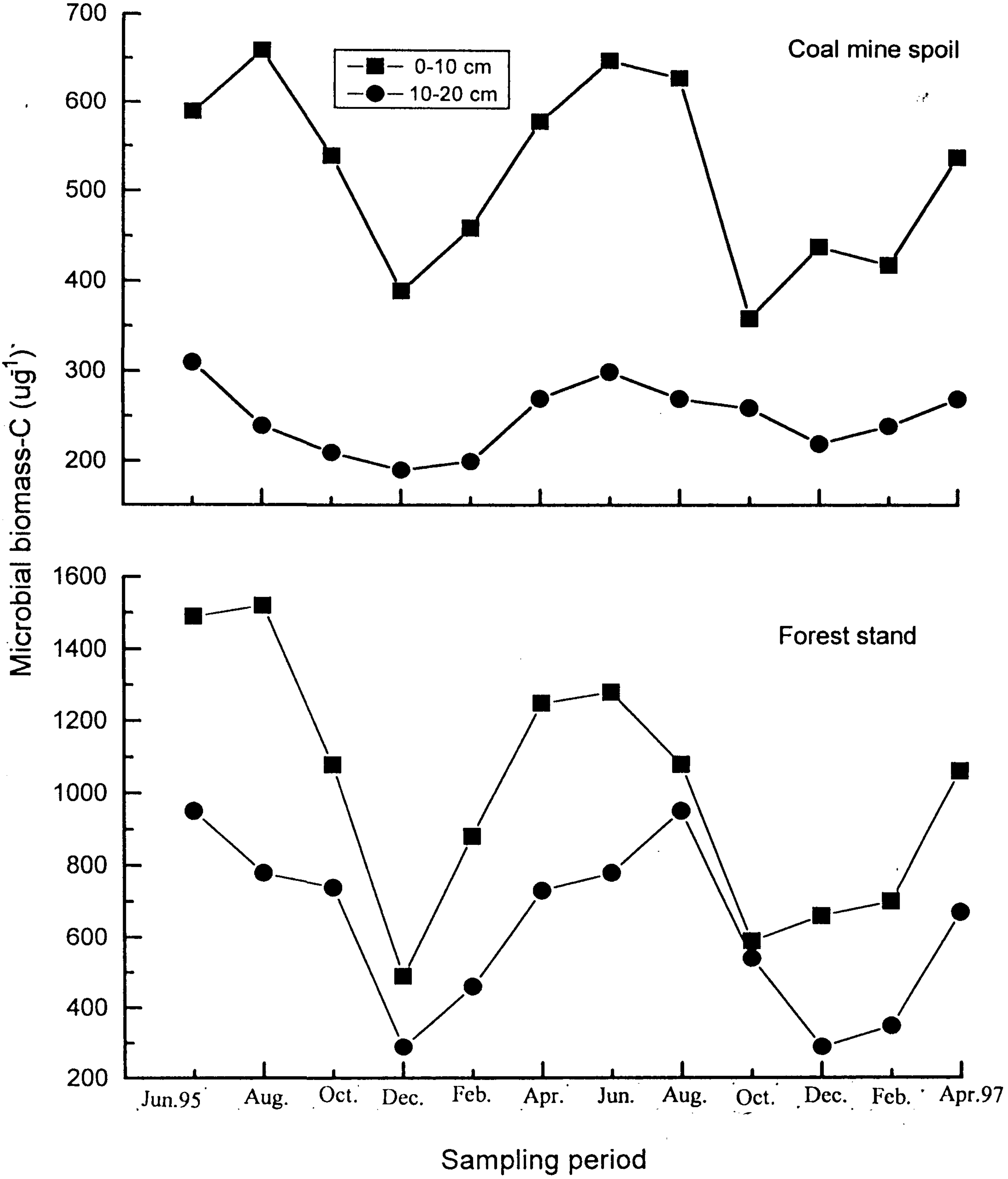

Fig.4.3. Bimonthy variation in microbial biomass-C in coal mine spoil and theforest stand

Table 4.5 . Correlation co-efficient values (r) for various parameters in 0-10 cm soil layer in the forest stand.

Source of variation	Fungal population	Bacterial population	Microbial Biomass-C	Soil respiration	Dehydrogenase	Urease	Phosphatase
Moisture content	NS	0.654*	NS	NS	NS	NS	NS
pH	NS	NS	-0.677*	-0.649*	NS	NS	NS
Nitrogen	NS	NS	NS	NS	NS	NS	NS
Phosphorus	0.734**	NS	NS	NS	NS	NS	NS
Potassium	NS	0.753**	NS	NS	NS	NS	NS
Rainfall	NS	0.553*	NS	NS	NS	NS	NS
Temperature	-0.553*	NS	NS	NS	NS	0.702**	NS
Relative humidity	NS	0.639*	NS	NS	NS	0.683*	NS
Dehydrogenase	NS	NS	NS				
Urease	NS	NS	NS				
Phosphatase	NS	NS	NS				
Soil respiration	NS	-0.559*	NS				

** P<0.01
* P<0.05

Plate 4.1. *Pseudomonas* sp.

Plate 4.2. *Bacillus* sp.

Plate 4.3. *Thiobacillus* sp.

microorganisms.The peak in bacterial population during rainy season in both the coal mine spoil and the forest stand could be due to the favourable soil moisture and temperature during the periods. Schnurer *et al.* (1986) reported that the bacterial population was high during rainy season.High bacterial population and a low fungal population during rainy season may be ascribed to the competition among these two types of organisms and the concentrate materials presence in the fungal hyphae were utilized by bacteria (Cromak *et al.* 1975). Bacteria have the ability to grow under conditions of high water potential. When the conditions are not favourable for the growth of heterotrophic bacteria, they can utilized CO_2 as a source of cell carbon and energy. Both bacteria and fungi showed positive reaction to soil mositure (Soderstrom, 1979, Clarholm and Rosswall, 1980, Baath and Soderstrom, 1982, Lundgren and Soderstrom, 1983, Schnurer *et al.*, 1986, Ohtoner and Markola, 1991). The litter fall with its higher moisture and nutrient contents stimulated microbial activity (Kauri, 1981). Changes in the properties of organic matter due to decomposition may influence seasonal patterns of microbial abundance. Seasons indirectly affect plant productivity and thus the seasonal patterns of organic matter release. This in turn affects the microbial population in soil (Berg *et al.* 1998). Seasonal changes were attributed to periodic inputs of organic matter and nitrogen. Environmental factors are also important in controlling the size of bacterial population in soil (Stotzky 1972).

The reduction in population from surface to sub-surface soil was ascribed to the high organic matter, nutrient status and better aeration in the surface layer (Balasubramanium *et al.*, 1972) and moisture regime (Selvraj and Rangaswamy, 1978, Clarholm and Rosswall, 1980). The reaction of the bacteria to fluctuations in water content seemed to depend on the micro-stratification in the organic profile, as the strength diminished with depth (Berg *et al.*, 1998). Mishra and Kanaujia (1972) reported that organic matter, pH, soil depth and season played a crucial role in the distribution of mycoflora. With increase in depth, O_2 content declined which is necessary for growth. Physical and chemical condition of soil in the surface soil layer favour the growth of microorganisms. Highly acidic condition in coal mine spoil reduced the microbial population. Soil microorganisms under low pH, metal or stress condition were low because they diverted their energy from growth to cell maintenance (Killham, 1985). Microorganisms subjected to stress may have a direct interference with enzymes systems which reduced the availability of metabolites to the microbial population (Witter and Dahlin, 1995). N saturation may very well be a factor that imposes a stress on the microflora (Berg *et al.* 1998). The higher count of bacteria and fungi in forest soil as compared to the coal mine spoil was due to the greater availability of nutrients in forest soil on account of greater accumulation of litter and fine roots (Maithani., 1996). The texture of soil contain more clay content in forest stand and it can retain moisture which favour the growth of microorganisms.

Bacterial population showed positive correlation ($P<0.05$) between rainfall and relative humidity and could explain the ability of bacteria to grow under conditions of high water potential. Moisture content and potassium showed a positive ($P <0.01$) correlation in the forest stand (Table 4.5).

Soil microbial biomass, a living part of soil organic matter, is responsible for the transformation of the added and native organic matter (Dalal *et al.*, 1991). Litter and soil physical factors play a role in determining the microbial biomass

(Bosatta and Agren, 1994). In forest, the concentration of microbial biomass was high during spring and rainy season and minimum during winter is similar from those reported for tropical deciduous forests, savanna and temperate pastures where peak values for microbial nutrients were observed during early spring and summer (Sarathchandra *et al.*, 1984 and Diaz-Ravina *et al.*, 1993). The peak microbial biomass during spring and rainy seasons indicated the period of favourable growth of microorganisms due to the increase in temperature and favourable moisture. Microbial biomass was affected by varying soil moisture content (Wardle and Parkinson, 1990) because it influenced the rate of decomposition of litter and fine roots. Temperature-moisture regimes influenced microbial dynamics as it is evident by the extremely low microbial biomass during winter. Decomposition of organic detritus is mainly caused by microbes (Singh and Gupta, 1977) and any change in the quality and quantity of detritus may influence the soil microbial biomass. Growth of the microbial biomass and the activity were favoured during spring and rainy season which may be due to greater release of microbial nutrients through microbial mineralization.

In coal mine spoil, the microbial biomass-C was very low as compared to forest soil because of the soil disturbance in those areas. Extreme low pH values can result in slow rate of microbial biomass built up (Cerri and Jenkinson, 1981) indicating that the stress factor reduces the populations of organic matter immobilized in the microbial biomass and microorganisms exist most of the time in dormant state (Gray and Williams, 1971). Low pH as stress of heavy metals toxicity in soils can result in a reduced size of the soil microbial biomass (Brookes and McGrath, 1984, Chander and Brookes, 1993 and Witter *et al.*, 1993). Biomass exchange capacity(CEC) and pH (Wolter and Jorgenson, 1991). Microbial biomass varied according to the climatic conditions and soil properties (Ladd *et al.*, 1981).

The decrease in the concentration of biomass with the increase in soil depth in both the sites may be attributed to the reduction in organic matter and nutrients. Bio-chemical activities of Jogeford soil declined with soil depth (Ross *et al.*, 1982). Similar results have been recorded with other soils (Ross and Roberts, 1968 and Tabatabai and Bremner, 1970). The soil depth effects may have been associated with differences in gaseous diffusion. Loss of this surface soil through human or natural disturbance could be detrimental to the functioning of ecosystem (Bolton Jr *et al.,* 1993). Kaiser and Heinemeyer (1993) also observed higher soil microbial biomass at the soil surface in the sagebrush stoppe of wyoming.

Changes in soil management processes cause variation in microbial biomass to increase or decrease much faster than the total amount of soil organic matter so that microbial biomass as a percentage of total organic carbon can provide a sensitive indicator of less detectable trends in total soil organic-C loss or accumulation (Ayanaba *et al.*, 1976 and Powlson *et al.*, 1987). Biomass-C follows changes in carbon input to soil (Haron *et al.*, 1998). The size of the biomass carbon pool may partly reflect the recalcitrance of the soil organic matter pool with a smaller proportion of the biomass in soil organic matter pool with more recalcitrant organic matter (Witter , 1996).

The high concentration of biomass in forest soil than coal mine spoil is due to greater accumulation of litter and fine roots in the forest stand which favoured the growth of microbial population and accumulation of microbial biomass. Biomass increased with higher organic matter content (Schnurer *et al.*, 1985). Arunachalam (1996) reported an increase in organic matter content in forest stand owing to the greater accumulation of plant derived organic matter and microbial products. A reduced metabolic efficiency is probably the reason for the lower microbial content at the acidic site (Anderson and Domsch, 1993).

CHAPTER V

SEASONAL CHANGES IN MICROBIAL ACTIVITY AND CO_2 EVOLUTION IN COAL MINE SPOIL AND THE FOREST SOIL

Introduction

Mining operation leads to pollution of soil with heavy metals, such contamination of soil have adverse effect on soil microbial and biochemical processes due to inhibition of enzymes by metals (Tyler, 1974, 1975, 1976, Jordan and Lechevalier, 1975 and Freedman and Hutchinson, 1980). Soil enzyme activities are affected by high concentration of trace elements (Frankenberger, *et al.,* 1983). Chemical reaction in soil is highly influenced by enzymatic activity. A low level of biological activity is associated with soil deterioration (Gildon and Rimmer, 1993). The biological activity in soil provides better insight in understanding the transformation of organic matter (Pietkainen and Fritze, 1995). Soil enzyme activity estimates are often used as indices of microbial activity and fertility of soil (Skujins, 1978). Microbial processes play an important role in changes of soil structure. As microorganisms are important source of soil enzymes, the activity of these enzymes correlates with microbial activity.

Measurement methods of biological activity have been used induced respiration as CO_2 production (Stroo and Jencks, 1982 and Chichenstar and Smith, 1983) enzyme activity (Stroo and Jencks, 1982).

The method induced respiration as CO_2 production has been used to measure biological activity (Lundegardh, 1927). Soil microorganisms such as bacteria and fungi play a major role in releasing CO_2 by metabolizing organic debris. Total soil respiration is an important attribute that provides an estimate of the turnover of soil organic matter (Parker et al., 1983). Soil microbes are the major contributors to the CO_2 flux from soils. Temperature, soil aeration and nutrient availability are important factors in regulating the rate of organic matter decomposition in peat (Silvola *et al.*, 1985). Silvola *et al.* (1996) reported that cool and humid conditions favour carbon accumulation in soil.

