EFFECT OF HEAVY METALS ON THE STRUCTURE AND FUNCTION OF ECTOMYCORRHIZAE OF PINE

(Pinus kesiya Royle Ex. Gordon)

By

TALIJUNGLA

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DEDICATED
TO
MY PARENTS

NORTH-EASTERN HILL UNIVERSITY
SHILLONG

CERTIFICATE

I, Talijungla, hereby, declare that the subject matter of the thesis entitled "Effect of heavy metals on the structure and function of ectomycorrhizae of pine (*Pinus kesiya* Royle Ex Gordon)" 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.

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

17/3 — (Head of the Department)

Dkhar 16.3.99 — (Joint Supervisor)

G. D. Sharma — (Supervisor)

16.3.99 — (Candidate)

Head
Department of Botany
School of Life Science
N. E. H. U.
Shillong-793022

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GENERAL INTRODUCTION

General Introduction

Most of the world's terresterial ecosystems are dominated by plants that require association with mycorrhizal fungi to achieve optimum productivity. Such mutualistic, symbiotic association has been recognised for more than a century. Recently, methods have been developed to employ them in 1. forestry and in agriculture, of the several types of mycorrhizae. Ectomycorrhizae are characteristics of woody perennials in families such as Betulaceae, Fagaceae, Dipterocarpaceae, Myrtaceae, Pinaceae and Salicaceae. These families account for much of the world's wood production. Mycorrhizal fungi act as highly efficient extensions of the host root systems to explore the soil for nutrients and water absorption and translocate them to the host. The host, in turn, provide energy to fungi in the form of photosynthates.

The present scantiness of understanding of the complex array of interactions, mechanism and strategies retard optimal use of mycorrhizae in efforts to improve forest productivity. The physical and chemical characteristics of the soil influence both the fungus and the host plant roots in establishing their mycorrhizal association. More imformations are to be gathered about the ecology and physiology of different mycorrhizal fungi to better predict where and when a specific fungus-host combination is likely to meet the objective of each mycorrhizal management programme. Ectomycorrhizal basidiomycetes constitute an important element of forest fungal communities (Villeneuve *et al.*, 1989 and Vogt *et al.*, 1991) and their unique characterstics as symbionts make them an interesting subject for study on population ecology. Their mycelia maintain a close physiological link to host-tree feeder roots (Read, 1992). Conidia are absent among most species (Hutchisons, 1989), with only few forming chlamydospores (Agerer,1991). These fungi therefore rely upon basidiospores, mycelial fragmentation and occasionally sclerotia (Molina *et al.*, 1992) as their principal means of dispersal and reproduction. These fungi have been demonstrated to possess substantial intraspecific variability for a number of traits, including the morphology of ectomycorrhizal roots (Wong *et al.*, 1989; Gardes *et al.*, 1990), their physiological characters (Ho, 1989; Kropp 1990b; and Gay*et al*, 1993) and their influence on host plant growth and mycorrhizal development (Kropp, 1990b; Marx, 1991).

In addition to its symbiotic functions (Read, 1991) mycorrhizal mycelium also furnishes inoculum to available root system (Brown Lee *et al.*, 1983 and Flemming, 1983) and potentially estabilishes interplant connections (Read, 1992).

Ectomycorrhizae include a great diversity of association. An estimated 2000 fungi can form mycorrhizae with Douglas fir alone (Trappe, 1977) and similar member can be postulated for other host genera , such as pines, oaks and beeches. A few are zygomycetes (Gerdemann and Trappe, 1974). The majority of ectomycorrhizal fungi are ascomycetes and basidiomycetes that fruit as mushrooms,truffles, or cup fungi (Trappe, 1962).

The ectomycorrhizal fungi show various degree of host specificity. Many can form mycorrhizae with nearly any ectomycorrhizal host (Molina and Trappe, 1982a). Others are specific to a particular host, such as pines or even a sub-group within that genus. Some hosts show more selectivity towards mycorrhizal fungi than others. Genera in the Pinaceae will form mycorrhizae with many wide ranging as well as host specific fungi (Molina and Trappe, 1982b).

The Khasi Pine (*Pinus kesiya* Royle ex. Gordon), an indigeneous timber yielding species is dominant at the higher altitude of North-Eastern Himalayas in natural forest ecosystem. Under natural conditions, the roots of pine form a symbiotic mycorrhizal association with fungi. *P.kesiya* harbours ecto or ectendomycorrhizal association (Sharma, 1981b; Kumar,1990; Jha, 1990) which helps in the establishment, survival and growth of pine seedlings (Kropp *et al.*,1990b and Koide and Lu 1995). The selection of suitable ectomycorrhizal fungi for seedlings inoculation can enhance the success of afforestation programmes (Harley and Smith, 1983). Many tree species including the conifers are able to grow in poor soil condition due to their extensive mycorrhizal root system (Koide and Lu, 1995).

Ectomycorrhizal association is specially adapted to nutrient stress condition. Their beneficial role in absorption of inorganic nutrients, production and supply of growth regulators, decreasing of soil toxicity and increasing resistance to extreme soil temperatures is known (Heinrich *et al.*,1989). In absence of mycorrhizae, tree species become stunted and yellow or they may even die (Sharma, 1981 b). Ectomycorrhizal fungi modify the morphology of roots and this change is correlated with the production of cytokinin and other growth regulators which increase the physiological process of the roots. Non-mycorrhizal seedlings however, fail to survive in natural planting sites, specially during the period of environmental stress (Anderson and Rygiewiez, 1991).

Climatic factors may affect the growth and development of ectomycorrhizal fungi. The studies carried out in laboratory conditions on ectomycorrhizal fungi may, however, show some differences in response to the natural environmental conditions due to complexity and interaction between soil,climatic and biological components (Jha, 1990).

Though, studies on mycorrhizae have received enough attention during the past few years, however, their interaction in relation to environmental factors need careful investigation, particularly in relation to the soil pollution caused by heavy metals.

It has been found that tree seedlings lacking ectomycorrhizal show severe nutrient deficiencies until mycorrhizae are formed (Trappe and Strand, 1969). The fungi play a vital role in nutrient cycling, productivity and plant succession in the ecosystem. Mycorrhizal inoculation improves plant productivity specially in soils with low nutrient status. Artificial inoculation of host plants with specific mycorrhizal fungus may depend upon the efficiency of mycobiont on to improve the growth of host under varied ecological conditions (Browning and Whitney, 1993). The disinfection of soil often leads to low mycorrhizal association causing retarded plant growth due to low water and nutrient availability which can be often over come by introducing suitable mycorrhizal fungi into the soil (Sharma *et al.*, 1995).

Ectomycorrhizae are structurally modified rootlets surrounded by mycobiont and they function as an nutrient absorbing organs. The extramatrical phase extends and function as link between the root system and the soil.

The activity of any particular enzyme in soil is a composite of activities associated with various biotic and abiotic factors (Tiwari *et al.*,1987a).Dehydrogenase activity in soil provides correlative information on the biological activity and microbial population. Microbial biomass and their dehydrogenase activity have been observed to be influenced by rhizosphere of the host plant (Speir *et al.*, 1980). Urease activity generally correlates with organic matter content. Various factors such as pH, moisture content, temperature and number of micro-organisms affect the urease activity in soil (Jha *et al.*, 1992). Phosphatase is thought to be directly related to the level of organic phosphorus in the soil. Acid phosphatase is responsible for hydrolysis of organic phosphorus in the soil (Rogers *et al.*, 1942). Therefore, the activity of this enzyme is significant in P-cycling and in plant nutrition. Phosphatases are also produced by ectomycorrhizal fungi (Dighton, 1983). Acid phosphatase activity and fungal mantle mycelium hydrolyse the complex organic phosphorus which improve the nutrient uptake. Root surface phosphatase may be more important in the organic phosphorus mobilization and making it available to the plants (Antibus *et al.*, 1992). Root surface phosphatase activity has its relationship with mycorrhizal association and P-uptake (Dodd *et al.*, 1987). The number of mycorrhizal rootlets and fungal mycelium associated with them as determined by the specific mycobiont is important. Variation in the efficiency of mycorrhizae for metabolic activities and NPK uptake may depend on the specific mycorrhizal fungi. The benefit of mycorrhizae for improving status of trees are largely related to hyphal net of extramatrical mycelia in the soil providing a large surface for the absorption and uptake of nutrients (Rousseau *et al.*, 1994). The use of heavy metals has been increased in various forestry practices in the form of fungicides and pesticides and application of municipal and industrial discharges. Heavy metals like lead and zinc are found inhibitory to microbial growth and decomposition (Joshi, 1993). However, many fungi show remarkable ability to survive and grow in high concentrations of heavy metals.

Toxic metals are numerous and vary in their action (Gadd, 1993). Metal ions can be bound to wall polymers at the cell wall, such as chitin or melanin and inside the fungal cells they might be rendered harmless to complexation, compartmentation or volatilization. Some evidences that ectomycorrhizal fungi can evolve metal tolerance (Colpaert and Van Assche, 1992, 1993; and Egerton-Warburton *et al.*, 1995) and that some ecotypes are constitutively tolerant are known (Denny and Wilkins,1987). Infra and inter-specific variation may exist in the metal sensitivity of ectomycorrhizal fungi (Brown and Wilkins, 1985; Colpaert *et al.*, 1987; 1992; Jones *et al.*, 1988; Egerton-Warburton *et al* 1995 and Hartley *et al.*, 1997).

Several ectomycorrhizal fungi may affect the survival of plants and protect them against metal contamination in soil by influencing the uptake and accumulation of toxic ions (Jones and Hutchinson, 1986, 1988b). Successful tree establishment on contaminated sites has economic benefits (Denny and Wilkins, 1987).

Atmospheric input of heavy metals can lead to their accumulation in the soil of forest ecosystems, specially of Pb and Cd (Zottl, 1985 and Kahle *et al.*,1992). Heavy metals may reduce root growth, alter distribution of cations in the plants (Godbold *et al.*, 1988 and Kahle *et al.*, 1992) and reduce mycorrhizal infection (Dixon *et al.*, 1988). Higher concentration of certain metals such as Cu, Pb and Zn act as environmental stress factors, as their toxic influences may bring about physiological reactions in the host plant (Levitt, 1980) resulting in reduction in vigour and growth, reproduction and in the extreme, even death. The survival of seedlings on contaminated sites determines its sensitivity to metal toxicity. Mycorrhizal fungi may bind metals and thus detoxify them.

High concentration of dissolved Al has resulted in the screening of many forest tree species for Al sensitivity (Anderson,1988 and Shaedle *et al.*, 1989). Mycorrhizal fungi may affect the response of trees to Al (Harley and Smith, 1983).

Zn is an essential element required at all concentration by all fungi (Carlile and Watkinson, 1994). By contrast, Cd and

Pb are non-essential elements which can be toxic above threshold concentrations (Smith and Read, 1997). The translocation and accumulation of heavy metals may be concentrated in tissues.

Copper acts as cation-forming element and serve as Co-enzymes that activate an enzyme but is not an integral part of the molecule. Hence, the right amount of the nutrient element must be applied and uniformly distributed. The accumulation of pollutants on the plant surface is a contributing factor to the forest decline and is very important to understand the early growth and development of tree seedlings (Simmleit *et al.*,1986). In the last decade, however the focus has been laid to study the toxicity of heavy metals to the plants to an increase in industrial activity, excessive use of fertilizers and pesticides, application of sewage and waste disposal on land and mining (Hutton and Symeon, 1986). The interaction between these contaminants and plants with respect to toxicity have been studied (Symeonidis and Karataglis, 1992). However, informations on the heavy metal toxicity to the mycorrhizal system are scarce. Therefore, the present investigation was carried out to study the effect of heavy metals on the structure and function of ectomycorrhizae of pine. The approach of the present study to deal with the problem was as follows:-

- Effect of heavy metals on the synthesis of ectomycorrhizae.
- Accumulation of heavy metals in ectomycorrhizae of pine.
- Effect of heavy metals on metabolic activity of ectomycorrhizal roots and rhizospheric soil of pine seedlings.
- Effect of heavy metals in establishment and growth of the pine

seedlings.

- Effect of heavy metals on the uptake of NPK by mycorrhizal and non-mycorrhizal seedlings of pine.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

EFFECT OF HEAVY METALS ON THE SYNTHESIS OF MYCORRHIZAE ITS STRUCTURE AND MAINTENANCE OF ECTOMYCORRHIZAL FUNGI

Frank (1885) coined the term mycorrhiza for a fungus root organ and later on in 1887 he distinguished two types of mycorrhizae ie ectrotrophic and endotrophic.

Singer and Morello (1960) have reported that ectomycorrhizae occur at higher altitude of 25^{O}N and 35.55^{O}E.

Fontana (1962) in a survey of mycorrhizal association noticed that 14 tree species from the plain, hill and mountains of Italy possessed ectotrophic mycorrhizae.

Trappe (1967) demonstrated pure culture synthesis of Douglas fir mycorrhizae with species of *Hebeloma*, *Suillus*, *Rhizopogan* and *Astraeus*.

Pachlewska (1967a) augmented that a starvation medium consisting of agar, thiamine and water was more suitable for synthesis of mycorrhizae with *P. sylvestris* seedlings.

Bowen (1965), Mikola (1973), Trappe (1977) and Marx (1980) have used the pure mycelial cultures of fungi for inoculation of the seedlings of trees.

Warcup (1971) isolated 25 mycorrhizal fungi from their sporocarps growing in Eucalypt forest and identified them.

Hutchinson and Whitby (1974) reported considerable reduction in forest productivity due to a combination of SO_2 acidity and metals.

Lamb (1979) isolated 20 different mycorrhizal species from the sporocarps of *P. radiata* stand.

Marx (1980) suggested that ectomycorrhizal fungus inoculation is a better tool for forestry practices.

Sharma (1981) isolated more than 10 fungal species from the *P. kesiya* stand using agar thiamine medium and synthesised mycorrhizae.

Malajezuk *et al. (1982) isolated some fungi from sporocarps.* They isolated *Cenococcum geophilum* from sterilised sclerotium.

Ng *et al.* (1982) have isolated *R. luteolus* and *B. elegans* from the sporocarps collected from *P. radiata* woodland.

Palmer and Molina (1982) studied the isolation, maintenance and pure culture manipulation of ectomycorrhizal fungi and also the methods and principle of mycorrhizal research.

Danielson *et al.* (1984) confirmed the effectiveness of mycelial slurries of mycorrhizal fungi for the inoculation of container grown Jack pine seedlings.

Sharma (1981) and Chu Chou and Grace (1988) used different media to isolate the fungi from the mycorrhizal roots and suggested that Hagem (Modess, 1941) and MMN (Marx, 1969) agar media were best for the fungal growth.

Denny and Wilkins (1987) argued that the extramatrical mycelium plays a key role in retention of high amount of Zn ions.

Sahardi (1988) suggested a spore inoculum technique for pine stand under natural condition.

Sharma and Mishra (1988) were of the opinion that fast growing fungi can produce more inoculum within short duration. They improved the growth of fungi by adding Sphagnum to the MMN vermiculate medium and found it better over other sources of inocula.

Raman (1988) found sorghum grain superior than wheat grains for the growth of fungus.

Jha (1990) isolated 5 ectomycorrhizal fungi from sporocarps in *P. kesiya* stand.

Allen (1992) observed that plant roots produce stimulatory exudates (volatile and non-volatile), which are important when mycorrhizal fungus is penetrating and colonizing the host plant roots.

McQuattie and Schier (1992) found increased vacuolation and swelling of nuclear membrane of root meristem and intracellular penetration of fungal hyphae in cortical cells in ectomycorrhizas of Al-exposed seedling root.

Colpaert *et al.* (1993) studied the effect of Cd on the *in vitro* growth effects of Cd on ectomycorrhizal *Pinus sylvestris.*

Rao (1994) used MMN medium for the synthesis of ectomycorrhizal fungi.

Gadd (1993) observed the interaction of fungi with toxic metals.

Carlile and Watkinson (1994) reported that Zn is an essential element required at low concentration by all fungi.

ACCUMULATION OF HEAVY METALS IN ECTOMYCORRHIZAL FUNGI

Barber (1974) observed that toxicity of metals is first evident in root tips, followed by subsequent inhibition of lateral root development resulting at a rate insufficient for normal growth.

Jackson and Watson (1977) found that depletion in fine roots in litter polluted by lead smelter emissions have high concentration of heavy metals in the root tissue.

Kelly *et al.* (1979) investigated the heavy metal accumulation in fine roots and growth of seedlings are influenced by soil Cd level.

Jones and Hutchinsons (1988) have demonstrated that *Lactarius rufus* increaesd the Ni tolerance of its host plants for a short time. They further found that the amount of fungal tissue produced by a mycobiont is positively correlated with the protection of the host plant against metal toxicity.

Darlington and Rauser (1988) have suggested that Cd might induce an increase in mycelial strand density around mycorrhizal roots and consequently change in Cd supply to both the symbionts.

Duddridge (1980), Heinrich *et al.*(1988) have shown that ectomycorrhizal fungi could survive in water defficient soils and can translocate stored water to the host.

Tyler (1988) proposed a mechanism of avoidance of toxicity in some vascular plants. The high accumulation of toxic metal encountered in the fine roots of certain species necessitate a more rapid replacement of these roots.

Colpaert and Van Assche (1992a) found an ameliorating effect in case of Zn toxicity and clearly indicated that several ectomycorrhizal fungi can protect their host at its elevated concentration in soil. They further (1993) investigated that seedlings were less susceptible to toxic concentration of Zn in the growth medium when mycorrhizae were present.

Bender *et al.* (1989) observed the content of Cd concentration in stems and needles of pine and assigned it to the compartmentalization effect.

Giller *et al.* (1989) concluded that the effective clover rhizobia were unable to survive in the free living state outside the protected root-nodule in the metal contaminated soil. They suggested that Cd, Zn and Cu are the most toxic metals to rhizobia.

Shaedle *et al.* (1989) reported that high concentration of Al has resulted in the screening of many forest tree species for Al sensitivity.

Tyler *et al.* (1989) observed that ectomycorrhizal fungi play a large role in nutrient uptake under natural conditions suggesting that mycorrhizal fungi might influence the uptake and accumulation of toxic ions. Furthermore, development of fungi might be affected by high Cd concentration since they are probably more exposed to toxic enviroment than the roots of vascular plants. Mycorrhizal fungi were strongly supprressd by Cd in some forest tree seedlings inoculated with *Suillus luteus.*

Koomen *et al.* (1990) confirmed that mycorrhizal infection is delayed in soils contaminated with heavy metals from sewage sludge applications.

Andre *et al.* (1991) have successfully studied foliar absorption, translocation and distribution of metal elements in crops and trees with radioisotopes.

Bennet and Breen (1991) observed the recovery of the roots of *Zea mays* from various Al treatments toward elucidating the regulatory process that underline root growth control.

Berg *et al.* (1991) have shown that heavy metal accumulation disturbes decomposition and nutrient mineralisation processes in the forest soil leading to low nutrient availability.

Gabrielli *et al.* (1991) investigated the accumulation mechanism and heavy metal tolerance of a Ni hyper accumulater.

Ownby *et al.* (1991) reported quantative changes in cytoplasmic and microsomal proteins associated with Al toxicity in two cultivars of winter wheat.

Villeneuva (1989) *and* Vogt *et al. (1991) concluded that* ectomycorrhizal basidiomycetes constitute an important element in forest fungal communities and their unique characteristics as root symbionts make them an interesting subject for studies of population ecology and genetics.

Wilkins (1991) studied the influence of sheathing ectomycorrhiza of trees on the uptake and toxicity of metals. They further observed the localization of Al in the roots of Norway spruce (*Picea abies*) inoculated with *Paxillus involutus*.

Crowder and Griepson (1992) observed amelioration of Cu and Ni toxicity by iron plaque on roots of rice.

Ye *et al.* (1992) reported the distribution and accumulation of heavy metals in *Typha latifolia* from Pb or Zn mine waste water.

Sweet *et al.* (1993) analysed multi-element airborne particulates containing trace elements for detecting potentially toxic metals.

Turnau *et al.* (1993) observed the element localization in mycorrhizal roots of *Pteridium aquilinum*(L) Kuhn collected from experimental plots treated with Cd dust.

Brunold *et al.* (1994) reviewed metal binding by mycorrhizal fungi. The nature of protection provided by mycorrhizal fungi against high concentration of Zn and was showed that binding of Zn to extramatrical hyphal wall and to extra-hyphal slime was a major importance. Alva and Chen (1995) observed the effects of external Cu concentration on uptake of trace elements by citrus seedlings.

Ye (1995) studied heavy metal tolerance, uptake and accumulations in populations of *Typha latifolia* and *Phragmites australis*.

Haimii *et al.* (1996) and Fritz *et al*, (1996) found that heavy metals have direct toxic effects on soil fauna and soil microbes.

Baker *et al.* (1997) investigated the tolerance, uptake and accumulation of Zn, Pb and Cd by *Typha latifolia*.

Evans *et al.* (1997) studied the Cu binding proteins in ectomycorrhizal fungi.

Simon *et al.* (1997) investigated the heavy metal accumulation in soil; a comparasion of methods illustrated by a case study on compost application.

DEHYDROGENASE, UREASE AND PHOSPHATASE

Lenhard (1956) for the first time observed the dehydrogenase activity in soil by using Triphenyl-tetrazolium chloride salt.

Doelman and Haanstra (1979a) studied the effect of Pb on the soil respiration and dehydrogenase activity.

Stott *et al.* (1980,1985) investigated the interrelations between selected soil characteristics and arylsulphatase and urease activities. They further studied the inhibition of pyrophophatase activity in soil by trace elements.

Frankenberger *et al.* (1983) studied relationship between enzyme activities and microbial growth and activity indices in soil.

Rother *et al.* (1983) observed the decrease in plant nodule size and nitrogenase (acetylane reduction) activity in white clover grown in soil highly contaminated with Cd, Pb and Zn.

Shkolnik *et al.* (1984) reported that excess of Ni depresses catalyse activity in mature plants which interferred with iron nutrition and produced other specific signs of toxicity.

Rao and Ghai (1985) found that urease activity was positively correlated with organic carbon and nitrogen and negative correlation was observed with soil pH and calcium carbonate content of the soil.

Tiwari *et al.*(1987) mentioned that moisture plays a significant role in the variation of dehydrogenase activity. They further noted higher urease activity during summer season on the surface soil which was attributed to high organic carbon and favourable moisture content of the soil.

Heyser *et al.* (1986), Deighton *et al.* (1987) and Bresser *et al.* (1988) concluded that 'acid rain'caused soil chemical changes and acidified stem flow which was correlated with a decrease freguency of coralloid *mycorrhizae.*

Ho (1988) studied a simple method for assessing acid phophatase activity of ectomycorrhizal fungi.

Siegel *et al.* (1988) showed that large concentration of heavy metals was harmful to the growth, morphology and metabolism of micro-organism *in vitro.*

Marscher and Haussling (1989) have noticed that phosphatase activity of mycorrhizal roots was inversely proportional in P-deficient soil and that acid phosphatase activity was 2.5 times more in rhizosphere in Norway spruce than in the bulk soil.

Kropp (1990) observed some mycorrhizal fungi may improve the host ability to utilise insoluble phosphatase by producing acid phophatase enzyme.

EFFECT OF HEAVY METALS ON GROWTH AND ESTABLISHMENT

Macleod and Jackson (1965) studied effect of concentration of the Al ion in root development and establishment of legume seedlings.

Marx *et al.* (1971) found the influence of ectomycorrhizae on survival and growth of septic seedlings of Lobolly pine at high temperature.

Hutchinson and Whitby (1974) demonstrated a relative reduction of root elongation by tomato plants in solution of Ni, Cu and Al.

Jordan (1975) conclated that seedlings in Zn-contaminated soils produced significantly less root and shoot growth than did controls.

Marx and Bryan (1975, 1976. 1978) studied the growth and ectomycorrhizal development of Lobolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. They further investigated the sewage sludge and *Pisolithus tinctorius*, their effect on growth of pine seedlings. They also studied the growth and ectomycorrhizal development of lobolly pine seedlings in fumigated and non-fumigated nursery soil infested with different fungal symbionts.

Levitt (1980) suggested that high concentrations of metals such as Cd, Cu, Pb and Zn are enviromental stress factors as their toxic influence may bring about physiological reaction change resulting in reduction in vigour and growth and reproduction or in the extreme even death.

Bradley *et al.* (1981) studied mycorrhizal infection and resistance to heavy metal toxicity in *Callunia vulgaris*.

Ross (1982) observed the effect of Cu, Cd and Zn on germination and mycelial growth of *Candida albicans.*

Alexander and Fairly (1983) have shown that ectomycorrhizal roots in forest ecosystems are more intimately associated with litter than the humified horizon.

Brown Lee *et al.* (1983) showed that ectomycorrhizal mycelial strands can extend from plant to plant thus, initiating infection in seedlings and provide functional pathways for the transfer of labelled assimilate between individual.

Brookes and McGrath (1984) reported the effect of metal toxicity on the size of the soil microbial biomass.

Peterson *et al.*(1984) reported that ectomycorrhizal fungi stimulate growth of many trees and protect them from many diseases.

Ford *et al.* (1985) reported that after 10 months of pine seedlings transplantation in green house, mycorrhizal fungi enhanced the seedling growth significantly than control. The performance of *S. aurantium* was superior over the other mycorrhizal fungi in increasing P-uptake.

Brook and McGrath (1987) concluded that heavy metal contamination of land by past application of sewage sludge can have adverse effects on the soil microbial biomass. They also found significant decrease in white clover yield in an experiment to which sewage sludge contaminated predominantly by Zn was added. Cu also decrease the yield of white clover to some extent whereas Ni had no effect on yield.

Cline *et al.* (1987) concluded that climatic factors may effect the growth and development of ectomycorrhizal fungi and acquired some information on inter and intra specific growth variation of ectomycorrhizal fungi in responce to different temperature.

Schlegal *et al.* (1987) observed that Cd treatment had no effect on the shoot biomass production of pine seedlings,though the water use of the seedlings decreased. Cd toxicity often result in an increase in water stress. A decrease in transpiration is a early sign of Cd toxicity.

Danielson (1988), Le Tacon *et al.* (1988) and Marx *et al.* (1988) found that seedling growth response vary according to soil characteristics as well as fungal inoculum used.

Elis *et al.* (1988) found that pine root exudates stimulate the synthesis of antifungal compounds by ectomycorrhizal fungus *Paxillus involutus*.

Balsberg *et al.* (1989) showed that Cd often induces an inhibition of photosynthesis in an imbalance in essential micronutrients. It is more toxic than most other heavy metals i.e, Zn, Cu, Pb and Ni.

Alloway (1990) clearly observed that metal smelting, mining and manufacturing process more often results in environmental contamination with a mixture of potentially toxic metals.

Cumming and Weinstein (1990) investigated the ability of mycorrhizal fungus *Pisolithus tinctorius* (Pers) Coker and Couch to modulate Al toxicity in Pitch pine (*Pinus rigida* Mill) and concluded that mycorrhizae increase Al tolerance.

Cole *et al.*(1990) found that atmosphere is a key medium in the transfer of trace elements pollutants from urban pollution sources to rural forest ecosystems. Comparing natural and anthropogenic sources, industrial emmissions are seen to be primarily responsible for most trace elements in the air.

Earnst *et al.* (1990) have shown that tree suffers very quickly from metal surplus, but much less is known about the metal tolerance of trees than that of herbaceous plant species.

Mc Laughlin *et al.*(1990) reported that air pollution is considered to be a major pollutants and potential contributer to the decline of conifers in high elevation forest of Eastern North-America.

Nriagu (1990) estimated that the burning of fossil fuels accounted for more than 90% of V and 80% of Ni pollutants discharged in the environment.

Anderson *et al.*(1991) showed stress response interaction in mycorrhizal plant growth.

Dalberg (1991) studied the mycorrhizae in coniferous forest; structure and dynamic of population and communities.

Macfall and Slack (1991) studied the importance of ectomycorrhizal fungi in the establishment of conifer seedlings.

Kasuya *et al.* (1991) investigated the influence of Al on the vitro formation of mycorrhizae in *Pinus caribea.*

Kinraide (1991) recognised Al as most limiting factors in many acid soils throughout the world possibly affecting about 40% and perhaps upto 70% of the world's land that is potentialy usable for food and biomass production. Al reduced root growth, rhizotoxicity of various mononuclear and polynuclear species.

Deacon and Flemming (1992) studied competitive interactions among ectomycorrhizal fungi during root colonization and are thought to occur primarily by competitive exclusion, with mycelial growth rate, inoculum potential and other biotic and abiotic factors influencing the successof suitable mycobionts.

Godbold (1992) found that Al decreases root growth and Ca and Mg uptake in *Picia abies* seedlings. They also studied metal toxicity in mycorrhizal Norway spruce seedling in response of forest ecosystems to enviromental changes.

Symeonidis *et al.* (1992) reported the effects of Cd, Pb and Zn on root growth of two metal tolerant genotypes of *Holcus lanatus* L.

Read (1993) concluded that the ectomycorrhizal are crucial biological factors for stability of woods and forest in temperate regions.

Fox *et al.*(1994) investigated the ectomycorrhiza and rhizosphere micro-organisms of seedlings of *Pseudotsuga menzeisu* (merb) Franco planted on a degrated site and inoculated with forest soils pretreated with selected biocides.

Loranger and Zayed (1994) investigated Mn and Pb concentration in ambient air and emission rates from unleaded and leaded gasoline between 1981 and 1992 in Canada: a comparative study.

Molina *et al.*(1994) observed the population responses of target and non-target forest soil organisms to selected biocides.

Greipson (1995) studied the effect of iron plaque on roots of rice growth in excess Zn and in accumulation of phosphorus in plants in excess Cu or Ni.

Tam (1995) reported heavy metals tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*.

Salemma *et al.* (1995) confirmed a forest vegetation change along a pollution gradient in South Western Finland. They also investigated Cu in Scots pine stand around a heavy metal smelter in South Western Finland.

Frietz *et al.* (1996) studied vitality fertilization of Scots pine stands growing along a gradient of heavy metal pollution; short term effect on microbial biomass and respiration rate in the humus layer.

Harley *et al.* (1997) reported that ectomycorrhizal fungi have essential role in ameliorating metal toxicity to their host. They also studied the intra and inter specific variation exist in the metal sensitivity of ectomycorrhizal fungi and also observed that ectomycorrhizal fungi exhibit adaptive tolerance to potentialy toxic metals in the environment.

Moolenaar *et al.* (1997) investigated the indicaters of the substainability of the heavy metals management in agro-ecosystems.

Smith and Read (1997) investigated that Cd, Pb and Sb are non-essential elements which can be toxic above threshold concentrations. They also reported that ectomycorrhizal fungi colonize the roots of most woody plants and improve their growth and survival by enhancing nutrient, especially phosphate and nitrogen uptake.

EFFECT OF HEAVY METALS ON THE UPTAKE OF N,P,K.

Clarkson (1967) investigated the interaction between Al and phosphorus on root surface and cell wall materials.

Bond (1971) studied fungi of Oak and North Carolina and their influence on uptake of K by Oak seedlings.

Ho and Zak (1971) found that ectomycorrhizal fungi enhance absorption of inorganic nutrients, particularly phosphorus.

Bowen (1973) confirmed that ectomycorrhizae enhance the uptake of NPK apart from a few other minerals by the plants.

Rauser (1978) observed that in addition to the disturbance in the carbohydrate metabolism, excess of Co, Ni and Zn also interfered with the translocation of the sugars from the source to the sink like roots.

Wallace *et al.* (1978) observed the influence of phosphorus on Zn, Fe, Mn and Cu on phosphorus uptake by plants.

Mayer and Khanna (1979) found that the atmospheric pollutants also effect plants, which act as nutrient sinks by influencing the species composition and physiology of mycorrhizae.

Mezal (1980) observed that the ectomycorrhizal association is adapted to nutrient stresses and is an ecological aid to plant growing in a temperate condition.

Rhodes *et al* (1980) found that the fungal sheath together with fungal hyphae acts as an extension of the root system, thus can explore a greator volume of soil for nutrients, than the ordinary roots can do.

Harley and Smith (1983) observed that mycorrhizal fungi influenced the uptake of mineral elements from the soil, they may affect the responce of trees to Al.

Thompson and Medre (1984, 1985) observed the consequent potential loss in the ability of the mycorrhizal system to sequester nutrients from the organic resources in the soil may be due to mobilization of Al. Al toxicity reduces protein synthesis in mycorrhizal fungi.

Read *et al.* (1985) observed the extramatrical mycelium extends out into the soil from ectomycorrhizal roots and provides a network for nutrient uptake and transport. It also connects fungal fruit bodies with host roots, and carries carbohydrate from the root necessary for their development. The nutrient uptake capacity of fungus is likely to be correlated with the amount of mycelium colonising the soil.

Finlay *et al* (1989) found that the mycorrhizal roots take up several times more phosphorus per unit length than non-mycorrhizal roots.

Keltjens (1987, 1988, 1990) studied the nitrogen source of Al toxicity of two sorghum genotypes differing in Al susceptibility, short term effect of Al on nutrient uptake, root respiration and nitrate reductase activity. He also studied the effect of Al on growth, nutrient uptake, proton efflux and phosphorus assimilation of Al tolerant and sensitive sorghum.

Kropp *et al.* (1990) reported that ectomycorrhizal fungi play an important role in the phosphorus nutrition of their host plants.

Berg *et al.* (1991) studied reduction of decomposition rates of Scot pine needle litter due to heavy metal pollution.

Cakmak and Horst (1991b) analysed effect of Al on net efflux of nitrate and potassium from root tips of soyabean (*Glycine max*)

Fink (1991) observed structural changes in conifer needles due to Mg and K defficiency.

Godbold*et al.* (1991a) reported the effect of nitrogen and Al on the culture of mycorrhizal tree seedlings under controlled conditions.

Vitousek and Howarth (1991) confirmed that N was the most limiting nutrient in many plant communities.

Cui and Nobel (1992)studied nutrient status, water uptake and gas exchange for three desert succulent infected with mycorrhizal fungi.

Read (1992) investigated in addition to its role in root colonization, mycelial growth rate may also affect the ability of the extramatrical mycelium to exploit available soil resources and transport them to the host.

Twiss *et al.* (1993) studied the influence of Cu toxicity on phosphorus nutrition in three strains of *Scenedesmus acutus.*

Smith and Read (1997) observed that ectomycorrhizal fungi play an important role in enhancimg uptake of mineral nutrients for many plant species.

MATERIALS AND METHODS

MATERIALS AND METHODS

1.Effect of heavy metals on the synthesis of ectomycorrhizae of pine

(a) Selection of mycorrhizal fungi

Four ectomycorrhizal fungi ie, *Boletus sp.*, *Cenococcum graniforme* , *Scleroderma aurantium* and *Suillus luteus* were selected based on earlier studies on their efficiency in p-uptake. Young fruiting bodies were collected from the pine forest of North Eastern Hill University campus, East Khasi Hills, Shillong and brought to the laboratory on the same day and identified as outlined by Singer (1962).

(b) Isolation of mycorrhizal fungi from the Sporocarps

The young sporocarps were preferred for isolation of fungi. Their abexial surfaces were sterilized with 90% alcohol for one to two minutes. After that fruiting bodies were cut into two halves with the help of sterilized scalpel. Small piece of tissue was separated from the inner side of pieced cap of fruit body and inoculated onto modified Melin-Norkran's agar medium (Marx, 1969a) in 5 replicates. The Petri plates were incubated at 23^{o}C (± 2^{o}C) in B.O.D incubator. The whole isolation process was carried out under aseptic condition. The plates were checked regularly and the contaminated ones were discarded. The cultures were examined under microscope for clamp connections and were sorted out.

(c) Maintenance of the ectomycorrhizal fungi

The ectomycorrhizal fungi were purified by hyphal cut method and were multiplied on modified Melin-Norkran's nutrient agar medium (MMN). The pure culture of fungi were then transferred onto the slants of MMN medium in culture tubes and allowed to grow for five to six days at ($23 \pm^{o}$ 2^{o}C) in the BOD incubator. Twenty culture tubes in each case were taken out after six days of incubation and kept at 5^{o}C in refrigerator. At a regular interval of 60 days, tubes were replaced by freshly prepared MMN medium.

(d) Synthesis of ectomycorrhizae by isolated cultures

To confirm the isolated fungi as mycorrhizal, their symbiotic association was resynthesised under laboratory condition.

The pine seeds were soaked in sterilised water for 24 hours and surface sterilized in 5% sodium hypochlorite for 5 minutes. The seeds were washed with sterilized water 5-6 times and transferred to Petri plates containing water-agar meduim (2 g agar in 100 ml distilled water) and were kept at 25^{o}C in dark condition for seed germination. After 20 days, seedlings of 2 cm long radicle were transferred to the culture tubes containing water-agar medium. Fungi isolated earlier from sporocarps were grown on MMN medium at 23^{o}C (± 2^{o}C) for 30 days.

After 10 days of seedling transplantation in culture tubes, the selected isolates of fungi were inoculated (5mm diameter block) near the roots of Pine seedlings in 3 replicates and incubated at 20^{o} (± 2^{o}C) under light (2500 lux) of 12 h photo-period. The relative humidity was maintained (70-90%) in the growth chamber, thereafter the seedlings were examined for mycorrhizal symbiosis.

(e) Application of heavy metals

Treatment of different heavy metals was followed after 30 days of inoculation of fungi and formation of mycorrhizae. Six different concentrations namely 0 ppm (control), 10 ppm, 50 ppm, 100 ppm, 200 ppm and 500 ppm of Pb [$(CH_3COO)_2$ Pb OH], Zn ($ZnSO_4$, $7H_2O$), Al (Al Cl_3), Ni (Ni Cl_2, $6H_2O$), Cu ($CuSO_4$, $5H_2O$) and cadmium metals were selected, 5ml of each concentration was applied to each culture tubes. The experiment was continued for 6 months and seedlings roots were taken for SEM (Scanning Electron Microscopy).

(f) Specimen (mycorrhizal roots) preparation for SEM

(a) Fixation: It was the first step in the preparation of specimen for electron microscopy. Through this process, cells were put to death instantaneously using fixative to prevent autolysis and preserve various chemical constituents of the cell. Since affinity of different fixatives to react with various chemical constituents of the cell varies and hence to obtain a complete picture, several fixative procedures were followed concurrently.

The hand sectioned roots (approx. 2-3mm) were fixed in 2.5% gluteraldehyde (prepared in 0.1M Na-cacodylate buffer) for 4 h at $4^{o}C$ and washed in the buffer for 15-20 minutes, and secondary fixation in 1% buffered OSO_4, then washed with distilled water. Dehydration of sample was done in acetone grades 30, 50, 70, 80, 90 and 95% with 2 changes of 15 minutes in each grade. After that, samples were taken for critical point drying using acetone as intermediate fluid and liquid CO_2 as transitional fluid. Samples were then mounted on aluminium copper stubs with double sided adhesive tape, and samples were sputter coated with gold palladium alloy for five minutes and photograped on a JEOL-35 JSMCF-SEM at an accelerating voltage of 15 KV. 1100 Ion Sputter (Jeol, Japan).

2. Studies on accumulation of heavy metals in ectomycorrhizae of pine (*P. kesiya*)

For the study of accumulation of heavy metals in ectomycorrhizae of pine, the seeds of *P. kesiya* were collected and sterilized in 5% Sodium hypochlorite for 5 minutes and were germinated in Petri dishes on water-agar medium for 20 days at 25°C in the dark under sterile conditions. The seedlings (approx. 2cm radicle) were transferred to pots (26cm x 16cm) containing sterile sand and soil mixture (1:3). The seedlings were inoculated with four different ectomycorrhizal fungi isolated earlier.The plants were grown with fungi for 30 days and confirmed to the mycorrhizal association. Fifteen seedlings were maintained in each pot.

(a) Application of heavy metals

Six different concentrations namely 0 ppm (control), 10 ppm, 50 ppm, 100 ppm, 200 ppm and 500 ppm of Pb, Zn, Ni,Cu, Cd metal and Al were selected, and treated (10 ml each) artificially to each pots. Control pots were maintained without heavy metal treatment in 12 pots and in another 30 pots, only heavy metals of different concentrations were applied separately to seedlings without inoculating the ectomycorrhizal fungi. The experiment was run in a net house (4.6m x 2.15 m). Average temperature during the study period varied between 20-29^{o}C during the day, light intensity 1,000-1,5000 Lux; and photoperiod 8-12h a day. The average relative humidity was ranging between 67-83% during the experimental period. Seedlings were watered daily. A dish was placed below each pot and great care was taken durind watering to avoid cross-contamination. The experiment was continued for 1 year.

(b) Analysis of total metal concentration in pine roots

One year old seedlings were harvested and washed carefully. The roots were collected from each treatment separately and oven dried at 60°C for 48 h. The samples were ground to pass through a 2mm sieve. About 0.2g of the fine powder sample was digested with a triacid mixture (HNO_3: H_2SO_4:$HClO_4$; 3:1:1) at 65^{o}C (all acids used were of analytical grade). It was then filtered through Whatman filter paper (No. 1) and the final volume was made upto 50 ml with distilled water. The concentration of the element was detected by using an Atomic Absorption Spectrophotometer (AAS), (Perkin Elmer Model 2380). Distilled water sample was used as blank.

The concentration of metal was calculated using the formula:

$$\text{Total metal } (\mu g^{-1}) = \frac{C(\text{ppm}) \times \text{solution volume (ml)}}{\text{Sample weight (g)}}$$

C = ppm metal obtained from the standard curve.

Statistical analysis The data was processed by analysis of variance (ANOVA) and significant differences among means were identified with Duncan's multiple range test at P = 0.05 and 0.01

level. Correlation coefficient was also calculated.

3. Effect of heavy metals on enzyme activities in rhizospheric soil and in roots of pine

(i) Study under field condition

The study was conducted at the Pine stand of Permanent Campus, North- Eastern Hill University, Shillong (altitude 1500m,msl latitude $25^{o}34'$N, longitude $91^{o}54'$E) in the East Khasi Hills District, Meghalaya. Two sites were selected on a gentle slope facing eastward and one located at 1m higher than the other. 36 microplots (1 cm^2 each) were prepared for both fields (plate 1 and 2) and were irrigated as needed. The seeds of *P. kesiya* were collected in December, 1995 and soaked in water for 24 hours and surface sterilised in 5% sodium hypochlorite for 5 minutes. The seeds were then washed with sterilised water 5-6 times and sown in each microplot. 15 seedlings were maintained in each plot. when the seedlings were 1 month old the mixed ectomycorrhizal fungi slurry (10 ml each) was inoculated to each plot. Four ectomycorrhizal fungi i.e. *Scleroderma aurantium, Cenococcum graniforme, Suillus luteus* and *Boletus* sp. were collected and the sporocarps were dried in air. The Sporocarps were then squeezed with hand and mixed with distilled water and inoculated (20 ml) in each plot near the seedlings root. Periodically 2-3 seedlings from each plot were randomnly harvested and roots were examined under binocular microscope for mycorrhizal colonization. After confirming the mycorrhizal establishment, the seedlings were treated with different concentrations of heavy metals. Six different concentrations namely 0 ppm (control), 10 ppm,100 ppm,200 ppm, 500 ppm of Pb, Zn, Cu, Ni, Cd and Al were selected and applied (10 ml each) in each plot. A control set of plot was also maintained in the same manner except that the seedlings were not inoculated with mycorrhizal fungi. The experiment was set in March, 1996 and the study continued till February 1997. Sampling was done in every season to analyse the activities of enzyme.

(ii)Study under pot culture

Earthen pots (26cm x 16cm) were filled with sterilised sand and soil mixture (1:3). In each pot approximately 2 cm long seedlings which were germinated in water-agar medium (2g agar in 100 ml dist.water) were transferred. 15 seedlings were maintained in each pot. The seedlings were inoculated with four different ectomycorrhizal fungi separately such as *S. aurantium*,*S. luteus*, *C. graniforme* and *Boletus* sp. The inoculation was done using pure culture inoculation technique (Marx *et al.*,1971).

After confirming the mycorrhizal colonization the seedlings were treated with different concentrations of heavy metals. Six different concentrations such as 0 ppm (control), 10 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm of Pb, Zn, Ni, Cd, Al and Cu were selected and 10 ml of each concentration was applied in each pot. Control pots were maintained for each concentration without inoculation of ectomycorrhizal fungi. The experiment was run in a net house (4.6m x 2.15m), average temparature in net house varied between 20-29°C during the experimental period, light intensity, 1000-1500 Lux; and photoperiod 8-10 h average relative humidity from 67-83%. Seedlings were watered daily with sterile water. The experiment was set in March, 1996 and sampling for different observations was done in every season. The study was continued till February 1997.

(iii) Dehydrogenase activity in rhizospheric soil

2,3,5-Triphenyl Tetrazolium Chloride (TTC) reduction technique was adopted for the evaluation of dehydrogenase activity in rhizosphere soil (Casida, 1977). 0.5 g of fresh soil from the field and from the pots was taken in test tube for each treatment separately and to it was added 0.1 g of calcium carbonate and mixed properly. 1 ml of 10% TTC solution was added to it. Thereafter, the test tubes were plugged tightly with cotton plug and shaken well for proper mixing of the contents and were incubated at 37°C for 24 h. Three replicates were maintained for each sample. After 24 h of incubation, the slurry was filtered through Whatman filter paper (No 1) and washed with small amount of methanol. The volume of filtrate was made upto 50 ml by adding methanol. The optical density of pink coloured solution was measured on a spectrophotometer (Hitachi 220) at 485nm using methanol as blank. The optical density was calculated with the help of standard curve prepared by known amount of Triphenyl formazon in methanol. The values of dehydrogenase activity were converted into per gram dry soil taking into consideration the moisture content and expressed in terms of µg formazon per gram dry weight of soil per 24 h.

(iv) Dehydrogenase activity in mycorrhizal and non-mycorrhizal roots

2,3,5 Triphenyl Tetrazolium Chloride (TTC) reduction technique was adopted for the evaluation of dehydrogenase activity of roots (Casida, 1977). 0.5g of fresh roots were taken from each treatment performed both in field and in pots in the test tubes and to it 0.1g of calcium carbonate was added and mixed properly. 1 ml of 10 % TTC solution and 3 ml of distilled water were added to it. Thereafter, the test tubes were plugged tightly with the cotton and shaken well and incubated for 37°C for 24h in 3 replicates for each sample. The volume of filtrate was made upto 50 ml by adding methanol. The optical density of the solution was measured on spectrophotometer (Hitachi 220) at 485 nm using methanol as blank. The optical density was calculated with the help of standard curve prepared by known amount of Tri-phenyl formazon in methanol. The value of dehydrogenase activity was converted into per gram dry sample taking into consideration the moisture content of roots and expressed in terms of mg formazon per gram dry weight of sample per 24h.

(v) Urease activity in rhizospheric soil

McGarity and Myers (1967) method was followed to measure the urease activity in rhizospheric soil. 10 g of fresh soil was taken from each treatment maintained in field and pot cultures separately into 100 ml volumetric flask and 1ml of toluene was added and left for 15 minutes. Thereafter, 10 ml of acetate buffer (pH 7.0) and 5ml of (10%) urea solution were added. The flasks were swirled well for proper mixing and incubated at 37°c for 3h. In control instead of 5ml urea solution, 5ml of distilled water was added. After incubation, the flasks were taken out and the volume was made upto 50ml with distilled water. The mixture was shaken properly and was filtered through Whatman filter paper (No.1). Thereafter, 1ml filtrate was taken into 50 ml volumetric flask and to it 9 ml distilled water was added. 5 ml phenolate solution along with 3ml of sodium hypochlorite solution (0.9% active chlorine) was also mixed. After 20 minutes, the solution was made upto 50 ml by adding distilled water. The intensity of blue colour was read out on a spectrophotometer (Hitachi 220) at 630nm. Similar observations were taken for solution without soil as control.The amount of NH4-N released was caculated by the standard caliberation curve and expressed in terms of NH_4-N per gram dry sample per 3h.

Preparation of phenolate solution

62.5g phenol was dissolved in 20 ml methanol. Thereafter, 18.5ml acetone was added and the volume of mixture was made upto 100 ml with ethyl alcohol giving it as a phenol solution. 27g of sodium hydroxide was dissolved in 100 ml distilled water in separate bottle .The phenolate solution was prepared by mixing phenol soluiton , caustic soda solution and distilled water (20:20:60;V/V/V). The solution was always prepared fresh at the time of use.

(vi) **Phosphatase activity in rhizospheric soil**

Phosphatase activity was assayed by the method of Tabatabai and Bremmer (1969). Rhizospheric soil sample was collected from each treatment maintained in field and pot culture separately and was air dried and sieved (2mm). 0.1g of soil powder was taken from each treatment seperately into a 50 ml conical flask. 0.25

ml toluene, 4 ml of MUB (Modified universal buffer, pH 6.5 prepared as described by Skujins *et al.* (1962) and 1 ml of 0.115M p-nitrophenyl phosphate (PNP) solution was added to the flask. The flasks were shaken well to mix the contents and incubated at 37°C for 1h. After incubation, the cotton plugs were removed and 1 ml of 0.5M calcium chloride and 4 ml of 0.5M sodium hydroxide solutions were added. The suspension was filtered through Whatman filter paper (No. 1). The optical density of filtrate was measured at 430nm wavelength on a spectrophotometer (Hitachi 220). For the control, similar procedure was followed without the soil sample. The concentration of phosphatase activity in terms of p-nitrophenyl in each sample was calculated by a standard curve of PNP in water and was expressed as mg p-nitrophenol released per gram of dry soil per hour.

(vii) Phosphatase activity in mycorrhizal and non-mycorrhizal root surface

Phosphatase activity was assayed by the method of Tabatabai and Bremmer (1969). 0.1g of dried and powdered sample of mycorrhizal and non-mycorrhizal roots from each treatment maintained in field and pot cultures was taken into a 50ml conical flask separately. 0.25ml of toluene, 4ml of MUB (Modified universal buffer, pH 6.5 prepared as described by Skujins, *et al* (1962) and 1ml of 0.115M p-nitrophenyl phosphate (PNP) solution were added to the flask. The flasks were shaken well to mix the contents and incubated at 37°C for 1h. After incubation the cotton plugs were removed and 1ml of 0.5M calcium chloride and 4ml of 0.5M sodium hydroxide solution were added. The slurry was filtered through Whatman filter paper (No 1). The optical density of filterate was measured at 430 nm wavelength on spectrophotometer (Hitachi 220). For the control similar procedure was followed without the root sample.The concentration of phosphatase activity in terms of p-nitrophenyl in each sample was calculated by a standard curve of PNP in water and was expressed as mg p-nitrophenol released per gram of dry sample per hour.

The data was processed by analysis of variance (ANOVA) and significant differences among means were identified with Duncan's multiple range test at P = 0.01 and 0.05 level. Correlation coeffecient was also calculated.

4. Effect of heavy metals on establishment and growth of pine seedlings

To study the effect of heavy metals on the establishment and growth of pine seedlings for both field and pot experiment the same procedure was followed as mentioned earlier. 3 seedlings from each plots were harvested every four months after heavy metal treatment and were brought to the laboratory for observations. Roots of seedlings were washed under running water and the percentage of infection of ectomycorrhizal short roots was determined by the method of Beckjord *et al*(1984). Shoot height, root length, root collar diameter, needle length, number of needles were measured every 4 months. The study was continued for one year. The seedlings volume was calculated as [(root collar diameter)^{2}X height or D^2H] (Marx,1983). Percentage of survival of seedlings was determined by the following formula:

$$\text{Survival of seedlings (\%)} = \frac{\text{Total number of seedlings at the time of harvesting Survival}}{\text{Total number of seedlings at the time of germination.}} \times 100$$

The population of ectomycorrhizae/cm was calculated by the following formula (Sharma, 1981).

$$\text{Mycorrhizae \%} = \frac{\text{Total number of mycorrhizal rootlets}}{\text{Total number of rootlets}} \times 100$$

5.Effect of heavy metals on the uptake of NPK by mycorrhizal and non-mycorrhizal seedlings

To study the effect of heavy metals on the uptake of NPK by mycorrhizal and non-mycorrhizal seedlings in both field and the pot experiment the same procedure was followed as mentioned earlier.

The seedlings were harvested. Shoot and root dry weight were determined by drying them in the oven at $80^{\circ}C$ for 24 h to determine their biomass. Analysis of different elements in dried seedlings sample was done.

(a)Determination of total nitrogen

Kjeldahl's digestion method was followed to determine the total nitrogen in plant tissue as desribed by Mishra (1968). 0.2g of powdered and sieved seedling tissue (.2mm mesh) was taken in a 100 ml micro-Kjeldahl flask. To it, 6ml of concentrated sulphuric acid and one Kjeltab were added. The digestion was carried out in a digestion unit. At the end of digestion when the colour of the solution turned milky white the heating was stopped and flasks were allowed to cool. The content was diluted with 50 ml distilled water in a volumetric flask.

Distillation was done in Kjeldahl distillation set, with 10 ml digested solution and 10ml of 40% NaOH. Distillation was continued for 15-20 minutes and the distillate was collected in a beaker with 5 ml boric acid indicator (100g boric acid in 10 litre of distilled water +100 ml bromocresol green i.e, 100 g in 100 ml of methanol, + 70 ml of methyl red i.e, 100 mg in 100 ml methanol). The distillate collected in the beaker (about 50 ml) was titrated against N/14 HCl. Percentage of total nitrogen was calculated by the following formula:

$$N(\%)=\frac{\text{T-blank X Solution volume (ml)}}{\text{10 X aliquot volume(ml)X sample weight (g)}}$$

(b) Determination of total phosphorus and potassium

For the estimation of total phosphorus and potasium in plant tissue the wet triacid digestion procedure was followed as sugested by Allen (1974). 0.2g of powdered plant material was taken into 100ml digestion flask. Thereafter, 1ml of 60% $HClO_4$, 5ml $HNO_3$and 0.5ml H_2SO_4 were added. The flasks were swirled gently to mix powder with acid solution. The mixture was digested for a period of 40 minutes till the material became colourless. The digested mixture was cooled and diluted to 100 ml with distilled water and filtered through Whatman filter paper (No. 1). The filtrate was used for estimation of phosphorus and potassium.

Phosphorus was analysed by molybdenum blue method (Jackson, 1973). 5 ml of digested sample was pipetted out and transferred to 50 ml volumetric flasks. 2 ml of ammonium molybdate and 1 ml of stannous chloride were mixed and total volume was prepared upto 50 ml by adding double distilled water and left for 30 minutes. The optical density of the solution was measured on a spectrophotometer (Hitachi 220) at 700 nm wave length and converted into known units through standard calibration curve and calculated into percentage phosphorus by the following formula:

$$P(\%) = \frac{C\ (mg) \times \text{Solution volume (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample weight (g)}}$$

where C = mg P obtained from the graph.

For the analysis of the potassium, the filtrate was directly read out in flame-photometer (Jackson, 1973), and converted into a known unit through standard curve and percentage potassium was calculated as per the following formula:

$$K(\%) = \frac{C\ \text{(ppm) from graph} \times \text{solution volume (ml)}}{10^4 \times \text{Sample weight (g)}}$$

where C = ppm of K obtained from the solution.

The data was processed by analysis of variance (ANOVA) and significant differences among means were identified with Duncan's multiple range test at P = 0.01 and 0.05 level. Correlation coeffecient was also calculated.

RESULTS

Results

Effect of heavy metals on the synthesis of ectomycorrhizae

Modified Melin Norkhran's medium (MMN) was used for culture of symbiont and growth of ectomycorrhizal fungi. Four ectomycorrhizal fungi were isolated and tested for the synthesis of mycorrhizal association .*Cenococcum graniforme* isolated from sclerotia while *Boletus* sp., *Scleroderma aurantium* and *Suillus luteus* were isolated from sporocarps (Table 1.1). The morphological characters of sporocarps (Plates 1 i,ii) and radial growth were used for identification of fungi using the keys outlined by Singer (1962, 1975). The characteristic features of fungi on MMN media are described here.

Cenococcum graniforme: Fungal colony was black and rough in texture, round with regular margin; aerial hyphae was absent and growth of fungus was slow, wet colony on the upper surface, mycelia were black in colour, compactly interwoven, formed sclerotia under dried condition, hyphal size 8.3 ± 0.31 µm; mycorrhizae black with digitate rough texture.

Scleroderma aurantium: Peridium thick, split into polygonal scales, coarse in texture; sub-globose; gleba blackish; strong odour; spores brown, carpophore diameter 7-10 cm, spiny, 4-9 µm. Fungal colony grey rough in texture, mycelia closely interwoven, aerial hyphae absent, warty appearance with irregular margin, hyphal size 7.5- ±0.26 µm; mycorrhizae dichotomously branched and white to yellow with smooth texture.

Boletus sp: Sporophore 5-10 cm high; cap 5-7 cm, convex, smooth,

Plate 1: (i) Sporocarps of *Suillus luteus*.
(ii) Sporocarps of *Scleroderma aurantium*.

PLATE 1

(i)

(ii)

viscous in damp weather, pale yellow in young stage and light brown towards maturity; pore small; circular; stipe smooth; flesh white and smooth; odour pleasant; spore olive brown, fusiform, 4-6 µm. Fungal colony light brown; aerial hyphae present; mycelium closely interwoven; hyphal size 9.1 ± 0.38 µm; mycorrhizae coralloid and creamy with smooth texture.

Suillus luteus: Sporophore 4-8 cm high, cap size 5-6 cm, convex, presenting conical appearance; margin regular with partial velar remains; cuticle viscous in damp weather, greyish brown; pore tiny polygonal. Fungal colony light brown with aerial hyphae; mycelium closely interwoven; hyphal size 9.4 ± 0.2 µm; mycorrhizae white and dichotomously branched with smooth texture.

The mating type and mycelial growth rate are summarised, (Table 1.1) indicating the relative size of colonies, sector formation, zones of migration of hyphal development.

Structural characteristics

Observation of ectomycorrhizal and non-mycorrhizal short roots by electron microscopy showed variation in the cells due to high concentration of metals. Cytological characteristics of the mycorrhizal fungus altered with increased concentration of Cd, Cu, Pb, Zn, Ni and Al. The fungal cytoplasm appeared more disrupted at 200 ppm and 500 ppm of metals. The hyphae penetrated the root directly, with comparatively few infection points, between the anticlinal walls of epidermal cells or through the root hairs across their distal or basal part. Dense fungal structures are formed mainly in the inner layer forming branches penetrating into deeper cortex (Plate 1 a). White fungal patches, interwoven strands were formed extending out from mycorrhizal

Table 1.1 Source, place of collection and host of the ectomycorrhizal fungi.

Ectomycorrhizal fungi	Source of isolation	Place of collection	Host
Cenococcum graniforme	Sclerotia	NEHU campus pine forest	*Pinus kesiya*
Scleroderma aurantium	Sporocarp	NEHU campus pine forest	*Pinus kesiya*
Boletus sp.	Sporocarp	NEHU campus pine forest	*Pinus kesiya*
Suillus luteus	Sporpcarp	NEHU campus pine forest	*Pinus kesiya*

Table 1.2 Morphological characteristics of mycorrhizae formed by different ectomycorrhizal fungi.

