ECOLOGICAL STUDIES ON THE EFFECT OF AIR POLLUTION ON THE LEAF SURFACE MICROORGANISMS AND LITTER DECOMPOSITION

By

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CERTIFICATE

We certify that the thesis entitled **ECOLOGICAL STUDIES ON THE EFFECT OF AIR POLLUTION ON THE LEAF SURFACE MICRO-ORGANISMS AND LITTER DECOMPOSITION**, submitted by Mr. Santa Ram Joshi for the degree of **Doctor of Philosophy** of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under our supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the **Ph.D. degree**. This work has not been submitted for any degree of any other university.

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GENERAL INTRODUCTION

North-eastern region of India is undergoing industrial development at a faster rate. Roads form the main system of communication owing to the hilly topography of the region. Automobiles discharge a number of gaseous and trace metal contaminants due to incomplete combustion of petroleum fuels. Certain other human activities like stone grinding, road construction, sand milling etc. also increase the atmospheric dust and heavy metal contaminant level. The dust and contaminants ultimately get settled on the leaf surfaces of roadside trees and soils. Such activities may affect the composition and activity of microorganisms in soil environment and change the rates of litter mineralization and immobilization near highways.

Pollution and leaf surface microbes:

The term 'phyllosphere' was introduced by Last (1955a, b) and Ruinen (1961) and subsequently used as 'phylloplane' by Dickinson (1965) for the study of leaf surface microflora. It has attracted the attention of several workers (Lamb and

Brown, 1970; Sinha, 1971; Dickinson, 1971, 1976; Mishra and Srivastava, 1971, 1974; Mishra and Tewari, 1976b; Sharma and Mukherji, 1976; Gupta and Dixit, 1983; Cabral, 1985; Vardavakis, 1988; Elsharouny, 1988; Legault et al., 1989; Kinkel et al., 1989; Adhikari, 1990). Most of these studies deal with the structural analysis of microbial communities and not much attention has been paid to study the microbial communities with reference to air pollution and metal contaminants (Gingell et al., 1976; Smith, 1977; Smith et al., 1978, Bewley, 1980; Bewley and Campbell, 1980; Mowll and Gadd, 1985; Fenn et al., 1989; Helander and Rantio-Lehtimaki, 1990; Magan and McLeod, 1991).

Leaf surface holds a fascination especially for the microbial ecologist because of its complex nutrient spectrum which supports a rich microflora. The qualitative and quantitative analysis of the epiphytic micro-organisms on leaf surface as well as their activities impose an important problem concerning the inter-relationships between plants, micro-organisms and the environmental conditions.

Leaf surface harbours a definite microbial community by virtue of the presence of leachates (Godfrey, 1976; Irvine et al., 1978). The different microbial communities consisting of mycelial fungi, yeasts, bacteria, actinomycetes and algae in such a community interact for space and nutrients and may produce secondary metabolites and antibiotics (Fokkema, 1973; Hudson, 1978; Dickinson et al., 1975). Earlier workers (Last, 1955a; Ruinen, 1956, 1961; Dickinson 1965, 1967) studied the microflora of different plant species without laying much emphasis on the mutual interaction of the microorganisms associated with leaf surfaces and the changing environments.

Phylloplane microorganisms come into contact with a variety of gaseous and particulate air pollutants which may be deposited on the leaf surfaces. Disruption of phylloplane microbial communities by air pollutants could have a negative impact on biological control of pathogens which are less sensitive to air pollutants than antagonists (Hibben and Taylor, 1975). However, considerable interest has been generated recently in investigating the leaf surface microorganisms tolerant to the heavy metals and gaseous pollutants in roadside environments (Gingell, 1976; Smith, 1977; Bewley, 1980; Bewley and Campbell, 1980; Mowll and Gadd, 1985; Magan and McLeod, 1988; Fenn et al., 1989; Singh and Rai, 1989; Dean-Ross and Mills, 1989; Mansfield et al., 1991).