Phosphatase activity is directly related to the level of organic phosphorus in soil (Rao *et al.*, 1995). The rate of P mineralization depends on microbial activity and the free phosphatase (Dalal, 1977). Phosphatases are produced by microorganisms (Herbein and Neal, 1990). Appiah and Thomas (1982) reported that phosphatase activity is correlated with the content of organic phosphorus and the organic matter. In soil, microbial population participates in the process of organic phosphorus mineralization through the action of phosphatase enzymes, releasing available phosphorus (Islam and Ahmed, 1973). Microorganisms which contain part of the organic phosphorus of wastes within their structures, influence the transformation of the organic matter of a waste either during composting or when it is added fresh to the soils. Phosphatase activity in these kind of wastes might be an indicator of microbial action and evolution of organic matter in the composting process similar to that found for phosphatase in soil (Garcia *et al.*, 1993). Soil phosphatase activity and the general mineralization of organic-P have been reviewed extensively (Cosgrove, 1967, Ramirez-Martinez, 1968, Halstead and Mckercher, 1975, Haysman, 1975, Dalal, 1977 and Speir and Ross, 1978).

Dehydrogenase activity is more dependent upon the metabolic state of the microbial population of the soil than upon the specific free enzymes on particular substrate (Ross, 1970). Dehydrogenase activity is considered to be caused by a broad group of endocellular enzymes (Skujins, 1978) which transfer hydrogen and electrons from substrate to appropriate acceptors during the initial stage of oxidation of organic compounds. Dehydrogenase activity has been recommended as an index of general activity of soil microorganism (Skujins, 1967 and Casida, 1968) and catabolic activity of a soil.

Soil ureases are microbial products that can accumulate in cell free form in soil because they are highly resistant to environmental degradation (McNaughton, 1997). Urease activity generally correlates with organic matter due to its existence as a complex with organic constituents (Skujins, 1976). Temperature and other soil factors such as moisture content, pH, organic matter and numbers of microorganisms affect the urease activity in soil (Skujins, 1967 and O'Toole *et al.*, 1985).

This chapter aims to investigate the effect of coal mining disturbance on the soil enzyme activities of microorganisms and the importance of microbial activity in releasing the nutrients so as to understand their relationship with catabolic activity of soil.

Materials and methods

Soil samples were collected on a bimonthly basis from coal mine spoil and the forest stand. Soil samples were collected from two depths (0-10 cm and 10-20 cm) randomly from ten places and mixed thoroughly to obtain a homogeneous sample.

Soil respiration

The amount of CO_2 evolved was measured by following the method of MacFadyen (1970). 1 kg of soil from each sample was placed in a glass jar. one 100 ml beaker containing 20 ml of 0. 1 N KOH solution was kept inside the jar. The lid of glass jar was then sealed by grease to make it air tight and left for 24 hours. After 24 hours the jar was opened and CO_2 fixed by KOH was estimated by titrating with 0.1 N HCL solution using phenolphthalein as an indicator. For control, sterilised sand was used instead of soil samples. Three replicates were maintained for each sample. The soil respiration was expressed as CO_2 evolved in terms of mg CO_2 evolved/kg/day on dry weight basis.

Dehydrogenase Activity

Casida's (1977) 2,3,5 triphenyl tetrazolium chloride (TTC) reduction technique was followed for the estimation of dehydrogenase activity. 10 g of fresh soil was taken in a test tube. The soil was then mixed with 0. 1 g of $CaCO_3$ and 1 ml of 1% TTC solution. The content kept in the tube was mixed thoroughly and plugged with cotton and wrapped with aluminium foil. The test tubes were incubated at 37°C for 24 hours in an incubator. Three replicates were maintained in each case.

The resulting slurry was transferred on Whatman No 1 filter paper and extracted with successive aliquotes of concentrated methanol. The absorbance of the filtrate was read at 485 nm in Hitachi Spectrophotometer (220) using methanol as blank. The activity was represented in terms of concentration of formazan which was calculated by a standard curve of triphenyl formazan in methanol.

The dehydrogenase activity per gram dry soil was expressed in terms of mg formazan per gram dry soil per hour.

Urease Activity

Urease activity was estimated by Mc Garity and Myers (1967) method. 10 g of fresh soil was kept in test tube and was treated with 1 ml of toluene. It was allowed to stand for 15 minutes to permit the complete penetration of toluene into the soil. Thereafter, 10 ml of buffer (pH 7) and 5 ml of 10 % urea solution were added. The flask was shaken and incubated at 37°C for 3 hours. In control, 10 ml of distilled water was added instead of urea solution.

After incubation, the volume was made upto 100 ml by adding distilled water. The content in the flask was mixed thoroughly and was filtered through Whatman filter paper No.1.

Indophenol blue method was adopted for the measurement of ammonia released as a result of urease activity. 0.5 ml of filtrate was taken in to a 50 ml volumetric flask and to it 5 ml of distilled water was added. The mixture in flask was treated with 2 ml of phenolate solution and 1.5 ml of sodium hypochlorite solution containing 5% of active chlorine. The final volume was made upto 50 ml by adding distilled water. The absorbance was measured using Hitachi spectrophotometer at 630 nm. For control (without soil) similar procedure was followed. The amount of NH_4^+-N released was calculated as NH_4^+-N per gram dry soil per hour.

Phosphatase Activity

Phosphatase activity in soil was assayed by the method of Tabatabai and Bremner (1969). Air dried 1 g ground sieved (0.2 mm) soil was used for estimation of phosphatase activity. The soil was taken into a test tube. 4 ml of modified universal buffer (pH 6.5), 0.25 ml of toluene and 1 ml of 0.115 M P-nitrophenyl phosphate (PNP) solution were added to the flask (Skujins, 1967). The flask was swirled for few seconds and then incubated at 37^{0}C for one hour. 1 ml of 0.5 M calcium chloride and 4 ml of 0.5 M NaOH were added to it after incubation. The soil suspension was filtered through Whatman No.1 filter paper. The absorbance was measured at 430 nm on Hitachi (220) spectrophotometer. Blank was maintained similarly without soil.

Phosphatase activity in terms of concentration of P-nirophenol in each sample was calculated by a standard curve of P-nitrophenol per g dry soil per hour.

Results

CO_2 evolution in coal mine spoil and the forest soil varied significantly ($P<0.01$) between the sampling periods (Fig. 5.1). In both the cases high amount of CO_2 evolved during June and August and low during December and February. The amount of CO_2 evolution declined significantly ($P<0.05$) from the surface (0-10 cm) to the sub-surface (10-20 cm) soil layer in coal mine spoil and the forest stand. The amount of CO_2 evolution was much higher in the forest soil than the coal mine spoil.

Dehydrogenase activity also varied widely between the sampling period (Fig. 5.2). In both coal mine spoil and the forest soil dehydrogenase activity was high during June and August (Rainy) and low during December and February (winter) .The upper soil layer had higher dehydrogenase activity than the lower soil layer in both the coal mine spoil and the forest soil. Forest stand had a significantly ($P<0.01$) higher dehydrogenase activity than the coal mine spoil.

Urease activity also varied significantly ($P<0.05$) between the sampling period in both the coal mine spoil and the forest stand (Fig.5.3). High urease activity was observed during June and August (rainy) and low during December and February (winter) in both the coal mine spoil and the forest soil. Urease activity showed a declining trend with increasing soil depth from the surface to the subsurface soil layer in both the cases. High urease activity was observed in forest soil as compared to that of coal mine spoil.

Phosphatase activity varied significantly ($P<0.05$) between the sampling period in both the coal mine spoil and the forest stand (Fig. 5.4). In both the forest stand and the coal mine spoil it showed high phosphatase activity during April (Spring) and low activity during June, August (rainy)and December and February (winter). Phosphatase activity declined significantly ($P<0.05$) from the surface to the subsurface soil layer in both the cases and showed higher value in the forest stand.

Discussion

The amount of CO_2 evolution was more in the surface soil layer than in the subsurface soil layer in both coal mine spoil and the forest stand. The decline in CO_2 evolution with soil depth may be attributed to the decline in soil organic matter content (Vardakakis, 1989). Soil respiration is mainly mediated by soil

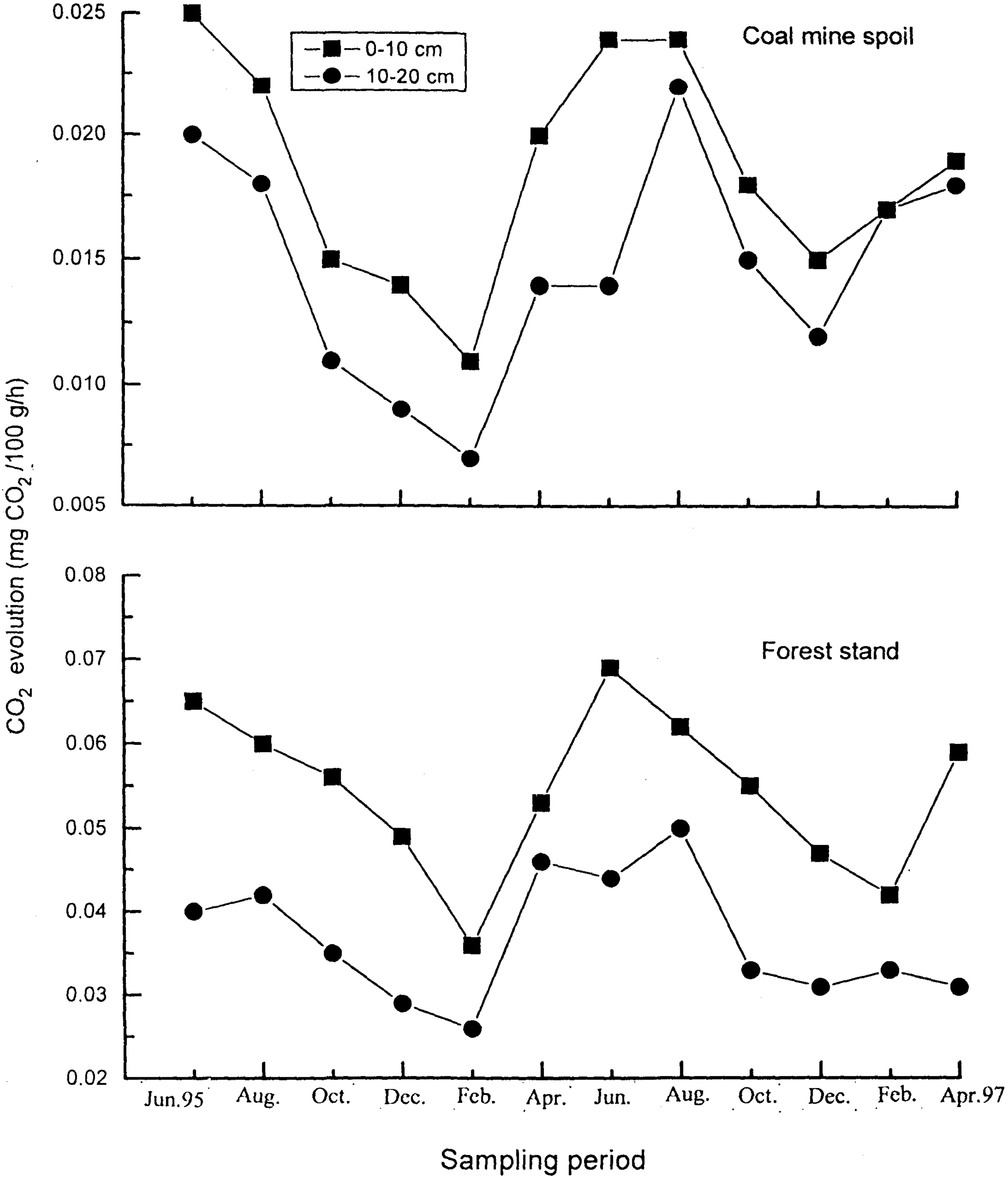

Fig.5.1. Bimonthly variation of CO$_2$ evolution in coal mine spoil and the forest stand.

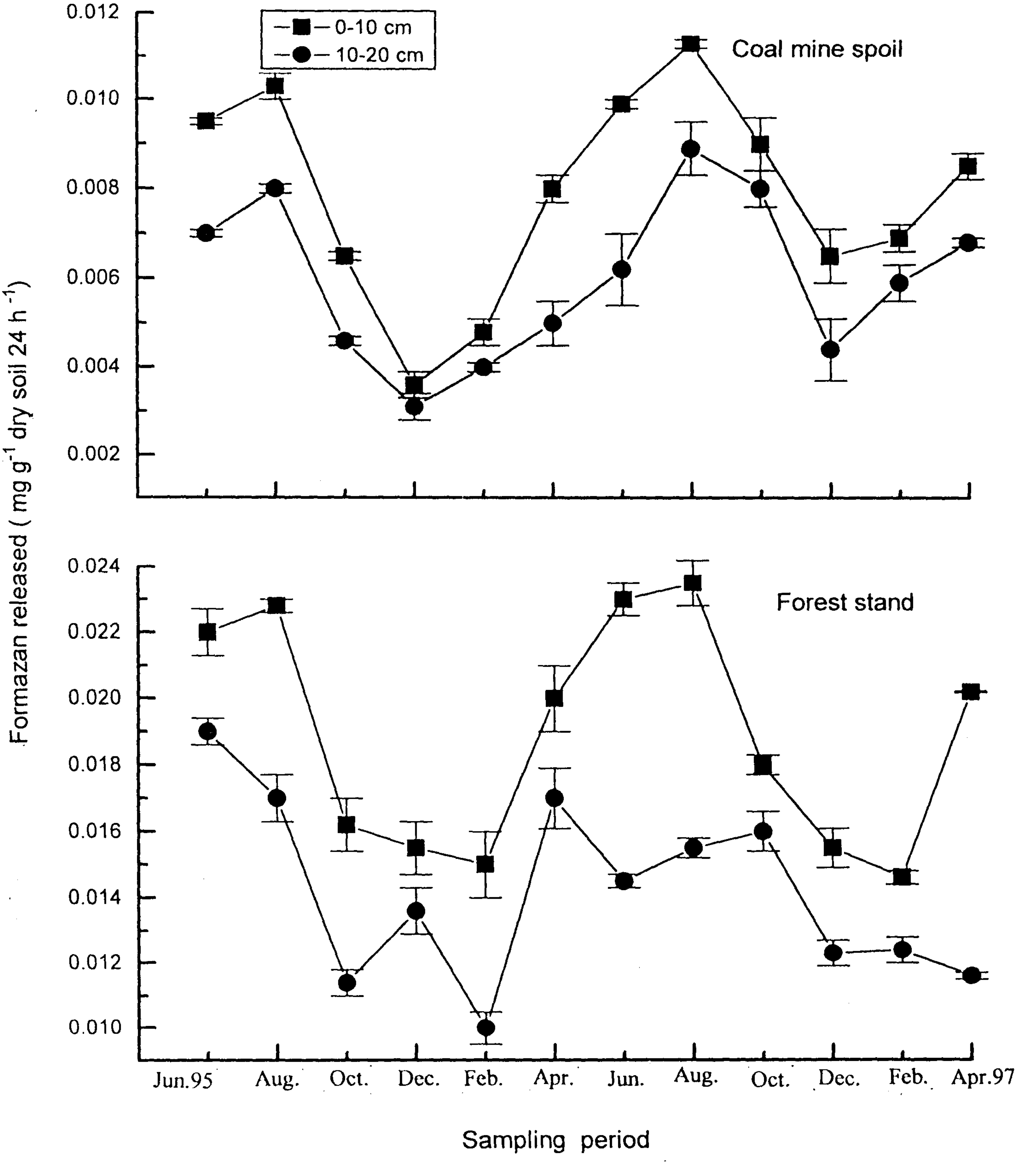

Fig. 5.2. Bimonthly variation of dehydrogenase activity in coal mine spoil and forest stand

