RECLAMATION AND REHABILITATION
OF
IRON ORE MINE WASTES

Thesis submitted to the Goa University
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of
Doctor of Philosophy
in
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By

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Dedicated to my
Untiring Beloved Mother CLEMENTINA,
Father MICHAEL
& family

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CERTIFICATE

As required under R. No. 0.19.8 (vi) of the **Goa University**, I certify that the thesis entitled **" Reclamation and Rehabilitation of Iron Ore Mine Wastes "** submitted by **Mr. Francis Lourdes Coelho** for the award of **Doctor of Philosophy** is a record of research work done by the candidate during the period of study under my guidance.

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STATEMENT

As required under R. No. 0.19.8 (ii), I state that the research work entitled " **Reclamation and Rehabilitation of Iron Ore Mine Wastes** " is my original contribution and the same has not been submitted for any degree of this or any other University on any previous occasion.

To the best of my knowledge, the present study is the first of this kind.

The research work comprising this thesis is my original contribution.

1 - Analysis of iron ore mine wastes indicate that it has poor soil structure and texture and soil genesis. It is deficient in macro-nutrients and has high concentration of iron, alumina and manganese which inhibits the plant growth.

2 - Establishment of the plants on the iron ore mine wastes can be successfully achieved by inoculation of seeds with micro-organisms, such as **Azotobacter, Rhizobium** and VA Mycorrhiza.

3 - Introduction of earthworm " **Pheretima orientalis** " can bring about changes in physical, chemical and biological properties of iron ore mine waste and can improve plant growth

4 - **Ipomoea pes-caprae** can help in stabilization of dumps and taillings.

5 - Native plant species showed higher survival percentage and good growth response over the exotic species. Ameliorating the iron ore mine wastes with sea-weed fertilizer has an advantage over the commercially available fertilizers.

The outcome of this research can contribute to our knowledge considerably in understanding the responses of plants grown in iron ore mine wastes, having higher concentration of iron, alumina and manganese and deficient in essential plant nutrients.

It would definitely aid in rapid reclamation and rehabilitation of iron ore mined land efficiently and economically, and help to blend the disturbed land area into the surrounding environment.

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INTRODUCTION

Much of the world's wealth is derived from mining activities. Land surfaces are inevitably disturbed in seeking to win ores from the earth. During the last century and half, industrialization has necessitated the winning of large quantities of materials from the earth. The economic grade of ore has declined as all rich easily worked ores have been exhausted. Mechanization and improved technology have brought increasingly large tracts of land into states of disturbance.

The environmental degradation due to large scale exploitation of mineral resources without adequately integrating the environmental concerns with exploitation was noticed by the advanced countries decades ago. The growing public awareness about environmental concerns, subsequently compelled several developed countries to launch extensive programmes for reclamation/rehabilitation of mined areas and for control of environmental pollution caused by whole gamut of mining operations. It is only in comparatively recent times that economic effort has been put into rehabilitation of mined lands. However, this problem has not gained much attention in our country.

Environmental problem of immense concern associated with the mining activity is the degradation of ecosystem and creation of unsighty landscape devoid of vegetation, especiallly due to open cast mining and heaping, pilling of rejects and mine spoil. Further contamination of water course and streams, ground water and adjoining lands due to toxic metals leached from soil aggravates the environmental pollution problems. Landscape created due to open cast mining and dumping of soil do not have a suitable surface of productive soil to provide bedding for anchorage and support the plants. Further lack of useful plant nutrients in newly developed landscape do not favour the proliferation of vegetative canopy. In othe cases even though the surface is porus, the presence of toxic heavy metals at high concentration makes the survival of vegetation more difficult due to severe phytotoxicity (Smith and Bradshaw, 1972).

Widespread creation of such landscape due to extensive open cast mining may not only affect the agro forest productivity but also the ecosystem and ecological balance, if appropriate measures for reclamation and rehabilitation of mined land and mine spoil are not taken seriously in time.

Reclamation of mine wastes has been attempted using physical, chemical, and vegetative methods with varying degrees of success (Dean **et al.** 1973; Brown **et al.** 1976; Shetron **et al.** 1977). Physical and chemical methods of stabilization are only effective in the short term and equally important in many eyes, do nothing to improve the visual appearance of the landscape. Vegetative stabilization is normally the most desirable technique due to long term impact, low maintenance costs and aesthetic appeal.

IRON ORE MINING INDUSTRY IN GOA AND IT'S IMPACT ON ENVIRONMENT

Goa the land of scenic beauty with an area of 3600 sq. km. lies on the west coast of India, in the cradle of high ranges of Western Ghats, between 15° 48'00"N and 14° 53'54" N Latitude and 74° 20'13" and 73° 40'33" East Longitude. Sandwiched between the states Maharashtra to the North and Karnataka to the East and South, Whereas to the West, the State is bounded by the blue waters of the Arabian Sea, has rich deposits of iron ore extending from South East to North West of the Territory.

Mining of iron ore in a small way has been reported to be going on in Goa since the year 1910. But the significant mining can be traced back only for the year 1947.

Goa had been a prime exporter of iron ore since 1950, as much as 300 million tonnes of iron ore has been exported during all these years. Present production of iron ore is of the order of 15 mt/yr which contributes 40% to the total iron ore production in the country and 50 % of it's export. The estimated reserves of iron ore as on today is around 400 mt and is expected to last for another 25 30 years, at the present rate of mining.

The open cast mining for this rich iron ore deposit have caused major disturbance to the landscapes. And the wastes produced by mining are a serious threat to our environment. Yet, mining is an important dominant industry and forms the backbone of Goa's economy, while fishing an agriculture come only next.

The excavation for iron ore exposes large volumes of earth's crust to the atmosphere that intrude upon the landscape, the mining operation is such that, two classes of waste are produced, (1) piles of surface overburden waste rock and lean ore, which constitute the reject dumps, and (2) a fine grained waste resulting from the ore beneficiation process and deposited in large man made basins called tailing ponds.

Indiscriminate mining since 1961 has destroyed 50,000 ha of forests in Goa(Times of India, 13.2.1984), and it has been estimated that, during all these years as much as 900 1000 mt of waste rock, low grade ores and tailings have been accumulated near mining areas. The waste materials consist mainly of laterites, phyllites, quartzites, manganiferous and various other types of clays, slimes etc.

With present annual production of 15 mt of iron ore, it is expected that 40 50 mt of wastes has to be stored per year, and approximately 150 m cubic meters of water is to be discharged from pits to the drainage system. Mining waste dumps are biggest manmade hillocks, volume and height of such dumps is increasing every year. Most of the waste dumps rises upto 50 60 m high with 50 55° angle of repose. This being unconsolidated are prone to slumps and slides due to heavy monsoon rains.

Damage to the environment by the mining activity has been caused largely by reject dumps, pumping out of muddy waters from the working pits including those where the mine working have gone below the water, and slimes from the beneficiation plant. The damage is more evidenced in monsoon, when the rain water carries the washed out material from the mine waste dumps to the adjoining agricultural fields and water streams. The slimes and silts, which enter the agricultural field are of such character, they get hardened on drying, thus making aeration and root penetration difficult. Indiscriminate dumping of rejects have killed the fertility of over 10,000 ha of agricultural land (Times of India, 13-2-1984). The washed out material from the dumps and the flow of slimes from the beneficiation plants besides polluting the springs and wells, also cause silting of water ways specially during monsoon. Such silting of waterways over the years caused even flooding of adjacent fields and inhabited areas during monsoon. During dry seasons dust from blasting and transportation vehicles is the major cause of air pollution around the neighbouring villages of mining areas, sometimes reaching miles away with increasing wind velocities. Diseases such as silicosis, tuberculosis and allergic disease such as asthama are frequently common in inhabitants of the area and mine workers (a personal communication).

THE PRESENT PROBLEM

The annual production of iron ore in Goa is around 15mt. A tonne of ore mined for instance, produces 2-3 tons of waste, which means accumulation of 30-45mt of waste/yr, as per the present rate of mining. Since the operation of Pale mines in 1954, the surface overburden, waste rock and lean ore has been piled into nine dumps spread over 30 ha and three tailing basins occupy an area of 10 ha of land around the mines.

The disturbance of land surfaces alters the potential for vegetative growth, such that it is not possible for the pre-existing plant community to be exactly recreated. **Should mankind wish to preserve examples of naturally occuring vegetation, then mining is one activity which should not intrude into designed areas.** Mining which disturbs large tracts of land (as in the present case) is of much greater significance and the areas of land involved are such that **the predominant end point**

must be a biological one. To be more precise, a true revegetation must be in terms of species, number and organisation complexity. One American expert's maxim was " **If you can't put it back like it was before you got it out, then don't do it.** "

Natural ecological processes are often slow, reflecting the extreme edaphic conditions which mining creates.

In the present work, one of the rejected dump and abandoned tailing pond at Pale-Goa, of Chowgule & Co. Ltd., was undertaken for reclamation and rehabilitation.

The notion of **"Reclamation and Rehabilitation"** require careful consideration. **Reclamation** in the present context is used to describe a return to use from a state of waste. It implies that the site will be suitable for those species originally present in similar composition and density after treatments. Thus the original native species should be used in post mining, but it is acceptable if the site is rendered suitable for organisms that closely resemble the original species. Box (1979) suggests that **rehabilitation** means the disturbed sites will be returned to a form and productivity where a stable condition is established, consistent with the adjoining aesthetic values. Rehabilitation allows alternative land uses to the pre-existing one. Attempts were therefore made in this direction to achieve these goals.

The major objective here was to encourage the establishment of a plant community that would evolve into an ecologically stable entity comparable with the surrounding natural systems. If plant species could be utilized that would benefit the surrounding landowner on a subsistence or cottage industry basis then it would be a bonus.

The present research was designed and concentrated to develop a rapid low maintenance cost reclamation and rehabilitation of iron ore mine wastes in Goa, that would be an eye opener for rest of the mine owners in the country.

A review of the available recorded literature suggest that there is no substantial work done on **"Reclamation and Rehabilitation of Iron Ore Mine Wastes."** Therefore, it was felt worthwhile to undertake the present study.

The thesis deals with (1) A brief description of study area, (2) Micro-organisms and the reclamation of iron ore mine waste, which comprises of (a) Studies on **Azotobacter** species in iron ore mine waste and (b) Establishment and selection of successful vesicular arbuscular mycorrhizal fungi for **Leucaena leucocephala** in iron ore tailings. (3) Impact of earthworm introduction on iron ore mine waste and plant growth. (4) The potential of **Ipomoea pes-caprae** for iron ore mine waste stabilization. (5) Establishing vegetation on iron ore mine wastes.

The available literature pertinent to the present subject has been reviewed under the title **" Previous Work "** at the beginning of the thesis.

PREVIOUS WORK

Many industrial and mining operations give rise to concentrations of waste with unique properties. The need to reclaim these waste speedily and efficiently with a reasonable expenditure of time and money has been demonstrated by Shetron and Duffek (1970); Jones (1972); Prather (1973). Reclamation has been attempted using physical, chemical and vegetative methods with varying degrees of sucess (Dean **et al.** 1973; Brown **et al.** 1976; Shetron **et al.**1977). Of these methods, vegetative stabilization is normally the most desirable technique due long term impact, low maintenance costs and rapid, and results in the most complete surface protection, against wind and water. In addition plant cover improves the aesthetic appearance of the waste area, which would otherwise remain barren.

Published results of research over the past two decades have defined problems associated with the growth of plants on wastes. Some of the problems associated with vegetation stabilization arise from lack fertility of these wastes , physical properties, diversity of mineralogy, environmental setting and beneficiation process for the extraction of the metal (Dean **et al.** 1969; Peters, 1970; Shetron and Duffek , 1970; Nielson and Paterson, 1972; Day and Ludeke, 1973) and the climate. Although the general problems may be similiar, solutions for one area may not be applicable to another. Each situation is unique.

Characteristics of Mine Wastes

Physical Prperties

The texture of soil influnces most of the physical and chemical properties. The three major wastes from metal mining are overburden, waste rocks and tailings. Generally all these materials present physical problems for plant growth but not sufficient to prevent vegetation establishment.

Shetron **et al.**(1977) carried out the physical analysis of iron tailings. Based on the particle size analysis, they classified tailings into three types-coares, stratified and slimes. They found that sand tailings retain less water than clay or stratified tailings,are structureless and have high (sand) to very low (clay) infiltration rates. James (1966) found with slime dams on the witwatersrand that permeability was extremely low. Murray (1977) reported high bulk density values in compacted layer of mine tailings. Shetron **et al.** (1977) reported high bulk density in case of iron ore tailings.

Wild and Wiltshire (1971) and MacLean and Dekker (1976) studied the pH of different mine waste and reported a large variation in acidity among different sites ranging from pH 1.5 to above 10. Wong **et al.**(1983) reported that the iron tailings were alkaline.

A review of the recorded literature reveals that no much work has been done to understand the physical properties of mine waste.

Chemical Properties

The chemical composition of waste is highly variable even within a particular mining operation, not only depend upon the nature of the original ore but also on the metals extracted, the method of treatment and disposal, climatic conditions and weathering reactions that follow disposal.

Nutrient deficiencies are widely reported as a major limitation, particularly in terms of a low, or complete lack of organic matter and nitrogen in mining wastes (Mitchell, 1959; James, 1966). Cope (1962) reported the deficiency of phosphorous as a common feature of mine wastes. Smith and Bradshaw (1970) stated that potassium and micronutrient deficiencies are frequently encountered in mine wastes.

Shetron and Caroll (1977) working on copper and iron mine wastes reported that tailings had high available calcium and magnesium and lowest phosphorous. Nitrogen was non-existent in all mine wastes. Available phosphorous and potassium were generally low.

Wong **et al.** (1983) carried out the chemical analysis of iron ore tailings and showed that the tailings were alkaline,lacking in organic matter and nitrogen but rich in metals such as sodium calcium, magnesium, manganese, iron, zinc and copper.

Shetron (1983) showed that, in iron ore tailings the organic matter and nitrogen are essentially non-existent, phosphorous levels are low, potassium, calcium, magnesium and metal range in availability, have alkaline pH and low cation exchange capacity.

Role of Micro-organisms in Reclamation of Mine Wastes.

Only a limited number of studies have been conducted on the biological properties of mine wastes (Jurgensen,1979), estimates of micro-organisms present in unvegetated mine wastes indicated values much lower than normally found in undisturbed or agricultural soils.

Shetron **et al.**(1977) estimated micro-orgnisms in the rhizosphere of alfalfa, grass (**Fescue** sp.) and willow in iron tailings. They found that the total rhizosphere population was much higher than the tailings.

Alexander (1965) and Clark (1967) reported that the primary environmental variables which would directly influence microbial development in mine waste include moisture,aeration, temperature, organic matter, acidity and inorganic nutrient supply.

Studies on natural and artificial colonization of sand waste from kaolin mining in the U. K. and on waste materials elsewhere revealed that nitrogen accumulation and build up of a nitrogen cycle is the most important factor in soil and vegetation development (Dancer **et al.** 1977; Marrs **et al.** 1981; and Roberts **et al.**1981).

Abd-el-Malek (1971) studied the free living nitrogen fixing bacteria in Egyptian soil and thier possible contribution to soil fertility. Studies on the occurrence of **Azotobacter** in some soil types in India have been done by Rangaswami and Sadasivam (1964).

Population of free living N-fixing bacteria in mine wastes are very low until plants become well established (Wilson,1965; Krol **et al.**1972; Cresswell, 1973; Muller, 1973). Smith **et al.**(1971) calculated that average N gains of 20 to 35 kgs/ha/yr. had to occur on 85 to 100 year old iron mine wastes to account for the N levels.

Increase and improvement of crop yield using **Azotobacter** have been reported by several workers, Rovira (1963) in maize, tomato amd wheat; Brown **et al.**(1964) in wheat and barley; Mehrotra and Lehri (1971) in wheat, paddy, brinjal, cabbage and tomato. Jackson **et al.** (1964) reported similar effects on tomato plants of **Azotobacter** inoculation and application of Gibberellins.

Mishustin and Shilnikova (1964 & 1971); Brown (1972) and Lakshmi Kumari **et al.** (1975) reported the ability of **Azotobacter** to produce growth substances and antifungal antibiotics.

Mikola (1973) demonstrated that in areas where soil lack the appropriate mycorrhizal fungi, afforestation without inoculation is generally unsuccessful.

Schramm (1966) has found that mycorrhizal fungi are an important component in establishing vegetation on anthrocite coal refuse. He reported that where mycorrhizae where absent plants showed negligible growth, chlorotic or subnormal colour and often complete necrosis.

Daft and Nicolson (1974) and Daft **et al.** (1975) found that all the coal wastes they examined in Pennsylvania and Scotland had appreciable endomycorrhizal infection on the colonizing plants.

Nicholas and Hutnik (1971) and Medve **et al.**(1977) showed that inoculation of coal spoils with certain mycorrhizal fungi, macerated roots or forest soil greatly increased tree survival and /or growth.

Harris and Jurgensen (1977) reported that plantings of willow (**Salix** spp.) and hybrid poplar (**Populus** spp.) in iron tailings gave extensive mycorrhizal development and vigorous tree growth.

Nicholas and Hutnik (1971) successfully innoculated both **Betula pendula** and **Pinus resinosa** with **Pisolithus tinotorius** and **Cenococcum graniforme** in coal spoils.

The selection of endomycorrhizae for the use with a particular plant species on certain sites has been receiving increased attention (Mosse, 1975; Marx, 1977).

Impact of Earthworm on Physical and Chemical Properties of Mine Waste.

The role of earthworms in soil has been studied for atleast a hundred years. Several workers, Wollney (1890) in Germany; Russell (1910) and Salisbury (1923) in England; Puh (1941) in China; Lunt and Jacobson (1944), Hopp and Salter (1948) in the U.S.A.; Barley and Jennings (1959) in Australia; Van Rhee (1965) in Netherland; Syers **et al.** (1978) in New Zealand; De Vleeschauwar and Lal (1981), Lal and Akinremi (1983) in Nigeria, have studied this problem of the role of earthworms in soil fertility and plant growth.

Nijhawan and Kanwar (1952) carried out the mechanical analysis of casts excreted by **Pheretima** sp. and **Euthyphoeus waltoni.** Mechanical analysis of casts were also carried out by Joshi and Kelkar (1952). De Vleeschauwer and Lal (1981) studied the properties of worm casts under secondary tropical forest regrowth with dominant earthworm spp. being **Hyperiodrilus, Dichogaster** and **Millsonia.**

The chemical composition of earthworm casting have been reported by many investigators like Wollney (1890); Puh (1941); Lunt and Jacobson (1944); Shrikhande and Pathak (1948).

Many studies, most from the temperate regions have indicated the importance of worm activity to soil productivity (Rusell, 1910 ; Hopp and Hopkins, 1946; Barley, 1961). Crossley and Hoglund (1962) and Edwards and Heath (1963) have shown that the rate of mineralization of crop residue is significantly influenced by the activity of earthworms.

Lunt and Jacobson (1944), Nijhawan and Kanwar (1952), Nye (1955) and Cook **et al.** (1979) have reported that the plant nutrients are generally more concentrated in casts than in their parent soils. Lal and De Vleeschauwer (1982) have shown that worm casts contain significantly most organic matter and other plant nutrient elements than does the uningested surface soil.

Puh (1941), Ponomareva (1962), Gupta and Sakal (1967) have shown that earthworm casts are enriched in nitrogen relative to the surface horizon. Studies of Ponomareva (1962), Sharpley and Syers (1976 and 1977) have indicated that surface casts contained greater amounts of phosphorous than underlying soil.

Stockli (1928) carried out bacteriological analysis of worm casts. He showed that virtually all species of bacteria and physiological groups sought were present in greater numbers in earthwrom castings than in soil. Dawson (1947) reported increase in bacterial count in worm casts. Barley and Jennings (1959) reported that earthworms in culture experiement promoted other decomposers.

Nijhawan and Kanwar (1952) studied the effect of earthworm castings (**Pheretima** sp. and **Euthyphoeus waltoni**) on the productivity of soil and reported an increase in yield of wheat. Joshi and Kelkar (1952) studied the beneficial effect of earthworms on crop yield and showed that the presence of earthworm increased the yield of jowar and wheat.

Ponomareva (1962) reported 400 percent increase in dry matter production from the pots with earthworm casts than on the controls. Nielson (1965) demonstrated an increase in production associated with worm activity in both turf and pot trials. Van Rhee (1965) working with **Lumbricids** showed that the dry matter production in grass, wheat and clover was on an average 287, 111 and 877 percent higher than in controls.

In India, investigators on this problem, however seems to have been carried out by a few investigators (Shrikhande and Pathak, 1948; Joshi and Kelkar, 1952; Nijhawan and Kanwar, 1952). It has been noted that the investigation for utilizing earthworms on a large scale for increasing crop yields is rather lacking and the question of utilization of earthworms does not appear to have received the attention it deserves in the country.

Vimmerstedt and Finney (1973) demonstrated the feasibility of introducing earthworms into revegetated acid coal spoils to increase the rate of incorporation of organic matter.

Introduction of earthworms and their development on wastes could be an important element of successful rehabilitation effort.

The Potential of Ipomoea pes-caprae for Iron Ore Waste Stabilization.

Extensive research into the problem of derelict land reclamation has now provided techniques to facilitate the successful establishment of vegetation on most spoils. However, little attention has been paid so far to the morphology, physiology and bio-chemical responses of planted species.

Ipomoea pes-caprae have invaded substantial area of iron ore mine waste at Pale-Mines (Goa) containing high levels of Fe,Mn and Al. There is no record of any work on this particular plant species.

Effects of heavy metals on plants has been studied extensively, toxic levels of some elements are common in metal mining wastes (James and Mrost,1965). Excesses or deficiencies of metals ions have effect on plant growth and morphology (Goodall and Gregory, 1966; Sauchelli, 1969; Epstein, 1972; Hewitt and Smith, 1975).

Studies on the effect of single toxic metal in plants or comparison of the toxicity of two metals have been frequently reported by various workers, Wu and Antonovics (1975) reported the effect of zinc and copper on **Agrostis stolonifera,** Agarwala **et al.**(1965) reported the effect of iron on growth of maize and radish. Investigation comparing the toxicity of a number of metals include copper, nickel, cobalt, zinc, chromium and manganese in reducing fresh weight in mustard (**Sinapis alba**) (Dekok,1956). Wong and Bradshaw (1982) studied the comparative effect of aluminium, cadmium, chromium, copper, iron mercury, manganese, nickel, lead and zinc on radicle of rye grass. Toxicities due to high levels of aluminium and manganese related to acidity are reported (Lowry, 1961).

Bradshaw and Chadwick (1980) reported that heavy metals cause virtual cessation of root growth and formation of short stumpy laterals and ultimately, although not immediately the death of plants.

Nag **et al.** (1981) have studied the heavy metals effects on plants tissues involving chlorophyll and proteins. Metals taken up by plants are incorporated into a tissue depending on its mobility within the plants, i.e. translocation (Ernst,1980). Surplus of heavy metals can severely reduce growth and biomass production of plants. The expression of a metabolic disorder may be the results of interaction of iron uptake and or transport (Ernst,1972).

Establishing Vegetation on Iron Mine Wastes.

Bradshaw (1970) has found that populations of species growing on toxic soils are able to continue rooting in conditions which are so toxic that ordinary species cannot produce roots at all. He claimed that these tolerant plants were unaffected by the toxicities and provided a rapid solution to derelict land problems. Being adapted to high mineral concentration and the climatic environment of the area, these plants would seem ideal pioneer species for establishment on mine wastes.

In selecting species for mined land reclamation in Goa, many local species which are already adapted to the climatic regimes and topography of the area are often overlooked in preference for the more sophisticated exotic species such as the Australian acacia (**Acacia auriculiformis**).

The majority of the reclamation schemes have been designed for the ultimate establishment of grasses and clovers and much research has been undertaken in this aspects. Sheldon (1974) stated that the use of trees in reclamation has however been neglected. Because of the appreciation that special problems are involved in the planting, the establishment and subsequent maintenance programmes of trees planted in degraded substrates, only small scale planting are undertaken. Much of this is of an experimental nature, but in the past little has been carried out on a scientific basis. Pasture establishment seemed to have been less successful and dependent upon factors such as the nature, composition and particularly the fertility status of the material.

James (1966) stressed the importance of bringing into being a plant community that would be self-perpetuating without further attention or artificial aid. LeRoy and Keller (1972) noted permanent and maintenance free vegetation is essential in reclamation. However, no efforts have been made to search for plants that would perform well and function normally under nutrient deficient soil conditions as existing in the iron ore mine wastes.

Most reports of successful revegetation indicates fertilizaton as an important establishment procedure. Mitchell (1959) stated that restoration of soil fertility on mined areas is an extremely slow process. Recolonization by plants is extremely slow. Therefore the establishment of vegetation with heavy fertilizer application speeds up the slow natural process, restoring fertility and natural cycles which were destroyed by the mining process.

Ludeke **et al.**(1974) reported that addition of organic matter, commercial fertilizer and supplemental irrigation water increase vegetation establishment on disturb sites. Bennet (1977) have shown that many plants can be grown on area disturbed by mining if environmental and nutritional requirements are met. Luellen (1977) reported that mine wastes should be conditioned with organic matter to improve soil structure and prevent surface crusting. Ludeke **et al.**(1974) and Aldon (1978) found that barely

(**Hordeum Vulgare**,L.) straw incorporated into disturbed soil materials was an effective source of organic matter to obtain satisfactory plant growth. Gommel (1975) noted that the establishment of grasses on iron smelting wastes was enhanced by the addition of nitrogen and phosphorous, however phosphorous was the most limiting nutrient.