Ectomycorrhizal fungi	Morphology of mycorrhizae	Colour of mycorrhizae	Texture of mycorrhizae
Cenococcum graniforme	Digitate type	Black	Rough
Scleroderma aurantium	Dichotomously branched	White to yellowish	Smooth
Boletus sp	Coralloid type	Creamy	Smooth
Suillus luteus	Dichotomously branched	Yellowish white	Smooth.

mantle (Plate 1 b). Several fruiting bodies were formed and hyphal strands were clearly visible. *Scleroderma aurantium* colonised the root withen 6-7 weeks and formed extensive extramatrical mycelium (Plate 1 c) and thick mycelial strands were developed in all root chambers but were lacking in seedlings with higher concentrations of metals. *Boletus* sp. was also a rapid colonizer. The ectomycorrhizal mycelium, however, was very diffused particularly in the early stages of colonization. *Suilus luteus* and *Cenococcum graniforme* colonised the root intensively after 4-6 weeks (Plate 2 a). Growth of these species was more visible from the early stage of seedling growth after intensive colonization of the root the growth of mycelium slowed down, particularly of *C. graniforme*. However, small strands were formed during last period specially on the plants treated with 10 ppm and 50 ppm of heavy metals.

The effect of Cd and Ni on the mycorrhizal fungi was diverse. Some species seemed to be extremely sensitive whereas others remained unaffected, as measured by the presence of the living extramatrical mycelium at the end (Plate 2 b). Although, these fungi were seriousely damaged by addition of high concentration of heavy metals, however, the mycorrhizal association did not disappear completely from the root system (Plate 2 c). The density of mycelium greatly reduced in comparision with heavy metals untreated control mycorrhizal plants. Dead mycorrhizal mycelium was quite often colonised by saprophytic micro-organisms, which may increase the values of fungal biomass.

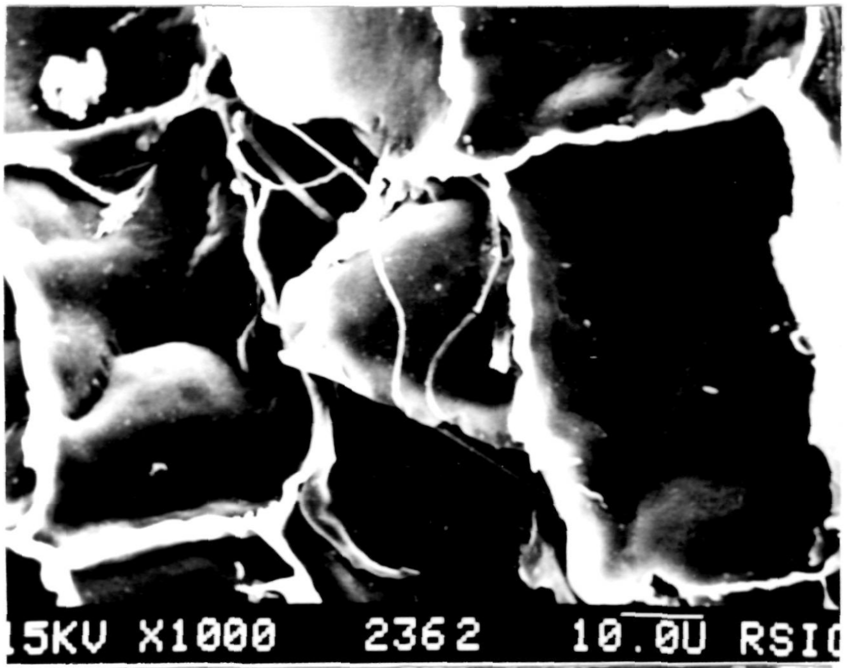
The effect of Cu, Pb and Ni on the mycorrhizal fungi was variable. In some plants the mycelium was able to persist and to plate 2 (a) Infected with *Cenococcum graniforme* and treated with Al 10 ppm showing interwoven mycelial strands.

(b) Infected with *Suillus luteus* treated with Ni 10 ppm showing extramatrical mycelium.

(c) Infected with *Boletus* sp. and treated with Al 500 ppm showing damaged by Al toxicity.

PLATE 2

a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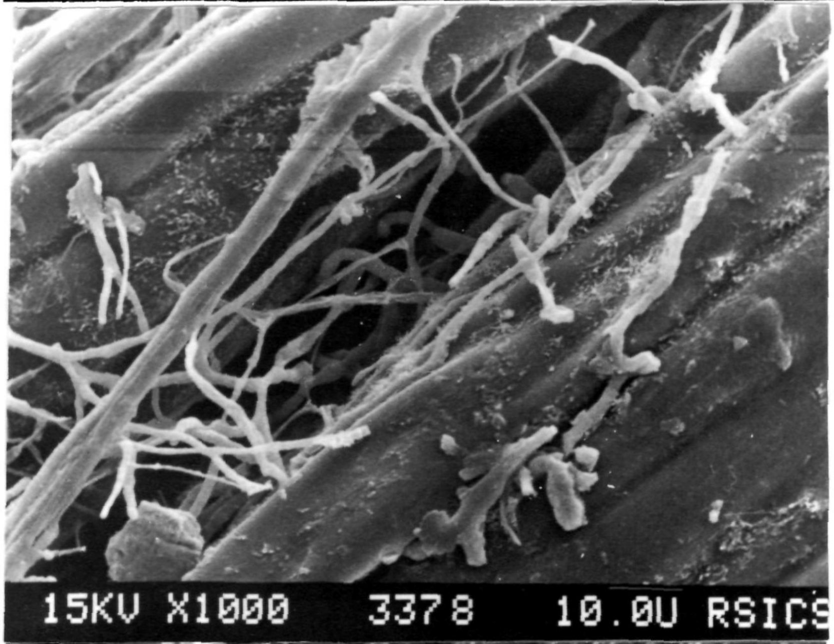
b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c

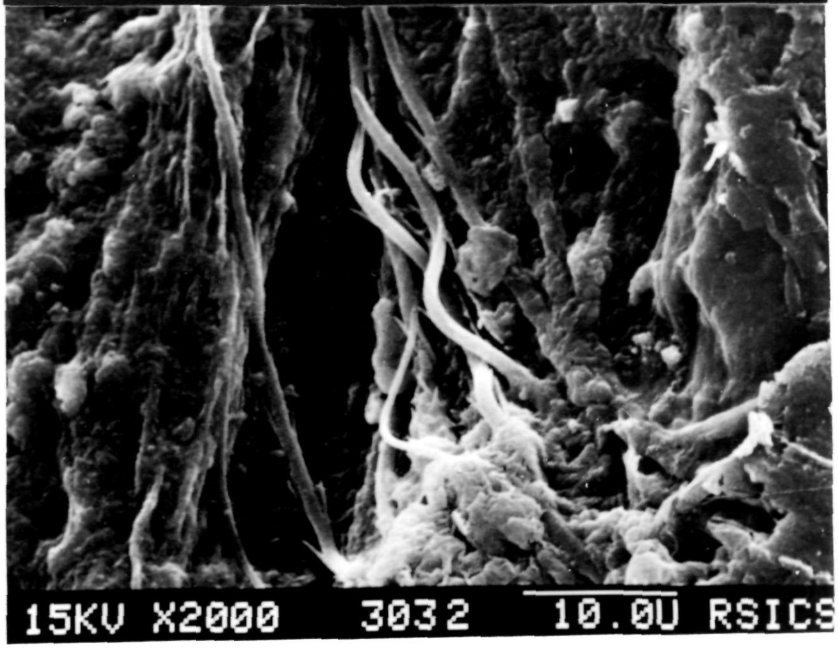

15KV X2000 3032 10.0U RSICS

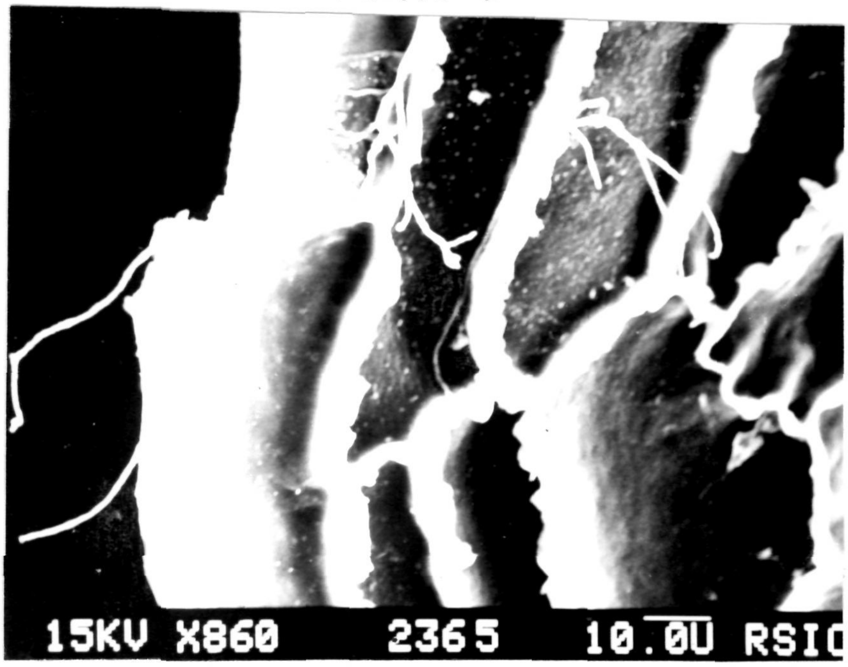
Plate 1 (a) Infected with *Suillus leutus* and treated with Zn 10 ppm showing dense fungal structures penetrating into deeper cortex.

(b) Infected with *Boletus* sp. and treated with Cd 10 ppm showing mantle (M) and interwoven hyphae (H).

(c) Infected with *Scleroderma aurantium* and treated with Cu 10 ppm showing extensive extramycelial strands penetrating into deeper cortex.

PLATE 1

a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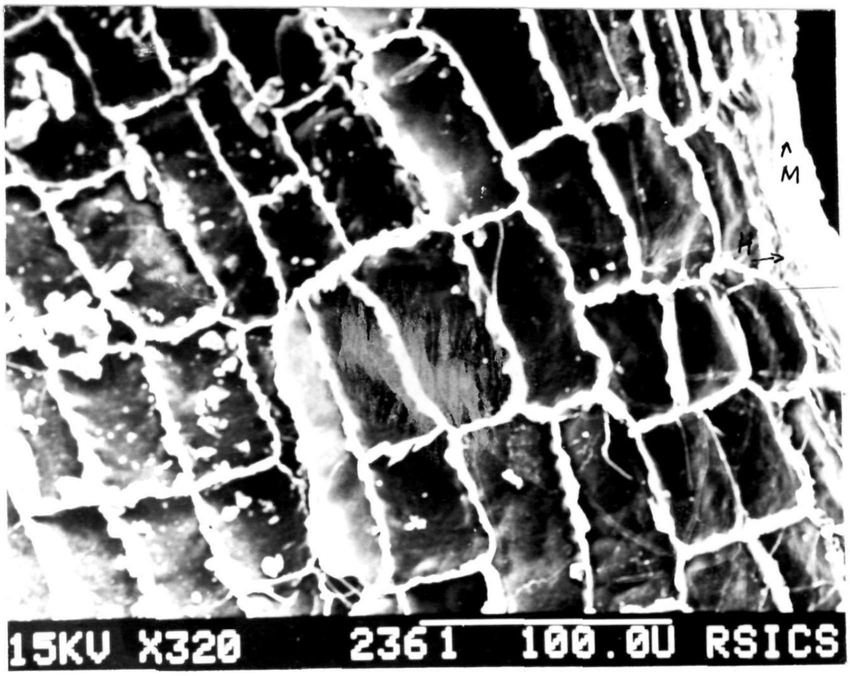
M
H
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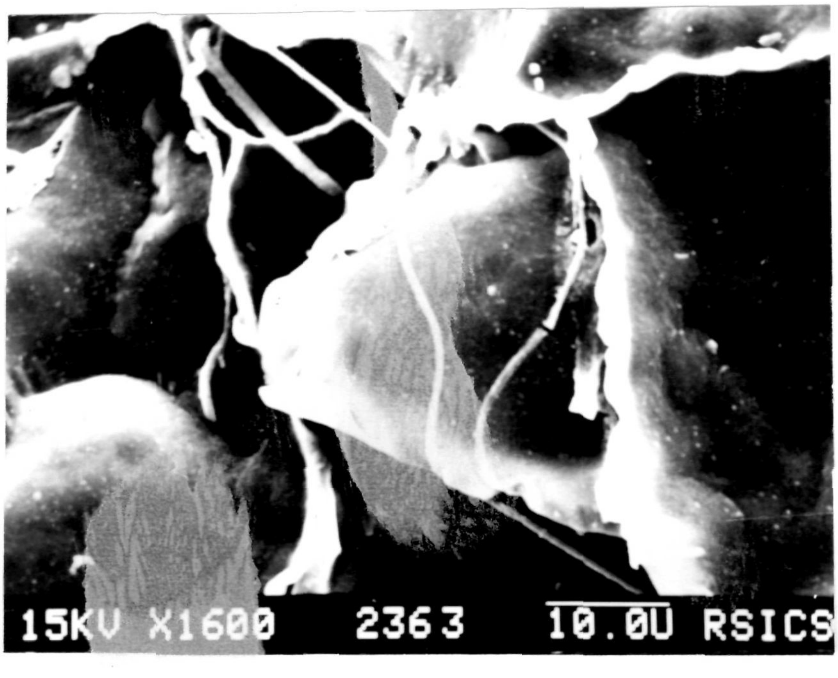
c


15KV X1600 2363 10.0U RSICS

recover somewhat whereas in others the condition of the mycelium did not ameliorate (Plate 3 a).

In case of Al treated seedlings visual symptoms of Al toxicity in non-mycorrhizal roots such as thickened and dark root tips were seen at all concentration of 50 ppm and above. In ectomycorrhizal roots these symptoms occurred only in small part of the root systems that had not become mycorrhizal. Although mycorrhizal infection was observed at all concentrations in mycorrhizal roots, short root bifurcations were less at 100 ppm and above than at lower concentration resulting in less penetration of fungi inside the cortical cells. The ectomycorrhizal and non-mycorrhizal roots exposed to high dose of heavy metals had all distorted cytoplasm (Plate 3 b).

The presence of Pb (50 ppm and above) also significantly reduced mycorrhizal infection. Level of Cd and Ni (100 ppm and above) checked the mycorrhizal formation on seedlings root system and significantly decreased ectomycorrhizal infection by *S. luteus*, *Boletus sp*, *S. aurantium* and *C. graniforme* in association with *P. kesiya*. Ectomycorrhizae were not observed on the non-inoculated seedlings in the control sets. Stunted seedlings which resulted due to high concentration of heavy metals had several cells thick of electron dense cells through which there was no penetration of hyphae. The fungal cells had thin walls, and there was no Hartig net. This was in marked contrast to untreated heavy metals seedlings, in which the electron dense cells were widely scattered and fungal hyphae readily entered the cortex of the host to form a well developed Hartig net (Plate 3 c). These morphological and cytological effects may be due to the toxic

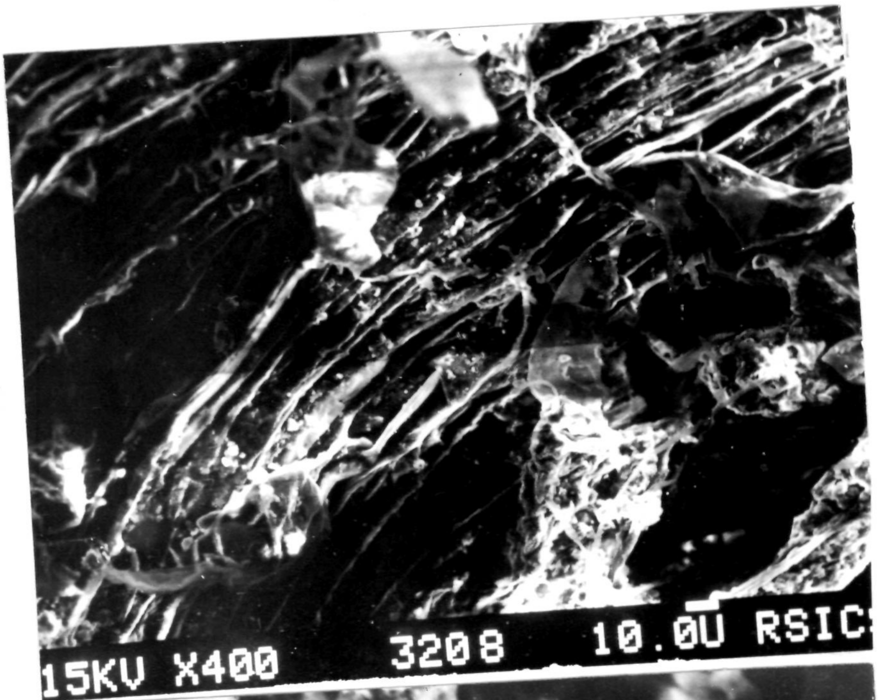
Plate 3 (a) Infected with *Cenococcum graniforme* and treated with Ni 500 ppm showing distorted cytoplasm.

(b) Non-mycorrhizal root exposed to Pb 500 ppm showing damaged by Pb toxicity.

(c) Control ectomycorrhizal root showing well developed Hartig net and mantle.

PLATE 3

a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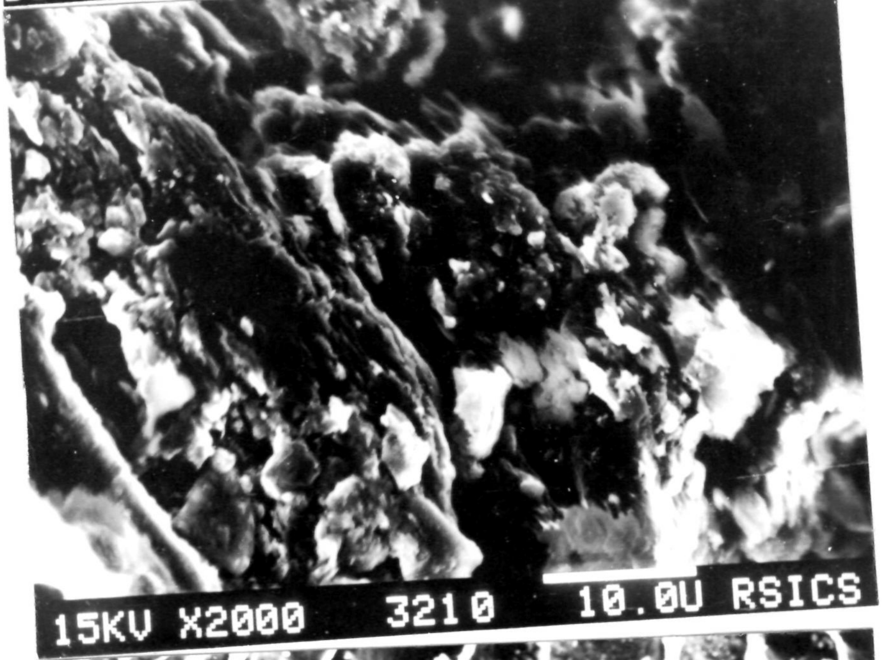
b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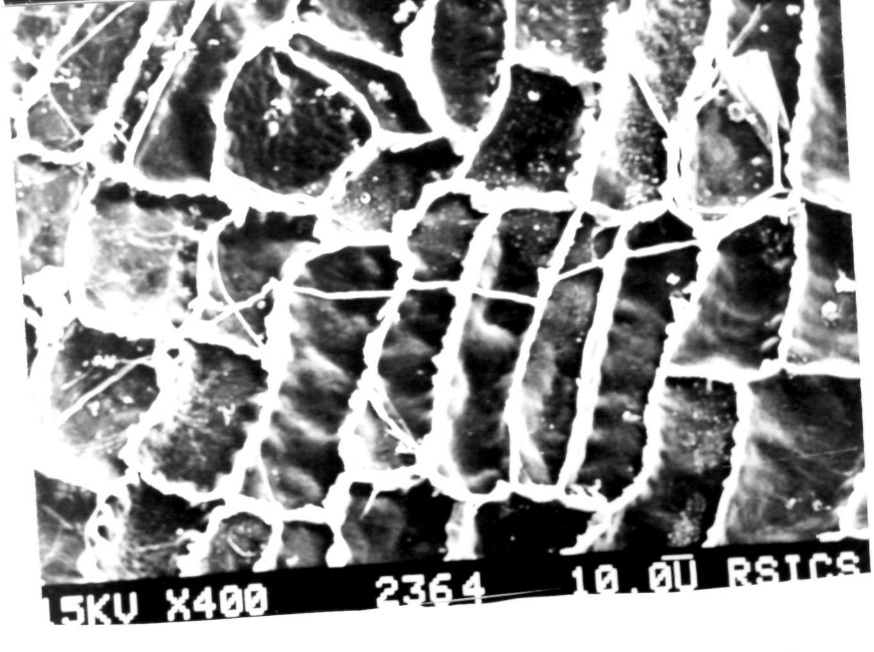
c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nature of Cd, Pb, Ni, Zn, Cu and Al. The inhibition of specific enzymes that are functional in forming Hartig net and hyphal development during mycorrhizal synthesis were suprressed. This observation on cortex cells in the Hartig net association of mycorrhizae was quite unlike those treated with low concentration of heavy metals.

All the heavy metals induced cellular changes in the symbiont, appearing as a faint appearance of plasma membrane, increasing transparency and gradual in granulation of cytoplasm and swelling of mitochondria and cristae. These changes were first observed in the mantle.

The fungal mantle of the ectomycorrhizae varied from 23-26 µm in thickness and the hyphae of the Hartig net freguently penetrated between all cortical cells upto endodermis in case of 10 ppm and 50 ppm concentration heavy metal treated ectomycorrhizal seedlings roots. In case of seedlings treated with heavy metals of 100 ppm and above of Ni, Zn, Pb, Cu, Cd and Al, the fungal mantle was from 11 to 18 µm in thickness, and the hyphae of Hartig net rarely penetrated deeper between the first layer of cortical cells.

Accumulation of heavy metals in ectomycorrhizae of pine

Accumulation of Zn, Ni, Cd, Cu, Pb and Al in ectomycorrhizal and non-mycorrhizal roots was estimated (Table 2.1). Considerable differences were observed in the level of metals accumulated in the root tissue. The degree of accumulation became greater as concentration of Zn, Ni, Cd, Cu, Pb and Al increased in soil. Zn accumulation was highest followed by Cu. The concentration of Zn

Table 2.1. Concentration of heavy metals (ppm) in the root tissue of mycorrhizal and non-mycorrhizal roots.

Element concentration (ppm) in soil	Concentration of heavy metals in different mycorrhizal roots				
	M1	M2	M3	M4	Control
Zn 10	3.4	2.4	2.4	3.0	5.0
Zn 50	24.5	23.7	25.0	20.0	35.0
Zn 100	42.0	39.0	37.0	40.0	45.0
Zn 200	47.0	61.0	55.0	62.0	67.0
Zn 500	95.0	69.0	75.0	85.0	97.0
Ni 10	2.0	2.0	2.0	2.0	3.0
Ni 50	8.0	12.0	13.0	10.0	15.0
Ni 100	22.0	21.0	20.0	30.0	35.0
Ni 200	32.0	29.0	35.0	41.0	44.0
Ni 500	47.0	47.0	40.0	48.0	57.0
Al 10	2.9	2.4	2.1	2.8	5.2
Al 50	3.4	3.5	4.5	5.2	12.0
Al 100	20.0	14.0	13.0	18.0	28.0
Al 200	28.0	28.0	31.0	29.0	35.0
Al 500	52.0	52.0	43.0	40.0	55.0
Cd 10	2.0	1.0	1.0	1.0	3.0
Cd 50	3.0	3.0	2.0	2.0	4.0
Cd 100	7.1	7.0	5.4	4.9	8.1
Cd 200	15.0	9.0	8.3	8.3	15.8
Cd 500	20.0	21.0	17.0	12.0	30.0
Cu 10	5.1	5.8	5.3	4.9	6.1
Cu 50	19.0	10.0	21.0	13.0	27.0
Cu 100	30.0	24.0	23.0	21.0	37.0
Cu 200	39.0	26.0	27.0	26.0	45.0
Cu 500	61.0	45.0	45.0	40.0	85.0
Pb 10	1.6	1.6	1.5	1.4	2.1
Pb 50	2.0	4.0	4.0	2.9	7.0
Pb 100	4.5	10.0	10.0	8.0	12.0
Pb 200	14.0	15.0	15.0	17.0	20.0
Pb 500	30.0	20.0	22.0	20.0	38.0

M1 = *S. aurantium*. M2 = *Boletus* sp., M3 = *C. graniforme*. M4 = *S. luteus*.

Table 2.2 Analysis of variance (F) of various mycobionts and mycorrhizal and non-mycorrhizal with metal concentration and mycorrhizal infection

Source of variation	Variation between various mycobionts	Variation between mycorrhizal and non-mycorrhizal root tissue.
Metal concentration	NS	7.61*
Mycorrhizal infection	4.22**	4.5*

* = significant at p< 0.01 level
** = significant at p< 0.05 level

in roots increased with increasing concentration of Zn in the rhizosphere but such increase was reduced by ectomycorrhizal fungi. The results indicates that the presence of ectomycorrhizae has affect on the lowering of heavy metals in the root tissue. The concentration of metals in root was significantly lower in ectomycorrhizal roots than non-mycorrhizal ones at all levels.

It was found that Zn accumulated most in non-mycorrhizal roots (7 ppm), while minimum accumulation was found (2.4 ppm) in *Boletus* formed mycorrhizal roots. Highest accumulation of Cd was found in non-mycorrhizal roots (30 ppm) followed by *S.aurantium* mycorrhizal roots (25 ppm), minimum accumulation of Cd was found in *Boletus* sp and *S.luteus* inoculated roots (1.05 ppm each). Accumulation of Cd in root tissue was lowest than other heavy metals.

Uptake of Pb and its accumulation also varied at different levels of Pb treatments in ectomycorhizal and non-mycorrhizal seedlings. Maximum accumulation of Pb was observed in non-mycorrhizal roots (38 ppm) followed by *S.aurantium* treated seedlings (30 ppm) and minimum (1.4 ppm) in *S.luteus* treated seedlings. Highest accumulation of Cu was noticed in non-mycorrhizal roots (61 ppm), minimum accumulation of Cu was observed in *C.graniforme* inoculated roots (4.9 ppm). Maximum accumulation of Ni was observed in non-mycorrhizal seedlings (57 ppm) followed by *Boletus* sp. treated roots (47.3 ppm), while minimum accumulation was observed in *C.graniforme* treated seedlings (1.2 ppm). Accumulation of Al was also higher in non-mycorrhizal roots (55 ppm) followed by *Boletus* sp. treated roots (53.4 ppm), minimum accumulation of Al was observed in *C* *graniforme* treated roots (2 ppm). The accumulation of Cd, Cu, Zn, Ni, Pb and Al was more in non-mycorrhizal roots than ectomycorrhizal roots.

There was a significant variation ($P < 0.05$) in the metal concentration between various mycobionts and in mycorrhizal and non-mycorrhizal roots ($P < 0.01$) (Table 2.2).

Effect of heavy metlas on enzyme activities in rhizospheric soil and in roots of pine

Results showed that the enzyme activity was subjected to seasonal variation. The effect of heavy metals on urease, phosphatase and dehydrogenase activity differed considerably.

Dehydrogenase activity was apparently less at the high concentrations of Zn, Cu, Ni, Cd, Pb and Al. Addition of ectomycorrhizal fungi to seedlings increased the activities of dehydrogenase. Highest activity was observed during summer and minimum was observed during winter. Dehydrogenase activity was more in root region than in rhizospheric soil in all the four seasons. The seasonal fluctuations followed divergent pattern during the year. During summer months, dehydrogenase activity remained almost constant, but after September it decreased considerably and reached its minimum and during this period soil temperature and moisture of the soil were also lowest. During winter and spring months variation was low. After May the activity started to increase gradually and reached a peak in August. Application of heavy metals inhibited the activity of dehydrogenase. It could be inferred that adverse affect due to heavy metals was severe and prolonged. In *Boletus* sp inoculated seedlings Cu and Zn did not reduce the dehydrogenase activity significantly both in rhizosphere soil and in root region (Figs.3,6, 27,30). Highest percentage of inhibition of dehydrogenase activity Cd (23 %), Pb (25 %) and Ni (25 %) treated rhizospheric soil. In case of roots, Al and Cd (19 %) inhibited maximum activity than Zn, Cu, Pb and Ni treated roots. More inhibition of activity was observed (36 %) in Ni treated rhizosphere soil as compared to the inhibition in Al treated and infected with *C. graniforme* . The inhibition percentage of rest of the metals did not vary much. Among four ectomycorrhizal fungi, *S.aurantium* treated roots showed the least seasonal variation but had highest activity (Figs. 37-42).

In case of rhizospheric soil highest percentage of inhibition was observed in *S. aurantium* inoculated seedlings treated with Pb, Cd and Ni. Dehydrogenase activity in *S. luteus* inoculated rhizospheric soil and mycorrhizal roots showed almost the similar pattern at different metal treatments (Figs. 43-48, 55-60). Maximum inhibition of activity was observed in Al treated soil and roots. The non-mycorrhizal roots and its rhizospheric soil showed high percentage of inhibition of dehydrogenase activity. Zn and Ni caused upto 50% inhibition in rhizospheric soil wheras in roots Cu and Pb had caused 44 % inhibition in the enzyme activity. It was observed that in absence of ectomycorrhizae, Pb, Zn, Ni, Cd, Cu and Al drastically reduced the enzyme activity at higher concentration of metals.

Dehydrogenase activity was more in mixed mycobionts inoculated root surface and rhizosphere soil than non-mycorrhizal ones (Figs.61-72). In case of roots the presence of heavy metals did not appreciably affect the dehydrogenase activity. Highest percentage of inhibition in enzyme activity was observed in Al (39%) and Pb (31%) treated soil, where as Zn, Cd, Ni and Al showed an average of 10-15% inhibition in the activity. Dehydrogenase activity in rhizospheric soil was inhibited more by the presence of metals with an average percentage ranging from 35-40%. Maximum inhibition (38 %) was observed in Pb treated rhizosphere soil in contrast to Zn treated soil which had only 25% inhibition. In control, non-mycorrhizal roots activity was severly inhibited by Cd which showed upto 54 % inhibition and 46 % inhibition was observed in Zn and Pb treated rhizosphere soil. Zn and Cu treated roots showed 26 % and 39 % inihbition respectively. The effect of heavy metals on urease activity differed considerably. Inoculation of ectomycorrhizal fungi harboured increased activity of urease enzyme than uninoculated ones. The urease activity was apparently less at 100 ppm and above of Zn, Ni, Cd, Pb, Cu and Al treated pots. The activity of urease was less in rhizospheric soil as compared to the roots. During spring to summer urease activity remained almost constant but after September the activity started to increase gradually and reached its peak in autumn by contrast, in winter it decreased and remained constant throughout. The highest urease activity was observed in autumn. The monthly fluctuations of urease activity was stronger than dehydrogenase and phosphatase. Among the six metals studied Al, Cu and Zn were less inhibitory for urease activity as compared to Cd, Ni and Pb. Metals like Ni and Cd were found more toxic than the rest of the metals. Highest

Fig.1-6 Dehydrogenase Activity on Root Surface Inoculated with *Boletus sp.*

Formazan released mg/g dry wt/24 h

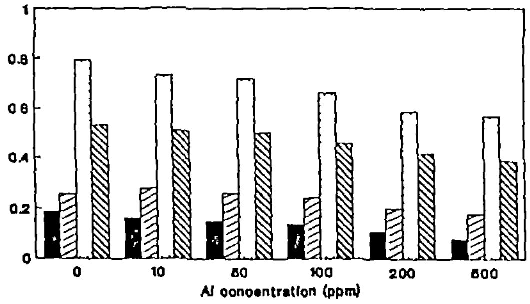


Fig.1

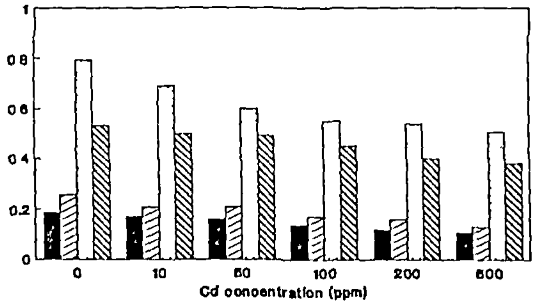


Fig.2

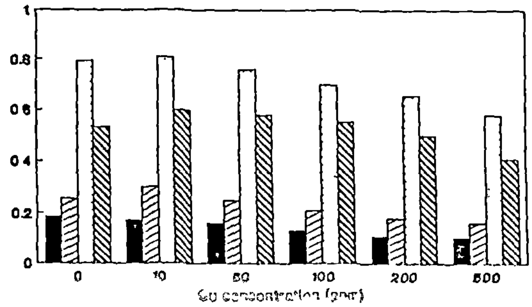


Fig.3

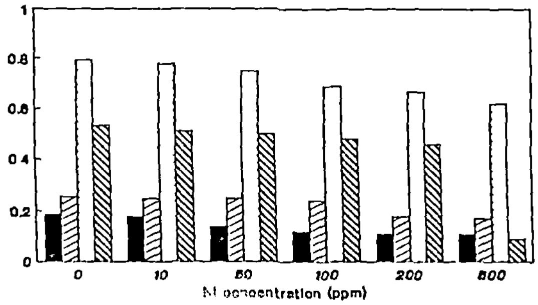


Fig.4

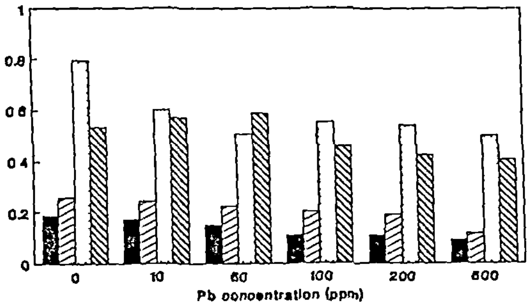


Fig.5

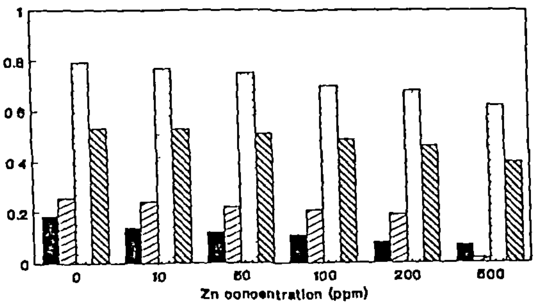


Fig.6

Fig.7-12 Dehydrogenase Activity on Root Surface Inoculated with *C.graniforme*

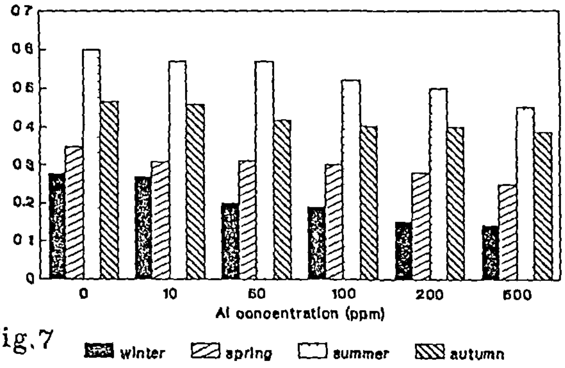


Fig.7

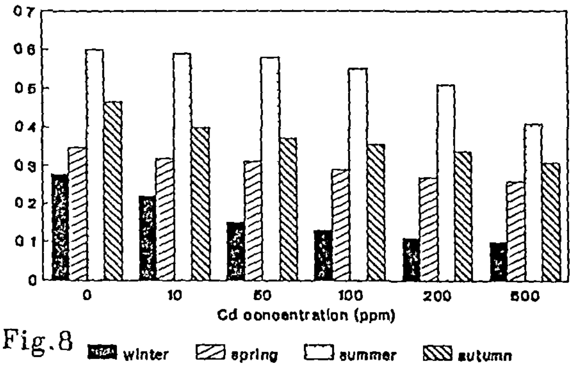


Fig.8

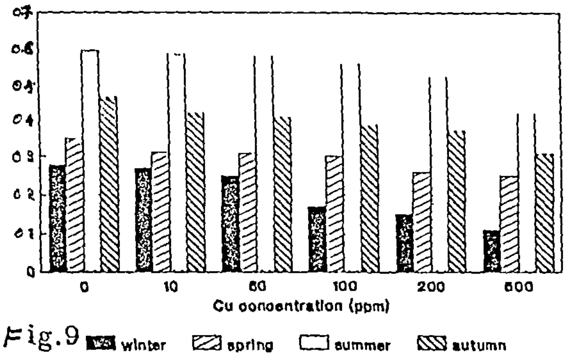


Fig.9

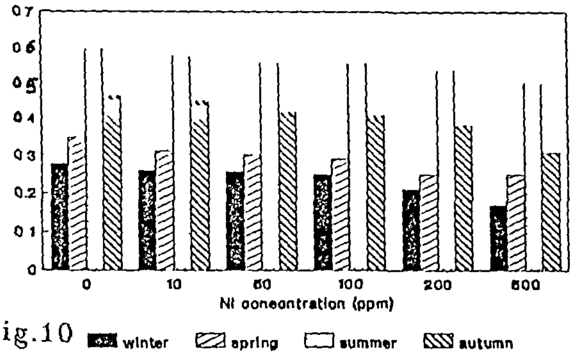


Fig.10

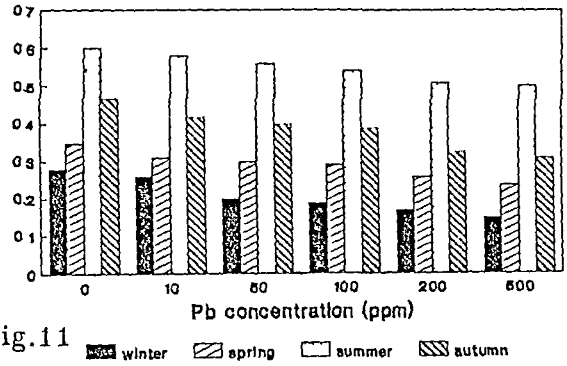


Fig.11

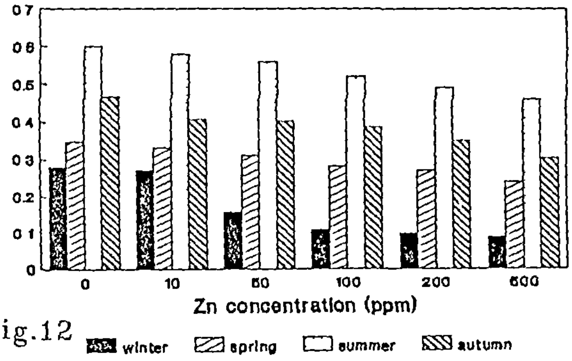


Fig.12

Fig.13-18 Dehydrogenase Activity on Non-mycorrhizal Root Surface

Formazan released mg/g dry wt/24 h

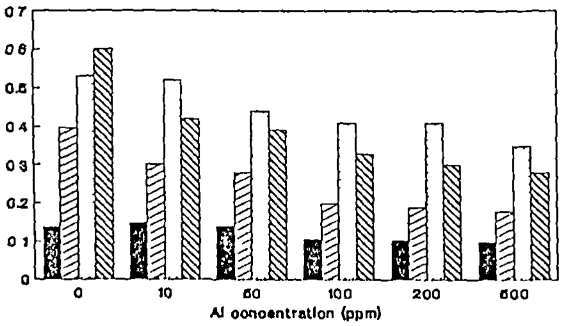


Fig.13

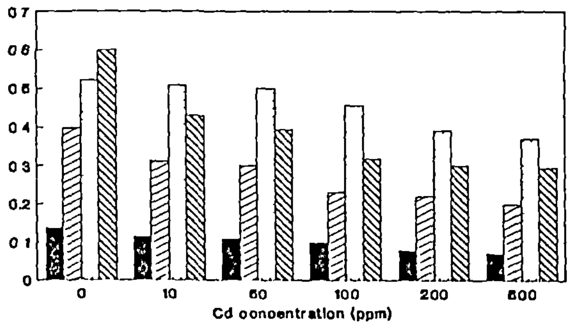


Fig.14

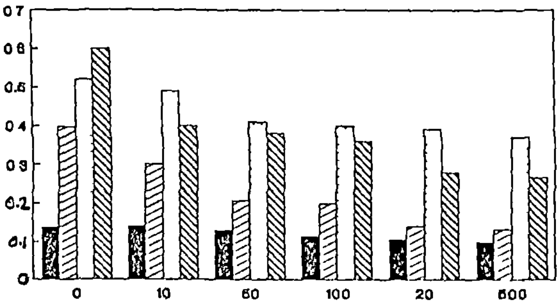


Fig.15

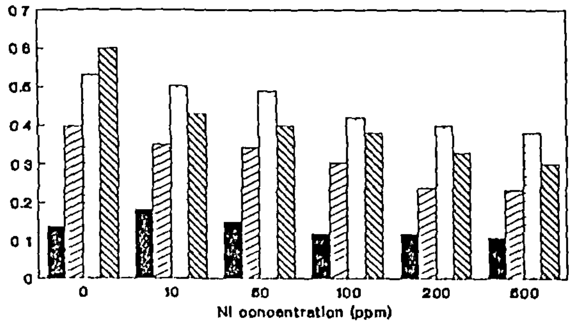


Fig.16

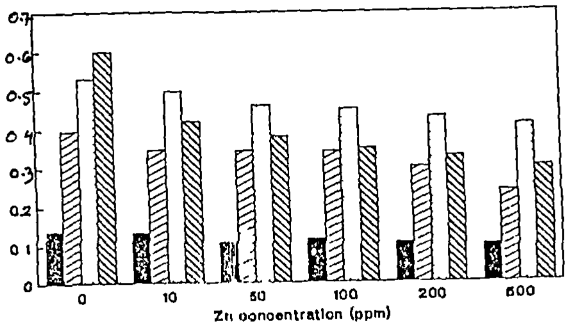


Fig.17

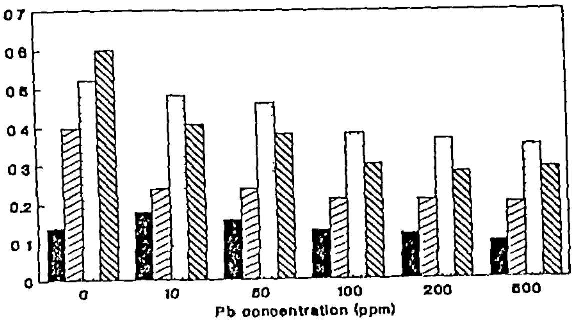


Fig.18

Fig.19-24 Dehydrogenase Activity on Non-mycorrhizal rhizospheric soil

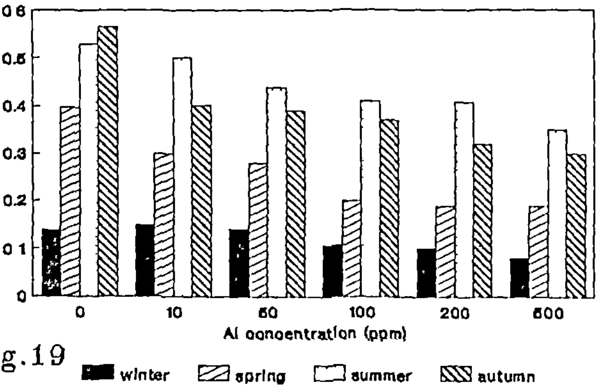


Fig.19

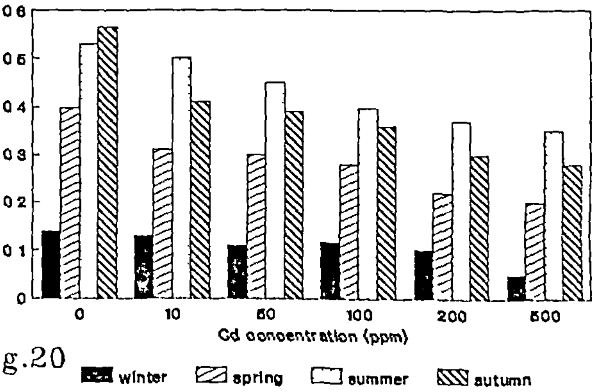


Fig.20

Formazan released mg g dry wt.24 h

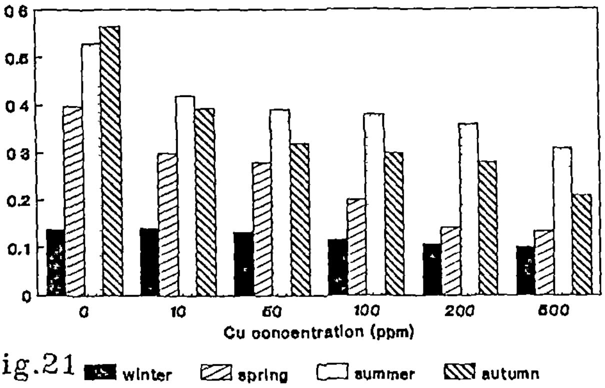


Fig.21

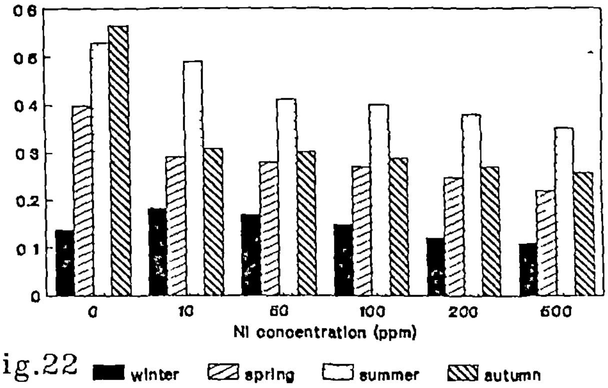


Fig.22

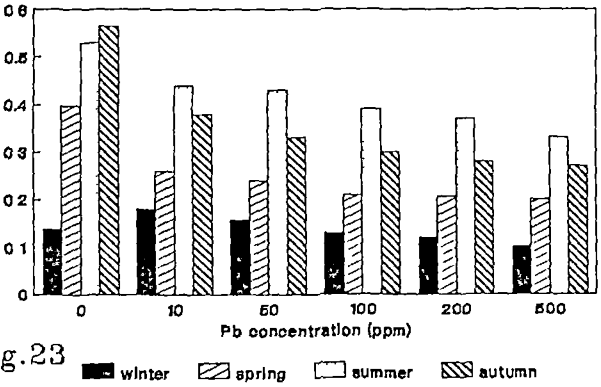


Fig.23

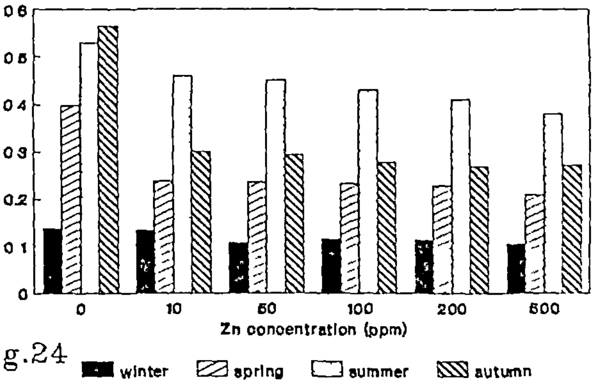


Fig.24

Fig.25–30 Dehydrogenase Activity on rhizospheric Soil Inoculated with *Boletus sp.*

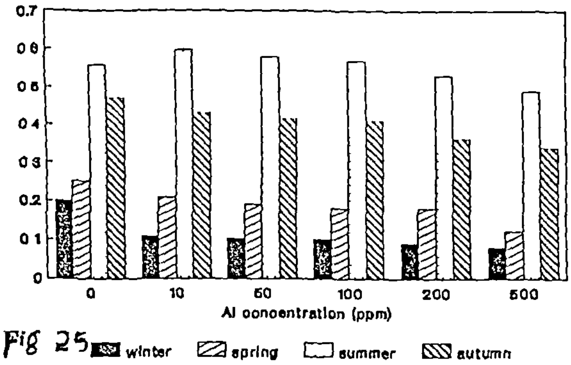


Fig 25

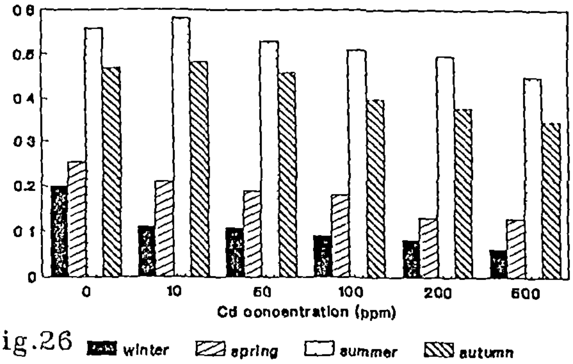


Fig.26

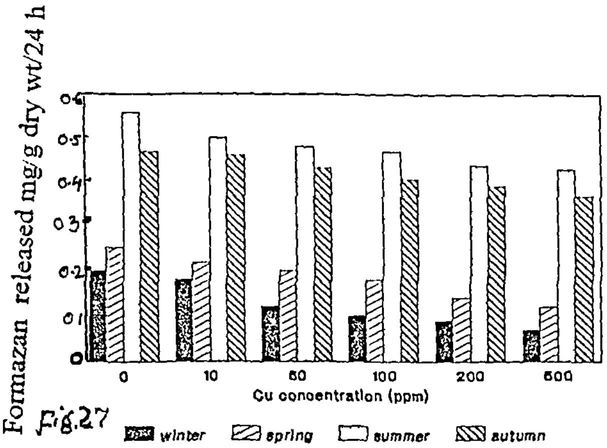
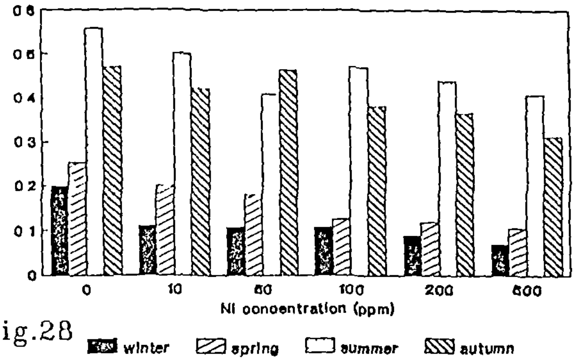


Fig.27



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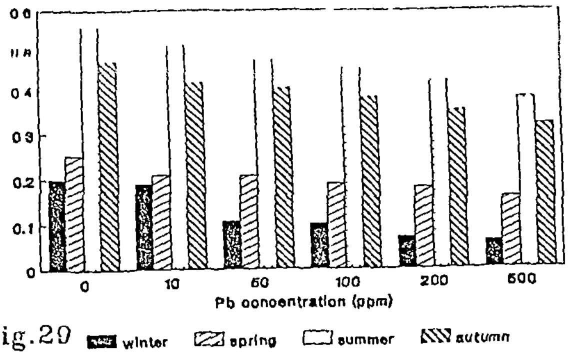

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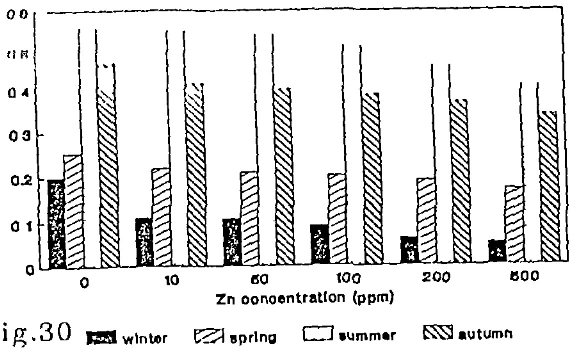


Fig.30

Fig.31-36 Dehydrogenase Activity on rhizospheric soil Inoculated with

C.graniforme

Formazan released mg/g dry wt/24 h

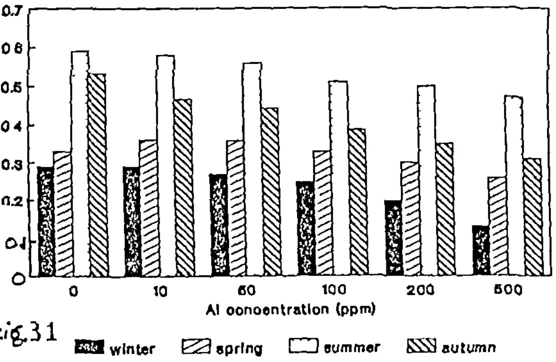


Fig.31

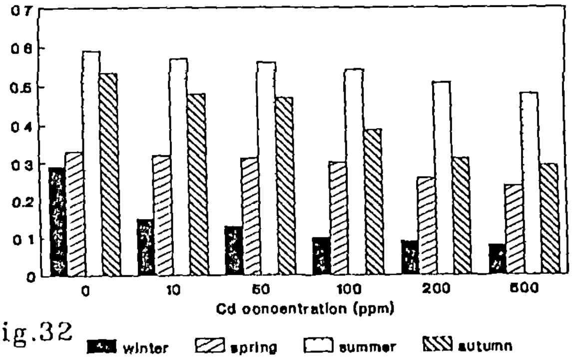
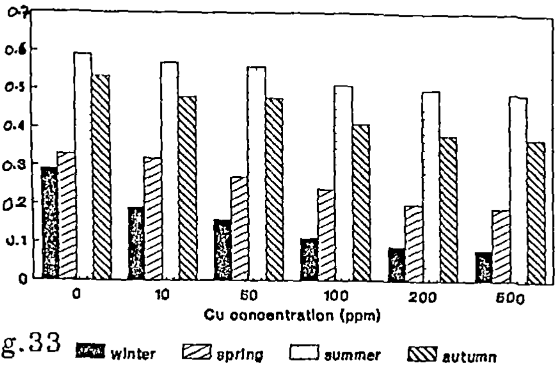


Fig.32



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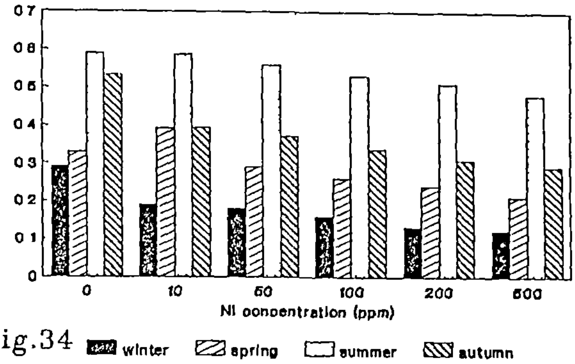
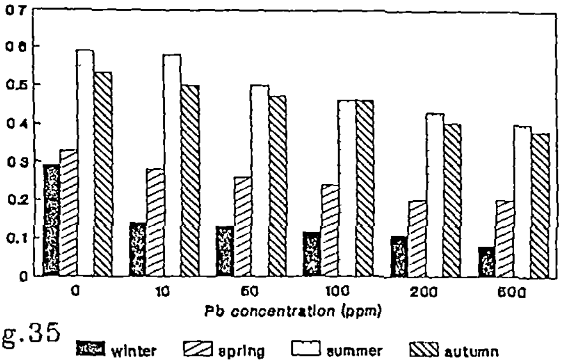


Fig.34



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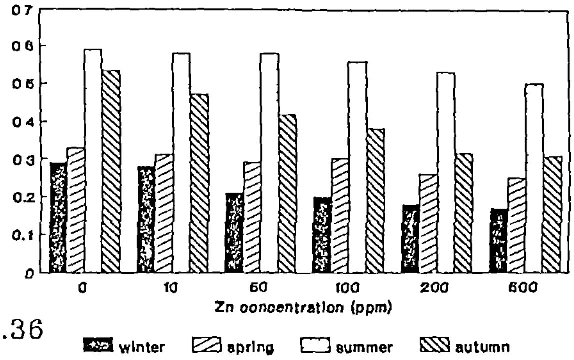
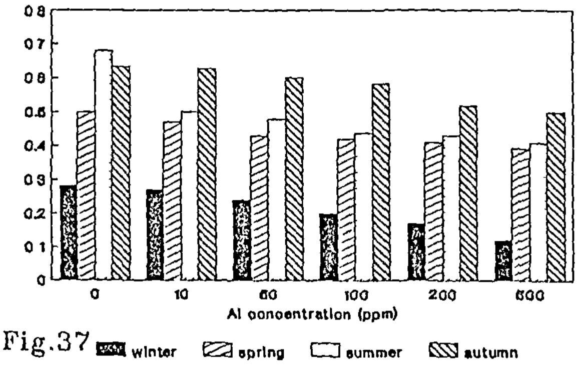


Fig.36

Fig.37–42 Dehydrogenase Activity on Root Surface Inoculated with *S.aurantium*

Formazan released mg/g dry wt/24 h



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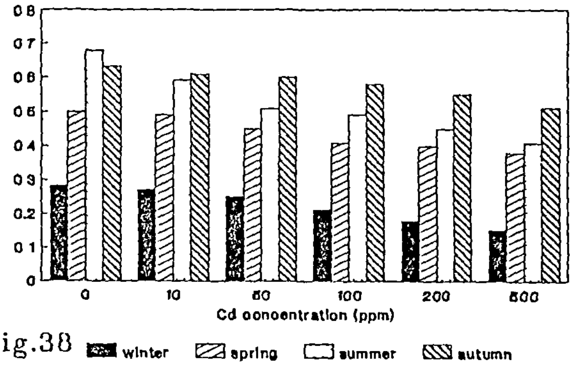

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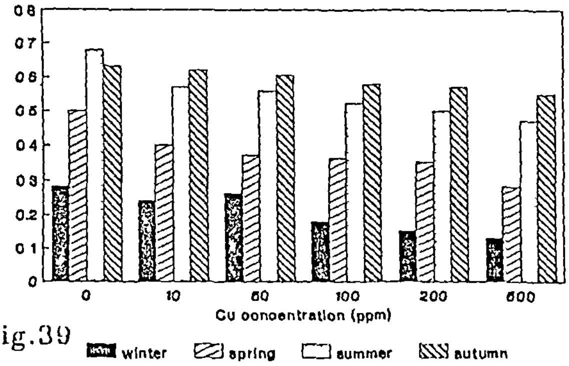

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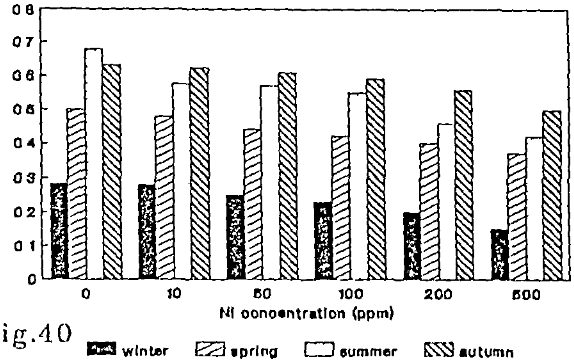