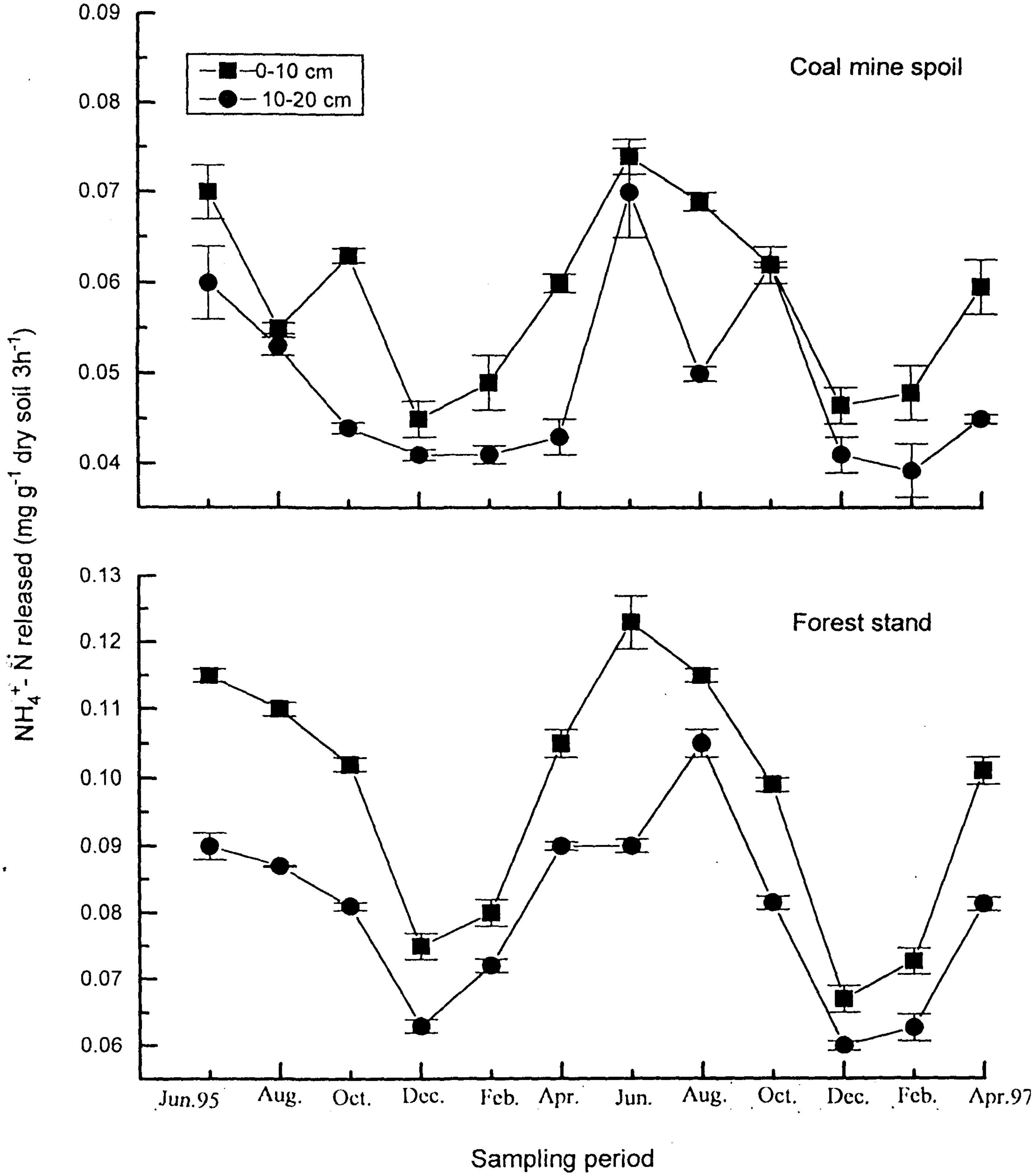

Fig.5.3. Bimonthly variation in urease activity in coal mine spoil and forest stand.

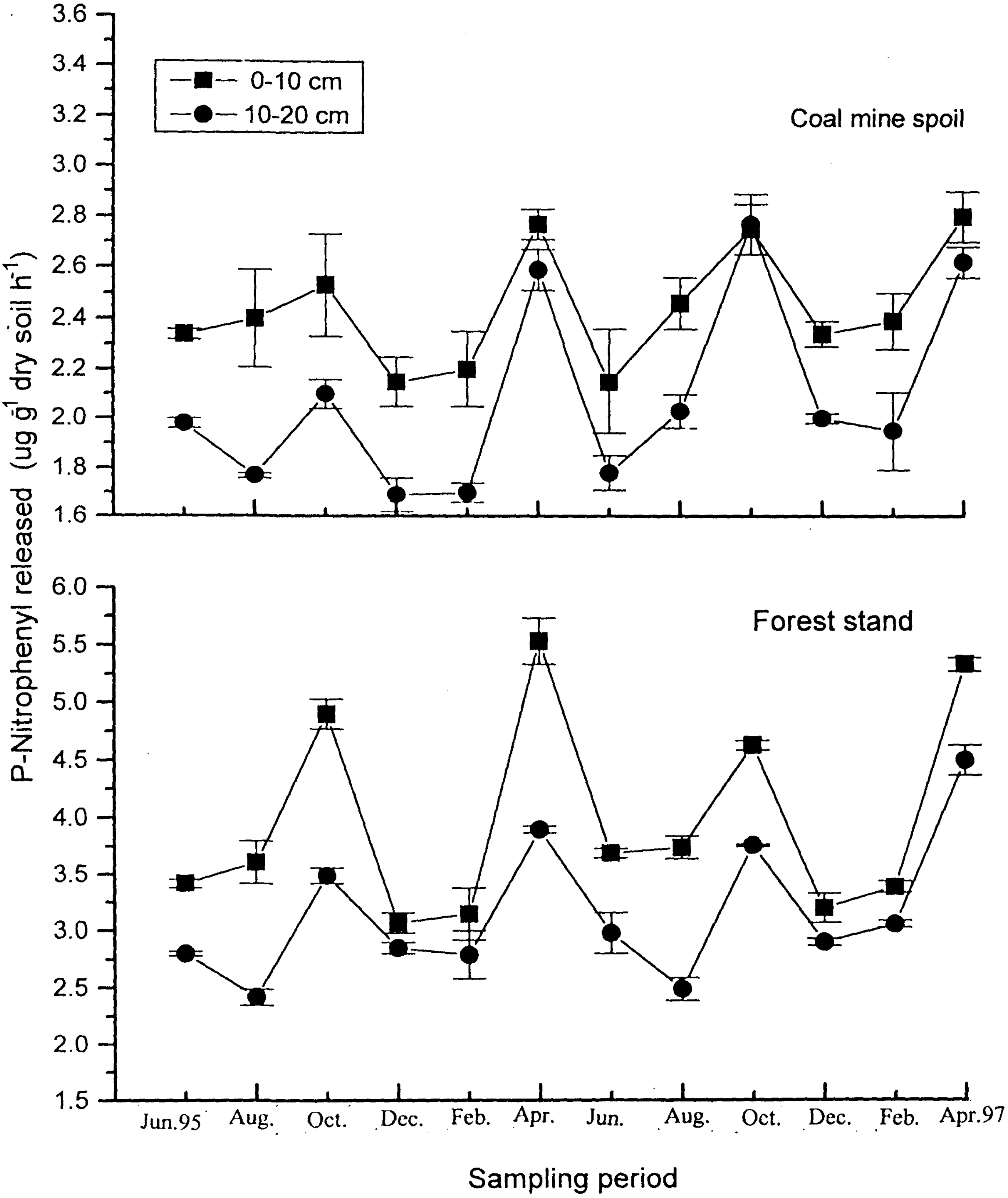

Fig.5.4. Bimonthy variation in phosphatase activity in coal mine spoil and the forest stand

microbes and roots. Majority of the fine roots were restricted in the top soil layer in forest stand. Microbial population declined with increase in soil depth (Chapter IV). As a result the CO_2 evolution rate declined from the surface to the subsurface soil layer in both the cases. In forest soil and coal mine spoil less amount of CO_2 evolved during winter was due to low organic matter content, low microbial population , low moisture content and temperature in the soil which resulted in the decrease of soil metabolic activities during winter months. The high rate of activity during rainy season reflected the favourable effect of soil moisture and moderate temperature on microbial activity (Gupta and Singh, 1980). It was also due to high organic matter content and high microbial population during rainy season which resulted in the increased CO_2 evolution . Increase in soil CO_2 concentration could result from a slight increase in soil moisture (Kusar, 1989). The lowering of the rate of CO_2 evolution at high moisture level is probably due to decrease in respiration caused by a lowering of diffusion of O_2 to the organisms (Boddy, 1983). Rise in temperature and draw-down of water table thus increase CO_2 fluxes from peat. Increased microbial oxidation in the soils could also result in increased emission of CO_2. Soil CO_2 evolution was generally found to increase with soil temperature.

The high CO_2 evolution rate in forest soil compared to the coal mine spoil may be attributed to high microbial population (Dkhar and Mishra, 1987) and increased organic matter (Ewel *et al.*, 1988). Wicklow *et al.*(1973) reported that the different pattern of CO_2 evolution is due to the variation in microbial population and substrate quality. Soil nitrogen and moisture were the positive factors which enhanced CO_2 evolution (Tiwari *et al.*, 1987). Soil moisture enhance the rate of soil respiration by affecting microbial activity and making available the organic carbon to them and nitrogen provided protein source for microbial growth (Tiwari *et al.*, 1987). The high CO_2 evolution in forest soil in the present investigation may be ascribed to the high organic matter content in overlying litter which governed the soil respiration by affecting microbial activity. The soil which received nitrogen rich litter respire more (Tiwari *et al.*, 1987). Besides, thick mat of fine roots were present in the forest floor. Singh and Gupta (1977) reported that roots contribute about 70 % to the soil respiration.

Seasonal variation in enzyme activity appears to be dependent on many factors such as soil aeration , soil moisture, vegetation and microflora as well as soil temperature (Aseeva and Vanyarkho, 1969). The soil nutrients were the most important factor likely to regulate microbial activity (Wynn-Williams, 1982).

In both forest soil and the coal mine spoil, high dehydrogenase activity in rainy season was the result of high population of bacteria encountered. Skujins (1976, 1978) observed a correlation between the dehydrogenase activity and microbial population. Ross (1970), Baruah and Mishra (1984), Dkhar and Mishra

(1987), Tiwari *et al.* (1987) reported that the bacterial population and the soil moisture content are the main factors which determine the dehydrogenase activity in soil. Dehydrogenase activity is related to the moisture content and temperature of the soil (Ortem and Neuhaus, 1970). An increase in soil moisture content enhanced the availability of organic carbon to heterotrophs which favoured the growth of microorganism and increased their population and are responsible for the increased microbial activity as indicated by a positive correlation between organic matter and dehydrogenase (Rao *et al.*, 1995). Less activity during autumn may be due to sub-optimal microbial population and activity of low carbon content. The heavy precipitation washed away the organic carbon and microbial propagules which may temporarily reduce the dehydrogenase activity (Deka *et al.*, 1989). During December and February, reduction of soil moisture and temperature caused dryness of soil which became unfavourable for bacterial and fungal growth and reduced the dehydrogenase activity (Shukla *et al.*, 1987).

Lower dehydrogenase activity in coal mine spoil could be due to the heavy metal concentration (Reddy *et al.*, 1987). Soil dehydrogenase activity was also apparently less in the high metal soil than in the low metal soil. The low activity is also due to the low organic matter content. High dehydrogenase activity in forest soil was related to high nitrogen , organic carbon content and increased bacterial and fungal populations.

In both the coal mine spoil and the forest soil, high urease activity in rainy season was related to the concentration of organic carbon (Rao *et al.*, 1995). Higher organic carbon, bacterial population and favourable moisture enhanced urease activity (Dalal, 1975, Speir, 1977, Dkhar and Mishra, 1983 and O'Toole *et al.*, 1985). Highest urease activities were found in rainy season and declined towards winter which was also reported by Stott and Hagendorn (1980). Low activity during winter could be due to low organic matter, low temperature and low moisture content which reduced the microbial population in soil. Less activity was found in coal mine spoil as compared to forest soil which could be due to the difference in the chemical composition of the vegetational debris causing changes in the composition of the microorganisms of the soil. This change in addition to changes in microhabitats induced by different types of vegetation, may be one of the causes of the observed differences in enzymatic activity (Palma and Conti, 1990). High soil urease activity in the forest stand may be attributed to the faster rate of turnover of organic matter.