However the sea-weeds as organic amendments and rich source of micro-nutrient and plant growth factors remains to be unexploited in mined land reclamation programmes.

Shetron and Duffek (1970) evaluated the potential of iron tailings as a medium for plant growth. Shetron and Carroll (1977) studied the performance of trees and shrubs on iron ore tailings. Their selection was based on performance of trees and shrubs on similar sites, local species growing on similar soil material and the ease of obtaining planting stock.

The work reported in these thesis was designed (1) to collect information and study plant responses and find out solution to alleviate the problems, that would be faced by the recreated plant community in confirmity with the surrounding natural systems, (2) to assess the costs and benefits of rapid low costs reclamation and rehabilitation of iron ore mine wastes in Goa, that could form the basis for future planning of reclamation schemes for mined land in the country.

CHAPTER ONE

DESCRIPTION OF STUDY AREA AND CHARACTERISTICS OF IRON ORE MINE WASTES

1.1. LOCATION AND GEOLOGY

The Pale-Iron Ore Mines of Chowgule and Co. Ltd., are situated 17 km. North of Ponda town at Eastings 04-07 and Northings 35-08 in Bicholim-Satari taluka in the Western region of North Goa District. The mines are accessible by the Margao-Ponda-Mapusca road and are about 2 km. from Ambegale-Pale Village (Fig.1.1).

The mine was put into operation in 1954, and now comprises of two leases, Pale Dongor and Kuntichem Tollem, spread over a lease area of approximately 152.63 ha. The current area under active mining for an annual iron ore production of 1.5 million tonnes is around 100 ha.

This exposes and accumulates 3-5 million tonnes of reject and overburden, which is piled into nine dumps and three tailing basins which occupies around 27 percent of the total lease area. The height of the dumps range from 50-60 meters with 50-55° angle of repose.

The deposits are precambrian in age, equivalent to Chitradurga Schist belt of Karnataka. The rocks appear to be Banded Hematite Quartzite (BHQ) which were leached by the meteoric waters and consequently SiO_2 and Al_2O_3 in the original rock was washed away leaving the residue rich in Iron.

The formation have been subjected to laterization during recent to sub-recent times, resulting in a cover of laterite of varying thickness, because of the typical tropical climate.

Major part of the area is covered with sandy and reddish brown laterite soil. Texturally, the soil varies from sandy to silty clay and loam. The soils are essentially of acidic type (with pH value 6-6.8) and low to medium in organic matter (0.3-1.9 percent)and have a high water holding capacity (21-44 percent) with the salt content of less than 0.2 percent.

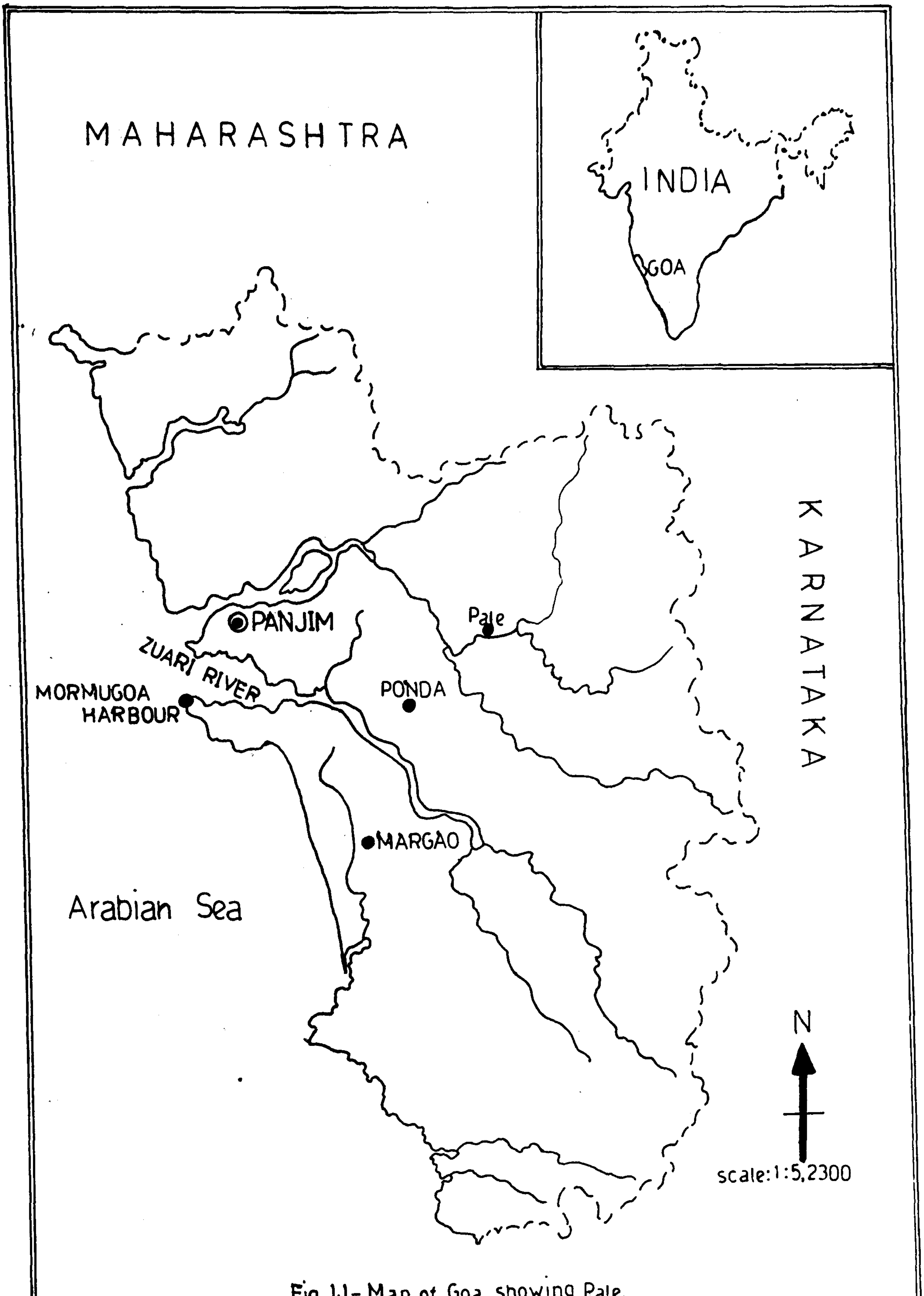


Fig. 1.1- Map of Goa showing Pale.

1.2. ENVIRONMENT

1.2.1. Altitude and Temperature

The elevation of the mines vary from 170 to 210 meters above mean sea level. The climate is tropical/monsoonal with hot wet summers and dry mild winters.

Climatological data for the three years from 1987 through 1989 indicates (Fig.1.2a, b & c) that the mean minimum temperature in winter is 20.2°C (January,1987), while it reaches an average maximum of 34.6°C (May, 1988).

During the hot season temperature rises slowly from March and later part of April, and May forms the hottest period, with onset of monsoons temperature drops considerably by 3-5°C. Day temperatures during monsoons are lower than those in the cold seasons. In the post monsoon months of October and November, day temperatures increase gradually and days in winter are comparatively very hot. Night temperatures are lowest in January.

1.2.2. Rainfall

The average annual rainfall over the three years (Fig.1.3a, b & c) at Pale ranges from 2374.9 mm (1987) to 4119.5 mm (1989), which is markedly seasonal. The area receives about 85 percent of the rainfall from the South-West monsoon between June and September. The monsoon usually set in around the last week of May and in early November, this gives an appreciable cooling to the hot summer teperatures. The rainiest month is June-July, when nearly a third of the annual rainfall is recieved accompanied by light to heavy gusty winds reaching 20-60 km/hr.

1.2.3. Humidity

During June to October the humidity rises to 90-95 percent, while for rest of the year it ranges between 74 - 89 percent (Fig.1.4a, b & c).

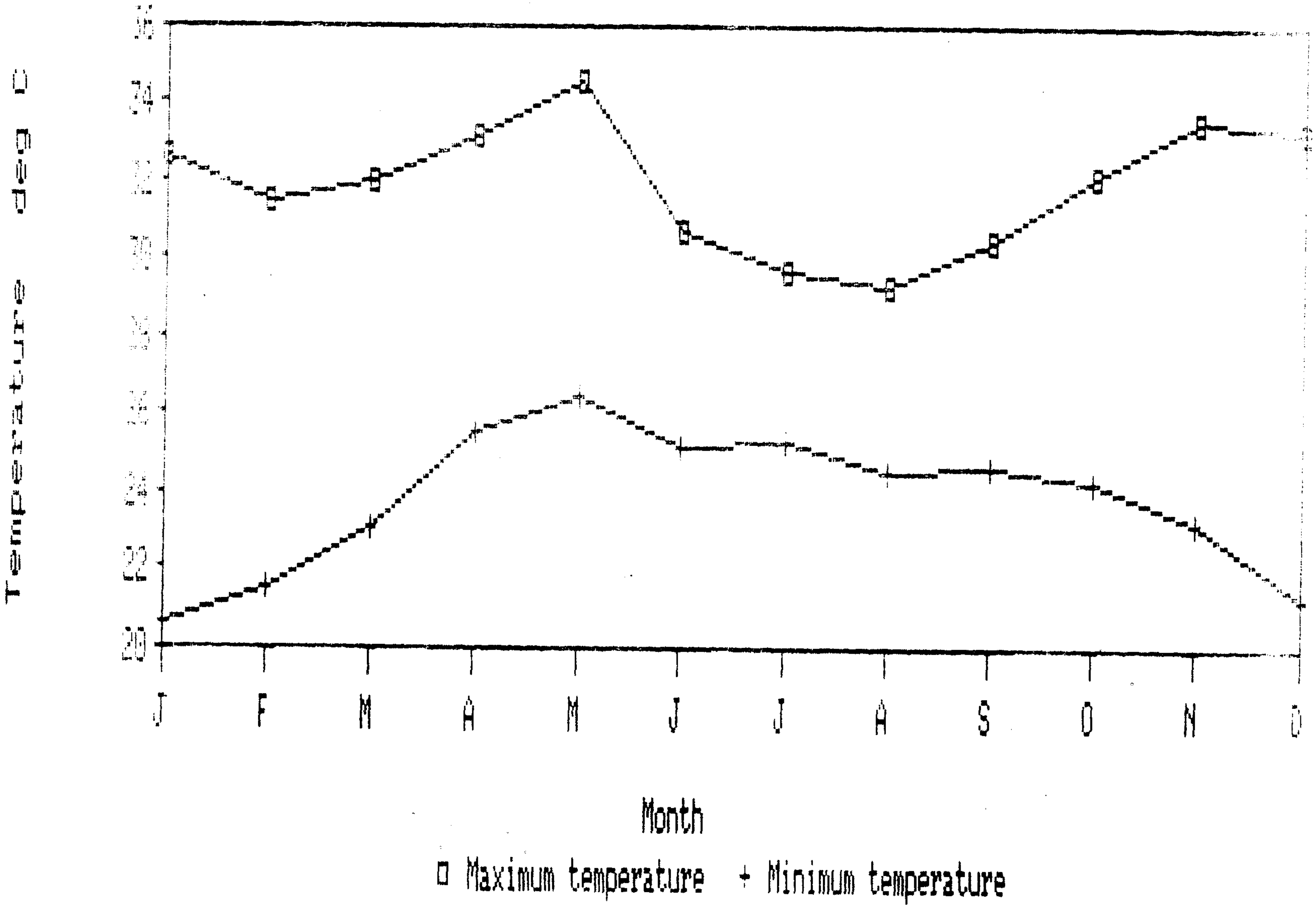


Fig. 1.2a - Average monthly maximum and minimum temperature at Pale - Goa, during 1988.

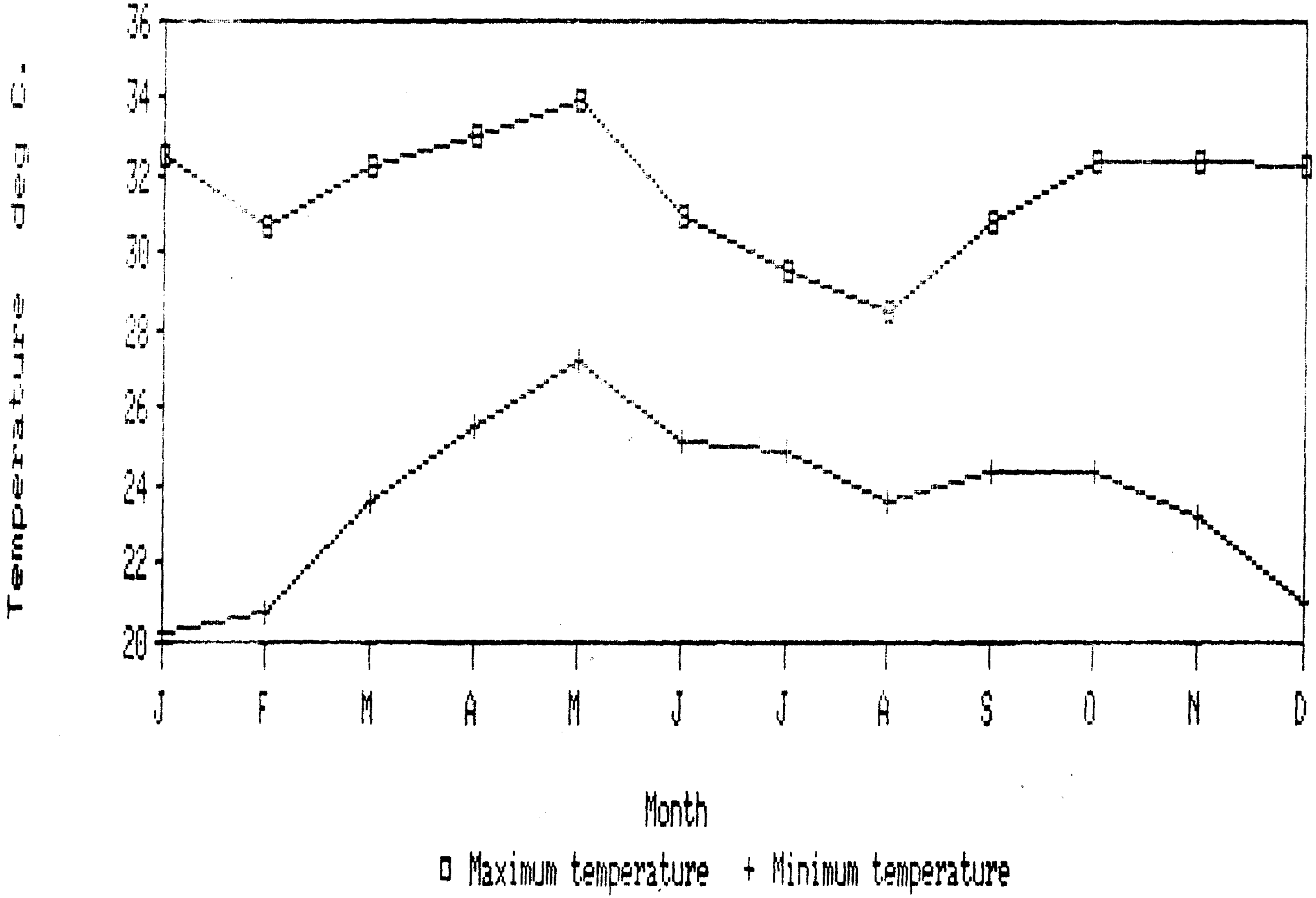


Fig. 1.2b - Average monthly maximum and minimum temperature at Pale - Goa, during 1987.

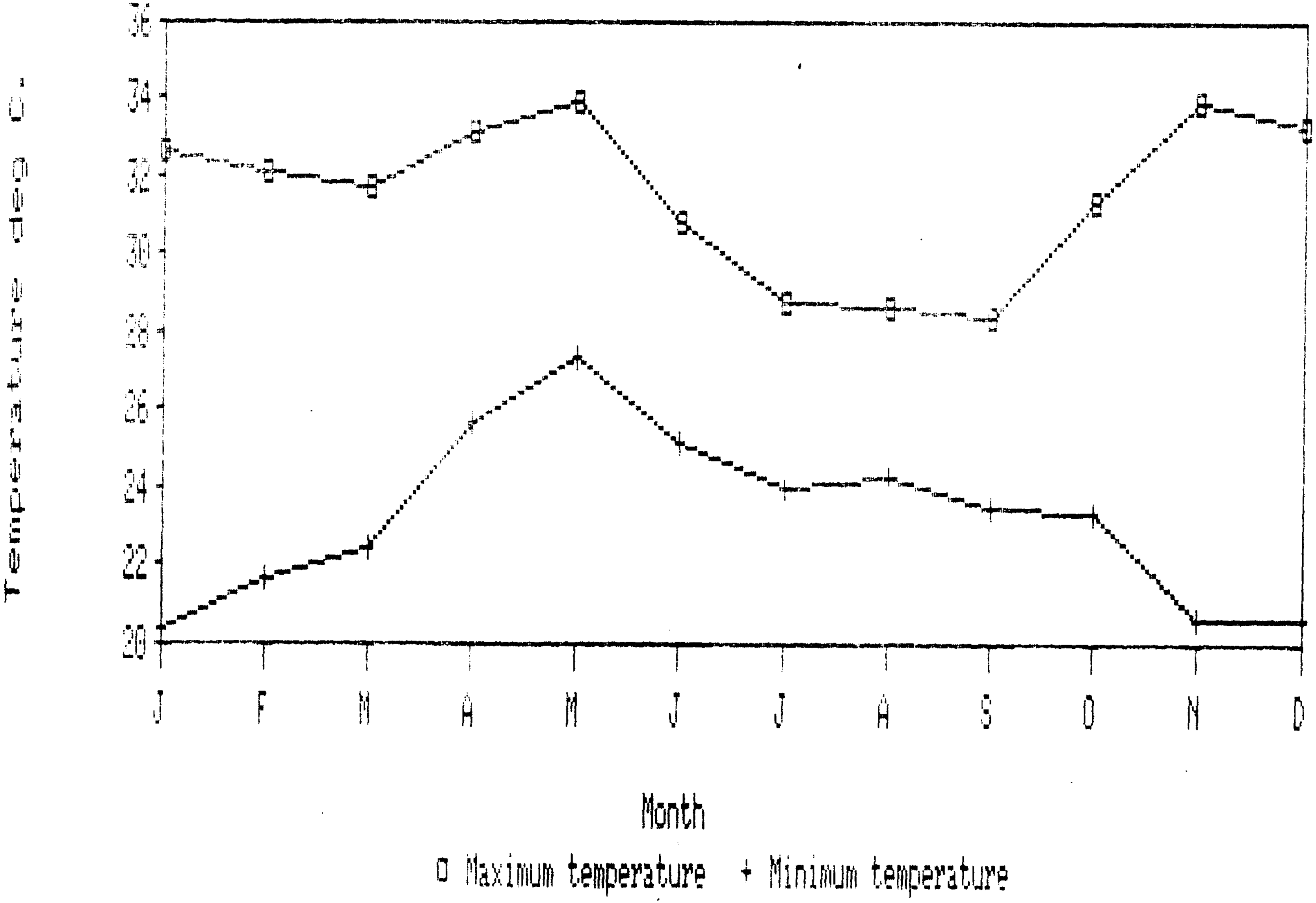


Fig. 1.2c - Average monthly maximum and minimum temperature at Pale - Goa, during 1989.

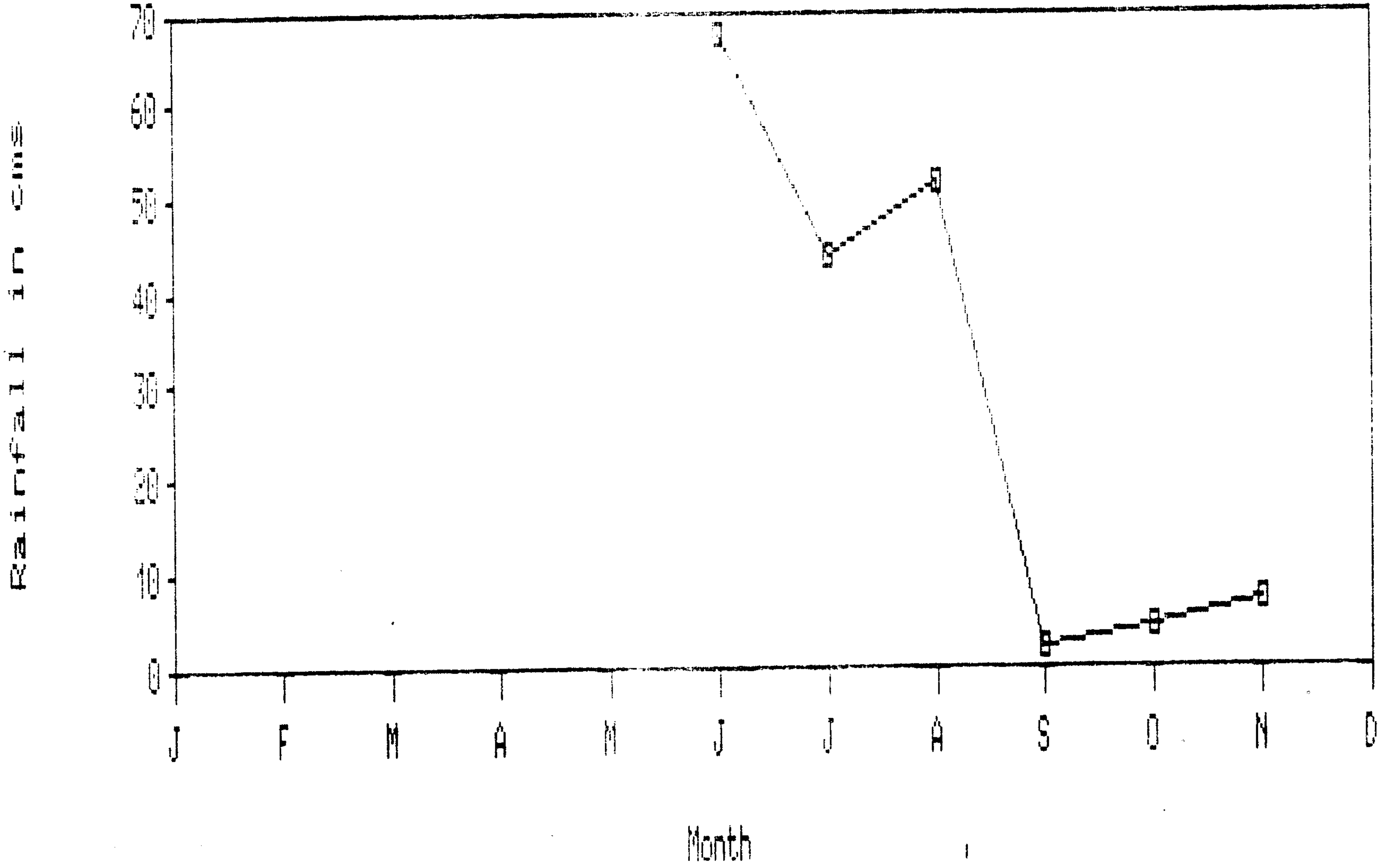


Fig. 1.3a - Average monthly rainfall at Pale - Goa, during 1987.