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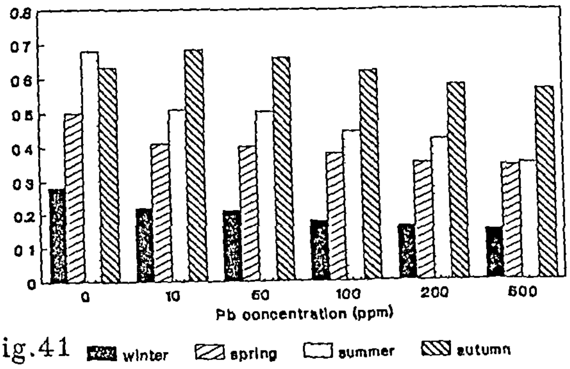

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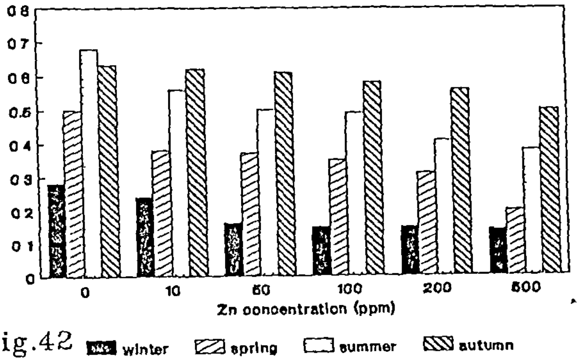


Fig.42

Fig.43-48 Dehydrogenase Activity on Root Surface Inoculated with *S.luteus*

Formazan released mg/g dry wt/24 h

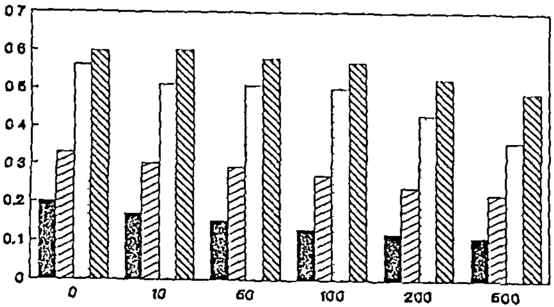


Fig.43

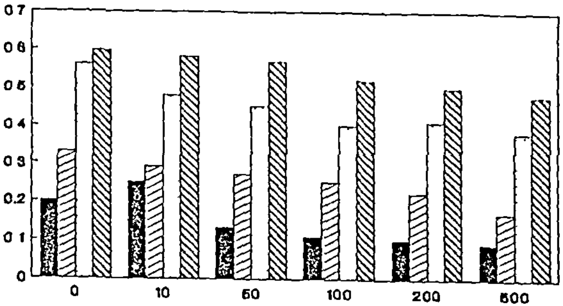


Fig.44

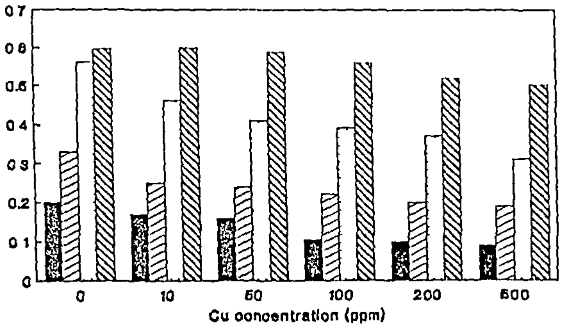


Fig.45

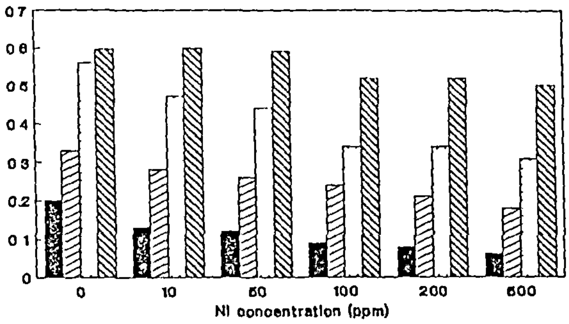


Fig.46

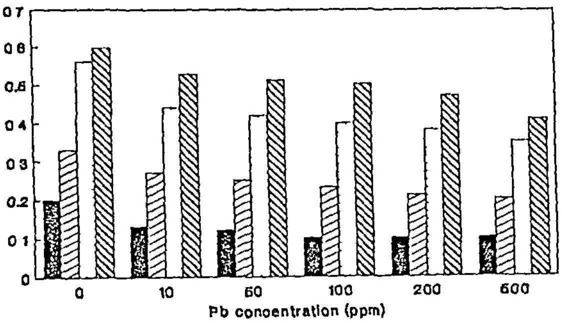


Fig.47

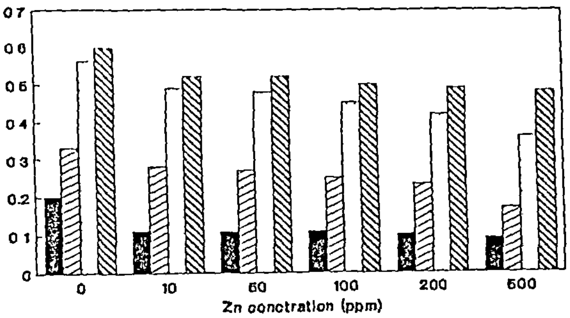


Fig.48

Fig.49-54 Dehydrogenase Activity on rhizospheric Soil Inoculated with *S. aurantium*

Formazan released mg g dry wt/24 h

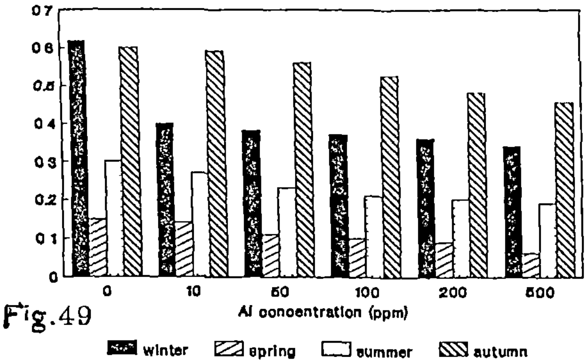


Fig.49

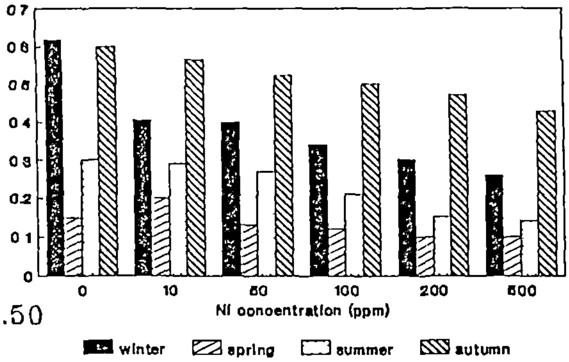


Fig.50

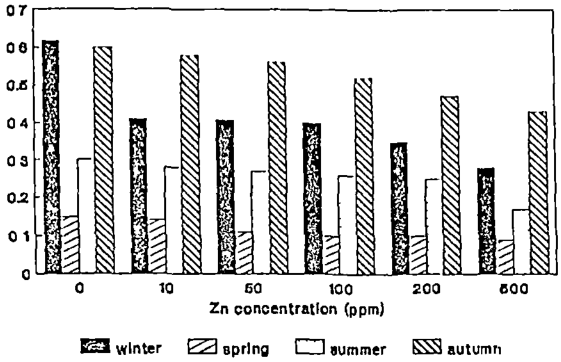


Fig.51

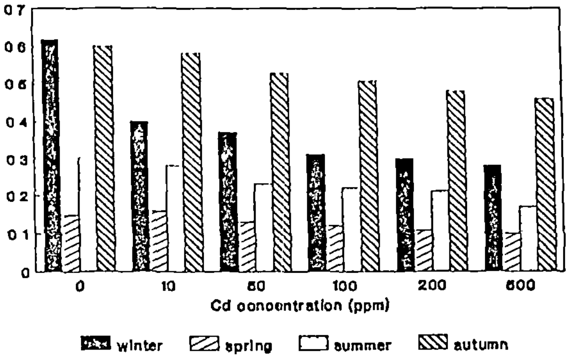


Fig.52

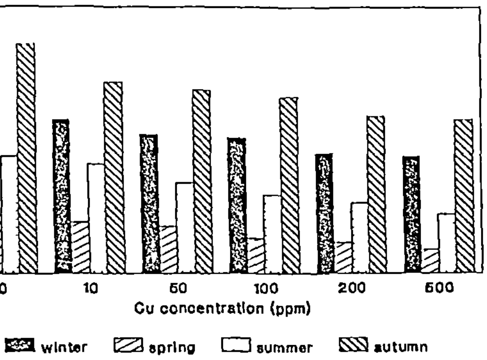


Fig.53

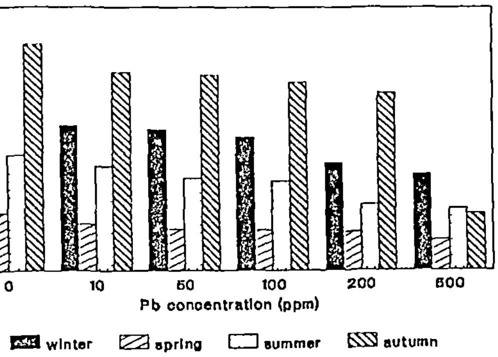


Fig 54

Fig.55–60 Dehydrogenase Activity on rhizospheric Soil Inoculated with *S.luteus*

Formazan released mg/g dry wt/24 h

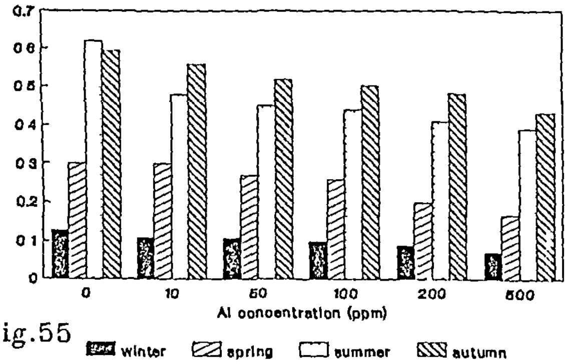


Fig.55

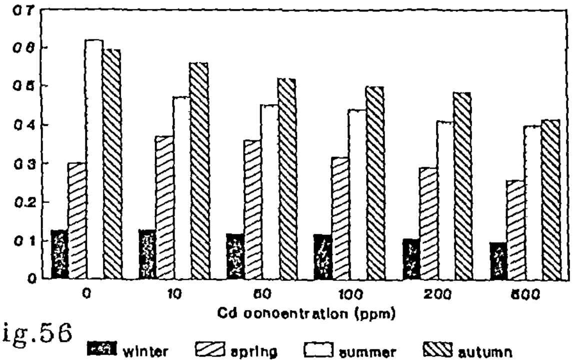


Fig.56

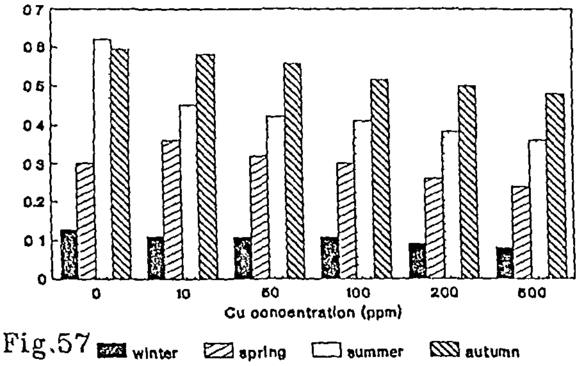


Fig.57

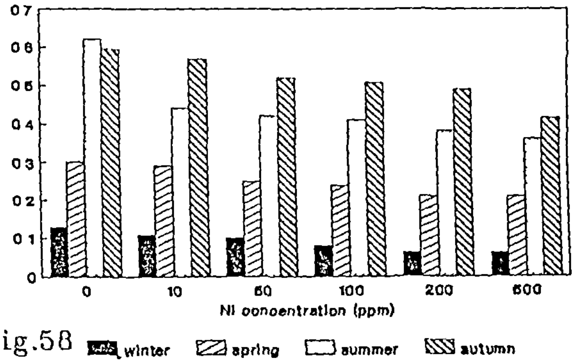


Fig.58

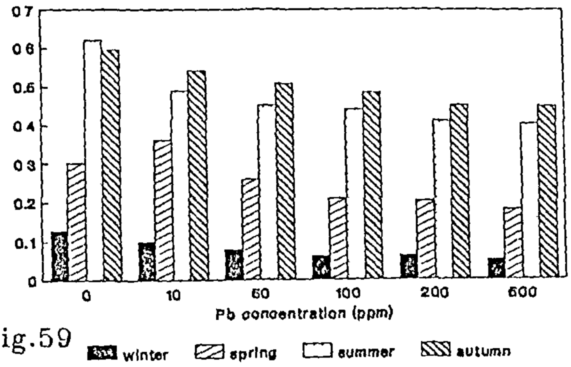


Fig.59

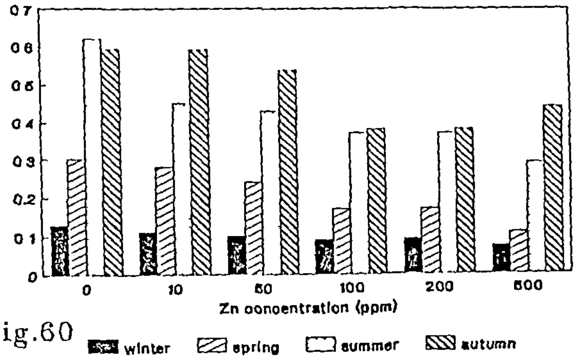


Fig.60

Fig.61–66 Dehydrogenase Activity on Root Surface Inoculated with mixed mycobionts

Formazan released mg/g dry wt/24 h

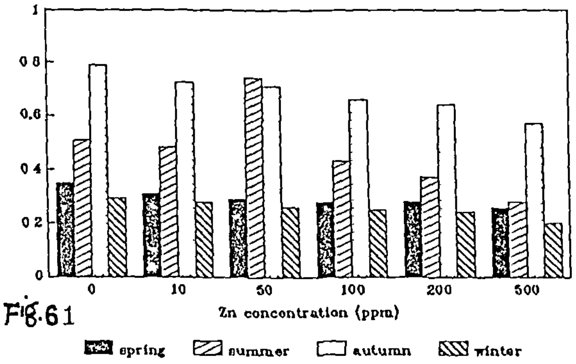


Fig.61

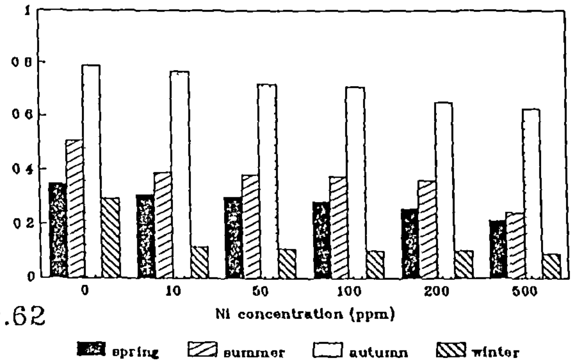


Fig.62

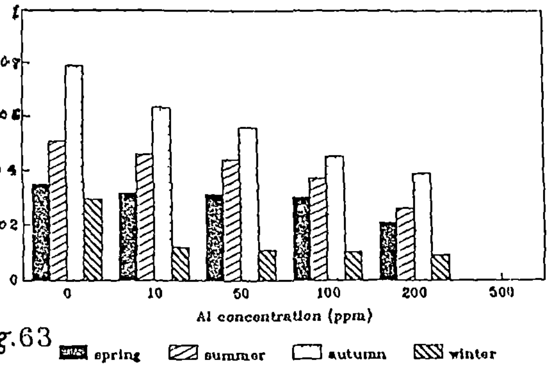
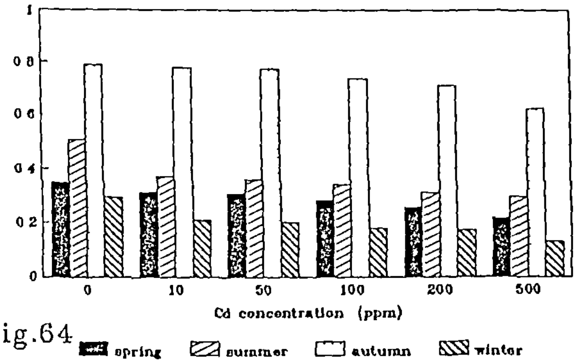


Fig.63



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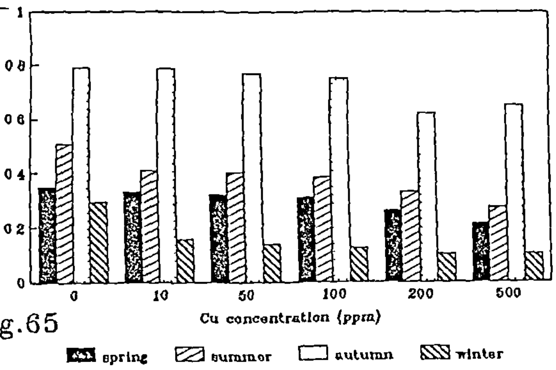


Fig.65

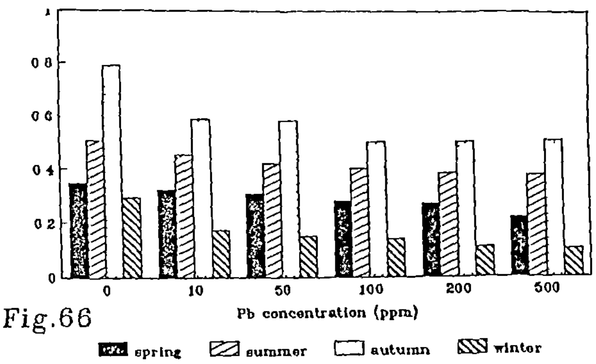


Fig.66

Fig.67-72 Dehydrogenase Activity on rhizospheric Soil Inoculated with mixed mycobionts

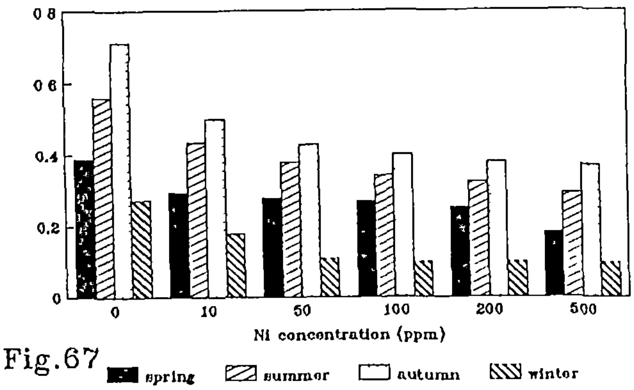


Fig.67

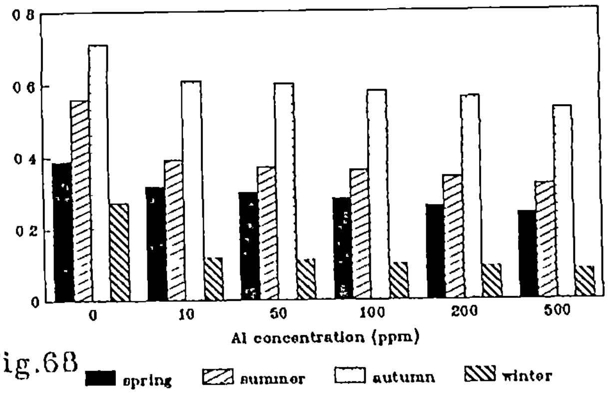


Fig.68

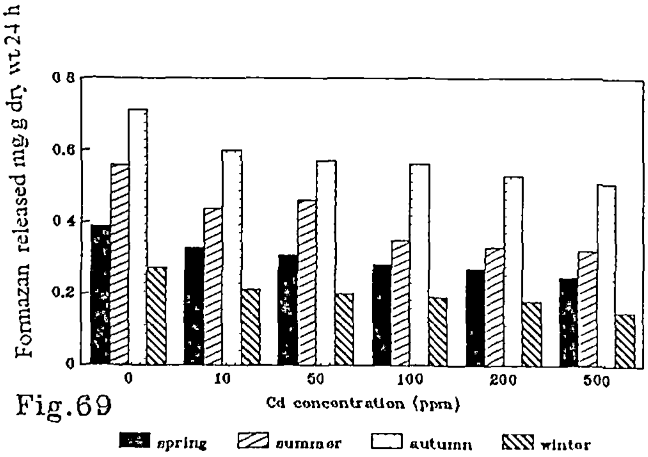


Fig.69

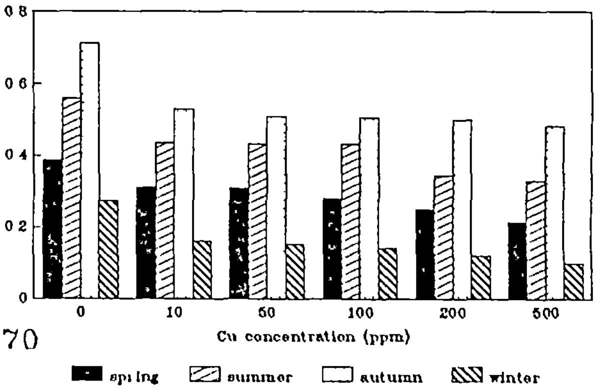


Fig.70

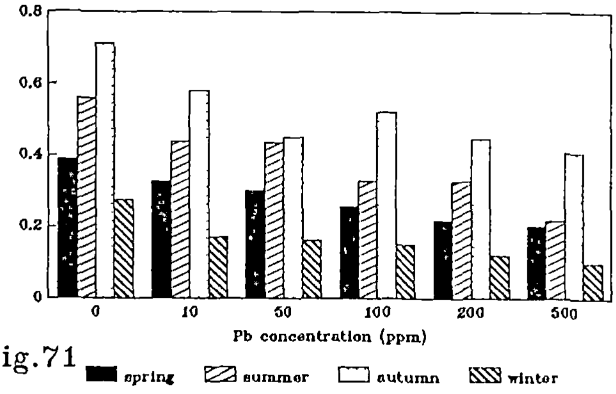


Fig.71

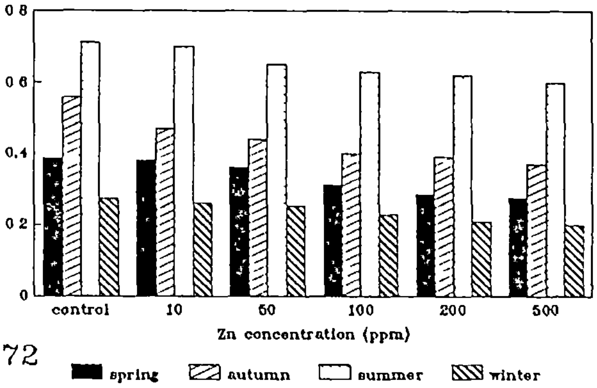


Fig.72

Fig.73–78 Dehydrogenase Activity on Non-mycorrhizal Root Surface

Formazan released mg/g dry wt/24 h

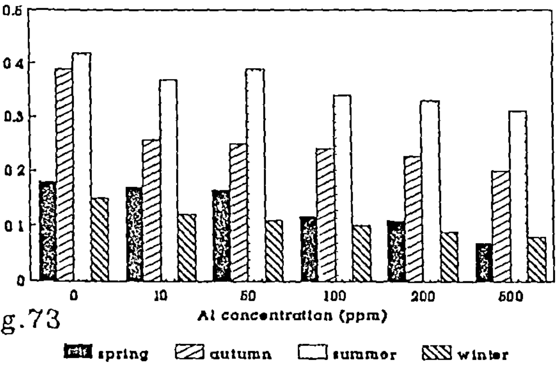


Fig.73

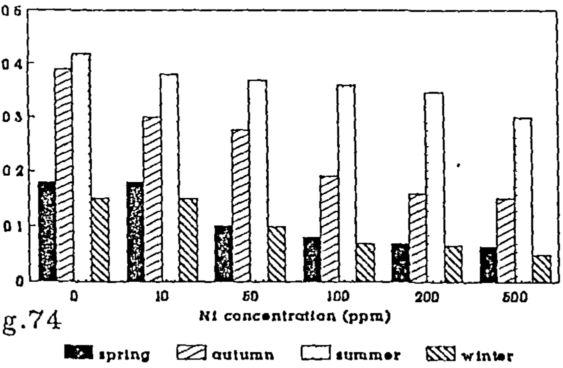


Fig.74

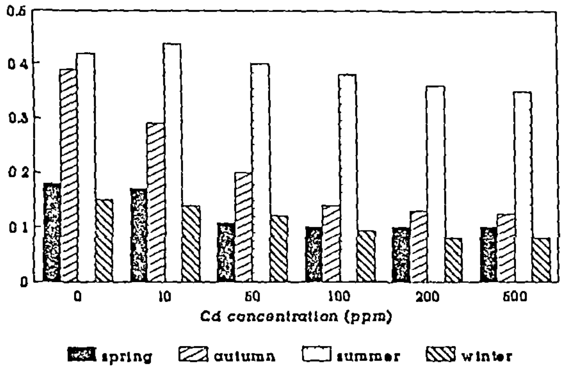


Fig.75

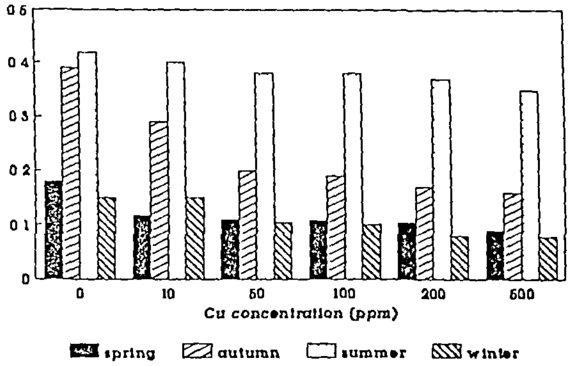


Fig.76

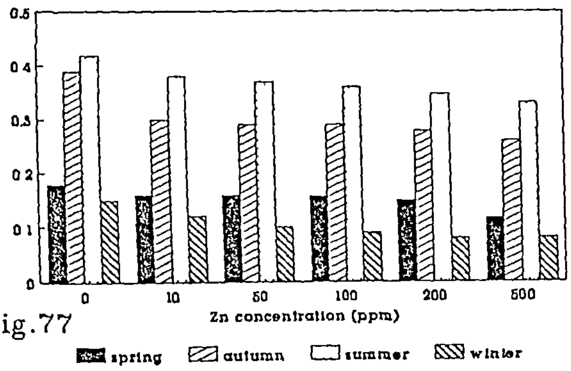


Fig.77

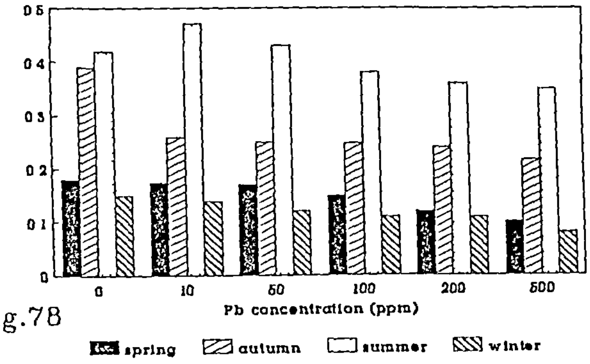


Fig.78

Fig.79–84 Dehydrgenase Activity on Non-mycorrhizal rhizospheric soil

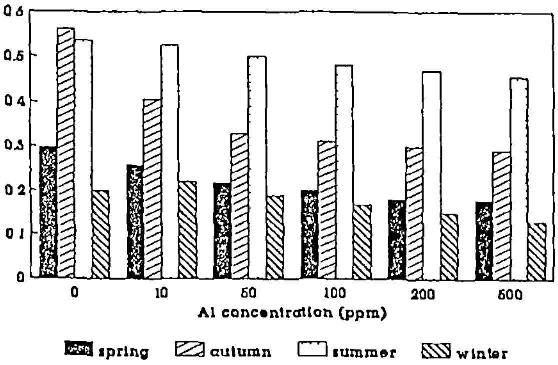


Fig.79

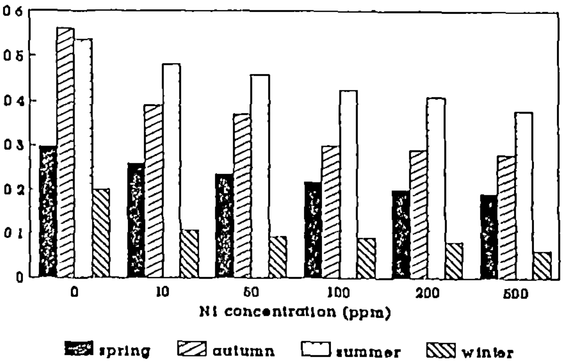


Fig.80

Formazan released mg/g dry wt/24 h

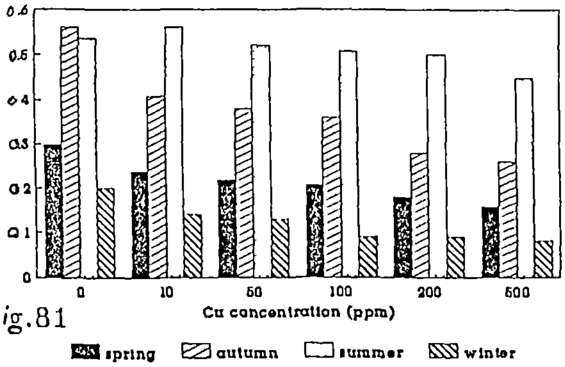


Fig.81

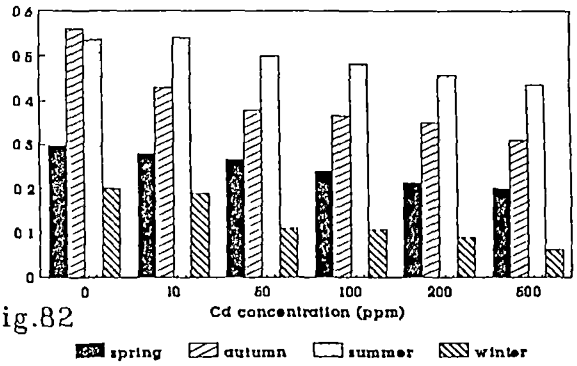


Fig.82

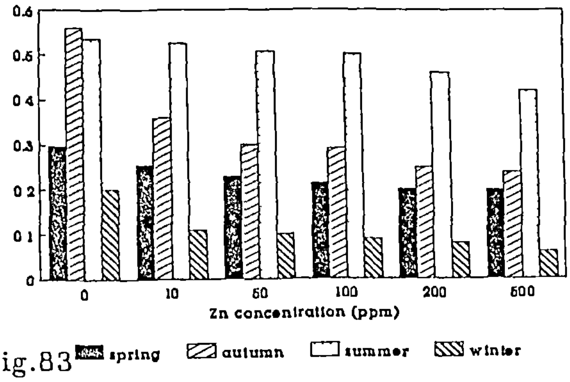


Fig.83

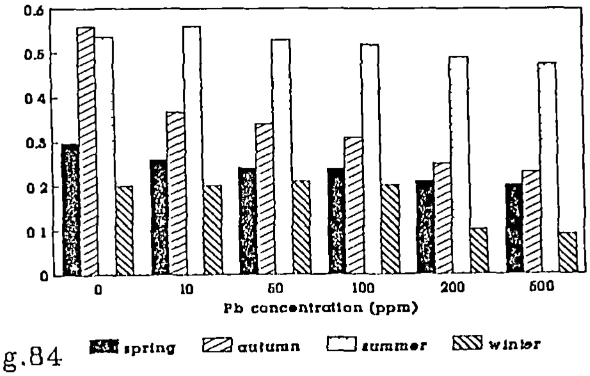


Fig.84

percentage of inhibition of urease activity was observed in *S.aurantium* treated seedlings with an average of 47 % inhibition whereas seedlings treated with Ni exhibited minimum inhibition. Cu and Al had inhibited upto 24 % in rhizospheric soil treated with *C. graniforme*. In case of non-mycorrhizal roots the percentage of inhibition was high with an average of 45-50 % in all the six metals studied (Figs. 217-228).

Study in field condition revealed that the urease activity was higher in seedlings infected with mixed inoculum compared to the single inoculum in pots and the inhibition rate of enzymes by heavy metals was less both in rhizospheric soil as well as in root tissue (Figs. 169-252). Minimum inhibition (18 %) in root surface was observed in Cu treated and inoculated with mixed ectomycorrhizal fungi (Figs. 171) and a maximum inhibition (35 %) was found in Zn treated mycorrhizal roots and rhizospheric soil (Figs. 174, 180). The rhizospheric soil of non-mycorrhizal seedlings exhibited highest inhibition (48 %, 45% and 44 %) in Cd, Ni and Pb treated soil respectively (Figs.224, 225, 228) and a minimum inhibition (22 %) was observed in Zn treated ectomycorrhizal roots. Phosphatase activity showed the marked seasonal variation and highest enzyme activity as compared to urease and dehydrogenase (Figs. 85-178). Two periods of phosphatase activity were distinguishable, one with a a higher activity in summer and another with a markedly low activity in winter and spring. During the summer months its activity remained constant, and it increased slightly in September and reached its peak in autumn, but in November it

Fig.85–90 Phosphatase Activity on Root Surface Inoculated with mixed mycobionts

Formazan released mg/g dry wt/24 h

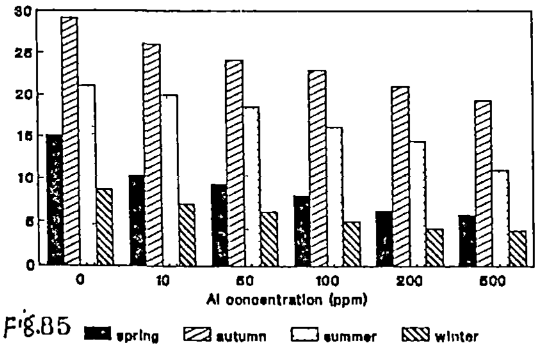


Fig.85

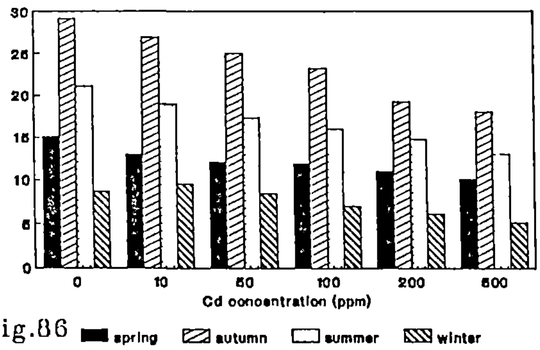


Fig.86

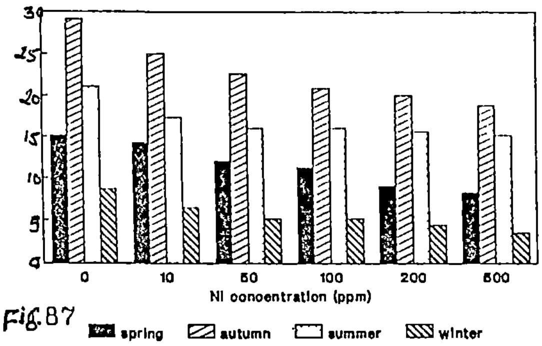


Fig.87

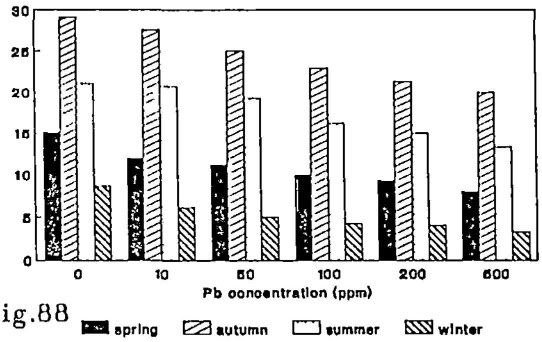


Fig.88

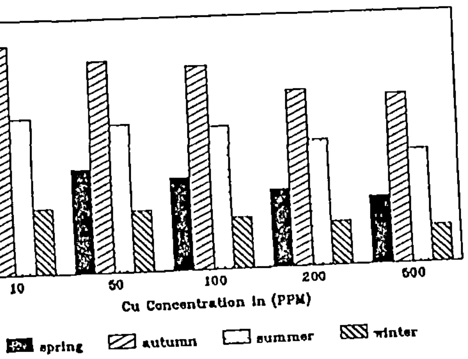


Fig.89

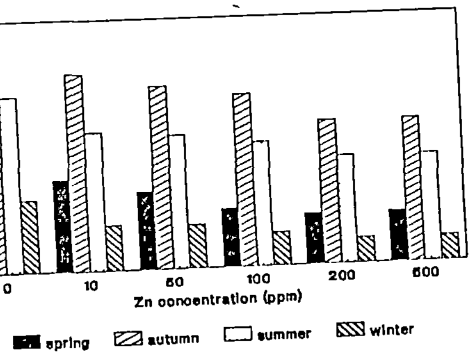


Fig.90

Fig.91–96 Phosphatase Activity on rhizospheric Soil Inoculated with mixed mycobionts

P-nitrophenol released mg/g dry wt/h

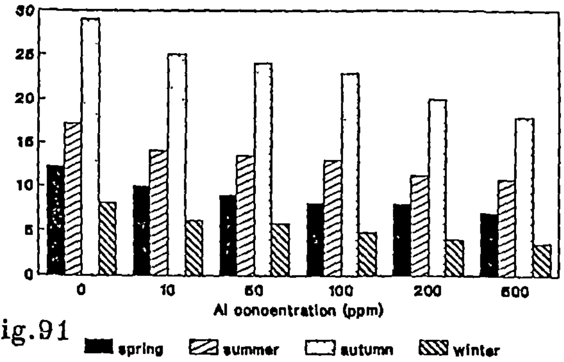


Fig.91

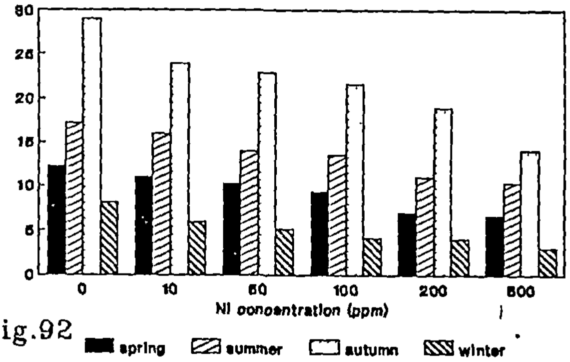


Fig.92

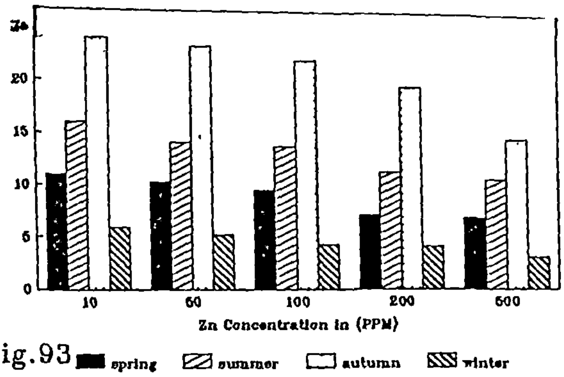


Fig.93

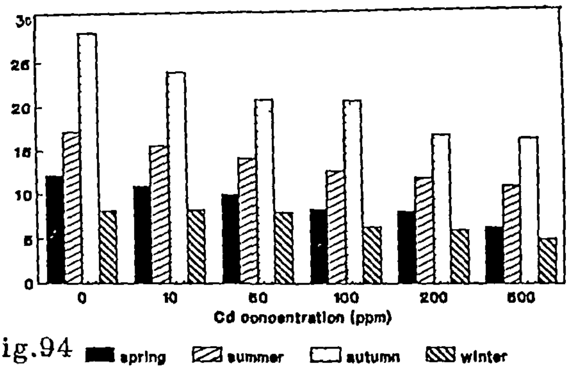


Fig.94

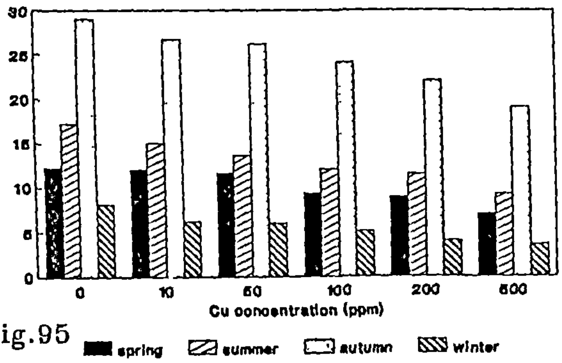


Fig.95

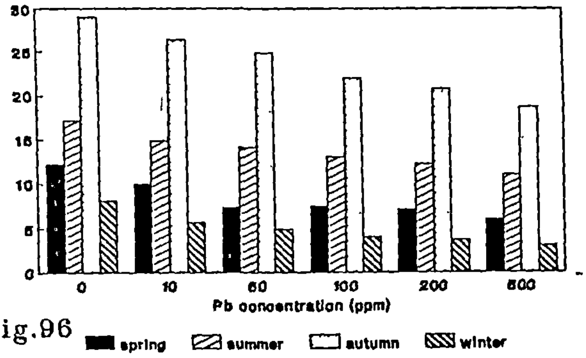


Fig.96

Fig.97–102 Phosphatase Activity on Root Surface Inoculated with *Boletus sp.*

P-nitrophenol released mg/g dry wt/h

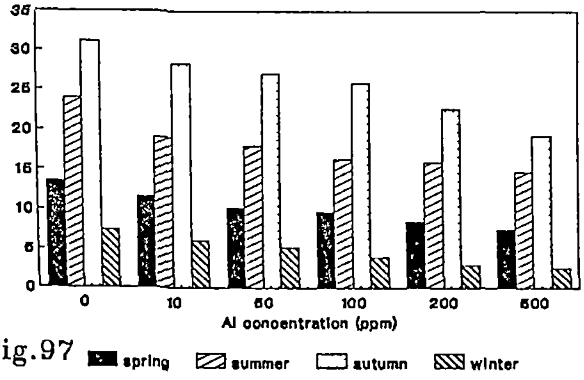


Fig.97

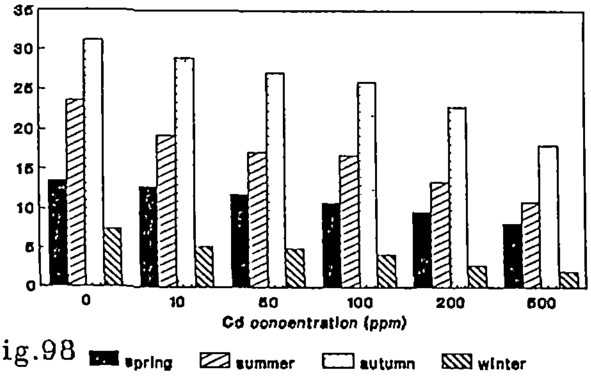


Fig.98

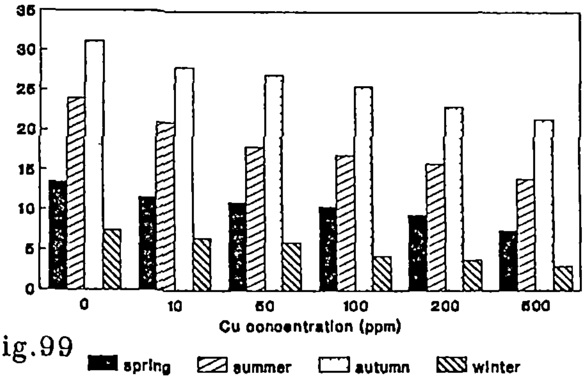


Fig.99

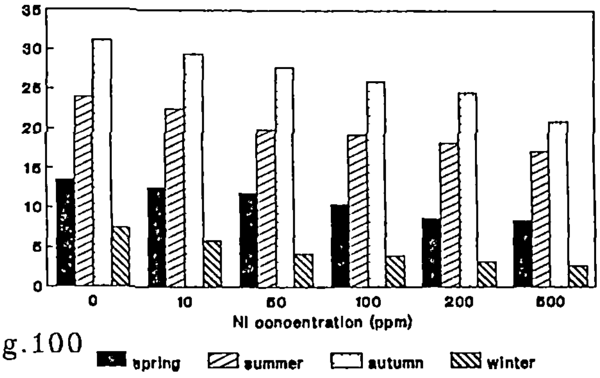


Fig.100

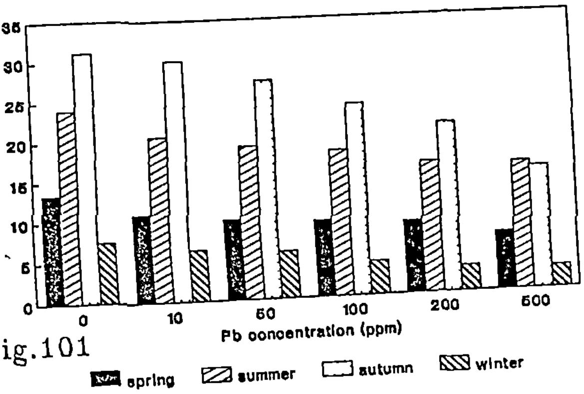


Fig.101

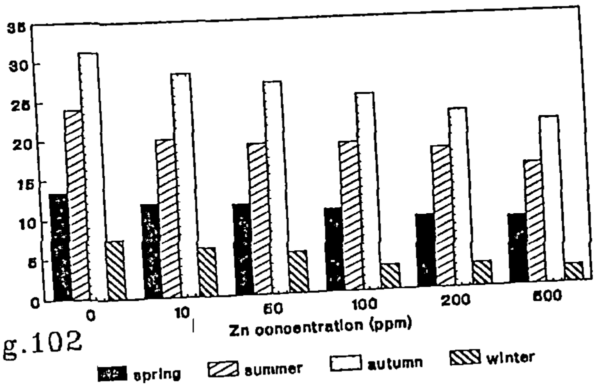


Fig.102

Fig.103–108 Phosphatase Activity on rhizospheric Soil Inoculated with *Boletus sp.*

P-nitrophenol released mg/g dry wt/h

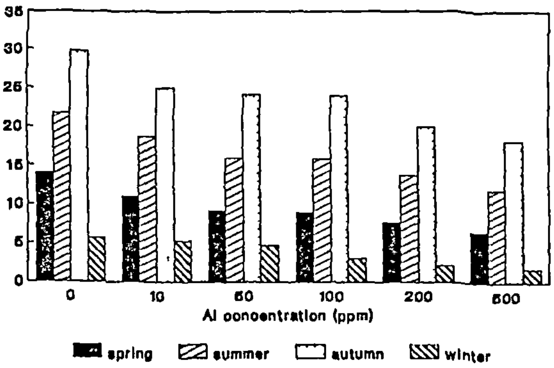


Fig.103

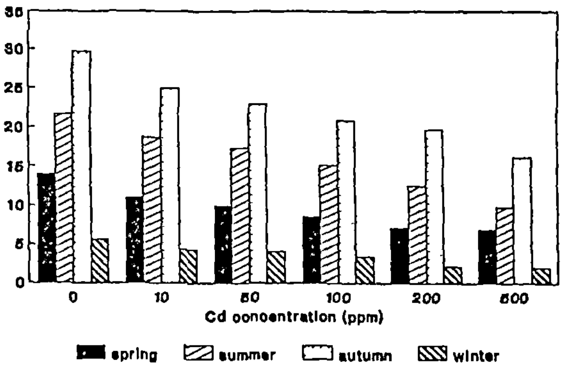


Fig.104

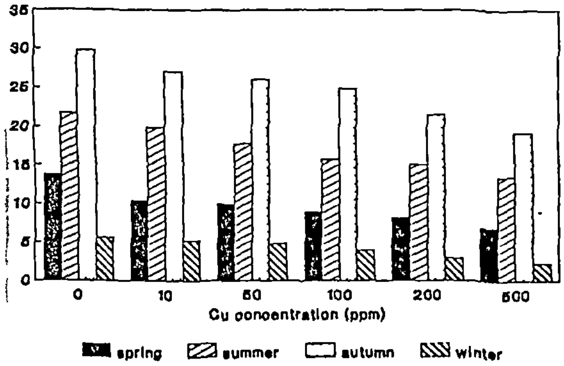


Fig.105

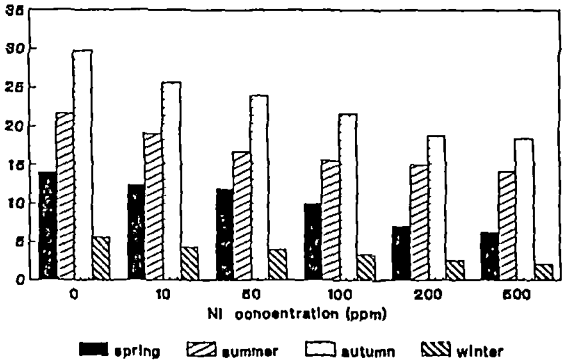


Fig.106

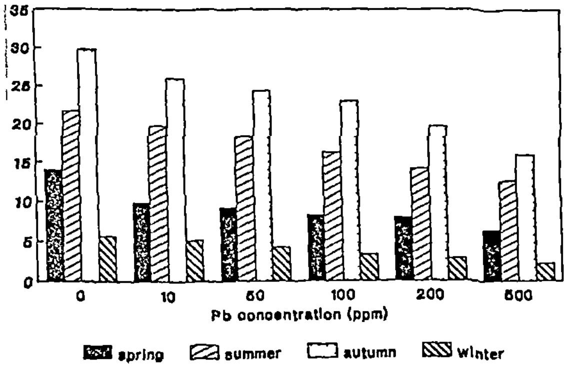


Fig.107

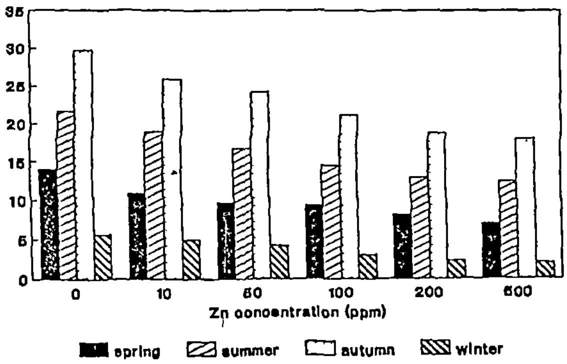


Fig.108

Fig.109–114 Phosphatase Activity on Root Surface Inoculated with *C.graniforme*

P-nitrophenol released mg/g dry wt/h

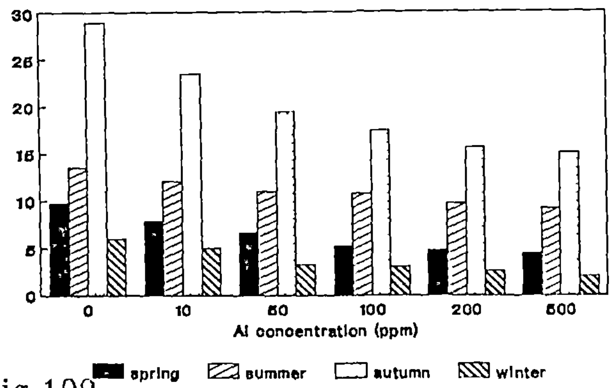


Fig.109

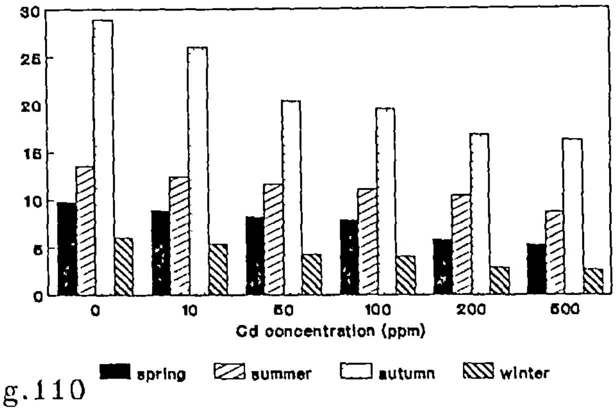


Fig.110

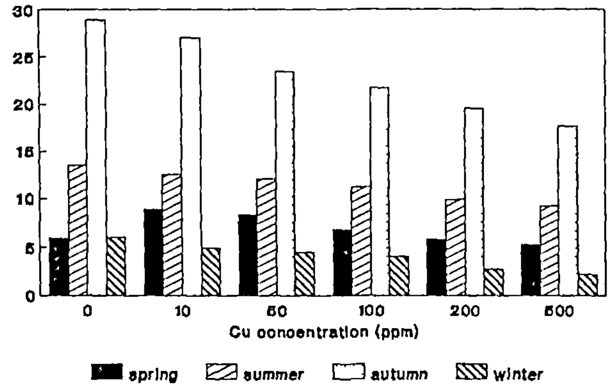


Fig.111

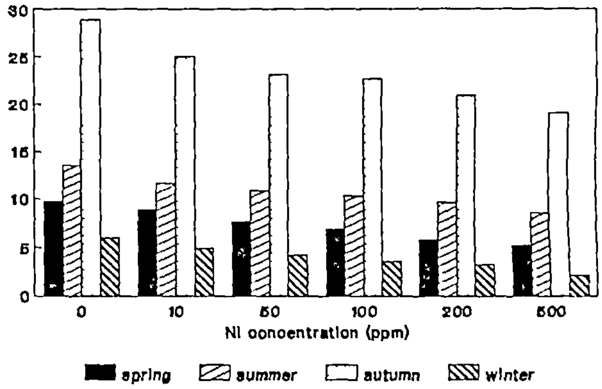


Fig.112

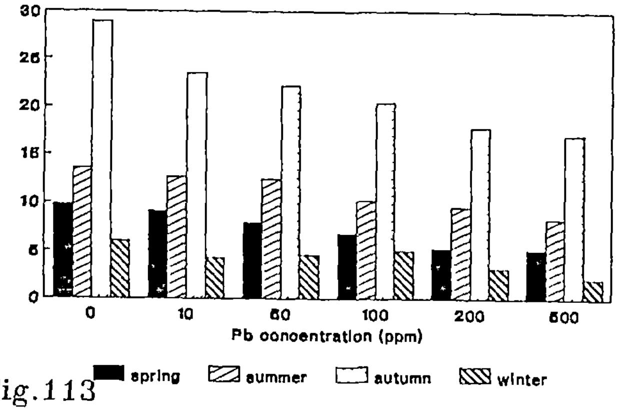


Fig.113

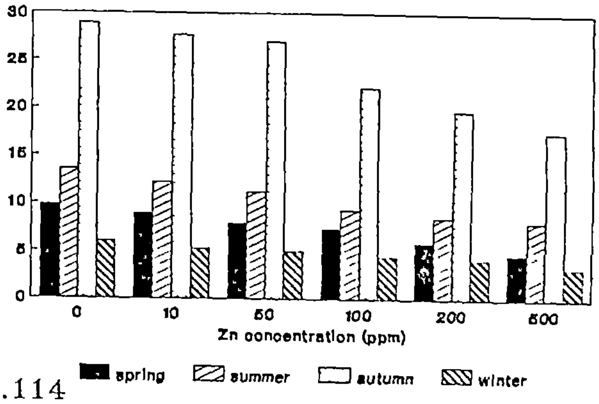


Fig.114

Fig.115-120 Phosphatase Activity on rhizospheric Soil Inoculated with *C.graniforme*