Phosphatase activity was high in forest soil as compared to coal mine spoil. This could be due to the presence of vegetation and thick accumulation of litter which contributed to the high organic matter content in the forest soil. Pancholy and Rice (1966) and Ross (1973) reported that there is a close relatioship between enzyme activity and vegetation. Saratchandra and Perrott (1981) reported that the enzyme activities increased with increase in organic matter content. Soil fertility with regard to phosphorus is correlated with phosphatase activity. The soil phosphatase mediates the release of inorganic P from organically bound P in litter, dead root system and other organic debris (Harrison, 1983). A relationship between phosphatase activity and phosphorus release in soils was observed. Production of P depended on phosphatase activity. Less activity in coal mine spoil was due to low organic matter content, low microbial population. The enzyme activity varied in different seasons as well as with types of plant species (Beck, 1974).

The declining trend of phosphatase activity with increase in soil depth may be attributed to the decrease in root density and soil microbial activity with increasing depth (Parkinson *et al.*, 1968, Harrison, 1983 and Acea and Carballas, 1985). Soil phosphatase is inactivated or degraded as it penetrates the soil. In both forest soil and the coal mine spoil increased soil phosphatase activity in spring season was due to the increased temperature, moisture and high fungal population (Nakas *et al.*, 1987).The low activity during rainy and winter seasons was due to low level of fungal population and other microbes resulting into low level of phosphorus. Fungal isolates produced high level of phosphatase as compared to bacterial isolates (Nakas *et al.*, 1987). Soil fungi exhibited considerable level of phosphatase activity (Speir and Ross, 1978).High phosphatase activity observed during spring season was in agreement with the report of Ramirez and McLaren (1966b) and Rastin *et al.* (1988). It is clear that soil disturbance has marked affect on the microbial communities and their activities. The high concentration of various soil enzymes like dehydrogenase, urease and phosphatase in forest soil which is less disturbed than the coal mine spoil was due to high organic-C and mineral nutrients and microbial population. The reduced microbial population and their activities in the more degraded site may lead to decreased mineralization.

CHAPTER VI

ROLE OF MICROBES IN REGENERATION OF NUTRIENTS

Introduction

Coal mining activity generates huge ecological disturbances and negative environmental impacts due to deforestation for exploitation of coal. Degradation of mineral resources leads to loss of gene pool and biological diversity. Forest cutting has been reported to cause a net loss of soil organic carbon (Nye and Greenland, 1960). This loss affects the soil fertility and plant regeneration in the degraded sites. Degradation of the soil system through soil organic matter loss results from soil tillage (Follelt and Schimel, 1989) and clearing of natural vegetation (Srivastava and Singh, 1989). Soil degradation, tillage and severe erosion leading to the loss of natural soil fertility. These degradation processes of soil are often associated with marked changes in the activity and diversity of soil biota (Lal, 1986). Physically, nutritionally and microbiologically impoverished coal mine spoils present rigorous condition for both the plant and the microbial growth (Jha and Singh, 1993b).

Soil is inhabited by a large group of microorganisms viz., bacteria, fungi, actinomycetes, arthropods and protozoa etc. Among these, bacteria and fungi play a more important role in nutrient cycling and in influencing the biological properties of soil. The microbial communities constitute one of the most important component of the soil. They are responsible for the break down of organic matter and nutrient transformation.

The activities of microorganisms and soil fauna serve to promote soil aggregation processes and the maintenance and stabilization of soil structure (Oades, 1984 and 1993), reducing erosion (Lal, 1986) and greater moisture infiltration, maintenance of soil organic matter, induce nutrient retention and storage (Russsel, 1973). Distribution of organic substrate, microbial biomass and microbial activity in soils are the major determinant in nutrient cycling and organic matter stabilization (Ladd *et al.*, 1993).

Nutrient mineralization from soil organic matter is the primary source of nutrients for plant growth in man y ecosystems (Berendse *et al.*, 1987 b). Soil organic matter largely originates from plant litter and root residue which differ widely in decomposition rate (Swift *et al.*, 1979). In this way, plant species affect the formation of soil organic matter and nutrient availability (Van Vuuren and Berendse, 1993). Absence of litter in coal mine spoils leads to the organic matter deficiency. Nitrogen and phosphorus are the two limiting nutrients in coal mine spoils (Williams, 1975 and Wittwer et al., 1981). Soil at open cast sites are characterised by low soil N status (McRae, 1989). S levels increase due to oxidation of pyrite in coal mine spoils (Shankar et al., 1993). Wali and Freeman (1973) working on coal mine spoils of North Dakota found that Bo, Cu, Fe, Li, Sr and Zn contents were much greater in spoils as compared with unmined sites.

The importance of microbial community in regeneration of nutrient and in the re-establishment of a functioning ecosystem in strip mined land has not been studied in detail (Cundell, 1977). Therefore, the present study was carried out to investigate the nutrient regeneration and their relationship with microbes.

Materials and Methods

Soil sampling was done bimonthly by collecting five soil cores from two soil layers (0-10 cm and 10-20 cm). The soil samples from each depth were mixed thoroughly to make a composite sample. They were air dried, ground and sieved through a 0.2 mm mesh sieve. The samples were then kept in polythene bag for analysis.

Soil Analysis

Soil texture was determined by Bouyoucos Hydrometer Method (Allen *et al.*, 1974). Soil moisture content was determined gravimetrically by taking 10 g of unsieved soils and the results were expressed on oven dry weight basis (Allen *et al.*, 1974). Soil pH was determined electronically by digital systronics-335 pH meter in 1:2.5 suspension of soil in deionised water (Anderson and Ingram, 1993).

Organic carbon was determined by rapid titration method (Walkley and Black, 1934). soil organic matter content was obtained by multiplying the organic carbon concentration by 1.724 basing carbon (Allen *et al.*, 1974). Available phosphorus was determined by ammonium molybdate blue method after extracting soil P in 0.5 M sodium bicarbonate (Anderson and Ingram, 1993). Total Kjeldahl nitrogen (TKN) was determined by digesting air-dried soil samples with concentrated sulphuric acid using kjeltab (Tecator) as a catalyst, on a block digester. Distillation was carried out in a semi-microdistillation set and the distillate was titrated with N/140 HCl.

Exchangeable potassium was determined by flame photometer method after extracting in ammonium acetate solution pH = 7 (Allen et al., 1974).

Results

Soil Texture

The texture of the soil was sandy in both coal mine spoil and the forest stand. In forest stand, the clay content increased significantly ($P<0.01$) from 1.08% in the upper 0-10 cm soil layer to 5.32% in the 10-20 cm soil layer. Whereas in coal mine spoil a reverse trend was observed with a decrease in clay content from the surface to the sub-surface soil layer. The percentage of sand was more or less same in both the coal mine spoil as well as in the forest stand. However, the clay content was highest in the forest stand (Table 6.1).

Soil pH

pH showed wide fluctuations between the sampling period throughout the study (Fig. 6.1). pH was acidic in nature in both the coal mine spoil and the forest stand. In coal mine spoil, the pH was more acidic in nature. pH declined from the surface soil layer to the sub-surface soil layer in both the cases.

Moisture content

The soil moisture content varied significantly ($P<0.01$) between the sampling period (Fig. 6.2).The surface soil layer had a high moisture content than the sub-surface soil layer in forest stand except in the 2nd year of study during spring the subsurface soil layer had high moisture content than the surface soil layer. In the coal mine spoil during April (spring) December and February (winter) the surface soil layer had low moisture content than the subsurface layer whereas during October (autumn) and June and August (rainy) the surface layer had high moisture content than the subsurface layer.

Organic Carbon

Organic carbon also varied significantly ($P<0.05$) between the sampling period in both the coal mine spoil and the forest stand (Fig. 6.3). In coal mine spoil,, organic carbon was less in the Ist year of the study and it varied from 1.5% to 2.31% in the upper (0-10 cm) and 1.14% to 2.22% in the lower (10-20 cm) soil layer. In the 2nd year there was slight increase and the value varied from 2.35% to 3.11% in the upper layer and 1.7% to 3.04% in the lower soil layer. In the forest stand, the value varied from 3.15% to 4.35% in 0-10 cm soil layer to 2.30% to

3.84% in the 10-20 cm soil layer. The organic matter content was high during June in forest stand. In coal mine spoil, organic matter increased slightly in the 2nd year of the study and it was high during June and April. Organic matter content declined significantly f ($P<0.05$) from the surface to the sub-surface soil layer in both the cases and also there was a significant ($P<0.05$) decline inthe organic matter content from forest to coal mine spoil.

Total Kjeldahl nitrogen (TKN)

TKN was significantly ($P<0.05$) higher in the forest stand. It declined significantly ($P<0.01$) with increasing soil depth in both the cases (Fig. 6.4). TKN showed variation between the sampling periods. In forest, the values ranged from 0.36% to 0.39% in the surface layer and 0.17% to 0.23% in the sub-surface soil layer. High value was observed during June and August. Whereas, in coal mine spoil, it ranged from 0.11% to 0.19% in the surface layer and 0.06% to 0.16% in the sub-surface layer. In coal mine spoil, TKN showed a minor increase in the second year of the study period.

Exchangeable potassium

Exchangeable potassium declined significantly ($P<0.05$) with increasing depth and it also declined significantly ($P<0.01$) from forest to coal mine spoil (Fig. 6.5). In coal mine spoil the concentration varied from 2.01 mg/g and 1.73 mg/g respectively and the amount increased in the 2 nd year of study as compared to the 1st year of the study. Forest showed a high value and it varied from2.54 to 3.2 mg/g. High concentration was observed during June , August (rainy) and low concentration during December and February (winter).

Available phosphorus

Available-P concentration was very low in the coal mine spoil as compared to the adjacent forest stand. It showed a declining trend from surface to sub-surface soil layer (Fig. 6.6). It declined from 9.68 mg/g in the surface soil layer to 7.79 mg/g in the sub-surface soil layer in coal mine spoil. Whereas in forest soil, the corresponding values were 23.5 and 17.1 mg/g respectively. High phosphorus was observed during April in both forest soil and coal mine spoil.

Sulphur

Sulphur content was much higher in the coal mine spoil than that of the forest stand (Table 6.2). It declined form 123.33 mg/g in the 0-10 cm soil layer to 117.50 mg/g in the 10-20 cm soil layer in the coal mine spoil. Whereas, in forest, the corresponding values were 51.60 mg/g and 49.10 mg/g respectively.

Iron

Iron concentration was more in the coal mine spoil than in the forest stand (Table 6.2). It declined from 478.90 mg/g in the 0-10 cm soil layer to 471.50 mg/g in the 10-20 cm soil layer. Whereas in forest the values were 383.20mg/g and 377.50 mg/g respectively.

Table 6.1 Texture of soil in coal mine spoil and soil of the forest stand.

Site	Soil depth (cm)	Clay (%)	Silt (%)	Sand (%)	Textural class
Forest stand	0-10	1.08±0.00	6.95±0.69	91.97±0.69	Sandy soil
	10-20	5.32±0.00	10.52±1.22	84.16±1.22	Sandy soil
Coal mine spoil	0-10	0.5±0.00	3.81±0.47	95.69±0.69	Sandy soil
	10-20	0.36±0.00	5.78±0.57	94.10±0.69	Sandy soil

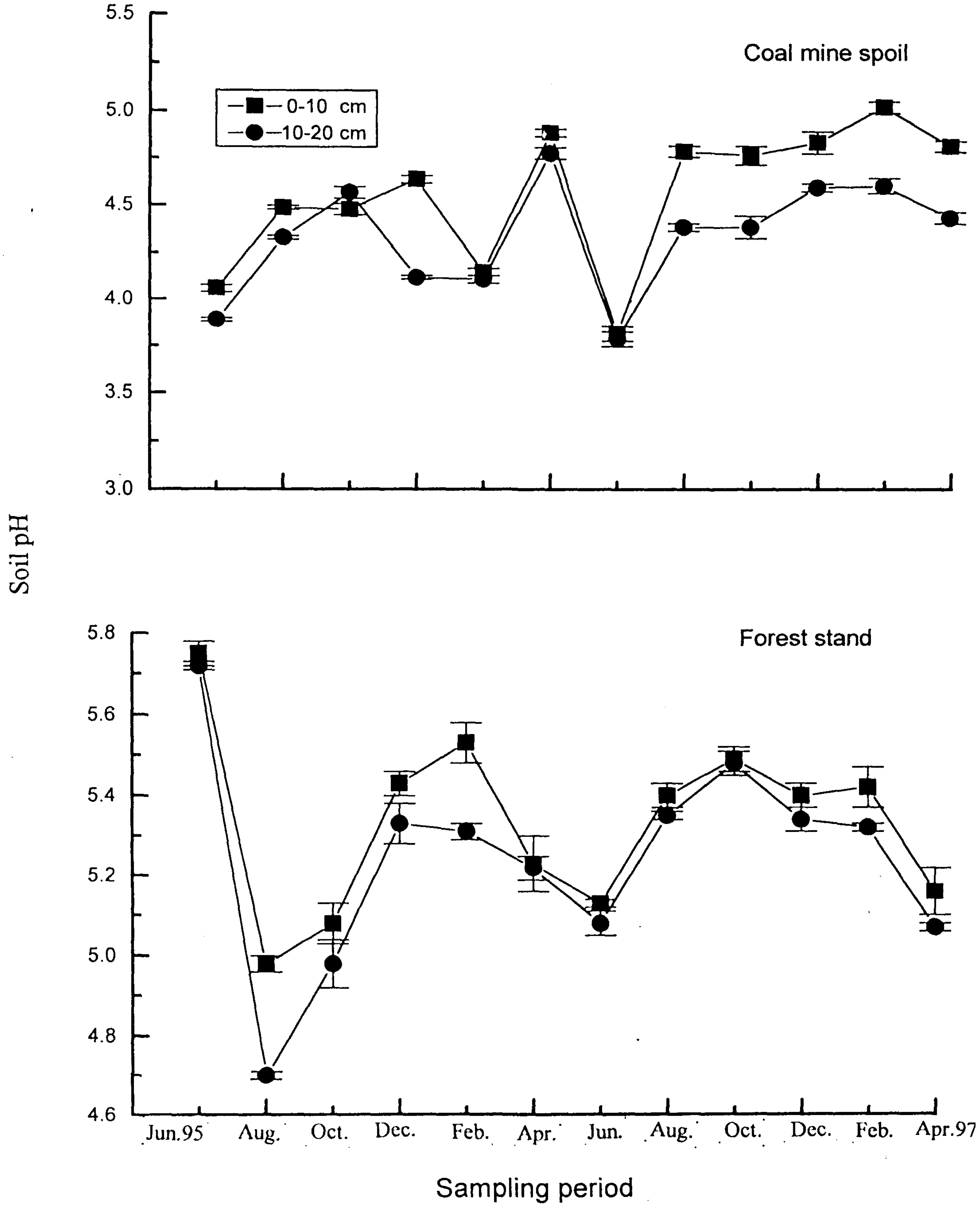

Fig. 6.1. Bimonthy variation of pH in coal mine spoil and the forest stand.