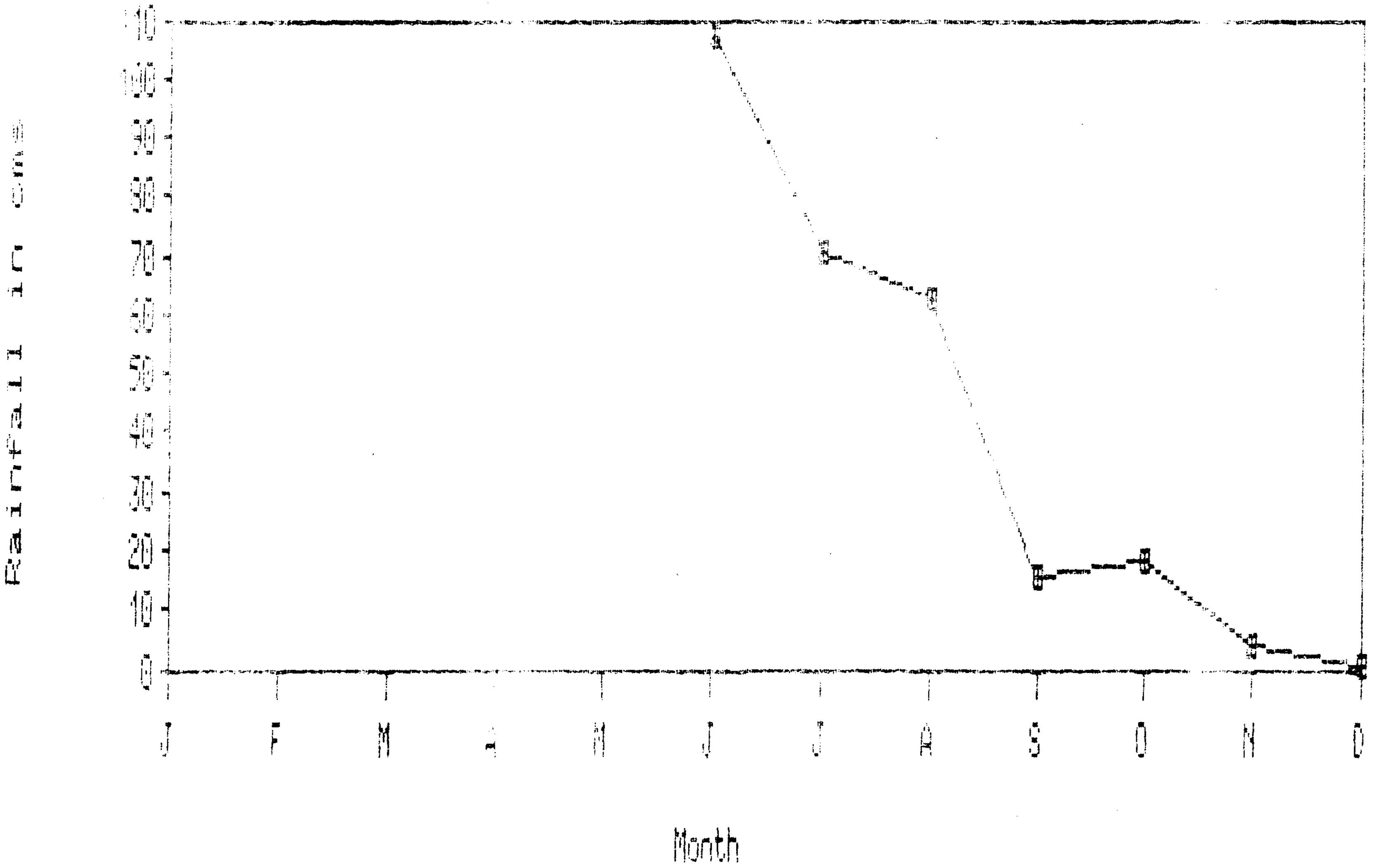


Fig. 1.3b - Average monthly rainfall at Pale - Goa, during 1988.

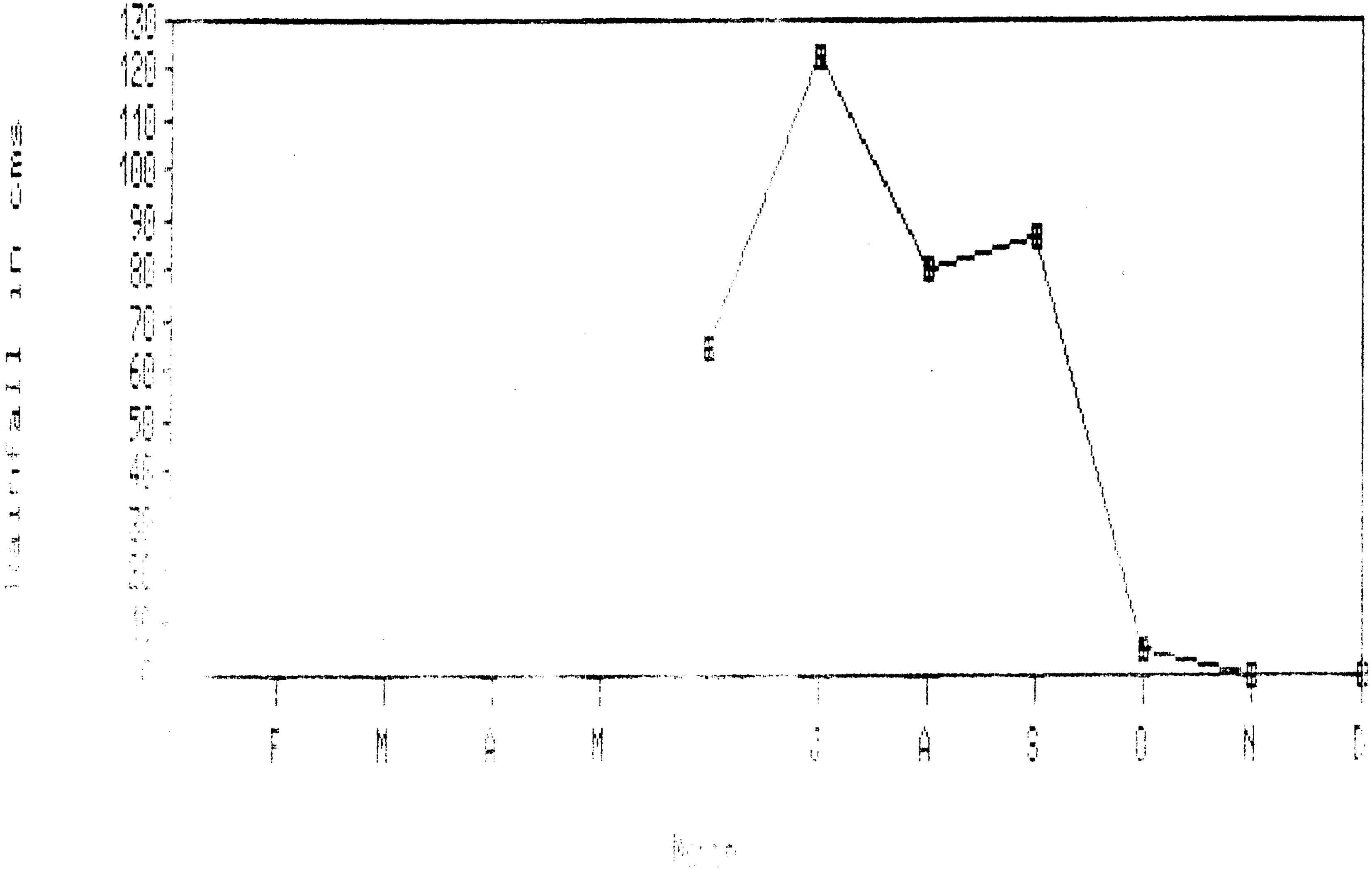


Fig. 1.3c - Average monthly rainfall at Pale - Goa, during 1989.

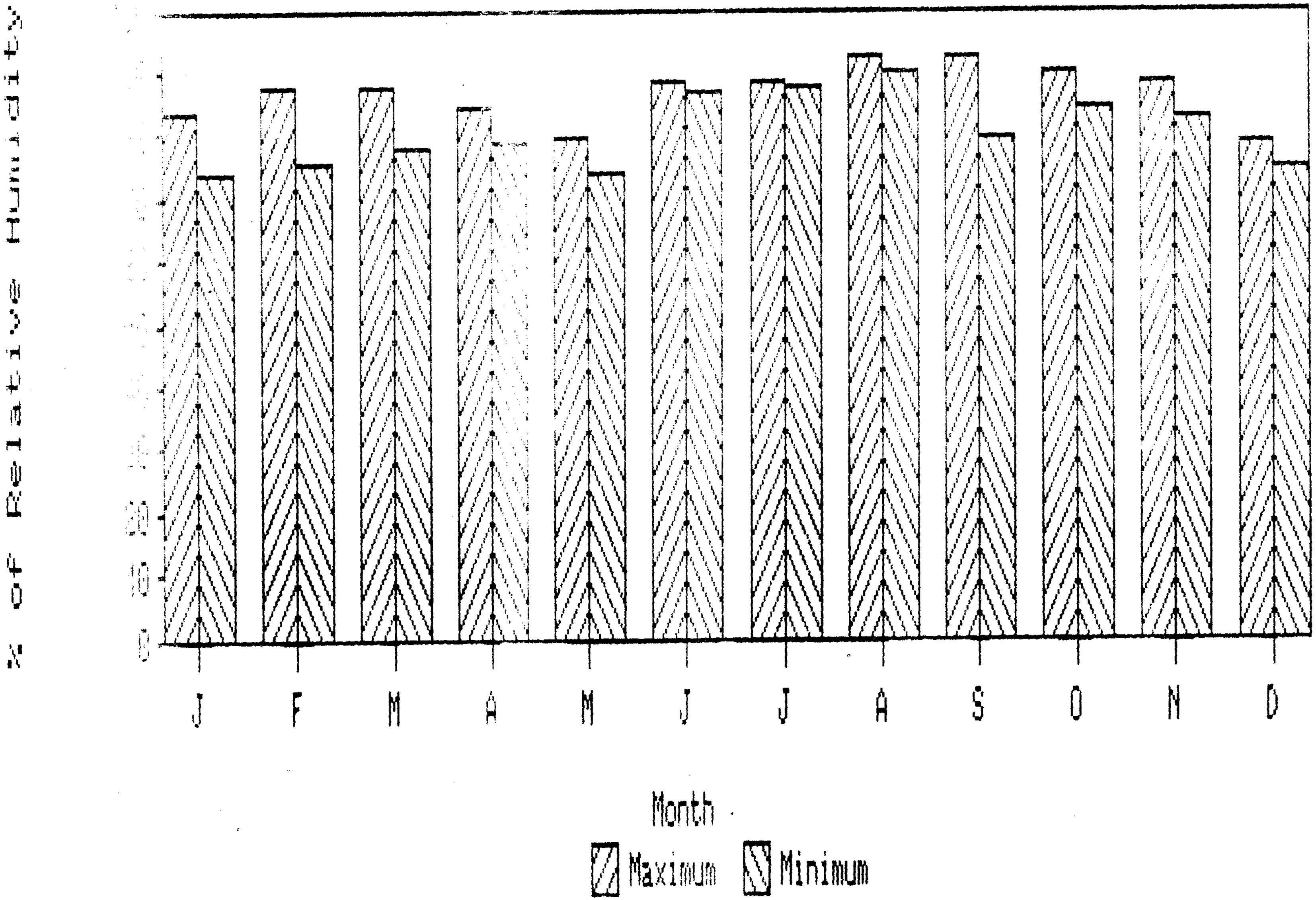


Fig. 1.4a - Average monthly maximum and minimum relative humidity at Pale - Goa, during 1987.

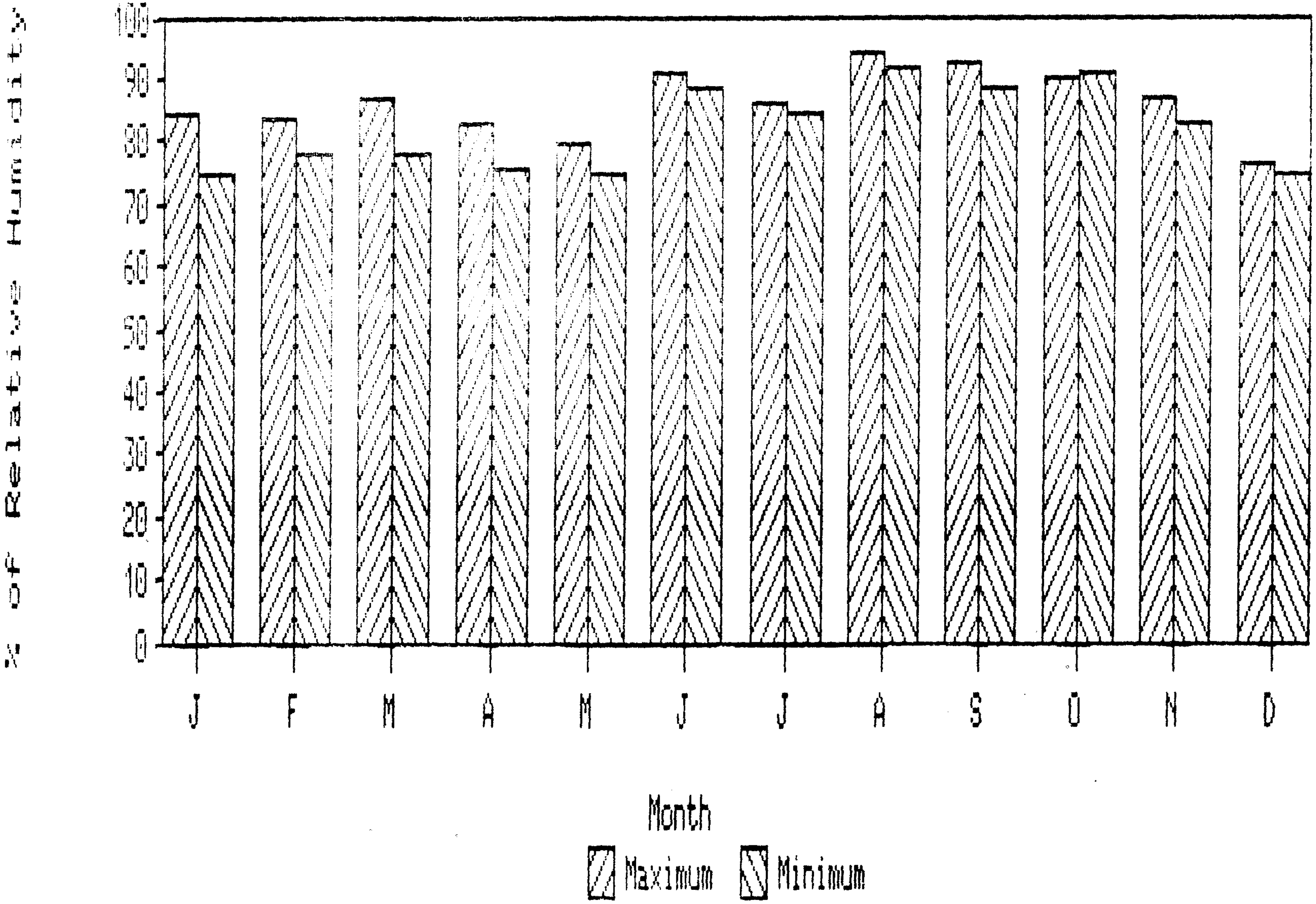


Fig. 1.4b - Average monthly maximum and minimum relative humidity at Pale - Goa, during 1988.

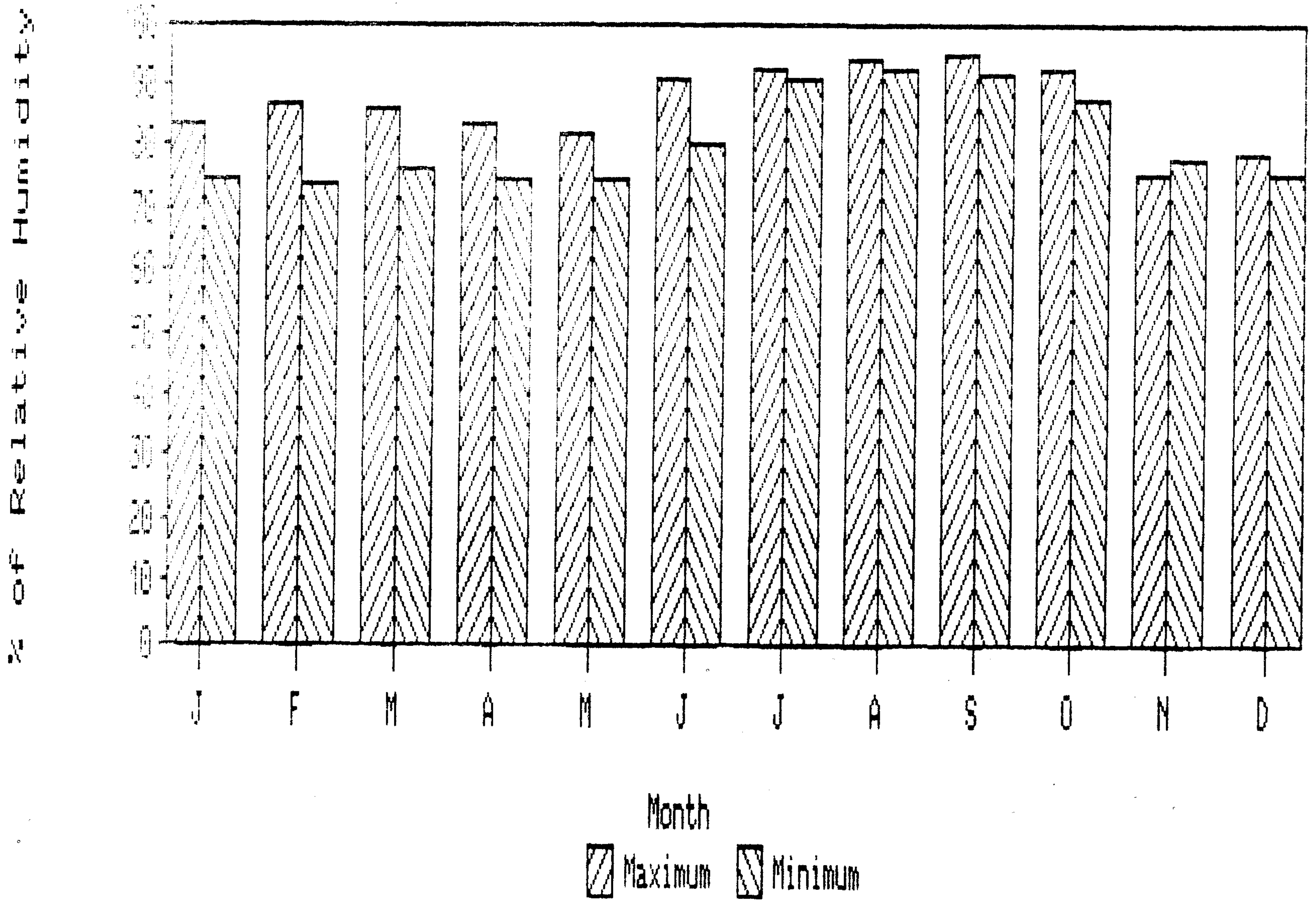


Fig. 1.4c - Average monthly maximum and minimum relative humidity at Pale - Goa, during 1989.

1.3. VEGETATION

The area is mainly of undualating rocky plateau with few hills and substantial area made barren for mining after millable timber and minor forest produce have been removed (Fig. 1.5a & b). A survey of natural vegetation around the mining sites shows that it is semi-deciduous and potentially evergreen open scrub forests with a more or less similar floral composition.

Four distinctive patterns or forms are visible and they are as follows;
1 - Moist fields which are often under cultivation.
2 - Open rocky plateau with scanty vegetation.
3 - Slopes of undisturbed mine areas which are more shrubby in nature with a few trees.
4 - Relatively dense forest areas facing a bank stream. This region is with maximum floral diversity.

The climax evergreen vegetation in this region comprises of plant species easily noticed are, **Alstonia scholaris**, **Mallotus albus** with broad leaves, **Garcinia indica**, **Xylia xylocarpa**, **Bauhinia purpurea**, **Pterocarpus marsupium**, **Tamarindus indica**, **Syzygium cumini**, **Syzygium zylenica** and **Mangifera indica** as trees.

However, some deciduous plant species are frequent like, **Streculia urens**, **Sapium isegne**, **Strychnos nux-vomica**, **Terminalia bellerica**, **Terminalia arjuna** and **Terminalia paniculata** along the drier rocky terrain.

There are two types of **associations** in broad aspects which seem to be governed by moisture content or humidity.
1 - Open semi-deciduous scrub forest on dry plains and slopes generally have vegetation of the same physiognomy and nearly same or uniform floristic composition. Plants of this association are; **Sterculia urens**, **Alstonia scholaris**, **Terminalia arjuna**, **Terminalia bellerica**, **Strychnos nux-vomica**, **Bombax ceiba** and **Careya arborea**.

EXPLANATION OF PLATE - I

VEGETATION

Fig. 1.5a & b - Substantial forest area made barren for mining of iron ore

Fig. 1.5a

Fig. 1.5b

2 - Open semi-deciduous forests on moist fileds and slopes facing river banks. This association is made up of **Artrocarpus arnothians**, **Psychotria dalzellii**, **Tamarindus indica**, **Neonauclea purpurea** and **Caryota urens**.

There are many types of **Consociations** as there are **dominants**. On the slopes of the undisturbed mine region there are two consociations; **Alstonia scholaris** with **Terminalia arjuna** and **Terminalia chebula** as invaders. Second tier is of **Garcinia indica** on steep slopes. Third tier is of **Mallotus albus**.

The drier plains exhibit several consociations; **Strychnos nux-vomica** along with shrubs of **Memecylon wightii**, **Sterculia urens** dominates on the open plains with scanty vegetation. **Terminalia bellerica** dominating on areas that are relatively moist towards Upla-Velge stream. **Terminalia arjuna** is dominant on areas facing the drier plains of Ambegale.

The relative distribution of **Hydnocarpus laurifolia** within the Chowgule's quarters at Pale is quite spectacular. This gives a clear indication that this tree was one time dominating on moist fields, but due to frequent felling its population gradually declined. Invaders are **Mangifera indica** and **Artocarpus heterophyllus** in this areas.

Moist fields in Upla-Velge show the dominance of **Tamarindus indica**. The distribution of **Artocarpus heterophyllus** along with **Mangifera indica** and **Holigarna arnottiana** as invaders in moist open forest near streams is undisputed.

A few pioneer tree species like **Alstonia scholaris**, **Mallotus albus**, **Terminalia paniculata**, **Terminalia bellerica**, **Strychnos nux-vomica**, **Trema orientalis** have invaded 20-25 years old dumps and among the shrubs **Memecylon wightii** and **Calicopteris floribunda** are sparsely common.

1.4. CHARACTERISTICS OF IRON ORE MINE WASTES

The potential for revegetating mine wastes varies considerably depending on the physical and chemical characteristics of mined rock, mineral extraction process used and climate of the area. Problems inherent in establishing vegetation on many mine wastes are:

1) deficiencies of plant nutrients
2) high salt level and heavy metal toxicity
3) unconsolidated sands which destroy plants by sand blasting or burial and
4) have a mineralogy upon weathering which will affect levels and availability of plant nutrients and possibly toxic minerals (Dean et al._1973)_

The physical, chemical and bilogical properties of mine wastes depend on the type of mining operation involved. LeRoy and Keller (1972) grouped such wastelands into two broad classes:

1) those resulting from strip and surface mining which consist of variable-textured mixtures of subsoil and rock
2) those resulting from the accumulation of tailings.

During the iron ore mining operations at Pale-Goa, different types of clays are obtained , viz; Intrusive, Lateritic, Limonitic (Foot wall clays), Manganiferous and Phyllitic (Hanging wall clays), Fig. 1.6.

These clays form the bulk of the overburden/reject during the process of ore extraction from the earth's crust. The random dumping of these wastes constitute the dumps (Fig. 1.7). The characteristics of these clays along with the range of the major elements found in them are given in Table-1.1.

The nature of the iron ore mining operation is such that two main types of materials require revegetation, viz; the unconsolidated dumps and tailing basins resulting from the beneficiation process of mined ores.

EXPLANATION OF PLATE - II

CHARACTERISTICS OF IRON ORE MINE WASTE

Fig. 1.6. Section of mine showing the different bands of clays

Fig. 1.7. Formation of iron ore mine waste dumps

Fig. 1.6

Fig. 1.7

The first stage in considering the revegetation of any site, whether planning the eventual reclamation of a new site, or dealing with an already derelict site, will be to characterise its physical, chemical and biological properties. In this way all the plant growth and after use limiting factors can be identified, and the correct remedial measures designed.

Therefore in th present chapter, the dump and tailing basin undertaken for revegetation were analysed for its physical and chemical characteristics.

1.5. MATERIALS AND METHODS

Soil samples were collected within the area of the dump and tailing basin demarcated for revegetation, from a depth of 0-30 cm. Twenty sub-samples collected from each of these areas were then separately bulked and mixed thoroughly on a rotatory drum to provide a composite sample representing that area.

The composite sample was air dried in shallow wooden trays and screened through 2 mm mesh sieve. The soil was analysed for its moisture content, pH, particle size, bulk and particle density, porosity and water holding capacity by standard methods.

The methods outlined by Piper (1966), Jackson (1973), Moore and Chapman (1976) were employed for chemical analysis, wherever necessary. Organic carbon was determined by Chromic acid method, iron, alumina and manganese percentage was determined by the methods described in "Manual of Procedures for Chemical and Instrumental Analysis of Ores, Minerals and Ore Dressing Products (1979)."

Silica content of the waste was determined gravimetrically, using sodium carbonate fusion mixture. The fused mixture was then dissolved in dil. HCl and filtered through Whatman no.1 filter paper. The residue was washed thoroughly with hot water and ashed along with the filter paper in an electric muffle furnace to obtain silica.

Nitrogen was estimated by Kjeldhal's method, available phosphorous by Bray's method. Available potassium, calcium, magnesium was determined by neutral normal ammonium acetate method and soluble salts by electrical conductivity method.

1.6. OBSERVATIONS

1.6.1. Soil Analysis

Dumps are formed due to the random dumping of surface overburden and waste rock, which is comprised of top soil and the types of clays encountered as enlisted in Table-1.1, as ingredients, which is heterogenous and with non-uniform textural and structural properties. The physical and chemical properties of the dump waste is governed by the proportion of each type of clay in the reject. The major metal and non-metal elements present in the rejects are Fe, Al, Mn and Si, in the form of Fe_2O_3, Al_2O_3, MnO and SiO_2 respectively, whose percentage vary from clay to clay.