P-nitrophenol released mg/g dry wt/h

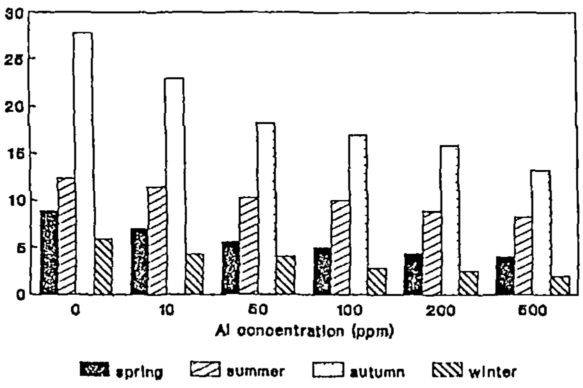


Fig.115

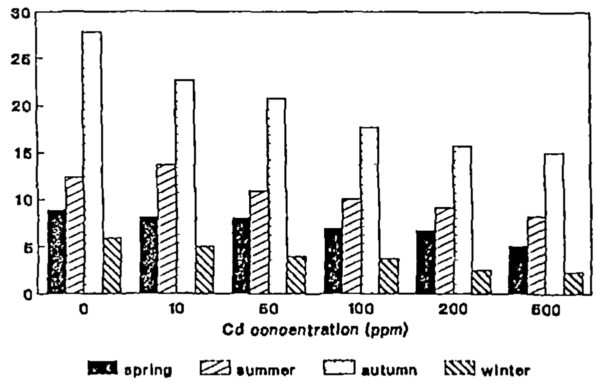


Fig.116

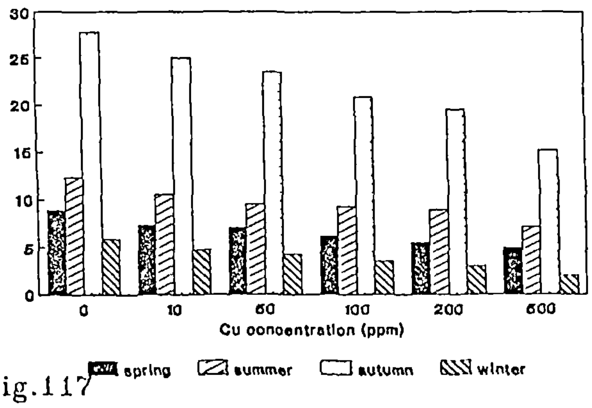


Fig.117

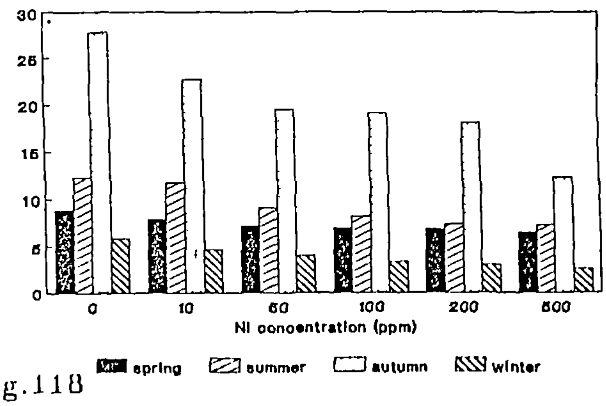


Fig.118

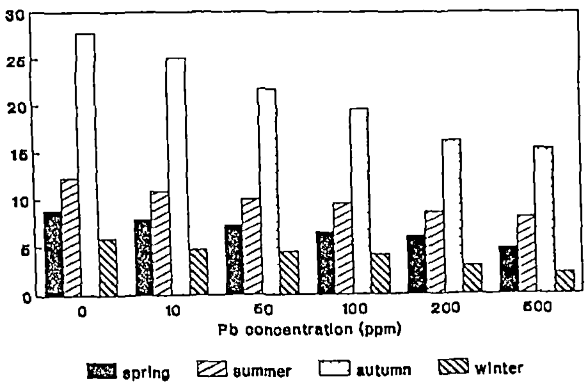


Fig.119

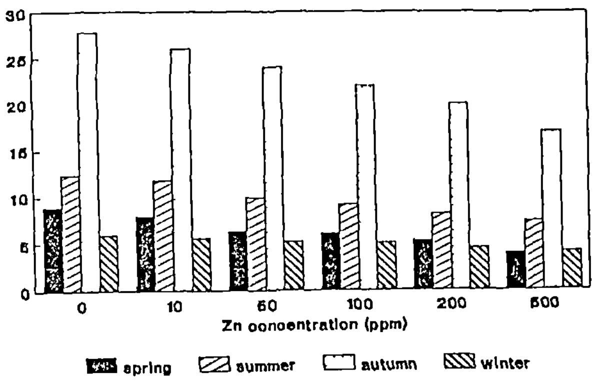


Fig.120

Fig.121–126 Phosphatase Activity on Root Surface Inoculated with *S.aurantium*

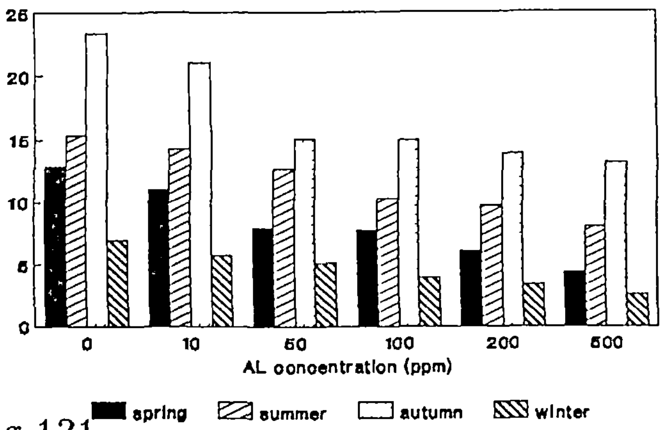


Fig.121

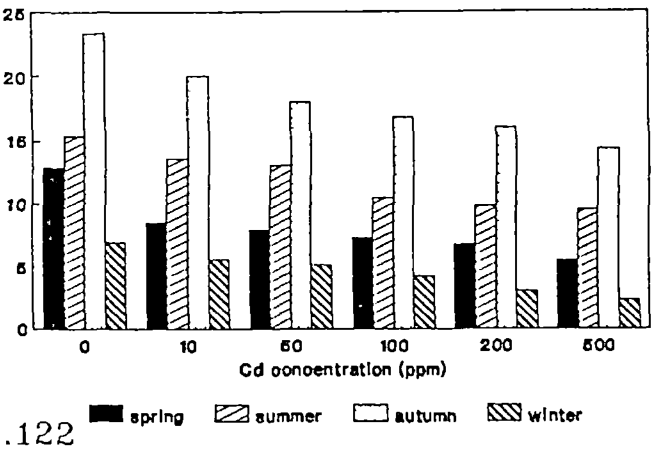


Fig.122

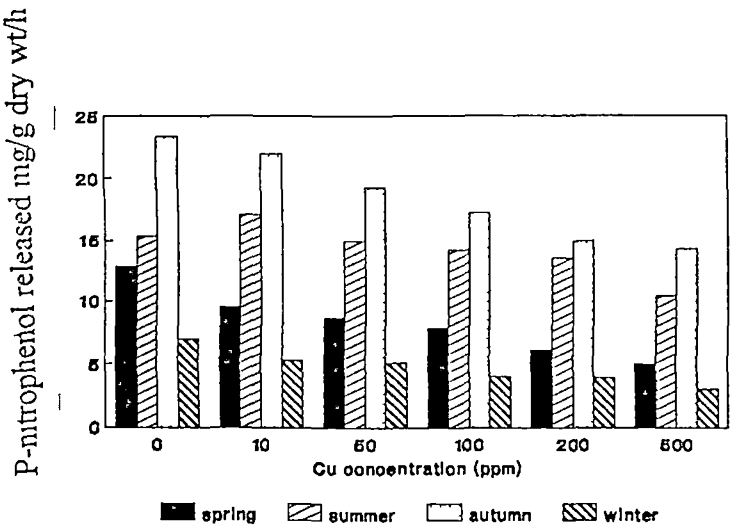


Fig.123

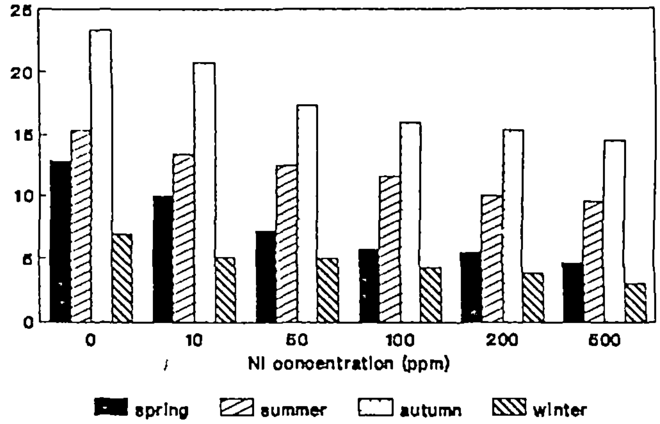


Fig.124

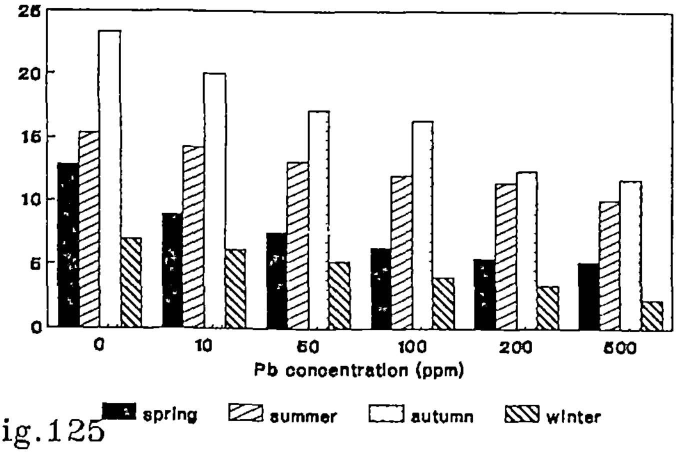


Fig.125

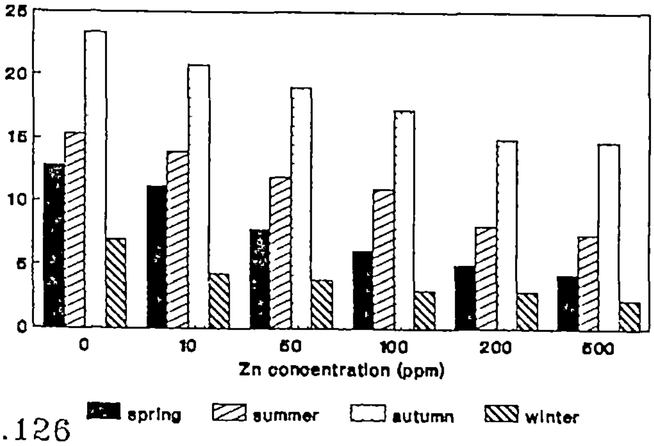


Fig.126

Fig. 127–132 Phosphatase Activity on rhizospheric Soil Inoculated with *S. aurantium*

P-nitrophenol released mg/g dry wt/h

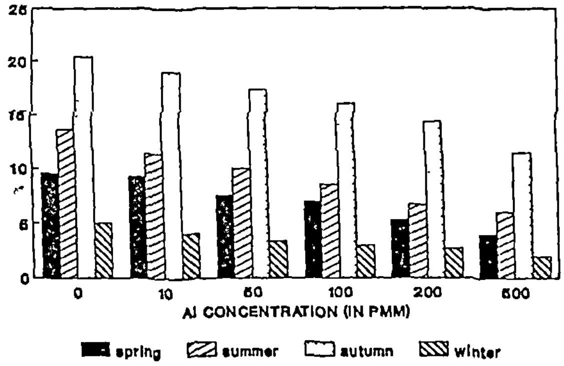


Fig.127

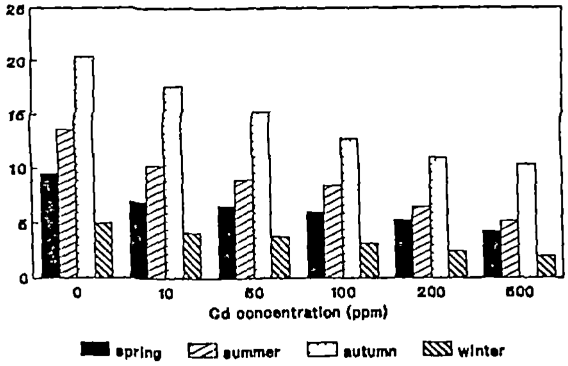
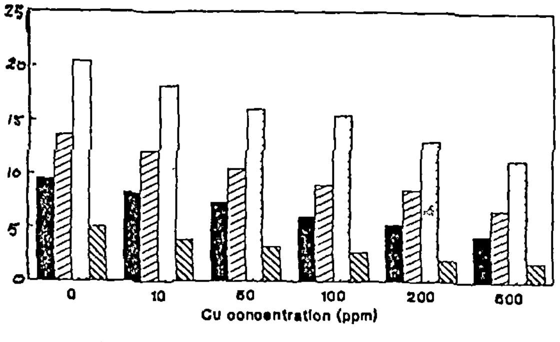


Fig.128



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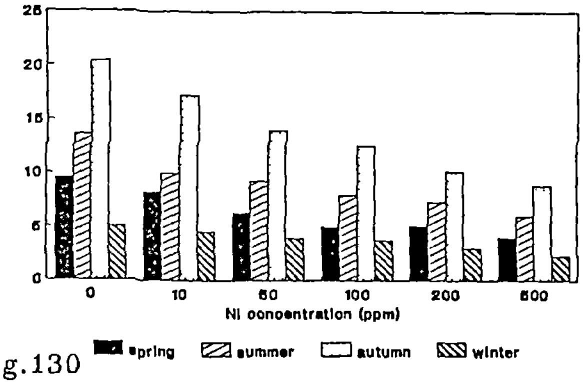


Fig.130

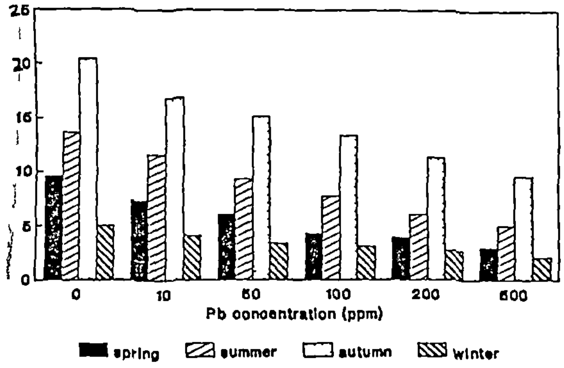


Fig.131

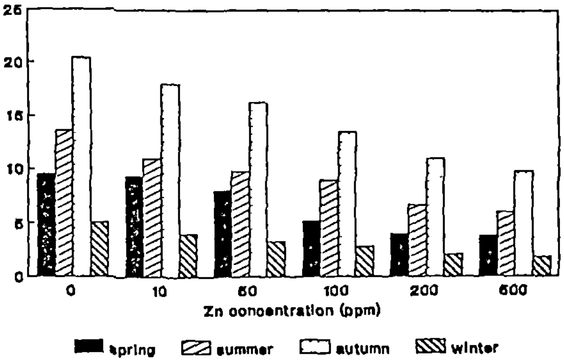


Fig.132

Fig.133–138 Phosphatase Activity on rhizospheric Soil Inoculated with *S. luteus*

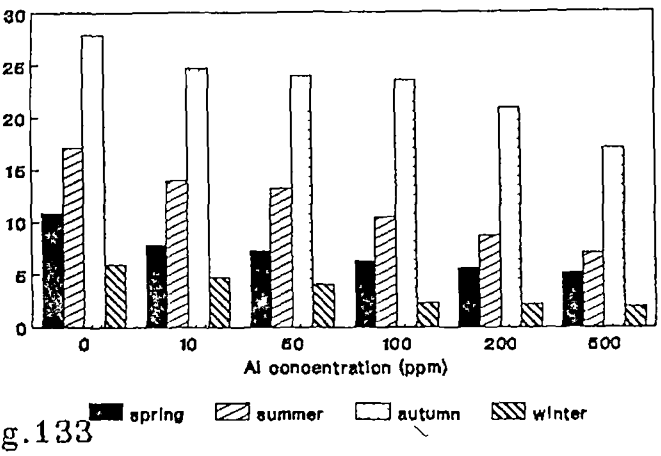


Fig.133

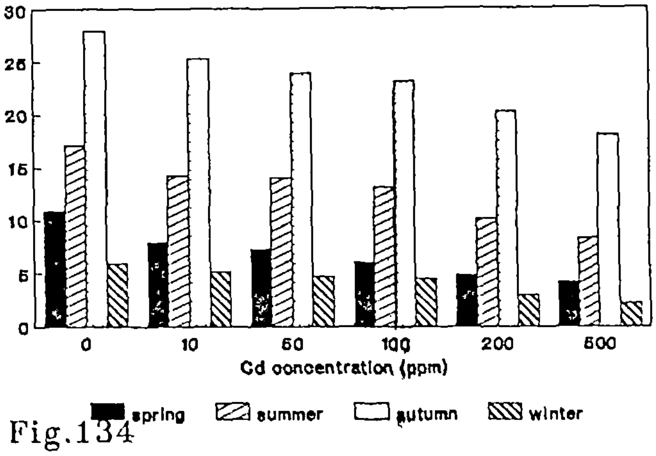


Fig.134

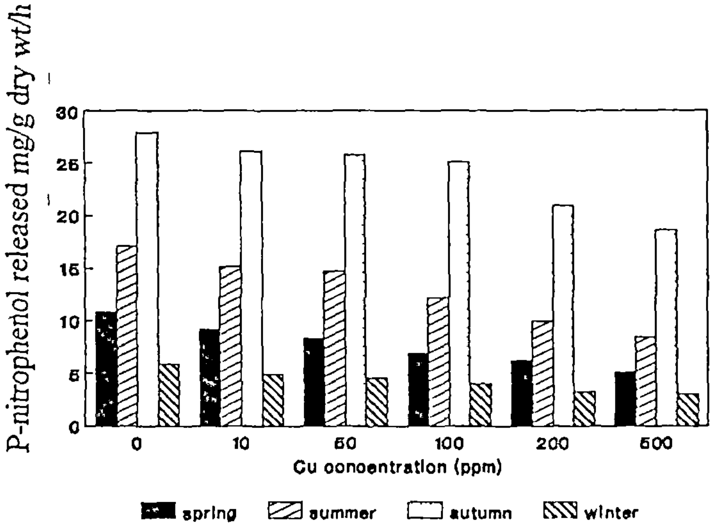


Fig.135

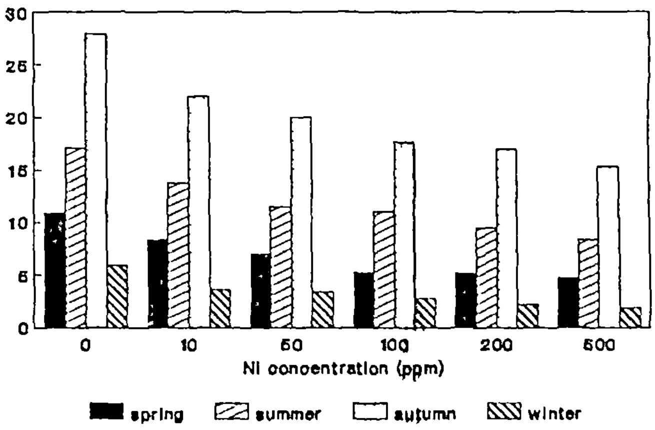


Fig.136

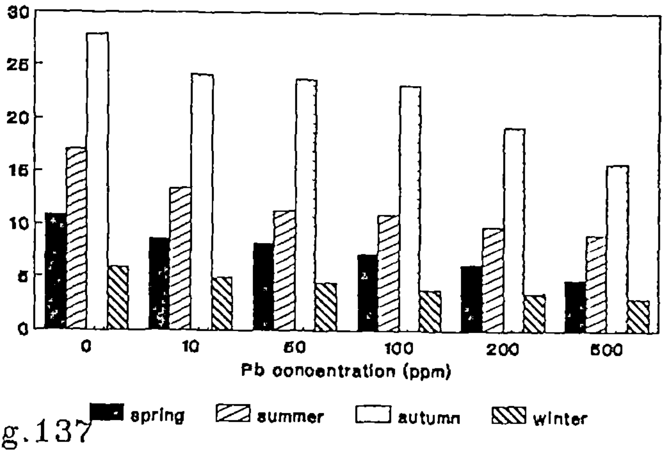


Fig.137

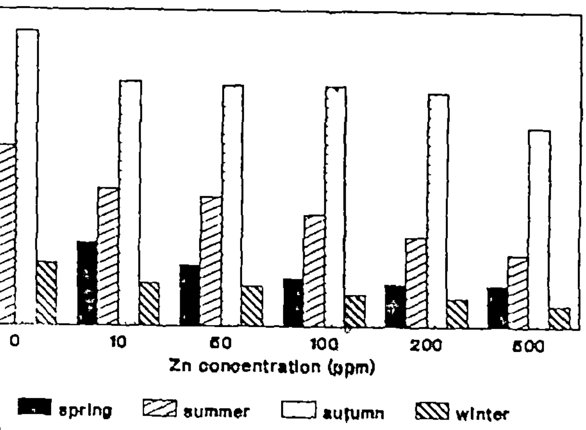


Fig.138

Fig.139–144 Phosphatase Activity on Root Surface Inoculated with *S.luteus*

P-nitrophenol released mg/g dry wt/h

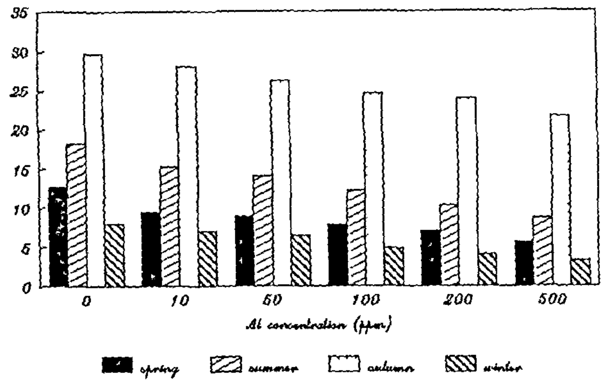


Fig.139

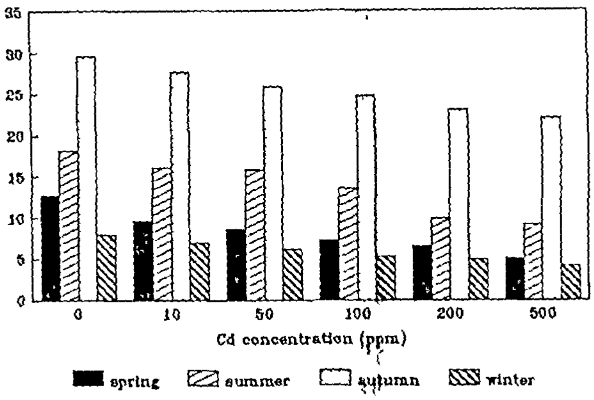


Fig.140

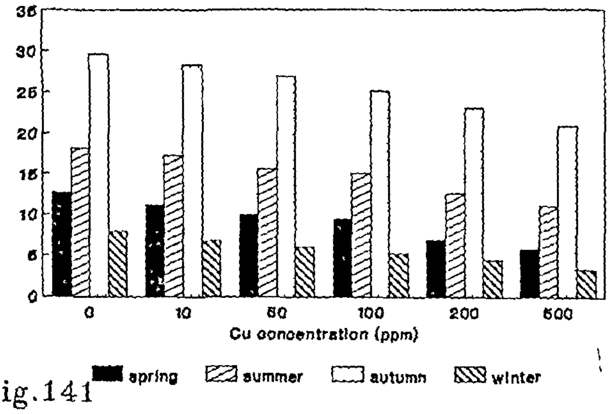


Fig.141

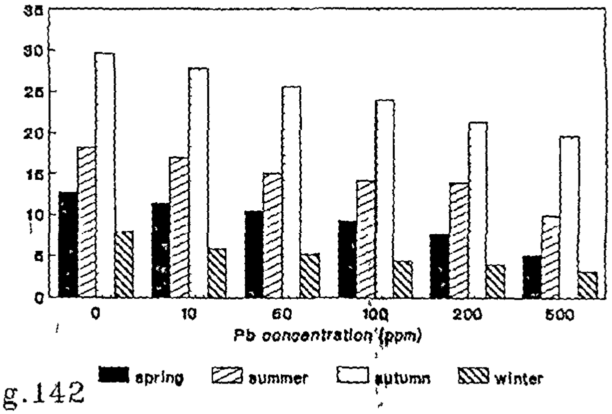


Fig.142

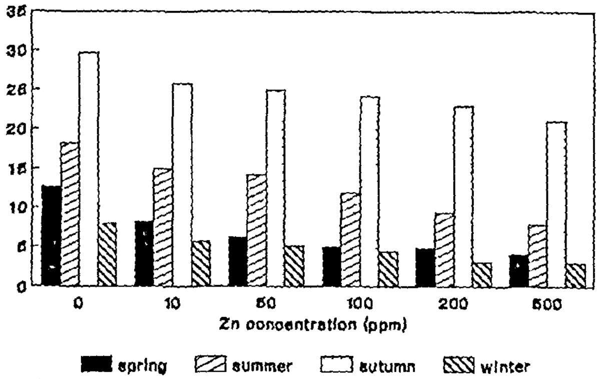


Fig.143

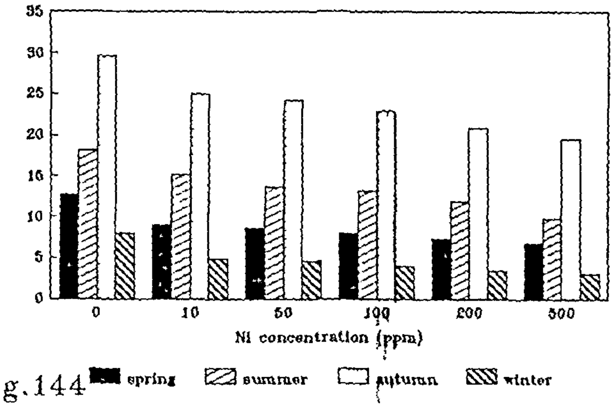


Fig.144

Fig.145–150 Phosphatase Activity on Non-mycorrhizal Root Surface

P-nitrophenol released mg/g dry wt/h

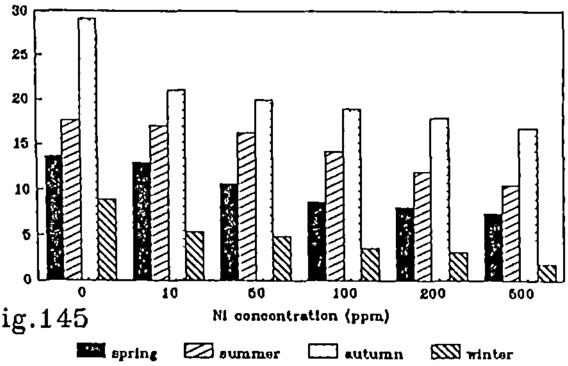


Fig.145

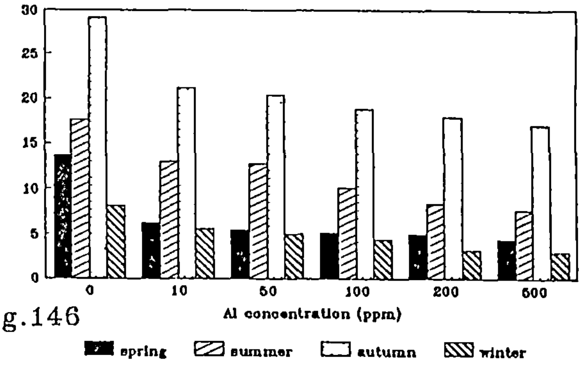


Fig.146

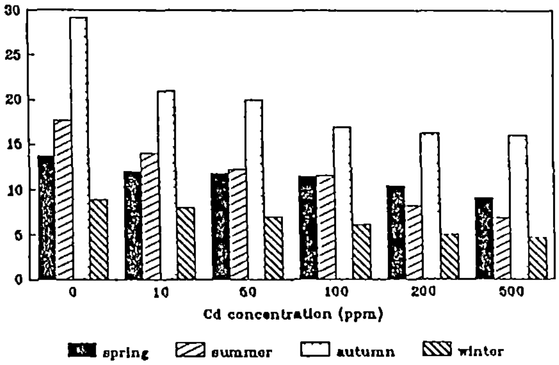


Fig.147

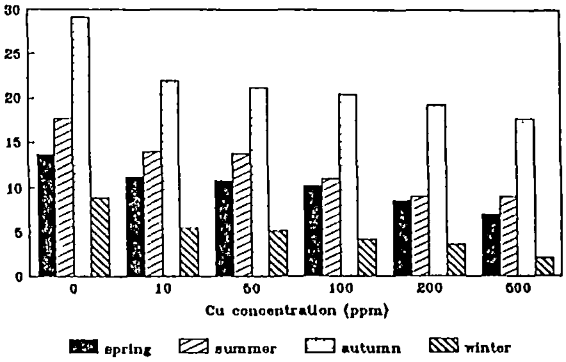


Fig.148

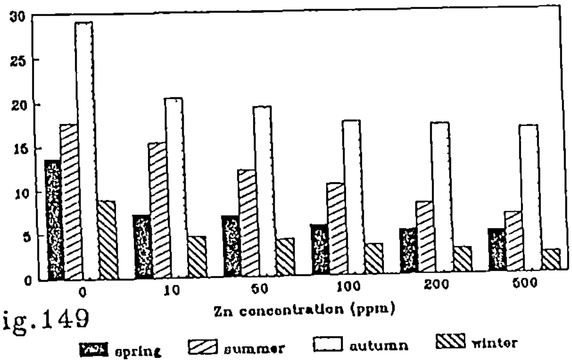


Fig.149

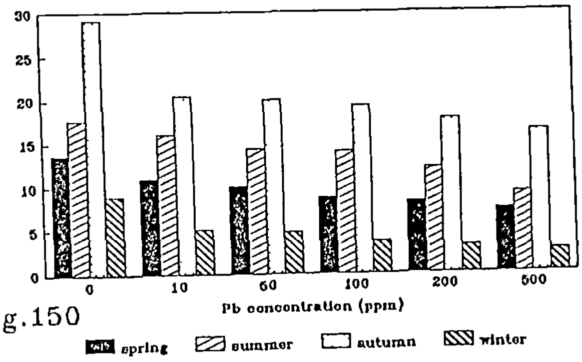


Fig.150

Fig.151-156 Phosphatase Activity on Non-mycorrhizal rhizospheric Soil

P-nitrophenol released mg/g dry wt/h

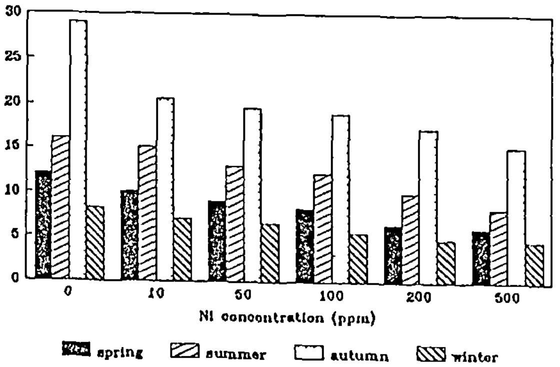


Fig.151

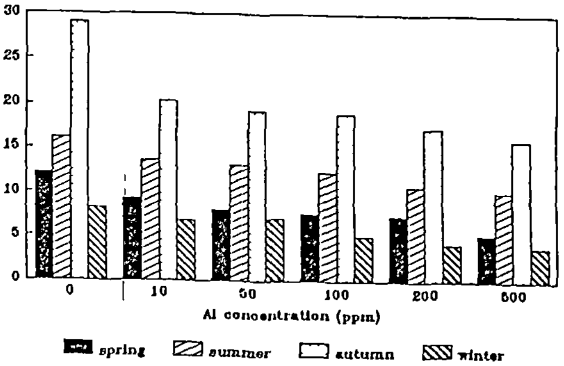


Fig.152

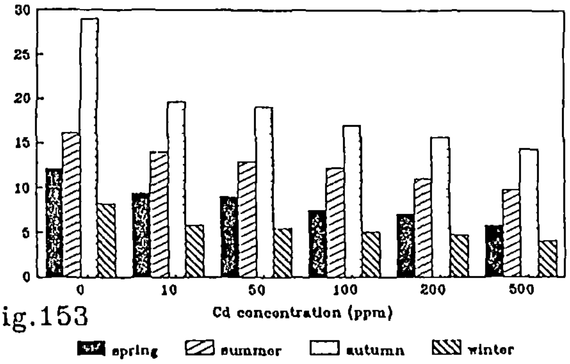


Fig.153

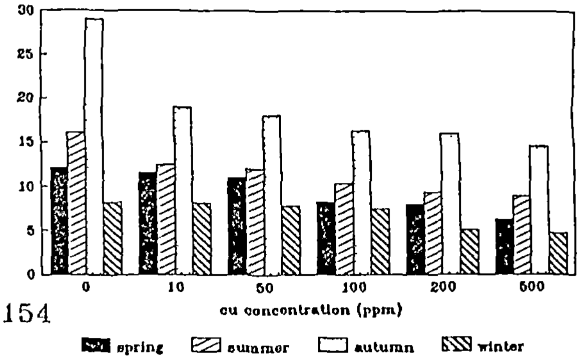


Fig.154

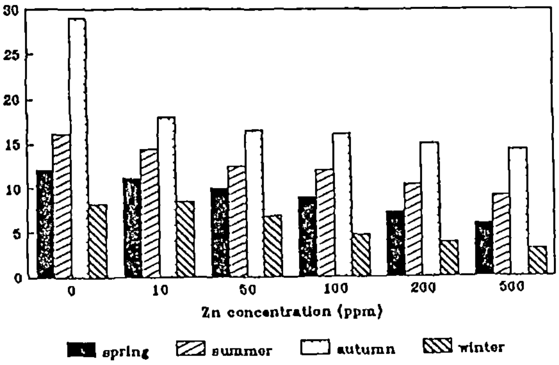


Fig.155

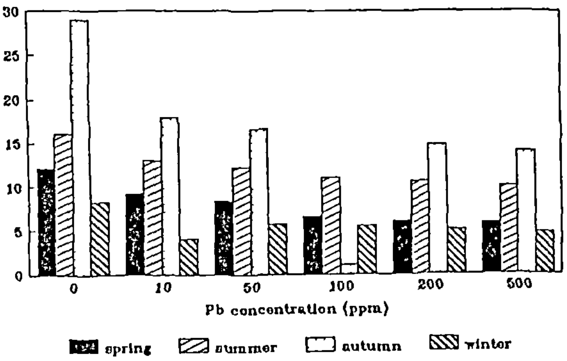


Fig.156

Fig.157–162 Phosphatase Activity on Non-mycorrhizal Root Surface

P-nitrophenol released mg/g dry wt/h

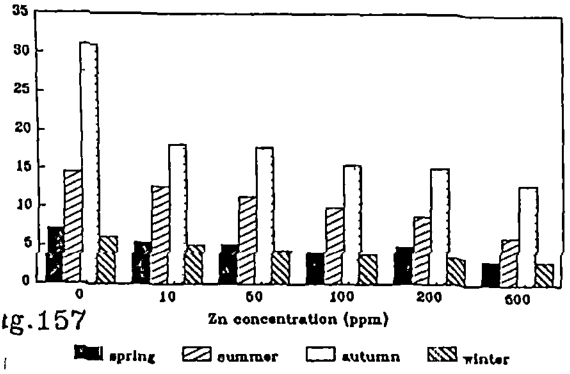


Fig.157

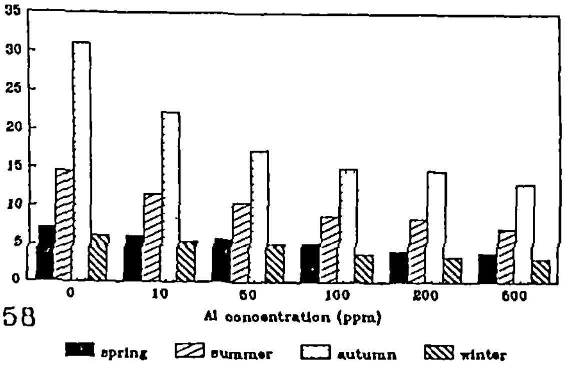


Fig.158

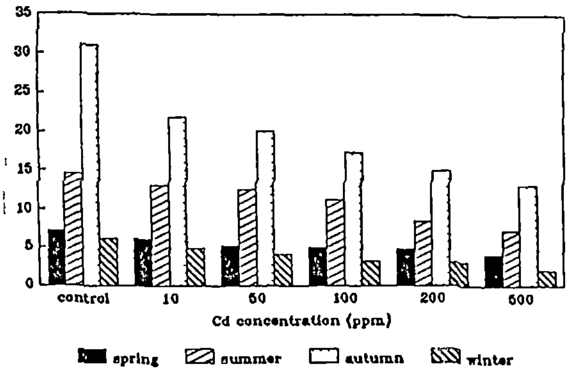


Fig.159

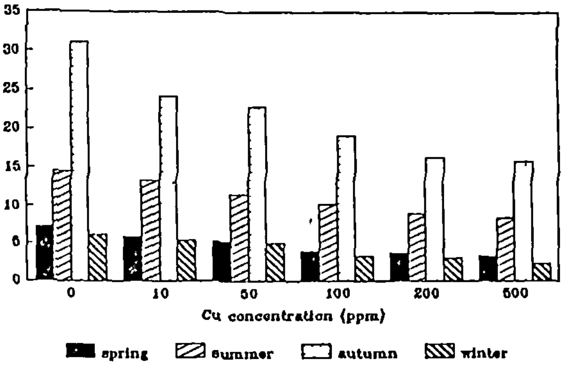


Fig.160

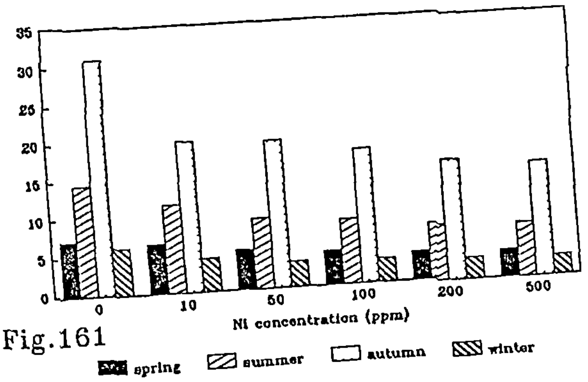


Fig.161

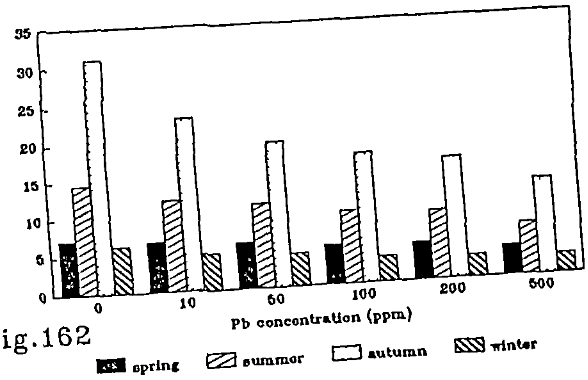
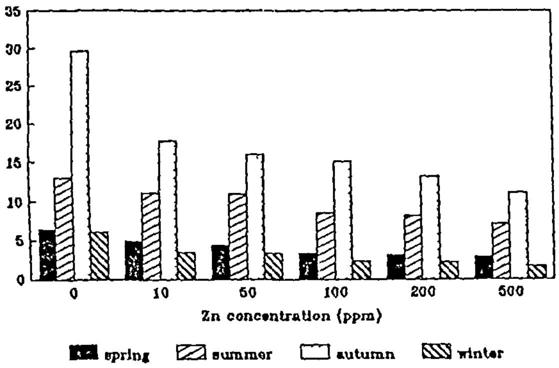


Fig.162

Fig.163-168 Phosphatase Activity on Non-mycorrhizal rhizospheric Soil

P-nitrophenol released mg/g dry wt/h



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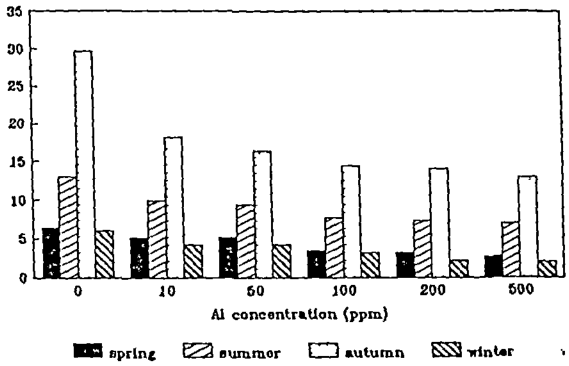
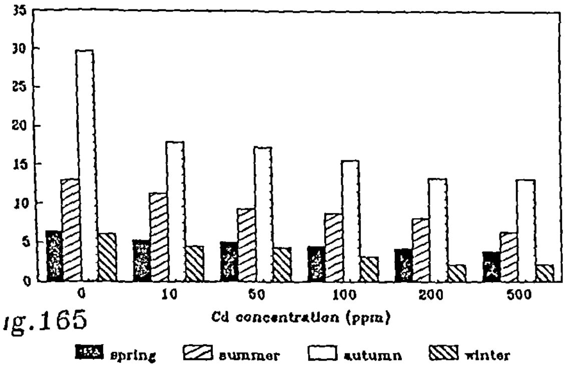


Fig.164



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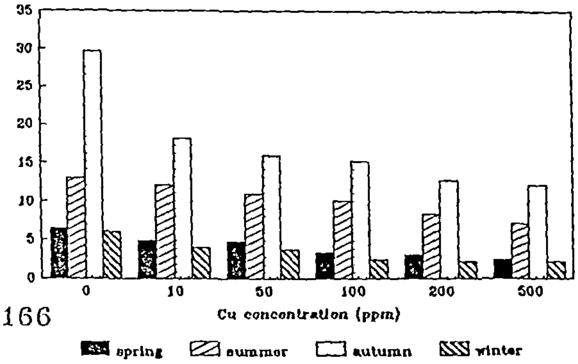

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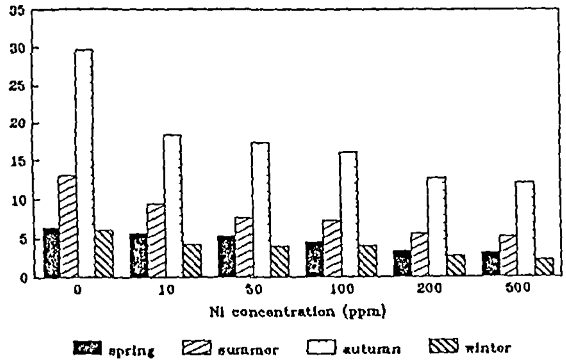

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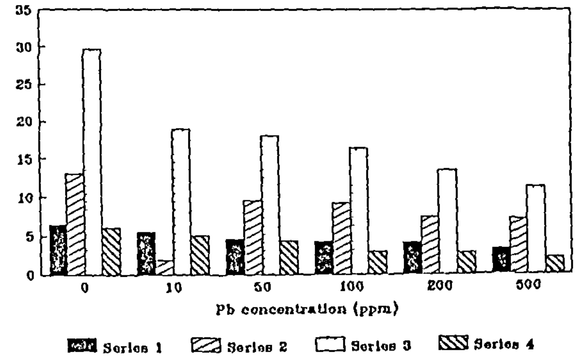


Fig.168

again decreased and remained constant during winter. It increased again in April and the highest phosphatase activity was observed in early summer and in early autumn.

Phosphatase activity was inhibited by Cd, Ni, Cu, Zn, Pb and Al treatments. Increased inhibition in its activity was observed at higher concentration of Pb, Ni and Al than Cu, Zn and Cd metal treated seedlings. The highest percentage of inhibition (50%) was observed in *S.aurantium* inoculated and Pb treated seedlings (Fig. 131) followed by Ni treated *S. aurantium* inoculated rhizospheric soil (Fig. 130). Minimum inhibition of phosphatase activity in roots and soil was observed in *Boletus* sp. inoculated seedlings. The lowest rate of inhibition was observed in Cd and Cu treated seedlings (Figs. 98-99, 104-105). The non-mycorrhizal seedlings exhibited an inhibition rate of phosphatase ranging from 45-50% both in roots as well as in rhizospheric soil (Fig. 157-168).

The activity of phosphatase was higher in case of mixed mycobionts inoculated roots and rhizospheric soil. The percentage of inhibition of enzyme activity was lower in field condition compared to seedlings grown in pots. Phosphatase activity was highest in autumn but from November onward it started to decline and remained constant during the winter months. It again increased gradually in spring season. The application of Cd, Cu, Ni, Zn, Pb and Al has inhibited the phosphatase activity with an average percentage of 19% in Cu treated roots and 18% in rhizospheric soil. Maximum average percentage of inhibition was observed in Zn treated roots with 29% and 36% in rhizospheric soil. Phosphatase activity was higher in mycorrhizal roots than

Fig.169–174 Urease Activity on Root Surface Inoculated with mixed mycobionts

NH$_4$-N released mg/g dry wt/3h

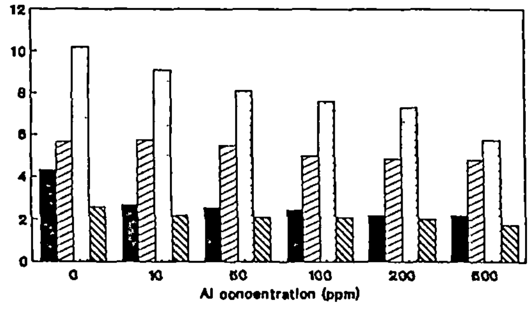


Fig.169

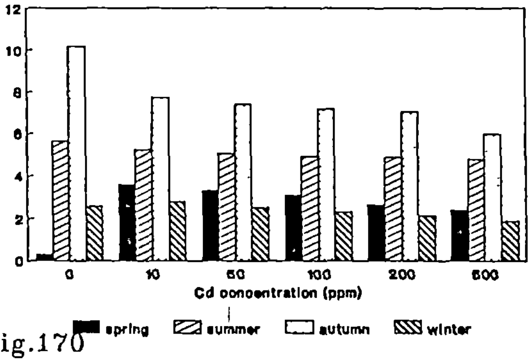


Fig.170

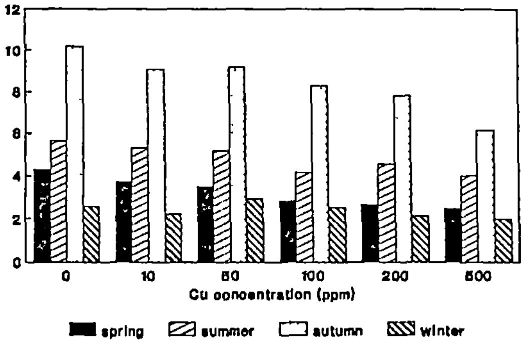


Fig.171

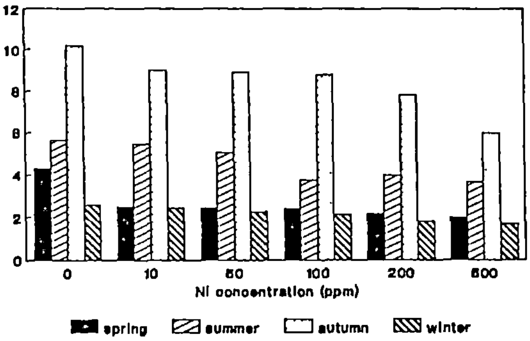


Fig.172

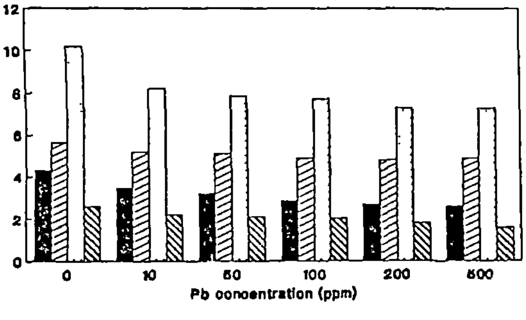


Fig.173

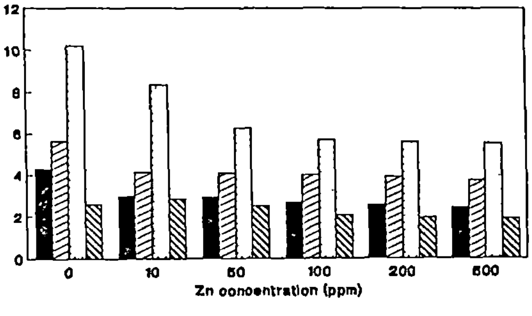


Fig.174

Fig.175–180 Urease Activity on rhizospheric Soil Inoculated with mixed mycobionts