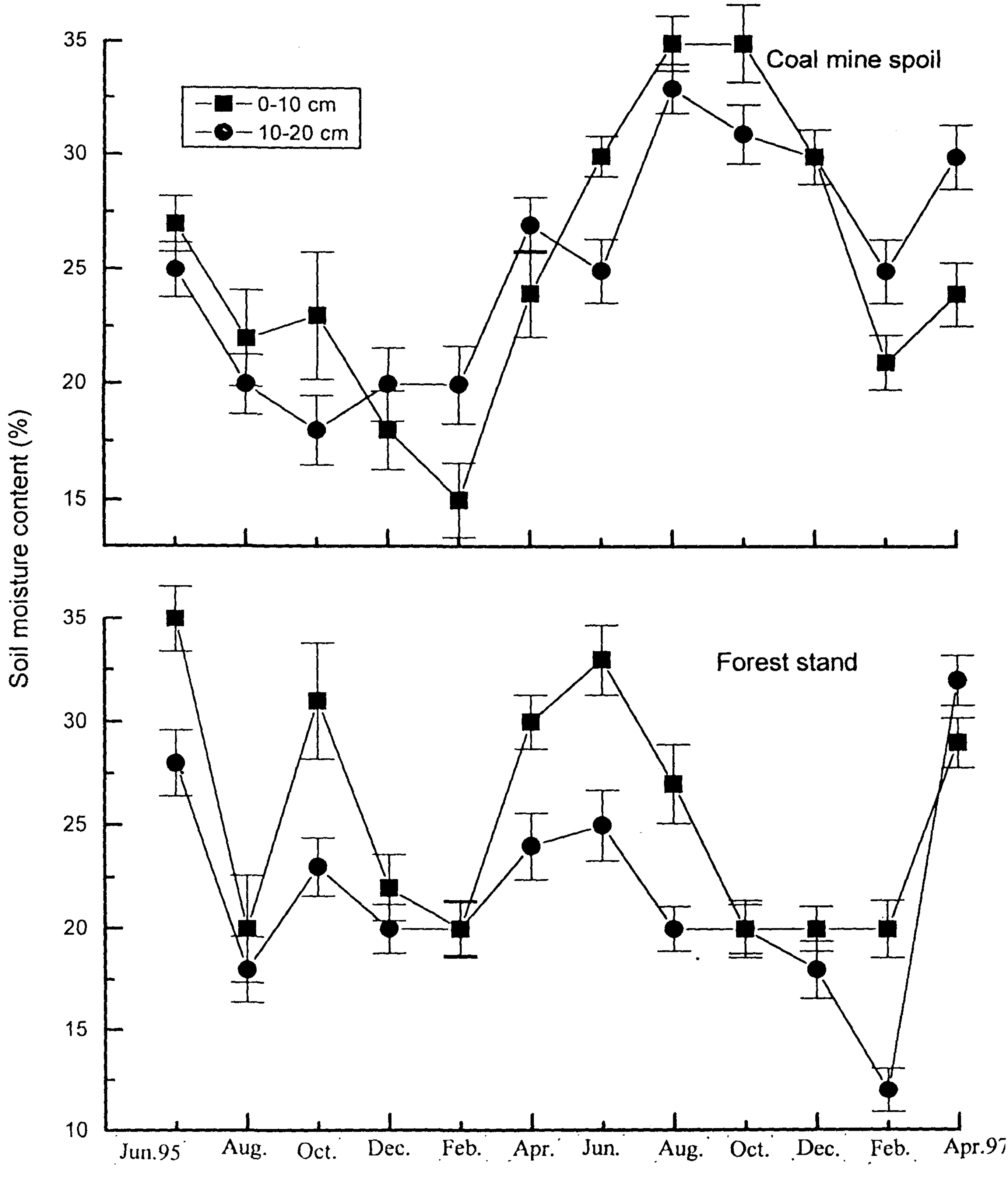

Fig. 6.2. Bimonthly variation in moisture content in the coal mine spoil and the forest stand

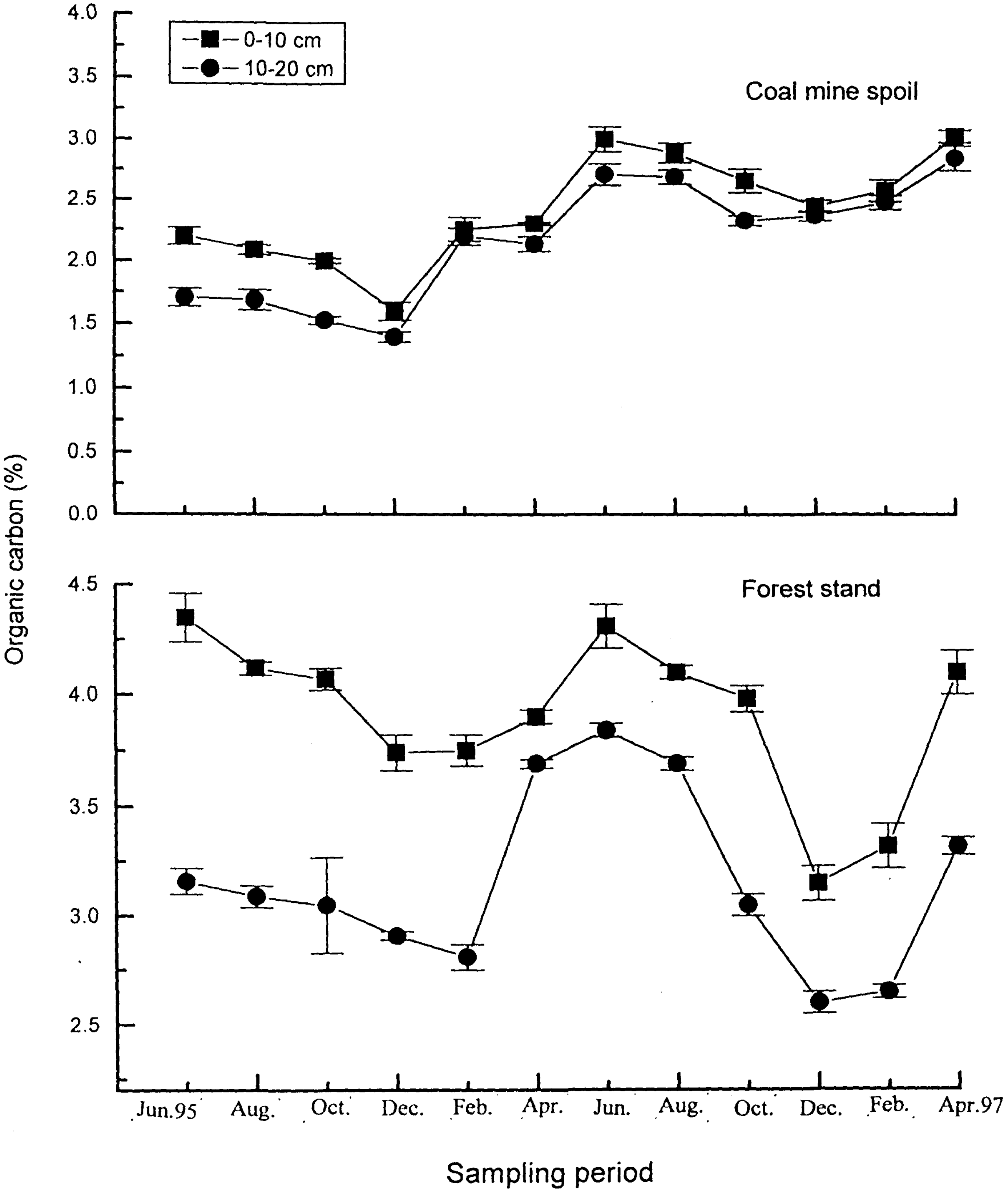

Fig. 6.3. Bimonthly variation in organic carbon in coal mine spoil and the forest stand.

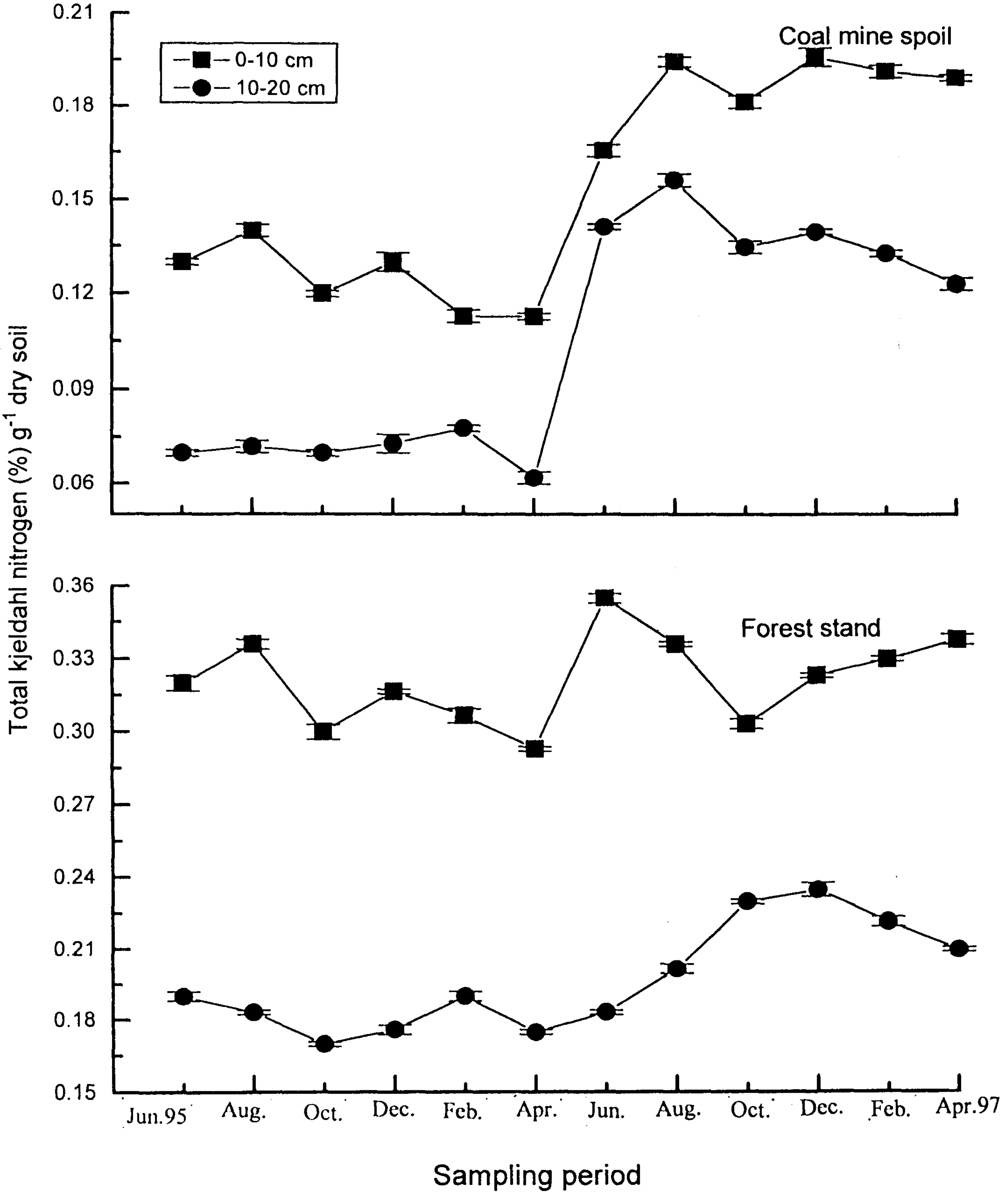

Fig. 6.4. Bimonthly variation in TKN in the coal mine spoil and the forest stand.