The relationship between air pollutants and microorganisms is an important area of research to investigate their ecological balance where microbes complete partial or whole life cycle in such vulnerable environment (Saunders, 1971, 1973; Heagle, 1973; Babich and Stotzky, 1974; Manning, 1975; Smith, 1976a). The leaves and twigs of urban and roadside plants are superficially contaminated with numerous trace metals (Smith, 1971, 1972, 1973, 1976b) and several of these are essential or toxic to microbes under certain conditions

(Ross, 1975). Such conditions make it appropriate to examine the effects of particulate metal contaminants on plant surface fungi.

The physical and chemical properties of the phylloplane, which are characteristics of a plant species (Hallam and Juniper, 1971) and can be modified by environmental factors (Ruinen, 1961; Tukey, 1971; Baker, 1974) determine the distribution and composition of the resident microflora. Some gaseous and particulate atmospheric pollutants may therefore alter the phylloplane microflora by eliminating sensitive species (Saunders, 1971).

Little work has been done on the effects of vehicular air pollution on phyllosphere microflora in urban environments, despite the considerable deposition of metal from petrol combustion (Smith, 1976b, Mowll and Gadd, 1985). Besides, heavy metals, several other contaminants are deposited on the plant surfaces of roadside trees and soils. Most of these pollutants are of anthropogenic origin generated from automobile exhaust, fossil fuel combustion, road construction, stone grinding, sand milling and other technological activities. The increasing number of vehicles running on diesel oil and incomplete combustion of fuel on account of their defective engines produce excessive exhausts containing tar particles and other elements which may be injurious to plant life and microorganisms.

Pollution, microbial succession and litter degradation:

Decomposition of litter is one of the most important phenomenon in the ecosystem. It is a process in which dead plant materials are biologically degraded from a stage where it is attached to the living plant upto the formation of humus. Forest litter is an unique component of the forest biogeocoenosis and is one of the major indicators of energy transfer. The biological degradation of plant litter represents one of the essential links in the natural process of carbon circulation (Vladimir, 1970). To sustain the integrity of an ecosystem, hormonious transfer of matter and energy among the primary producers, consumers and decomposers is necessary. On soils of low nutrient status, the role of microorganisms in the decomposition of plant material is of immense importance where the litter on the soil surface acts as an input-output system in the nutrition of woodlands and plants rely to a great extent, upon the recycling of nutrients.

The litter which falls seasonally or continuously is added to the soil through leaffall and death of plants. The leaf litter in deciduous forest ecosystem constitute about 70% of the litter on the soil surface (Jensen, 1974).

The pattern of leaffall and subsequent nutrient release are important determinants to understand the functioning of forest ecosystem. In forest ecosystem, the bulk of the above ground annual net productivity is transformed directly to the decomposer subsystem via litterfall (Swift *et al.*, 1979; Seastedt and Crossley, 1987; Crawford *et al.*, 1990).

Biological decomposition is influenced by different environmental conditions like climate (Shukla and Singh, 1984), soil microbes (Witkamp, 1963), litter moisture and pH (Khasttriya, 1990; Griffith and Boddy, 1991). Besides, various pollutants generated by the industries and vehicles may reduce decomposer populations and decomposition rates (Ruhling and Tyler, 1973; Jordan and Lechevalier, 1975; Doelman and Haanstra, 1979; Freedman and Hutchinson, 1980; Khan and Frankland, 1984; Minnich and McBride, 1986; Neuvonen and Suomela, 1990). These factors may affect the colonization of microbes, their activity and mechanisms of degradation, besides the nature of the products formed. Obviously, any influence of these factors during decomposition affects the cycling of nutrients. Besides, being dependent upon the immobilization of nutrients by microbes (Gosz *et al.*, 1973; Swift *et al.*, 1979; Singh *et al.*, 1989; Shukla *et al.*, 1990; Suberkropp, 1991), the rate of decomposition depends upon the quality and quantity of organic constituents of litter (Christensen, 1986), age of litter and the nutritional requirements of the heterotrophs.

Nitrogen and lignin are the major substrates controlling the rate of decomposition (Berg and Wessen, 1984). The nitrogen rich litter decomposes at a faster rate than the lignin rich one. Nitrogen is retained in the litter to a certain concentration and thereafter is released at the same rate as organic matter loss, while the elements in non-limiting concentrations are released throughout the decomposition phase (Berg and Staff, 1981).