NH$_4$-N released mg/g dry wt/3h

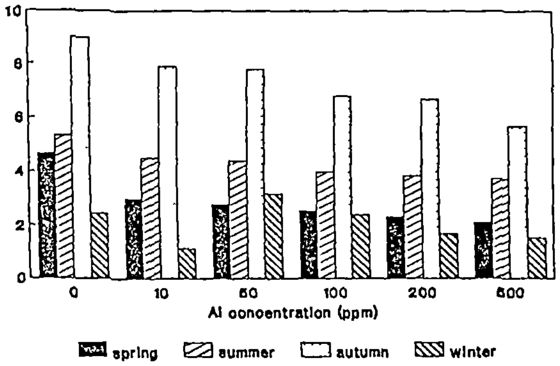


Fig.175

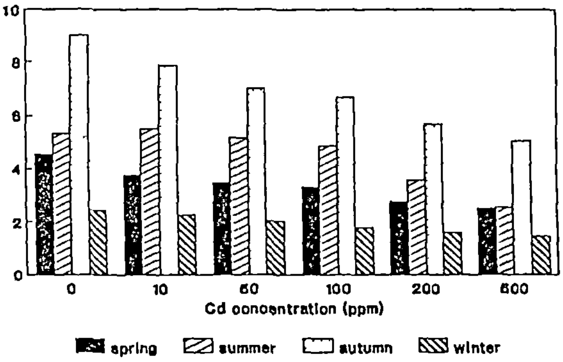


Fig.176

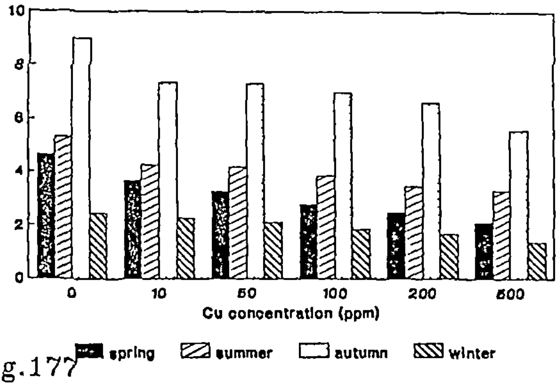


Fig.177

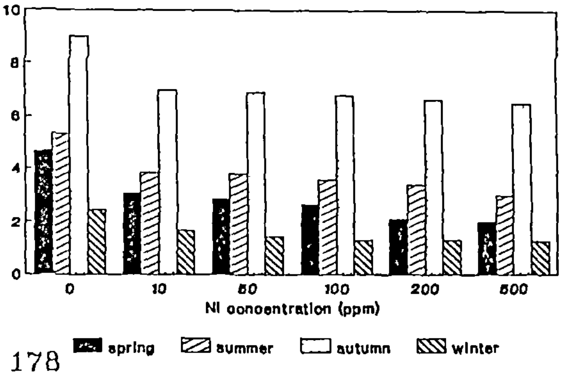


Fig. 178

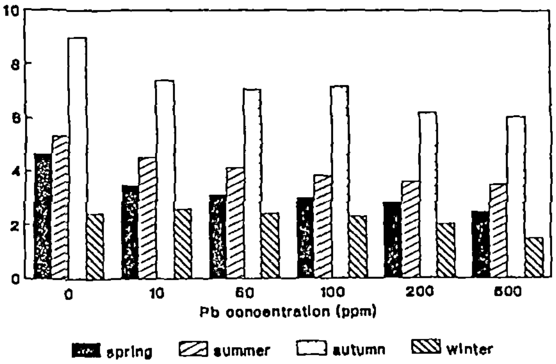


Fig.179

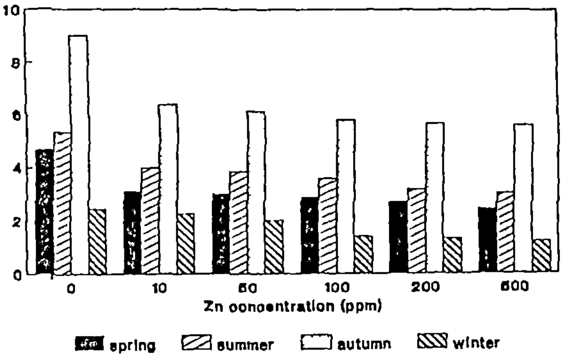


Fig.180

Fig.181-186 Urease Activity on rhizospheric Soil Inoculated with *S.aurantium*

NH$_4$-N released mg/g dry wt/3h

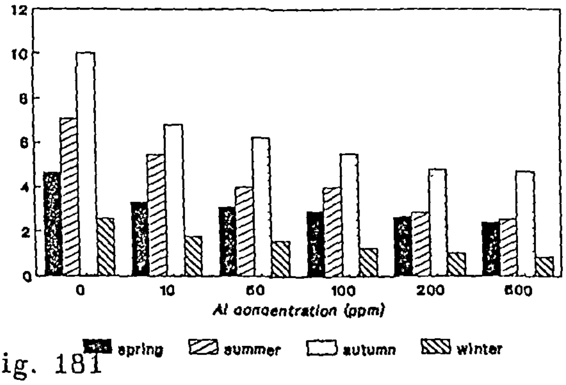


Fig. 181

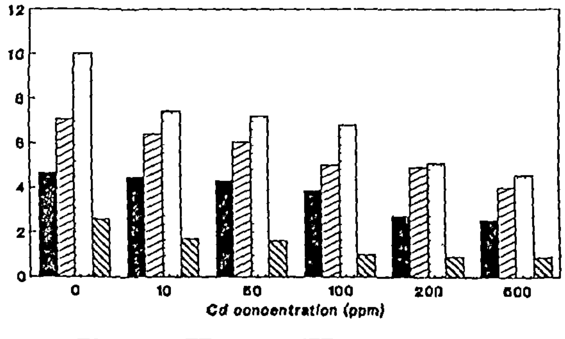


Fig.182

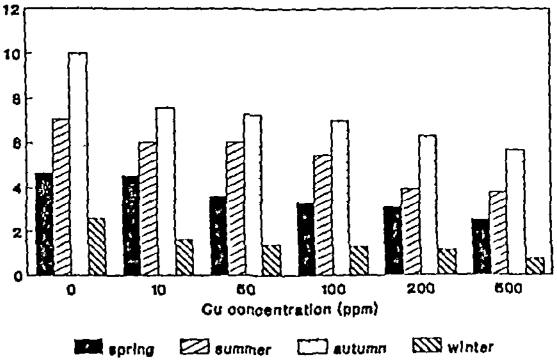


Fig.183

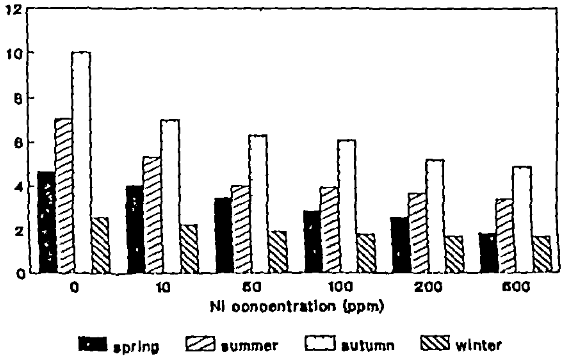


Fig.184

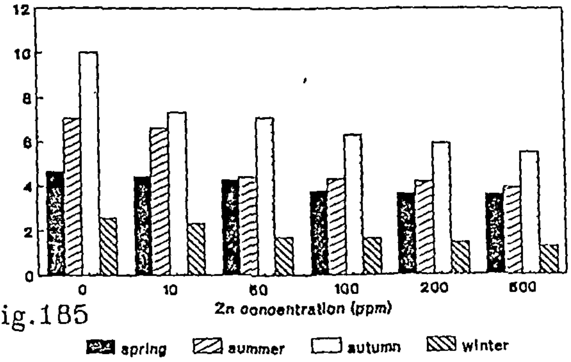


Fig.185

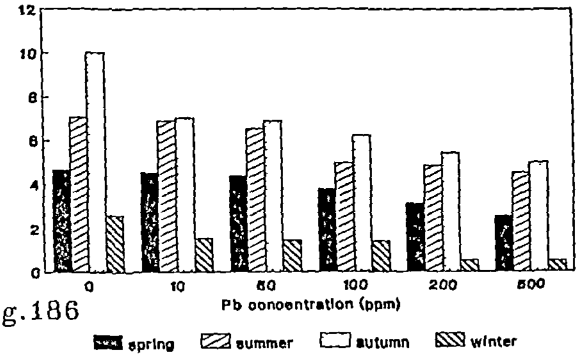


Fig.186

Fig.187-192 Urease Activity on rhizospheric Soil Inoculated with *S.luteus*

NH_4-N released mg/g dry wt/3h

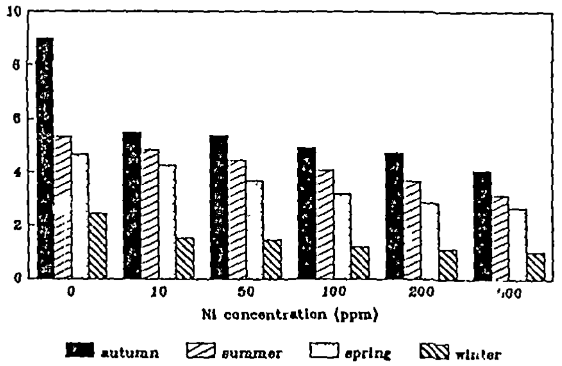


Fig.187

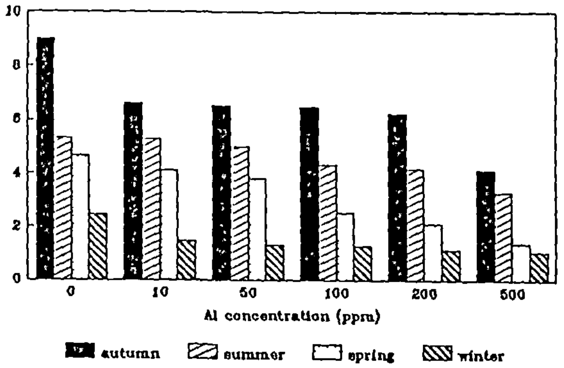


Fig.188

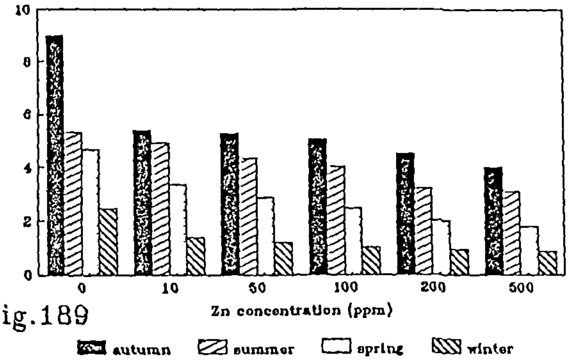


Fig.189

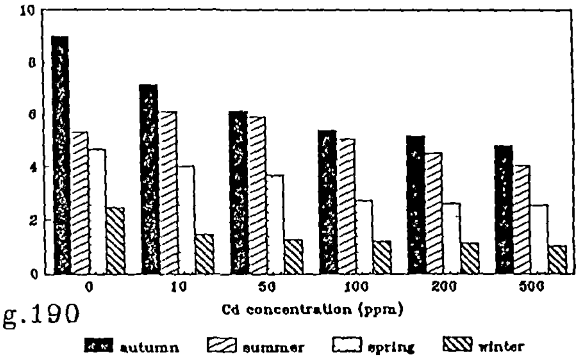


Fig.190

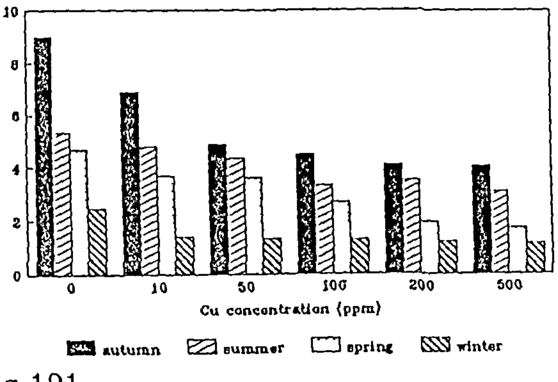


Fig.191

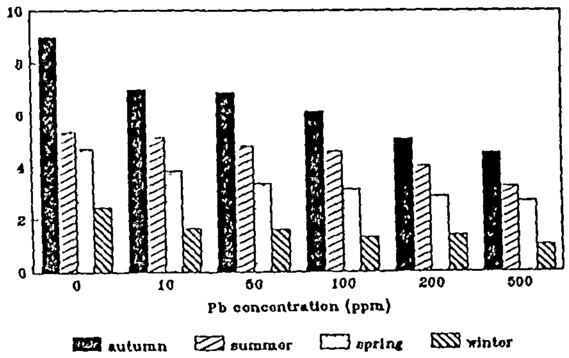


Fig.192

Fig.193–198 Urease Activity on Root Surface Inoculated with *C.graniforme*

NH_4-N released mg/g dry wt/3h

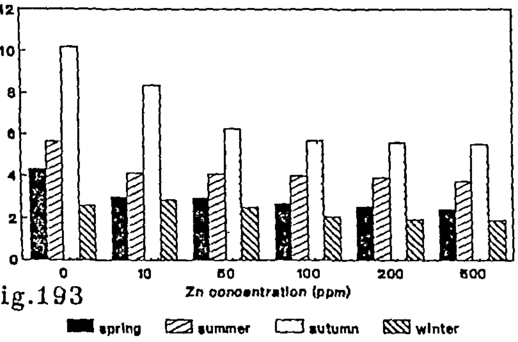


Fig.193

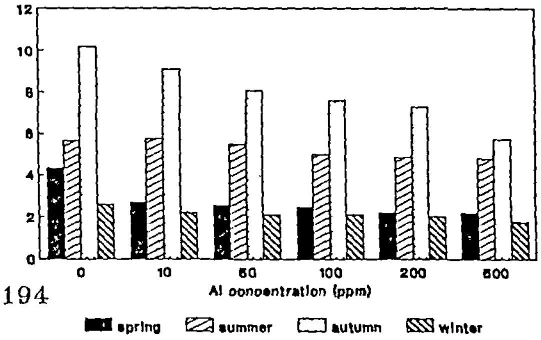


Fig.194

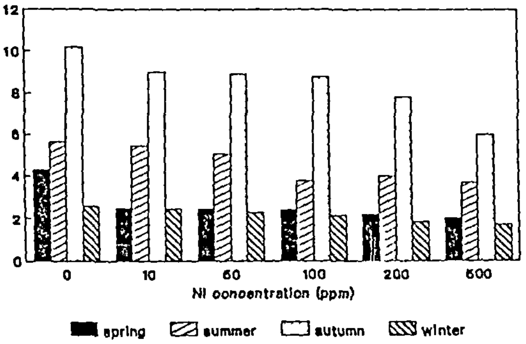


Fig.195

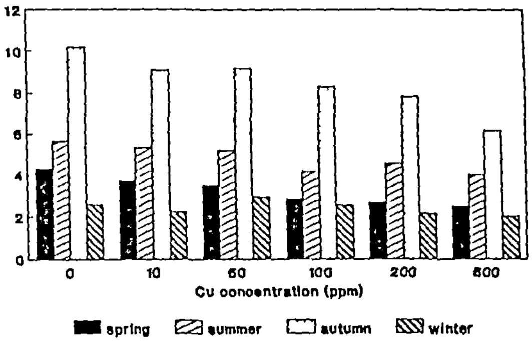


Fig.196

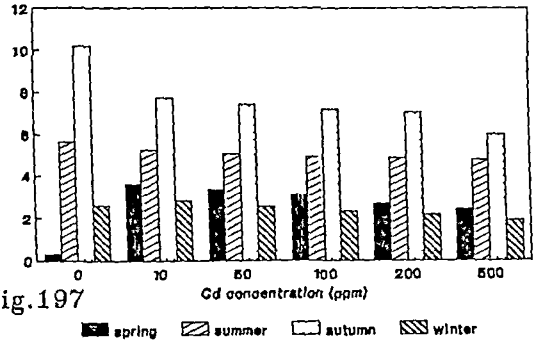


Fig.197

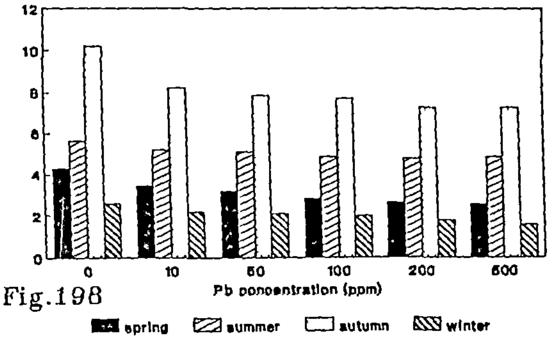


Fig.198

Fig.199–204 Urease Activity on rhizospheric Soil Inoculated with *C.graniforme*

NH_4-N released mg/g dry wt/3h

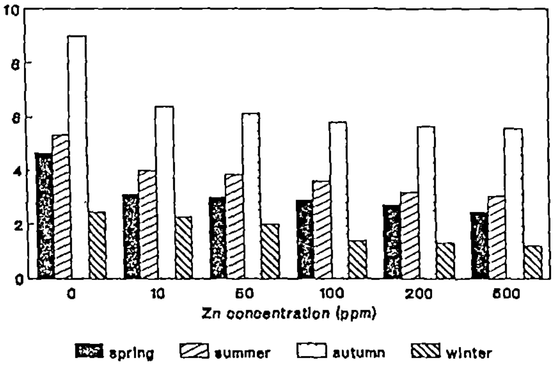


Fig.199

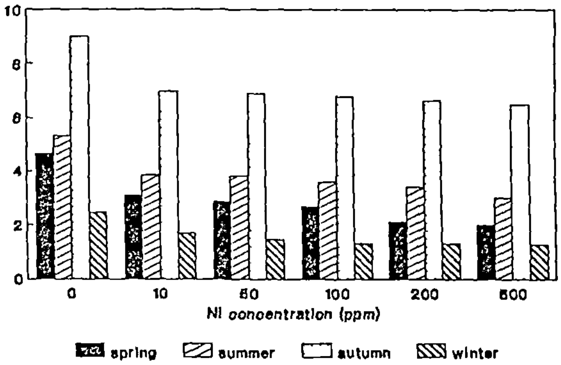


Fig.200

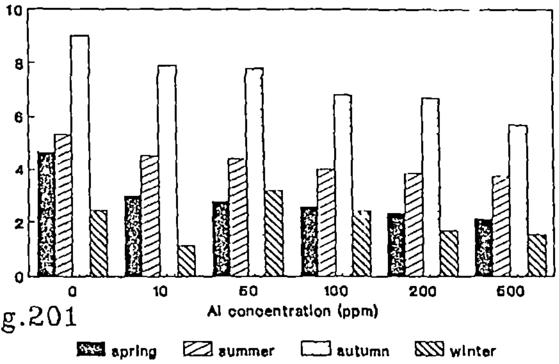


Fig.201

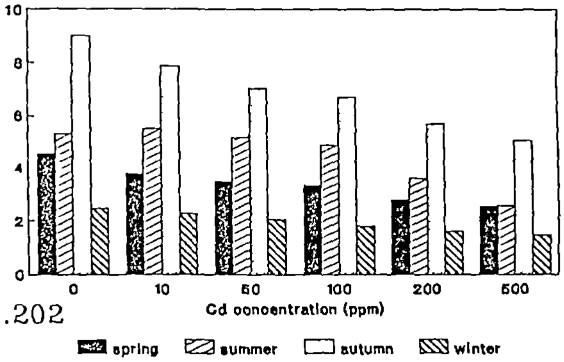


Fig.202

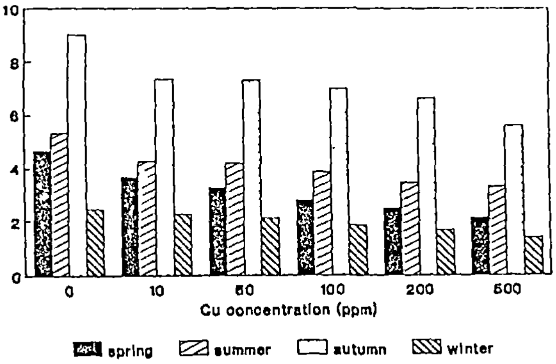


Fig.203

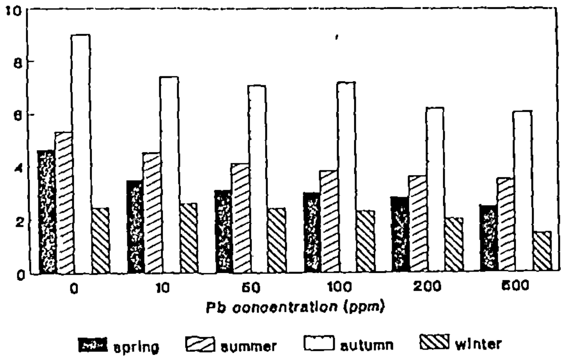


Fig.204

Fig.205–210 Urease Activity on Root Surface Inoculated with *S.aurantium*

NH$_4$-N released mg/g dry wt/3h

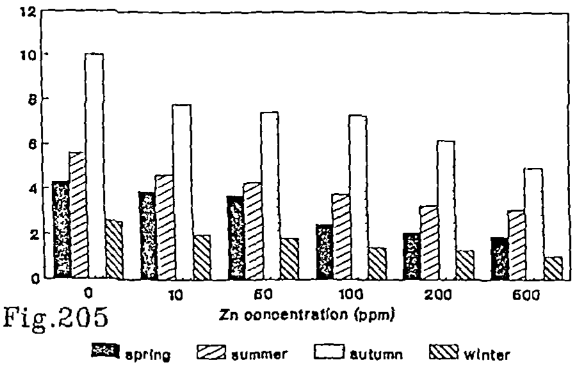


Fig.205

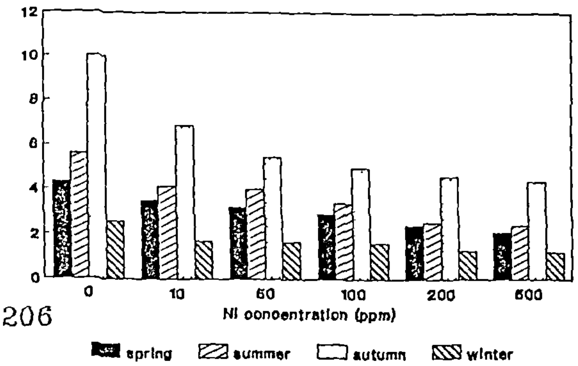


Fig.206

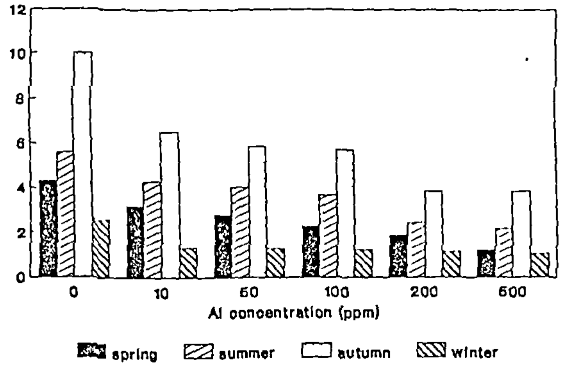


Fig.207

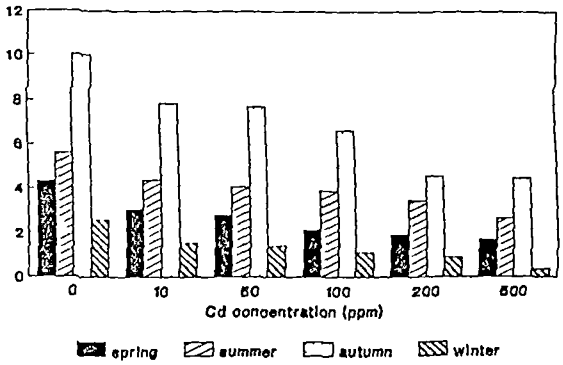


Fig.208

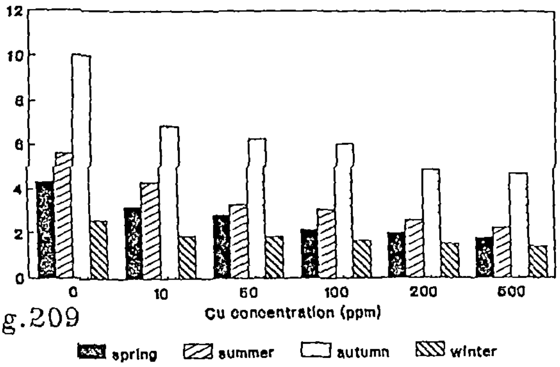


Fig.209

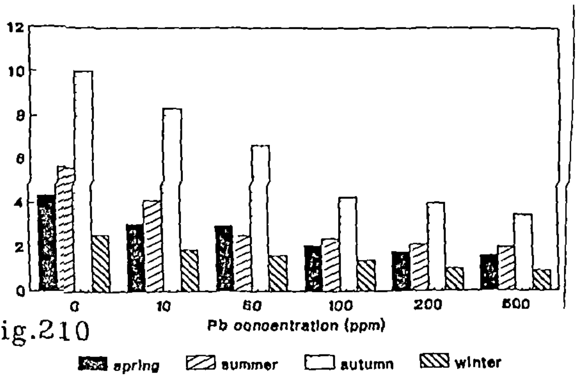


Fig.210

Fig.211–216 Urease Activity on rhizospheric Soil Inoculated with *S.aurantium*

NH$_4$-N released mg/g dry wt/3h

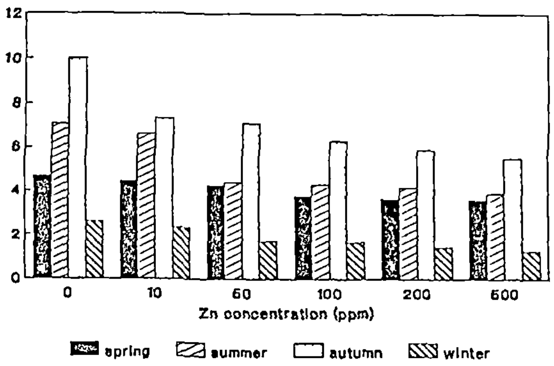


Fig.211

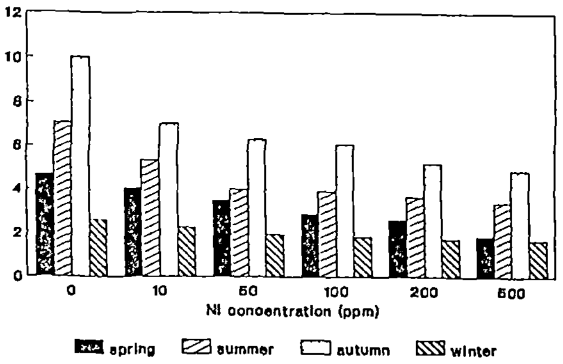


Fig.212

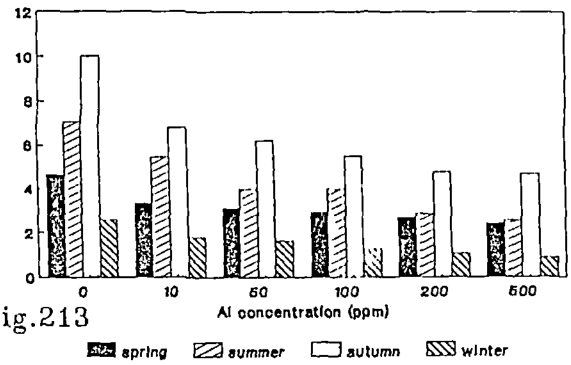


Fig.213

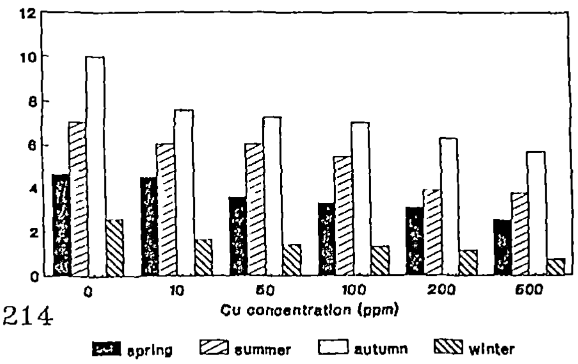


Fig.214

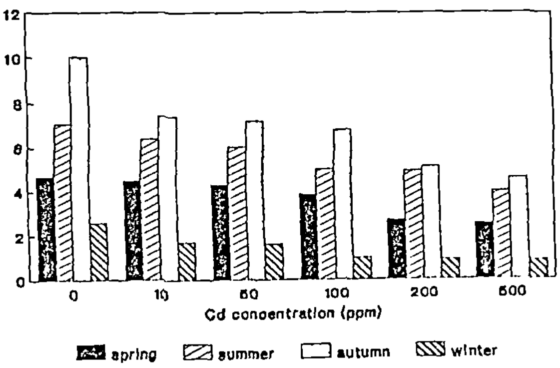


Fig.215

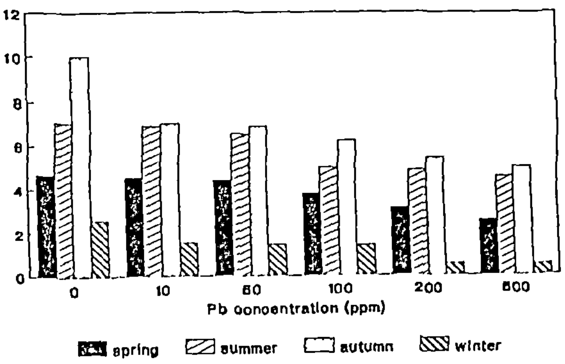


Fig.216

Fig.217−222 Urease Activity on Non−mycorrhizal Root Surface

NH_4-N released mg/g dry wt/3h

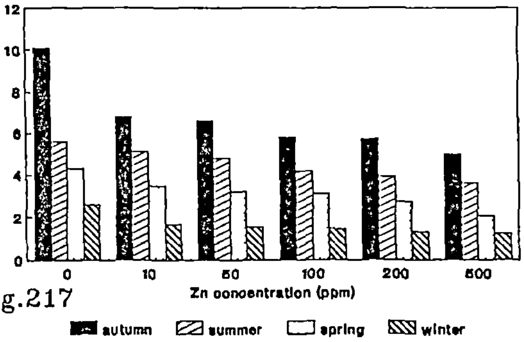


Fig.217

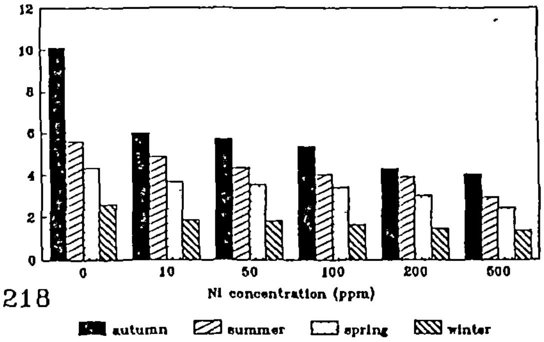


Fig.218

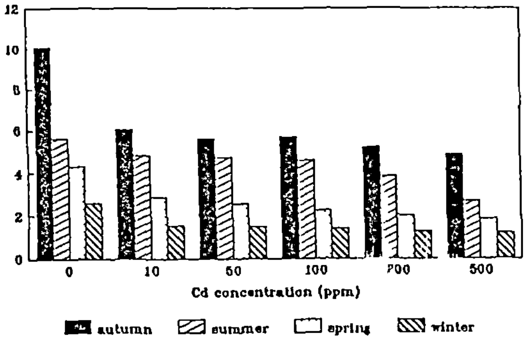


Fig.219

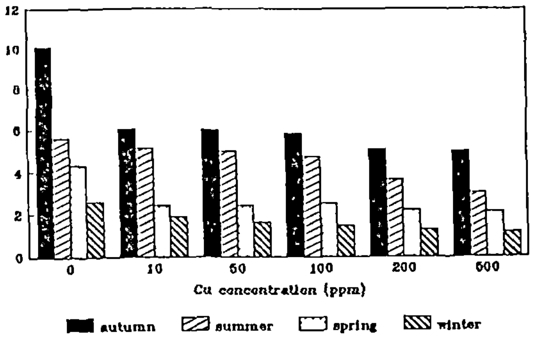


Fig.220

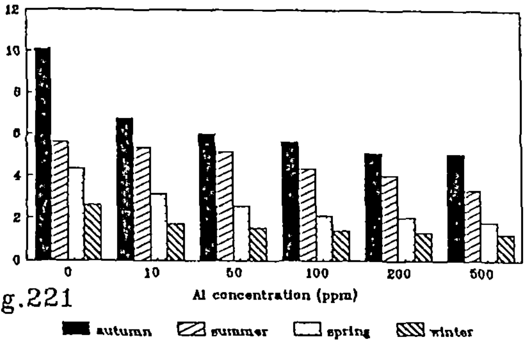


Fig.221

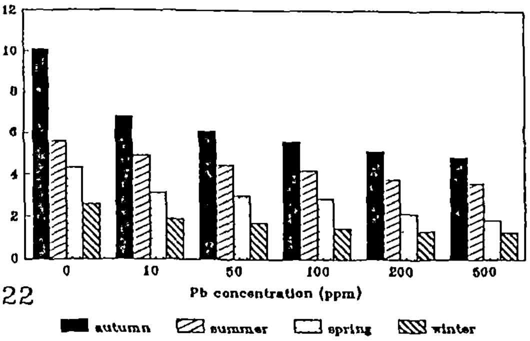


Fig.222

Fig.223-228 Urease Activity on Non-mycorrhizal rhizospheric Soil

NH_4-N released mg/g dry wt/3h

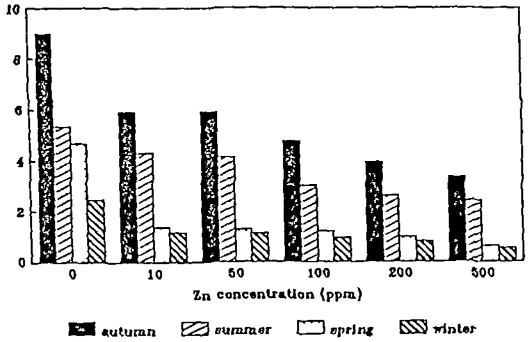


Fig.223

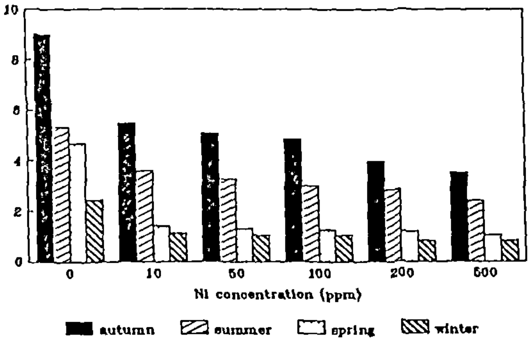


Fig.224

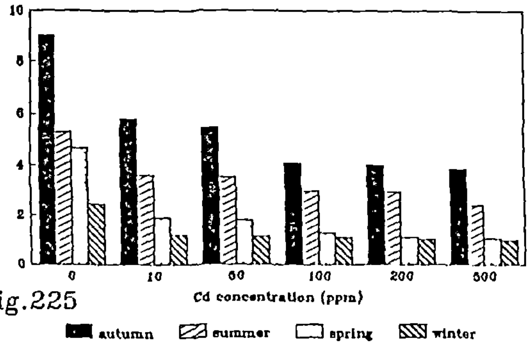


Fig.225

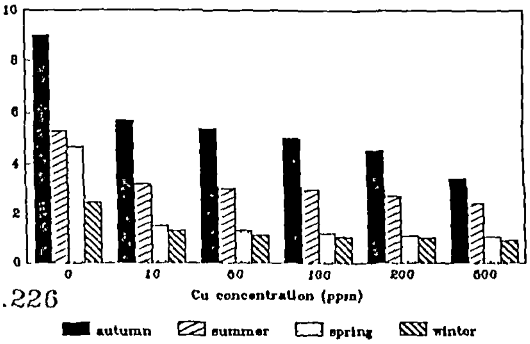


Fig.226

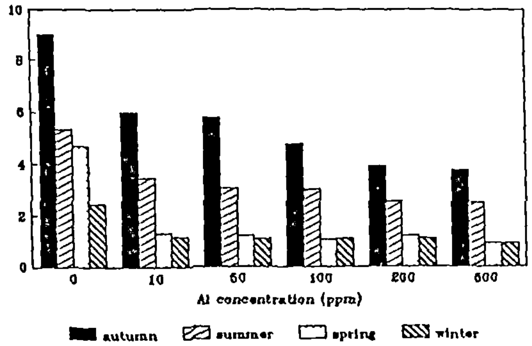


Fig.227

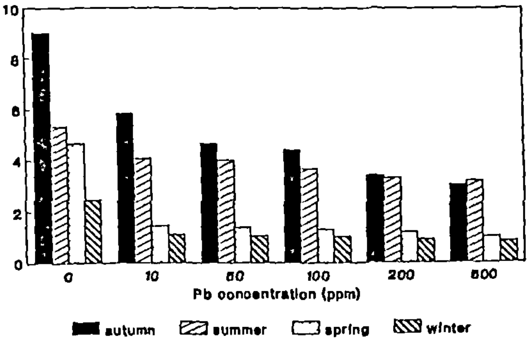


Fig.228

Fig.229-234 Urease Activity on Non-mycorrhizal Root Surface

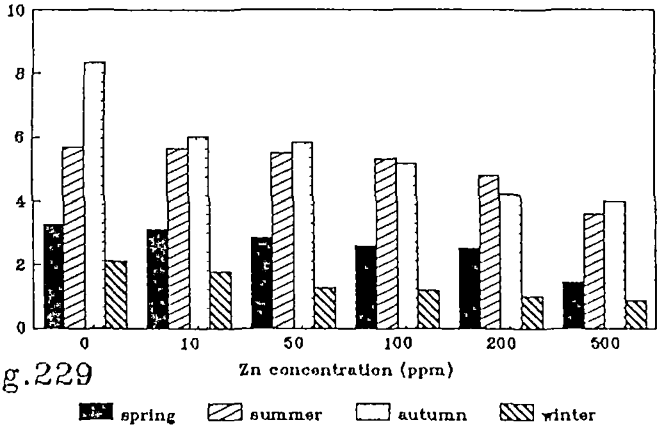


Fig.229

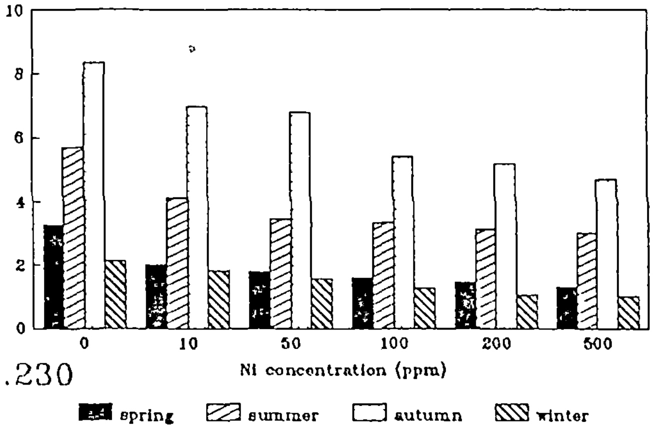
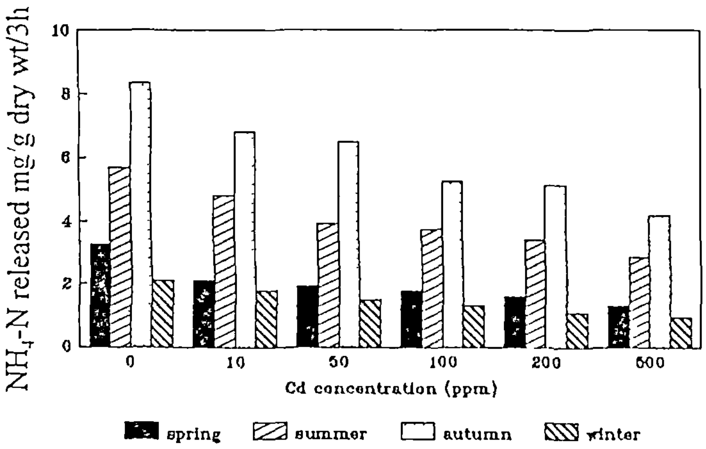


Fig.230



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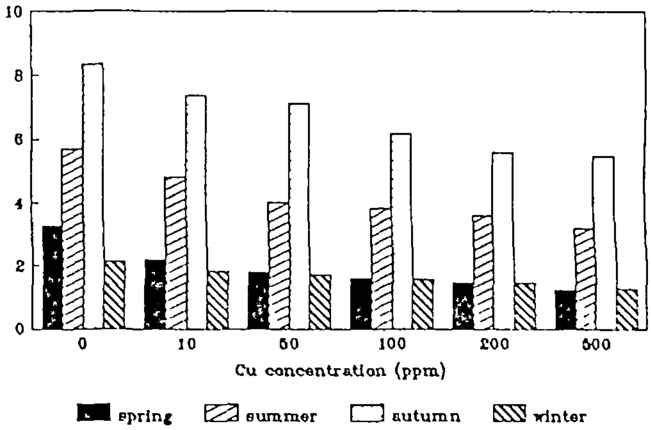


Fig.232

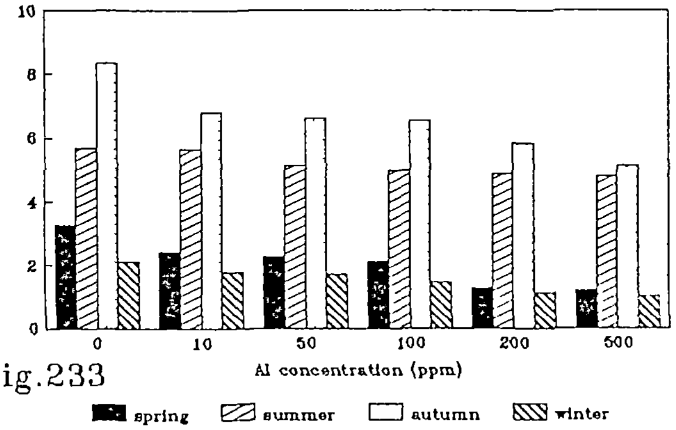


Fig.233

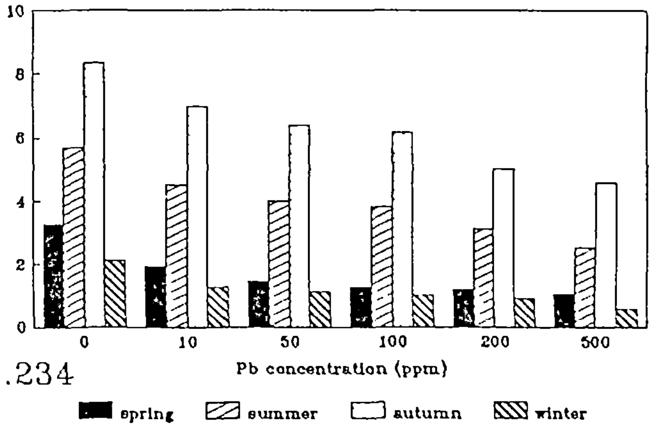


Fig.234

Fig.229-234 Urease Activity on Non-mycorrhizal Root Surface

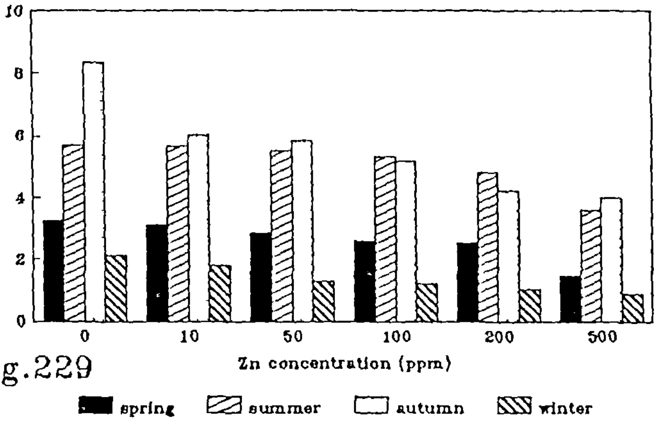


Fig.229

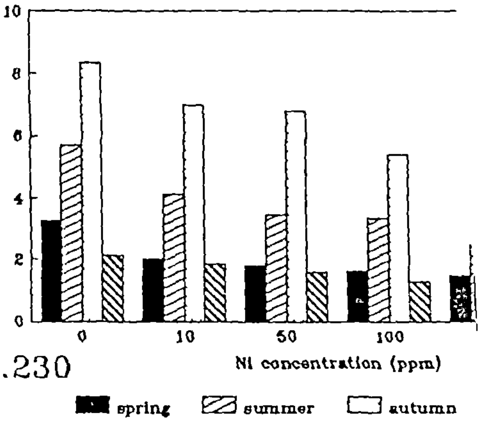


Fig.230

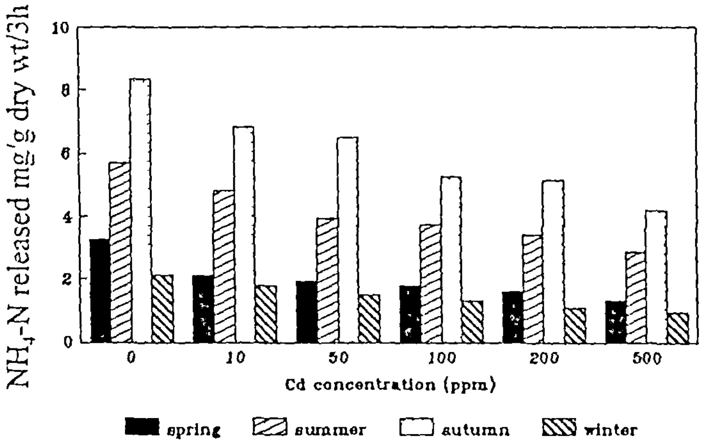
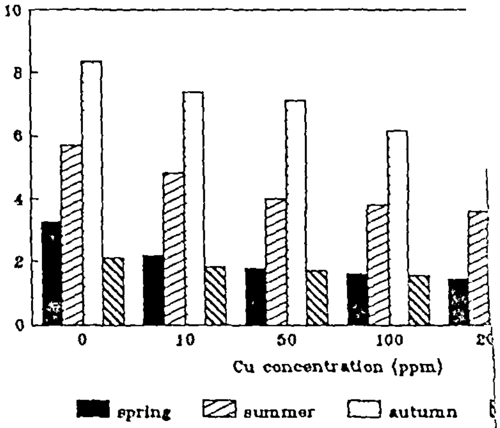


Fig.231



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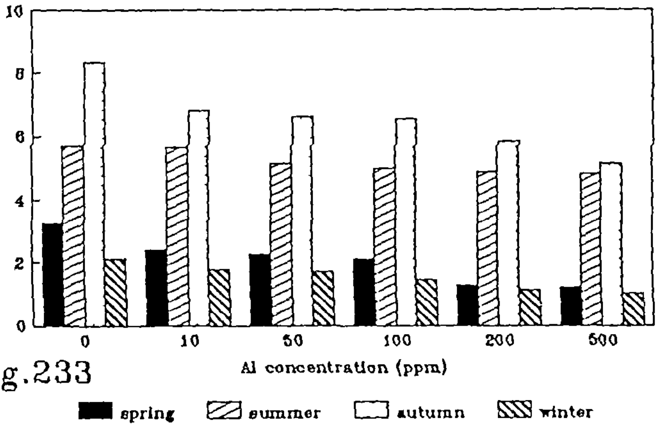

Fig.233

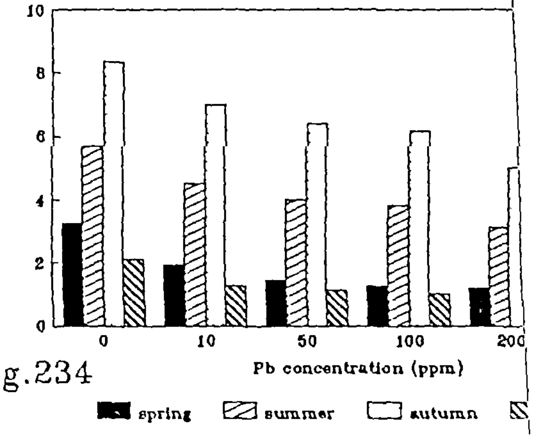

Fig.234

Fig.235–240 Urease Activity on Non-mycorrhizal rhizospheric Soil

NH$_4$-N released mg/g dry wt/3h

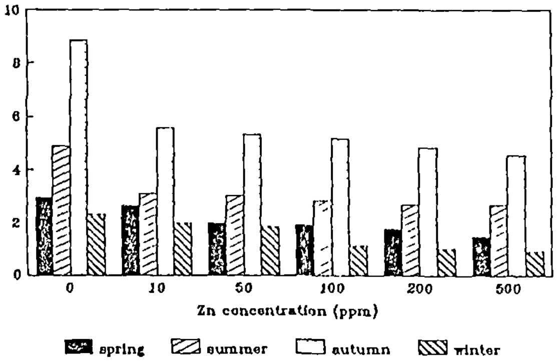

Fig.235

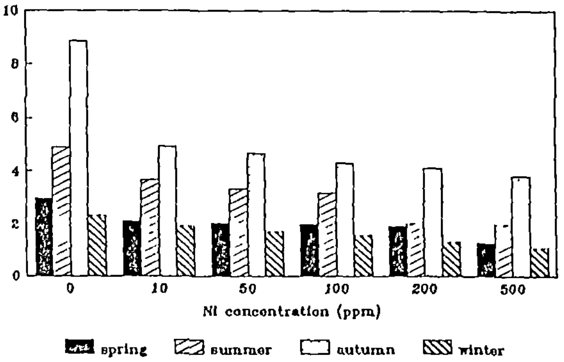

Fig.236

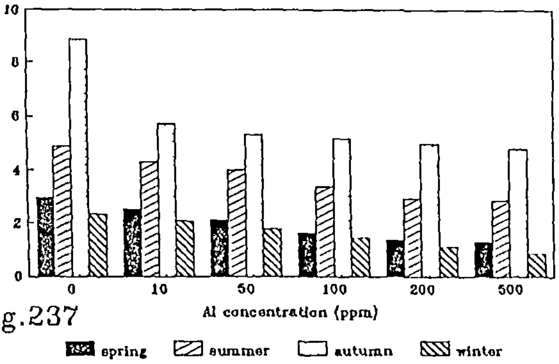

Fig.237

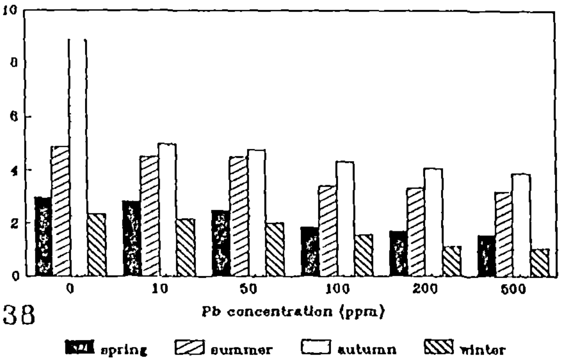

Fig.238

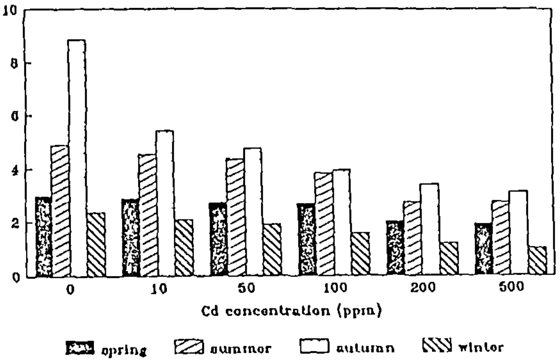

Fig.239

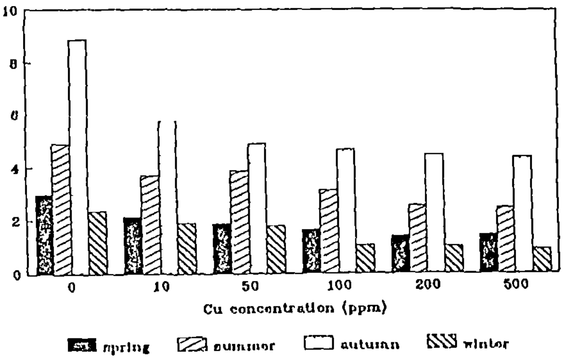

Fig.240

Fig.241–246 Urease Activity on rhizospheric Soil Inoculated with *Boletus sp.*

NH_4-N released mg/g dry wt/3h

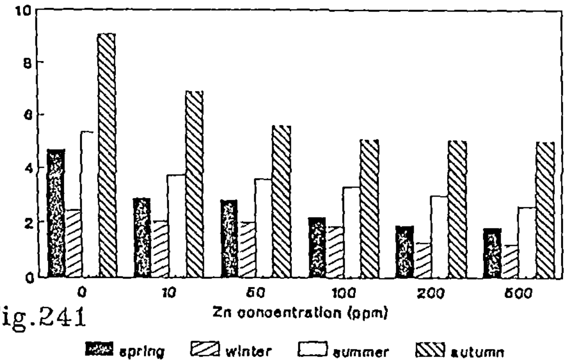

Fig.241

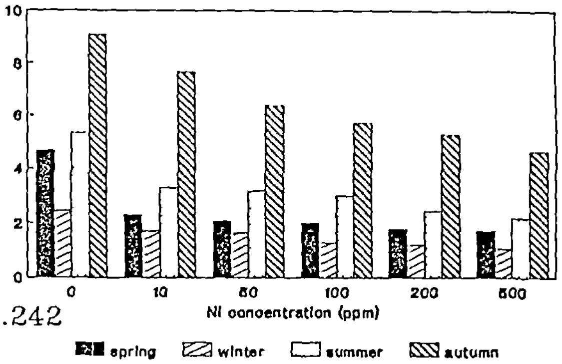

Fig.242

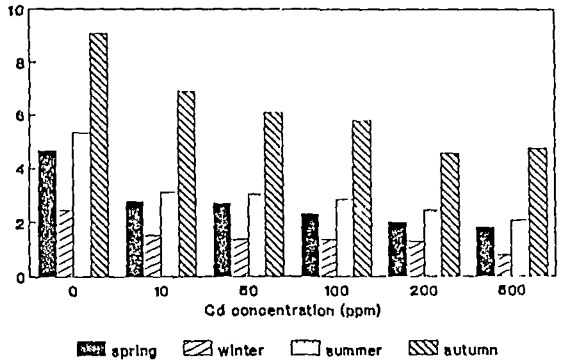

Fig.243

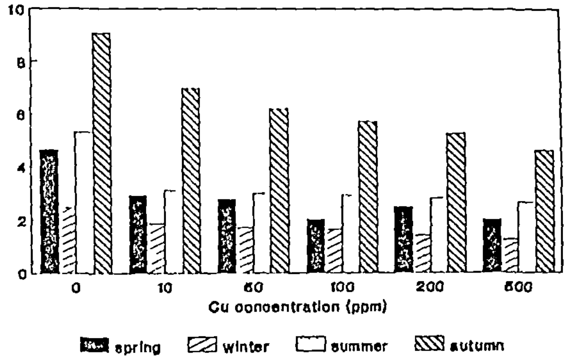

Fig.244

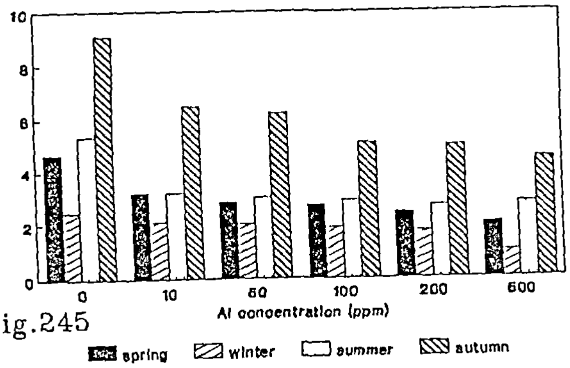

Fig.245

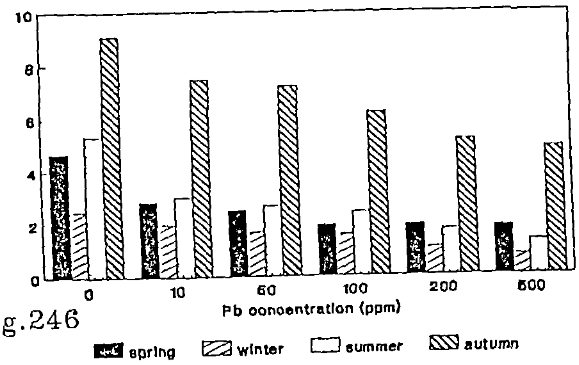

Fig.246

Fig.247–252 Urease Activity on Root Surface Inoculated with *Boletus sp.*

NH$_4$-N released mg/g dry wt/3h

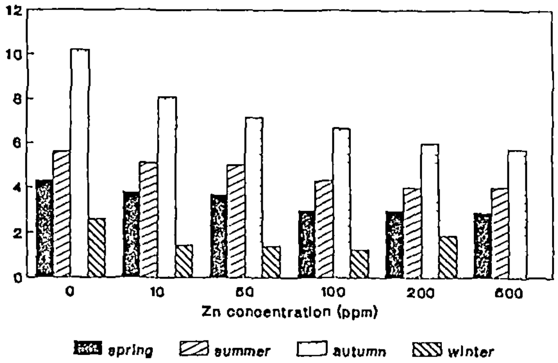

Fig.247

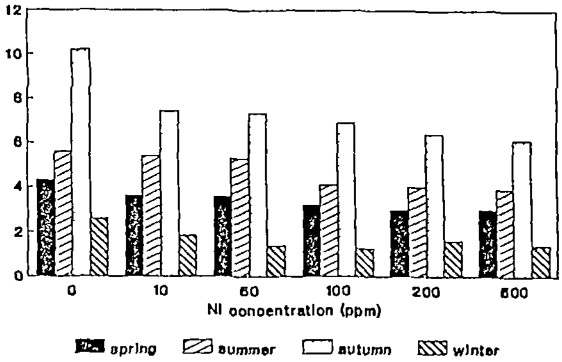

Fig.248

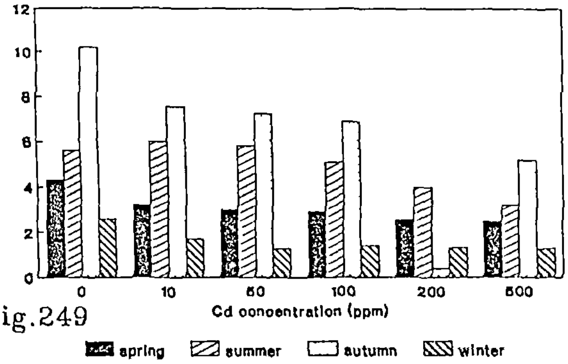

Fig.249

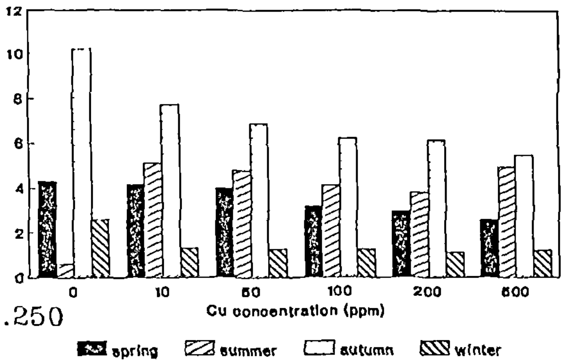

Fig.250

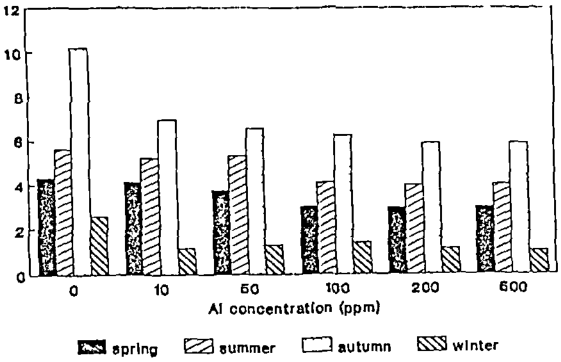

Fig.251

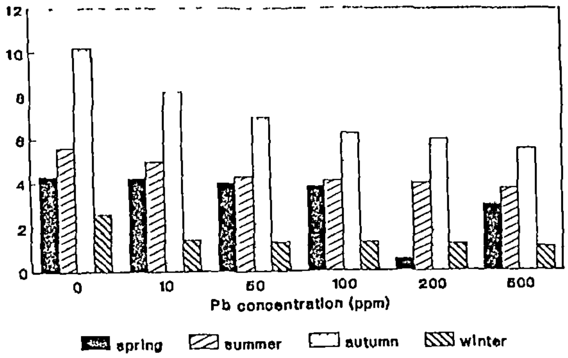

Fig.252

Table 3.1 Correlation coefficient (r) values of mycorrhizal infection with enzyme activities of pine seedlings in pots and in field condition.

		Mycorrhizal infection						
Parameters		M1	M2	M3	M4	NM	MMF	NM
Phosphatase	1	0.91^{*}	0.89^{*}	0.88^{*}	0.80^{**}	NS	0.90^{**}	NS
	2	0.92^{*}	0.93^{*}	0.96^{*}	0.95^{*}	NS	0.98^{*}	NS
Dehydrogenase	1	0.87^{*}	0.81^{**}	0.91^{*}	0.89^{*}	NS	0.91^{*}	NS
	2	0.96^{*}	0.97^{*}	0.93^{*}	0.90^{*}	NS	0.93^{*}	NS
Urease	1	0.98^{*}	0.91^{*}	0.87^{*}	0.80^{**}	NS	0.92^{*}	NS
	2	0.90^{*}	0.94^{*}	0.96^{*}	0.86^{**}	NS	0.90^{*}	NS

M1= *C.graniforme*, M2 = *S.luteus*, M3 = *Boletus* sp.,

M4 = *S.aurantium.*

NM = Non-mycorrhizal, MMF = Mixed mycorrhizal fungi.

$*$ = significant at $p<0.01$ level
$**$ = significant at $p<0.05$ level.

Table 3.2 Analysis of variance (F) values with the sampling periods, mycorrhizal and non-mycorrhizal and pots and field with various parameters in pine seedlings.

Source of variance		Variation between sampling periods	Variation between mycorrhizal and non-mycorrhizal roots	Variation between pots and field conditions
Phosphatase	1	13.32**	10.84**	12.31**
	2	7.3*	7.91*	5.58**
Urease	1	3.5**	7.9*	7.25*
	2	3.43**	3.13*	4.57**
Dehydrogenase	1	7.59*	6.33**	9.18**
	2	6.71*	6.28**	7.93*

1 = root surface
2 = rhizospheric soil

* = significant at 0.01 level
** = significant at 0.05 level

in rhizospheric soil.

In case of non-mycorrhizal seedlings the phosphatase activity was less and inhibition rate was high. Cd and Pb inhibited the rate of phosphatase activity from 42% to 40% respectively in roots while 45% and 44% inhibition in rhizospheric soil was observed in Zn and Pb treated seedlings respectively. There was a negative correlation between heavy metals *concentration and the activity of urease, phosphatase* and dehydrogenase activity. A positive correlation was found between mycorrhizal infection and the activity of urease, phosphatase and dehydrogenase ($P < 0.05$ and $P < 0.01$ level) (Table 3.1). There was a significant variation between sampling periods of *phosphatase, urease and dehydrogenase* ($P < 0.05$) in both rhizospheric soil as well as in root region. A significant variation was also found between the mycorrhizal and non-mycorrhizal ones ($P < 0.01$) and between pots and field condition (Table 3.2).

Effect of heavy metals on the establishment, survival and growth of pine seedlings

Tables 4.1-4.26 show the effect of ectomycorrhizal fungi on pine seedlings, shoot height, number of needles, root length, needle length recorded at the 4th month, 8th month and 1 year old seedlings.

Effect of heavy metals on the growth of seedlings of *P. kesiya* inoculated with different ectomycorrhizal fungi was assessed for 1 year. Initial average shoot height, needle length, number of needles, root length and root colar diameter ranged from 4-7 cm, 2-3 cm, 16-18, 3-8 cm and 0.10-0.12 cm respectively (Tables 4.1-4.5). In case of uninoculated seedlings the reduction in growth rate was observed as shown in Plate 2 (2). There was no significant difference in the growth of seedlings between the four ectomycorrhizal fungi inoculated seedlings but the control seedlings showed much lower growth rate as compared to ectomycorrhizal seedlings. However, inspite of the heavy metals contaminations,*C. graniforme*, (Plates 3 i- vi) *Boletus* sp. *S. aurantium*, and *S. luteus* significantly improved the growth of seedlings than non-mycorrhizal ones.

Inoculation of pine seedlings with ectomycorrhizal fungi consistently stimulated an increase in shoot height, number of needles, lateral root length throughout the growing seasons as compared with non-mycorrhizal seedlings. Shoot height was improved in *S.luteus* inoculated seedlings with an average of 7 cm followed by *Boletus* and *Cenococcum* treated seedlings (Tables 4.17, 4.18, 4.20). Minimum shoot height was observed in Pb and Al treated ectomycorrhizal seedlings. In case of non-mycorrhizal seedlings the shoot height was an average of 4.5 cm (Table 4.5). The growth of mycorrhizal plants without any heavy metal treatment was vigorous as shown in plate 2 (1) as compared with that of non-mycorrhizal plants exposed to heavy metals at different levels of Cd, Cu, Pb, Zn, Ni and Al. Root length was reduced by the 100 ppm and more concentration of these metals in the potting media. Such effect was decreased by the ectomycorrhizal fungi. No ectomycorrhizal formation was observed in non-inoculated seedlings in pot cultures. Seedlings inoculated with *C. graniforme* and treated with Cu showed

Table 4.1 Effect of heavy metals on the growth in 4 months old pine seedlings inoculated with *Suillus luteus* in pot culture.