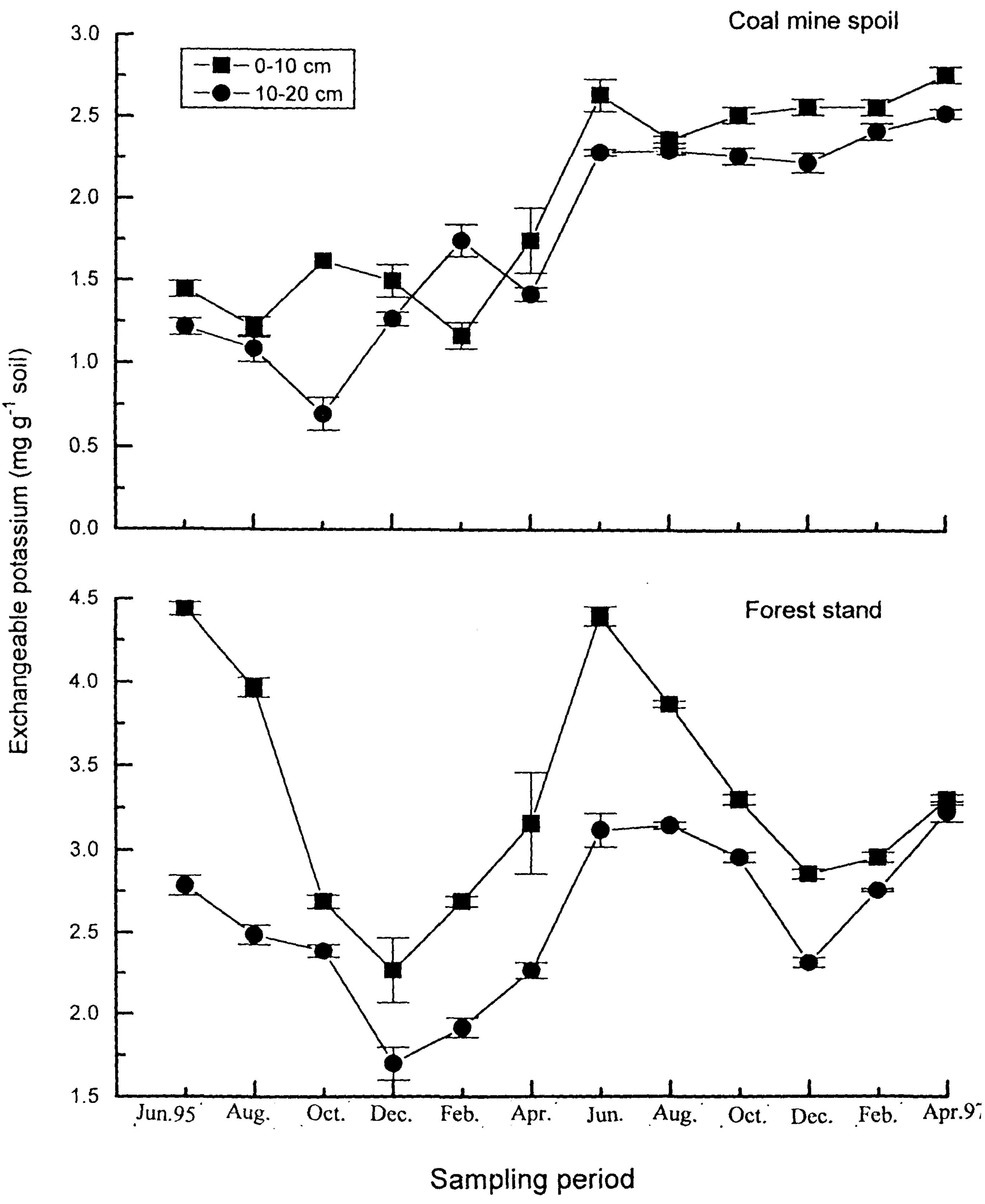

Fig. 6.5. Bimonthly variation in exchangeable potassium concentration in coal mine spoil and the forest stand.

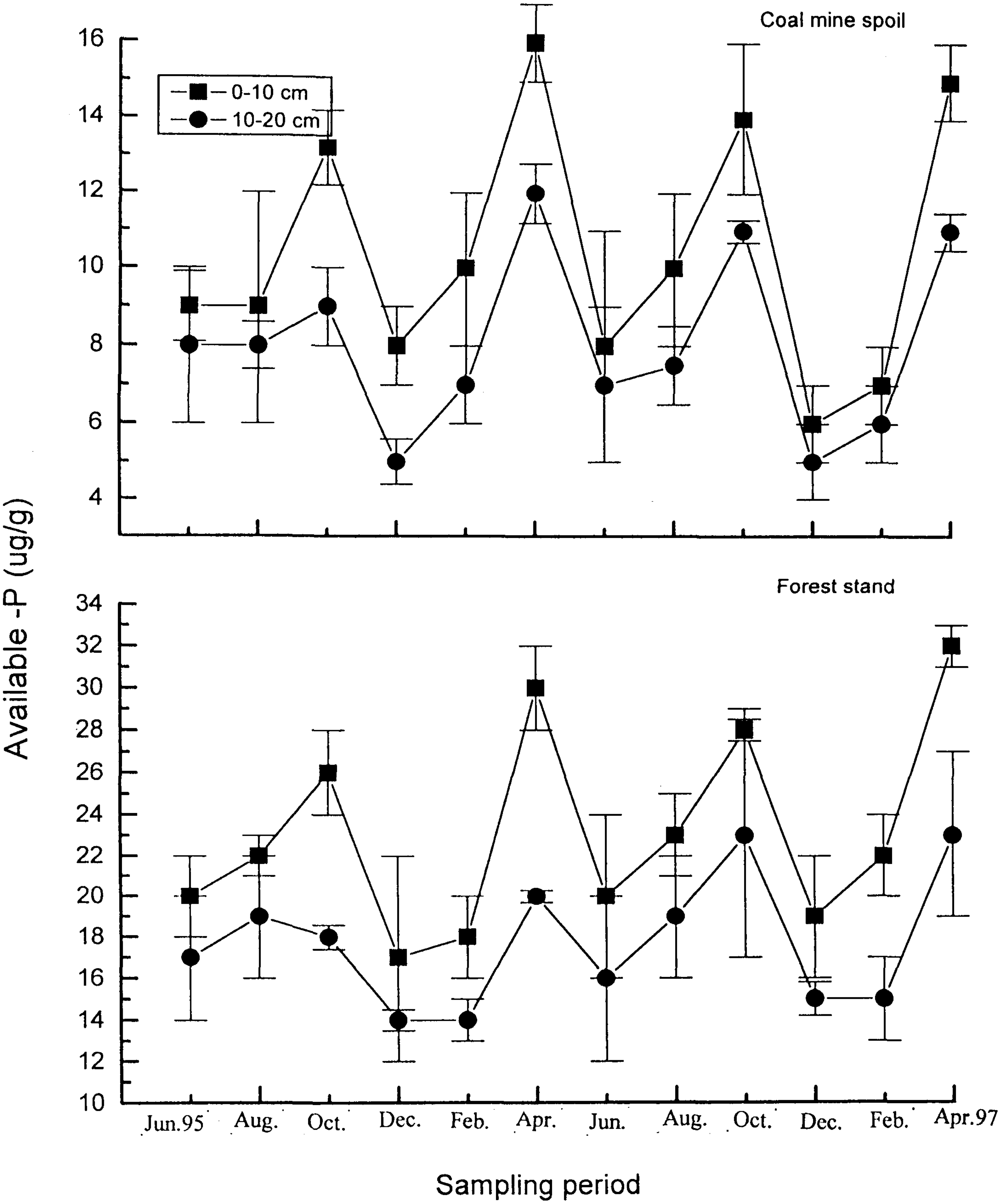

Fig. 6.6. Bimonthly variation in available-P in the coal mine spoil and the forest stand.

Table 6.2. Sulphur and Iron contents in the coal mine spoil and the forest stand.

Site	Depth (cm)	Sulphur (mg 100 g^{-1})	Iron (ppm)
Coal mine spoil	0-10	123.33 ±10.38	478.90±14.67
	10-20	117.50±7.98	471.50±19.18
Forest stand	0-10	51.60±4.32	382.20±18.23
	10-20	49.10±2.98	377.50±15.32

Discussion

The result revealed that physico-chemical properties of coal mine spoil varied widely with the forest stand and also with the seasons.The high acidic nature of coal mine spoils may be attributed to the oxidation of iron pyrites (Caruccio, 1975 and Johnson and Bradshaw, 1979). Forest stand had a much higher pH as compared to the coal mine spoil. Low pH is detrimental to plant growth. The availability of certain element such as Fe, Al and Mn to the plant increases as the soil pH decreases. Under such conditions these elements become toxic to plant (Berg and Vogel, 1973 and Inverson and Wali, 1992). The loss of finer particles , especially clay component increase the proportion of sand in the soil during early developmental stages after disturbance (Eyre, 1968). Maximum retention of moisture in the surface soil layer might be due to greater accumulation of litter on the forest floor (Maithani, 1996).

The high organic carbon content during rainy season in the forest stand may be attributed to the high microbial activity and the release of nutrients during decomposition (Deka, 1981 and Dkhar, 1983).The slight increase in organic matter content in coal mine spoil in the 2nd year of the study may be attributed to the increase in organic matter with the increase in age of the spoil due to the initiation of colonization of plant species and accumulation of litter from the surrounding vegetation. Down (1975) reported increase in organic matter concentration with the age of mine spoils at Somerset coal field. Total nitrogen was more or less of the same trend in all the seasons in forest stand which may be due to its undisturbed condition and the soil was not exposed due to thick canopy and it can retain moisture due to high accumulation of litter.Nitrogen deficiency in coal mine spoil could be due to its great susceptibility to leaching loses (Richardson and Dicker, 1972 and Gemmell, 1973). In coal mine spoil the TKN increased slightly during the second year of the study. With increasing age of the spoil there was a slight improvement in the nutrient status. Jencks *et al.* (1982) reported an increase in soil nitrogen concentration with age in coal mine spoils of west Virginia. Li and Daniels (1994) also reported increased N accumulation with age in Appalachian mine spoils. The increase in N concentration with age presumably because of its release during partial destruction of the silicate lattices by acid generated during spoil weathering (Reeder and Berg, 1977a). Phosphorus was low in coal mine spoils. Inverson and Wali (1992) reported that phosphorus was a limiting nutrient in mined spoils. It may also be due to the low content of inorganic P in the original material in mined spoils. In forest stand highest level of phosphorus was observed in April. Ahlgren and Ahlgren (1965) suggested that greater microbial activity released P at faster rate which may be the possible reason for increased P content during April.

The increase in soil organic matter content during the second year of the study may be due to increasing age of the spoils (Lyngdoh, 1995). Soil water content, temperature, acidity and substrate quality are the primary factors affecting the rate of organic matter decomposition in the wetlands (Clymo, 1984b and Hogg, 1993). There was also a relationship between organic matter content and litter accumulation in forest stand (Maithani, 1996). The declining trend of organic matter content with increasing soil depth may be attributed to the maximum microbial activity (Mishra, 1966 and Deka, 1981) and thick accumulation of litter on the forest floor and dense growth of fine roots in the surface soil layer (Babu John, 1998). Maximum retention of moisture in the surface soil layer might be due to greater accumulation of litter on the forest floor (Maithani, 1996) and higher soil organic matter content in this layer.

Potassium was also less in coal mine spoil as compared to forest stand. The high potassium value during rainy season in forest stand may be ascribed to the rapid release of nutrients from decomposing litter (Tukey *et al.*, 1958). In coal mine spoil potassium increased in the 2nd year of study which may be due to the increase concentration of organic matter. Marrs *et al.* (1980) reported an increase in soil potassium with the age of the spoil.

Jha (1990) reported that nitrogen, phosphorus and exchangeable potassium increase in concentration with increasing age of the spoil.Shankar *et al.* (1993)

also reported that increase in N, P and K cocncentration with spoil age.

Sulphur concentration was much higher in coal mine spoil than the forest stand. It may be due to acidic precipitation which represent a major input of sulphur adsorption (Likens and Borman, 1974). As the pH decreased sulphur adsorption potential increased accordingly. S levels increase due to oxidation of pyrite in coal mine spoil (Shankar *et al.*, 1993)

In the present investigation, Fe concentration was more in the coal mine spoil than in the forest stand. Wali and Freeman (1973) also reported a similar pattern. High iron concentration in coal mine spoil may be due to the high acidic condition of spoil. When the pH decreases, the concentration of Fe increases (Berg and Vogel, 1973 and Inverson and Wali, 1992).This may be due to greater accumulation of litter and availability of moisture in forest stand. The increased Fe concentration may be attributed to microbial activity (Opakunle, 1991). Anaerobic microbial respiration can be significant which reduces Fe^{3+} and Fe^{2+} leached from the soil (Heyes and Moore, 1992).

CHAPTER VII

THE EFFICIENCY OF MICROBES IN UPTAKE OF SULPHUR

Introduction

Coal mine spoils harbour extreme acidic environment due to the oxidation of iron pyrites (FeS_2). Pyrites when exposed to the atmosphere release sulphuric acid, besides contain toxic levels of certain soluble elements such as Fe, Al, Mn and Cu (Chadwick, 1973 and Caruccio, 1975). Kimber *et al.* (1978) and Wali and Freeman (1973) reported the presence of a greater quantity of metals like Bo, Cu, Fe, Li, Sr and Zn which could be toxic to plants while working on coal mine spoils of Scotland and North Dakota. Sulphur level also increased due to oxidation of pyrite in coal mine spoils (Shankar e*t al,*. 1993). Fungi and bacteria play a more important role in environmental sulphur oxidation (Wainwright, 1984a).

Soil microorganisms and soil microbial process become disrupted by elevated metal concentration, causing ecosystem disturbance (Giller *et al.*, 1998). Iron oxidising bacteria *Thiobacillus ferro-oxidans* reduced iron pyrites to $FeSo_4$ and H_2So_4 and cause extreme acidification (Giller *et al.*, 1998). Development of tolerance and shifts in microbial community structure could be expected to compensate for loss of more sensitive microbial population (Giller *et al.*, 1998). The ability of some fungi to survive and grow in such conditions has been reported by Ross (1975) and microorganisms may be used as toxicity indicators (Diaz-Baez and Rolder, 1996).