1.6.2. Mechanical and Physical Analysis of Natural soil and Iron Ore Mine Wastes.

The natural soil taken for comparison is a loam with 51.2% sand,24.7% silt and 20.3% clay and having bulk density 0.96g/cm^3, particle density 1.9g/cm^3, porosity 49.48%, water holding capacity 44.1% and moisture content 12.67%. Whereas, the dump waste come under the textural class of sandy loams, containing 56.9% sand, 21.9% silt and 18.1% clay and having bulk density 1.3g/cm^3, particle density 2.8g/cm^3, porosity 53.5%, water holding capacity 40.6% and moisture content 8.72%. similarly, the tailings are made up of 48.6% sand, 25% silt and 26.1% clay and can be classified as sandy clay loams, having bulk density 1.7g/cm^3, particle density 3.0g/cm^3, porosity 44.4%, water holding capacity 31.7% and moisture content 13.9% respectively, Table-1.2.

The tailings have low porosity as compared to natural soil and dump waste, but however the porosity of dump waste is much higher as compared to natural soil. The sands of dump waste are

Table 1.1. Types of Clays and their Composition found in Iron Ore Mine (Pale Goa)

Name of Clay	Colour	Percentage of Metal Elements
Intrusive	Pale Pink with yellow spots	Fe:18 25; Al_2O_3; 30 35; MnO: 0.02 0.5 SiO_2 : 10 15
Lateritic	Brown pink	Fe: 40 45; Al_2O_3 : 20 25 MnO: 0.25 0.5; SiO_2:10 20
Limonitic (Footwall clays)	Yellowish orange	Fe: 45 56; Al_2O_3 : 14 17 MnO : 0.25 0.5; SiO_2: 7 9
Manganiferous	Black,yellow,brown sticky with oily appearance	Fe: 35 43; Al_2O_3 : 5 10 MnO: 5 12; SiO_2 : 5 9
Phyllitic (Hanging Wall Clays)	Pink	Fe: 11 12; Al_2O_3 : 17 25 MnO : traces; SiO_2 : 25 30

Table 1.2. Selected Physical Properties of Natural Soils Dump Waste and Tailings.

Textural Class	**Particle size diameter (mm)**
Sand	2 0.02 mm
Silt	0.02 0.002 mm
Clay	0.002 mm

Soil Sample	**Particle Size Analysis** Sand	Silt	Clay	Bulk Density	Particle Density	Porosity	Water holding capacity	Moisture
	%	%	%	g/cm^3	g/cm^3	%	%	%
Natural Soil (Loam)	51.2	24.7	20.3	0.96	1.9	49.48	44.1	12.67
Dump Waste (Sandy Loams)	56.9	21.9	18.1	1.3	2.8	53.5	40.6	8.72
Tailings (Sandy Clay Loam)	48.6	25.0	26.1	1.7	3.0	44.4	31.7	13.9

EXPLANATION OF PLATE - III

Fig. 1.8. Dewatered iron ore tailings with large shrinkage cracks.

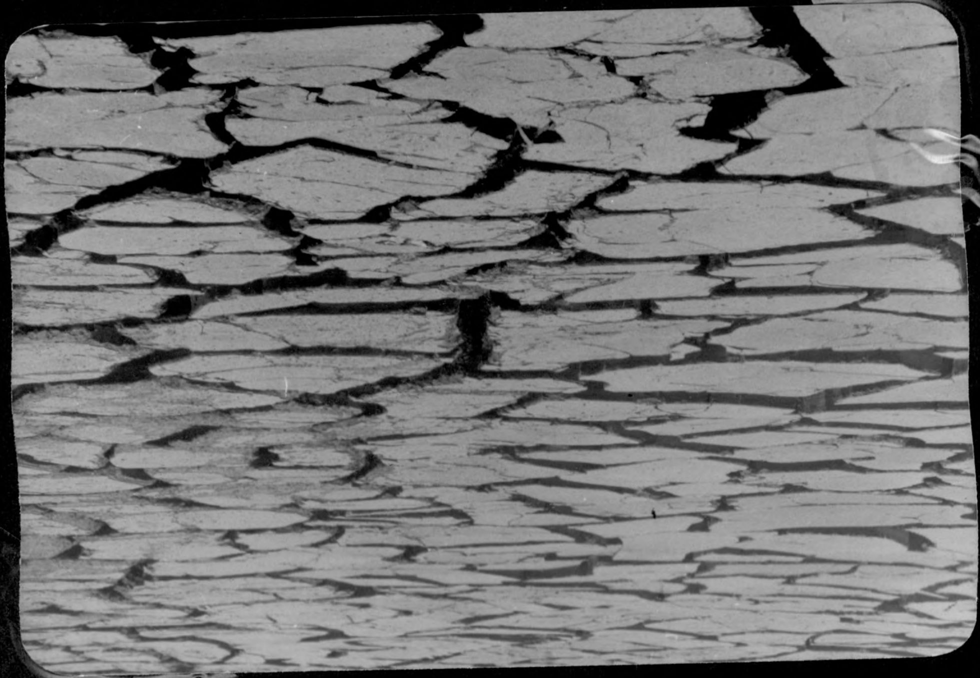


Fig. 1.8

unstructured with a loose consistency. Maximum water holding capacity was observed in natural soil and minimum for tailings.

The tailings are massive and plastic when wet, dewatered tailings become hard, developing 1-3 inch wide shrinkage cracks (Fig. 1.8) in the surface two feet. The shrinkage cracks do not close upon re-wetting. Moist tailings are coffee brown in colour and become greyish-brown on drying.

The dump waste and tailings are young and represent fresh materials lacking the effects of soil formation and genetic processes common to natural soils.

1.6.3. Chemical Analysis of Natural Soil and Iron Ore Mine Wastes.

The chemical composition of mine wastes is mainly affected by the origin of the parent ore bodies and by the efficiency of the extraction process. The content of metal would reflect the type of mine.

The chemical characteristics of natural soil, dump waste and tailings is presented in Table-1.3. The natural soil and dump waste are acidic in nature, pH is 6.2 and 6.6 respectively. The tailings tend to be alkaline with pH 7.2.

Low concentration of major plant nutrients and high concentration of heavy metals in the waste reflect the low content of organic matter, which is 0.48% in dump waste and 0.2% in tailings as compared to the natural soil (1.8%) which is a common source of nitrogen in natural soils.

Similarly, there is a severe deficiency of nitrogen, phosphorous, potassium, magnesium and calcium in both dump waste and tailings, compared to the natural soil. It was found that the dump waste had available nitrogen (15kg/ha), available potassium (85kg/ha), available phosphorous (6kg/ha), magnesium (0.02%) and calcium(0.09%) and high concentration of major metal elements present were Fe (46.2%), Al_2O_3 (17.9%), Mn (0.2%) and SiO_2 (21.9%) respectively. Whereas, in tailings the available nitrogen was (8kg/ha), phosphorous (4kg/ha), potassium (40kg/ha), magnesium

Table 1.3. Selected Chemical Properties of Natural Soils, Dump Waste & Tailings.

Chemical Composition	Soil Sample		
	Natural Soil	Dump Waste	Tailings
pH	6.2	6.6	7.2
Organic carbon %	1.8	0.48	0.22
Cation exchange capacity meq/100g	5.6	3.8	2.5
Total exchangeable bases %	6.0	5.6	3.3
Electrical conductivity m mhos, 1:2.5 soil to water	0.46	0.18	0.16
Iron (Fe) %	35.04	46.2	51.5
Alumina (Al_2O_3) %	19.11	17.9	8.65
Manganese (Mn) %	Traces	0.2	0.25
Silica (SiO_2) %	25.6	21.9	7.95
Calcium (Ca) %	0.25	0.09	0.061
Magnesium (Mg) %	0.04	0.02	0.01
Available nitrogen kg/ha	46.0	15.0	8.0
Available phosphorous kg/ha	12.0	6.0	4.0
Available potassium kg/ha	120.0	85.0	40.0
LOI %	17.15	8.5	7.4

(0.01%) and calcium (0.06%), these values are much lower as compared to the dump waste and natural soil. Tailings had high content of Fe (51.5%) compared to dump waste and natural soil (34.04%). But however, alumina (8.65%) and silica (7.95%) content was comparatively lower than that present in dump waste and natural soil. Moreover manganese content of tailings was higher (0.25%) as compared to natural soil (traces) and dump waste(0.2%).

The tailings had lower cation exchange capacity (2.5meq/100g) as compared to natural soil (5.6 meq/100g) and dump waste(3.8meq/100g). The electrical conductivity which determines the total soluble salt content was higher in natural soil (0.46 mmhos), lower in dump waste (0.18 mmhos) and tailings (0.16 mmhos).

1.7. DISCUSSIONS

Physico-chemical factors at the iron ore mining areas under go change due to the removal of vegetation cover, top soil and the disturbance of the soil horizons. Leaching and erosion wich occur later, contribute greatly to the degradation.

For the reclamation of any degraded area knowledge of physico-chemical parameters of degraded and undegraded area in the locality is essential. However, the exact assessment of these parameters at the entire area is difficult, as the constitution of the soil varies even at the close proximity of the sampling sites due to the random dumping of the top soil overburden, waste rock and due to the interaction of various factors.

Normal soils consists of an inorganic frame work of sand, silt and clay particles, intimately mixed with organic material, produced by degradation of animal and plant remains, which greatly govern the physical, chemical and biological properties of the soil. The physical and chemical properties of the mine wastes are important parameters in considering the most appropriate revegetation scheme.

Physical properties have an important influence on the ultimate biological productivity of the mine waste sites. There are three major wastes obtained from iron ore mining operation, overburden, waste rock and tailings, which present physical problems for plant growth but not sufficient to prevent vegetation establishment.

The composition of the waste is mainly affected by the origin of the parent ore bodies and by the efficiency of the extraction and beneficiation process, and there may be always a considerable variation within site. The content of metal in waste reflect the type of mine from where the waste is generated. The dump waste and tailings are young and represent fresh material lacking the effects of soil formation and genetic processes common to natural soils.

Thus, they lack organic matter and normally have low levels of available nitrogen (N), phosphorous (P) and sometimes potassium (K) (Czapowskyj, 1973; Jeffrey **et al.** 1975, Grandt, 1978). The rock origin and negligible organic matter content of mine wastes are also reflected in higher bulk densities, lower cation exchange capacity (CEC) values and poorer structure than undisturbed soils (Smith **et al.** 1971; Shetron **et al.** 1977).

The physical analysis of iron ore wastes revealed that the wastes had high bulk and particle density compared to the natural soils. The bulk density for natural soils falls within the range of 1.0-1.5g/cm^3 (Williamson **et al.** 1982) and particle density 2.65g/cm^3 (Waddington, 1969) whereas the bulk density and particle density of dump waste and tailings are much above these values as seen in Table-1.2, which is normally the characteristic feature of metalliferous mine waste. The results of physical analysis of iron ore mine wastes obtained here are consonant with the observations made by Murray (1977) with other mine wastes.

The porosity of the soil is regulated by its bulk and particle density. The texture and structure of waste materials have profound effects on water filtration and storage. Infiltration is reduced on steep slopes and compacted materials and is increased on well structured or sandy soils or materials rich in organic matter. The dump waste had a high porosity due to the sandy materials, unstructured, unconsolidated having loose consistency, has large pores between the particles, which allow good aeration and rapid infiltration but retention of water is poor and it dries out easily and rapidly. The passage of water rapidly reduces its nutrient content. Tailings in contrast has very small particles with little pore space and poor aeration. It tends to be dense with a high proportion of moisture bound to the particles and thus unavailable to plants.

The low water holding capacity of the waste can be attributed to the poor texture, structure and low organic matter content which is known to be responsible for improving the water holding capacity.

The wastes are deficient or are very poor in organic matter as seen in Table-1.3. Normal soils have 2-5% organic matter. Mine materials have none or it may be so low that they cannot sustain vegetation on it. Besides supplying nutrients, organic matter contributes considerably to soil physical properties.In natural soils, particles of various size cluster together as a result of organic matter and activities of micro-organisms to form aggregates which confer good porosity, aeration and drainage condition to the medium.

Mine waste lack organic matter and suitable micro-organisms, therefore minimal aggregation of particles occur and the material lacks structure. This is aggravated by the total absence of larger organisms such as worms which in normal soil are responsible for mixing and distribution of decaying plant materials (Williamson et al. 1982) which greatly contribute to the organic matter of soil.

Concentrations of major plant nutrients and heavy metals in the iron ore mine wastes reflect the absence of organic matter, severe deficiencies of nitrogen, phosphorous and potassium, and phytotoxic levels of iron and manganese. The iron and manganese concentration of the wastes are variable but consistently beyond the range within which varieties of plant species are capable of normal growth and development.

The values obtained here are typical of mine wastes and the low fertility is clear for N, P and K. The pH of the waste determined indicate the deficiency of primary essential nutrients and an additional limitation of plant growth on the unamended waste materials. The data shows that the dump waste and tailings are less fertile than natural soils. The deficiency of these elements have been reported in different other mine waste (Dean **et al.** 1973; Bradshaw and Chadwick, 1980; Williamson **et al.** 1982 and Shetron, 1983).

Investigation into the vegetative stabilization of iron ore mine waste should include an examination of the physical and chemical properties to assess the suitability of the materials for plant growth. The physical nature of waste can sometimes be assessed by an experienced eye, without recourse to formal tests, but the chemical content of the waste requires analytical investigatons. Biological characteristics are rarely investigated but are improved indirectly as the physical and chemical problems are overcome.

A primary requirement for planning a suitable revegetaton program for these iron ore mine wastes is to determine the amount of organic matter, N, P, K, Ca and Mg available to plants. These nutrients are consumed in large quantities by the plants and unless they are restored or are present in sufficient amounts plants establishment and growth will be difficult.

CHAPTER TWO

MICRO-ORGANISMS AND THE RECLAMATION OF IRON ORE MINE WASTES

2.1 STUDIES ON *AZOTOBACTER* SPECIES IN IRON ORE MINE WASTE

Successful revegetation after mining will depend upon the re-establishment of nutrient cycling. Even though many workers have recognised the importance of microbial activity in mined land reclamation (Wild and Wiltshire,1971; Jonas, 1973; Goodman, 1974; Down, 1975; Cundall, 1977), most work has been centered on identifying and alleviating the revegetation problems, like nutrient deficiencies and imbalances, toxicities, moisture deficits and wind erosion (Hutnik and Davis, 1973). The significance of microbial activity to vegetation establishment on mine wastes has generally been ignored.

Numerous studies have shown that soil microflora is a crucial factor in plant nutrient availability and uptake, either through organic matter decomposition or mineral weathering. The micro-organisms also can influence plant root morphology, root to shoot ratios, as well as supplying essential growth factors and vitamins to the plants (Nicholas,1965;Barber,1968; Bowen and Rovira, 1976).

In successional terms the soil microflora is important in nutrient cycling. Development of a suitable microflora on waste could be an important aspect of a successful rehabilitation or stabilization scheme. As many micro-organisms require organic carbon their increase follows the success of plant growth. It is well established fact that some plant species will not grow well if particular micro-organisms are lacking.

Lack of plant nutrients is a universal feature of mining waste and is likely to cause a level of expenditure that will rule out commercial forestry. In natural soils and in mine wastes, the nutrients which has the greatest effect on plant growth is nitrogen. All mine wastes are generally deficient in nitrogen (Bradshaw and Chadwick,1980) and nitrogen levels are invariably inadequate for plant growth.

Studies on natural and artificial colonisation of sand waste from Kaolin mining in the U.K. and on waste materials elsewhere reveal that nitrogen accumulation and the build up of a nitrogen cycle is the most important factor in soil and vegetation development (Dancer **et al.** 1977; Marrs **et al.**1981 and Roberts **et al.** 1981).

Although extensive research has been carried out on non-symbiotic nitrogen fixers, ecological and nitorgen fixing potentiality of **Azotobacter** species in improving plant growth in mine wastes remain still unexplored.

Revegetationists still depend upon commercial fertilizers, which is not only costly but inadequate and can be leached out from the soil. Under such circumstances the utilization of natural sources of nitrogen like **Azotobacter** to improve soil fertility and plant growth would help many fold.

In the present study, ecological survey of **Azotobacter** from iron ore mine waste dumps was carried out. The waste from different dumps were analysed for physical, chemical and biological properties. Potential nitrogen fixing **Azotobacter** species from rhizosphere of **Cassia tora**,L. were isolated, identified, characterized and thier nitrogen fixing ability was determined. The best strain was used for inoculation tests.

2.2 MATERIALS AND METHODS

Ecological survey of nine different iron-ore waste dumps was carried out in Jan'87. Soil samples were collected in a sterile conical flask (250 ml), upto a depth of 15 cm, at different spots of the waste dumps and these were mixed together to form a composite sample, which would represent the dump. The samples were immediately shifted to the laboratory for microbiological, physical and chemical analysis. Immediate analysis avoids and reduces the changes due to aeration, moisture content and also different temperatures in the laboratory environment.

The total microbial population of the soil sample was estimated by the dilution plate technique, using nutrient agar. After estimating the moisture content, the population per gram of soil on dry weight basis was calculated (Anonymous, 1957). **Azotobacter** population in the soil was estimated also by the dilution plate method using Norris (1959) medium, prepared with double distilled water. The colonies on the agar plates were differentiated into various morphological types and the total number under each type was counted. Representative colonies from each type were isolated and examined in detail for morphological, cultural and biochemical properties, on the basis of which they were classified into species (Breed **et al.** 1957).

2.2.1 Physical and Chemical Analysis of Natural Soil and Mine Waste.

The iron ore mine waste samples were analysed for moisture content, pH, organic matter, total nitrogen, phosphorous, potassium and magnesium contents according to standard methods as described in Chapter - I.

2.2.2 Isolation of Azotobacter from the Rhizosphere of Cassia tora, L.

Five different soil samples from the rhizosphere of **Cassia tora** which was found growing around the iron ore mine and natural soil were collected separately. Rhizosphere samples were prepared by shaking the excess soil from the roots, which were then shaken in sterile distilled water for 15 min. Rhizosphere soil samples were passed through a 2 mm sieve and 10g fresh weight suspended in water and the suspension diluted. **Azotobacter** and total bacterial population from these samples were counted by the standard agar plate method as described earlier. The cultures isolated from the mine waste rhizosphere were screened for their ability to fix atmospheric nitrogen using Jensen's medium, contained in a 250 ml Erlenmeyer flasks, at the rate of 100 ml per flask, maintained at 28°C. The nitrogen content of the medium was estimated by the micro-Kjeldahl method. The best strain was used in plant growth experiment in mine waste.

2.2.3 Effect of Azotobacter species on Growth of Cassia tora, L. in Iron Ore Mine Waste.

The mine waste was screened through 2mm sieve and was thoroughly mixed with 0,1,2 and 3% of dried ground rice straw containing 0.55% N as a energy source. This was sterilized after a month in an autoclave for one hour at 120°C and 20 lbs pressure. Approximately 4 kgs of amended sterile waste was filled in black polybags (6 x 10") size. Healthy seeds of **Cassia tora** were surface sterilized with acidified 0.1% $HgCl_2$ for 4-5 min. and washed several times with sterile distilled water. Seeds were given boiling water treatment to break dormancy. The treated seeds were kept for 2 days aseptically at 29-30°C for germination. The germinating seeds were carefully transferred to pots filled with sterlized amended waste with a sterile forceps. Two seedlings were maintained per pot. Two ml cultures of **A.chroococum** a highly efficient strain was used as inoculant. Corresponding uninoculated controls were maintained with 1,2 and 3% dried ground rice straw and were treated with 2 ml of sterile nitrogen deficient medium but without sucrose. Each treatment was replicated ten times.

Growth parameters and yield were recorded at maturity, shoot and root dry weight were obtained, nitrogen percentage in dry material was measured by Kjeldahl's method.

2.2.4 Establishment of Azotobacter chroococum in the Rhizosphere of Cassia tora, L.

The ability of **Azotobacter chroococum** to establish in the rhizosphere of **Cassia tora** was tested by re-isolating the bacteria and estimating the total population.

At 30 days, 45 days and at maturity, two inoculated plants from each treatment were removed and suitable amount of rhizosphere soil was suspended in known volume of sterile distilled water. The soil suspension was shaken on a reciprocating shaker for 10 min. Serial dilutions were plated on nitrogen deficient Norris medium, counts were made after 3 days and were related to one gram of dry rhizosphere soil (Barea and Brown,1974).

2.3 OBSERVATIONS

2.3.1. Physical and Chemical Analysis of Iron Ore Mine Waste from Various Dumps at Pale-Mines

Nine iron-ore waste dumps were designated as P_1, P_2, P_3, P_4, P_5, P_6 ..... P_9. Soil samples from these dumps were analysed for its physical and chemical characteristics. The exact age of the dumps could not be acertained at the time of analysis, because the dumping plan and pattern changed every year.

Physical and chemical analysis of waste is embodied in Table - 2.1. The table shows that the wastes are either slightly acidic or near neutral in nature, the pH ranges from 6.0 - 6.5.

The wastes vary in the total organic carbon and values for various dumps lie between 0.3-0.48%, there is relatively poor concentration of total nitrogen and ranges from 0.038-0.069 percent which can be attributed to the lack of organic matter and poor soil fertility. Moisture content varies considerably from dump to dump and fluctuates between 5.5 to 10.5 percent.

Available phosphorous and potassium are much below the normal limits of fertility, phosphorous ranges from 18.4-64.4 kg/ha. and potassium 45-82 kg/ha.

There is significiant variability in the Fe, Al_2O_3, SiO_2, and MnO content of the waste. The iron ore content ranges from 11.2-56%, Al_2O_3 from 4.57-17% , SiO_2 from 6.55-58.58% and similarly, MnO varies from 0.15-0.40%. The wastes are deficient in calcium and magnesium. Calcium and magnesium ranges from 0.8-0.18% and 0.022-0.043% respectively.

2.3.2 Microbiological Analysis of Iron Ore Mine Waste from Various Dumps at Pale-Mines.

The total bacterial population is indicated in Table-2.2. There was a wide variation in the total count of bacteria on

Table 2.1. Physical and Chemical Analysis of Iron Ore Mine Waste from Various Dumps at Pale-Mines.

Source of sample	pH	Organic carbon %	Moisture %	Nitrogen %	Available P_2O_5 Kg/ha	Available K_2O Kg/ha	Fe %	Al_2O_3 %	SiO_2 %	MnO %	CaO %	MgO %
Field Soil	6.8	1.5	15.39	0.137	48.0	120	35.04	19.1	25.60	traces	0.25	0.09
Dump No.P_1	6.4	0.44	5.5	0.047	20.2	45	41.0	10.24	21.96	0.20	0.08	0.032
P_2	6.0	0.39	10.5	0.045	23.0	53	11.2	17.0	58.58	0.31	0.10	0.04
P_3	6.2	0.44	9.65	0.069	27.6	49	43.0	3.50	9.29	2.40	0.17	0.04
P_4	6.5	0.39	9.00	0.042	20.2	51	46.0	15.19	9.82	0.20	0.09	0.029
P_5	6.3	0.48	8.00	0.041	18.4	53	47.2	11.74	9.75	0.21	0.15	0.031
P_6	6.0	0.44	10.1	0.056	20.2	47	56.0	4.57	6.55	0.25	0.13	0.041
P_7	6.4	0.39	7.65	0.038	64.4	56	46.2	12.6	12.40	0.20	0.11	0.05
P_8	6.5	0.32	9.86	0.064	23.0	82	35.8	3.32	41.27	0.15	0.11	0.027
P_9	6.5	0.39	6.35	0.051	27.6	70	48.2	14.31	8.55	0.25	0.18	0.043

Table 2.2 Microbiological analysis of Iron Ore Mine Waste from various Dumps at Pale - Mines.

Source of Sample	pH	Total bacterial population/g of dry soil	Total *Azotobacter* population/g of dry soil
Field Soil	6.8	61.2×10^6	40.9×10^2
Dump no. P_1	6.4	9.3×10^4	3.1×10^2
P_2	6.0	8.7×10^4	2.97×10^2
P_3	6.2	9.0×10^4	2.91×10^2
P_4	6.5	7.28×10^4	2.5×10^2
P_5	6.3	9.8×10^4	2.43×10^2
P_6	6.0	9.30×10^4	2.6×10^2
P_7	6.4	6.98×10^4	2.37×10^2
P_8	6.5	3.78×10^4	2.2×10^2
P_9	6.5	7.2×10^4	3.4×10^2

Population counts are mean of replicate samples.

nutrient agar as well as on nitrogen free medium. The population showed varied fluctuations from dump to dump.

The normal total bacterial population in field soil was 61.2 $x10^6$/g of soil and **Azotobacter** population 40.9 x $102_{/g}$ of soil. Whereas, the total population in various dumps varied from 3.78 x 10^4- 9.8 x 10^4/g of soil and **Azotobacter** population varied from 2.6 x 10^2- 3.4 x 10^2/g of soil respectively. The highest total bacterial population was recorded in P_9 dump and lowest in P_8 dump. The highest **Azotobacter** population was observed in P_9 dump, and lowest in P_6 dump.

2.3.3. Rhizosphere Population of Cassia tora, L. in Natural soil and Mine Waste.

The distribution of **Azotobacter** studied in ten rhizosphere samples of **Cassia tora** from natural soils and from plants growing around the mines is listed in Table - 2.3.

The total bacterial population in the rhizosphere of **Cassia tora** growing around the mines was comparatively lower than the natural rhizosphere soil. The undisturbed natural rhizosphere of **Cassia tora** had a total bacterial population of 72 x 10^6/g of soil and **Azotobacter** population 1.21 x 10^3/g of soil. Whereas, in the one growing around the mine, it was 25 x 10^4 and 3.5 x 10^2 /g of soil respectively. However, there was no wide change between the pH of both the types of samples analysed.