Metal conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needl-les	Seedl-ings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.3	3.1	18	0.105	0.12	6.9
Zn10	6.1	2.8	18	0.08	0.10	6.4
Zn50	5.9	2.6	17	0.06	0.10	6.9
Zn100	5.5	2.4	17	0.055	0.10	6.0
Zn200	5.3	2.1	17	0.053	0.10	5.8
Zn500	5.0	2.0	18	0.052	0.10	5.3
Ni10	7.0	2.8	18	0.10	0.12	4.1
Ni50	6.9	2.9	18	0.096	0.12	3.9
Ni100	6.4	2.7	19	0.06	0.10	3.7
Ni200	5.7	2.4	18	0.057	0.10	3.3
Ni500	5.2	2.4	17	0.054	0.10	3.2
Pb10	6.2	2.8	16	0.089	0.12	6.5
Pb50	5.8	2.6	17	0.083	0.12	6.5
Pb100	5.6	2.5	18	0.056	0.10	6.3
Pb200	5.3	2.5	18	0.053	0.10	5.9
Pb500	5.1	2.2	18	0.051	0.10	5.5
Cu10	6.4	2.7	17	0.064	0.10	5.1
Cu50	6.0	2.6	17	0.085	0.12	5.5
Cu100	5.5	2.4	17	0.055	0.10	4.8
CU200	5.4	2.3	18	0.054	0.10	4.6
Cu500	4.8	2.1	18	0.048	0.10	4.6
Al10	6.5	3.1	17	0.065	0.10	6.7
Al50	6.2	3.0	18	0.09	0.10	6.6
Al100	5.7	2.8	18	0.082	0.12	6.1
Al200	5.6	2.6	18	0.056	0.10	5.8
Al500	5.4	2.4	17	0.054	0.10	5.7
Cd10	6.9	2.8	17	0.099	0.12	6.8
Cd50	6.4	2.4	18	0.092	0.12	6.5
Cd100	6.4	2.4	17	0.064	0.10	6.1
Cd200	6.1	2.3	18	0.061	0.10	5.8
Cd500	5.7	2.1	18	0.057	0.10	5.5

Table 4.2 Effect of heavy metals on the growth in 4 months old pine seedlings inoculated with *Boletus. sp* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.32	3.7	19	0.105	0.12	7.6
Zn10	6.5	3.3	18	0.093	0.12	6.5
Zn50	6.5	3.2	18	0.093	0.12	6.1
ZN100	6.4	2.9	18	0.092	0.12	5.0
ZN200	5.8	2.3	18	0.058	0.10	5.0
ZN500	5.0	2.1	17	0.051	0.10	4.2
Ni10	6.4	3.1	18	0.092	0.12	7.1
NI50	6.3	2.8	19	0.090	0.12	6.8
NI100	5.9	2.5	16	0.084	0.12	5.1
Ni200	6.0	2.4	17	0.086	0.12	5.0
NI500	6.0	2.3	17	0.061	0.10	4.0
Al10	7.0	3.4	18	0.100	0.12	3.4
Al50	6.1	3.3	18	0.089	0.12	3.3
AL100	5.1	2.6	18	0.073	0.12	2.8
Al200	5.0	2.5	17	0.051	0.10	2.5
Al500	4.3	2.4	17	0.043	0.10	2.4
Cd10	6.0	2.8	19	0.086	0.12	7.3
Cd50	5.8	2.6	18	0.083	0.12	7.1
CD100	5.3	2.3	16	0.053	0.10	6.7
Cd200	5.0	2.3	16	0.051	0.10	6.6
Cd500	4.3	2.0	17	0.041	0.10	5.9
Cu10	5.5	3.1	19	0.079	0.12	7.2
CU50	4.9	2.8	19	0.070	0.12	6.9
Cu100	4.8	2.4	18	0.053	0.12	5.9
Cu200	4.3	2.3	17	0.043	0.10	5.5
Cu500	4.1	2.1	17	0.041	0.10	5.1
Pb10	6.3	2.7	18	0.090	0.12	7.1
Pb50	6.2	2.5	18	0.089	0.12	6.5
Pb100	5.9	2.3	18	0.085	0.12	5.2
Pb200	4.9	2.2	16	0.049	0.10	5.1
Pb500	4.5	2.2	17	0.045	0.10	4.1

Table 4.3 Effect of heavy metals on the growth in 4 months old pine seedlings inoculated with *S. aurantium* in pot culture

Metals conc. (ppm)	Shoot heigth (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.3	3.7	19	0.105	0.12	7.6
Zn10	6.5	3.3	18	0.093	0.12	6.5
Zn50	6.5	3.2	18	0.093	0.12	6.1
Zn100	6.4	2.9	18	0.092	0.12	5.0
Zn200	5.8	2.3	18	0.053	0.10	5.0
Zn500	5.0	2.1	17	0.050	0.10	4.2
Ni10	6.4	3.0	18	0.092	0.12	7.1
Ni50	6.3	2.8	19	0.090	0.12	6.8
Ni100	5.9	2.5	16	0.084	0.12	5.1
Ni200	6.0	2.4	17	0.086	0.12	5.0
Ni500	6.0	2.3	17	0.061	0.10	4.0
Al10	7.0	3.4	18	0.100	0.12	3.3
Al50	6.1	3.3	18	0.087	0.12	3.3
Al100	5.1	2.6	18	0.073	0.12	2.6
Al200	5.0	2.5	17	0.050	0.10	2.5
Al500	4.3	2.4	17	0.043	0.10	2.4
Cd10	6.0	2.8	19	0.086	0.12	7.3
Cd50	5.8	2.6	18	0.083	0.12	7.1
Cd100	5.3	2.3	16	0.053	0.10	6.8
Cd200	5.0	2.3	16	0.051	0.10	6.6
Cd500	4.3	2.0	17	0.043	0.10	5.9
Cu10	5.0	3.1	19	0.079	0.12	7.2
Cu50	4.9	2.9	19	0.070	0.12	6.9
Cu100	4.8	2.4	18	0.048	0.10	5.9
Cu200	4.3	2.3	17	0.043	0.10	5.5
Cu500	4.1	2.1	17	0.041	0.10	5.1
Pb10	6.3	2.7	18	0.090	0.12	7.1
Pb50	6.2	2.5	18	0.089	0.12	6.5
Pb100	5.9	2.3	18	0.085	0.12	5.2
Pb200	4.9	2.2	16	0.049	0.10	5.0
Pb500	4.6	2.2	17	0.045	0.10	4.1

Table 4.4 Effect of heavy metals on the growth in 4 months old pine seedlings inoculated with *C. graniforme* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.3	3.8	18	0.105	0.12	9.6
Zn10	5.9	2.4	19	0.084	0.12	9.0
Zn50	5.6	2.3	18	0.080	0.12	7.7
Zn100	5.6	2.2	18	0.078	0.12	7.1
Zn200	5.5	2.2	18	0.075	0.10	7.0
Zn500	5.1	2.2	17	0.073	0.12	7.0
Ni10	6.0	2.5	19	0.101	0.12	6.7
Ni50	5.4	2.4	18	0.083	0.12	6.6
Ni100	5.1	2.3	18	0.051	0.10	6.2
Ni200	4.1	2.3	18	0.041	0.10	6.0
Ni500	4.1	2.1	17	0.039	0.12	5.2
Al10	6.0	3.5	19	0.086	0.12	6.3
Al50	5.9	3.3	19	0.084	0.12	6.2
Al100	5.5	3.2	18	0.050	0.12	5.9
Al200	5.3	3.1	17	0.052	0.10	5.8
Al500	5.2	2.8	17	0.052	0.10	5.1
Cd10	6.2	3.0	19	0.089	0.12	8.3
Cd50	6.1	2.7	18	0.087	0.12	8.1
Cd100	5.7	2.4	18	0.082	0.12	8.0
Cd200	5.6	2.3	18	0.056	0.10	7.1
Cd500	5.3	2.1	17	0.053	0.10	6.3
Cu10	5.9	2.8	18	0.084	0.12	9.0
Cu50	5.8	2.6	17	0.083	0.12	8.2
Cu100	5.5	2.4	18	0.079	0.12	8.1
Cu200	5.5	2.4	18	0.055	0.10	7.1
Cu500	5.3	2.2	17	0.052	0.10	6.3
Pb10	5.7	2.8	19	0.082	0.12	6.8
Pb50	5.5	2.6	18	0.079	0.12	6.6
Pb100	5.2	2.4	19	0.074	0.12	5.8
Pb200	5.0	2.4	18	0.052	0.10	5.2
Pb500	4.8	2.3	18	0.069	0.12	5.1

Table 4.5 Effect of heavy metals on the growth of 4 months old pine seedlings in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.0	3.8	19	0.104	0.12	7.1
Zn10	4.0	2.8	18	0.04	0.10	4.8
Zn50	4.0	2.6	18	0.04	0.10	4.7
Zn100	3.7	2.5	17	0.037	0.10	4.4
Zn200	3.1	2.4	17	0.031	0.10	4.2
Zn500	3.0	2.1	17	0.030	0.10	4.0
Ni10	5.4	3.2	18	0.077	0.12	6.5
Ni50	5.1	2.8	17	0.051	0.10	6.0
Ni100	4.5	2.6	17	0.06	0.10	5.5
Ni200	4.4	2.3	17	0.044	0.10	5.1
Ni500	4.0	2.0	17	0.04	0.10	4.2
Al10	4.5	3.1	17	0.064	0.12	6.0
Al50	4.2	2.4	17	0.06	0.12	5.2
Al100	4.1	2.3	17	0.045	0.10	5.1
Al200	4.0	2.2	16	0.041	0.10	4.3
Al500	3.4	2.1	16	0.035	0.10	4.1
Cd10	4.6	3.9	18	0.047	0.10	6.2
Cd50	3.6	3.5	18	0.038	0.12	5.9
Cd100	3.2	3.1	17	0.032	0.10	5.4
Cd200	2.3	2.5	17	0.033	0.12	5.2
Cd500	2.2	2.1	16	0.023	0.10	5.0
Cu10	5.3	3.2	18	0.076	0.12	7.0
Cu50	5.1	3.0	17	0.073	0.12	6.7
Cu100	5.0	2.4	16	0.072	0.12	6.1
Cu200	4.8	2.1	16	0.028	0.10	5.2
Cu500	3.2	2.1	16	0.032	0.10	5.1
Pb10	5.0	3.0	19	0.042	0.10	5.2
Pb50	4.2	2.6	18	0.042	0.12	5.0
Pb100	4.0	2.4	18	0.041	0.10	4.3
Pb200	4.3	2.2	18	0.43	0.10	4.2
Pb500	3.2	2.1	18	0.032	0.10	4.1

Table 4.6 Effect of heavy metals on the growth in 8 months old pine seedlings inoculated with *S. luteus* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.9	3.0	58	0.255	0.18	8.8
Zn10	7.7	3.0	55	0.277	0.18	8.6
Zn50	7.3	2.9	51	0.236	0.18	8.3
Zn100	6.8	2.9	54	0.220	0.18	8.0
Zn200	6.5	2.8	50	0.166	0.16	6.1
Zn500	5.9	2.6	46	0.132	0.15	6.0
Ni10	7.5	3.3	44	0.270	0.19	5.8
Ni50	7.3	3.0	39	0.235	0.18	5.7
Ni100	7.1	3.0	38	0.189	0.16	5.6
Ni200	6.4	2.6	36	0.163	0.15	5.6
Ni500	5.7	2.5	31	0.128	0.15	5.2
Al10	7.6	3.6	48	0.246	0.18	8.0
Al50	7.2	3.4	47	0.234	0.18	7.9
Al100	7.0	3.2	39	0.157	0.15	7.9
Al200	6.5	3.0	39	0.146	0.15	7.3
Al500	6.1	2.9	31	0.137	0.15	6.1
Cd10	7.3	3.1	49	0.263	0.19	7.9
Cd50	7.2	3.0	44	0.259	0.19	7.3
Cd100	6.8	2.9	43	0.153	0.15	6.1
Cd200	6.7	2.5	39	0.150	0.15	6.0
Cd500	6.2	2.4	36	0.089	0.12	5.3
Cu10	6.9	2.9	55	0.249	0.19	6.1
Cu50	6.7	2.8	51	0.171	0.16	6.1
Cu100	6.3	2.7	40	0.161	0.16	5.9
Cu200	6.0	2.7	33	0.86	0.12	5.1
Cu500	5.7	2.4	32	0.82	0.12	5.1
Pb10	7.1	3.4	58	0.230	0.18	8.0
Pb50	6.9	2.9	58	0.176	0.16	7.9
Pb100	6.4	2.7	41	0.086	0.12	6.0
Pb200	6.0	2.7	40	0.006	0.12	6.0
Pb500	5.7	2.5	32	0.082	0.12	5.3

Table 4.7 Effect of heavy metals on the growth in 8 months old pine seedlings inoculated with *Boletus. sp* in pot culture

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.9	4.3	57	0.255	0.18	8.2
Zn10	6.9	3.9	44	0.233	0.18	6.9
Zn50	6.7	3.5	43	0.217	0.18	6.2
Zn100	6.6	3.4	34	0.168	0.16	5.3
Zn200	6.0	3.0	33	0.135	0.15	5.2
Zn500	5.5	2.8	28	0.123	0.15	4.9
Ni10	6.9	3.2	39	0.223	0.18	8.1
Ni50	6.6	2.9	38	0.148	0.15	7.3
Ni100	6.5	2.7	38	0.146	0.15	5.8
Ni200	5.5	2.8	29	0.123	0.15	5.5
Ni500	6.1	2.4	26	0.137	0.15	5.6
Al10	7.0	4.1	52	0.252	0.18	7.6
Al50	7.0	3.5	42	0.226	0.18	7.0
Al100	6.3	3.4	39	0.141	0.15	6.3
Al200	5.4	2.8	29	0.123	0.15	5.0
Al500	5.0	2.5	28	0.072	0.12	4.3
Cd10	6.5	3.5	42	0.210	0.18	8.0
Cd50	6.2	3.1	40	0.158	0.16	7.4
Cd100	5.8	2.9	38	0.130	0.15	7.1
Cd200	5.0	2.5	26	0.112	0.15	5.5
Cd500	5.0	2.4	33	0.073	0.12	6.3
Cu10	5.8	3.2	41	0.171	0.18	7.3
Cu50	5.8	3.1	41	0.170	0.18	7.2
Cu100	5.1	2.9	38	0.131	0.16	5.8
Cu200	5.0	2.5	26	0.112	0.15	5.5
Cu500	4.7	2.4	25	0.067	0.12	5.6
Pb10	6.9	3.4	55	0.155	0.15	7.6
Pb50	6.6	2.9	52	0.148	0.15	6.7
Pb100	6.3	2.8	46	0.090	0.12	5.3
Pb200	5.9	2.6	37	0.084	0.12	5.3
Pb500	4.7	2.4	36	0.064	0.12	4.6

Table 4.8 Effect of heavy metals on the growth in 8 months old pine seedlings inoculated with *S. aurantium* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.9	3.6	53	0.255	0.18	8.5
Zn10	6.2	3.1	36	0.158	0.16	7.0
Zn50	6.0	2.9	24	0.153	0.16	6.9
Zn100	5.5	2.6	24	0.123	0.15	5.0
Zn200	5.3	2.6	25	0.135	0.16	5.0
Zn500	5.1	2.3	22	0.114	0.15	4.1
Ni10	7.1	3.2	32	0.230	0.18	8.3
Ni50	6.5	3.1	28	0.210	0.18	8.1
Ni100	5.5	2.6	24	0.123	0.15	5.0
Ni200	5.3	2.6	25	0.076	0.16	5.0
Ni500	5.1	2.3	22	0.075	0.15	4.1
Al10	6.1	3.2	43	0.156	0.16	8.0
Al50	5.3	3.2	42	0.119	0.15	7.1
Al100	4.6	2.9	43	0.103	0.15	5.3
Al200	4.6	2.8	38	0.077	0.13	4.8
Al500	5.4	2.5	35	0.064	0.12	4.7
Cd10	6.5	3.3	37	0.234	0.18	7.3
Cd50	6.2	3.1	37	0.158	0.16	7.2
Cd100	5.9	3.0	33	0.084	0.12	7.0
Cd200	5.2	2.9	30	0.074	0.12	6.0
Cd500	4.4	2.7	29	0.060	0.12	5.8
Cu10	6.7	4.0	41	0.171	0.16	6.6
Cu50	6.3	3.9	40	0.141	0.15	6.5
Cu100	6.0	3.9	35	0.086	0.12	5.1
Cu200	5.2	3.2	38	0.074	0.12	5.0
Cu500	5.0	3.1	33	0.084	0.13	4.6
Pb10	6.3	3.4	34	0.204	0.18	8.2
Pb50	6.0	3.3	45	0.194	0.18	6.0
Pb100	5.0	2.9	42	0.128	0.16	7.0
Pb200	4.8	2.8	42	0.069	0.12	6.1
Pb500	4.6	2.8	36	0.077	0.13	4.3

Table 4.9 Effect of heavy metals on the growth in 8 months old pine seedlings inoculated with *C. graniforme* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.9	4.0	46	0.255	0.18	9.8
Zn10	6.1	3.1	32	0.220	0.18	8.6
Zn50	5.8	3.0	30	0.209	0.18	8.2
Zn100	5.7	2.5	28	0.143	0.16	7.5
Zn200	5.6	2.3	27	0.143	0.16	7.4
Zn500	5.3	2.3	21	0.119	0.15	7.3
Ni10	6.3	2.8	34	0.204	0.18	8.1
Ni50	6.0	2.6	29	0.153	0.16	7.0
Ni100	5.4	2.4	24	0.121	0.15	7.6
Ni200	4.2	2.4	23	0.094	0.15	7.0
Ni500	4.2	2.2	22	0.094	0.15	6.8
Al10	6.2	3.6	36	0.158	0.16	7.3
Al50	6.1	3.4	32	0.156	0.16	7.1
Al100	5.7	3.3	29	0.145	0.16	6.8
Al200	5.4	3.2	27	0.121	0.15	6.3
Al500	5.3	3.0	26	0.076	0.12	6.1
Cd10	6.4	3.2	35	0.207	0.18	8.9
Cd50	6.2	2.9	33	0.179	0.17	8.6
Cd100	5.8	2.6	31	0.083	0.12	8.4
Cd200	5.7	2.4	28	0.082	0.12	7.8
Cd500	5.4	2.3	24	0.077	0.12	6.8
Cu10	6.3	3.1	38	0.161	0.16	9.5
Cu50	6.0	2.9	36	0.153	0.16	9.1
Cu100	5.7	2.7	35	0.128	0.15	8.6
Cu200	5.6	2.5	36	0.080	0.12	7.5
Cu500	5.3	2.3	31	0.076	0.12	6.5
Pb10	6.0	3.1	40	0.143	0.16	7.3
Pb50	5.7	2.9	36	0.164	0.17	7.1
Pb100	5.4	2.6	32	0.077	0.12	6.8
Pb200	5.1	2.4	30	0.073	0.12	6.2
Pb500	4.9	2.4	25	0.070	0.12	6.0

Table 4.10 Effect of heavy metals on the growth in 8 months old pine seedlings in pot culture

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol. (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.7	3.8	65	0.223	0.18	7.6
Zn10	4.1	3.0	42	0.092	0.15	4.8
Zn50	4.1	2.9	40	0.080	0.14	4.7
Zn100	3.3	2.4	31	0.054	0.12	4.1
Zn200	3.3	2.4	30	0.047	0.12	4.1
Zn500	3.2	2.3	30	0.046	0.12	4.0
Ni10	5.5	3.2	62	0.123	0.15	7.1
Ni50	5.2	3.2	59	0.117	0.15	7.0
Ni100	4.6	2.9	56	0.103	0.15	6.3
Ni200	4.5	2.7	59	0.101	0.15	5.4
Ni500	4.2	2.4	50	0.060	0.12	4.3
Al10	4.4	3.4	52	0.112	0.16	6.8
Al50	4.3	2.5	51	0.096	0.15	6.7
Al100	4.2	2.4	46	0.060	0.12	5.1
Al200	4.1	2.4	42	0.059	0.12	5.0
Al500	3.4	2.3	39	0.034	0.10	4.3
Cd10	4.7	4.0	60	0.120	0.16	7.4
Cd50	3.7	3.7	59	0.094	0.16	7.1
Cd100	3.6	3.3	58	0.081	0.15	6.8
Cd200	2.4	2.8	41	0.054	0.15	6.8
Cd500	2.3	2.2	41	0.033	0.12	5.0
Cu10	5.4	3.3	58	0.138	0.16	7.4
Cu50	5.2	3.1	57	0.133	0.16	7.1
Cu100	5.1	2.5	51	0.130	0.16	7.0
Cu200	4.9	2.3	46	0.110	0.15	5.8
Cu500	3.3	2.3	45	0.074	0.15	5.1
Pb10	5.1	3.1	66	0.130	0.16	5.7
Pb50	4.4	2.7	51	0.112	0.16	5.6
Pb100	4.1	2.5	50	0.059	0.12	5.2
Pb200	4.4	2.5	42	0.063	0.12	5.0
Pb500	3.7	2.3	40	0.056	0.12	4.4

Table 4.11 Effect of heavy metals on the growth in 4 months old pine seedlings inoculated with mixed mycorrhizal fungi in field condition.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length(cm)
0	13.3	5.0	102	0.492	0.19	9.5
Zn10	11.3	4.4	96	0.366	0.18	9.3
Zn50	11.2	4.0	83	0.227	0.15	7.3
Zn100	10.1	3.3	80	0.227	0.15	7.0
Zn200	9.2	3.0	68	0.132	0.12	6.9
Zn500	8.9	2.8	59	0.128	0.12	6.8
Ni10	10.1	3.9	92	0.258	0.16	8.3
Ni50	10.0	3.4	91	0.225	0.15	8.0
Ni100	9.3	3.3	82	0.209	0.15	7.6
Ni200	7.3	2.9	74	0.105	0.12	7.5
Ni500	7.0	2.8	60	0.070	0.10	7.3
Al10	12.0	4.3	89	0.388	0.18	6.7
Al50	9.3	4.1	83	0.238	0.16	6.0
Al100	9.2	4.0	76	0.235	0.16	5.1
Al200	9.0	3.8	73	0.202	0.15	5.0
Al500	6.0	2.8	65	0.086	0.12	4.6
Cd10	7.5	4.3	98	0.243	0.18	9.0
Cd50	7.0	4.2	90	0.226	0.18	8.4
Cd100	5.5	4.1	83	0.107	0.14	8.1
Cd200	5.3	4.1	60	0.076	0.12	5.9
Cd500	5.2	3.6	51	0.074	0.12	5.5
Cu10	9.2	3.9	92	0.332	0.19	6.8
Cu50	9.0	3.8	82	0.291	0.18	6.0
Cu100	8.9	3.4	76	0.227	0.16	5.8
Cu200	8.3	3.0	71	0.212	0.15	5.5
Cu500	8.0	2.9	62	0.180	0.15	4.9
Pb10	11.0	4.9	91	0.281	0.16	6.6
Pb50	9.0	4.0	84	0.230	0.16	6.3
Pb100	8.0	3.9	68	0.180	0.15	6.1
Pb200	7.1	3.5	61	0.102	0.12	5.2
Pb500	7.0	3.4	54	0.100	0.12	5.0

Table 4.12 Effect of heavy metals on the growth in 8 months old pine seedlings inoculated with mixed mycorrhizal fungi in field condition.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings Vol. (cm^3)	Root colar dm(cm)	Root Length (cm)
0	16.5	5.1	168	0.872	0.23	16.9
Zn10	12.5	4.5	161	0.661	0.23	16.0
Zn50	11.1	4.1	151	0.537	0.22	15.2
Zn100	10.0	3.4	116	0.529	0.23	11.5
Zn200	9.8	3.1	111	0.317	0.18	11.0
Zn500	9.5	3.0	108	0.213	0.15	9.0
Ni10	11.9	4.3	136	0.575	0.22	14.0
Ni50	11.7	3.6	125	0.618	0.23	10.1
Ni100	11.6	3.5	121	0.561	0.22	10.0
Ni200	7.8	3.1	106	0.312	0.20	8.3
Ni500	7.1	3.0	102	0.197	0.16	8.0
Al10	11.0	4.6	142	0.581	0.23	9.8
Al50	10.2	4.2	139	0.493	0.22	9.7
Al100	10.0	4.1	103	0.484	0.22	9.1
Al200	9.8	3.9	102	0.432	0.21	9.0
Al500	7.6	3.1	87	0.246	0.18	8.7
Cd10	9.6	4.7	130	0.464	0.22	15.1
Cd50	9.5	4.4	121	0.549	0.22	14.2
Cd100	8.0	4.2	119	0.423	0.23	14.0
Cd200	7.3	3.8	110	0.321	0.21	12.0
Cd500	7.0	3.7	101	0.179	0.16	11.5
Cu10	9.6	4.2	142	0.479	0.22	13.4
Cu50	9.2	4.1	134	0.445	0.22	13.0
Cu100	9.0	3.8	124	0.360	0.20	11.2
Cu200	8.9	3.2	112	0.283	0.18	11.0
Cu500	8.4	3.1	92	0.272	0.18	9.2
Pb10	11.2	5.0	140	0.552	0.21	14.2
Pb50	10.3	4.2	132	0.498	0.22	14.2
Pb100	9.5	4.1	129	0.380	0.20	13.5
Pb200	9.3	3.7	120	0.238	0.16	13.0
Pb500	9.1	3.6	112	0.240	0.15	11.1

Table 4.14 Effect of heavy metals on the growth in 4 months old pine seedlings in field condition.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	9.5	4.8	61	0.220	0.19	7.3
Zn10	5.4	3.6	40	0.121	0.15	5.2
Zn50	5.1	3.0	36	0.073	0.21	5.1
Zn100	5.0	2.1	33	0.072	0.12	4.6
Zn200	4.1	2.0	30	0.041	0.10	4.1
Zn500	4.2	2.1	30	0.042	0.10	4.0
Ni10	6.1	3.3	41	0.119	0.14	5.1
Ni50	5.9	2.3	34	0.099	0.13	5.0
Ni100	5.2	2.1	34	0.062	0.11	4.1
Ni200	5.1	2.1	33	0.051	0.10	4.0
Ni500	4.9	2.1	30	0.049	0.10	3.3
Al10	5.9	3.9	45	0.170	0.17	4.1
Al50	5.2	2.5	44	0.101	0.14	4.1
Al100	5.1	2.4	32	0.073	0.12	3.6
Al200	4.9	2.5	32	0.070	0.12	3.2
Al500	4.1	2.2	30	0.041	0.10	3.4
Cd10	5.9	3.0	36	0.132	0.15	6.8
Cd50	4.9	2.9	33	0.070	0.12	6.7
Cd100	5.0	2.8	33	0.072	0.12	6.1
Cd200	5.1	2.1	30	0.051	0.10	5.0
Cd500	5.0	2.0	21	0.050	0.10	5.0
Cu10	5.2	3.9	40	0.168	0.18	4.4
Cu50	5.0	3.1	38	0.162	0.18	4.0
Cu100	5.0	3.0	36	0.110	0.15	3.9
Cu200	5.0	2.9	34	0.072	0.12	3.3
Cu500	4.1	2.8	32	0.059	0.12	3.1
Pb10	5.7	4.7	45	0.128	0.15	5.2
Pb50	5.6	3.3	42	0.080	0.12	5.0
Pb100	5.3	3.0	43	0.076	0.12	4.1
Pb200	5.2	2.8	32	0.074	0.12	3.1
Pb500	5.1	2.7	31	0.051	0.10	3.0

Table 4.15 Effect of heavy metals on the growth in 8 months old pine seedlings in field condition.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	12.0	5.0	110	0.405	0.20	11.1
Zn10	5.8	3.7	91	0.187	0.18	6.8
Zn50	5.3	3.1	80	0.171	0.18	6.2
Zn100	5.2	2.4	52	0.101	0.14	5.6
Zn200	5.0	2.2	48	0.069	0.12	4.4
Zn500	4.8	2.1	45	0.067	0.12	4.3
Ni10	6.3	3.4	121	0.227	0.19	5.8
Ni50	6.0	2.5	98	0.194	0.18	5.6
Ni100	5.3	2.3	86	0.119	0.15	4.9
Ni200	5.2	2.2	80	0.074	0.12	4.8
Ni500	5.0	2.2	73	0.072	0.12	4.7
Al10	6.1	4.0	114	0.197	0.18	5.6
Al50	5.5	2.6	69	0.178	0.18	5.2
Al100	5.3	2.5	68	0.171	0.15	4.6
Al200	4.9	2.6	51	0.070	0.12	4.1
Al500	4.4	2.5	50	0.074	0.13	4.0
Cd10	6.0	3.2	89	0.194	0.18	7.4
Cd50	5.4	3.0	85	0.138	0.16	7.2
Cd100	5.2	3.9	82	0.133	0.16	6.9
Cd200	5.1	2.3	73	0.073	0.12	5.8
Cd500	5.1	2.1	62	0.073	0.12	5.6
Cu10	7.6	4.0	80	0.194	0.16	5.6
Cu50	7.0	3.2	78	0.178	0.16	4.3
Cu100	6.4	3.2	76	0.150	0.13	4.2
Cu200	6.3	3.1	76	0.090	0.12	4.2
Cu500	6.0	2.9	69	0.086	0.12	4.0
Pb10	6.3	4.9	98	0.204	0.18	8.1
Pb50	6.1	3.5	82	0.156	0.16	6.4
Pb100	5.6	3.2	80	0.143	0.16	5.3
Pb200	5.4	2.9	72	0.121	0.15	4.9
Pb500	5.3	2.9	65	0.076	0.12	4.3

Table 4.17 Effect of heavy metals on the growth in 1 year old pine seedlings inoculated with *S. luteus* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	8.2	4.5	70	0.512	0.25	12.3
Zn10	7.8	3.2	68	0.412	0.23	11.0
Zn50	7.5	3.0	60	0.363	0.22	10.3
Zn100	7.0	3.0	56	0.338	0.22	9.8
Zn200	6.7	2.9	51	0.295	0.21	9.1
Zn500	6.0	2.7	49	0.194	0.18	8.2
Ni10	7.6	3.5	59	0.402	0.23	9.3
Ni50	7.4	3.1	51	0.391	0.23	9.0
Ni100	7.2	3.0	46	0.317	0.21	8.2
Ni200	6.5	2.7	41	0.286	0.21	7.0
Ni500	5.9	2.6	39	0.260	0.21	6.9
Al10	7.7	3.7	61	0.372	0.22	11.2
Al50	7.3	3.5	56	0.353	0.22	9.8
Al100	7.2	3.3	48	0.317	0.21	8.1
Al200	6.7	3.1	41	0.241	0.19	7.9
Al500	6.3	3.0	38	0.161	0.16	6.9
Cd10	7.5	3.6	62	0.363	0.22	9.3
Cd50	7.3	3.3	53	0.353	0.22	8.0
Cd100	7.0	3.0	44	0.338	0.22	7.5
Cd200	6.8	2.7	42	0.220	0.18	7.0
Cd500	6.4	2.5	39	0.163	0.16	6.9
Cu10	7.1	3.6	69	0.343	0.22	10.1
Cu50	7.0	3.3	61	0.370	0.23	9.3
Cu100	6.5	3.0	58	0.286	0.21	8.2
Cu200	6.2	2.8	51	0.273	0.21	8.0
Cu500	5.9	2.5	48	0.260	0.21	7.1
Pb10	7.3	3.5	69	0.386	0.23	11.4
Pb50	7.1	2.9	63	0.256	0.19	10.3
Pb100	6.6	2.8	59	0.238	0.19	8.0
Pb200	5.9	2.6	47	0.191	0.18	7.4
Pb500	5.2	2.4	41	0.133	0.16	6.1

Table 4.18 Effect of heavy metals on the growth in 1 year old pine seedlings inoculated with *Boletus sp.* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	8.2	4.5	78	0.512	0.25	12.0
Zn10	7.3	4.0	69	0.353	0.22	8.1
Zn50	7.1	3.6	62	0.343	0.22	7.3
Zn100	6.8	3.5	53	0.299	0.21	7.0
Zn200	6.2	3.1	48	0.223	0.19	6.2
Zn500	5.7	2.9	33	0.205	0.19	5.3
Ni10	7.4	3.4	71	0.236	0.21	9.3
Ni50	6.8	3.1	62	0.299	0.21	8.3
Ni100	6.7	2.8	53	0.295	0.21	7.3
Ni200	6.2	2.6	50	0.139	0.15	6.1
Ni500	6.2	2.5	42	0.121	0.14	5.2
Al10	7.7	4.2	69	0.407	0.23	8.4
Al50	7.3	4.0	61	0.321	0.21	8.1
Al100	6.5	3.6	53	0.234	0.19	7.3
Al200	5.7	3.1	42	0.184	0.18	6.7
Al500	5.3	2.7	32	0.171	0.18	5.6
Cd10	6.7	3.6	70	0.324	0.24	9.3
Cd50	6.4	3.4	62	0.309	0.22	8.2
Cd100	6.0	3.0	49	0.290	0.22	7.7
Cd200	5.5	2.7	41	0.140	0.16	7.4
Cd500	5.4	2.7	32	0.171	0.18	5.6
Cu10	6.6	3.6	63	0.380	0.24	8.7
Cu50	6.1	3.3	52	0.269	0.21	8.0
Cu100	5.4	3.1	42	0.238	0.29	6.1
Cu200	5.2	2.6	39	0.229	0.29	6.0
Cu500	5.0	2.5	32	0.162	0.18	5.8
Pb10	7.2	3.6	62	0.248	0.22	8.7
Pb50	7.0	3.2	59	0.338	0.22	8.1
Pb100	6.4	3.0	55	0.207	0.18	7.0
Pb200	6.0	2.9	47	0.194	0.18	6.8
Pb500	5.1	2.7	41	0.099	0.14	6.1

Table 4.20 Effect of heavy metals on the growth in 1 year old pine seedlings inoculated with *C. graniforme* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings$_3$voc (cm^3)	Root colar dm(cm)	Root length (cm)
0	8.2	4.5	78	0.512	0.25	12.5
Zn10	6.3	3.3	76	0.333	0.23	9.3
Zn50	6.0	3.1	73	0.317	0.23	9.0
Zn100	5.8	2.6	60	0.255	0.21	8.3
Zn200	5.6	2.4	53	0.246	0.21	8.1
Zn500	5.4	2.4	44	0.238	0.21	7.4
Ni10	6.5	3.1	73	0.374	0.24	9.6
Ni50	6.4	3.0	70	0.309	0.22	9.2
Ni100	6.0	2.7	63	0.264	0.21	9.1
Ni200	5.5	2.5	51	0.242	0.21	8.3
Ni500	5.0	2.5	46	0.162	0.18	8.0
Al10	6.3	3.7	76	0.304	0.22	8.9
Al50	6.2	3.5	73	0.300	0.22	8.3
Al100	6.0	3.4	59	0.290	0.22	7.1
Al200	5.5	3.3	51	0.140	0.16	7.0
Al500	5.4	3.1	47	0.138	0.16	6.7
Cd10	6.6	3.5	72	0.238	0.19	9.8
Cd50	6.3	3.2	71	0.227	0.10	8.8
Cd100	6.0	3.0	68	0.216	0.18	8.7
Cd200	5.9	2.8	51	0.236	0.20	8.0
Cd500	5.5	2.5	50	0.140	0.16	7.1
Cu10	6.5	3.6	71	0.286	0.21	11.0
Cu50	6.2	3.2	66	0.273	0.21	10.7
Cu100	6.0	3.0	60	0.264	0.21	10.0
Cu200	5.7	2.7	51	0.128	0.15	9.3
Cu500	5.5	2.5	47	0.123	0.15	8.0
Pb10	6.2	3.2	59	0.300	0.22	9.8
Pb50	6.0	3.0	55	0.264	0.21	9.6
Pb100	5.6	2.7	51	0.202	0.19	9.2
Pb200	5.2	2.5	46	0.133	0.16	8.0
Pb500	5.0	2.5	40	0.112	0.15	7.3

Plate 2: Difference in growth rate

1 Mycorrhizal seedlings

2 Non-mycorrhizal seedlings

PLATE 2.

1

2

Plate: 3 Seedlings treated with different concentration of heavy metals and inoculated with *Cenococcum graniforme*

(i) Treated with Cu

(ii) Treated with Pb

(iii) Treated with Cd

(iv) Treated with Zn

(v) Treated with Ni

(vi) Treated with Al.

PLATE 3


(i)


(ii)


(iii)

(iv)


Zn 10 ppm
9.G. grani-
forme
50 ppm
100 ppm
200 ppm
500 ppm

(v)


Ni-10 PPM
9-C. grani-
forme
50 PPM
100 PPM
200 PPM
500 PPM

(vi)


Al 10 PPM
9-C. grani-
forme
50 PPM
100 PPM
200 PPM
500 PPM

higher increase of root length as shown in Plate 4 (1, 2) with an average of 9.8 cm (Table 4.20) followed by *S. luteus* inoculated seedlings treated with Zn which had an average of 9.5 cm (Table 4.17). Minimum root length was observed in non inoculated seedlings treated with Zn with an average of 5.6 cm (Table 4.21). Mycorrhizal infection was maximum in *S. luteus* inoculated seedlings (68 %) treated with Pb (Table 4.24). *Minimum infection was noticed with C. graniforme (29 %)* treated with Al (Table 4.24 and Plates 5 1,2). Above 100 ppm concentration of heavy metals, the elongation of primary root was distorted, the number of ectomycorrhizal roots was markedly reduced and lateral root development was arrested (Plate 6 1) resulting in short thickened to about 1-2 mm long. The intensity of abberation was related to the heavy metals treated concentrations. In lower concentrations of heavy metals, 50 ppm and below (Plates 6 2,3) there were no obvious effects on root morphology and it was less severe than non-mycorrhizal ones. The percentage of mycorrhizal short root generally exceeded 45 % regardless of the heavy metal treatment. The deleterious effect of heavy metals was ameliorated by the presence of ectomycorrhizal fungi. The inhibitory effects of heavy metals on growth were greater for roots (Plate 7 1,2) than for shoots. Ni (10 ppm) reduced root growth by 22 % but shoot growth was reduced only by 7 %. Qualitatively, however, the responses for roots and shoots to different treatments were similar.

Metal toxicity in non-mycorrhizal roots, such as thickened and dark root tips were seen at all concentrations of Cd, Cu, Zn,

Plate 4: 1. Treated with Cu and inoculated with*Cenococcum graniforme*

2. Treated with Zn and inoculated with *Suillus luteus*.

Plate 5: 1. Treated with Al and infected with *Cenococcum graniforme*

2. Treated with Pb infected with *Suillus luteus*

PLATE 4:

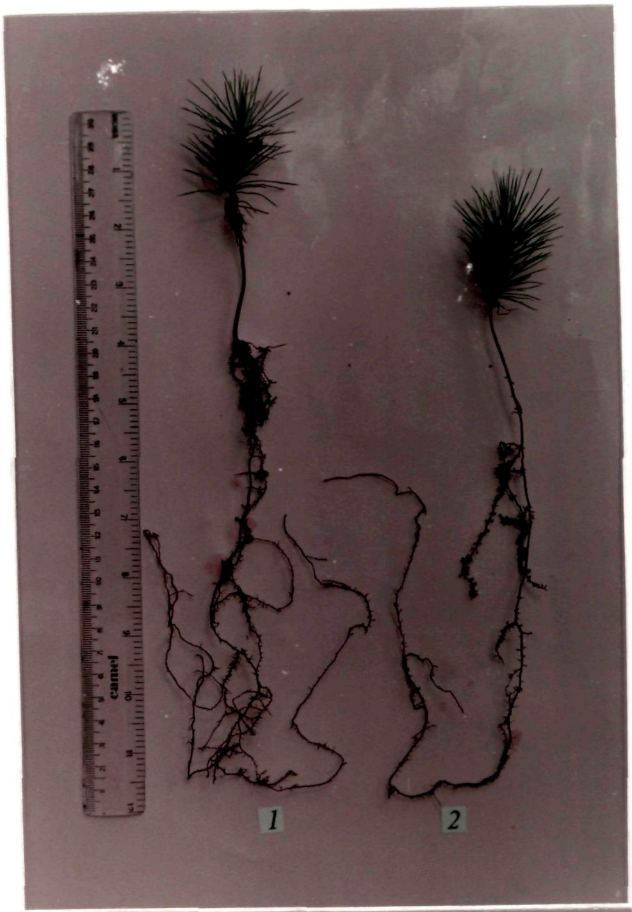

PLATE 5:

Plate 6: Mycorrhizal roots treated with :

1. Ni 200 ppm
2. Cd 10 ppm
3. Al 50 ppm

Plate 7 : Untreated (1) and treated (Ni 10 ppm) (2) mycorrhizal roots

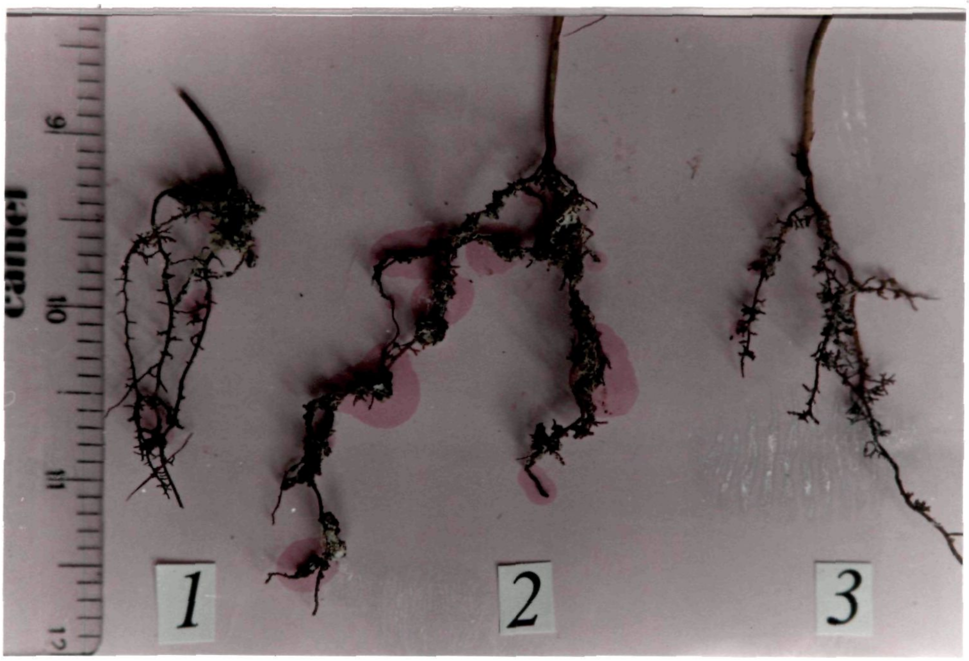

PLATE 6

PLATE 7

Ni, Pb and Al at 50 ppm and above while in ectomycorrhizal roots these symptoms occurred only in small part of the root system at higher concentrations (100 ppm and above). Although mycorrhizal infection was observed at all concentrations of different metals in ectomycorrhizal roots. Compound causing short roots bifurcations were less numerous at 100 ppm and above of Cu, Cd, Ni, Zn, Pb and Al than at lower concentrations. In general, ectomycorrhizal fungi exhibited a resistance to different heavy metals resulting better growth of pine seedlings than non-mycorrhizal ones.

Seedling volume was maximum in *S.luteus* inoculated seedlings treated with Zn and Cu and minimum in *S.aurantium* and *C. graniforme* inoculated ones treated with Pb (Tables 4.17, 4.19 and 4.20).

Number of needles was more in ectomycorrhizal seedlings.*C.graniforme* inoculated seedlings had maximum needles (Table 4.20). Seedlings treated with Zn, Ni, Al and Cd had almost equal number of needles with an average of 60-65. Minimum number of needles was observed in *S.aurantium* inoculated seedlings treated with Zn with an average of 42 followed by *S.luteus* inoculation treated with Ni with an average of 47 (Tables 4.19, 4.17). Non-mycorrhizal seedlings treated with Al had only an average number of 35 needles, which was very less compared to ectomycorrhizal Al treated seedlings (Table 4.21).

Length of needle did not vary significantly in all different treatments of heavy metals in ectomycorrhizal and non-mycorrhizal seedlings. *S.aurantium* inoculated and Cu treated seedlings had an average needle length of 3.7 cm (table

Table 4.19 Effect of heavy metals on the growth in 1 year old pine seedlings inoculated with *S. aurantium* in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	8.2	4.5	78	0.512	0.25	9.2
Zn10	6.5	3.3	59	0.343	0.23	8.3
Zn50	6.3	3.1	51	0.277	0.21	8.1
Zn100	5.8	2.7	43	0.255	0.21	7.6
Zn200	5.4	2.6	32	0.238	0.21	7.2
Zn500	5.2	2.4	29	0.229	0.21	7.0
Ni10	7.2	3.4	61	0.348	0.22	8.5
Ni50	6.8	3.2	56	0.245	0.19	8.4
Ni100	5.5	3.0	52	0.290	0.23	8.0
Ni200	5.5	3.0	46	0.178	0.18	7.8
Ni500	5.3	2.9	42	0.135	0.16	7.2
Al10	6.5	3.4	63	0.338	0.23	8.8
Al50	6.1	3.3	61	0.322	0.23	8.4
Al100	5.5	3.0	52	0.290	0.23	8.0
Al200	5.1	2.0	50	0.246	0.22	7.3
Al500	4.7	2.7	47	0.120	0.16	7.0
Cd10	6.7	3.6	60	0.354	0.23	8.6
Cd50	6.4	3.2	55	0.282	0.21	8.1
Cd100	6.1	3.1	51	0.197	0.18	8.0
Cd200	5.8	3.0	46	0.187	0.18	7.4
Cd500	5.2	2.8	41	0.168	0.18	7.0
Cu10	6.9	4.1	59	0.333	0.22	7.7
Cu50	6.4	4.0	55	0.309	0.22	7.7
Cu100	6.2	4.0	52	0.273	0.21	7.8
Cu200	5.3	3.4	43	0.233	0.21	7.1
Cu500	5.1	3.2	40	0.224	0.21	6.3
Pb10	6.5	3.6	69	0.314	0.22	8.6
Pb50	6.2	3.5	66	0.223	0.19	8.5
Pb100	5.3	3.1	62	0.191	0.19	8.1
Pb200	5.1	3.0	52	0.130	0.16	7.3
Pb500	4.8	3.0	50	0.069	0.12	7.0

Table 4.21 Effect of heavy metals on the growth in 1 year old pine seedlings in pot culture.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	7.7	4.4	73	0.452	0.23	10.1
Zn10	4.2	3.2	57	0.185	0.21	6.7
Zn50	4.2	3.0	56	0.106	0.16	5.3
Zn100	3.9	2.7	50	0.099	0.16	5.0
Zn200	3.4	2.5	40	0.066	0.14	4.7
Zn500	3.3	2.5	33	0.064	0.14	4.5
Ni10	5.6	3.4	57	0.202	0.19	8.5
Ni50	5.3	3.3	57	0.191	0.19	8.1
Ni100	4.7	3.0	46	0.079	0.13	7.3
Ni200	4.6	2.8	36	0.077	0.13	6.3
Ni500	4.3	2.5	35	0.061	0.12	5.1
Al10	4.5	3.4	46	0.180	0.20	7.4
Al50	4.4	2.6	46	0.142	0.18	7.1
Al100	4.3	2.5	35	0.084	0.14	6.3
Al200	4.2	2.4	29	0.082	0.14	5.7
Al500	3.5	2.4	23	0.050	0.12	5.1
Cd10	4.8	4.1	49	0.211	0.21	8.9
Cd50	3.9	3.8	41	0.140	0.19	8.6
Cd100	3.7	3.4	39	0.133	0.19	8.3
Cd200	2.5	3.0	36	0.064	0.16	7.3
Cd500	2.4	2.6	37	0.086	0.19	7.0
Cu10	5.5	3.5	53	0.266	0.22	8.0
Cu50	5.3	3.3	53	0.256	0.22	8.1
Cu100	5.2	2.6	44	0.168	0.18	7.6
Cu200	5.0	2.4	43	0.162	0.18	7.6
Cu500	3.5	2.3	41	0.059	0.13	6.0
Pb10	5.3	3.3	60	0.233	0.21	7.9
Pb50	4.5	3.1	59	0.198	0.21	7.7
Pb100	4.2	2.7	50	0.107	0.16	7.1
Pb200	4.4	2.4	47	0.112	0.16	6.8
Pb500	3.8	2.4	46	0.085	0.15	6.2

4.19) which was maximum followed by Al treated and *Boletus* inoculated seedlings (Table 4.18). Minimum length of needle was observed in control Al treated seedlings (Table 4.21). After a period of one year, pine seedlings showed 78 % and 73 % survival in *C.graniforme* treated with Al and Cu respectively (Table 4.24). On the other hand, non-mycorrhizal seedlings showed 38% survival in Al, Cd and Pb treated seedlings (Table 4.26).

Ectomycorrhizal infection was visible after 2 months of fungal inoculation. On the fourth month, percentage of infection ranged between 25-40% in seedlings treated with Pb, Zn, Al, Cd, Cu and Ni (Table 4.22). Whereas untreated mycorrhizal seedlings had 45% of mycorrhizal infection. After 8th months colonization increased to 40-60% in heavy metal treated seedlings (Table 4.23) and control seedlings showed 73% infection. Highest percentage of infection was observed in Pb treated and *S.luteus* infected seedlings after one year (Table 4.24). In other treatments colonization of mycorrhizal decreased as the concentration of metals increased.

In case of field condition, untreated control seedlings showed 90 % mycorrhizal infection (Table 4.21). The percentage of inhibition of mycorrhizal infection also increased with increasing the metal concentration. Highest percentage of inhibition was observed in Al, Cu and Pb treated seedlings. Stem height was highest in Zn and Ni treated seedlings on the fourth month and lowest in Cd treated seedlings (Table 4.25). After 1 year, the highest shoot length was observed in Cd treated seedlings and minimum was in Pb and Ni treated seedlings (Table 4.13). Control uninoculated seedlings showed

Table 4.13 Effect of heavy metals on the growth in 1 year old pine seedlings inoculated with mixed mycorrhizal fungi in field condition.

Metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length(cm)
0	16.7	5.8	219	1.00	0.25	48.7
Zn10	13.6	4.7	201	0.783	0.24	44.0
Zn50	12.2	4.2	190	0.702	0.24	41.0
Zn100	11.0	3.6	189	0.581	0.23	38.0
Zn200	10.3	3.2	172	0.498	0.22	20.0
Zn500	10.0	3.1	156	0.484	0.22	18.0
Ni10	12.6	4.5	173	0.725	0.25	32.0
Ni50	12.2	3.7	168	0.624	0.21	31.0
Ni100	11.9	3.6	153	0.575	0.22	26.0
Ni200	8.7	3.2	115	0.383	0.21	25.0
Ni500	7.9	3.1	111	0.285	0.19	21.0
Al10	13.1	4.7	204	0.754	0.24	39.5
Al50	11.2	4.3	169	0.592	0.23	37.0
Al100	11.0	4.2	146	0.581	0.23	33.0
Al200	10.0	4.0	140	0.484	0.22	30.0
Al500	9.8	3.3	131	0.432	0.21	29.0
Cd10	15.3	4.8	198	0.809	0.23	30.0
Cd50	15.1	4.5	183	0.798	0.23	22.0
Cd100	14.0	4.4	169	0.677	0.22	18.0
Cd200	13.3	3.9	143	0.643	0.22	13.9
Cd500	12.0	3.8	121	0.388	0.18	12.9
Cu10	15.5	4.4	201	0.892	0.24	41.0
Cu50	12.1	4.2	151	0.698	0.24	40.0
Cu100	10.6	4.0	139	0.513	0.22	39.0
Cu200	10.1	3.4	133	0.445	0.21	32.0
Cu500	9.6	3.2	119	0.423	0.21	28.0
Pb10	12.0	5.1	207	0.691	0.24	31.0
Pb50	11.3	4.3	206	0.650	0.24	26.0
Pb100	10.0	4.2	183	0.529	0.23	23.0
Pb200	9.8	3.8	178	0.432	0.21	19.0
Pb500	9.4	3.7	169	0.240	0.16	16.9

Table 4.22 Effect of heavy metals on survival (%) and mycorrhizal infection (%) of 4 months old pine seedlings in pot culture.

Metal conc. (ppm)	Survival (%)					Mycorrhizal infection (%)			
	M1	M2	M3	M4	Control	M1	M2	M3	M4
0	100	100	100	100	100	42	42	42	42
Zn10	93	93	100	92	100	30	31	31	29
Zn50	100	90	93	90	93	29	30	30	27
Zn100	100	100	90	86	90	27	26	26	22
Zn200	90	90	100	83	90	24	22	22	16
Zn500	90	90	93	80	86	15	15	14	12
Ni10	100	100	93	93	93	38	29	29	32
Ni50	100	100	90	86	90	38	27	27	30
Ni100	93	70	86	86	86	25	27	26	27
Ni200	100	80	83	73	80	21	23	24	20
Ni500	90	80	80	70	70	17	18	19	15
Al10	100	80	100	100	100	33	28	29	32
Al50	100	100	100	93	100	28	25	27	29
Al100	100	100	100	90	90	23	25	26	23
Al200	93	93	94	80	80	16	20	20	16
Al500	90	90	90	80	70	13	12	14	12
Cd10	100	100	100	100	100	41	31	30	30
Cd50	100	93	93	92	90	32	26	28	27
Cd100	90	100	90	90	90	29	20	23	25
Cd200	90	100	90	86	90	21	16	18	21
Cd500	86	90	90	70	80	18	15	15	17
Cu10	100	100	100	94	100	36	27	29	37
Cu50	100	100	100	90	90	31	27	27	35
Cu100	100	100	93	86	90	23	21	24	28
Cu200	92	93	90	80	80	19	20	21	17
Cu500	90	90	86	76	73	17	17	18	14
Pb10	100	100	100	93	100	40	31	33	31
Pb50	100	92	100	90	91	38	25	27	30
Pb100	100	90	90	90	90	27	21	23	26
Pb200	90	90	82	86	90	27	19	19	20
Pb500	84	83	80	80	83	26	16	15	18

M1=*S. luteus*, M2=*Boletus sp*, M3=*S. aurantium*, M4=*C. graniforme*

Table 4.23 Effect of heavy metals on survival (%) and mycorrhizal infection (%) in 8 months old pine seedlings in po culture

Metals conc. (ppm)	Survival (%)					Mycorrhizal infection (%)			
	M1	M2	M3	M4	Control	M1	M2	M3	M4
0	93	93	94	93	93	68	68	68	68
Zn10	90	93	76	90	90	63	53	50	48
Zn50	90	90	73	86	80	38	51	44	46
Zn100	80	90	67	86	76	21	41	42	40
Zn200	80	90	70	73	73	19	28	31	32
Zn500	73	86	60	70	60	18	24	31	30
Ni10	93	93	93	93	90	60	56	53	51
Ni50	83	90	86	86	83	53	43	41	43
Ni100	80	80	76	83	70	40	43	40	41
Ni200	80	80	70	80	70	25	31	35	39
Ni500	80	70	60	70	63	25	24	31	39
Al10	100	93	86	93	90	62	51	43	47
Al50	100	86	86	93	83	62	43	36	46
Al100	93	84	80	90	73	40	37	23	30
Al200	86	80	73	80	70	26	25	22	23
Al500	73	72	72	73	70	21	24	19	20
Cd10	100	100	93	93	93	58	50	53	53
Cd50	93	93	86	93	90	55	31	47	51
Cd100	93	86	74	86	80	30	28	41	43
Cd200	86	83	72	83	70	30	21	37	35
Cd500	83	76	70	73	66	22	21	35	32
Cu10	93	92	93	93	88	59	43	48	49
Cu50	90	90	90	90	80	51	43	43	48
Cu100	90	83	83	80	76	42	41	40	32
Cu200	76	80	80	76	73	36	32	33	29
Cu500	70	70	70	70	67	24	22	22	26
Pb10	86	93	93	93	90	47	64	51	51
Pb50	76	76	93	93	86	47	60	42	47
Pb100	73	73	76	83	73	35	61	40	33
Pb200	66	70	73	77	60	28	43	31	31
Pb500	66	70	70	73	63	23	40	25	26

M1=*S. luteus*, M2=*Boletus sp.*, M3=*S. aurantium*, M4=*C. graniforme*

Table 4.24 Effect of heavy metals on survival (%) and mycorrhizal infection (%) in 1 year old pine seedlings in pot culture.

Metals conc. (ppm)	Survival (%)					Mycorrhizal infection (%)			
	M1	M2	M3	M4	Control	M1	M2	M3	M4
0	93	93	93	93	90	88	88	88	88
Zn10	76	93	76	83	70	83	64	61	64
Zn50	76	86	70	80	66	60	61	61	53
Zn100	73	83	66	80	63	40	52	52	49
Zn200	70	80	63	73	60	37	39	39	46
Zn500	60	70	60	73	58	29	36	37	39
Ni10	86	86	93	86	90	78	71	65	59
Ni50	80	86	86	80	80	66	70	61	55
Ni100	80	83	73	80	73	51	55	56	54
Ni200	76	80	73	73	73	43	46	53	38
Ni500	73	73	66	73	64	28	42	42	36
Al10	86	80	90	86	86	81	72	88	54
Al50	86	80	80	86	80	75	63	52	45
Al100	80	80	80	84	76	44	59	36	41
Al200	80	76	73	80	73	30	55	31	33
Al500	76	73	73	76	70	29	32	28	32
Cd10	93	86	86	86	90	69	59	61	63
Cd50	93	80	80	80	80	63	55	62	61
Cd100	86	80	80	80	76	42	42	54	53
Cd200	80	76	80	80	73	33	32	46	42
Cd500	80	73	73	76	70	31	31	41	35
Cu10	86	93	86	86	86	66	63	63	71
Cu50	86	86	80	80	80	64	54	60	66
Cu100	80	80	76	80	73	50	51	56	49
Cu200	80	80	73	80	73	39	37	46	43
Cu500	73	80	73	73	70	33	31	35	41
Pb10	93	86	86	86	80	82	58	59	61
Pb50	86	80	73	80	80	80	55	67	56
Pb100	86	80	73	80	73	68	51	49	49
Pb200	73	73	70	73	70	60	41	47	40
Pb500	72	73	66	73	60	50	33	31	33

M1=*S. luteus*, M2=*Boletus sp*, M3=*S. aurantium*, M4=*C. graniforme*

Table 4.25 Effect of heavy metals on survival (%) and mycorrhizal infection (%) in pine seedlings inoculated with mixed mycorrhizal fungi in field condition.

Metals conc. (ppm)	Survival (%)			Mycorrhizal infection (%)		
	a	b	c	a	b	c
0	100	93	93	45	73	90
Zn10	100	94	86	41	64	72
Zn50	86	86	80	40	61	70
Zn100	80	80	73	35	53	69
Zn200	80	76	66	30	50	62
Zn500	73	73	50	30	42	53
Ni10	100	90	86	43	62	73
Ni50	93	86	80	39	55	64
Ni100	90	86	80	36	51	61
Ni200	86	80	73	36	43	59
Ni500	80	80	73	30	43	56
Al10	100	90	90	38	58	70
Al50	90	80	86	36	57	63
Al100	80	80	74	30	49	60
Al200	73	76	73	30	42	52
Al500	73	60	60	28	41	48
Cd10	93	93	86	40	61	71
Cd50	90	90	80	38	59	65
Cd100	86	86	80	32	51	63
Cd200	86	80	73	31	43	60
Cd500	80	76	66	25	40	51
Cu10	100	93	86	41	59	68
Cu50	90	90	80	37	56	66
Cu100	86	80	80	37	51	63
Cu200	80	80	76	31	43	55
Cu500	73	73	66	26	39	50
Pb10	93	91	86	42	60	67
Pb50	88	86	85	39	58	63
Pb100	81	77	80	36	50	56
Pb200	76	73	71	33	42	51
Pb500	71	70	62	27	36	48

a=120 days, b=240 days, c=360 days.

Table 4.26 Effect of heavy metals on survival (%) of pine seedlings in field condition.

Metals conc. (ppm)	Survival (%) a	b	c
0	100	93	90
Zn10	90	93	86
Zn50	80	83	76
Zn100	76	73	73
Zn200	73	70	66
Zn500	70	66	63
Ni10	100	93	80
Ni50	94	86	76
Ni100	85	80	66
Ni200	80	73	60
Ni500	73	70	53
Al10	100	86	73
Al50	86	80	66
Al100	76	73	66
Al200	70	67	60
Al500	73	66	53
Cd10	93	80	73
Cd50	90	80	73
Cd100	80	73	70
Cd200	76	70	65
Cd500	73	66	60
Cu10	100	80	76
Cu50	76	73	73
Cu100	73	70	66
Cu200	73	66	66
Cu500	70	60	53
Pb10	100	80	73
Pb50	93	73	73
Pb100	80	70	65
Pb200	80	70	60
Pb500	73	66	50

a=120 days, b= 240 days, c= 360 days.

Table 4.16 Effect of heavy metals on the growth in 1 year old pine seedlings in field condition.

metals conc. (ppm)	Shoot height (cm)	Needle length (cm)	No.of needles	Seedlings vol (cm^3)	Root colar dm(cm)	Root length (cm)
0	14.0	5.3	167	0.590	0.25	30.5
Zn10	6.6	3.8	113	0.319	0.22	27.1
Zn50	5.5	3.5	89	0.266	0.22	20.8
Zn100	5.4	2.5	78	0.194	0.19	16.1
Zn200	5.2	2.5	57	0.168	0.18	14.8
Zn500	5.0	2.4	54	0.162	0.18	10.6
Ni10	9.5	3.8	146	0.418	0.21	26.0
Ni50	8.2	3.0	112	0.296	0.19	21.1
Ni100	7.0	2.7	102	0.252	0.19	15.8
Ni200	6.1	2.6	92	0.156	0.16	15.1
Ni500	5.9	2.4	87	0.132	0.15	14.8
Al10	9.1	3.8	136	0.481	0.23	16.1
Al50	8.5	3.6	78	0.411	0.22	16.0
Al100	6.0	2.9	77	0.290	0.22	15.6
Al200	5.8	2.8	74	0.209	0.18	15.0
Al500	5.5	2.7	58	0.178	0.18	14.5
Cd10	9.5	3.5	120	0.419	0.21	16.3
Cd50	5.8	3.0	119	0.255	0.21	16.0
Cd100	5.4	2.7	91	0.138	0.16	15.4
Cd200	5.0	2.5	83	0.128	0.16	14.8
Cd500	4.5	2.3	66	0.064	0.12	14.6
Cu10	8.2	3.5	116	0.361	0.21	26.4
Cu50	6.8	3.4	106	0.174	0.16	23.0
Cu100	6.0	3.1	105	0.150	0.14	16.0
Cu200	5.5	3.1	105	0.079	0.12	15.0
Cu500	5.4	2.5	85	0.077	0.12	13.8
Pb10	7.0	4.5	111	0.308	0.21	18.9
Pb50	6.5	3.7	98	0.286	0.21	18.0
Pb100	6.1	3.2	81	0.156	0.16	17.1
Pb200	5.8	2.8	76	0.148	0.16	17.0
Pb500	5.7	2.7	59	0.082	0.12	15.9

retarded shoot height in all the different metals treatments (Plate 8 i, ii) inoculated seedlings. After 1 year the seedlings height was maximum in Ni and minimum in Zn treated seedlings (Table 4.16).

Needle length did not vary much among different metal treatments. Mycorrhizal inoculated seedlings however, showed better growth than non-inoculated ones. The length of needle was minimum and maximum in Zn and Pb treated non-mycorrhizal seedlings respectively (Table 4.16). In case of Pb treated mycorrhizal seedlings, the average needle length was 4.2 cm and in Ni ones 3.6 cm. While non-mycorrhizal seedlings showed 3.3 cm and 3.1 cm in Pb and Ni treated seedlings respectively (Tables 4.13 and 4.16).

Number of needles was highest in Pb treated mycorrhizal seedlings and minimum in control non-mycorrhizal seedlings (Tables 4.13, 4.16). Untreated mycorrhizal seedlings showed better growth than heavy metal treated ones. Root length was maximum in mycorrhizal seedlings treated with Cu and Zn (Table 4.13). Control mycorrhizal seedlings showed better root length than heavy metal treated ones. In control non-mycorrhizal seedling, the elongation of primary and lateral roots was reduced by addition of Zn, Ni, Pb, Cd, Cu and Al specially at the concentration of 50 ppm and above the root length ranged from 15 to 18 cm. Seedling volume was maximum with Cd treated mycorrhizal seedlings (Table 4.13) and in control non-mycorrhizal Al and Cu treated seedlings showed maximum and minimum volume respectively (Table 4.16).

Plate 8 : (i) Non-mycorrhizal seedlings treated with different heavy metals.

(ii) Mycorrhizal seedlings treated with different heavy metals.

PLATE 8

(i)

(ii)

Table 4.27 Correlation coefficient (r) of mycorrhizal infection (%) with various parameters in pots and in field mycorrhizal and non-mycorrhizal seedlings.

	Pots					Field	
	Mycorrhizal infection						
Parameters	M1	M2	M3	M4	NM	MMF	NM
Shoot height	0.98*	0.99*	0.95*	0.95*	NS	0.98*	NS
Needle length	0.91*	0.84*	0.92*	0.96*	NS	0.97*	NS
Number of needle	0.93*	0.96*	0.97*	0.96*	NS	0.91*	NS
Seedlings volume	0.81**	0.79**	0.91*	0.85**	NS	0.96*	NS
Root length	0.95*	0.90*	0.89*	0.99*	NS	0.93*	NS
Root colar diameter	0.81**	0.79**	0.86**	0.90*	NS	0.91*	NS
Survival	0.93*	0.94*	0.88*	0.96*	NS	0.98*	NS

M1= *C.graniforme*,M2=*S.luteus*, M3=*Boletus* sp, M4= *S.aurantium.*

NM= Non-mycorrhizal fungi, MMF = Mixed mycorrhizal fungi.