Materials and methods

Martin rose bengal broth was used for isolation of fungi from different concentration of coal samples i.e. 100%, 75%, 50% and 25%. Two fungi namely *Pencillium brevicompactum* and *Trichoderma koningii* isolated earlier from different concentraion of spoils samples were maintained on Martin Rose Bengal broth. Different concentration of sulphuric acid (H_2So_4) i.e. 5%, 10%, 15%, 20%, 30% ,35% and 40% were used to study the growth to these fungi and also to assess their ability in uptake of sulphate from the substrate. One millilitre of different concentrations of acid was mixed with 50 ml of Rose Bengal broth and the concentration of sulphate was analysed. Thereafter, fungal isolates i.e. *P. brevicompactum* and *T. koningii* were inoculated into liquid medium and incubated at 25^{0}C for 15 days to measure the growth. Then the fungal cultures were filtered through whatman filter paper No.42 and oven dried at 60^{0}C for 36 hours. The dry weight of fungi was measured. The filtrate was used to analyse the amount of sulphate.

Thiobacillus sp and *Bacillus* sp grown on nutrient broth were used to test the ability of bacteria for the uptake of sulphate isolated from coal sample earlier.

To test the ability of bacteria to tolerate high concentration of sulphate i.e. 10 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm of sodium sulphate (Na_2So_4) and Pyrite sulphur (FeS_2) were used. Ten millilitre of different concentration of FeS_2 and Na_2So_4 were mixed with nutrient broth separately and the initial concentration of sulphate was analysed. Thereafter bacteria i.e. *Thiobacillus* sp and *Bacillus* sp were inoculated into the nutrient broth and incubated at 30°C for 12 days. The amount of sulphate content was analysed. For determination of sulphate in substrate was done following the methods of Allen *et al.* (1974).

Results

From coal samples *Penicillium brevicompactum* and *Trichoderma koningii* were isolated (Table 7.1) .*P. brevicompactum* and *T. koningii* were used to study their growth in extreme acidic conditions. It was observed that at low concentration of acid causing slight pH change in the medium the growth of fungi was high but as the acidity increased, the growth of fungi reduced. In extreme acidic condition (pH1.2), it became toxic to the fungi (Table 7.2).

Dry mass of the fungal species was more at the low concentration of acid and as acidity increased the dry mass of fungi decreased (Table 7.2).

Sulphate content after 15 days of fungal growth was analysed. It was found that at low concentration of H_2So_4, the uptake of sulphate by fungi was high. Little amount of sulphate was left in the filtrate. When the acidic conditions were increased, the break down and uptake of sulphate were slow (Table 7.3).

Sulphate content was analysed after 12 days of bacterial growth. It was found that *Bacillus* sp. utilized the sulphate from the culture medium, whereas a contrasting finding was observed in case of *Thiobacillus* sp.where the concentration of sulphate was more than the initial amount of sulphate content in the medium (Table 7.4 and 7.5).

Discussion

It was observed that the bacterial growth was better than that of fungi in extreme acidic conditions. Both the *Thiobacillus* sp and *Bacillus* sp could grew chemotropically at pH 2.5. In natural condition, the association of *Penicillium brevicompactum, Trichoderma koningii, Thiobacillus* sp and *Bacillus* sp within the coal and the coal mine spoils indicated their sulphur oxidising capability and tolerance to the acidic conditions. The oxidation of pyrite sulphur (FeS_2) and Sodium sulphate was more by *Thiobacillus* sp than the *Bacillus* sp.indicating that the utilization of elemental sulphur by the earlier organisms was due to high bacterial cell adsorption than the later bacterium (Konishi *et al.*, 1995, Suzuki, 1974 and Ehrlich, 1990). More uptake of sulphate by oxidation of sulphuric acid at low concentration by *P. brevicompactum* and *T. koningii* suggested their adaptability to moderate acidic conditions, however, they could survive in extreme

Table 7.1. Isolation of fungi from coal samples

Concentration of coal (%)	Fungal species
100	*Penicillium brevicompactum*
75	*P. brevicompactum* and *Trichoderma koningii*
50	*P. brevicompactum* and *T. koningii*
25	*P. brevicompactum, T. koningii* and *T. viride*

Table 7.2. Dry mass (mg) of fungi in liquid medium supplemented with H_2So_4 (%) after 15 days

Con. of H_2So_4	Initiial pH	Initial weight	Dry mass		Dry mass	
			Penicillium brevicompactum	Final pH	*Trichoderma koningii*	Final pH
5	4.40	0.5	275.9	6.25	584.2	6.65
10	3.69	0.5	217.1	6.07	422.5	6.23
15	2.77	0.5	190.8	5.85	343.2	6.10
20	2.41	0.5	152.7	5.47	245.4	5.85
25	2.16	0.5	114.7	5.25	231.2	5.62
30	2.02	0.5	106.8	5.10	193.5	5.40
35	1.88	0.5	103.5	4.85	123.2	5.2
40	1.2	0.5	-	-	-	-

Table 7.3. Sulphate content in culture after 15 days of fungal growth

Conc.of H_2So_4	So_4^{-2} (mg $^{-1}$) in culture filterate			
	Penicillium brevicompactum	% uptake	*Trichoderma koningii*	% uptake
5	4	96	11.6	86
10	5.36	95.3	11.8	85.2
15	27.97	82.15	31.8	79.7
20	37.72	79.6	37.4	79.8
25	44.2	76.7	39.6	79.1
30	56.2	75	53.2	76.6
35	61.56	75	63.2	74.4

Table 7.4 . The sulphate content in culture containing Na_2So_4 after 12 days of bacterial growth

Na_2So_4 (ppm)	Initial pH	So_4^{2}-S (mg^{-1})					
		Bacillus	Uptake (%)	Final pH	*Thiobacillus*	Release (%)	Final pH
10	3.9	1.4	86	5.9	23	121	4.90
50	3.69	1.8	83	5.31	15.8	43	5.01
100	3.01	4.4	63.3	5.80	40.6	238	4.95
250	2.98	4.8	61.2	5.74	28.0	125	5.03
500	2.75	7.6	50.6	5.62	18.3	49	5.05
1000	2.15	10.6	47.5	5.50	10.2	18	5.00

Table 7.5. . The sulphate content in culture containing FeS_2 after 12 days of bacterial growth

FeS_2 (ppm)	Initial pH	So_4^{2}-S (mg^{-1})					
		Bacillus	uptake (%)	Final pH	*Thiobacillus*	Release (%)	Final pH
10	3.7	0.6	94.8	4.91	12.3	84.8	5.0
50	3.4	0.8	93.0	5.31	9.5	69.3	5.1
100	2.99	1.0	91.9	5.90	18.4	142.6	5.08
250	2.71	1.2	90.6	5.87	11.2	90.3	5.2
500	2.5	1.4	89.7	5.81	8.5	70.8	5.2
1000	2.01	1.8	87.5	5.75	7.4	63.7	5.21

acidic conditions but within low metabolic activities. A relationship was observed between the sulphate uptake and growth of bacterial cell and fungal mycelium . The volume of sulphur-liquid mixture, mass of microbial cell and mass of initial sulphur particle were important factors to regulate the growth of bacteria. However, oxidation rate and growth of different microbes varied. *P. brevicompactum* and *Thiobacillus sp.* had a better growth than *Bacillus* sp and *T. koningii* indicating their better ability to withstanding environmental stress. Survival of the of microbes in extreme acidic condition may also be influenced by the acid tolerance acquisition capacity of the organisms (Davis *et al.*, 1996). The oxidation capacity of the bacteria (*Thiobacillus* sp) can further be investigated for removal of metal toxicity from coals and metal recovery in mining (Norris andKelly, 1982). The preference of sulphate source over pyrite one by the bacteria was observed. Sulphate substrate preference for sulphur assimilation indicated that *Thiobacillus* sp was more efficient in oxidising the sulphate than pyrite source (Beil *et al.*, 1996). The differential sulphate reducing capacity of bacteria and fungi may further be used in bioprecipitation of toxic metals and reduce the toxicity (White and Gadd, 1996) and acidity. Thus leacheate of coal and spoils may be converted to less toxic compounds. The conversion of sulphate to sulphide simultaneously may be responsible for the raising pH of the substrate (White and Gadd, 1996). Analysis of bacteria and fungi with coal and spoils suggest their possible role in sulphur and iron cycling in subsurface (Ulrich *et al.*, 1998).

CHAPTER VIII

GENERAL DISCUSSION

Coal mine activities in Jaintia Hills, Meghalaya have resulted into the large areas that are devoid of vegetation and soil. Mining operations have also affected the population and activity of microorganisms. Microorganisms play a major role in mineral cycling and transformation of elements like carbon, phosphorus, nitrogen and potassium. Microbiological studies of these spoils have provided an ideal opportunity to understand the importance of microorganisms in stabilization of the coal mine spoils. They also serve an important role in the establishment of early colonization of plant species.

The results presented in the foregoing chapters showed that coal mine spoil affected microbial community adversely than forest floor. The coal mine spoils were extreme acidic and had low content of organic matter and other mineral nutrients which affected the growth and activity of microorganisms. Present findings were supported by other workers in different parts of the world (Chadwick, 1973, Caruccio, 1975, Johnson and Bradshaw, 1979, Jha and Singh, 1991 and Inverson and Wali, 1992).

Fungal population was high during spring season and low during rainy and winter. Bacterial population was maximum during rainy season which could be due to the favourable soil moisture and temperature during these periods.High bacterial population during rainy season and low population of fungi may be due to more competitive ability of the earlier in high moist conditions over the other ones and also the degradation of fungal hyphae by the bacteria (Cromack *et al.*, 1975). The population of bacteria was favoured by high soil moisture, temperature and organic matter content of the soil. The growth of microbes and their multiplication by moderate moisture content and temperature have been reported by Soderstrom (1979), Clarholm and Rosswall (1980), Baath and Soderstrom (1982), Lundgren and Soderstrom (1983), Schnurer *et al.* (1986) and Ohtonen and Markola (1991). Organic matter content influenced the microbial population. High population of microorganisms in forest stand as compared to coal mine spoils was due to high organic matter and other mineral nutrients in the forest stand (Lynch, 1981, Guidi *et al.*, 1988). The acidic condition of coal mine spoils reduced the population of microorganisms. Bacterial population was significantly ($P<0.05$) correlated with rainfall, temperature and raltive humidity in the surface soil layer (Table 8.1) In both forest soil and coal mine spoils, the microbial biomass-C was high during spring and rainy seasons and low during winter and autumn. The concentration of microbial biomass-C was affected by soil moisture content (Wardle and Parkinson, 1990). The temperature and moisture regimes influenced the microbial dynamics as was evidenced by the extremely low

Table 8.1 . Correlation co-efficient values (r) for various parameters in 0-10 cm soil layer in the coal mine spoil.

Source of variation	Fungal population	Bacterial population	Microbial Biomass-C	Soil respiration	Dehydrogenase	Urease	Phosphatase
Moisture content	NS	NS	0.553*	0.553*	0.839**	NS	NS
pH	NS	-0.659*	NS	NS	NS	-0.713**	NS
Nitrogen	NS	NS	0.559*	NS	NS	NS	NS
Phosphorus	NS	NS	NS	NS	NS	NS	NS
Potassium	NS	NS	NS	NS	NS	NS	NS
Rainfall	NS	0.686**	NS	NS	NS	NS	-0.553*
Temperature	NS	0.559*	NS	NS	NS	NS	-0.824**
Relative humidity	NS	0.558*	NS	NS	NS	NS	-0.616*
Dehydrogenase	NS	NS	NS				
Urease	NS	NS	NS				
Phosphatase	NS	NS	NS				
Soil respiration	NS	NS	NS				

** $P<0.01$

* $P<0.05$

microbial biomass during winter. Coal mine spoils contained very low concentration of microbial biomass which was related to low pH of soil condition (Wolters and Joergenson, 1991 and Ladd *et al.*, 1981) and also to low concentration of organic matter content (Schnurer *et al.*, 1985). A reduced metabolic efficiency was due to lower microbial biomass content at the acidic site (Anderson and Domsch, 1993). Decrease in microbial biomass due to increase in soil depth was attributed to the reduction in organic matter and other mineral nutrients, which also caused a reduced biochemical activity. Ross *et al.* (1982) reported the decline in biochemical activities with increase in soil depth.

Mine spoil revealed very low CO_2 evolution. This may be due to highly disturbing conditions of spoils which changed its physical condition and that may have influenced the dynamics of soil biological activity (Frazluebbers, 1995). CO_2 evolution was maximum during the rainy season and minimum during the winter season . This was attributed to the increased soil moisture and temperature which resulted in an increase in CO_2 evolution (Gupta and Singh, 1980, Kursar, 1989). Increased CO_2 evolution in forest soil as compared to the coal mine spoils may be due to its undisturbing condition and also due to high moisture and high organic matter content (Tiwari *et al.*, 1987). And it was also due to the high microbial population and thick accumulation of fine root which contributed to the CO_2 evolution in soil.The amount of CO_2 decline with soil depth may be due to the reduction in organic matter (Vardavakis, 1989). Soil respiration was significantly P<0.05) correlated with moisture content, nitrogen, phosphorus and potassium in the subsurface soil layer (Table 8.2).

Enzyme activity was higher in forest soil as compared to that in coal mine spoil. This was due to more nutrient content in litter and its high moisture retention ability stimulated the microbial activity in forest soil (Kauri, 1981). The soil nutrients were the most important factors likely to regulate the microbial activity (Wynn-Williams, 1982). In forest stand and coal mine spoil, dehydrogenase activity was maximum during rainy season and minimum during the winter season. It was correlated to high population of bacteria during rainy season. Dehydrogenase activity was also positively correlated with the moisture content and temperature of the soil (Ortem and Neuhaus, 1970). Bacterial population and soil moisture content are the main factors which determined dehydrogenase activity in soil (Tiwari *et al.*, 1987).