The readily degradable or soluble compounds, insoluble polymer carbohydrates and insoluble phenolic compounds mostly lignins constitute the chemical constituents of fresh litter. Plant biomass is primarily cellulose (30-40%), hemicellulose (20-30%) and lignin (5-10%) (Ladisch et al., 1983).

Various saprophytic groups of microbes are involved directly or indirectly in the transformation of carbon, nitrogen, phosphorus, potassium and other elements, locked up in the organic matter. Though a large number of organisms are involved in decomposition process, fungi are the most active decomposers of the plant litters. The quality and quantity of microbes depend upon the nature of soil, plant vegetation and various environmental factors notably temperature, moisture (Rai, 1973; Jensen, 1974), aeration (Mikola, 1954; Witkamp, 1966) and soil pH (Baath et al., 1980; Berg, 1986). The marked toxicity of pollutants and trace contaminants like heavy metals severely affects litter decomposition processes (Jordan and Lechevalier, 1975; Watson et al., 1976; Strojan, 1978) which lead to appreciate litter accumulation, suggesting a major reduction in nutrient cycling in contaminated sites. The difference in the diversity of litter colonizers reflects the potential difference in the rate of decomposition (Hutchinson and King, 1989).

Pollution and microbial enzymes:

The biodegradation of litter is accomplished by the microbial enzymes complexes. The primary colonisers produce enzyme(s) necessary for the hydrolysis of polysaccharides localised at the micro-substrate interface. Organisms capable of producing required enzymes become dominant (Reese, 1968). Different enzymes are required for the hydrolysis of various substrates due to their various linkage types. The polysaccharases are of two general types, i.e. endopolysaccharases and exopolysaccharases. The endopolysaccharases are random acting enzymes hydrolysing the higher molecular weight long-chained polymeric carbohydrates. The exopolysaccharases act only after endo-enzymes bring about a considerable increase in the polymer chains. They act upon the non-reducing terminal units of the chain and one sugar unit is removed at a time. Like endo-enzymes, exoenzymes are also substrate specific (Reese, 1968). Other enzymes which are widely distributed are invertase and amylase (Skujins, 1978), involved in the hydrolysis of carbohydrates as a part of organic matter mineralization (Skujins, 1967; Hankin et al., 1976; Spalding, 1977). Effect of gaseous and trace metal contaminants on microbial growth and decomposition process may be the result of reduced microbial enzyme complexes.

The cycling of minerals, production of hydrolytic enzymes and activity of microbes on the leaf surface to a great extent may be influenced by host and climatic factors. Influence in the normal functioning of the microbes due to changed environment will affect the normal ecosystem functioning.

The fast industrial development coupled with urbanization has encouraged vehicular transport system in north-east India. The increased discharge of unburnt fuel and heavy metals in the atmosphere by vehicles and other human activities provide an opportunity to investigate the effect of vehicular pollution on leaf surface microbes, microbial litter decomposition and enzymes activities in decomposing litters. Most of the works on the effect of pollutants on phylloplane microbes and decomposition processes are carried out in temperate environments but information on colonization of leaf surface by microbes and the microbial decomposition of litter in polluted areas in sub-tropical and tropical conditions are scanty.

The present investigation was, therefore, undertaken to understand the population dynamics of leaf surface microflora of roadside trees and the microbial and biochemical aspects of litter decomposition as affected by vehicular pollution. The approach to deal with the problem was as follows:

- Bimonthly study of population dynamics of leaf surface microbes in polluted (roadside forest strip) and unpolluted (non-roadside forest) conditions.
- Succession of fungi on different leaf litters (alder and

pine) at polluted and unpolluted sites.

- Microbial decomposition of the tree leaf litters under field conditions at polluted and unpolluted sites.
- Microbial activities on litter in terms of their different enzymes viz; cellulase, amylase and invertase at two sites.
- Effect of certain pollutants on litter decomposition by dominant fungi and on microbial enzymes related to degradation of leaf litters *in vitro*.