2.3.4. Morophological, Cultural and Biochemical Characteristics of Azotobacter species Isolated from Rhizosphere of Cassia tora, L. Growing Around the Iron Ore Mines.

The occurrence of different species of **Azotobacter** in the rhizosphere of **Cassia tora** and their morphological, cultural and biochemical characteristics is summarized in Table - 2.4. Altogether twenty two cultures were isolated in Norris medium and were grouped initially according to their morphology and colony type. On this basis eleven cultures were tested for their capacity and ability to fix atmospheric nitrogen in Jensen's medium (1951).

The characterization of the isolates showed that all were

Table 2.3. Normal Rhizosphere Population of *Cassia tora*, L. in Natural soil and Mine waste.

Site	pH	Total bacterial population/g of soil	Total bacterial population/g of Rhizosphere soil	Total *Azotobacter* population/g of Rhizosphere soil.
Natural soil	6.5	86×10^5	72×10^6	1.21×10^3
Mine Waste	6.7	49×10^3	25×10^4	3.5×10^2

Population counts are mean of replicate samples.

Table 2.4. Morphological, Cultural and Biochemical Characteristics of Isolates from Rhizosphere of Cassia tora, L.

Isolate no.	Microscopic characteristics	Cultural characteristics	Carbohydrate utilization			Catalase test	Pigmentation	Identification Remarks.
			Starch	Mannitol	Rhamnose			
1.	Gram negative, motile, cyst formation not observed.	Colonies large, slimmy Producing purplish pigment.		+	–	+	Purplish water soluble	**A.macrocytogenes**
2.	Gram negative, motile forming cyst	Capsular slime produced with green pigment	–	+	+	+	Green water soluble	**A. vinelandii**
3.	" "	" "	–	+	+	+	"	"
4.	Gram negative, motile forming cyst	Capsular slime produced Brown pigment turning black on ageing	+	+		+	Brown insoluble	**A. chroococum.**
5.	Gram negative motile cyst formation not observed	Colonies large, slimmy, producing purplish pigment		+	–	+	Purplish water soluble	**A. macrocytogenes**
6.	Gram negative, motile forming cyst	Capsular slime produced Brown pigment turning black on ageing	+	+	+	+	Brown insoluble pigment	**A. chroococum**
7.	" "	" "	+	+	-	+	"	"
8.	Gram ngative, motile, forming cyst	Capsular slime produced with green pigment	–	+	+	+	Green water soluble	**A. vinelandii**
9.	Gram negative, motile, cyst formation not observed	Colonies large, slimmy producing purplish pigment.	–	+	–	+	Purplish water soluble	**A. macrocytogenes**
10.	Gram negative motile forming cyst	Capsular slime produced with green pigment	–	+	+	+	Green water soluble	**A. vinelandii**
11.	Gram negative, motile forming cyst	Capsular slime produced	+	+	–	+	Brown insoluble pigment	**A. chroococum**

chemo-organotrophs, aerobic, gram negative organisms, with variable morphology. All grew well on nitrogen free mannitol medium. They were all motile and formed cyst, except isolates no. 1, 5 and 9, but no endospores. All produced distinct spherical colonies with profuse capsular slime and different coloured pigments specific to their species on glucose-nitrogen free agar slants. Isolates no. 1,5 and 9 produced purplish water soluble green pigments and utilized mannitol and rhamnose but not starch. Similarly, isolates no. 4, 6, 7 & 11 produced brown pigment which turned black on ageing after 15 days. All isolates were catalase positive. On the basis of morphological, cultural and biochemical characteristics, the isolates were tentatively identified, according to Bergy's Manual (1957) and Gibbs and Shapton (1968).

2.3.5. Nitrogen Fixing Ability of Isolates.

It was considered important in this study that the non-symbiotic bacteria that are used, will favour the strain which is capable of colonizing the rhizosphere of **Cassia tora**. Therefore, cultures freshly isolated from rhizosphere were identified for their nitrogen fixing ability and the best culture was used in inoculation test.

Microbial growth in medium which is free of nitrogen provided as a proof of the ability to assimilate nitrogen. All the isolates were identified and characterized for fixed nitrogen in Jensen's medium (1951) utilizing sucrose as the energy source. Quantative determination during the growth of culture showed increase in the total nitrogen content of the medium. The nitrogen fixing ability of the isolates per gram of sucrose oxidized is given in Table-2.5.

It was observed that all the isolates had promising non-symbiotic nitrogen fixing ability. Isolates no. 4, 6, 7 and 11 possessed exceptionally good ability and the rest moderate. Isolate no. 7 showed the best nitrogen fixing ability among the four which fixed 17 mg/g of sucrose oxidized. The lowest nitrogen fixing capacity was observed among isolates no 1, 5 and 9. Among the different isolates of the same species there was no significant difference.

Table 2.5. Nitrogen fixing ability of Isolates

Isolate No.	**mg of N/g of sucrose in Jensen's medium**
1	12.5
2	13.7
3	14.0
4	16.7
5	12.0
6	16.0
7	17.0
8	15.1
9	12.7
10	14.5
11	16.8

Results are mean of three determinations.

2.3.6. Recovery of Inoculated Azotobacter chroococum from the Rhizosphere of Cassia tora, L.

As a result of inoculation large population of **Azotobacter** was established and maintained in the rhizosphere as indicated in Table-2.6. The table shows that the inoculation gave a partial increase in **Azotobacter** at the time of second sampling and at harvest. There was no marked difference between first sampling after 30 days of plantation and second sampling in the rice straw amended pots. However, the total **Azotobacter** count per gram of dry soil in case of unamended inoculated plant declined at the time of second and final sampling. It is quite probable that establishment may have occured earlier and later diminised with time due to absence or depletion of energy source from the initially high levels established by inoculation.

2.3.7. Effect of Azotobacter chroococum on Morphological and Growth Yield Characteristics of Cassia tora, L. in Iron Ore Mine Waste.

Pot trials were designed to examine the effects of inoculation on plant yield and the influence of different soil amendments. Inoculation with **A. chroococum** brought about changes in morphological and yield characteristics of plant. The differences between treatments at maturity were conspicous. **Azotobacter** inoculated plants were visibly taller and greener than their corresponding controls (Fig.2.1.). Inoculation increased the height of plant by 16.8 - 27.9 percent over their respective controls as indicated in Table-2.7a.

The beneficial effects of inoculation with **A. chroococum** were manifested in the dimension of the plant. Plant hieght was significantly increased by inoculation (Fig. 2.2). Inoculation enhanced the length of internode. The size of fifth internode in case of inoculated plants amended with 0, 1, 2 and 3 percent straw were 3.3, 3.5, 3.8 and 5.0 cm respectively. Whereas, it was 3.1, 3.1, 3.2 and 3.4 cm in thier respective controls. Similarly, the length of flag leaf in treated plants was 4.3, 4.3, 4.6 and 5.0 cm, whereas in their corresponding controls it was 4.1, 4.2, 4.3 and 4.3 cm respectively. The accelerated internodal length and leaf size were appreciably significant among the different treatments.

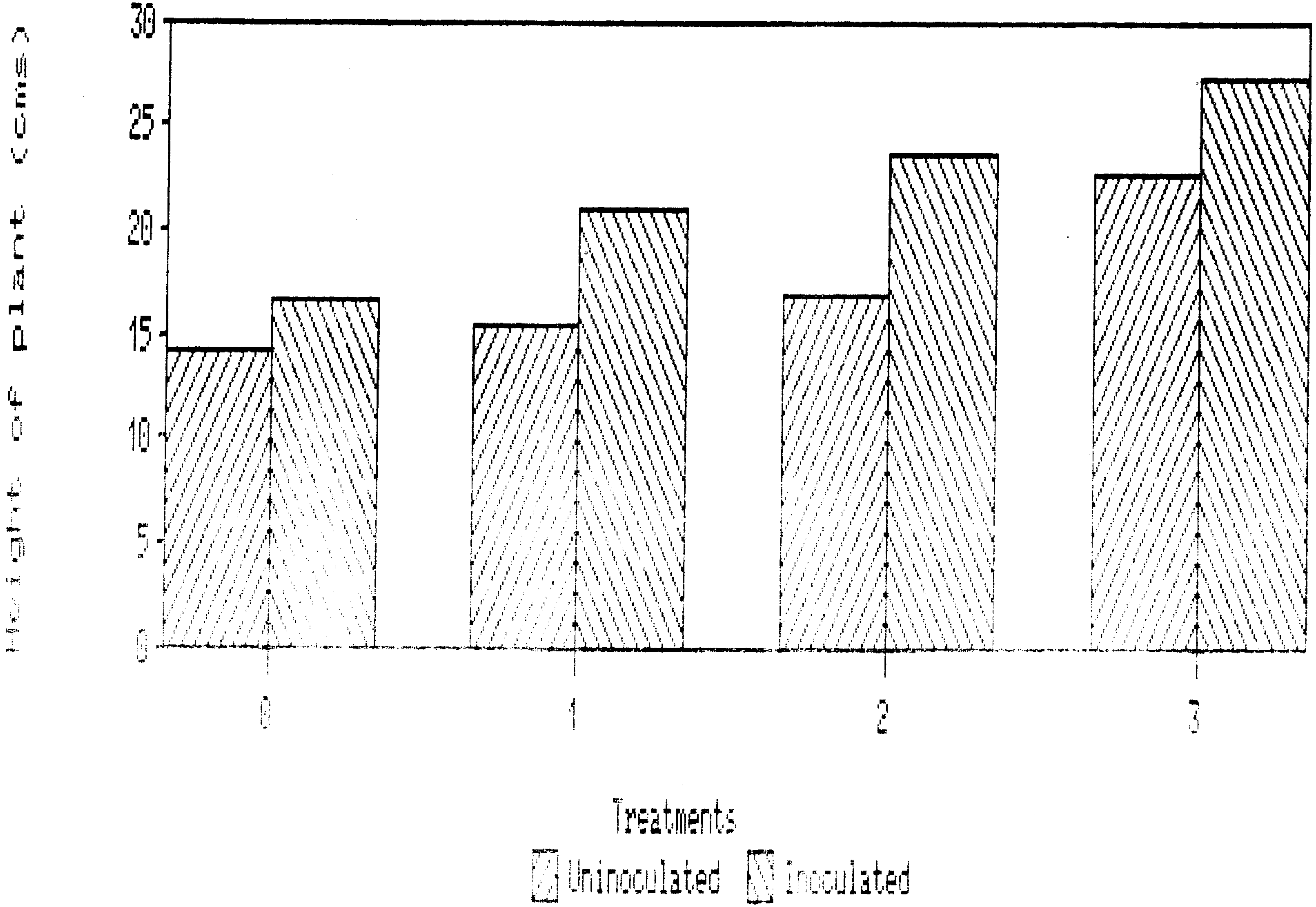
EXPLANATION OF PLATE - IV

MICRO-ORGANISMS AND PLANT GROWTH

Fig. 2.1. Effect of **A. Chroococum** inoculation on the growth of **C. tora**, L. in Iron Ore Mine Waste.

1. Iron ore mine waste (Control)
2. Iron ore mine waste + 1% straw (Control)
3. " + 2% "
4. " + 3% "
5. " + 1% straw + **A. Chroococum**
6. " + 2% "
7. " + 3% "

Fig. 2.1



0 - Unamended
2 - Amended with 2% dried ground rice straw

1 - Amended with 1% dried ground rice straw
3 - Amended with 3% dried ground rice straw

Fig. 2.2 - Effect of **Azotobacter chroococum** inoculation on the height of **Cassia tora**, L. in Iron Ore Mine Waste.

Table 2.6. Inoculated Population of Azotobacter chroococum from the Rhizosphere of Cassia tora, L. grown in Mine Waste

Treatment	**A.chroococum/g of dry soil** **30 days**	**45 days**	**At harvest**
Unamended inoculated (control)	13.5×10^4	9.2×10^3	5×10^2
Iron ore Waste + 1% rice straw + **A. chroococum**	15×10^4	16.5×10^4	16.6×10^4
Iron ore waste + 2% rice straw + **A. chroococum**	17×10^4	17.6×10^4	18.1×10^4
Iron ore waste + 3% rice straw + **A. chroococum**	20×10^4	21×10^4	21.5×10^4

Azotobacter counts are mean of replicate samples.

Table 2.7a. Effect of *A. chroococum* Inoculation on Morphological Characteristics of *Cassia tora,* L. in Iron Ore Mine Waste.

Treatment	Ht. of plant cm	Size of 5th internode cm	Length of flag leaf cm	Age of plant at flowering	No. of flowers/ plant	Flowering node no.	Size of pod cm
Iron mine waste	14.2 ± 1.71	3.1 ± 0.21	4.1 ± 0.25	99	2	13th	14.0 ± 0.39
Iron mine waste + 1% rice straw	15.5 ± 2.01	3.1 ± 0.25	4.2 ± 0.21	97	2	13th	14.3 ± 0.22
Iron mine waste + 2% rice straw	17.0 ± 1.8	3.2 ± 0.30	4.2 ± 0.39	97	2	13th	14.4 ± 0.09
Iron mine waste + 3% rice straw	22.7 ± 2.62	3.4 ± 0.28	4.3 ± 0.26	96	3	12th	14.5 ± 0.05
Iron mine waste + **A chroococum.**	16.8 ± 2.48	3.3 ± 0.39	4.3 ± 0.18	93	3	12th	14.5 ± 0.03
Iron mine waste + 1% rice straw + **A. chroococum**	21.1 ± 2.5	3.5 ± 0.41	4.3 ± 0.11	93	3	10th	14.6 ± 0.10
Iron mine waste + 2% rice straw + **A. chroococum.**	23.6 ± 1.87	3.8 ± 0.37	4.6 ± 0.31	90	4	11th	14.8 ± 0.10
Iron mine waste + 3% rice straw + **A. chroococum.**	27.3 ± 2.91	5.01 ± 0.72	5.0 ± 0.40	83	6	9th	15.2 ± 0.20

± Denotes standard deviation.

Flower emergence and fruit formation were advanced by a week or more in case of inoculated amended plants. The number of flowers varied in each treatment at flowering. The treatment with 3 percent straw and inoculated gave six flowers after 83 days, followed by in plants inoculated and amended with two percent and one percent respectively. However, there was no significant difference in early flower emergence and the number of flowers among the controls. Moreover, flowering occured at lower nodes and there was a difference in flowering node among the inoculated plants. But no significant change was observed in case of their corresponding controls.

In plants amended with 3 percent straw flowering was observed at ninth node, but in 2 percent straw it occured at 11th node, but again in 1 percent the flowering reverts to the 10th node.

There was an appreciable change in the size of pods in control, as well as in inoculated plants. Moreover, the difference among the control was not much significant, whereas in the treated plants there was relatively marked difference in size of pods. Similarly, there was an increase in number of seeds/pod by a factor of one in the inoculated plants. Such a difference was not observed among the controls. Number of seeds/pod were co-related to the pod size. Early fruit ripening was observed in treated plants and there was no variable change among the inoculated plants, whereas there was a delay in controls.

Inoculation increased yield in terms of plant dry weight by 7-22 percent. The increase in shoot and root dry weight was observed both in the control and inoculated treatments. The increase in shoot dry weight in control was relative to the increase in dry weight of inoculated plants. The dry weight in controls ranged from 2.738 -2.986 g/plant, whereas in inoculated it was 2.892 -3.610 g/plant. Similiar increase was observed in the root dry weight and ranged from 0.501 - 0.784 g/plant in control and 0.588 -1.261 g/plant in inoculated plants respectively (Table-2.7b).

Table 2.7b. Effect of A. chroococum Inoculation on Yield of Cassia tora, L. in Iron Ore Mine Waste.

Treatment	Mean Shoot dry wt. g.	Mean Root dry wt. g.	No. of pods/ plant	Nitrogen in shoots. mg/plant	Nitrogen in shoots. %	No. of seeds per/pod	% Effect on yield
Iron mine waste	2.738	0.501	7	11.5	2.38	25	-
Iron mine waste + 1% rice straw	2.790	0.596	7	11.6	2.405	25	-
Iron mine waste + 2% rice straw	2.845	0.705	10	11.6	2.452	26	-
Iron mine waste + 3% rice straw	2.986	0.784	11	11.8	2.53	26	-
Iron mine waste + **A.chrococum**	2.892	0.588	13	11.9	2.43	27	6.93
Iron mine waste + 1% rice straw + **A. chroococum.**	3.117	0.717	16	12.4	2.51	29	11.68
Iron mine waste + 2% rice straw + **A. chroococum.**	3.263	0.933	17	12.7	2.56	32	15.40
Iron mine waste + 3% rice straw + **A. chroococum.**	3.610	1.261	22	13.1	2.75	33	22.60

There was also an increase in pod yield at maturity. Although the number of pods increased from 7-11/plant in case of amended controls with increase in amendments, but there was significant increase for inoculated from 13-22/plant. There seems to be a co-relation between the number of pods and percentage of straw added, both in controls and treated plants. Levels of nitrogen in the shoots where markedly effected by inoculation. Total nitrogen content was much higher in the inoculated plants than their corresponding controls. It increased with increase in percentage of straw added and in controls it varied from 8.1-8.8 mg/plant. Whereas, in the inoculated it was 9.3-10.1 mg/plant.

2.4. DISCUSSIONS

2.4.1. Physical and Chemical Analysis of Iron Ore Mine Waste from Various Dumps at Pale-Mines.

The physical and chemical properties of various iron ore mine waste dumps differed considerably (Table-2.1). The variation in the waste's properties may be probably due to the nature of overburden and underlying clays encountered during the process of mining, their random and the changing patterns of dumping and piling. This is a common feature in any mining operation. The age of the dumps which is uncertain to determine, also contributes to the variation in properties of the waste.

Iron ore mine spoils are not true soils. Mine spoils lack organic matter and normally have low levels of available nitrogen(N), phosphorous(P) and sometimes potassium(K) (Cjapowskyj, 1973, Jeffrey **et al.** 1975, Grandt, 1978). The normal field soil taken for comparison had organic carbon (1.5%), nitrogen (0.137%), phophorous-P_2O_5 (48kg/ha) and potassium -K_2O (120kg/ha). The iron ore mine dump waste had values much lower than these normal values as can be seen in Table-2.1. The percentage of these macro-nutrients varied from dump to dump, and also the pH from 6.0 to 6.5. It is the unique physical and chemical characteristics of each mine waste which determine the degree that both plants and micro-organisms can become established on these sites.

2.4.2. Microbiological Analysis of Iron Ore Mine Waste from Various Dumps at Pale-Mines.

Bacterial population counts made from different dumps (Table-2.2) are not a measure of actual microbial activity but they do reflect overall site potential for micro-organisms. The result of bacterial population of different iron ore waste dumps of different ages indicated that the number was much lower (ranging from 9.3×10^4 to 3.7×10^4/g of dry soil) than normally found (61.2×10^6/g of dry soil) in undisturbed top soil or agricultural soil.

The values obtained in iron ore mine waste can be co-related to the organic matter content of the waste. Muller (1973) found in lignite coal spoils that even after 5-10 years of cultivation with crops, population of bacteria were estimated to be under 10^5/g of spoil. He attributed this low bacterial count to the poor organic matter of the waste. The iron ore mine waste dumps in the present investigation were barren and unvegetated. Therefore, age of the dumps or mine waste appeared to have little correlation with microbial population. However, besides organic matter content, moisture contents and pH and other soil conditions could probably be the most important bacterial population controlling factors in the iron ore mine waste.

The occurrence of non-symbiotic nitrogen fixing bactria like **Azotobacter** is known to depend on the variuos soil factors like pH, moisture content, depth, availability of nutrients, organic matter etc. (Krishnamurthi, 1962; Subramoney and Abrahim, 1962). This shows that the soil microflora are characteristically influenced by physical and chemical properties of thier soil milieu. The results of the present investigation, on the reduction in total bacterial population from 9.8×10^4 - 3.78×10^4 /g of soil and **Azotobacter** population from 3.4×10^4 - 2.2×10^2/g of soil (Table- 2.2) in iron mine waste compared to natural agricultural soil or field soil, which is 61.2×10^6/g of soil (total bacterial population) and 40.9×10^2/g of soil (total **Azotobacter** population) respectively, can be co related to the abnormal nutritional level, organic matter content and moisture percentage of the waste (Table- 2.1).

2.4.3. Rhizosphere Population of Cassia tora, L. in Natural Soil and Mine Waste.

The natural distribution of total bacterial population of rhizosphere of **Cassia tora** in natural soil was much higher (72 x 10^6/g of soil) than the one in mine waste rhizosphere (25 x 10^4/g of soil). Similarly, the **Azotobacter** population in mine waste rhizosphere was much lower (3.5 x 10^2/g of soil) than natural rhizosphere soil (1 21 x 10^3/g of soil) as can be seen in Table-2.3. Although age and health of the plant at sampling could be the controlling factors but the soil conditions, its physical and chemical properties play an important role.

Total bacterial population of the iron ore mine waste rhizosphere of **Cassia tora** is greater (25 x 10^4/g of soil) than the waste itself (49 x 10^3/g of soil). Similar is also the case with the natural rhizosphere soil of **Cassia tora** (Table 2.3). This concordant with the observations made by Shetron **et al.**(1977) in iron ore tailings with **Alfalfa** and grass (**Fescue** sp.) He showed that the total rhizosphere population of **Alfalfa** was (1.9 x 10^5/g of soil) and grass (2.86 x 10^5/g of soil). whereas, in tailings itself, was (1.140 x 10^3/g of soil) and (5.9 x 10^2/g of soil) for **Alfalfa** and grass, respectively.

Strzelezyk (1958) has shown that rhizosphere soil contained more micro-organisms antagonistic to **Azotobacter** than root-free soil, Brown **et al.** (1962) suggested that, there is also probably intense competition between many microbes for food oxygen, moisture etc. and the few **Azotobacter** may be suppressed. The low numbers observed in the present findings may not only be attributed to the variable physical and chemical conditions of the soil, but can be also attributed to the observations of the above workers.

2.4.4. Morphological, Cultural and Biochemical Characteristics of Azotobacter Isolated from Rhizosphere of Cassia tora, L. Growing Around the Iron Ore Mines.

Eleven isolates obtained from the rhizosphere of **Cassia tora** differed in thier morphological, cultural and biochemical characteristics (Table-2.4). According to Bergy's manual (1957) and

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EXPLANATION OF PLATE - V

IDENTIFICATION OF AZOTOBACTER SPECIES

Fig. 2.2. - **Azotobacter chroococum** (10 x 45u)
Fig. 2.3. - **Azotobacter vinelandii** (10 x 45u)

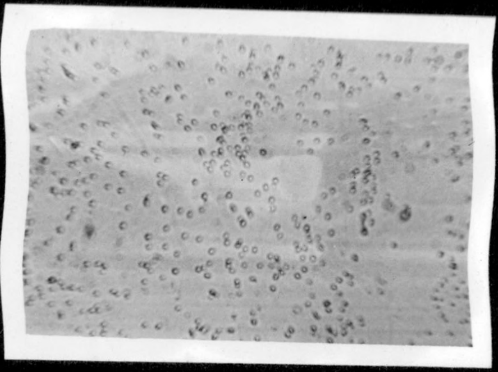


Fig. 2.2

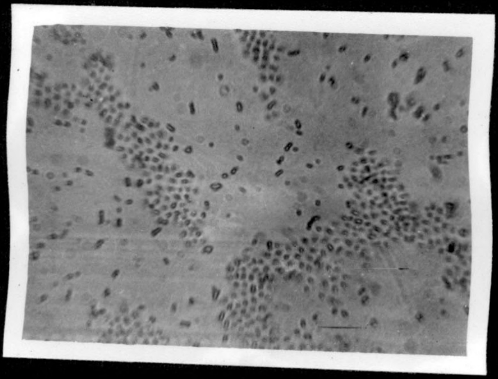


Fig. 2.3

Gibbs and Shapton (1968), the isolates no. 1, 5, and 9 were identified and classified to be **A. macrocytogenes** isolates no. 2, 3, 8 and 10 were **A. vinelandii** and isolates no. 4, 6, 7 and 11 were found to be **A. chroococum**. All isolates fixed nitrogen efficiently in nitrogen deficient liquid medium. The significant differences in nitrogen fixing capacities among all three species indicate the prevalence of strain variation (Table-2.5) in the species occuring in the iron ore mine wate. **Azotobacter chroococum** (Fig. 2.3) and **A. vinelandii**(Fig. 2.4) form short rods, which occur singly or in pairs and form cysts. **Azotobacter macrocytogenes** are coccoid cells produced slime and capsule formation, many tetrads and large clusters are formed. **Azotobacter chroococum** strain which has been found to fix 17 mg/g of carbon source could be suggested as a potential species in re-vegetation of iron ore mine waste for **Cassia tora** seed inoculation, as one of the initial step in reclamation of iron ore waste dumps.