Low dehydrogenase activity in coal mine spoil may be due to the environmental factors affecting the microbial population and also due to low organic matter content. Low dehydrogenase activity in coal mine spoils could also be due to the heavy metal concentration (Reddy *et al.*, 1987). In forest stand and coal mine spoil urease activity was maximum during the rainy season and minimum during the winter season.It was correlated with the temperature,

Table 8.2 . Correlation co-efficient values (r) for various parameters in 10-20 cm soil layer in the coal mine spoil.

Source of variation	Fungal population	Bacterial population	Microbial Biomass-C	Soil respiration	Dehydrogenase	Urease	Phosphatase
Moisture content	NS	NS	NS	0.793**	0.768**	-0.834**	NS
pH	NS	-0.742**	NS	NS	0.553*	NS	NS
Nitrogen	NS	NS	NS	0.553*	NS	NS	NS
Phosphorus	NS	NS	NS	0.631*	NS	NS	NS
Potassium	NS	NS	0.609*	0.553*	NS	NS	NS
Rainfall	NS	NS	NS	NS	NS	NS	-0.553*
Temperature	NS	NS	NS	NS	NS	NS	-0.729**
Relative humidity	0.553*	NS	NS	NS	NS	NS	-0.622*
Dehydrogenase	NS	-0.649*	NS				
Urease	NS	-0.652*	NS				
Phosphatase	NS	NS	NS				
Soil respiration	NS	NS	NS				

** $P<0.01$
* $P<0.05$

moisture content and the concentration of organic carbon in soil (Dalal, 1975, Spier, 1977, Beri et al., 1978, Dkhar and Mishra, 1983, Otoole et al., 1985 and Rao *et al.*, 1995). Due to low temperature and moisture stress during winter, low urease activity was observed (Ross *et al.,* 1984). High urease activity during the rainy season was due to high concentration of organic carbon, bacterial population and high moisture (Dalal, 1975, Speir, 1977, Beri *et al.*, 1978, Dkhar and Mishra, 1983 and O'Toole *et al.*, 1985). Low activity of urease was observed in coal mine spoils which was due to extreme low pH and low organic matter content of the spoil . These soil condition did not favour the growth of the microorganisms and caused differences in the enzymatic activity in the soils (Palma and Conti, 1990).

Low phosphatase activity in coal mine spoil was also due to low organic matter content in the spoil. Enzyme activities increased with increase in organic matter content (Sarathchandra and Perroth, 1981).

The low microbial activity in the coal mine spoils was attributed to the very low concentration of organic matter and mineral nutrients particularly phosphorus. Lyngdoh (1995) also observed that phosphorus was a limiting nutrient in mine spoils. Greater microbial activity in forest soil might be due to high organic matter and the release of phosphorus making it available for the uptake by plants (Ahlgren and Ahlgren, 1965).

Low organic matter content in coal mine spoils may be due to the absence of litter in the spoil. The high accumulation of litter in the forest soil contributed to the high concentration of organic matter thereby enhancing the growth of microorganisms by providing energy locked in different carbon molecules.

The concentration of total nitrogen, organic carbon and potassium were low in the coal mine spoils and it was found that these elements increased slightly in the second year of the study. This may be attributed to the increased age of the spoils which improved the nutrient status (Lyngdoh, 1995). Accumulation of N, P, and K was also probably related to the soil reaction, water holding capacity and soil moisture content (Lyngdoh, 1995). The low concentration of these elements may be due to the low clay content in coal mine spoils.

Fe concentration was more in coal mine spoils due to lateritic soil and presence of pyrite in coal. The absence of litter worked an additional factor to the low availability of moisture to microbes. Fe concentration in the litter layer might be attributed to microbial activity (O Pakunle, 1991). Increased soil moisture in soil favoured the solublization of ferrous ions and allowed the diffusion of Fe. Low concentration of Fe may also be due to high sand content (McLaughlin *et al.*, 1994).

High concentration of sulphur was observed in coal mine spoils. It may be due to acidic precipitation which represent a major input of sulphate (Likens and Borman, 1974). Studies on microbial population and the biomass, seasonal activity of dehydrogenase, urease , phosphatase, CO_2 evolution , total nitrogen, organic carbon, phosphorus, potassium, sulphur and iron revealed that the surface soil layer (0-10 cm) had more population, high activity, high concentration of different elements than the sub-surface soil layer (10-20 cm). Maximum biological activities takes place on the surface soil layer due to favourable temperature, aeration and moisture content (Appiah and Thomas, 1982, Ortem and Neuhaus, 1970). Besides accumulation of litter on the soil surface and the high concentration of fine roots in the surface soil layer contributed to the high concentration of nutrients in the surface soil layer (Babu John, 1998).

In natural condition, the association of *Penicillium brevicompactum, Trichoderma koningii, Thiobacillus* sp and *Bacillus* sp with coal spoils indicated their sulphur oxidizing capability an tolerance to acidic condition. The oxidation of pyrite sulphur (FeS_2) and sodium sulphate (Na_2So_4) was more by *Thiobacillus* sp than the *Bacillus* sp which indicate that the utilization of elemental sulphur by the earlier organisms was due to high bacterial cell adsorption than the later bacterium (Konishi et al., 1995, Suzuki, 1974 and Ehrlick, 1990). More uptake of sulphate by oxidation of sulphuric acid at low concentration by *P. brevicompatum* and *T. koningii* suggested their adaptability to moderate acidic condition, however, they could survive in extreme acidic conditions but with low metabolic activities. Survival and growth of microbes in extreme acidic condition may also influenced by the acidic tolerance acquisition capacity of the organism (Davis et al., 1996).

The present investigation highlights several microbiological changes associated with coal mining activity in the Jaintia Hills of Meghalaya. Microbial community, microbial biomass and activity have provided a greater insight on the importance of microorganisms in improving the condition of the soil. Daft *et al.* (1975) reported that microbial biomass in such stresses and degraded systems play a significant role in turnover of organic matter and cycling of nutrients which helped in establishment of pioneer plants on coal mine wastes in Scotland and Pennsylvania. It is hoped that the present investigation provides an important information for improving the degraded land with the help of microorganisms in the coal mine spoils of Jaintia Hills, Meghalaya.

CHAPTER IX

SUMMARY

The present investigation deals with the bimonthly assessment of microbial community, microbial biomass their activities and values of CO_2 evolution from coal mine spoils and unmined forest stand. It also deals with the role of microbes in regeneration of nutrients and the efficiency of some fungi such as *Penicillium brevicompactum* and *Trichoderma koningii* and bacteria such as *Bacillus* sp and *Thiobacillus sp.* in breaking down of sulphates to remove coal toxicity were also investigated.

The experimental sites were situated in Bapung latitude $25^0 24'35''$ N, longitude $92^0 18'30''$ E, altitude 1300 m asl of Jaintia Hills district in Meghalaya. The average annual rainfall was 2500 mm distributed over seven months of the year. The average maximum and minimum temperature was 24.8^0C and 11.6^0C respectively and the relative humidity was maximum during rainy season and minimum during winter season. Mine spoils of about three years old and unmined forest stand were selected for the present investigation. Soil samples were collected from two soil depths (0-10 cm and 10-20 cm). The soils were sandy in nature in both the sites. However, the clay content was higher in the forest.

Fungal population showed high value in the forest stand. Th efungal population varied significantly ($P<0.01$) between the sampling period in both the coal mine spoil and the forest stand. In both the sites fungal population was high during April and low during June, August and December. The fungal population at both the sites was greater in the surface soil layer than the sub-surface soil layer. In coal mine spoil, the dominant species in the surface layer were *Pythium intermedium* and *Penicillium* sp and in the sub-surface layer in addition to these *Trichoderma viride* was also another dominant fungi. In the forest stand *Pythium intermedium* was the dominant species at both the depths.

There was a difference in the composition of the fungal flora at both the sites. *Mamaria* sp., *Monocillium* sp, *Paeceolomyces carneus* and *Scopulariopsis brevicaulis* could be found in coal mine spoil whereas *Acremorium murorum*, *Cunninghamella elegans*, *Gliomastrx murorum*, *Torulomyces* sp and *Verticillium tennerum* could be found in forest soil.

Bacterial population was higher than the fungal population in both the coal mine spoil and forest stand. In both the sites, population was maximum during June whereas in the other months the population showed declining trend. It showed a high value in the forest stand. In forest soil *Pseudomonas* sp, and *Bacillus* sp were the dominant species whereas, *Thiobacillus* sp, *Micrococcus* sp were the dominant ones in the coal mine spoil.

Microbial biomass-C was significantly higher in the forest stand ($P<0.05$).

It also varied significantly ($P<0.01$) between the sampling periods and decline singnificantly ($P<0.01$) from the surface to the sub-surface soil layer in both the cases. A high values was observed during April and August and a low value during December in the 1st year of study and in the 2nd year it showed a high value during April, June and August and low value during October.

CO_2 evolution was much higher in the forest stand as compared to the coal mine spoil. It varied significantly between the sampling period ($P<0.01$) in both the cases. High amount of CO_2 evolved during June and August and low during December and February. The amount of CO_2 declined from the surface to the sub-surface soil layer in both the sites.

Dehydrogenase activity varied widely between the sampling periods. Forest stand had a significantly higer dehydrogenase activity than the coal mine spoil ($P<0.01$). At both the sites, dehydrogenase activity was high during June and August (rainy) and low during December and February (winter) and the upper soil layer had higher dehydrogenase activity than the lower soil layer.

High urease activity was observed in forest soil as compared to that of mine spoil. In both the cases, urease activity varied significantly between the sampling periods ($P<0.05$). High urease activity was observed during June and August (rainy) and low during December and February (winter). The activity declined from the surface to the sub-surface soil layer.

Phosphatase activity showed higher value in the forest stand as compared to that of coal mine spoil. In both, the forest stand and the coal mine spoil, phosphatase activity varied significantly between the sampling periods ($P<0.05$). It showed high value during April (spring) and low during June and August (rainy) and December and February (winter). Phosphatase activity decline significantly from the surface to the sub-surface soil layer ($P<0.05$).

Physico-chemical properties

Texture was sandy in both the coal mine spoil and the forest stand. However, the clay content was higher in the forest stand. The soil was acidic in both the sites, however, in the coal mine spoil, pH was more acidic in nature than that in the forest stand. There was a declining trend of pH from the surface to the sub-surface layer in both the cases. Soil moisture was more in the forest stand as compared to that of coal mine spoil.

Organic carbon content, total Kjeldahl nitrogen, available phosphorus and exchangeable potassium were higher in the forest stand as compared to that of coal mine spoil. In forest stand, high value was observed during June. In coal mine spoil, low value was observed during the 1st year of the study whereas in the 2nd year of study there was slight increase in concentration of organic carbon, TKN, available phosphorus and exchangeable potassium. The concentrations of sulphur and iron were higher in the coal mine spoil than the forest stand, whereas in the coal mine spoil the concentration of phosphorus and nitrogen were low than that of forest stand.

It was observed that the bacterial growth was better than that of fungi in extreme acidic conditions. Both the *Thiobacillus* sp and *Bacillus* sp could grow chemotropically at pH 2.5. In natural condition, the association of *Penicillium brevicompactum, Trichoderma koningii, Thiobacillus* sp and *Bacillus* sp within coal and the coal mine spoils indicated their sulphur oxidising capability and tolerance to the acidic conditions. The oxidation of pyrite sulphur (FeS_2) and Sodium sulphate was more by *Thiobacillus* sp than the *Bacillus* sp. indicating that the utilization of elemental sulphur by the earlier organisms was due to high bacterial cell adsorption than the later bacterium. More uptake of sulphate by oxidation of sulphuric acid at low concentration by *P. brevicompactum* and *T. koningii* suggested their adaptability to moderate acidic conditions, however, they could survive in extreme acidic conditions but within low metabolic activities. A relatioship between the sulphate uptake and growth of bacterial cell and fungal mycelium. The volume of sulphur-liquid mixture, mass of microbial cell and mass of initial sulphur particle were important factors to regulate the growth of bacteria. However, oxidation rate and growth of different microbes varied. *P. brevicompactum* and *Thiobacillus sp.* had better growth than others indicating their better ability of withstanding environmental stress. Survival of microbes in extreme acidic condition may also be influenced by the acid tolerance acquisition capacity of the organisms. The preference of sulphate source over pyrite one by the bacteria was observed. Sulphate substrate preference for S assimilation indicated that *Thiobacillus* sp was more efficient in oxidising the sulphate than pyrite source . The oxidation capacity of the bacteria (*Thiobacillus* sp) can further be investigated for removal of metal toxicity from coals and metal recovery in mining . The differential sulphate reducing capacity of bacteria and fungi may further be used in bioprecipitation of toxic metals and reduce the toxicity and acidity. Thus leachets of coal and spoils may be converted to less toxic compounds. The conversion of sulphate to sulphide simultaneously may be responsible for the raising pH of the substrate. Analysis of bacteria and fungi with coal and survive spoils suggest their possible role in sulphur and iron cycling in subsurface.

In present investigation revealed that coal mining activity in Jaintia Hills of Meghalaya generates huge ecological disturbance and negative environmental impacts due to deforestation for exploitation of coal . Coal mine spoil represented rigorous condition for both the microbial community and microbial activity and as such , only those species which can tolerate the extreme condition can colonise the mine spoil. The study was also revealed taht *Penicillium brevicompactum* and *Thiobacillus* sp had better growth than other inidicating their ability of withstanding environmental stress. The ability of bacteria and fungi in reducing the toxicity and acidity may help in improving the poor nutrient condition of coal mine spoil of Jaintia hills district of Meghalaya. Species which could tolerate the extreme acidic conditions colonised the mine spoil. From the present study it was observed that fungi such as *Penicillium brevicompactum* and T*richoderma koningii* and bacteria such as *Thiobacillus* sp and *Bacillus* sp are tolerant to the extreme condition of mine spoil.

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