* = significant at $p<0.01$ level
** = significant at $p<0.05$ level

Table 4.28 Analysis of variance (F) of sampling periods, mycorrhizal and non-mycorrhizal and various mycobionts with various parameters in pine seedlings.

Sources of variation	Variation between sampling periods	Variation between mycorrhizal and non-mycorrhizal	Variation between mycobionts
Shoot height	5.49*	13.7**	NS
Needle length	2.8**	6.13**	NS
Number of needle	13.53**	4.12**	NS
Root colar diameter	6.21**	4.52*	NS
Root length	3.73*	3.59*	NS
Seedlings volume	3.32**	4.23**	NS
Mycorrhizal infection	7.8*	2.11**	NS

* = significant at $p<0.01$ level

** = significant at $p<0.05$ level

The mycorrhizal fungi had improved the survival and growth of the seedlings,thus percentage of survival of seedling varied at different concentrations of metals. Higher percentage of survival was observed with Zn treated non-mycorrhizal seedlings (Table 4.26). Mycorrhizal seedlings had higher survival than non-mycorrhizal ones . Highest percentage of survival was observed with Ni treated mycorrhizal seedlings (Table 4.25).

A positive correlation was found between mycorrhizal infection and shoot height, needle length, number of needles, root length, survival ($P < 0.01$), seedlings volume and root colar diameter ($P < 0.05$) in all the ectomycorrhizal fungi treated seedlings (Table 4.27). There was a negative correlation between the growth parameters and metal concentration. A significant variation ($P < 0.05$) was found between the sampling periods of all the growth parameters. A significant variation ($P < 0.01$) was also found between mycorrhizal and non-mycorrhizal seedlings. There was an insignificant variation between the growth parameters of various mycobionts (Table 4.28).

Heavy metals on uptake of N, P, K

A trend in nutrient content of seedlings applied with different concentrations of heavy metals was observed. Both mycorrhizal and non-mycorrhizal seedlings had higher total N concentration than P and K. The treatment of Ni, Cd, Cu, Pb, Zn and Al adversely affected the total contents on N, P and K seedlings. More than 50 ppm of heavy metals doses appeared to have suppressive effects, specially on non-mycorrhizal seedlings. It was observed that contents of nutrients as a whole are restricted by the presence of heavy metals.

More nitrogen contents was found in pine seedlings infected with *C.graniforme, Boletus* sp.,*S.aurantium* and *S.luteus* treated with Pb, Cd, Ni, Zn Cu and Al compared to non-mycorrhizal seedlings (Table 5.1). The percentage of N was almost equal in all the four mycorrhizal fungi treated seedlings. The contents of N decreased with the increase of heavy metal concentration.

Seedlings infected with mixed mycobionts exhibited maximum uptake of nitrogen at 50 ppm and at lower concentrations of Zn, Cu, Cd and Al. Nitrogen contents was lower in Pb and Ni treated seedlings at higher doses compared to the other heavy metal treatments (Table 5.4). Non-mycorrhizal seedlings were with less nitrogen content than mycorrhizal ones (Table 5.4).

Phosphorus content was almost similar in all the ectomycorrhizal seedlings. However, P-content decreased with the application of increased concentration of Zn, Ni, Pb, Cu, Cd and Al. Maximum P-content was noticed in *Boletus* inoculated seedlings (Table 5.3) while non-mycorrhizal seedlings showed lowest P-content.

Seedlings infected with mixed inoculum of mycobionts showed more content of phosphorus than individual symbionts (Table 5.4). The percentage of phosphorus content decreased with increasing concentration of heavy metal in both mycorrhizal and non-mycorrhizal seedlings.

Potassium content was almost equal in all the four ectomycorrhizal fungi infected seedlings (Table 5.2). Non-mycorrhizal seedlings showed no marked differences in K-uptake.

Table 5.1 Effect of heavy metals on nitrogen content (%) in mycorrhizal and non-mycorrhizal seedlings.

Metals conc. (ppm)	Nitrogen content (%)				
	Control	M1	M2	M3	M4
0	0.99	2.00	2.00	2.00	2.00
Zn10	0.91	1.87	2.25	1.93	1.83
Zn50	0.80	1.80	2.19	1.86	1.72
Zn100	0.69	1.57	1.92	1.79	1.56
Zn200	0.66	1.54	1.75	1.72	1.51
Zn500	0.64	1.52	1.61	1.61	1.05
Ni10	0.97	1.72	1.88	1.90	1.87
Ni50	0.91	1.64	1.79	1.77	1.76
Ni100	0.78	1.52	1.72	1.72	1.66
Ni200	0.72	1.42	1.58	1.70	1.63
Ni500	0.70	1.41	1.47	1.61	1.52
Al10	0.98	1.65	2.03	1.95	2.04
Al50	0.92	1.62	1.91	1.92	1.94
Al100	0.85	1.59	1.65	1.75	1.91
Al200	0.80	1.48	1.56	1.72	1.80
Al500	0.79	1.47	1.35	1.66	1.78
Cd10	0.95	1.71	2.11	2.01	2.12
Cd50	0.92	1.68	1.92	1.90	1.90
Cd100	0.86	1.62	1.90	1.89	1.70
Cd200	0.85	1.58	1.80	1.83	1.70
Cd500	0.80	1.42	1.75	1.61	1.53
Cu10	1.01	1.53	1.92	2.09	2.11
Cu50	0.91	1.50	1.68	1.89	1.87
Cu100	0.81	1.48	1.60	1.69	1.80
Cu200	0.66	1.46	1.58	1.64	1.57
Cu500	0.63	1.34	1.55	1.59	1.50
Pb10	0.96	1.90	1.62	1.81	1.90
Pb50	0.85	1.75	1.67	1.70	1.90
Pb100	0.73	1.63	1.60	1.63	1.88
Pb200	0.68	1.38	1.58	1.44	1.81
Pb500	0.63	1.34	1.51	1.40	1.52

M1=*S. aurantium*, M2=*Boletus* sp, M3= *C. graniforme*, M$_4$=*S. luteus*

Table 5.2 Effect of heavy metals on potassium content (%) in mycorrhizal and non-mycorrhizal seedlings.

Metals conc. (ppm)	Potassium content (%)				
	Control	M1	M2	M3	M4
0	0.90	0.90	0.90	0.90	0.90
Zn10	0.70	0.78	0.84	0.89	0.84
Zn50	0.63	0.72	0.75	0.86	0.70
Zn100	0.60	0.68	0.78	0.72	0.78
Zn200	0.41	0.60	0.59	0.64	0.61
Zn500	0.33	0.52	0.57	0.58	0.45
Ni10	0.69	0.75	1.00	0.73	0.81
Ni50	0.47	0.70	0.84	0.56	0.59
Ni100	0.36	0.67	0.65	0.53	0.50
Ni200	0.31	0.58	0.61	0.40	0.42
Ni500	0.25	0.53	0.50	0.35	0.30
Al10	0.71	0.75	0.78	0.86	0.97
Al50	0.63	0.68	0.69	0.75	0.77
Al100	0.51	0.62	0.58	0.60	0.69
Al200	0.50	0.60	0.55	0.52	0.57
Al500	0.33	0.52	0.46	0.40	0.47
Cd10	0.75	0.76	0.82	0.97	0.68
Cd50	0.60	0.63	0.78	0.87	0.65
Cd100	0.49	0.56	0.67	0.65	0.65
Cd200	0.40	0.49	0.62	0.59	0.50
Cd500	0.41	0.45	0.47	0.56	0.45
Cu10	0.62	0.69	0.70	0.72	0.83
Cu50	0.40	0.65	0.64	0.68	0.76
Cu100	0.34	0.58	0.52	0.59	0.70
Cu200	0.30	0.50	0.48	0.51	0.52
Cu500	0.40	0.47	0.44	0.43	0.47
Pb10	0.58	0.68	0.68	0.82	0.72
Pb50	0.56	0.62	0.62	0.78	0.61
Pb100	0.51	0.55	0.57	0.71	0.54
Pb200	0.44	0.51	0.53	0.68	0.53
Pb500	0.40	0.47	0.50	0.61	0.51

M1=*S. aurantium*,M2=*Boletus* sp., M3=*C. graniforme*, M4=*S. luteus*.

Table 5.3 Effect of heavy metals on phosphorus content (%) in mycorrhizal and non-mycorrhizal seedlings.

Metals conc. (ppm)	Phosphorus content (%)				
	Control	M1	M2	M3	M4
0	0.88	0.88	0.88	0.88	0.88
Zn10	0.65	0.81	0.90	0.89	0.86
Zn50	0.59	0.86	0.82	0.82	0.70
Zn100	0.51	0.78	0.66	0.78	0.69
Zn200	0.39	0.66	0.63	0.62	0.45
Zn500	0.35	0.61	0.61	0.60	0.42
Ni10	0.67	0.86	0.70	0.87	0.89
Ni50	0.60	0.80	0.66	0.70	0.80
Ni100	0.55	0.79	0.61	0.65	0.67
Ni200	0.51	0.61	0.59	0.63	0.65
Ni500	0.48	0.56	0.50	0.56	0.59
Al10	0.69	0.73	0.68	0.88	0.79
Al50	0.61	0.68	0.63	0.71	0.74
Al100	0.49	0.55	0.55	0.62	0.72
Al200	0.49	0.53	0.50	0.63	0.59
Al500	0.48	0.51	0.49	0.55	0.55
Cd10	0.69	0.90	0.74	0.79	0.90
Cd50	0.61	0.89	0.66	0.69	0.80
Cd100	0.60	0.76	0.63	0.61	0.74
Cd200	0.51	0.59	0.55	0.55	0.66
Cd500	0.49	0.53	0.53	0.51	0.55
Cu10	0.73	0.89	0.83	0.87	0.84
Cu50	0.60	0.80	0.82	0.80	0.73
Cu100	0.58	0.70	0.72	0.69	0.69
Cu200	0.55	0.61	0.68	0.65	0.60
Cu500	0.50	0.60	0.59	0.55	0.54
Pb10	0.74	0.81	0.86	0.88	0.90
Pb50	0.61	0.72	0.80	0.86	0.84
Pb100	0.60	0.67	0.70	0.76	0.70
Pb200	0.51	0.60	0.63	0.73	0.65
Pb500	0.44	0.53	0.50	0.59	0.55

M1=*S. aurantium*, M2=*Boletus sp*, M3=*C. graniforme* M4=*S. luteus*

Table 5.4 Effect of heavy metals on nitrogen, phosphorus, potassium contents in mycorrhizal and non-mycorrhizal seedlings treated with mixed mycobionts.

Metals conc. (ppm)	Nitrogen (%)		Phosphorus(%)		Potassium (%)	
	M1	0	M2	0	M3	0
0	2.50	1.91	1.21	0.90	1.23	0.93
Zn10	2.31	1.91	0.96	0.79	1.14	0.88
Zn50	2.25	1.82	0.84	0.70	1.03	0.87
Zn100	2.20	1.74	0.80	0.62	0.97	0.76
Zn200	1.78	1.32	0.70	0.65	0.91	0.65
Zn500	1.62	1.09	0.67	0.50	0.80	0.59
Ni10	2.49	1.28	0.91	0.80	1.02	0.92
Ni50	1.98	1.21	0.80	0.69	0.96	0.90
Ni100	1.91	1.16	0.71	0.60	0.92	0.83
Ni200	1.84	1.08	0.69	0.51	0.87	0.80
Ni500	1.69	1.00	0.56	0.40	0.73	0.60
Al10	2.48	1.85	0.89	0.71	0.95	0.89
Al50	2.40	1.80	0.81	0.67	0.90	0.83
Al100	2.33	1.75	0.78	0.59	0.89	0.73
Al200	1.92	1.58	0.63	0.56	0.71	0.63
Al500	1.75	1.32	0.59	0.40	0.69	0.61
Cd10	2,29	1.92	0.93	0.74	1.07	1.00
Cd50	2.21	1.82	0.79	0.70	1.05	0.95
Cd100	1.89	1.10	0.73	0.67	0.83	0.76
Cd200	1.81	1.08	0.70	0.62	0.79	0.75
Cd500	1.68	1.04	0.60	0.42	0.66	0.60
Cu10	2.07	1.16	0.90	0.81	1.04	1.24
Cu50	1.98	1.05	0.86	0.69	1.07	0.85
Cu100	1.70	1.01	0.80	0.70	0.98	0.62
Cu200	1.67	0.97	0.73	0.61	0.93	0.61
Cu500	1.02	0.87	0.62	0.43	0.86	0.57
Pb10	1.61	1.18	0.94	0.85	1.17	1.10
Pb10	1.47	1.18	0.86	0.76	1.11	1.02
Pb100	1.39	1.08	0.80	0.70	0.81	0.78
Pb200	1.34	1.01	0.75	0.50	0.78	0.71
Pb500	1.34	0.99	0.67	0.35	0.73	0.70

Table 5.5 Correlation coefficient (r) of mycorrhizal infection (%) with various parameters in pots and field pine seedlings.

	Mycorrhizal infection						
Parameters	M1	M2	M3	M4	NM	MMF	NM
Nitrogen	0.89^{*}	0.96^{*}	0.80^{**}	0.92^{*}	NS	0.99^{*}	NS
Phosphorus	0.79^{**}	0.93^{*}	0.88^{*}	0.90^{*}	NS	0.91^{*}	NS
Potassium	0.92^{*}	0.93^{*}	0.96^{*}	0.97^{*}	NS	0.90^{*}	NS

M1 =*C.graniforme*, M2 = *S.luteus*, M3 = *Boletus* sp.,

M4 = *S.aurantium*.

NM = Non-mycorrhizal, MMF = Mixed mycorrhizal fungi.

* = significant at $p<0.01$ level
** = significant at $p<0.05$ level

Table 5.6 Analysis of variance (F) values of various mycobionts, mycorrhizal and non-mycorrhizal and pots and field with various parameters in pine seedlings.

Source of variance	Variation between mycobionts	Variation between mycorrhizal and non-mycorrhizal	Variation between pots and field.
Nitrogen	NS	2.97^{*}	5.49^{*}
Phosphorus	NS	2.76^{**}	2.52^{*}
Potassium	NS	2.48^{**}	4.27^{**}

* = significant at $p<0.01$ level
** = significant at $p<0.05$ level.

The uptake of K by seedlings was low at high concentration of heavy metals.

Higher content of K was observed in seedlings infected with mixed mycobionts than those infected with single infected fungus (Table 5.4).

There was a positive correlation between mycorrhizal infection and N, K ($P < 0.01$) and P ($P < 0.05$) uptake (table 5.5). A negative correlation was found between metals concentration and N, P and K uptake. There was a significant variation between mycorrhizal and non-mycorrhizal seedlings in N ($P < 0.01$), K and P ($P < 0.05$) uptake. A significant variation was also found ($P < 0.01$) between the contents of N, P and K in pots and field condition (Table 5.6). Insignificant variation in the content of N, P and K between the various mycobionts was found.

DISCUSSION

Discussion

Results have indicated that application of heavy metals to soil was toxic to the development of ectomycorrhizae with pine seedlings. Reduction of ectomycorrhizal development may be due to high concentration and effectiveness of Cd, Ni, Zn, Cu, Pb and Al which became toxic to the external mycelium. Root exudate is an important factor in governing mycorrhizal colonization (Duchesne *et al.*,1989). Perhaps heavy metal altered the root exudation in a way which did not favour the multiplication of mycorrhizal fungi (Schwab*et al.*,1982). The importance of ectomycorrhizal fungi and their ability to grow in presence of heavy metals though to a lesser extent could influence their root colonization. Deacon and Flemming (1992) considered that short roots are the primary resource unit for mycelial interaction and root colonization to be determined mainly by competitive exclusion because of superior colonization ability, with mycelial inoculum potential, growth rate, host specificity and abiotic factors influencing mycobiont success. All the four ectomycorrhizal fungi could withstand the heavy metals toxicity upto 50 ppm. They produced dense extramycelium which was damaged by higher concentration of metals. Denny and Wilkins (1987) argued that the extramatrical mycelium played a key role in retention of high amount of Zn ions. Jones and Hutchinsons (1988) found that the amount of fungal tissue produced by a mycobiont was positively correlated with the protection of host plant against metals toxicity.

It was observed that high concentration of Zn, Ni, Cd, Cu, Pb and Al limited the mycelium production and its colonization into deeper cortex. The cytological interactions in the fungal hyphae with increase in heavy metals concentration also suggested an accumulation of metals in the fungal mantle, however, this also may have occurred if mycorrhizal fungus were more sensitive to these metals than the host.

Cellular and structural changes in root of pine seedlings treated with heavy metals appeared at higher concentrations in both mycorrhizal and non-mycorrhizal roots which could be due to breakdown of cytoplasmic structure, disruption of cell walls and cortical cells and accumulation of dense compounds. Cytological changes observed in metals-stressed pine roots were similar to those that occurred in seedlings in which nutrient deficiency was induced deliberately (Fink, 1991). It was observed that non-mycorrhizal pine seedlings to be more sensitive to the toxic effects of heavy metals. It has been shown that in *Agrostis tenuis* the metal is confined to the roots the main site of complexing being cell wall (Wu *et al.*,1975). At high levels of metal, however, inhibition of root extension and hence complexing ability occurred. In these circumstances the presence of an ectomycorrhizal fungal symbiont could be of considerable importance. Fungal cell walls known to have strong affinities for metallic cations (Ashida *et al.*,1975) which provide much greater concentration of fungal material in the root. particularly efficient exclusion mechanism. Ellaborate hyphal coils occupy most of the cortical cells of pine roots with lower concentrations of metals through which absorptions occurs and these would provide a greatly increased surface for retention of metal ions.

The response of ectomycorrhizal fungi to heavy metals varied widely which was dependent on the ectomycorrhizal fungal species and the metal concentration. Metal content in non-mycorrhizal roots was higher than those of ectomycorrhizal roots. This suggested that the production of dense mycelium was an advantage for the seedlings by reducing the uptake Pb, Cd, Cu, Zn, Ni and Al by the host plant. However, once a saturation point was reached in the mycelium, the uptake of metals in the plants also increased, the rapid mycelial turnover which was associated with metal exclusion and hence avoidance of toxicity. This hypothesis was supported by Colpaert and Assche (1992) for Ni and Zn tolerance in plants.

Our results showed that Cd and Pb were most toxic to ectomycorrhizal fungi and the development of these fungi was inhibited in a moderately contaminated soil. The accumulation of metals in the mycelium and protecting root against metal toxicity was an important factor. Jones and Hutchinsons, (1988) have demonstrated that *Lactarius rufus* increased the Ni tolerance of its host plant for a short time. It is very unlikely that mycorrhizal fungi directly influenced the transport of heavy metals in the plants. However, they could influence the amount of metal transported to the roots. All mycorrhizal fungi had an ameliorating effect on the reduction of metal concentration in the root tissue. This suggested that the whole biomass in sporophore, the extramatrical mycelium as well as in the mantle mycelia, can complex a considerable amount of added metals and thus strongly reduce the availability of the toxic ions to the pine seedlings. High accumulation of Zn and Cu comparing to Ni, Cd, Pb and Al suggested that Zn and Cu are essential trace elements and are not toxic to the plants and fungal symbiont at lower concentrations. Seedlings root contained high concentration of Cu which is an essential micronutrient for plants and mycorrhizae are proved to sequester it in high quantities (Berthelson *et al.*,1995). This sequestration could prevent the deleterious effects of metals to the host to a certain extent. Denny and Wilkins (1987) also argued that the extramatrical mycelium played a key role in retention of high amount of Zn ions. Lindsay (1972) also observed high concentration of Zn in root tissue. The metals primarily acted on the mycelium, the fungal tissue might also provide the host with protection against heavy metals depending upon the metal accumulation capacity of the mycelium. The influence of metal uptake is presumed to be greatest for elements which have narrow diffusion zones around the plant root such as Cd, Pb and Ni (Harley and Smith, 1983). In this study, differential of metal uptake may be associated with the development of fungal mantle. Bradley *et al.*,1981 also confirmed binding of metals to roots or fungal structures . The relatively high tissue contents of heavy metals in non-mycorrhizal and low amount of Zn, Cu, Ni, Cd, Pb and Al in ectomycorrhizal seedlings supports this hypothesis.

The results suggested that Cu, Ni, Cd, Zn, Pb and Al had deleterious effects on activities of urease, dehydrogenase and phosphatase on the root surface of pine and in the rhizospheric soil. Possibly the presence of ectomycorrhizal fungi enabled the seedlings to have their increased metabolic activities. Jha (1991) corellated the high concentration of various enzymes like urease, dehydrogenase and phosphatase to the high microbial population in soil. The activity of enzyme was lower in rhizospheric soil than root surface which may be due to reduced number of micro-organisms in the rhizosphere region.

Seasonal variation of enzyme activities appears to be dependent on factors such as aeration, soil moisture, soil temperature and microflora (Burns, 1978). The activity of enzymes in roots of pine seedlings treated with metals is in contrary to the findings of Ruegsegger *et al.* (1990) who showed that the activity of enzyme in maize and pea roots increased as a result of Cd treatment. This could be due to their toxic nature when present in high amount.

The results indicated that there was a decrease in phosphatase, urease and dehydrogenase activity when treated with high concentration of heavy metals, the degree of inhibition is dependent on the amount of metals added and the intensity of inhibition among the factors may be enhanced due to low amount of organic matter. Soil organic matter is able to form complex metallic compound by cation exchange, adsorption or chellation. Inhibition of enzyme activity of phosphatase by Cu and Zn cations has been reported (Tabatabai *et al.*,1977). The micro-organisms on the root surface and in rhizosphere region may differ in their sensitivity to metal toxicity. High concentration of metal exposure may also cause death of cells due to disruption of membrane resulting into changes in viability or competitive ability of the microbes.

The dehydrogenase activity was considerably decreased in the rhizosphere soil as well as on the root surface with high metal doses. Reddy and Faza (1989) also reported the decreased dehydrogenase activity in soils containing heavy metals. The root growth was normaly low in winter which leads to the assumption that along with high metals, low temperature added to the reduced microbial activity. The appreciable decrease in urease activity in rhizospheric soils receiving high metal concentration suggested that it is derived from living cells in low quantity. Gianfreda *et al.* (1994) reported that application of Cu in the form of fungicides altered the urease activity in soil.

The greater inhibition of enzymes in the presence of heavy metals could be due to inhibition of enzyme sites that was not available for catalysis. Stott *et al.*1985 reported that metals like Cd, Ni, Zn Cu and Pb may inhibit enzyme reaction by complexing the substrate, or reaction with the enzyme substrate complex. The mode of inhibition is dependent on the type of reaction or complexation produced. Tyler (1981) also observed that heavy metals inhibited a variety of microbial enzymes, indicating that nature and type of inhibition varies among the metals used and the enzyme studied. However, little information is available on the inhibition of these enzymes on root surface and in rhizosphere soils by metal ions. The degree of inhibition of urease, dehydrogenase and phosphatase activity on root surface and rhizosphere soil of pine seedlings by heavy metal is much smaller in mycorrhizal roots than non-mycorrhizal ones.

This could be due to the binding of metal ions by fungal mycelium which consisted of mantle and Hartig net. This would explain why mycorrhizal plants had lesser inhibition compared to non-mycorrhizal ones. Denny and Wilkins (1987) also measured lower contents of Zn in the cortex of mycorrhizal than non-mycorrhizal birch plants.

Inoculation of pine seedlings with *Boletus* sp, *C. graniforme, S. aurantium,* and *S. luteus* consistantly stimulated shoot height, number of needles, needle length, root length and seedling volume as compared to non-mycorrhizal seedlings. Macfall and Slack (1991) also noted significant increase in pine (*Pinus resinosa* Ait) seedlings and root and shoot dry weights inoculated with ectomycorrhizal fungi as compared to non-mycorrhizal seedlings. Similarly, Gbdesgesin (1990) observed better survival and growth of pine (*Pinus oocarpa*) seedlings with *Pisolithus tinctorius* in field conditions after a period of four years. *Suillus luteus* inoculated seedlings showed highest percentage of survival and growth which was followed by *Boletus* sp inoculated seedlings. Mycorrhizal inoculated seedlings had better survival, greater shoot heights, more number of needles and greater needle length than did non-inoculated ones. This indicates that the mycorrhizal seedlings were more tolerant to the toxic effect of heavy metals.

The extent of growth suppression was clearly influenced by the treatment of heavy metals. The severity of growth inhibition in metal treated non-mycorrhizal and its association with a marked increase in metal concentration compared to mycorrhizal seedlings suggested that the metal accumulation is primarily responsible for seedlings damage. Our results indicated the inhibition of root elongation at higher concentration (100 ppm and above) of heavy metals. The capacity of mycorrhizae formation was the largest for *S. luteus* inoculated seedlings treated with Pb appeared to be less inhibitory for mycorrhizal formation. The fungus showed the rapid colonization and growth so that the toxic effects brought about by the metals were reduced quickly, therby allowing the root to retain its normal growth.

In this study plant roots were most susceptible to metal damage than other growth parameters, and all the four mycorrhizal fungi appeared to ameliorate this damage. Initially the plant roots became dark, short and thick, these symptoms were attributed to metal toxicity (Mengel *et al.*, 1982). However, mycorrhizal roots grew normally when compared with to brown, short and stubby roots observed in non-mycorrhizal plants. The presence of growing fungus appeared to reduce the toxicity of Cd, Cu, Ni, Zn, Pb and Al. The toxicity symptoms remained in non-inoculated plants. With time it appeared either that the plant itself or the inoculated fungus was able to bind the metals, mediate the rhizosphere pH or that the substrate or root exudates complex, the available metals so that the element was no longer a toxic. However, additional researches into understanding of the mechanism of protection of roots against metal toxicity are required. The inferior growth of seedlings may have been linked to their lower level of mycorrhization. Baar *et al.* (1994) also observed the reduction in growth of seedlings due to low mycorrhizal level. This lower level of mycorrhization could be due to the toxicity of Zn, Cu, Cd, Ni, Pb and Al when present at high concentration. Relatively undisturbed soils should contain host root systems and mycorrhizal mycelia (Last *et al.*, 1987). These mycelia should be favoured colonizers of new roots and young seedlings (Baar *et al* .,1994) The mycorrhizal inoculum treatment in this study produced superior growth as compared with the non-inoculated ones.

The mixed inoculum treatment to the pine seedlings was better than either single inoculum or non-inoculation of seedlings. This may be due to its greater inoculum potential, which could permit the fungi more effective host exploitation of resources and provide mycobionts needed to survive in the fluctuating environmental conditions. The single inoculum treatment showed less significant variation in their improvement of host growth. The strong impact of mixed inoculum on host plant and fungal growth rates may have major ecological implication, and will have a competitive advantage and probably were able to infect more root tips (Susanne *et al.*, 1990). The better growth of seedlings infected with mixed mycelia was also observed in jack pine by Gardes *et al.*(1990a) and De la Bastide *et al.* (1995a). Our results proved that more than one fungus inoculum can colonise host root system more extensively and produce more mycelia which proved advantageous to the plants growth and their establishment.

The ectomycorrhizal fungus increased the tolerance of pine seedlings to heavy metals, although the fungi did not preclude the toxic effect of metals. Hence, ectomycorrhizal seedlings grew better than non-mycorrhizal ones at all levels of heavy metals.

Analysis of mineral nutrients in seedlings of pine suggested that growth was greater in the mycorrhizal seedlings than in non-mycorrhizal ones which could be due to the increased uptake of nutrients especially of N and P. The greater uptake of P by mycorrhizal seedlings compared with non-mycorrhizal ones has been related to the increased root surface area (Harley and Smith, 1983). Increased shoot growth which occurred when pine seedlings became mycorrhizal, has also been observed (Rousseau and Reid, 1991). Contents of N in ectomycorrhizal seedlings were more than twice than in non-mycorrhizal ones at each metal treatment level. The cause of growth reduction in non-mycorrhizal plants may be due to their N status. Since, N is the growth limiting factor, any change in N uptake and transport would alter the growth of plant. Nitrogen contents in the shoot is directly related to the relative growth rate of plants (Ingestad *et al* ., 1986) as was clear from the increased uptake of nitrogen, potassium and phosphorus by mycorrhizal seedlings at different levels of metals. Study demonstrates that the inoculation of mixed inoculum of mycobionts may be more advantageous to both plants and fungus, which could permit the host to exploit more effectively of mineral resources. There is evidence that Cd and other heavy metals influenced the translocation of essential elements (Smith and Brennan, 1984). Cd, Ni and Pb are considered extremely toxic metals in plants causing disorders in photosynthetic machinery even at relatively low concentration resulting the growth reduction of seedlings (Tinker, 1981).

The greater root development in the seedlings treated with 50 ppm and below of heavy metals might have accounted for higher N, P and K accumulation. The enhanced metal accumulation in non-mycorrhizal roots may be partially responsible for poor root development (Tan *et al.*, 1990b) which will lead to the less exploitation of mineral nutrients due to their reduced root system. Mycorrhizal association is specially adapted to nutrient stress condition (Heinrich *et al.*, 1989), it may be possible that under these circumstances, ectomycorrhizal roots and their mycelia function mineral source-sink than non-mycorrhizal ones. Reduced growth of extramycelium resulting from high concentration of heavy metals is likely to reduce the potential of the fungus to take up minerals and water and transfer them to the seedlings. Mycorrhizal development might be a more important factor than soil nutrient availability for determining nutrient levels especially for P due to its low mobility in the soil. Reduced mineral availability ot the plants was due to the reduced absorbing surface of the mycorrhizae caused by pollution which may result in a rapid decline of forest (Dighton *et al.*,1990). Similar decrease in nutrient concentration in seedlings has been reported due to Ni toxicity (Cataldo *et al.*, 1978). The main cause of reduced nutrient content of the seedlings was due to the high concentration of Cu, Zn, Ni, Cd, Pb and Al in soil which restricted the mycorrhizae in the root system. The nutrient concentration in the seedlings has shown that the lower levels of heavy metals were able to sustain growth of non-mycorrhizal and mycorrhizal seedlings. The main effect of metal treatment in non-mycorrhizal seedlings was directly correlated to the nutrient availability. At high levels of metals, however, inhibition of nutrient contents may be due to complexing ability, in these circumstances the presence of an ectomycorrhizal fungal symbiont could be of considerable importance. Kumar *et al.* (1991) observed that the mycorrhizal seedlings have more extensive root system than non-mycorrhizal ones.

SUMMARY

Summary

The present investigation was undertaken to study the toxicity of heavy metals on the structure and function of ectomycorrhizal fungi and their ability to colonize the pine seedlings.

Four mycorrhizal fungi forming ectomycorrhizae with Khasi pine (*Pinus kesiya* Royle ex Gordon) were selected. Six heavy metals were applied separately in pots as well as in field condition to the mycorrhizal and non-mycorrhizal pine seedlings. The study sites were located in pine forest stand of permanent campus, North Eastern Hill University, Shillong (1500, m msl). One site was located 1 m higher than the other. Pine seedlings inoculated with mycorrhizal fungi and treated with different concentrations were studied for one year to study the structure of mycorrhizae, enzyme activity in root region and nutrient uptake by the seedlings.

The fungal colonization was maximum in seedlings treated with lower concentration of metals as compared to higher concentration.

Heavy metals concentration showed negative correlation with mycorrhizal colonization.

Heavy metals were toxic to the development of ectomycorrhizae. High concentration of metals was specially toxic to the external mycelium, which ultimately resulted in reduction of ectomycorrhizal development. All the four ectomycorrhizal fungi could withstand the heavy metal toxicity upto 50 ppm.

However, the high concentration of metals limited the production of mycelium and its colonization. It was observed that non-mycorrhizal pine seedlings were more sensitive to the toxic effect of heavy metals than ectomycorrhizal ones.

Considerable differences were found in the levels of metals deposition in different ectomycorrhizal fungi inoculated seedlings. Significantly greater quantity of heavy metals was analysed in non-mycorrhizal roots than in mycorrhizal ones. Maximum concentration of Zn and Cu as compared to Cd, Pb, Ni and Al was observed. Cd and Pb were most toxic to ectomycorrhizal fungi than other heavy metals. The response of ectomycorrhizal fungi to heavy metals varied and that the response dependent on the ectomycorrhizal fungal species and the metal concentrations. The accumulations of metals in the mycelium and protecting root against metal toxicity was an important factor. All mycorrhizal fungi tested had an ameliorating affect on the reduction of metal concentration in the root tissue. There was a significant variation in the metal contents ($P < 0.01$) between mycorrhizal and non-mycorrhizal seedlings.

Heavy metals had deleterious effect on the activities of urease, dehydrogenase and phosphatase in the rhizosphere soil and root surface of pine seedlings. The enzyme activity was lower in rhizospheric soil than the root surface.

There was a decrease in urease, dehydrogenase and phosphatase activity when treated with high concentration of heavy metals. The degree of inhibition was dependent on the amount of metals added and the contents of soil organic matter. The degree of inhibition of enzymes was smaller in ectomycorrhizal roots

~~Pictorat~~
Pictorial Atlas of Soil and Seed fungi by
Tsuneo Watanabe - Lewis Publishers
Boca Raton Boston London New York Washington DC
1994.

Study on Soil and Seed Fungi

1 FUNGI IN SOIL AND FUNGUS FLORA

In soil there live numerous kinds of bacteria, actinomycetes fungi, algae and various other plants, amoebas and various other protozoa nematodes earthworms, mites and other soil animals which keep the individual units and populations in balance

Some of them disintegrate dead plants carcasses of dead animals, and organic matter forming humus and maintaining soil fertility

Some bacteria, including ammonifying bacteria, *Nitrosomonas* bacteria and nitrifying bacteria fix free nitrogen and are involved with the nitrogen cycle on the earth In addition there are some soilborne plant pathogens and parasitic harmful animals, although these organisms are few in number and small in population

In this text, fungi that live in soil and are detected or isolated from soil are tentatively termed as soil fungi Among such soil fungi some are typical which have never been isolated from other atmospheres excluding soil, and the other atypical which are readily and frequently isolated from various kinds of organic matter found in other atmospheres

Fungi from underground parts, and especially associated with soilborne diseases appear to be typical 'soil fungi' but some of them are also often isolated from seed, and thus named as seed fungi

Some fungi may live in soil associated with the organic matter derived from the aboveground living entities after their deaths

Some airborne fungi may be contaminated with soil and may be casually isolated from soil In addition, some fungi may be isolated from soil animals and they may also be classified as one of the soil fungi For example, 63 fungal species are isolated from cysts of soybean cyst nematode, *Heterodera schachtii* (Carris et al , 1989)

In this text no rigid definition of soil fungi is given but fungi detected or isolated from soil seeds and roots of plants are tentatively termed as soil fungi

The science of soil microbes has begun to study mechanisms of nitrogen fixation and other biochemical reaction in soil During the course of study, various bacteria yeasts, and fungi were isolated and described

Adametz (1886) was the pioneer of soil fungus study, isolating and naming four species of yeasts and 11 fungal species including *Aspergillus glaucus*, *Penicillium glaucus* and *Mucor stolonifer*

Jensen (19.2) isolated 5 species of soil fungi during the course of study on classification of soil microbes their morphology distribution and ecology such as survival in winter

In Holland Oudemans and Koning isolated 45 fungal species including *Trichoderma* [illegible]

Butler (1907) English mycologist isolated six *Pythium* species and some pythiaceous fungi in India and Hagem (1907) isolated 16 species belonging to Mucorales, including eight [illegible] species

In Japan Takahashi (19.9) isolated some fungi from field soils at Komaba in Tokyo identified 25 species belonging to 13 genera and reported in the first volume of *Ann Phytopathol Soc Jpn* He must be the pioneer in soil fungal study in Japan although some other early works on this line may have been conducted (note the early issues of *J Agrochem Jpn*)

Waksman is famous for his discovery of streptomycin, and he published a series of papers on soil fungi since 1916 and advocated a theory that common fungi live in any soil To demonstrate this, he isolated more than 200 fungal species belonging to 42 genera from 25 locations in the U S Among these fungi, four genera, — *Aspergillus, Mucor, Penicillium,* and *Trichoderma* — live commonly in soil of any location Especially, he noted that *Mucor* and *Penicillium* commonly occur in soil of temperate or cool-climate areas, *Aspergillus* in tropical soil, and *Trichoderma* occur frequently in wet or acidic soils In addition to the four genera mentioned above, the following eight genera were pointed out as dominant fungi *Acrostalagmus* (syn *Verticillium*), *Alternaria, Cephalosporium* (syn *Acremonium*), *Cladosporium, Fusarium, Rhizopus Verticillium* and *Zygorhynchus* He further found that soil fungi tend to colonize on moribund plant residues, that many kinds and numerous populations of bacteria and actinomycetes live in soil, and he further demonstrated that the more fertile the soils, the more numerous and massive are the fungi

Although there are differences in fungal species among soil isolates by different isolation methods, 12 out of 17 dominant fungi were listed by Waksman, and the other five, namely *Absidia, Botrytis, Chaetomium Cylindrocarpon,* and *Stemphylium*, are common in soil, according to Burges (1965)

Some fungi such as *Pythium Mortierella, Rhizoctonia,* and basidiomycetous fungi had not been listed or were very rarely listed as members of soil fungi before 1949 (Chesters, 1949), but by the hyphal isolation method devised by Warcup (1959), these fungi became commonly known as soil fungus members Especially, about 40 species of basidiomycetous fungi have been isolated from soil and were identified in his work

According to Burges (1965), over 600 fungus species including 200 phycomycetous species (Mastigomycotina and Zygomycotina), 32 ascomycetous fungi, and 385 deuteromycetous fungi were recorded as soil fungi in the first edition of "A Manual of Soil Fungi" written by Gilman in 1945 Since then, soil fungus study has been serious and active, but nearly 1200 species must be considered reasonable as the total number of soil fungi so far described, although such a figure was derived from doubling the figure in 1945 This figure is nearly 2% of 64,200 species of total fungi recorded (Hawksworth et al , 1983) This small figure must be due to the lack of attention to individual organisms, because previous workers have studied soil microorganisms en masse in the categories of bacteria, actinomycetes, fungi, algae, and so on

Researchers like to know the types of fungi, the number of genera and species, and the common and dominant species in the surrounding soil, although research purposes may vary considerably Concomitantly, it is helpful to compare fungi or fungus floras in different soils, paying attention to the characteristics of the floras, and their similarity rates To get this information, various isolation methods and media have been used, including soil dilution and soil plate methods, often with rose bengal streptomycin agar medium

For a particular genera or species, we would like to compare their occurrence or detection frequency (isolation frequency) and coefficient of similarity Values of coefficient of similarity are obtained, following the equation $S = 2W/(a + b)$, where "w" is the number of common species in two fungal populations and "a" and "b" are the numbers of species in both populations, namely, as the similarity between two populations increases, the value of the coefficient of similarity approaches one

By using these approaches, interrelationships among habitats of higher plants, soil fungus floras, many different soils including forest, grassland, uncultivated and cultivated soils, and comparison of soil fungus floras in diseased vs healthy soils, various soil atmospheres including soil pH, soil type, organic contents, soil depth in different seasons, and factors influencing soil fungus floras become subjects of study, and through the knowledge of soil fungus floras we are able to understand the various habitats of higher plants and their activities, soil fertility and disease occurrence areas and some environmental problems

2 RELATIONSHIPS BETWEEN SOILBORNE PLANT PATHOGENIC FUNGI AND OTHER SOIL MICROORGANISMS

Although fungi resting or dormant in soil may be affected by soil temperatures, water content, pH, and physical or chemical soil elements, no competitive relationships may exist among these organisms However, they are active and become cooperative with or antagonistic against other organisms with complicated relationships For example, on water agar plated with a part of the washed roots of any plants without sterilization, some fungus organs such as conidia, chlamydospores, and sporangia or fruiting bodies such as pycnidia and perithecia and others may be readily observed in an elapse of time Concomitantly bacteria and actinomycetes growing around the hyphae penetrate, parasitize, and disintegrate the hyphae, or the hyphae themselves intermingle with each other

Antagonism among amoeba, bacteria, or fungi under the microscope, or in nematodes escaped from the root tissues, may be observed on agar plates, being trapped by a kind of nematode trapper of the genus *Arthrobotrys*, and wriggling All these phenomena may always happen, especially in the vicinity of plant roots For example, *Rhizoctonia solani*, the notorious soilborne plant pathogen, may be parasitized by some typical soil fungi including *Trichoderma viride, Aspergillus,* and *Penicillium* spp , whereas *R solani* itself may parasitize *Pythium debaryanum*, the damping-off fungus (Butler, 1957)

Verticillium species isolated from strawberry roots was endoparasitic to nematode and antagonistic against many soilborne plant pathogenic fungi (Watanabe, 1980) It is generally easy to detect and isolate *in vitro* soil fungi which are parasitic on any soilborne plant pathogens or inhibitory against them However, their antagonistic activities appear to be very much limited in natural soil For example, about 40% of 3500 isolates of bacteria and actinomycetes obtained from soils of 60 locations in Australia were shown to be antagonistic against one, or more than two species of nine soilborne plant pathogens *in vitro*, but only 4% were antagonistic in soil (Broadbent et al , 1971) Antibiotic substances have been extracted from these antagonistic organisms, since Weindling in 1932 obtained viridin and gliotoxin from *T viride* (if possible, from *Gliocladium virense*, Webster and Lamos, 1964) antagonistic against *R solani*

On one hand, there are some organisms that are antagonistic, but on the other hand, some organisms are known to influence the morphogenesis of other organisms For example, *Phytophthora cinnamomi* are induced and stimulated for the formation of sporangia by some bacteria such as *Chromobacterium violaceum, Pseudomonas* spp (Zentmyer, 1965, Marx and Haasis, 1965), its oospores — which are not formed in single cultures because of their heterothallic nature with different mating types — are induced to form singly by the influence of volatile substances excreted by *T viride* and *Trichoderma* spp This phenonomenon is called the "Trichoderma effect" (Brasier, 1971) Furthermore, chlamydospores of *Fusarium solani* f *phaseoli* and sclerotia of *R solani* and *Sclerotium rolfsii* are induced or stimulated to form by some bacteria and actinomycetes including *Arthrobacter* sp , *Bacillus subtilis, B licheniformis,* and *Bacillus* spp , *Protaminobacter* sp , and *Streptomyces griseus* (Ford et al , 1970, Henis and Inbar, 1968)

Differentiation of the rhizomorph and its growth of *Armillaria mellea* are stimulated by the activity of *Aureobasidium pullulans*, and its activity is due to the ether effect (Pentland, 1967) A similar stimulative effect was shown by the culture broth of *Macrophoma* sp (Watanabe, 1986)

Sphaerostilbe repens, the root rot pathogen of tea and various plants in the tropics, is induced to form hyphal bundles and synnema by the influence of *Aspergillus* spp , *Penicillium* spp , and *Verticillium lamelicola* (Botton and El-Khouri, 1978) All these are examples of the morphogenesis of certain fungi under the influence of activities of other organisms, and these activities are related to alcohols including hexanal, ethyl alcohol, and methyl alcohol, fatty acids including linoleic acid, oils and fats and unidentified substances excreted by micro organisms

3 RESEARCH PROBLEMS ON SOIL FUNGUS FLORAS

Fungus floras present in soils or associated with plant roots have been studied worldwide by compiling fungal members together with their isolation or detection frequencies As one of the problems related to these studies, mycologists tend to specialize in particular fungi, whereas pathologists study the pathogenicities of any of the organisms, and soil microbiologists pick up microbial problems en masse Whatever approaches may be taken, we have to study fungus floras at first for the etiological study of plant diseases, and especially, to understand environmental factors for soilborne disease occurrence, the study of fungus floras is the most important

With different purposes in research, particular groups of fungi or all fungi may be studied Kinds of isolated fungi and their frequencies of isolation (or detection) may be studied at first, but treatment of each fungus may be different, for example, some of them may be described together with the process of identification, but others may just be listed without any detailed descriptions of identification procedures Therefore, it is almost impossible to refer to publications equally

Therefore, a list of fungal members in fungus floras may be of no use for some mycologists because of incomplete descriptions of the fungi in the list, for plant pathologists because of the lack of inoculation experiments, and for ecologists because of too many superfluous mathematical treatments of individual populations

However, by accumulating data on this line, knowledge may be increased, and based on this knowledge, natural phenomena may be understood and clarified

Technical problems such as isolation methods media, and differences in isolation and incubation temperatures may occur for any particular fungus floras For example, *Mortierella* spp and *Pythium* spp a kind of zygomycetous and mastigomycetous fungi, respectively, may be detected from almost any soil by a trapping method using cucumber seeds as a trapping substrate, but they were not listed in some literature where other isolation methods were used (see the Supplement at the end of this chapter) We often find that some 10 genera may be isolated from any plant root tissue plated on any nutrient rich media after surface sterilization with chemicals, but more than 30 genera may be isolated by single hyphal tippings from the same sample plated on water agar after just washing without sterilization

Many fungi remain unidentified because of the lack of the technology to induce sporulation

Synonyms may be another troublesome problem for the study of fungal floras For example, *Acrostalagmus Hormodendrum* and *Papulaspora* recorded as members of fungus floras in old literature are now believed to be synonyms of *Verticillium, Cladosporium* and *Arthrinium*, respectively These problems often bring some contradictions and disorder to the study of soil fungi

In Japan, there have been published some works on soil fungus floras, but in the U S and Europe abundant data and knowledge have been accumulated For example, many different researchers, using different research methods or technologies, in different times and locations clarified and found that there live some soil fungi in sand and frozen soil, and in addition that some common fungi are also present in such soils To my experience, the common fungi happened to be isolated in soils of both Japan and South and North America

Generally speaking, as research on soil fungus floras increases, soil fungi may be observed more individually, rather than en masse, and new fungi may be discovered and knowledge on their classification will be more and more increased

4 PROBLEMS ON CLASSIFICATION OF ROOT-INHABITING FUNGI

Among various fungi observed on plant roots, there are pathogens inhibiting plant growth damaging or collapsing them but some fungi are mycorhizal, which may stimulate plant growth However, the activities of many fungi are unknown Most of these fungi after penetrating through natural openings and wounds, colonizing the roots, take nutrients from substances excreted by the roots

We find numerous fungal genera associated with plant roots For example, 58, 46, and 38 genera were found to be associated with roots of strawberry, sugarcane, and paulownia, respectively (Watanabe, 1977c, Watanabe et al , 1974, 1987a)

The number of genera isolated is different on the basis of sample size, time of sample collection, isolation methods, and media used, or may be influenced by disorder or the development of taxonomy itself or recognition of the study of fungus floras Namely, when we pay attention to particular groups of fungi, we tend to neglect other individual fungi, without further doing identification, thus, the number of known genera is limited

For mastigomycetous fungi, *Pythium* spp are generally isolated from old or declining roots, occasionally with plant pathogenic *Aphanomyces*, and *Phytophthora*, and aqueous fungi such as *Dictyuchus, Saprolegnia,* and *Pythiogeton* Among zygomycetous fungi, the genus *Mortierella* is the most common, followed by *Mucor* and *Rhizopus* In addition, *Absidia, Gongronella*, and *Syncephalastrum* may often be isolated *Saksenaea* is not so often recorded as a member of soil fungus floras, but it was isolated from sugarcane roots at Okinawa *Helicocephalum*, one of the zygomycetous fungi which are not cultured singly and purely *in vitro* was detected on agar cultures together with various organisms, and it lived for more than 6 months by the mixed culture These are rather rare fungi, because for the past 30 years I have only once isolated them *Chaetomium*, the ascomycete, is most commonly isolated, and the number of its species isolated from soil is numerous In addition, *Thielaviopsis* and seven others are often isolated from soil Basidiomycetous fungi have not been commonly isolated from soil but *Coprinus* and *Thanatephorus* are examples of fruiting *in vitro* but most of the basidiomycetous isolates are not successful for fruiting *in vitro* They are just judged to be basidiomycetous because of their having clamp connections

Deuteromycetous fungi are most frequently isolated, and they are rich in many kinds of species *Alternaria* and *Penicillium* are always listed as the members of fungus floras Generally speaking, a total of 7 to 74 fungal genera have been isolated from soil of one location (see the Supplement at the end of this chapter)

Unsporulated sterile, and unidentified fungi are different in their treatment in the literature, and they are classified as independent items

It is not clear if these fungi do not sporulate because of their innate nature, or if we could not induce their sporulation because of lack of technique In addition, there are a few unidentified fungi, although they sporulated

In fungal taxonomy, fungi are classified, identified, and described mainly based on morphology observed in nature For example, fungi belonging to the genus *Pestalotia* (syn , *Pestalotiopsis*) form sporodochia with morphologically very characteristic conidia, but they do not usually form acervuli on agar cultures, the morphology of which has often been used as criteria for taxonomy It appears to be difficult to induce morphogenesis by inoculation in nature, because of the excessive labor and time required However, with the increase of knowledge on agar cultures of various fungi, unknown fungi may be identified more readily

5 STUDY OF SOIL FUNGI IN RELATION TO SEED FUNGI

Unemerged seeds in soil or pre emergence damping-off may often be caused by seedborne plant pathogens, and even after emergence, young seedlings often become collapsed by damping-off or root rots Most fungi associated with seeds, including plant pathogens, are similarly inhabitants of soil, and therefore the study of seed fungi is "the study of soil fungi" Among these fungi associated with commercial kidney bean seeds, *Colletotrichum lindemuthianum*, *Macrophomina phaseolina*, and *Rhizoctonia solani* were isolated from the seeds at the rates of 1 per 22 to 41 seeds (Watanabe, 1972b)

Christensen and Kaufmann (1965), working with the fungi associated with seeds, classified them into two groups, i e , "field fungi", which must be contaminated with seeds in the field during harvest, and "storage fungi", which must be contaminated during storage We can not specify when and how the contamination occurred on these seeds, but most of these fungi can live under both seed and soil conditions There must be a few fungi only limited to living in seed, which infect only seeds and complete their life cycle on the seeds Many organisms may be introduced into soil by sowing, but on the other hand, some organisms penetrate, contaminate, and colonize plant tissues directly or indirectly in various growth stages, repeatedly Among these fungi, some influence seed quality and reduce germinability Aflatoxin and toxic substances are produced from toxicogenic fungi and effect the health of animals and human beings. Therefore, the study of seed fungi is also very important as it concerns our health In addition, there are many mycologically interesting fungi to which we have to pay attention

SUPPLEMENT Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no of genera, species, and remark)[a]	Ref
North America			
Canada Manitoba, 75 samples	Dilution plate with two media	Total 64 g, 177 spp (*Zygomycotina* 7 g, 22 spp *Ascomycotina* 9 g 13 spp , *Basidiomycotina* 2 g 2 spp *Deuteromycotina* 46 g, 141 spp)	Bisby et al (1933, 1935)
Canada Rhizosphere soil of wheat and other crops	Dilution plate	Total 17 g 38 spp (*Zygomycotina* 3 g, 3 spp , *Deuteromycotina* 14 g 35 spp) Fungus floras of rhizosphere and nonrhizosphere soils compared	Timonin (1940)
Canada 10 forest soils	Dilution with soil extract agar	Total 20 g, over 56 spp (*Zygomycotina* 2 g, 12 spp *Ascomycotina* 1 g, 1 spp *Deuteromycotina* 19 g 43 spp) Dominant genera *Mortierella Pullularia* *Trichoderma Penicillium*	Morrall and Vanterpool (1968)
Canada Ontario four conifer forest soils	Soil washing	Total 41 g 68 spp (*Zygomycotina* 5 g 10 spp *Ascomycotina* 5 g 5 spp *Deuteromycotina* 31 g 60 spp) Dominant genera *Mortierella Penicillium*, *Trichoderma*	Widden and Parkinson (1973)

SUPPLEMENT (continued) Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no of genera, species, and remark)[a]	Ref
Canada Tundra at the North Pole	Soil washing and plate	Total 33 g, 46 spp (*Zygomycotina* 1 g 1 sp *Ascomycotina* 2 g, 2 spp , *Deuteromycotina* 23 g 38 spp) Dominant genera Sterile fungus, *Penicillium*, *Chrysosporium*, *Cylindrocarpon*, *Trichoderma* not detected Soil and leaf fungus floras compared	Widden and Parkinson (1979)
U.S New Jersey grassland and others, eight samples	Dilution and direct inoculation with four media	Total over 29 g, 94 spp (*Zygomycotina* 4 g 19 spp , *Ascomycotina* 2 g 5 spp *Deuteromycotina* 23 g 82 spp) Dominant genera *Penicillium*, *Mucor Aspergillus* *Trichoderma Cladosporium*	Waksman (1916)
U S New Jersey, grassland and others, 25 samples	Dilution and direct inoculation with four media	Total 42 g, 94 spp (*Zygomycotina* 4 g 18 spp *Ascomycotina* 5 g, 7 spp *Deuteromycotina* 33 g, 112 spp)	Waksman (1917)
U S. Texas, forest soils four positions	Dilution with Waksman's medium	Total 13 g, 32 spp (*Zygomycotina* 3 g, 5 spp , *Ascomycotina* 1 g, 1 sp *Deuteromycotina* 9 g 26 spp) Dominant genera *Penicillium Aspergillus*	Morrow (1940)
U S. Wisconsin southern hardwood forest soils, 13 locations	Dilution with soil extract agar	Total 20 g, 50 spp (*Zygomycotina* 4 g 9 spp , *Ascomycotina* 1 g, 1 sp *Deuteromycotina* 15 g, 40 spp) Dominant genera *Absidia Mucor Mortierella* *Zygorhynchus* Fungus floras reflected in higher plant vegetation	Tresner et al (1954)
U S. Georgia forest and cultivated soils 45 samples	Dilution direct inoculation and others with rose bengal streptomycin agar and various media	Total 63 g 165 spp (*Zygomycotina* 11 g, 22 spp *Ascomycotina* 8 g 10 spp *Basidiomycotina* 2 g 2 spp, *Deuteromycotina* 42 g, 131 spp) Dominant genera *Penicillium Aspergillus* *Cunninghamella Trichoderma* *Rhizopus* Fungus floras compared between summer and winter Fungus floras became poorer with soil depth	Miller et al (1957)

SUPPLEMENT (continued) Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no of genera, species, and remark)[a]	Ref.
U.S. Wisconsin forest soils, five locations	Dilution	Total 36 g, 199 spp (*Zygomycotina* 4 g, 9 spp, *Ascomycotina* 2 g, 2 spp, *Deuteromycotina* 30 g, 83 spp) Dominant genera *Penicillium, Gliocladium, Trichoderma* Rates of similarity among the respective samples, 18 9–40 7	Christensen et al (1962)
U.S Southern 10 states, 30 nursery soils	Dilution and soil plate with rose bengal streptomycin agar	Total 45 g, 121 spp (*Zygomycotina* 8 g, 16 spp, *Ascomycotina* 4 g, 11 spp, *Deuteromycotina* 33 g, 94 spp) Dominant genera *Aspergillus, Penicillium, Trichoderma, Fusarium* *Pythium* and *Rhizoctonia* not isolated	Hodges (1962)
U.S. Northern Wisconsin bogs and swamps, 15 locations	Dilution	Total 57 g, 130 spp (*Zygomycotina* 2 g, 11 spp., *Ascomycotina* 8 g, 14 spp, *Deuteromycotina* 47 g 105 spp)	Christensen and Whittingham (1965)
U.S. and Mexico Arizona Mexico Sonoran desert, 24 locations, 30 samples	Soil dilution and soil plate with five media	Total. 104 g, 230 spp (*Mastigomycotina* 1 g, 1 sp, *Zygomycotina* 7 g, 15 spp, *Ascomycotina* 21 g, 77 spp, *Basidiomycotina* 1 g, 4 spp, *Deuteromycotina* 74 g, 133 spp) No specific fungus flora in desert *Curvularia*, colored fungi, and Pycnidium-forming fungi frequently isolated	Ranzoni (1968)
U.S. Alaska, uncultivated soils, 19 samples	Dilution with ether treatment	Total 14 g, 23 spp (*Zygomyconia* 3 g 3 spp. *Ascomycotina* 2 g, 3 spp, *Deuteromycotina* 9 g, 17 spp) Dominant genera *Mortierella, Penicillium*	Yokoyama et al (1979)
U.S. South Dakota, grassland 10 samples	Dilution	Total 13 g, 62 spp (*Zygomycotina* 1 g, 1 sp, *Deuteromycotina* 12 g, 40 spp) Dominant genera *Penicillium, Acremonium Aspergillus, Chrysosporium, Fusarium* Fungus floras in grassland compared domestically and internationally	Clarke and Christensen (1981)

SUPPLEMENT (continued) Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no. of genera, species, and remark)[a]	Ref.
Central and South America			
Panama and Cost Rica Panama and Costa Rica 31 samples	Direct inoculation with 17 media	Total 73 g, 135 spp (*Mastigomycotina* 1 g, 1 sp, *Zygomycotina* 8 g, 12 spp, *Ascomycotina* 9 g, 20 spp *Deuteromycotina* 39 g, 100 spp) Dominant genera· *Penicillium, Aspergillus, Fusarium, Cunninghamella, Trichoderma* *Mortierella* and basidiomycetous fungi not isolated	Farrow (1954)
Panama and Cost Rica Banana field soils, 30 locations	Dilution with rose bengal streptomycin agar	Total 33 g, 47 spp (*Zygomycotina* 4 g, 5 spp, *Ascomycotina* 3 g, 6 spp, *Deuteromycotina* 21 g, 35 spp) *Mortierella Pythium*, and basidiomycetous fungi not isolated	Goos (1960)
Honduras Banana rhizosphere soils	Dilution with rose bengal streptomycin agar	Total 37 g, 51 spp (*Zygomycotina* 2 g, 2 spp *Ascomycotina* 4 g, 4 spp, *Basidiomycotina* 1 g, 1 sp, *Deuteromycotina* 30 g, 45 spp) *Mortierella* and *Pythium* not isolated Fungus floras on roots, rhizoplanes, and rhizospheres compared	Goos and Timonin (1962)
Honduras Banana plantation soils	Dilution with rose bengal streptomycin agar	Total 48 g, 64 spp, (*Zygomycotina* 6 g, 6 spp, *Ascomycotina* 8 g, 10 spp, *Basidiomycotina* 1 g, 1 sp, *Deuteromycotina* 33 g, 47 spp) *Pythium* and *Mortierella* not isolated	Goos (1963)
Trinidad Fallow and sugarcane field soils	Dilution and direct inoculation	Total 40 g, 44 spp (*Mastigomycotina* 1 g, 1 sp, *Zygomycotina* 2 g, 2 spp, *Ascomycotina* 4 g, 8 spp, *Deuteromycotina* 33 g, 33 spp)	Mills and Vlitos (1967)
Jamaica Sugarcane rhizosphere soils	Dilution and soil plate	Total 68 g, 91 spp (*Mastigomycotina* 1 g, 1 spp, *Zygomycotina* 6 g, 6 spp, *Ascomycotina* 10 g, 10 spp, *Deuteromycotina* 50 g, 50 spp) Dominant genera *Aspergillus Penicillium Paecilomyces, Cephalosporium*	Robison (1970)

SUPPLEMENT (continued) Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no. of genera, species, and remark)[a]	Ref.
Australia			
Australia Southern Australia, wheat field soils	Dilution, soil plate, and hyphal isolation	Total 57 g, 94 spp (*Mastigomycotina* 3 g, 4 spp *Zygomycotina* 9 g, 19 spp , *Ascomycotina* 11 g, 13 spp , *Basidiomycotina* 3 g, 3 spp , *Deuteromycotina* 31 g, 53 spp) Isolation methods compared Basidiomycetous fungi isolated only by hyphal isolation method	Warcup (1957)
Australia *Wheat field* soils	*Dilution, soil* plate, and plant residue	*Total 54 g, 94 spp* (*Mastigomycotina* 1 g, 8 spp , *Zygomycotina* 10 g, 11 spp , *Ascomycotina* 6 g, 8 spp , *Basidiomycotina* 5 g, 5 spp , *Deuteromycotina* 32 g, 42 spp) Effect of soil fumigation on fungus floras studied	*Warcup (1976)*
Asia			
India Tea rhizosphere soils	Dilution with rose bengal streptomycin agar	Total 26 g, 50 spp (*Zygomycotina* 4 g, 5 spp , *Ascomycotina* 4 g, 5 spp , *Deuteromycotina* 18 g, 40 spp) More numerous species isolated from charcoal stump roots infected by *Usulina zonata* than from healthy roots	Agnihothrudu (1960)
Malaysia Forest and cultivated soils in the west	Dilution plate	Total 26 g, 54 spp (*Zygomycotina* 3 g, 4 spp , *Ascomycotina* 4 g, 5 spp , *Basidiomycotina* 1 g, 7 spp,, *Deuteromycotina* 18 g, 38 spp) *Pythium* and *Mortierella* not isolated	Varghese (1972)
Japan Osaka paddy field soils, four locations	Dilution plate, isolated at 42°C	Total 21 g, 37 spp (*Zygomycotina* 2 g, 3 spp , *Ascomycotina* 9 g, 16 spp , *Deuteromycotina* 10 g, 18 spp) Fungus floras in both meso and thermophilic fungi	Ito et al (1981)

SUPPLEMENT (continued) Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no. of genera, species, and remark)[a]	Ref.
Europe			
England Brown forest and podozol soils	Sieve soaking plate and hyphal isolation	Total 22 g, 50 spp (*Zygomycotina* 3 g, 16 spp , *Deuteromycotina* 19 g, 33 spp) Dominant genera *Trichoderma, Mucor* *Penicillium, Mortierella*	Thornton (1956)
England Grassland, five locations	Soil plate	Total 48 g, 148 spp (*Mastigomycotina* 1 g, 6 spp , *Zygomycotina* 10 g, 29 spp , *Ascomycotina* 9 g, 62 spp , *Deuteromycotina* 30 g, 24 spp) Dominant genera *Penicillium, Mortierella, Absidia, Cephalosporium, Fusarium*	Warcup (1951a)
England Kidney bean root and its rhizosphere soils	Soil plate	Total 17 g, 52 spp (*Mastigomycotina* 1 g, 1 sp,, *Zygomycotina* 3 g, 5 spp , *Ascomycotina* 1 g, 1 sp , *Deuteromycotina* 12 g, 19 spp)	Dix (1964)
Sweden Forest soils in the south	Soil washing	Total 21 g, 90 spp (*Zygomycotina* 3 g, 18 spp , *Deuteromycotina* 18 g, 36 spp) Dominant genera, *Mortierella, Penicillium, Trichoderma* Three dominant genera occupied more than 71% of total isolates	Soderstrom (1975)
Africa			
Nyasaland Coffee field	Soil plate	Total 39 g, 81 spp (*Zygomycotina* 7 g, 8 spp *Ascomycotina* 3 g, 4 spp , *Basidiomycotina* 3 g, 3 spp , *Deuteromycotina* 26 g, 34 spp) Dominant genera *Aspergillus Trichoderma Cephalosporium Fusarium*	Siddiqi (1964)
Israel Peanut fields, 12 locations	Dilution with two media	Total 42 g, 95 spp (*Zygomycotina* 8 g, 14 spp , *Ascomycotina* 6 g, 30 spp , *Deuteromycotina* 27 g, 50 spp) Dominant genera *Mucor Rhizopus, Aspergillus, Penicillium Cephalosporium*	Joffe and Borut (1966)

SUPPLEMENT (continued) Examples of Studies of Soil Fungus Floras

Sample	Isolation (method and media)	Fungi (no. of genera, species, and remark)[a]	Ref.
Israel Cultivated soils	Dilution and direct inoculation with 13 media	Total 46 g, 147 spp (*Zygomycotina* 7 g, 13 spp ; *Ascomycotina* 7 g, 60 spp ; *Deuteromycotina* 32 g, 72 spp.) Dominant genera *Rhizopus*, *Aspergillus*, *Penicillium*, *Alternaria* Effect of fertilization and vegetation on fungus floras studied	Joffe (1963)

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[a] *Aspergillus*, *Penicillium*, and *Rhizoctonia* are variously treated for their anamorph or teleomorph in the original literature The total number of genera and species of the respective classes in each work do not always coincide because figures of unknown and sterile fungi may be included in one work, but not in another

2

Materials and Methodology

All fungi in the text are isolated, identified, and described on the basis of the following experimental methods and samples.