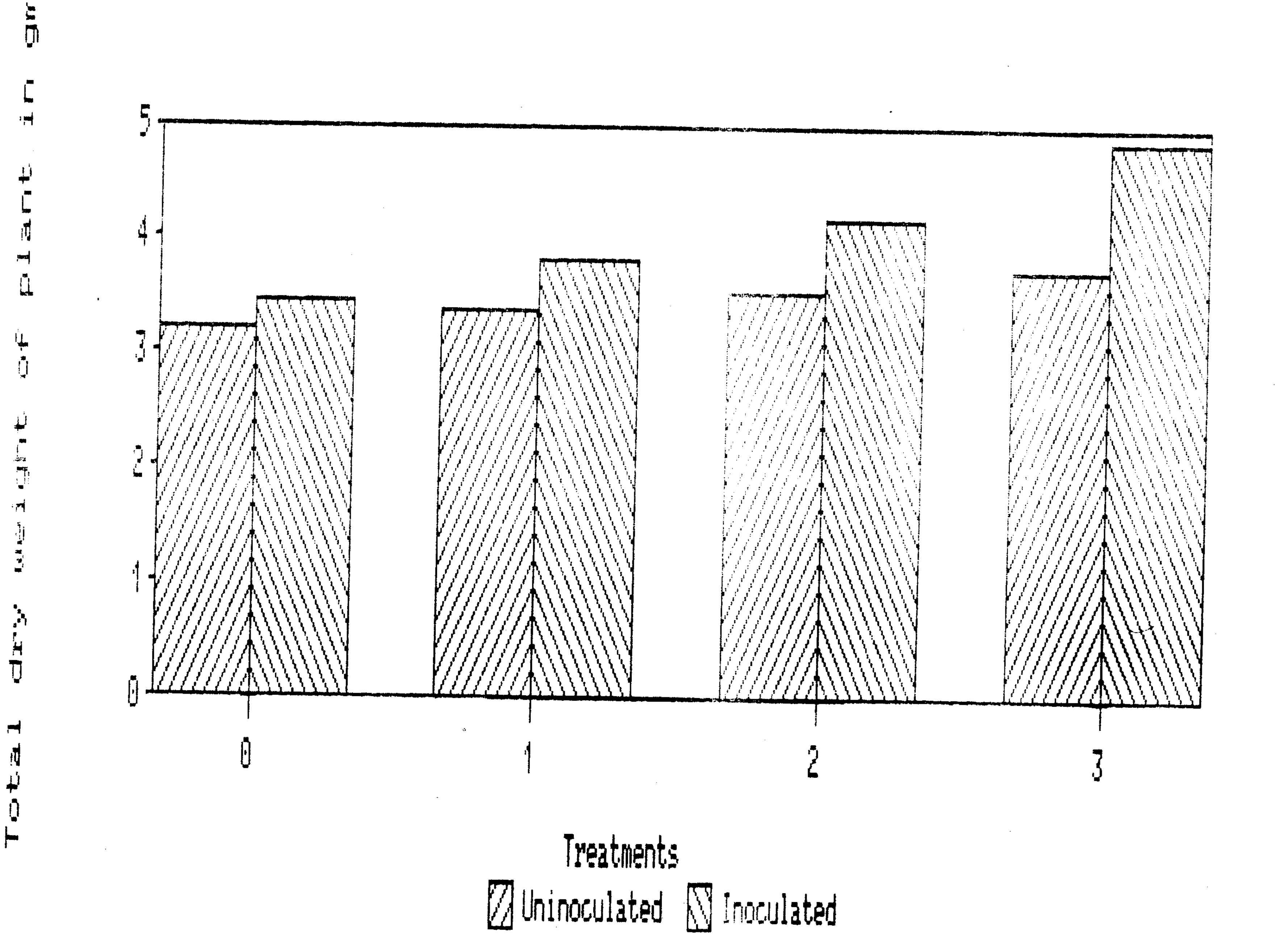
2.4.5 Recovery of Inoculated Azotobacter chroococum from the Rhizosphere of Cassia tora. L.

The low numbers of **Azotobacter** (3.5×10^2/g of soil) as seen in Table-2.3, can be substantially increased by inoculating either seeds, roots or soil. **Azotobacter** which was inoculated to the seeds established in the rhizosphere of seedlings and remained throughout the plant life, under favourable conditions. **Azotobacter** colonizes the whole root system from an initial seed inoculum, indicating continuous multiplication of cells.

The present result showed that under suitable conditions, **A. chroococum** grew in plant rhizosphere. The initial decline in the numbers of **A. chroococum** at 30 days, and rise at maturity (Table-2.6) may be due to the time lag for adjusting to the supplied energy source that is apparently available.

2.4.6. Effect of A. chroococum on Morphological and Growth Yield Characteristics of Cassia tora. L. in Iron Ore Mine Waste.

The result embodied in Table 2.7a and b show that inoclation of germinating seeds with **A. chroococum** had a high potential of accelerating the yield of **C. tora** in iron ore mine waste. The



0 - Unamended
1 - Amended with 1% dried ground rice straw
2 - Amended with 2% dried ground rice straw
3 - Amended with 3% dried ground rice straw

Fig. 2.5 - Effect of **Azotobacter chroococum** inoculation on the total dry weight of **Cassia tora**, L. in Iron Ore Mine Waste.

overall increase in yield was pronounced in inoculated plants amended with 1, 2 and 3 percent ground rice straw. The dry matter yield increased by 6-22 percent over the amended uninoculated control. It is evident from the result that adding suitable substrate to the waste resulted in an appreciable increase in growth yield (Fig. 2.5) and nitrogen content of **C. tora**.

In experiments conducted at Rothamsted a significant increase in crop yield was obtained due to **Azotobacter** inoculation (Jackson **et** al.1960). In green house experiments with plants grown in vermiculite or sand, it has been demonstrated that inoculation of **Setaria italica** with **A. chroococum** enhanced root development. It has been shown recently that this positive effect on roots result in increased mineral (N, P and K) uptake by the roots of corn and sorghum (Lin **et al.** 1983). In addition, the time required for heading and inflorescence development was shortened in **Setaria** inoculated with either **Azospirillum** or **Azotobacter** as compared to non-inoculated control. This phenomenon is observed in wheat, sorghum and **Panicum** in the green house, and in the field (Kapulnik **et al.** 1981; Kapulnik **et al.** 1983; Lin **et al.** 1983 and Sarig **et al.** 1984). It may be caused by better mineral nutrition and water potential in the inoculated plant (Yahalom **et al.** 1984). The present findings in **C. tora** grown in iron ore mine waste confirms these earlier reports.

The significant accelerated plant growth internodal elongation of stem, leaf length, early flowering shoot and root dry weight, number of pods total nitrogen in shoots etc. has been reported by earlier workers by inoculating **Azospirillum** or **Azotobacter** sp. in wheat sorghum and **Panicum** (Kapulnik **et al.** 1981 Yahalom **et al.** 1984) and Jackson **et al.** (1964) with **Lycopersicum escullentum** using **A. chroococum**. The present investigations are concordant with the findings of above workers.

The present results together with the previous reports clearly indicate that inoculation with **A. chroococum** enhances plant growth and N content, it can be proposed that the increase in growth and total N content resulted from the utilization by plants the nitrogen fixed by the bacteria. Brown (1982) suggested that the inoculation of **A. chroococum** occasionally promoted yield probably by mechanisms, other than biological nitrogen fixation.

The present work clearly suggests that the benefits of **A.chroococum** inoculation in **Cassia tora** hastens the plant growth and partially overcomes the universal nutrient budget deficiency of wastes like nitrogen.

Thus **A.chroococum** associated with the roots of **Cassia tora** may benefit the plant both by producing growth hormones (Brown,1963) and by nitrogen fixation, particularly at later stages of growth when the plant needs for nitrogen increases during flowering and seed formation. Growth regulators produced by the rhizosphere bacteria may help in modulation of growth and hence to overcome the stunted growth of plants observed in mine waste reclamation.

The growth enhancing effects are of interest because of their potential significance for yield increases in agronomic system in which the use of fertilizers is the limiting factor for their development.

Probably the most important factors which will limit the N fixation in mine waste is the lack of suitable organic substrate. The experiment showed clearly that substrates such as rice straw can support bacterial growth and nitrogen fixation in iron ore mine waste.

In conclusion the outcome of present study would help to avoid the ever growing danger of nitrate pollution through indiscriminate use of mineral nitrogen fertilisers, and save energy costs. A problem which is of much concern to the mining industry desiring to stabilize the mine wastes by revegetation.

The success of any reclamation scheme whether it involves agricultural restoration or natural vegetation will depend on a continous supply of nutrients especially nitrogen to the plant. Such a large capital of nitrogen that is required can however be most economically achieved through development of a nitrogen cycle rather than continual reliance on chemical fertilizers so much so that the defifiencies or nutrient losses (nitrogen) can be made good substantially by stimulating a natural source.

2.5. ESTABLISHMENT AND SELECTION OF SUCCESSFUL VESICULAR - ARBUSCULAR MYCORRHIZAL FUNGI FOR LEUCAENA LEUCOCEPHALA (LAM.) DE WIT. IN IRON ORE TAILINGS.

It is well known that vesicular arbuscular (VA) mycorrhizas can play an important role in the nutrition of plants growing in soils of low fertility (Mosse 1973; Tinker 1975). Successful revegetation after mining will depend upon the re establishment of nutrient cycling. Vesicular arbuscular mycorrhizas are likely to be important in nutrient cycling in native ecosystem (Sward 1978; Titze **et al.** 1979; Malajezuk **et al.** 1981; Warcup and McGee 1983; Peterson **et al.** 1985). However, factors associated with surface mining i.e removal of growing plants soil disturbance and soil storage / dumping could each be expected to decrease the number of viable propagules of VA mycorrhizal fungi.

Wilson (1965) and Dean and Havens (1970) have postulated that the lack of suitable micro organisms might be a deterrent to the development of vegetation on mine tailings The importance of mycorrhizal fungi in the establishment and development of forest trees on such adverse sites has been widely acknowledged (Harley 1969;Bjorkman 1970 and Hackskaylo 1972). Schramn (1966) has found that mycorrhizal fungi are an important component in establishing vegetation on anthrocite coal refuse Deliberate re establishment of effective mycorrhizal fungi could reduce the need for fertilizers (Jehne & Thompson 1981). Schramn (1966) noted that where mycorrhizae were absent plants showed negligible growth chlorotic or subnormal color and often complete necrosis

The selection of endomycorrhizae for use with a particular plant species on certain sites has been receiving increased attention (Mosse 1975; Marx 1977). Selection can only be achieved by means of comparisons performed in untreated waste with phosphorous supply limiting plant growth But however available literature reveals that there are no the need for fertilizer application, particularly phophorous, in iron ore mine waste reclamation. Helyer and Godden (1977) have estimated that the introduction of VA mycorrhizal fungi would decrease the amount of fertilizer required in the establishment phase.

Leucaena leucocephala is an important fodder tree legume, also used for land reclamation, erosion control, re-forestation, shade and hedge in many parts of the world (Duke, 1981). It is extensively used in the revegetation of iron ore mine waste in Goa. **Leucaena** has virtually no root hairs and strongly mycorrhizal (Munns and Moses, 1980). **Leucaena** plants growing in iron ore tailings show stunted and negligile growth probably due to the absence of suitable host-fungi specificity. Inoculation with **Rhizobium** and VA mycorrhiza is known to improve growth, nodulation and nitrogen fixation in **Leucaena** species and several leguminous crops (Redente and Reeves, 1981; Azcon-Aguilar **et al.** 1982; Manjunath **et al.** 1984).

It is transplanted legume tree and hence would be easier to raise mycorrhizal seedlings with samll quantities of inocula before transplanting to the adverse sites. Hence in the present study it was considered worthwhile to (1) isolate~~s~~ an effective native rhizobial strain and (2) examine the establishment and selection of successful Vesicular Arbuscular Mycorrhizal (VAM) fungi for **Leucaena** plants in iron ore tailings.

2.6. MATERIALS AND METHODS

2.6.1. Isolation of an Effective Native Rhizobia for Leucaena leucocephala (Lam.) de Wit.

Healthy pink root nodules were collected from **Leucaena** plants (3-4 months old) which were growing in and around the iron ore mines at Pale. The nodules were washed thoroughly with distilled water to remove gross surface contaminants. They were surface sterilized in 95 percent ethanol and in 0.1 percent acidified $HgCl_2$ for five minutes. The acidified nodules were again washed several times with sterile water. The nodules were crushed aseptically and milky fluid obtained was diluted with sterile water and spread over the surface of plates of yeast extract mannitol agar (YEMA) containing 0.002 percent actidione added separately before plating. The inoculated plates were incubated at room temperature for 48 hours and distinct individual colonies were isolated on YEMA. The isolates were characterized on the basis of morphological, cultural characteristics and biochemical properties. Isolated **Rhizobium** cultures were maintained on YEMA slants. Inocula were grown in YEMA broth having same composition as YEMA excluding agar.

For plant infectivity test and screening an effective strain, four kilograms of coarse river sand was washed and sterilized for one hour at 20 lbs pressure to eliminate contaminants. This was later taken in black polybags (8 x 12").

Healthy, uniform size seeds of local **Leucaena** cultivar were rinsed in 95 percent alcohol and were immersed for 3 min. in 0.2 percent of acidified $HgCl_2$. The seeds were washed thoroughly with sterile water and were exposed to hot water at 60°C, till the water attained room temperature.

Seeds were allowed to germinate aseptically on plain (water) agar in petri-dishes at 29°C. The germinated seeds were carefully transfered to polypots (one seed/pot) filled with sterile sand. Five days old seedlings were inoculated with heavy 2 ml suspension of five days old cultures of test strains, control was maintained. The plants were watered regularly to field capacity with distilled water and received equal quantity of Fahraeus medium (nitrogen-free) once a week. Five plants of each treatment were selected at random after 60 days of inoculation. Efficient nodules were observed on the roots. Plant and nodule dry weight were recorded. Total nitrogen in the shoots was determined by micro Kjeldahl's method. The best strain was used for inoculation in iron ore tailings.

2.6.2. Establishment and Selection of Successful VAM Fungi for Leucaena leucocephala (Lam.) de Wit in Iron Ore Tailings.

Four different VAM fungi, viz; **Gigaspora margarita, Glomus**

fasciculatum; Glomus macrocarpum and **Glomus mossae** (maintained as pot cultures using **Panicum maximum** Jacq,as the host) were obtained from the University of Agricultural Sciences, Bangalore (Courtesy, Dr. D.J. Bagyaraj, Assoc. Prof. of Microbiology), was used as an inoculum and were screened against the local cultivar of **Leucaena leucocephala** for their symbiotic response, in phosphorous deficient iron ore tailings, with pH 6.7. Mycorrhizal spore numbers in the inoculum were estimated by wet sieving and decantation technique (Gerdeman and Nicolson, 1963). The iron ore talings were screened through 2 mm sieve and sterilized at 20 lbs pressure for one hour. Six kilograms of sterilized tailings were filled in black polythene bags (8 x 12"). Fifty grams of the inoculum was placed in the planting hole of tailings. Sterilized and pre-germinated seeds of local **L. leucocephala** were then sown. Effective native **Rhizobium** strain LL-3 isolated from the local **L. leucocephala** was inoculated at sowing by adding 2 ml suspension of 6 days old culture with a population of 1.68×10^8 cells / ml suspension in planting point. Plants were treated as below:

1 - Uninoculated control.
2 - **L.leucocephala** + **Rhizobium** (LL-3 strain).
3 - **L.leucocephala** + **Rhizobium** + **Giagaspora margarita**
4 - **L.leucocephala** + **Rhizobium** + **Glomus fasciculatum**
5 - **L.leucocephala** + **Rhizobium** + **Glomus macrocarpum**
6 - **L.leucocephala** + **Rhizobium** + **Glomus mossae.**

Each treatment was replicated five times. Two seedlings were maintained per pot. Height of the plant, size of leaf and chlorophyll was determined after hundred days. Mycorrhizal spore numbers in soil were estimated by wet sieving and the decantation method of Gerdemann and Nicolson (1963). The percentage mycorrhizal infection of the roots was determined by clearing the roots with ten percent KOH and staining with trypan blue (Phillips and Hayman, 1970).

Dry weights of shoot, root and nodules were recorded. Total nitrogen in the shoots was determined by micro-Kjeldahl's method and phosphorous content of the plants was determined by the vanadomolybdate phosphoric yellow colour method (Jackson, 1973).

2.7. OBSERVATIONS

2.7.1. Isolation of an Effective Native Rhizobia for Leucaena leucocephala (Lam.) de Wit.

In all twelve isolates were obtained from the pink and healthy nodules of local **Leucaena** plants growing around the mines. Among them only eight isolates were identified and confirmed as strains of **Rhizobium**, based on thier morphological and physiological characters (Table-2.8). All strains isolated were motile, gram negative and grew luxuriantly on yeast extract mannitol agar (YEMA). Strains no. 1,2,5,9 and 11 formed medium size, circular, watery white and gummy colonies, certain strains no.3,6 and 10 produced large, circular, watery white and gummy colonies, strain no. 4 formed small irregular, creamy and gummy colonies, strains no. 7,8 and 12 formed small circular creamy and gummy colonies. All strains showed positive growth on congo red YEMA plates with no absorption of the dye. All strains except no. 4,7,9 and 12 were lactose negative. Similarly, strains no. 4,7,9 and 12 gave negative results for Bromocresol purple test, but rest of the strains were all positive, strains no. 5,10 and 12 showed mild growth on peptone glucose agar whereas, the remaining strains did not show any positive indication. Hydrogen sulphide (H_2S) gas formation was observed only in strains no. 4,7 and 12. All mature cells of strains except strains no. 4,7,10 and 12 produced distinct and prominent granules of polymer of B-hydroxybutyrate.

Results on the performance of effective strains of **Rhizobium** from confirmed inoculants are depicted in Table-2.9. All strains tested in sand cultures with **Leucaena** as a host produced nodules. No nodules were observed in the control plant. Visual observations show that nodules produced by strains LL-2, LL-5 and LL-8 were smaller to medium size and effective, and that of strains LL-6 and LL-7 were small, minute ineffective and lacked distinct pink colour. Whereas, nodules produced by strains LL-1, LL-3 and LL-4 were larger in size, bulky and sometimes bilobed and rich in leghemoglobin. The results clearly indicates that, of the three effective strains of **Rhizobium**, viz; LL-1, LL-3 and LL-4, plants inoculated with LL-3 showed a significant increase in the total

Table 2.8. Characteristics of *Rhizobium* strains isolated from *Leucaena Leucocephala* (Lam) de Wit.

Rhizobium Strain	Colony characters on YEMA plates.	Gram staining.	Growth on Congo Red YEMA plates	Lactose medium test	Bromocresol purple test	Growth on Peptone Glucose Agar.	H_2S formation	Polyhydroxy butyrate test
1*	Medium size circular watery white and gummy	-	+	-	+	-	-	+
2*	-do-	-	+	-	-	-	-	+
3*	Large,circular,watery white and gummy	-	+	-	+	-	-	+
4	Small,irregular,creamy and gummy	-	+	+	-	-	+	-
5*	Medium size, circular watery white and gummy	-	+	-	+	+	-	+
6*	Large,circular,watery white and gummy	-	+	-	+	-	-	+
7	Minute, circular,creamy and gummy	-	+	+	-	-	+	-
8*	Small,creamy,circular and gummy	-	+	-	+	-	-	+
9*	Medium size, circular watery white and gummy	-	+	+	-	-	-	+
10	Large,circular,watery white and gummy	-	+	-	+	+	-	-
11*	Medium size, circular watery white and gummy	-	+	-	+	-	-	+
12	Small, creamy, circular and gummy	-	+	+	-	+	+	-

* Identified to be **Rhizobium**

Table 2.9. Screening of Effective Native *Rhizobium* strains for *Leucaena leucocephala* (Lam.) de Wit.

Rhizobium strains.	Population/ml. of inoculum.	No. of nodules/ plant.	Nodule dry wt. per plant (mg)	Plant dry wt. (g)	Total nitrogen mg/plant.
Control	-	-	-	1.98 ± 0.17	18.1 ± 2.3
LL-1	1.61×10^6	25.5 ± 1.3	175.7 ± 2.9	2.8 ± 0.16	34.3 ± 3.1
LL-2	1.56×10^6	20.0 ± 1.0	167.1 ± 1.5	2.4 ± 0.29	30.9 ± 2.7
LL-3	1.73×10^6	38.1 ± 1.7	205.0 ± 2.0	3.2 ± 0.25	40.1 ± 3.0
LL-4	1.43×10^6	31.5 ± 0.5	182.0 ± 2.3	3.0 ± 0.37	36.7 ± 1.2
LL-5	1.65×10^6	19.3 ± 1.1	150.0 ± 0.9	2.3 ± 0.09	27.0 ± 1.6
LL-6	1.70×10^6	12.1 ± 1.8	85.0 ± 2.7	2.0 ± 0.25	17.2 ± 2.1
LL-7	1.75×10^6	18.5 ± 0.5	93.0 ± 2.9	2.1 ± 0.16	21.5 ± 0.9
LL-8	1.67×10^6	22.1 ± 0.9	149.0 ± 3.2	2.5 ± 0.21	23.6 ± 3.2

± Denotes Standard Deviation

plant biomass(3.2g/plant) over all other strains and control. Maximum nodulation (38 nodules/plant) and nodule dry weight (205mg/plant) was observed in plants inoculated with strain LL-3.

2.7.2. Establishment and Selection of Successful VAM Fungi for Leucaena leucocephala (Lam.) de Wit. in Iron Ore Tailings.

Results of establishment and selection of successful VAM Fungi for L. **leucocephala** is given in Table-2.10. All the four VAM Fungi tested hav been found to infect the local **Leucaena** cultivar and established well in tailings. Inoculation with endophyte greatly improved all plant characteristics i. e. healthy growth, large size leaf and darker foliage was observed.

All the four endophytes introduced accelerated plant height which increased markedly in all treatments over the controls (Fig.2.6). Maximum height (38.2 cm)was observed in plants treated with **Rhizobium** and **G. fasciculatum** had larger leaves (leaf length-5.1 cm) compared to the other three species tested, moreover increase in leaf length was also observed in the other three species over the controls. Increase in total chlorophyll content was observed in all treatments, but the chlorophyll content (383 mg/100g) was found to be maximum in plants inoculated with **G. fasciculatum**, as compared to the other treatments. These were closely followed by **G. mossae, G. margarita, G. macrocarpum** and plants inoculated with only **Rhizobium**. Inoculation did not stimulate nodulation, the differences in the number of nodules were not significant, but however appreciable differences were observed in nodule dry weight among all the treatments. Maximum nodule dry weight (78.3 mg/plant) was observed in plants inoculated with **Rhizobium** and **G. fasciculatum**. Increase in shoot and root dry matter was observed in all the **Leucaena** plants treated with VA endophytes.

Maximum dry weight of shoot (3.40 g/plant) and root (1.801g/plant was observed in plants inoculated with **Rhizobium** and **G. fasciculatum** and this was followed by **G. mossae** (shoot dry wt.-2.85 g/plant and root dry wt.-1.20g/plant), **G. margarita**

EXPLANATION OF PLATE - VI

Fig. 2.6. Selection of successful vesicular arbuscular mycorrhizal fungi for **Leucaena leucocephala** (Lam.) de Wit in iron ore tailings.

1. Uninoculated control
2. Inoculated with **Rhizobium** (LL-3)
3. **Rhizobium** + **Gigaspora margarita**
4. " + **Glomus fasciculatum**
5. " + " **macrocarpum**
6. " + " **mossae**

Fig. 2.6

Table 2.10. Screening of an Effective VAM Fungus for <u>Leucaena leucocephala</u> in Iron Ore Tailings.

Treatment	Plant height (cm)	Size of 4th leaf (cm)	*Chlorophyll mg/ 100g	No.of spores in inoculum / 50 g.	No.of nodules per plant	Nodules dry wt. per plant (mg)	Shoot dry wt. per plant (g)	Root dry wt. per plant (g)	% colonization.	No.of spores per 50g of root zone tailings.	+Nitrogen content of shoot mg/plant	Total P mg/plant
1-Uninoculated control.	25.30 ± 1.2	4.5 ± 0.12	225.32	–	–	–	1.37 ± 0.16	0.791 ± 0.05	–	–	23.10	3.2
2-Inoculated with **Rhizobium** (LL-3)	28.39 ± 1.73	4.7 ± 0.02	273.37	–	15.1 ± 2.3	60.3 ± 3.7	1.85 ± 0.05	0.935 ± 0.110	–	–	38.65	3.7
3-**Rhizobium** + **Gigaspora margarita**	31.4 ± 2.1	4.8 ± 0.17	337.08	256	16.7 ± 2.0	70.9 ± 2.9	2.55 ± 0.23	1.117 ± 0.07	51.0	196	56.32	5.1
4-**Rhizobium** + **Glomus fasciculatum**	38.2 ± 2.3	5.1 ± 0.3	383.0	297	16.6 ± 1.0	78.3 ± 3.0	3.40 ± 0.18	1.801 ± 0.06	60.3	215	68.10	6.0
5-**Rhizobium** + **Glomus macrocarpum**	30.5 ± 1.5	4.7 ± 0.15	282.01	263	14.9 ± 2.0	63.1 ± 1.5	2.17 ± 0.07	1.019 ± 0.03	49.6	173	47.91	4.7
6-**Rhizobium** + **Glomus mossae**	35.7 ± 1.9	4.8 ± 0.25	354.6	274	16.5 ± 1.7	71.7 ± 2.6	2.85 ± 0.11	1.20 ± 0.04	55.9	185	65.70	5.6

* Chlorophyll is the mean of three determinations.
+ Nitrogen content is the mean of three determinations.
± Denotes Standard Deviation.