1 COLLECTION SITES AND SAMPLES

Soil samples were collected from cultivated and uncultivated soils with various habitats including paddy field soil, grassland, and forest soil in Japan, sugarcane field soil in Taiwan, and paulownia plantations in Paraguay.

As plant samples, more than 100 plants were assayed including agricultural crops, flower plants, fruit and forest trees, and seeds of agricultural crops including pea and radish, and five forest seeds including Japanese black pine and flowering cherry seeds.

2 PRINCIPLES OF ISOLATION METHOD

After plating samples on agar cultures and incubating under certain conditions for a given period, samples may develop elongated hyphae and finally result in sporulation or fruit body formation.

By single hyphal tippings or isolation of spores directly at the tips of a transfer needle, or directly from fruiting bodies, pure cultures are established. For single hyphal tip isolation, a single hyphal tip elongated from substrates on water agar, less than 2 mm long in one piece, is selected and cut with agar blocks for establishment of pure cultures under the dissecting microscope at 30×.

At least ten needles may be prepared for fungal isolation, making them ready to use one by one after heat sterilization and cooling. As isolation media, 2% water agar has been frequently used. The agar plates should be thin, containing 7-ml/9-cm petri dishes. This is to avoid extra hyphal growth in thick agar and to isolate single hyphal tips readily. Bacteria are rather difficult to grow on plain water agar. Therefore, it is not necessary to prepare antibiotic- or acetic acid-containing agar.

In the single isolation method, spore suspensions appropriately diluted are poured onto plain water agar, and left for over 20 min for spore sedimentation and subsequently to remove extra water. After incubation for more than 2 h, single spores often germinated are dissected together with tiny agar blocks under a dissecting microscope. Spores with germ tubes are rather readily distinguished on agar. However, it is better to check and confirm single spores on each agar block under a compound microspope at 100× or more.

The best isolation techniques may be performed to use clean petri dishes without scratches or pits, and to prepare thin, rather solid plain-water agar plates and appropriate spore suspensions.

Using glass needles with capillary tips, single spores may be separated from a spore mass on water agar under a dissecting microscope. In this technique, thick plates (5 mm thick) of 3 to 4% plain water agar may be prepared. Isolations may be practiced with aseptic glass needles sterilized by soaking in boiled water for each isolation procedure.

3 ISOLATIONS AND CULTURES OF SOILBORNE FUNGI FROM PLANTS

Methods of isolation of soil fungi are different, according to the research purposes and samples used Here, I would like to discuss the technique to isolate possible plant pathogenic fungi from diseased plants This may be the general method for isolation of any fungi from plant materials The general procedures are as follows

1 Wash plant materials under running tap water for at least 30 min

2 For isolation from diseased plants, freshly infected parts may be selected, and they are cut into tissue segments of less than 5 mm From the aged infected tissues the more numerous saprophytic fungi may be isolated

3 Sterilized plant tissues may be prepared with antiformin or ethanol, together with unsterilized ones Concentrations of chemicals may be different, according to the samples used, but generally they may be soaked in 1 to 5% antiformin or 70% ethanol for 30 s to 5 min Unsterilized samples may be included to reduce failure of isolation of the fungi susceptible to such chemicals

Part of the samples used for microscopic examination may also be used for samples for fungal isolation

4 Plant tissue segments placed on isolation media are incubated at the appropriate temperature for 1 to 7 days The isolation media specific for individual fungi, may be used but for the general purposes, plain water agar may be one of the best media because bacteria and some contaminated fungi may be suppressed for lack of nutrition, and individual hyphae can be observed readily during isolation procedures The treated plates may be incubated, under lower temperatures below 15°C, at 20 to 25 C, the optimum for most fungi, and at higher temperatures above 34°C On the plates incubated under variable temperatures, morphogenesis may be stimulated, resulting in swift sporulation Identification practices may become easy

5 Hyphae may be elongated from the tissue segments plated within a few days, and single hyphal tippings may be practiced as soon as possible to get rid of extra contamination The plates used may be further incubated for continuous observations and, finally, ascocarps and other fruiting structures may be formed on such plates

6 For isolation of Pythiaceous fungi, including *Aphanomyces* spp the fungi may be trapped initially by susceptible plants or some other trapping substrates and these materials may be soaked further under water for observation and isolation

4 ISOLATION OF FUNGI FROM SOIL

For ecological studies of soil fungi such as distribution, populations, and activities of fungi in soil and subsequent use for prediction of soilborne disease occurrence, fungi have been isolated from soil

There are many isolation methods, including dilution plate, soil plate, immersion tube, plant debris, hyphal isolaton, flotation, and trapping methods

In this study, direct inoculation, trapping, soil dilution and flotation methods have been used for soil fungus isolations

The kinds of soil fungi and their isolation frequencies are different according to the isolation method, the media, and the temperatures during isolation procedures, and good results may be obtained by a combination of a few methods

4.1 Direct Inoculation Method

The direct inoculation method may be best for isolating various and general soil fungi simply, readily, and economically

The method is to isolate pure cultures by single hyphal tippings from hyphae grown out of soil particles sprinkled over agar media (Waksman, 1916) Czapek agar was originally used as an isolation medium by Waksman, but plain water agar is equally well suited and recommendable after comparisons of different kinds of fungus genera and isolation frequencies in potato dextrose agar (PDA), Czapek, and various agar media No addition of streptomycin or other antibiotics is necessary to avoid bacterial contamination The drawback of this method is that fast-growing fungi are isolated selectively while more slowly growing fungi are neglected

4.2 Dilution (Plate) Method and Isolation Media

In this method, diluted soil samples are plated onto isolation media, and pure cultures are obtained from colonies that have appeared on the selective media incubated for a few days at the appropriate temperatures

As selective media of *Fusarium* spp , pepton-pentachloronitrobenzene (PCNB) media (Nash and Snyder, 1965), V 8 juice-dextrose-yeast extract agar (VDYA)-PCNB (Papavizas, 1967), and Komada's synthetic agar medium (Komada, 1972) have been often used

For isolation of *Phytophthora*, the pimaricin-vancomycin-PCNB ($P_{10}VP$) (Tsao and Ocana, 1969) and hymexazol (3-hydroxy-5 methylisoxazole, HMI) containing $P_{10}VP$ or PDA at concentrations of 25 to 50 μm/ml, together with various antibiotics were devised by Masago et al (1977) and Tsao and Guy (1977)

The selective medium for *Pythium aphanidermatum* was also devised by Burr and Stanghellini (1973)

A monograph of chemicals and selective media for various fungi was summarized by Tsao (1970)

4.3 Trapping Method and the Substrates

This method is often used for isolation of mastigomycetous fungi The substrates mixed with wet soil samples and incubated below 10°C or at 25°C for 1 to 7 d are removed from soils and subsequently washed under running tap water These substrates are then placed on plain water agar and incubated for more than 1 d Then pure isolates are obtained by cutting single hyphal tips grown out of the substrate together with agar blocks The substrates used are various according to the individual research purposes, but potato tubers, apple fruit, roots of sweet potato and carrots, and seeds of cucumber, corn, and lupinus are often used

5 PRESERVATION OF CULTURES

The preservation of cultures is basic and important for mycological work The method the author has been using for the past 20 years consists of preparation of potato dextrose broth in tubes (7 ml per tube) and pouring the medium into old and shrinked cultures in tubes whenever necessary Without any particular experience it is possible to pour the medium into nearly 150 cultures in tubes within an hour, and it is not necessary to paste new labels for each test tube

The transfer medium is economically prepared because it does not contain agar However, it is rather difficult to observe colony characteristics as compared with slant agar cultures The author has been maintaining nearly 10 000 cultures in tubes using this method for the past 10 years

6 MORPHOGENESIS ON AGAR CULTURES AND THEIR OBSERVATIONS

For particular fungi, sporulation and formation of sclerotia may occur on hosts or on soil surfaces, and it may be possible to identify them on the basis of such morphologies However, no signs of morphologies may be observed in most of the etiological work of plant diseases, and therefore, cultures obtained from plant tissues or soils may be directly observed on agar cultures and identified on the basis of morphologies on agar cultures Therefore, it is prerequisite to induce sporulation for the cultures on agar

Most of deuteromycetous fungi may sporulate on rich agar media such as PDA, but others are rather difficult to sporulate Therefore, the following trials may be recommended to obtain successful sporulation

1 Some rich agar media including cornmeal agar, Czapek (Dox) agar, malt agar, oatmeal agar, PDA, and V 8 juice agar may be conventionally used
   Some ascomycetous fungi may often form fruiting structures in agar after a long incubation period, and therefore the quantity of media in the plates should be increased
2 The cultural environment including light conditions and temperatures should be altered for successful sporulation
3 Change the balance of nutrition in agar For example, cultures may be drastically changed from nutrient rich cultures to cultures poor in nutrients, including plain water agar culture
4 Use natural media which are prepared by mixing dried and propyren oxide–treated plant tissues into agar (Hansen and Snyder 1947)
5 In inducing zoospore discharge for some mastigomycetous fungi soak a bit of culture or infected plant tissue or substrate in pond water well water or Petri s salts solution
6 For some basidiomycetous fungi, successful fruiting may be obtained by casing (covering) cultures with soils or growing in wood chip medium including rice bran

3

Identification of Fungi

Species, the taxon (pl taxa) of any fungus may be named on the basis of morphological characteristics The fungus may be "identified" in determining its name by comparing the already known species in morphologies Generally speaking, identification of any fungus may be possible if their morphologies are observed clearly

Observations may be conducted at various levels from naked eye level through stereomicroscope or compound microscope to electron microscope However, on the basis of satisfactory observations by compound microscope, identification of any fungus may be possible At any rate, identification may be more correctly conducted due to individual abilities of observation of morphologies, or technical abilities for inducing sporulation in agar cultures

1 BASAL KNOWLEDGE FOR IDENTIFICATION

Spores may be one of the most important morphological characteristics for identification There are various spores including oospores, zygospores, ascospores, basidiospores, conidia, chlamydospores, sporangiospores, and so on, and based on spore morphology, fungi are easily differentiated into mastigomycetous, zygomycetous, ascomycetous, basidiomycetous, and deuteromycetous fungi

Without direct observation of spores, some fungi may be identified on the basis of some other morphological characteristics For example, mastigomycetous or zygomycetous fungi may be differentiated from other fungi because of the lack of hyphal septum Furthermore, after soaking cultures in water, some mastigomycetous fungi may emit zoospores, whereas most zygomycetous fungi may form sporangiospores

Some basidiomycetous fungi may be readily differentiated on the basis of clamped hyphae

For any fungi, particular representative isolates are selected at first for identification and observed for their morphological characteristics of certain organs in relation to other organs Fruiting structures, spores, mycelia, growing habits, and morphologies of various organs in nature may be observed initially and morphologies in cultures similarly observed subsequently

Observations under a dissecting microscope are conventionally conducted If observations are conducted directly under a compound microscope without placing a cover slip, the habit of sporulation, spores in chains, or the spore head may be readily observed Observations with an oil lens are also essential

Specialists may identify some fungi by the partial observation of those fungi on the basis of acumulated experience and knowledge, but observations must be repeated to know morphological characteristics in detail On the basis of the knowledge on morphological characteristics we may access the most suitable taxon

Keys must be prepared at various levels, including division, class, order, family, and genus Therefore, after trial and error, the most suitable taxon must be accessed Occasionally we may get to the species level successfully After consulting the literature related to the expected taxon and rechecking morphologies, we may finish the identification

Keys are based on the standard and general characteristics, but some keys are too artificial by nature including some exceptions Therefore, we just refer to such selections after keying out

Some fungi may be named without detailed studies, and therefore, with further study, other significant characteristics may be found and added, or old literature may be referenced later, resulting in reclassification or the formation of synonyms or new combinations

All these things may occur routinely, and we understand this readily because there are numerous synonyms Therefore, without completing identification just by following the keys, we have to check and consult fungi on the basis of the original descriptions, often comparing them with the type of specimen At any rate, overall judgment is essential for identification

2 NECESSITY OF EXPERIMENTATION

All descriptions, classifications, and naming may be based on the morphologies formed in nature However, some fungi may exceptionally form some morphological characterstics just by culturing There are many fungi, the names of which are coined on the basis of the names of host plants, and such customs may be still present For example, several synonyms are combined into one species on the basis of the results of inoculation experiments (namely, *Exobasidium* or rust)

Numerous heterothallic fungi may be classified based on the anamorphs, but by observations of the teleomorphs artificially produced by fertilization experiments, more scientifically reliable identification may be achieved Therefore, we have to get accustomed to observe morphologies formed on the host by inoculation tests or culturing

3 SELECTION OF APPROPRIATE BINOMIALS

It is quite difficult to select the best binomial among various synonyms For example, the binomials of the rice blast fungus, *Pyricularia oryzae* Cavara, have been traditionally used, but we are now at a loss whether we should use *P grisea* which has priority mycologically, or *Magnaporthe grisea* (Hebert) Barr based on the recent work of the teleomorph However, this fungus generally does not form the teleomorph in nature and *in vitro*, and therefore, on the basis of the rules of nomenclature, we can use either name scientifically (Rossman et al , 1990)

For the fungus with a few synonyms currently used, the best binomial must be selected on the basis of the individual scientific sense with future prospect If possible, synonyms may be included in any scientific descriptions for such fungal names, but to adopt the most suitable name, recent literature should always be consulted

For example, for the charcoal rot fungus, there are three currently used binomials, i e , *Macrophomina phaseolina* (Tassi) Goid , *Sclerotium bataticola* Taubenhouse, and *Rhizoctonia bataticola* (Taub) Butl , but the first name is now most commonly used

Verticillium albo atrum sensu lato had been commonly used as the wilt pathogen of various plants, but after controversy for recognition of *V dahliae* for a long time, the latter name has been used, together with *V albo atrum* sensu-strict

4 MORPHOLOGIES TO BE OBSERVED FOR IDENTIFICATION

Although observations of the morphologies are most important for identification, cultural characteristics are similarly emphasized for some fungi Therefore, such characteristics should not be neglected For identification, it is important to observe morphologies of individual fungi and to understand them

For conducting the observation, the following points should be paid attention, although there are some differences in individual fungi In addition, physiological characteristics such as temperature responses and host ranges are included in the keys, and some physiological characteristics should be similarly studied, together with morphological characteristics

A Cultural Characteristics

1 Color and tint in colony surface and reverse
2 Smell or fragrance
3 Quantity of aerial hyphae
4 Colony surface texture cottony, shrinked slopy, resupinate, velvety, powdery (floury), crustaceous, water soaked, embedded, yeast like, sticky, homogeneous or heterogeneous, presence or absence of elevation
5 Colony margin smooth, irregular
6 Pattern zonate, radiate, flowery, arachnate
7 Pigment exudated color watery
8 Organs formed fruiting structures sclerotia, rhizomorphs, synnema, sporodochia, stroma, setae

B Morphology

1 Size length, width, thickness, etc
2 Color
3 Shape
   a General characteristics for all fungi
      Hyphae (septate, aseptate, location of septum, clamp connection hyphopodia), ap pressoria, chlamydospores, rhizomorphs, synnema (pl -ta) and others
   b Differences in the respective classes
      Mastigomycetous fungi oogonium (pl a), antheridium (pl a), oospores (plerotic, aplerotic), sporangium (pl a) or hyphal swellings (hypha-like sporangia, sphaerosporangia, lobate sporangia)
      Zygomycetous fungi sporangium (columella, apophysis), sporangiola, sporangio spores, zygospores, suspensors, rhizoid, creeping hyphae
      Ascomycetous fungi ascocarp (naked ascocarp perithecium, apothecium etc), ascoma wall (peridium) stroma ascus (pl asci) (disposition, apical structure, evanescent or nonevanescent) paraphysis (pl paraphyses) ascospores
      Basidiomycetous fungi basidiocarp (volva stipe anulus, umbrella hymenium (location, shape, gill, pore, needle shaped, cystidia), basidia, spore print, basidiospores
      Deuteromycotina pycnidium (pl a), apothecium (pl a), sporodochium (pl -a), conidiophore (erect, resupinate, simple or branched, branching pattern), conidial types (aleuriosporae, annellosporae, arthrosporae, blastosporae, phialosporae, porosporae, radulasporae sympodulosporae), papulaspore
4 Number septum number of conidia, number of zoospore flagellum number of oospores in an oogonium antheridium number per oogonium number of oil globules per oospore, number of ascospores per ascus, number of basidiospores per basidium, etc
5 External and internal structures of tissues smooth, echinulate, warty, presence or absence of hair, textura (hyphal tissue) of peridium, component tissues of ascocarp or pycnidia (conidiocarp), component hyphae in basidiomycetes (presence or absence of primary hyphae, skeleton hyphae and/or uniting hyphae)
6 Presence or absence of protuberances number and shape

7 Positions of organs monoclinous or diclinous paragynous amphigynous hypogynous, etc, in positions of sexual organs in mastigomycetous fungi

8 Relations to other organs sexual patterns (conjugations of aplanogametangia, conjugation of aplanogamentangium and gametangium conjugation of gametangia) location

9 Proliferation internal proliferation, external proliferation

10 Germination pattern direct germination (by germ tubes), indirect germination (by zoospores)

11 Swimming pattern monoplanetism diplanetism

12 Connection conditions presence or absence of catenulation in conidia and chlamydospores and number and origin of catenulate spores

13 Shapes, formation order, and arrangement of appendaged hair setae, and others and/or supplementary organs

14 Formation pattern formation of fruiting structures (discrete, aggregated caespitose)

15 Positions of occurrence (aerial hyphae embedded, erumpent)

C Physiological Characteristics

1 Temperatures growth temperatures, optimum temperature for growth, temperatures for limited growth, growth rate

2 Growth media and nutritional requirements suitable media for sporulation and growth

3 Reaction for reagent and staining lactophenol, cotton blue, acid fuchsin KOH, $FeSO_4$, Melzer reagent

4 Resistance against chemicals

5 Hyphal anastomosis

6 Co culture reaction

7 Parasitic nature, pathogenicity

5 REFERENCES FOR FUNGAL TAXONOMY AND IDENTIFICATION

General: Ainsworth et al (1973) Alexopoulos and Mims (1979), Aoshima et al, Eds, (1983), Domsch and Gams (1972), Arx (1981a), Bessey (1961), Domsch et al (1980a b), Farr et al (1989), Hasegawa (Ed) (1984) Hawksworth et al (1983), Hiura (1967), Kobayashi et al (1992), Miyaji and Nishimura (1991), Webster (1980), Udagawa et al (1978)

Zygomycetes: Zycha et al (1969), Ito (1936)

Mastigomycetes. Middleton (1943), Plaats Niterink (1981), Erwin et al, Eds (1983), Katsura (1971) Ito (1936)

Ascomycetes: Dennis (1978) Hanlin (1989)

Basidiomycetes Ito (1955) Imazeki and Hongo (1987 1989)

Deuteromycetes Barron (1968), Barnett and Hunter (1987), Ellis (1971 1976), Matsushima (1975), Sutton (1980)

4

Key to Soil Fungus Classes

Key words hypha (pl e) clamp connection, sporangiospore(s), zygospore(s), oospore(s) ascospore(s), basidiospore(s), conidium (pl a), zoospore(s), sclerotium (pl a), rhizomorph

1	Hyphae	septate	6
		aseptate	2
2	Sporangiospores	formed	Zygomycetes
		none	3
3	Oospores	formed	Mastigomycetes
		none	4
4	Zoospores	formed	Mastigomycetes
		none	5
5	Zygospores	formed	Zygomycetes
		none	Mastigo or Zygomycetes
6	Hyphae	with clamp connection	Basidiomycetes
		without clamp connection	7
7	Spores	formed	8
		none	9
8	Ascospores	formed	Ascomycetes
	Basidiospores	formed	Basidiomycetes
	Conidia	formed	Deuteromycetes
9	Sclerotia and other organs	formed	10
		not formed	Deuteromycetes and others
10	Sclerotia	well differentiated (differentiation of rind and medulla)	*Sclerotium*
		not well differentiated	11
11	Hyphae	constricted near branching area	*Rhizoctonia*
		not so	12
12	Papulaspores	formed	*Papulaspore*
		none	13
13	Rhizomorph	formed	*Armillaria* and others
		none	Unidentified fungi

KEY TO MASTIGOMYCETES

Key words zoosporangium (pl -a), sporangium (pl a) sporangiospore(s), zoospore differentiation, zoospore(s), vesicle, oogonium (pl a), antheridium (pl -a), oospore(s), chlamydospore(s)

1	Sexual organs	formed	2
		none	8
2	Oogonia	amphigynons	*Phytophthora*
		not so	3
3	Sporangia	globose	4
		not so	5
4	Zoospores	differentiated inside sporangia	*Phytophthora*
		differentiated in vesicles developed outside sporangia	*Pythium*
5	Zoosporangia	both lobate and hypha-like	*Plectospira*
		either lobate or hypha-like	6
6	Zoosporangia	lobate	*Pythium*
		hypha-like	7
7	Zoospores	encysted, forming a mass at the tip of hypha-like sporangia	*Aphanomyces*
		not so	*Pythium*
8	Sporangia	large, mostly cylindrical	*Pythiogeton*
		not so	Heterothallic isolates (*Phytophthora* and others)

KEY TO ZYGOMYCETES

Key words sporangiophore(s), hyphal swellings (vesicles), sporangium (pl -a), merosporangia, apophysis (pl ses), columella, rhizoid(s), chlamydospores, zygospores, suspensors, homothallic heterothallic

1	Vesicles	formed between sporogiophore and sporangia	2
		not formed	4
2	Sporangia	formed directly on vesicle	3
		at apexes of branches developed on vesicle	*Umbelopsis*
3	Conidia	formed	*Cunninghamella*
	Merosporangia	formed	*Synephalastrum*
4	Sporangia	globose	5
		flask-shaped	*Saksenaea*
5	Sporangia	with apophysis	6
		without apophysis	7
6	Apophysis	globose	*Gongronella*
		not so	*Absidia*

7	Sporangia	well columellate	8
		without columella or poorly columellate	*Mortierella*
8	Columella	twisted or coiled	*Helicocephalum*
		not so	9
9	Rhizoid	formed just below sporangiophore	*Rhizopus*
		not formed	10
10	Sporangiophores	partially twisted	11
		not so	*Mucor*
11	Homothallic, Zygospores with unequal suspensors		*Zygorhnchus*
	Heterothallic		*Circinella*

KEY TO ASCOMYCETES

Key words ascocarp papilla, ostiole, seta (pl e), ascus (pl asci), uni- or bitunicate, evanescent or non evanescent, ascospores, gelatinous sheath

1	Ascocarp	ostiolate or papillate	2
		not so	9
2	Ascus	1 spored	*Monosporascus*
		4 to 8-spored	3
3	Ascospores	1 celled	4
		over 2 celled	7
4	Ascocarp	setose	*Chaetomium*
		without setae	5
5	Ascospore	with gelatinous sheath	*Sordaria*
		not so	6
6	Asci	globose	*Microascus*
		cylindrical	*Glomerella*
7	Ascospore cells	composed of dark and hyaline unequal cells	*Apiosordaria*
		with two homogenously collored cells	8
8	Ascus	bitunicate	*Didymella*
		unitunicate	*Nectria*
9	Ascospores	1 celled	10
		over 2-celled	11
10	Asci	evanescent	*Thielavia*
		non evanescent	*Anixiella*
11	Asci	bitunicate	12
		unitunicate	13

12. Ascospores	lunar-shaped	*Massarina*
	cylindrical	*Preussia*
13. Ascospores	2-celled, hyaline	*Eudarluca*
	over 2-celled, composed of hyaline and dark cells	*Zopfiella*

KEY TO BASIDIOMYCETES

Key words: basidiocarp, volva, stipe (stalk), annulus (ring), pileus (cup), hymenium (pl. -a) (arrangement: shape, lamellae (gill); pore, echinulate), cystidium (pl. -a), basidium (pl. -a), basidiospore(s)

Key: abbreviated

KEY TO DEUTEROMYCETES

Key words: conidiomata, conidiocarp, pycnidium (pl. -a), acervulus (pl. -li), sporodochium (pl. -a), synnema (pl. -mata), spore type (aleuriospore, annellospore, arthrospore, blastospore, phialospore, porospore, sympodulospore).

1. Conidiocarp or sporodochia	formed	2
	not formed	3
2. Pycnidia	formed	A. Pycnidium-former
Sporodochia (or acervuli)	formed	B. Sporodochium-former
Synnemata	formed	C. Synnema-former
3. Conidia	formed	4
	not formed	Sterile fungi
4. Conidia:	Aleurospore-type	D. Aleuriosporae
	Arthrospore-type	E. Arthrosporae
	Blastospore-type	F. Blastosporae
	Phialospore-type	G. Phialosporae
	Porospore-type	H. Porosporae
	Sympodurospore-type	I. Sympodurosporae
	Others	J. Annellosporae and others

A Pycnidium-Forming Fungi

Key words: pycnidia, ostiole, setae, conidia, filiform appendage, aggregated, discrete

1. Pycnidia	globose	2
	occasionally cup-shaped	*Hainesia*
2. Conidia	1-celled	3
	over 2-celled	7
3. Conidia	hyaline	4
	pigmented	11
4. Conidia	two types	*Phomopsis*
	one type	5

5. Pycnidia	ostiolate	6
	papillate	*Cytospora*
6. Setae	formed around ostioles	*Pyrenochaeta*
	not so	*Phoma, Macrophomina*
7. Conidia	2-celled	8
	over 3-celled	*Stagonospora*
8. Conidia	hyaline	9
	pigmented	10
9. Conidia	with filiform appendages	*Robillarda*
	not so, 2-celled with unequal size	*Apiocarpella*
10. Pycnidia	aggregated	*Botryodiplodia*
	discrete	*Diplodia*
11. Conidia	6 to 7-angled	*Microsphaeropsis*
	ovate or elliptical	12
12. Conidia	curved significantly	*Selenophoma*
	not so	*Coniothyrium*

B Sporodochium-Forming Fungi

Key words: sporodochia, setae, conidia, filiform appendages, vesicle, conidiophores

1. Setae	on sporodochia formed	2
	not formed	4
2. Setae	curved	*Sarcopodium*
	not curved	3
3. Sporodochia	well differentiated	*Volutella*
	not so	*Colletotrichum*
4. Conidia	simple	5
	complicated	11
5. Conidia	with filiform appendages	6
	not so	7
6. Conidia	cylindrical, concolor	*Hyphodiscosia*
	elliptical with central dark cells and hyaline end cells	*Pestalotia*
7. Conidia	1-celled	*Myrothecium*
	over 2-celled	8
8. Conidia	lunar-shaped,with foot cell	*Fusarium*
	cylindrical	9
9. Conidiophores	penicillate with stipe and terminal vesicle	*Cylindrocladium*
	simple, nonpenicillate	10

10. Conidia	mainly 3-septate	*Cylindrocarpon*
	long-cylindrical or thread-like, many-septate	*Gloeocercorpora*
11. Conidia	one type, branched	*Tetracladium*
	more than two types, smooth or echinulate	*Spegazzinia*

C Synnema-Forming Fungi

Key words: synnema (pl.-mata), setae, conidia

1. Synnemata	formed *in vitro*	2
	not formed, with pear-shaped hyphal swellings	*Dematophora*
2. Setae	formed among synnema, conidia 1-celled	*Trichurus*
	not so, conidia 2-celled	*Didymostilbe*

D Aleuriosporae

Key words: conidia, hyphae, clamp connection, conidiophores, synnema (pl.-ata), setae, septa, sterigma (pl.-ata)

1. Conidia	1-celled	2
	over 2-celled	8
2. Hyphae	with clamp connection	*Sporotrichum*
	not so	3
3. Conidia	hyaline	*Sepedonium*
	pigmented	4
4. Conidiophores	well developed	5
	not so	6
5. Synnemata and setae	formed	*Botryotrichum*
	not formed	*Staphylotrichum*
6. Conidiophores	globose, hyaline	*Nigrospora*
	not so	7
7. Conidia	globose	*Humicola*
	elliptical	*Mammaria*
8. Conidia	hyaline	9
	pigmented	10
9. Conidia	elliptical	*Monacrosporium*
	cylindrical, often radiately branched with two to three arms	*Trinacrium*
10. Conidia	longitudinally and transversely septate (muriform)	11
	mainly transversely septate	12
11. Conidia	globose	*Epicoccum*
	widely elliptical	*Pithomyces*

12. Conidia	branched	*Tetracladium*
	not so	13
13. Synnemata	conspicuous	14
	not so	*Trichocladium*
14. Conidiophores	with sterigmata	*Camposporium*
	not so	*Sporidesmium*

E Arthrosporae

Key words: conidiophore(s), conidia, catenulation

1. Conidiophores	well developed	2
	poorly developed	3
2. Conidia	1-celled	*Oidiodendron*
	2-celled	*Trichothecium*
3. Conidia	mainly cylindrical, truncate at both ends	*Geotrichum*
	mainly globose	4
4. Conidia	mainly catenulate	*Basipetospora*
	not catenulate	*Chrysosporium*

F Blastosporae

Key words: conidiophore(s), conidia, coniodiogenous cells, sterigma (pl.-ata)

1. Conidiophores	well developed	7
	poorly developed	2
2. Conidiogenous cells	globose, simple	*Arthrinium*
	not so	3
3. Hyphae and conidia	both hyaline	4
	not so	5
4. Sterigmata	developed on conidia	*Sporobolomyces*
	undeveloped	*Candida*
5. Conidia	1-celled	*Aureobasidium*
	over 2-celled	6
6. Conidia	branched to 3–4 directions	*Tetraploa*
	branched to 4–5 directions	*Tripospermum*
7. Conidiogenous cells	diffentiated, cylindrical or globose	8
	undifferentiated	11
8. Conidia	1-celled	9
	over 2-celled	*Cephaliophora*
9. Conidiogenous cells	formed limitedly at the apex	10
	formed everywhere	*Gonatobotrys*

10.	Conidia	formed at the apex of conidiogenous cell, 1–16 per cell	*Oedocephalum*
		formed apically and laterally, numerous	*Chromelosporium* (= *Ostracoderma*)
11.	Conidia	1-celled	12
		over 2-celled	15
12.	Conidia	hyaline	13
		pigmented	14
13.	Conidia	homogeneous in size, catenulate in a long chain	*Monilia*
		heterogeneous in size, catenulate in a short chain	*Hyalodendron*
14.	Conidia	globose	*Periconia*
		various including limoniform	*Cladosporium*
15.	Conidia	2-celled	16
		more than 2-celled	18
16.	Conidia	hyaline	*Trichothecium*
		pigmented	17
17.	Conidiophores	branched	*Cladosporium*
		almost unbranched	*Bispora*
18.	Conidia	cylindrical	*Septonema*
		globose	*Torula*
		taenia-like	*Taeniolella*

G Phialosporae

Key words: conidia, appendage, foot cell, catenulation, spore mass, fertile (conidium-forming) area, conidiophores, phialide(s), penicillate

1.	Conidia	over 2-celled	2
		1-celled	4
2.	Conidiophores	penicillate with stipe and terminal vesicles, conidia cylindrical	*Cylindrocladium*
		simple, conidia not cylindrical	3
3.	Conidia	lunate with a foot cell	*Fusarium*
		cylindrical, without a foot cell	*Cylindrocarpon*
4.	Conidiophores	with inflated apical cells bearing numerous phialides	*Aspergillus*
		without an inflated apical cell	5
5.	Conidia	pigmented	6
		hyaline	10
6.	Conidiophores	poorly developed	7
		well developed	8
7.	Conidia	aggregated in a mass	*Phialophora*
		catenulate	*Torulomyces*

8.	Conidia	branched	9
		almost unbranched	*Stachybotrys*
9.	Conidia	catenulate	*Phialomyces*
		aggregated in a mass	*Myrothecium*
10.	Conidia	globose	*Cladorhinum*
		not so	11
11.	Conidia	boat-shaped or lunate, with or without appendages	*Codinaea*
		not so	12
12.	Conidia	clavate	13
		not so	15
13.	Conidia	catenulate	14
		aggregated in a spore mass	*Stachybotryna*
14.	Chlamydospores	solitary	*Chalara*
		catenulate	*Thielaviopsis*
15.	Conidiophores	poorly developed, almost phialidic	*Acremonium* (= *Cephalosporium*)
		well developed	16
16.	Conidia	dry	17
		wet	20
17.	Conidiophores	pigmented, conidia catenulate	*Thysanophora*
		hyaline, spore aggregated in a row	18
18.	Conidia	cylindrical	*Metarhizium*
		not so	19
19.	Conidia	globose, conidiophores densely penicillate	*Penicillium*
		limoniform, conidiophores poorly penicillate	*Paecilomyces*
20.	Spore mass	only at the apex of conidiophores	*Gliocladium*
		formed at apical parts of conidiophores	21
21.	Conidiophores	hyaline	22
		pigmented	*Gonytrichum*
22.	Conidiophores	verticillate	*Verticillium*
		irregularly branched	*Trichoderma*

H Porosporae

Key words: conidia, longitudinally or transversely septate, beak, conidiophore(s), sympodulate

1.	Conidia	transversely and longitudinally septate (muriform)	2
		mainly transversely-septate	4
2.	Conidia	ovate or clavate, often well beaked	*Alternaria*
		eliptical, somewhat round without a beak	3

3	Conidiophores	proliferated apically developed sympodulally	*Stemphylium* *Ulocladium*
4	Conidia	catenulate not so	5 6
5	Conidia	globose long elliptical	*Torula* *Corynespora*
6	Conidia	mostly curved not so	*Curvularia* 7
7	Conidia	obclavate, germinated from any cell broadly fusiform, germinated from end cell	*Helminthosporium* *Biporalis*

I Sympodulosporae

Key words conidia, filiform appendages, hilum, biconical, conidiophore(s), sporogeneous (fertile) aeria

1	Conidia	coiled not so	*Helicomyces* . 2
2	Conidia	1-celled over 2 celled	3 7
3	Conidia	biconical not so	*Beltrania* 4
4	Conidiophores	with significant zigzag fertile area not so	*Tritirachium* 5
5	Conidiophores	almost simple branched	*Ramichloridium* (= *Rhinocladiella*) 6
6	Conidia	apiculate at one end with a hilum	*Hansfordia* *Nodulisporium*
7	Conidia	over 2 celled over 3 celled	8 11
8	Conidiophores	simple branched, candelabrum-like	9 *Candelabrella*
9	Conidia	cylindrical, 2 or over 2 celled ovate, 2 celled with unequal size	10 *Arthrobotrys*
10	Conidia	2 celled, long and cylindrical over 2 celled	*Dactylaria* *Scolecobasidium* (= *Ochroconis*)
11	Conidia	pluriseptate, clavate, conidiophores, simple and short 4 celled filiform appendages at both ends	*Mycocentrospora* *Hyphodiscosia*

J Annelosporae and Others

Key words conidiophores, conidiogenous cells, annellation

1	Conidiophores	with annellated conidiogenous cells not so	*Scopulariopsis* Others to be inspected

K Sterile (Non-Spore-Forming) Fungi

Key words sclerotia, rind, medulla, papulaspore

1	Sclerotia	well developed, composed of rind and medulla not so	*Sclerotia* 2
2	Hyphae	constricted near branching junction not so	*Rhizoctonia* 3
3	Papulaspores Microsclerotia (and pycnidia)	formed formed	*Papulaspora* *Macrophomina*

5

Fungus List

MASTIGOMYCOTINA

1 *Aphanomyces cladogamus*
2 *Phytophthora capsici*
3 *Phytophthora cryptogea*
4 *Phytophthora erythroseptica*
5 *Phytophthora megasperma*
6 *Phytophthora melonis*
7 *Phytophthora nicotianae* var *parasitica* (homothallic)
8 *Phytophthora nicotianae* var *parasitica* (heterothallic)
9 *Plectospira myriandra*
10 *Pythiogeton ramosum*
11 *Pythium acanthicum*
12 *Pythium acanthophoron*
13 *Pythium afertile*
14 *Pythium angustatum*
15 *Pythium aphanidermatum*
16 *Pythium apleroticum*
17 *Pythium carolinianum*
18 *Pythium catenulatum*
19 *Pythium conidiophorum*
20 *Pythium deliense*
21 *Pythium dissimile*
22 *Pythium dissotocum*
23 *Pythium echinulatum*
24 *Pythium elongatum*
25 *Pythium graminicolum*
26 *Pythium inflatum*
27 *Pythium intermedium*
28 *Pythium irregulare*
29 *Pythium myriotylum*
30 *Pythium nayoroense*
31 *Pythium oedochilum*
32 *Pythium paroecandrum*
33 *Pythium periplocum*
34 *Pythium rostratum*
35 *Pythium spinosum*
36 *Pythium splendens*
37 *Pythium sulcatum*
38 *Pythium sylvaticum*
39 *Pythium torulosum*
40 *Pythium ultimum*
41 *Pythium vexans*
42 *Pythium zinjeberum*

ZYGOMYCOTINA

1 *Absidia repens*
2 *Circinella muscae*
3 *Cunninghamella echinulata*
4 *Cunninghamella elegans*
5 *Gongronella butleri*
6. *Helicocephalum oligosporum*
7 *Mortierella ambigua*
8 *Mortierella chlamydospora*
9 *Mortierella elongata*
10 *Mortierella exigua*
11 *Mortierella humilis*
12 *Mortierella hyalina*
13 *Mortierella isabellina*
14 *Mortierella minutissima*
15 *Mortierella sclerotiella*
16 *Mortierella verticillata*

ZYGOMYCOTINA (continued)

17 *Mortierella zychae*
18 *Mucor circinoides*
19 *Mucor hachijoensis*
20 *Mucor hiemalis* f *luteus*
21 *Mucor microsporus*
22 *Mucor plumbeus*
23 *Rhizopus oryzae*
24 *Saksenae vasiformis*
25 *Syncephalastrum racemosum*
26 *Umbelopsis vinacea*
27 *Zygorhinchus moelleri*

ASCOMYCOTINA

1 *Anixiella reticulata*
2 *Apiosordaria verruculosa* var *maritima*
3 *Chaetomium aureum*
4 *Chaetomium brasiliense*
5 *Chaetomium cochliodes*
6 *Chaetomium dolichotrichum*
7 *Chaetomium erectum*
8 *Chaetomium funicola*
9 *Chaetomium fusiforme*
10 *Chaetomium globosum*
11 *Chaetomium reflexum*
12 *Chaetomium spirale*
13 *Didymella effusa*
14 *Eudarluca biconica*
15 *Glomerella glycines*
16 *Massarina* sp (73-225)
17 *Massarina* sp (73-463)
18 *Microascus longirostris*
19 *Monosporascus cannonballus*
20 *Nectria asakawaensis*
21 *Nectria fragariae*
22 *Nectria gliocladioides*
23 *Nectria hachijoensis*
24 *Preussia terricola*
25 *Sordaria fimicola*
26 *Sordaria nodulifera*
27 *Sordaria tamaensis*
28 *Thielavia terricola*
29 *Zopfiella curvata*
30 *Zopfiella latipes*
31 *Zopfiella pilifera*

BASIDIOMYCOTINA

Coprinus sp , *Armillaria mellea* and six taxa

DEUTEROMYCOTINA

1 *Acremonium* sp
2 *Alternaria alternata*
3 *Apiocarpella* sp
4 *Arthrinium* st (Teleomorph *Apiospora montagnei*)
5 *Arthrobotrys oligospora*
6 *Aspergillus brevipes*
7 *Aspergillus fumigatus*
8 *Aspergillus niger*
9 *Aspergillus parasiticus*
10 *Aspergillus* sp , Sect *Clavati*
11 *Aspergillus* sp , Sect *Wentii*
12 *Aureobasidium pullulans*
13 *Basipetospora rubra* (Teleomorph *Monascus ruber*)
14 *Beltrania rhombica*
15 *Bipolaris australiensis*
16 *Bipolaris holmii*
17 *Bipolaris sacchari*
18 *Bispora betulina*

DEUTEROMYCOTINA (continued)

19 *Botryodiplodia* sp
20 *Botryotrichum piluliferum*
21 *Camposporium laundonii*
22 *Candelabrella musiformis*
23 *Candelabrella* sp
24 *Candida* sp
25 *Cephaliophora irregularis*
26 *Cephaliophora tropica*
27 *Chalara thielavioides*
28 *Chromelosporium fulvum* (Teleomorph *Peziza ostracoderma*)
29 *Chrysosporium keratinophilum*
30 *Cladorrhinum bulbillosum*
31 *Cladorrhinum samala*
32 *Cladosporium cladosporioides*
33 *Codinea parva*
34 *Codinea* st (Teleomorph *Chaetosphaeria talboti*)
35 *Colletotrichum coccodes*
36 *Colletotrichum falcatum* (Teleomorph *Glomerella tucumanensis*)
37 *Colletotrichum lindemuthianum* (Teleomorph *Glomerella lindemuthiana*)
38 *Colletotrichum truncatum*
39 *Coniothyrium fuckelii* (Teleomorph *Diapleella coniothyrium*)
40 *Corynespora cassiicola*
41 *Corynespora citricola*
42 *Curvularia affinis*
43 *Curvularia brachyspora*
44 *Curvularia clavata*
45 *Curvularia lunata* (Teleomorph *Cochliobolus lunatus*)
46 *Curvularia pallescens* (Teleomorph *Cochliobolus pallescens*)
47 *Curvularia prasadii*
48 *Curvularia protuberata*
49 *Curvularia senegalensis*
50 *Curvularia tuberculata* (Teleomorph *Cochliobolus tuberculatus*)
51 *Cylindrocarpon destructans* (Teleomorph *Nectria radicicola*)
52 *Cylindrocarpon obtusisporum*
53 *Cylindrocarpon olidum*
54 *Cylindrocladium camelliae*
55 *Cylindrocladium colhounii* (Teleomorph *Calonectria colhounii*)
56 *Cylindrocladium floridanum* (Teleomorph *Calonectria kyotoensis*)
57 *Cylindrocladium parvum*
58 *Cylindrocladium scoparium*
59 *Cylindrocladium tenue*
60 *Cytospora sacchari*
61 *Dactylaria candidula*
62 *Dematophora necatrix* (Teleomorph *Rosellinia necatrix*)
63 *Didymostilbe* sp
64 *Diplodia frumenti* (Teleomorph *Botryosphaeria festucae*)
65 *Epicoccum purpurescens*
66 *Fusarium moniliforme* (Teleomorph *Gibberella fujikuroi*)
67 *Fusarium oxysporum*
68 *Fusarium roseum*
69 *Fusarium solani* (Teleomorph *Nectria haematococca*)
70 *Geotrichum candidum*
71 *Gliocladium catenulatum*
72 *Gliocladium roseum* (Teleomorph *Nectria gliocladioides*)
73 *Gliocladium virens*
74 *Gliocladium viride*
75 *Gloeocercospora sorghi*
76 *Gonatobotrys* sp
77 *Gonytrichum chlamydosporum*
78 *Hainesia lythri* (Teleomorph *Pezizella lythri*)
79 *Hansfordia biophila*
80 *Helicomyces roseus*
81 *Helminthosporium solani*
82 *Humicola dimorphospora*
83 *Humicola fuscoatra*
84 *Humicola grisea*
85 *Humicola tainanensis*
86 *Hyalodendron* sp
87 *Hyphodiscosia radicicola*
88 *Macrophomina phaseolina*

DEUTEROMYCOTINA (continued)

89 *Mammaria* sp
90 *Metarhizium anisopliae*
91 *Microsphaeropsis* sp
92 *Monacrosporium bembicodes*
93 *Monacrosporium ellipsosporum*
94 *Monilia pruinosa*
95 *Mycocentrospora acerina*
96 *Myrioconium* sp
97 *Myrothecium striatisporum*
98 *Myrothecium verrucaria*
99 *Nigrospora oryzae* (Teleomorph *Khuskia oryzae*)
100 *Nigrospora sacchari*
101 *Nodulisporium* sp
102 *Oedocephalum nayoroense*
103 *Oidiodendron cerealis*
104 *Oidiodendron citrinum*
105 *Oidiodendron flavum*
106 *Paecilomyces farinosus* (Teleomorph *Cordyceps memorabilis*)
107 *Paecilomyces inflatus*
108 *Paecilomyces javanicus*
109 *Paecilomyces persicinus*
110 *Paecilomyces puntonii*
111 *Paecilomyces roseolus*
112 *Paecilomyces variabilis*
113 *Paecilomyces victoriae*
114 *Papulaspora irregularis*
115 *Papulaspora nishigaharanus*
116 *Papulaspora pallidula*
117 *Papulaspora pannosa*
118 *Papulaspora* sp
119 *Penicillium corylophilum*
120 *Penicillium janthinellum*
121 *Penicillium lanosum*
122 *Penicillium nigricans*
123 *Penicillium resticulosum*
124 *Periconia byssoides*
125 *Periconia macrospinosa*
126 *Periconia saraswatipurensis*
127 *Pestalotia* spp
128 *Phialomyces macrosporus*
129 *Phialophora atrovirens*
130 *Phialophora cinerescens*
131 *Phialophora cyclaminis*
132 *Phialophora fastigiata*
133 *Phialophora malorum*
134 *Phialophora radicicola*
135 *Phialophora richardsiae*
136 *Phialophora* sp (73-410)
137 *Phialophora* sp (74-750)
138 *Phoma glomerata*
139 *Phoma medicaginis* var *pinodella*
140 *Phoma* sp
141 *Phomopsis* spp
142 *Pithomyces chartarum*
143 *Pithomyces maydicus*
144 *Pyrenochaeta globosa*
145 *Pyrenochaeta terrestris*
146 *Pyrenochaeta* sp
147 *Ramichloridium anceps*
148 *Ramichloridium subulatum*
149 *Rhizoctonia solani*
150 *Rhizoctonia* spp
151 *Robillarda agrostidis*
152 *Sarcopodium araliae*
153 *Sclerotium* spp
154 *Scolecobasidium constrictum*
155 *Scolecobasidium humicola*
156 *Scopulariopsis asperula*
157 *Scopulariopsis brevicaulis*
158 *Scopulariopsis canadensis*
159 *Selenophoma obtusa*
160 *Sepedonium chrysospermum*
161 *Sepedonium* sp
162 *Septonema chaetospira*
163 *Spegazzinia tessarthra*
164 *Sporidesmium bakeri*
165 *Sporobolomyces* sp
166 *Sporotrichum aureum*
167 *Sporotrichum* sp
168 *Stachybotrys bisbyi*
169 *Stachybotrys elegans*
170 *Stagonospora subseriata*
171 *Staphylotrichum coccosporum*

DEUTEROMYCOTINA (continued)

172 *Stemphylium botryosum*
173 *Taeniolella phialosperma*
174 *Tetracladium setigerum*
175 *Tetraploa ellisii*
176 *Thielaviopsis adiposa* (Telemorph *Ceratocystis adiposa*)
177 *Thielaviopsis paradoxa*
178 *Thysanophora penicilloides*
179 *Torula herbarum*
180 *Torula* sp
181 *Torulomyces lagena*
182 *Trichocladium pyriformis*
183 *Trichoderma aureoviride*
184 *Trichoderma hamatum*
185 *Trichoderma harzianum*
186 *Trichoderma koningi*
187 *Trichoderma pseudokoningi*
188 *Trichothecium roseum*
189 *Trichurus spiralis*
190 *Trimacrium viridis*
191 *Triposperm um myrti*
192 *Tritirachium* sp
193 *Ulocladium botrytis*
194 *Ulocladium chartarum*
195 *Verticillium dahliae*
196 *Verticillium fungicola*
197 *Verticillium lecanii*
198 *Verticillium nubilum*
199 *Verticillium* sp
200 *Volutella ciliata*

than those of non-mycorrhizal ones.

Dehydrogenase activity was highest during summer and lowest during winter. The activity of dehydrogenase was more in root surface than in the rhizosphere soil in all the four seasons. Cu and Zn did not reduce the dehydrogenase activity significantly in *Boletus* sp. inoculated seedlings. Highest percentage of inhibition of dehydrogenase was observed in Cd, Pb and Ni treated rhizosphere soil. Among the four ectomycorrhizal fungi, *S. aurantium* inoculated roots showed the least seasonal variation but highest activity. Pb and Ni showed maximum inhibition in dehydrodenase activity rhizosphere soil and the root surface. In non-mycorrhizal rhizospheric soil, Zn and Ni caused upto 50% inhibition and Pb and Cu caused upto 44% inhibition on root surface dehydrogenase activity. It was observed that in absence of ectomycorrhizae, heavy metals drastically reduced the enzyme activity. Dehydrogenase activity was more in mixed mycobionts inoculated roots and the rhizosphere soil than in single inoculum treated root surface and rhizospheric soil. Highest percentage of inhibition in enzyme activity was observed in Al and Pb treated root surface and its rhizosphere soil.

The urease activity was apparently less at 100 ppm and above of Zn, Cu, Ni, Cd, Pb and Al treated pots. Soil urease activity was less as compared to the root surface. The highest activity of urease was observed in autumn and lowest during winter. Ni and Cd were found more toxic to urease activity than rest of the metals. Highest percentage of inhibition of urease activity was observed in *S. aurantium* treated seedlings.

The degree of inhibition of urease activity was more on non-mycorrhizal root surface and its rhizosphere soil than in mycorrhizal ones.

The urease activity in root surface and rhizosphere soil was higher in mixed mycobionts inoculated seedlings than the single fungus inoculated ones. In Zn and Cu treated seedlings, root surface and rhizosphere soil showed maximum and minimum inhibition of urease activity respectively.

In two seasons, phosphatase activity was discernable, one with a higher activity in autumn and summer and another with a markedly low activity in winter and spring.

Increased inhibition in phosphatase activity was observed at higher concentration of heavy metal treated seedlings. The highest percentage of inhibition of phosphatase activity was observed in *S.aurantium* inoculated soil and Pb treated root surface followed by Ni treated rhizosphere soil. Minimum inhibition was observed in *Boletus* sp. inoculated seedlings treated with Cd and Cu.

Higher activity and lower inhibition percentage of phosphatase activity were observed in seedlings inoculated with mixed mycobionts. Maximum inhibition was observed in Zn treated seedlings and minimum in Cu treated seedlings.

There was a positive correlation between mycorrhizal infection and the activity of urease, phosphatase and dehydrogenase ($P < 0.01$). A negative correlation was found between the metal concentration and the activity of urease, phosphatase and dehydrogenase. A significant variation between the sampling periods ($P < 0.05$) and between mycorrhizal and non-mycorrhizal ($P < 0.01$)

was also found.

Insignificant difference in the growth of seedlings between the four ectomycorrhizal fungi inoculated seedlings was observed. The non-mycorrhizal seedlings showed significantly lower growth as compared to ectomycorrhizal seedlings. However, inspite of heavy metal contaminations, *C. graniforme*, *Boletus* sp. *S. luteus* and *S. aurantium* did significantly ($P < 0.01$ and $P < 0.05$) improve growth seedlings than non-mycorrhizal ones. There was a positive correlation between mycorrhizal infection and the growth of pine seedlings.

Inoculation of pine seedlings with ectomycorrhizal fungi consistently stimulated an increase in shoot height, number of needles, needle length, seedlings volume, root colar diameter, lateral root length and mycorrhizal infection throughout the growing seasons as compared to non-mycorrhizal ones. The growth of both mycorrhizal and non-mycorrhizal seedlings without any heavy metal treatment was vigorous as compared with that of plants exposed to heavy metals.

Shoot height was improved in *S. luteus* inoculated seedlings with an average of 7 cm followed by *Boletus* sp. and *C. graniforme* treated seedlings as compared to 4.5 cm in non-mycorrhizal seedlings. Minimum shoot height was observed in Pb and Al treated seedlings.

Root length was reduced by 100 ppm and more concentrations of all the heavy metals treated seedlings. *C. graniforme* infected seedlings treated with Cu showed maximum root length. Minimum root length was observed in Zn treated non-mycorrhizal seedlings.

Seedling volume was maximum in Zn and Cu treated and

S. luteus infected seedlings. Number of needles was more in ectomycorrhizal seedlings compared as to non-mycorrhizal ones. *C. graniforme* inoculated seedlings showed more number of needles and minimum in *S. aurantium* inoculated seedlings.

Length of needle did not vary significantly in different treatments of heavy metals in ectomycorrhizal and non-mycorrhizal seedlings.

Mycorrhizal infection was maximum in *S. luteus* inoculated and treated with Pb seedlings and minimum was noticed with *C. graniforme* inoculated and treated with Al.

C. graniforme inoculated seedlings showed highest percentage of survival followed by *Boletus* sp. and *S. aurantium* inoculated seedlings.

Seedlings survival and the growth were higher in mixed mycobionts inoculated seedlings as compared to single fungus inoculum seedlings. The percentage of inhibition of mycorrhizal infection also increased with increase in the metal concentration. Shoot height, needle length, number of needles, seedlings volume and seedlings survival were higher in mycorrhizal treated seedlings than non-mycorrhizal ones.

A trend in decrease in nutrient concentration of seedlings applied with different concentration of heavy metals was observed. The concentrations of nutrients as a whole were restricted by the presence in heavy metals.

More nitrogen contents was found in pine seedlings infected with *C. graniforme*, *S. luteus*, *S. aurantium* and *Boletus* sp. treated with Cd, Cu, Ni, Zn, Pb and Al compared to non-mycorrhizal ones. The concentration of nitrogen decreased with the increase in heavy metals.

Seedlings infected with mixed mycobionts exhibited maximum uptake of nitrogen. Pb and Ni treated seedlings showed less nitrogen content as compared to Zn, Cu, Al and Cd treated seedlings.

P-contents in pine seedlings was also decreased with increasing concentration of heavy metals. P-contents was noticed maximum in *Boletus* sp. inoculated seedlings, while non-mycorrhizal seedlings showed lowest p-contents. Seedlings infected with mixed mycobionts showed more P-contents than single inoculum inoculated seedlings.

K-contents in pine seedlings was also low at high concentration of heavy metals. Ectomycorrhizal seedlings had more K-contents than non-mycorrhizal ones.

Insignificant variation in the contents of N, P and K between the various mycobionts was observed . However, a significant variation was found in the contents of nitrogen ($P < 0.01$), potassium and phosphorus ($P < 0.05$) between mycorrhizal and non-mycorrhizal ones and also between pots and field condition ($P < 0.01$).

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