(shoot dry wt.-2.55g/plant and root dry wt.-1.117g/plant)and minimum in plants treated with **G.macrocarpum** (shoot dry wt.-2.17g/plant and root dry wt.-1.019g/plant), shoot dry weight (1.85g/plant) and root dry weight (0.935g/plant) was observed in plants inoculated with only **Rhizobium**. Whereas, the dry matter content in the uninoculated plants, it was shoots (1.37g/plant) and roots (0.791g/plant) respectively. Plants inoculated with VA endophytes greatly increased phosphorous uptake and phosphorous concentrations in shoots, in all the four treatments over the controls. Plants treated with **G. fasciculatum** showed maximum phosphorous (6.0 mg/plant) in shoots than control (3.2 mg/plant). However, the concentration of phosphorous in shoots of plants inoculated with only **Rhizobium** was 3.7 mg/plant. Nitrogen concentration in shoots of VAM inoculated plants relative to the controls was also variable. Plants inoculated with both **Rhizobium** and VA endophytes contained higher amount of nitrogen in their shoots as compared to the controls. Maximum nitrogen content was observed in plants inoculated with **G. fasciculatum** (68.10 mg/plant) and minimum in **G. macrocarpum** (47.91 mg/plant). Total nitrogen content with only **Rhizobium** showed an increase (38.65 mg/plant) over the uninoculated control (23.10 mg/plant).

Inoculation with VAM fungus resulted in infection and extensive colonization of roots of **L. leucocephala** cultivar in all four treatments. No infection was observed in the plants inoculated with only **Rhizobium** and uninoculated control. The plants inoculated with **G. fasciculatum** recorded the maximum percentage of root infection (60.3%), this was closely followed by plants inoculated with **G. mossae** (55.9%), **G. margarita** (51%) and **G. macrocarpum** (49.6%) respectively. However, there was no significant co-relation between the number of spores in the inoculum and percentage root infection in all the four treatments.

All the inoculated fungi produced mycorrhizal spores in the root zone of **Leucaena** plants. The highest count was recorded in the root zone of plants inoculated with **G. fasciculatum** (215 spores/50g of soil) whereas, the lowest was observed in **G.macrocarpum**(173 spores/50g soil). There was no distinct co-relation between the percentage infection and number of spores produced by the fungi in the root zone. Moreover there was a variability between the number of spores estimated in the root zone soil of **Leucaena** plants at the end of the experiment in all the treatments.

2.8. DISCUSSIONS

2.8.1. Isolation of an Effective Native Rhizobia for Leucaena leucocephala (Lam.) de Wit.

From the twelve isolates obtained, only eight isolates no. 1,2,3,5,6,8,9 and 11 were confirmed as strains of **Rhizobium** based on their morphological and physiological characters (Table 2.8).

According to Kleezouska **et al.** (1968) biochemical characters are not diagnostic in case of **Rhizobium**, but the ability to form nodules in the host is the most important characteristics to distinguish **Rhizobium** from other contaminants thus exhibiting a characteristic host specificity. Selection of strains of **Rhizobium** to form an effective nitrogen fixing nodules in the host depends on many criterion (Vincent 1956). Sen (1966) reported that strains of **Rhizobium** of a particular legume would cause the maximum amount of nitrogen fixation only when it is inoculated to the same legume, where edaphic and climatic conditions are almost similar to the place of origin.

The quality of legume inoculants depend on their effectiveness in fixing nitrogen with the intended host (Roughly 1976). Thus the ability of a strain to form nodules and fix nitrogen in particular localized conditions is an important requirement of inoculum quality. So to screen out an effective strain of **Rhizobium** first they must be evaluated based on its effectiveness in nitrogen fixation under laboratory conditions then finally under field conditions (Date 1976).

Although effectiveness in nitrogen fixation in sand culture experiment may not necessarily be upheld under field conditions, it is considered to be a basic and preliminary stage for selecting a cluster of best effective strains to put them under pot and field experiments in which the strains were tested with the host plant. In the present investigation, inoculation with **Rhizobium** strain LL-3 showed a significant increase in the total biomass (3.2g/plant) and nitrogen content (40.1 mg/plant) over all the other strains and uninoculated control (Table-2.9). The results showed that all the strains were not equally efficient.

Similar variations among the different strains of the same species to benefit the host have been reported by many workers in different leguminous crops (Chalal and Joshi, 1978; Vaishya **et al.**1983). Strain LL-3 was most efficient as it showed maximum number of nodules (38 1/plant) plant biomass and total nitrogen content , and can be exploited commercially for local **Leucaena** cultivar inoculation, in iron ore mined land reclamation.

2.8.2. Establishment and Selection of Successful VAM Fungi for Leucaena leucocephala (Lam.) de Wit. in Iron Ore Tailings.

Growth of **Leucaena** plants is severely retarded by basic soil conditions, and this situation may be aggravated particularly when phosphorous and micro-nutrient are limiting. Stunted growth observed in **Leucaena** plants growing in iron ore mine wastes may be probably due to the unavailability of essential plant nutrients. mycorrhiza play an essential role in the development of this legume tree on such sites. Establishment of vegetation on similar areas has depended largely upon the successful development of mycorrhizae (Schramm, 1966). The beneficial effects of inoculating forest trees with vesicular arbuscular mycorrhizal fungi to improve plant growth are well known (Kormanik **et al.** 1976; Janos, 1983; Jeffries, 1987). In several instances the inoculation of coal spoils with certain mycorrhizal fungi greatly increased the tree survival and growth (Nicholas and Hutnik, 1971; Medve **et al.** 1977). Investigators have demonstrated that in areas where soil lacks the appropriate mycorrhizal fungi afforestation without inoculation is generally unsuccessful (Mikola, 1973).

In the present study with iron ore tailings the introduced VA endohpytes established well and all plants inoculated with VAM showed infection and extensive colonization, produced spores in the root zone of **Leucaena** plants (Table-2.10). This indicates that the site has no much inhibitory effect on the symbiosis. Inoculation improved growth, plant height, plant dry matter yield, nodule dry weight, phosphorous uptake and nitrogen fixation, which indicates a better phosphate stimulated nitrogen nutrition. Synergistic effect of inoculation of VAM and **Rhizobia** have been reported by several workers (Smith and Daft, 1977; Azimi **et al.** 1980; Manjunath **et al.** 1984) in various leguminous crops. This is concordant with the present findings.

The increase in dry weight of nodule and stimulation of symbiotic nitrogen fixation by **Rhizobia** has been attributed to better phosphate nutrition of the host (Crush, 1974; Mosse **et al.** 1976; Smith and Daft 1977). Recent findings of Allen **et al.** (1980) suggest that growth hormones such as IAA and cytokinins may be present at higher concentrations in plants having mycorrhizal association than in those that are not. This would certainly contribute to the growth and yield of VAM inoculated crops. The chlorophyll content in the leaf increased (ranging from 273.37-383.0mg/100g) in all the inoculated plants over the control plants (225.32 mg/100). Allen **et al.** (1980) also reported increase in chlorophyll content of plants associated with VAM fungus, which confirms the present findings This may be possibly attributed to the availability of nutrients required for chlorophyll synthesis.

Inoculation with **G. fasciculatum** and **Rhizobium** greatly increased dry matter yield (5.20 g/plant), nodule dry weight (16.6/plant) percentage infection (60.3%) and nitrogen content (68.10 mg/plant) of plant as compared to the other species (Table-2.10). This indicated that the other three species were less efficient in stimulating plant growth. Differences among VAM fungi in their ability to increase nutrient uptake appear to be due to differences in their ability to form mycorrhizas rapidly and extensively (Abbott and Robson, 1982). Comparison of the effectiveness of VAM fungi in increasing plant growth show that there are differences among species. A wide variation among and within different species of VAM fungi in the ability for stimulating plant growth has been observed (Abbott and Robson, 1978; Mosse,1972, Govinda Rao **et al.** 1983).

Of all the four species tested, **G. fasciculatum** was most effecient and established successfully and adapted to the adverse conditions of the iron ore tailings. Hayman **et al.** (1981) pointed out the need for selection of VA endophyte suitable for a particular host, soil, climatic conditions and edaphic environment Although a few species have been tested, there is a need for screening a large number of species to obtain the most successful species for the right environment. Islam and Ayanaba (1981) working with cowpea, stressed the need for selection of a suitable strain of endophyte exogenous or indegenous, suitable for a particular environment.

A relatively high number (256-274/50g of inoculum) of spores were isolated in the inoculum, but the extent of VA mycorrhizal formation was low (49.6-60.3%) in all the treatments (Table-2.10). This means that the formation of VA mycorrhizae does not seem to be closely co-related with the spore count in the inoculum. Whether this poor co-relations are due to low spore viability, spore dormancy or adverse conditions in the tailings effecting germination is difficult to ascertain to accurately predict VA mycorrhizal infectivity. Poor co-relation between colonization by VA mycorrhizal fungi and spore formation has been reported by Allen and Allen (1980), Gould and Liberata (1981). The variability of infection and poor count of spores obtained from the different species indicates that probably a uniform inocula level has not yet been attained.

The number of spores isolated from each treatment did not give a good prediction of the extent of formation of VA mycorrhizae in roots of **L. leucocephala** in iron ore tailings. Porter **et al.** (1987) found that VAM when inoculated into the soil in which they did not occur naturally infected fewer roots and produced fewer spores than in their original soil. This must be perfectly true in the present experiment. It is therefore quite probable that the inability to efficiently sporulate and successfully infect in conditions as prevailing in the tailings might have resulted in production of few propagules in the root zone and poor colonization of roots. It is accepted that fungal spore germiation, hyphal growth and initiation of infection are strongly affected by chemical and/or physical factors of the soil (Munns and Mosse, 1980; Hayman, 1983). The high content of Fe Al and Mn generally present in the iron ore tailings may probably reduce plant growth, and also may reduce spore germination, germ tube growth and hence root colonization by VAM (Daniels and Trappe, 1980; Sequeira **et al.** 1984). Although available P is considered by most investigators to influence the degree of relative growth inhibition or enhancement in mycorrhizal symbiosis (Mosse, 1973), other factors of the environment probably influence plant response to VAM inoculation e.g. soil texture, pH, levels of N and other nutrients, soil water status and biotic factors (Lim and Cole 1984).

The species of introduced VAMF differed in their establishment and effectiveness. The extent of colonization of **L. leucocephala** by **G. fasciculatum** tended to be greater (60.3%) than the other three species tested (Table 2.10) The number of spores in the root zone and level of colonization of roots was also higher as compared to the other species. The increased colonization (60.3%) and greater spore count (215/50g of soil) in the root zone soil of **L. leucocephala** inoculated with **G. fasciculatum** may be due to high proportion of VA mycorrhizae being formed by the endophyte producing large number of spores infecting and re-infecting the roots. This implies that the edaphic conditions existing in the iron ore tailings must be conducive for its propagation establishment and survival, or in other words **G. fasciculatum** must be compatible with the local soil conditions. A successful inoculant fungus has to maintain a high inoculum potential defined by Garrett (1970) as the energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected.

For potential economic achievement with VAM inoculation, the conditions existing in the iron ore tailings may require to be made good or modulated, either by adjustment of adverse soil conditions and inoculation with propagules of VAMF suitable and selected for that site.

The experiment reported here have shown that VAM fungi can establish and succeed in iron ore tailings. **Glomus fasciculatum** can be recommended as inoculant to **Leucaena** seedling before transplantation, as it was found to be the best VAMF to obtain healthy and vigorous growth.

Mycorrhizal inoculation may substitute and reduce the need for phosphorous fertilization and help plants survive in adverse conditions thereby alleviating escalating energy costs.

CHAPTER THREE

IMPACT OF EARTHWORM INTRODUCTION ON IRON ORE MINE WASTE AND PLANT GROWTH

3.1. INTRODUCTION

It is well established that earthworms play a significant role in incorporation and decomposition of plant litter in pasture ecosystem (Sharpley **et al.** 1979). Many studies, most from the temperate regions have indicated the importance of earthworm activity to soil productivity (Barley, 1961). Most of these studies were restricted to pot culture experiments. In India, investigation on this problem, however seems to have been carried out by a few investigators (Nijhawan and Kanwar, 1952). The quantative work on the mechanisms by which earthworms bring about changes in the soil is relatively recent (Syers and Springett, 1984).

The Indian earthworm, **"Pheretima orientalis"** an oligochaete is predominately abundant and active in most moist soils of Goa during monsoons. It's burrowing habit and ingestion of soil along with plant litter churns huge earth during this wet season of the year, thus helping in soil tillage and fertility.

Each year strip mining for iron ore in Goa, exposes about 30-45 million tons of sterile wastes. Strip mining destroys much of soil flora and fauna. Readily dispersed organisms, such as bacteria, fungi, arthropods re-establish themselves soon after a source of food is provided, others such as earthworm repopulate more slowly.

Several surveys of the rejected iron ore waste dumps during monsoon of 1987, revealed that these infertile areas were devoid of this important soil macro-fauna, and there was total absence of this animal even on dumps which were 15-20 years old. Vimmerstedt and Finney (1973) demonstrated the feasibility of introducing earthworms into revegetated acid coal spoils to increase the rate of incorporation of organic matter.

Purposes of these investigations were (1) to determine whether earthworms could be established on iron ore mine waste after suitable amendment (2) to study the physical, chemical and biological properties of waste due to earthworm activity (3) effect of earthworm on plant growth.

3.2. MATERIALS AND METHODS

Earthworms were isolated along with humus during late June 1987, and were stored temporarily in plastic bucket for a week. Moisture was maintained at field capacity. Air dried iron ore waste from dumps was pounded and screened through a 2 mm sieve. Approximately 4 Kg. of iron ore waste was taken in clay pots (25 x 35 cm) thoroughly mixed with dry ground leaves of **Mangifera indica, L.** at the rate of 0, 100, 200 and 300 g. Each pot was inoculated with ten healthy and active earthworms, all of more or less the same size. Similarly, controls were maintained with 0, 100, 200 and 300g of dry ground leaves of **Mangifera indica,** without earthworms. There were five replicates for each treatment. Mouths of the pots were covered with a perforated polythene sheet and kept in a cool shady place at 20-25°C, for four months (July to October). The pots were watered to maintain field capacity. Their activity was occasionally observed at sunset and dawn.

Exactly after four months the survival percentage was determined, the castings were analysed for its physical, chemical and biological properties by standard methods.

All the casts and soils from their corresponding controls were examined microbiologically, by making suitable dilutions with sterile water. Bacterial counts were made after 48 hours, by plating one ml of each dilution in triplicate on nutrient agar plates and incubating at room temprature. Earthworm activated soil and that from the control clay pots was transferred into polybags (15 x 25cm) size. **Crotalaria retusa,** L. a nitrogen fixing leguminous plant, found growing around the iron ore mines was taken as a test plant for growth studies. Growth parameters were recorded at maturity.

3.3. OBSERVATIONS

3.3.1. Establishment of Earthworms on Iron Ore Mine Waste after Suitable Amendment.

At the end of four months, some earthworms were either missing or dead. In the controls the added leaf matter had remained unincorporated, while in the inoculated pots, where the earthworms were active, the leaf matter was found incorporated in the worm casts. The activity of the earthworms influenced the incorporation of plant litter. The ingestion and subsequent incorporation of litter by the earthworms indicated that optimum conditions prevailed.

The observations showed 15 percent survival in unamended control pots, indicating the unsuitability of the environment for its survival due to the absence of organic matter. Whereas, there was a marked improvement in survival percentage of 60, 75 and 80 percent in the pots amended with 100, 200 and 300g of dried ground leaves of **M. indica** (Table-3.1). There was a direct relationship between the survival percentage of earthworm and the quantity of ground plant litter with which the waste was amended. The survival percentage and hence the worm activity increased with increase in plant matter.

Examination of pots after dark showed that the earthworms were active in the soil. Immature earthworms were also observed which indicated that they were capable of reproducing successfully in the iron ore mine waste after suitable amendment with dried ground leaf matter. However, no worms were observed in the control pots

3.3.2. Change in Physical, Chemical and Biological Properties of Iron Ore Mine Waste due to Earthworm Activity.

Physical Properties

Mechanical and physical analysis of iron ore mine waste and worm casts is presented in Table-3.1. The casts sand, silt and clay contents were significantly different from the soils of unamended uninoculated controls. The percentage of sand in casts of unamended waste decreased from 57 to 54.2 percent. Whereas, it was 53.1, 52.6 and 50.6 percent in casts of waste amended with 100, 200 and 300g of leaf matter respectively. The high sand content of 57 percent in the unamended uninoculated control reflect the high sand content in the parent waste.

Conversely, there was an increase in silt content from 18 (in unamended uninoculated control) to 19.4, 19.6. 20.3 and 21.6 percent in casts from pots amended with 0, 100, 200 and 300g of leaf matter. Similarly, the clay percentage increased from 22 to 23.4, 23.7 and 25.9 percent respectively in casts from pots amended with 0, 100, 200 and 300g of dry leaf matter. It was observed that the cast sand content was lower and the silt and clay content were higher than the control iron ore waste. The decrease in content of sand in casts of different treatments is approximately equal to the increase in silt and clay content in the cast. The relative change in the textural composition of the earthworm casts however, was significantly different from one another.

The bulk density of uninoculated controls and casts of different treatments differed from one another, and ranged from 1.25 to 1.30g/cm^3 in controls and 1.35 to 1.45g/cm^3 in casts.

There was a co-relation between the different amendments and moisture content of the casts and their controls. The moisture content of soil increased from 8.7 to 9.5 percent in amended controls and 9.3 to 10.5 percent in casts. The moisture content

Table 3.1. Survival Percentage of Earthworms and Impact on Physical Characteristics of Iron-Ore Mine Waste.

Treatment.	Survival Percentage	Mechanical analysis of waste and casts			Bulk density g/ cm^3	Moisture percentage	Water holding capacity %
		Sand%	Silt%	Clay%			
1. Iron ore waste	-	57	18	22	1.30	8.7	41.6
2. Iron ore waste + 100g dry leaves	-	-	-	-	1.28	9.0	41.9
3. Iron ore waste + 200g dry leaves	-	-	-	-	1.27	9.1	43.1
4. Iron ore waste + 300g dry leaves	-	-	-	-	1.25	9.5	44.0
5. Iron ore waste + Earthworms	15	54.2	19.4	23.4	1.35	9.3	42.0
6. Iron ore waste + 100g dry leaves + Earthworms	60	53.1	19.6	23.7	1.38	9.8	44.7
7. Iron ore waste + 200g dry leaves + Earthworms	75	52.6	20.3	24.1	1.40	10.2	45.2
8. Iron ore waste + 300g dry Earthworms	80	50.5	21.6	25.9	1.45	10.5	47.1

was found to increase with increasing plant material from 0 to 300g in both control and casts. Similar observations were made in case of water holding capacity. The water holding capacity increased from 41.6 to 44.0 per cent in amended controls and 42.0 to 47.1 per cent in casts (Table-3.1).

Chemical Properties

Chemical analysis is embodied in Table-3.2. The pH of the amended controls and casts decreased gradually over the four months period. The pH of the control soils with 0, 100, 200 and 300g of dry leaf matter was 6.8, 6.7, 6.5 and 6.4 and that of the corresponding casts was 6.5, 6.3, 6.2 and 6.0 respectively. Although there was a fractional increase in pH with increase in plant material in the control, but there was a significant pH change in casts.

Earthworm casts had appreciably greater cation exchange capacity (CEC) than their corresponding controls and ranged from 3.9 to 4.5 meq/100g in controls and 5.1 to 5.9meq/100g in casts. It was observed that the differences in cation exchange capacity was fractional for control and casts of different treatments. Similar to the cation exchange capacity, earthworm casts had higher concentration of exchangeable Ca^{+2}, Mg^{+2} and K^{+} than their corresponding control. Exchangeable calcium ranged from 0.05 to 0.15 per cent in controls and 0.08 to 0.18 per cent in casts, exchangeable magnesium ranged from 0.02 to 0.03 per cent in control and 0.029 to 0.042 per cent in casts, whereas the potassium ranged from 0.13 to 0.16 per cent in control and 0.15 to 0.21 per cent in the casts.

Kjeldhal's nitrogen increased from 0.064 to 0.110 per cent in control soils and 0.069 to 0.141 per cent in casts of different treatments amended with 0, 100, 200 and 300g leaf matter. Similarly, it was found that the earthworm casts had appreciably greater Bray's Phosphorous than their corresponding controls, and ranged from 3.0 to 3.9 ppm in control and 3.8 to 5.6 ppm in casts of different treatments. Because earthworms feed on organic matter, most of the nitrogen and phosphorous of casts may have an

Table 3.2. Impact of Earthworms on Chemical Properties of Iron Ore Mine Waste.

Treatments.	pH	OC %	EC m mohos	CEC meq/100g	N %	P ppm	K %	Ca %	Mg %
1. Iron ore waste	6.8	0.35	0.14	3.9	0.064	3.0	0.13	0.05	0.02
2. Iron ore waste + 100g dry leaves	6.7	0.60	0.14	4.0	0.067	3.2	0.13	0.09	0.027
3. Iron ore waste + 200 dry leaves	6.5	0.65	0.16	4.1	0.098	3.7	0.15	0.13	0.028
4. Iron ore waste + 300g dry leaves	6.4	0.72	0.17	4.5	0.110	3.9	0.16	0.15	0.031
5. Iron ore waste + Earthworms	6.5	0.38	0.14	5.1	0.069	3.8	0.15	0.08	0.029
6. Iron ore waste + 100g dry leaves + Earthworms	6.3	0.68	0.15	5.2	0.077	4.0	0.17	0.15	0.035
7. Iron ore waste + 200g dry leaves + Earthworms	6.2	0.79	0.17	5.5	0.135	4.3	0.18	0.17	0.039
8. Iron ore waste + 300g dry leaves + Earthworms	6.2	1.1	0.20	5.9	0.141	5.6	0.21	0.18	0.042

pH - Determined by glass electrode using 1:2.5 soil to water ratio.

OC - Organic Carbon determined by Chromic acid method.

EC - Electrical conductivity in m mohos using 1:2.5 soil water ratio.

CEC - Cation Exchange Capacity by neutral normal ammonium acetate method, meq/100g

N - Nitrogen (%) determined by Kjeldhals' method.

P - Phosphorous (Bray P) in ppm

Ca^{+2}, Mg^{+2} percentage - Ammonium acetate extract method.

organic origin.

The casts were richer in soluble salt content as seen from their higher electrical conductivity figures than those of their corresponding controls. The electrical conductivity of control soil amended with 0, 100, 200 and 300g of leaf matter were 0.14, 0.14, 0.16 and 0.16 m mohos respectively, whereas that of the corresponding casts were 0.14, 0.15, 0.17 and 0.20 m mohos respectively.

Biological Properties

Biological properties indicated in Table-3.3, showed that the agar plates recorded an increase in total bacterial count with increasing plant litter but however, the total count was relatively higher in casts than their corresponding controls and varied from 3.7 x 10^3 to 8.2 x 10^3/g of soil in control and 3.9 x 10^3 to 5.9 x 10^4/g of casts. The casts may have probably made the environment more conducive for their successful proliferation

Effect of Earthworm on Plant Growth

The result of the effect of earthworms on growth of **Crotalaria retusa,** L. are shown in table 3.4. The observations showed an increase in yield in terms of height (Fig. 3.1), dry weight (Fig. 3.2) and pods (Fig. 3.3) in all the treatments. There was nearly 20-25 per cent increase over the controls. The growth in terms of height in control was 44.4, 48.0, 55.0 and 60.21 cm in pots amended with 0, 100, 200 and 300g of leaf matter and 48.5, 62.1, 73.5 and 84.5 cm respectively in earthworm inoculated pots. The dry weight ranged from 6.619 to 7.405g/plant in control and 6.800 to 8.162g/plant in the experimental pots. The experimental plants were dark green, broad leaved and bushy with profuse branching than their controls. Increase in root length in all the treatments was observed, moreover the root length of plants in experimental pots was relatively greater than their corresponding contols, and ranged from 12.30 to 19.17 cm in

Table-3.3. Impact of Earthworm on Bacterial Population in Iron Ore Mine Waste.

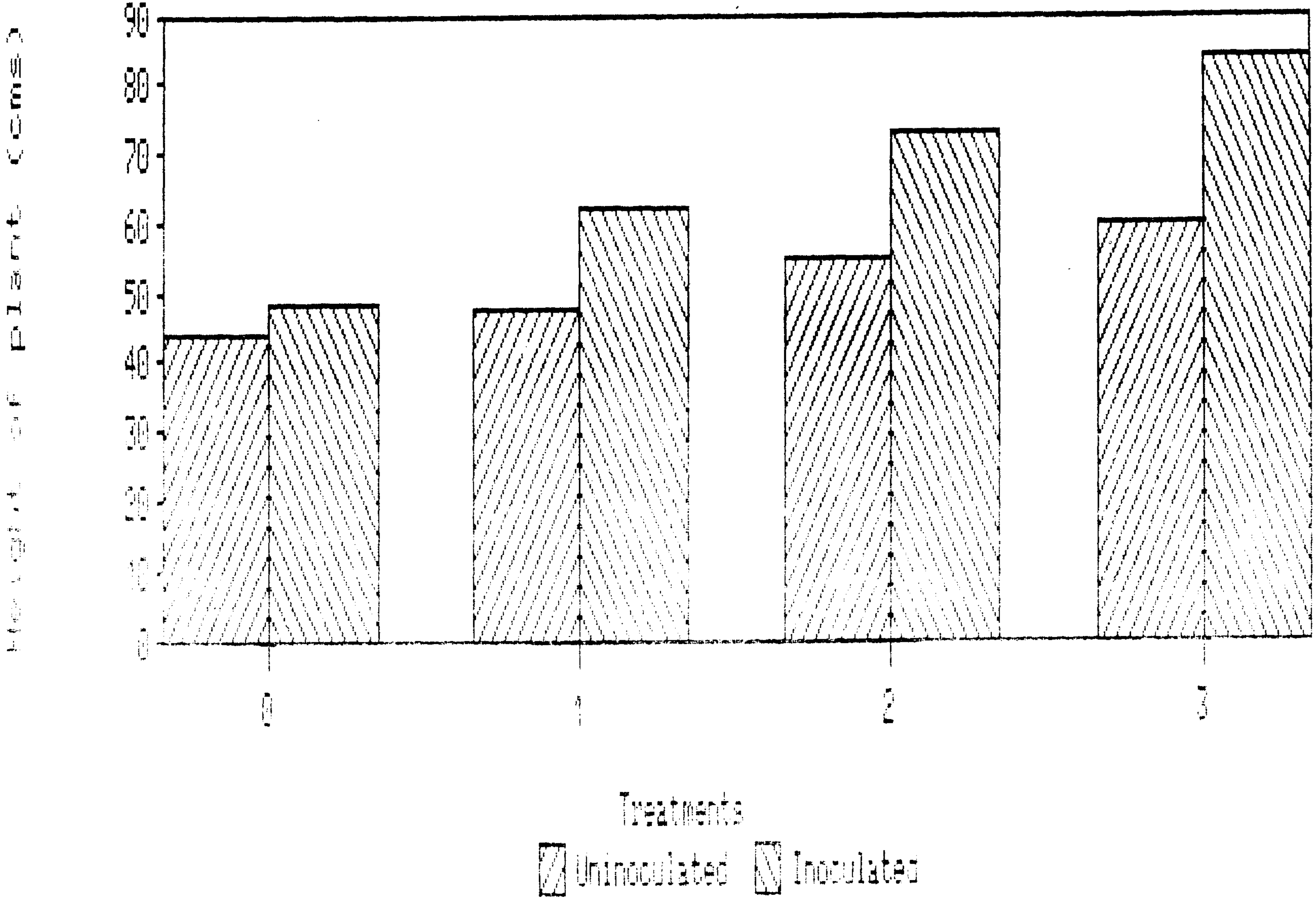
Treatments	Total bacterial count per g of soil
1. Iron ore waste	3.7×10^3
2. Iron ore waste + 100g dry leaves	4.6×10^3
3. Iron ore waste + 200g dry leaves	5.0×10^3
4. Iron ore waste + 300g dry leaves	8.2×10^3
5. Iron ore waste + Earthworms	3.9×10^3
6. Iron ore waste + 100g dry leaves + Earthworms	4.9×10^3
7. Iron ore waste + 200 g dry leaves + Earthworms	3.6×10^4
8. Iron ore waste + 300g dry leaves + Earthworms	5.9×10^4

Population counts are mean of replicate samples.

Table 3.4. Impact of Earthworm on Growth Response of *Crotolaria retusa* L. in Iron Ore Mine Waste.

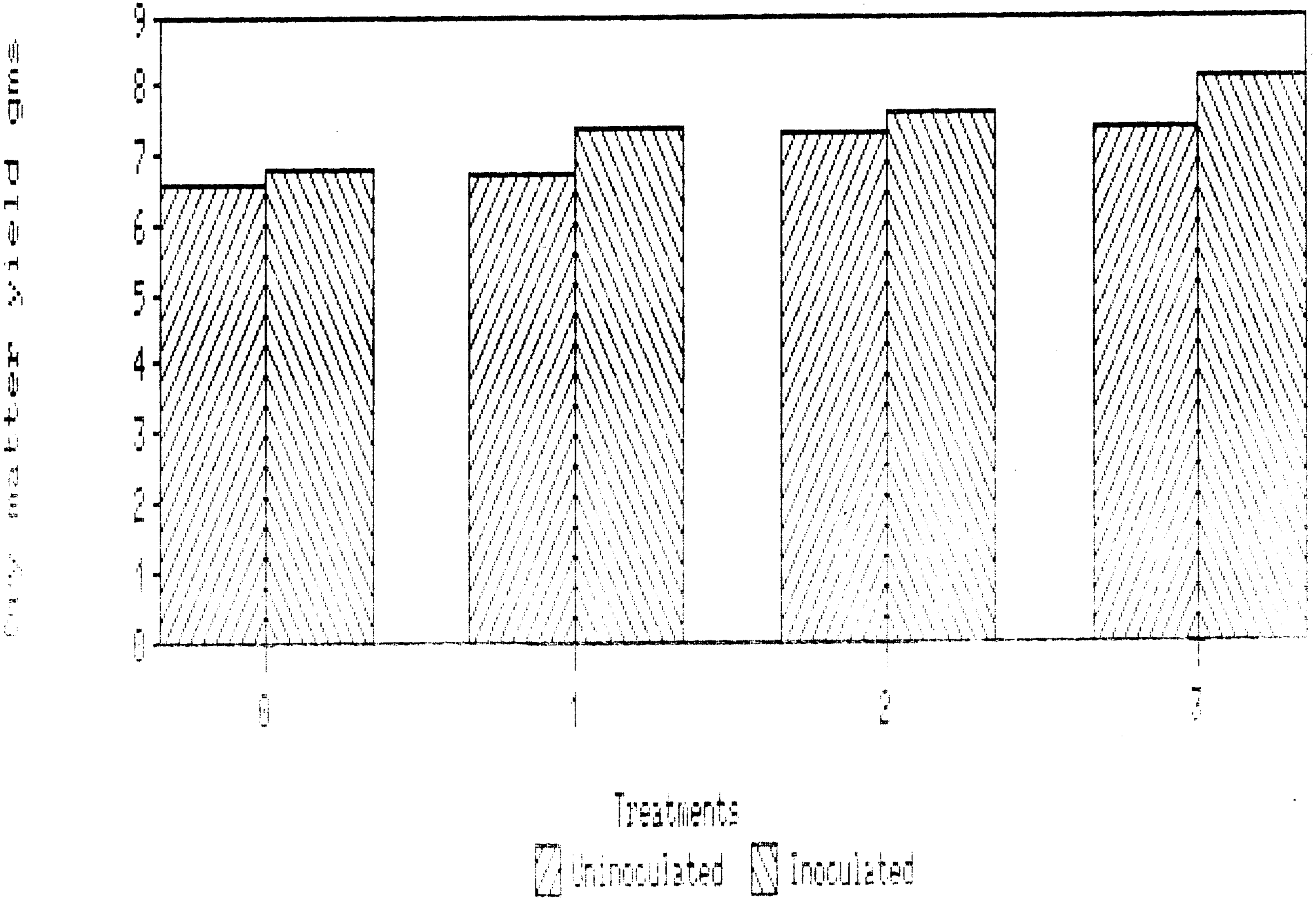
Treatments	Plant height cms.	No. of nodules per plant	Root length cms	Plant dry weight g	No. of pods per plant	Increase in dry weight %
1. Iron ore waste	44.4 ± 3.41	17	12.30 ± 3.15	6.619 ± 0.143	6	-
2. Iron ore waste + 100g dry leaves	48.0 ± 2.0	27	13.67 ± 2.97	6.753 ± 0.137	11	-
3. Iron ore waste + 200g dry leaves	55.0 ± 2.65	32	17.63 ± 3.76	7.312 ± 0.205	18	-
4. Iron ore waste + 300g dry leaves	60.21 ± 3.25	39	19.17 ± 4.58	7.405 ± 0.267	24	-
5. Iron ore waste + Earthworms	48.5 ± 3.10	24	12.93 ± 2.86	6.800 ± 0.317	10	2.7
6. Iron ore waste + 100g dry leaves + Earthworms	62.1 ± 2.45	53	18.23 ± 3.53	7.429 ± 0.219	29	9.9
7. Iron ore waste + 200g dry leaves + Earthworms	73.5 ± 3.72	67	21.07 ± 5.16	7.621 ± 0.173	32	4.2
8. Iron ore waste + 300g dry leaves + Earthworms	84.5 ± 2.13	81	23.14 ± 3.91	8.162 ± 0.249	37	10.2

± Denotes Standard Deviation.



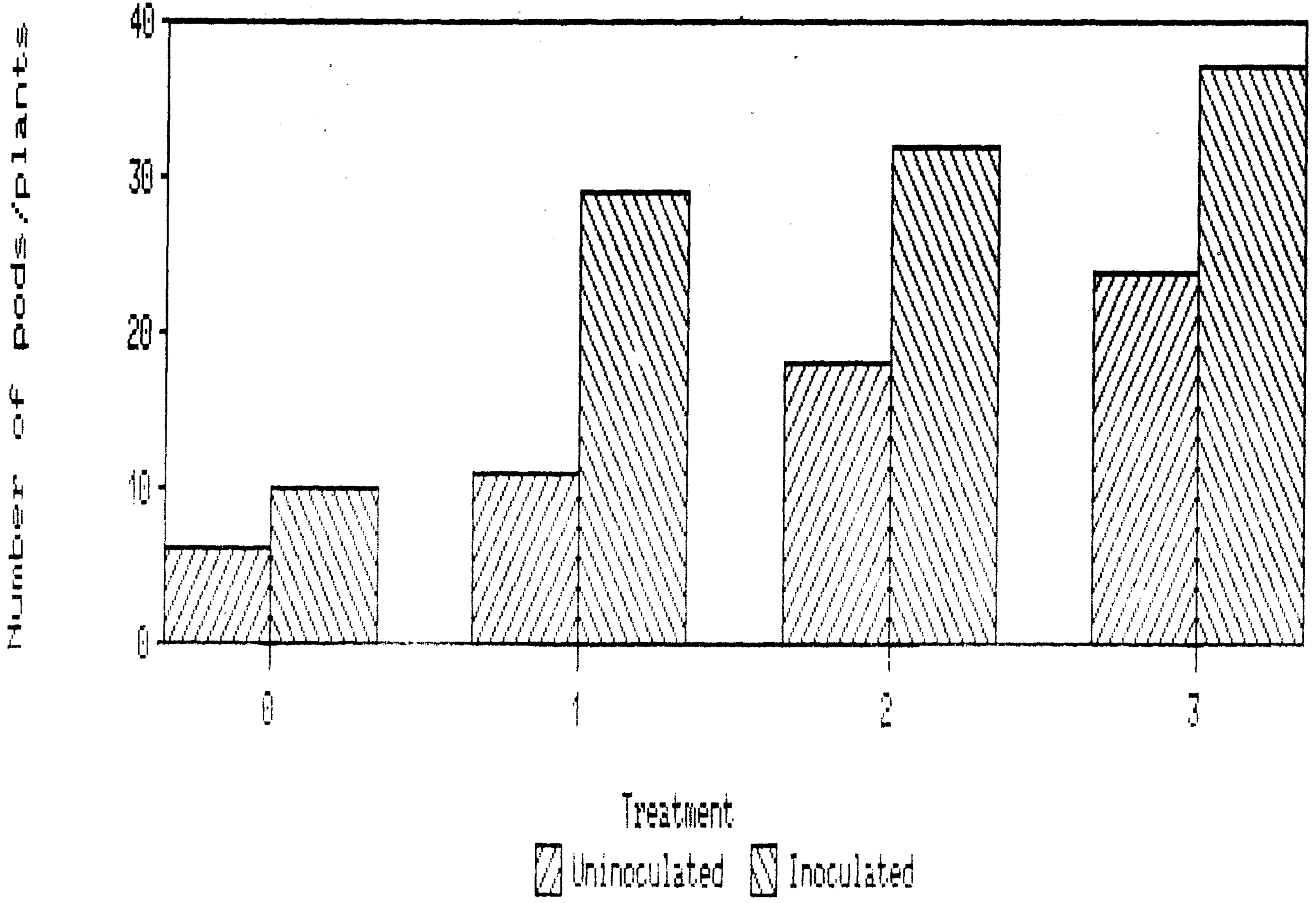
0 - Unamended
1 - Amended with 100g dried ground leaves of **M. indica.**
2 - Amended with 200g dried ground leaves of **M. indica.**
3 - Amended with 300g dried ground leaves of **M. indica.**

Fig. 3.1 - impact of earthworms on growth response of **C. retusa**, L. in Iron Ore Mine Waste.



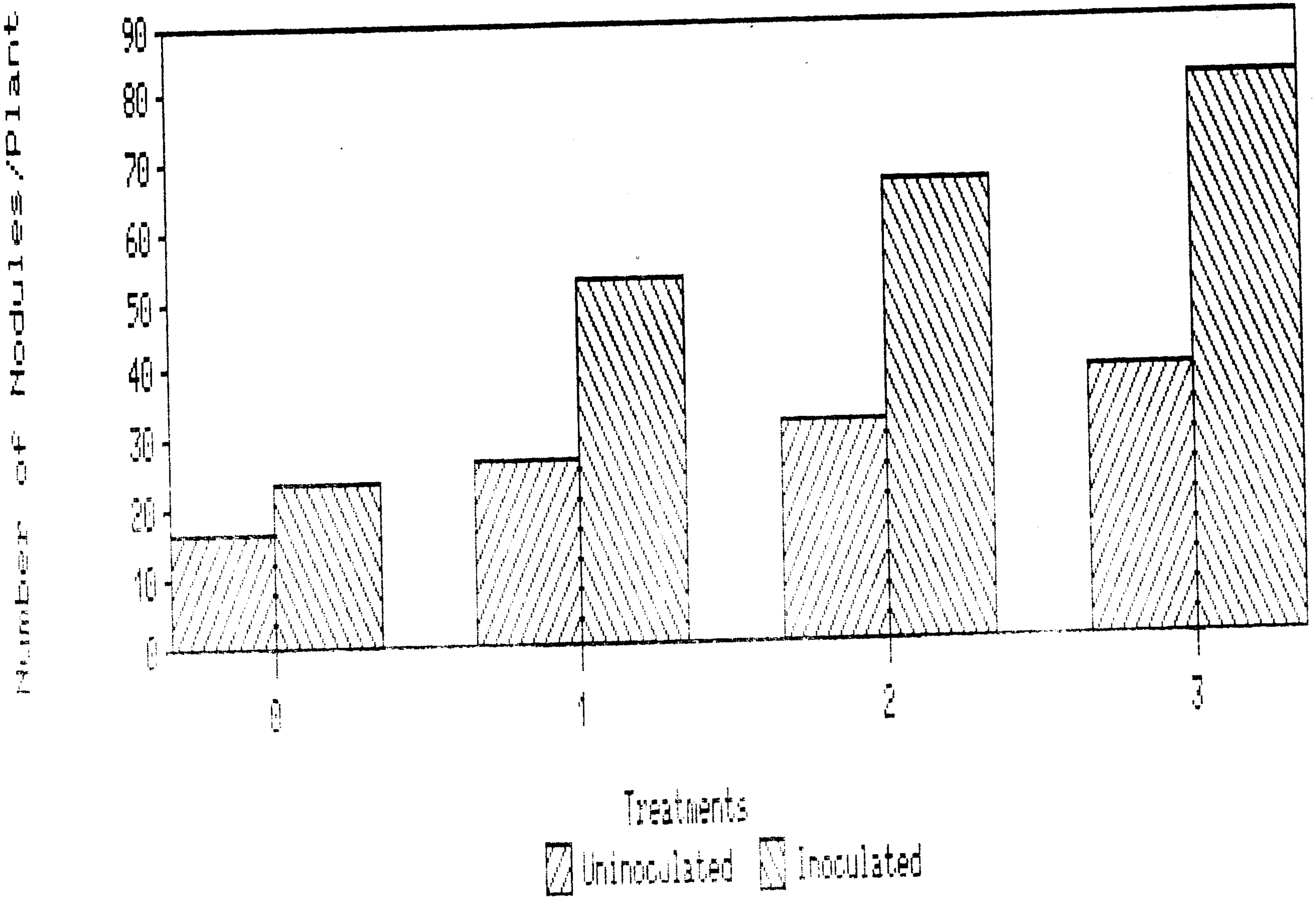
0 - Unamended
1 - Amended with 100g dried ground leaves of **M. indica.**
2 - Amended with 200g dried ground leaves of **M. indica.**
3 - Amended with 300g dried ground leaves of **M. indica.**

Fig. 3.2 - Impact of earthworms on dry matter yield of **C. retusa**, L. in Iron Ore Mine Waste.



0 - Unamended
1 - Amended with 100g dried ground leaves of **M. indica.**
2 - Amended with 200g dried ground leaves of **M. indica.**
3 - Amended with 300g dried ground leaves of **M. indica.**

Fig. 3.3 - Impact of earthworms on pod yield of **C. retusa**, L. in Iron Ore Mine Waste.



0 - Unamended
1 - Amended with 100g dried ground leaves of **M. indica.**
2 - Amended with 200g dried ground leaves of **M. indica.**
3 - Amended with 300g dried ground leaves of **M. indica.**

Fig. 3.4 - Impact of earthworms on nodulation of **C. retusa**, L. in Iron Ore Mine Waste.

controls and 12.93 to 23.14 cm in the experimental plants. A marked improvement in nodulation 17 to 39 nodules /plant in controls and 24 to 81 nodules/plant in earthworm inoculated plants was observed.

A higher pod yield, 6-24 pods/plant in control and 10-37 pods/plant was recorded in experimental pots, at the end of the experiment for growth studies (Fig. 3.4).

3.4. DISCUSSIONS

3.4.1. Establishment of Earthworms on Iron Ore Mine Waste after Suitable Amendment.

Earthworms are an important factor in soil economy, particularly in the breakdown of organic matter and in the maintenance and improvement of soil structure.

The results obtained here (Table-3.1 & 3.2) indicated varying survival percentage (ranging from 15-80%) of earthworms and significant differences between the physical, chemical and biological properties of casts and their corresponding controls for all treatments investigated. Some of the physical and chemical properties are affected by the textural composition of the waste and the quality and quantity of organic material added as an amendment. It is obvious that, the biochemical and physiochemical behaviour of waste are drastically affected by the intense biological activity of "**Pheretima orientalis**" (Fig.3.1).

Intense mining activity have disturbed the natural habitat of the earthworms, although conditions were purposely made highly favourable with the addition of dry ground leaves of **Mangifera indica**, L. the earthworms failed to show a very significant survival in treatments amended with 0,100 and 200g of dry leaf matter. This can be correlated to the absence of a well defined proportion of plant litter necessary for their survival. However, it is quite probable that earthworms obtain

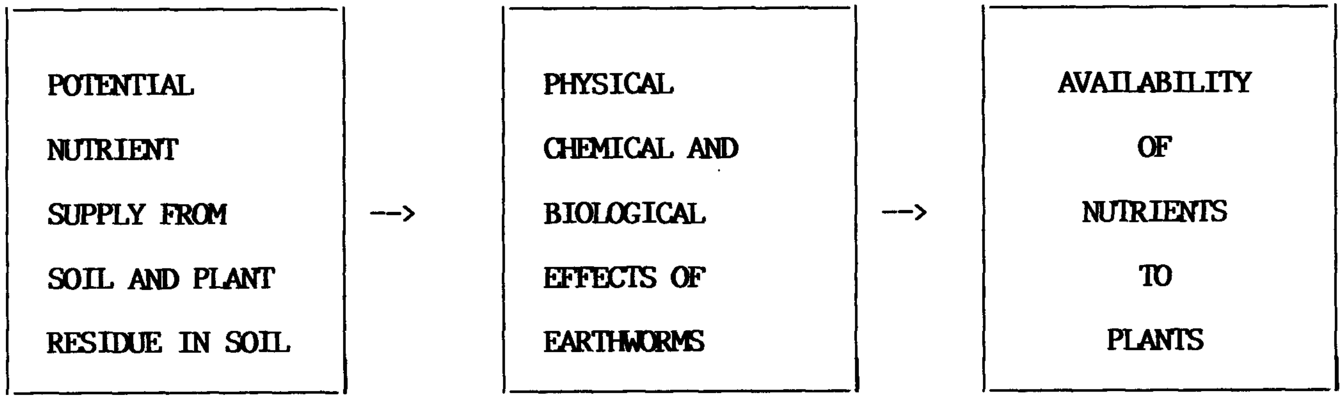


Fig.-1. Physical Chemical and Biological Effects on Soil Nutrient Supply which are Influenced by Earthworms in Iron Ore Mine Waste.

most of their mineral nutrients from ingested leaf litter and hence the food supply is one of the governing factor for their survival.

The present finding may well fit into that of Lofs-Holmin (1981), who showed that population of L. **terrestris** were mainly dependent on the organic content of the soil. The total absence of organic matter in the iron ore wastes make the environment hostile for their survival. Van der Drift (1955), also found that earthworms introduced to experimental plots failed to survive except where the plots had been marled. The absence of earthworms from soil is an indication that condition are unsuitable for them.

3.4.2. Change in Physical, Chemical and Biological Properties of Iron Ore Mine Waste due to Earthworm Activity.

Mechanical analysis of the casts showed that the casts contained less sand (ranging from 50.5-54.2%) more silt (ranging from 19.4-21.6%) and clay (ranging from 23.4-25.9%) than their corresponding controls (Table-3.1). This is consistent with the results obtained by De Vleeschauwer and Lal (1981), who showed that the casts contained less sand, more silt and clay than the surface soils, under secondary tropical forest regrowth. Similar observations were also made by Lal and Akinremi (1983).

Evans (1948) found that the amount of coarse sand relative to silt and clay in two old pastures with high earthworm populations increased with depth and suggested that this might be partly due to a reduction of the amount of sand in the upper layers by its comminution in the intestines of earthworms. The presence of the smaller fraction of coarse sand in worm casts than in soil nearby has been .taken as evidence of this process (Teotia **et al.** 1950; Shrikande and Pathak, 1951; Joshi and Kelkar, 1952). However more direct evidence of communition is available from experiments with earthworms kept in pots, in which measurable reduction in size of particles of granite (Bassalik, 1913), basalt (Meyer, 1943) and soil (Blank and Giesecke, 1924) were obtained. The change in mechanical composition of casts is either due to the grinding action of earthworms "gizzard" wherein the soil eaten along with other food material gets triturated and disintegrated into further fine particles, or it is likely that silt sized particles are preferentially ingested along with the plant material.

Physical Properties

Earthworm casts had appreciably higher (ranging from 1.35-1.45g/cm^3) bulk densities than their corresponding controls (ranging from 1.25-1.30g/cm^3) table-3.1. This is in agreement with the results obtained by De Vleeschauwer and Lal (1981) in worm cast under secondary tropical forest regrowth. They showed that the worm casts had a significantly higher bulk densities (1.15-1.40g/cm^3) than their surface soils (0.98-1.21g/cm^3) because of the casts lower porosity and cementing action of worms body fluid. However, they failed to suggest that the change in the bulk density was due to the mechanical composition of of casts. Such a change in bulk density of casts can be attributed to the increase in silt and clay and decrease in sand content of the casts.

There was gradual increase in moisture content (from 9.3-10.5%) and significant increase in water holding capacity (from 42-47.1%) of the casts, than their corresponding controls (Table-3.1). Evans (1948) showed that the casts had a higher proportion of structural pores finer than 50u which increased the volume of readily available water the soil could hold, he also showed that at Rothamsted the top few inches of pasture soils had a pore space of 67 percent where the earthworms were present but only of 40 percent where they were absent. The casts must be predominating in micro-pores. Similar conditions must have prevailed in the present experiment. The increase in moisture content and water holding capacity may also be probably due to the tendency of organic materials binding water molecules within its lattices. Lal and Akinremi (1981) reported that the water holding capacity of casts was about double than that of the soil.

Chemical Properties

Earthworm casts had fractionally lower pH (6.0-6.5) than their corresponding controls (6.4-6.8), table-3.2. Cohen and Lewis (1949) suggested that the ammonia which forms a large portion of nitrogenous matter excreted by earthworms may cause a temporary rise in soil pH. The increase in organic matter due to the increased addition of 100, 200 and 300 g of dry ground foliage of **M. indica** may have partly contributed to the lowering of pH in controls and casts.

Worm casts had significantly higher (ranging from 0.38-1.1) organic carbon content than their coresponding controls (ranging from 0.35-0.72%). It was expected that, the increasing addition of ground plant matter from 0-300g would gradually increase the organic carbon in the treatments. But, it is certainly true to say that the higher organic carbon observed in the casts was due to the contribution of earthworm activity, which remains concentrated in the casts. Joshi and Kelkar (1952), De Vleeschauwer and Lal (1981) also reported significant increase in organic carbon in the worm casts than their nearby surface soils.

A higher cation exchange (ranging from 5.1 - 5.9 meq/100g) was observed in the worm casts compared to their corresponding controls (ranging from 3.9-4.5 meq/100g). This confirms the finding of De Vleechauwer and Lal (1981). They obtained higher cation exchange (ranging from 8.9-17.7 meq/100g) in casts than their surface soils (ranging from 1.9-4.9 meq/100g). Similar observations were also made by several other workers, Powers and Bullen (1935); Lunt and Jacobson (1944); stockli (1949); Ponomareva (1950); Shrikhande and Pathak (1951) and Fink (1952). The higher cation exchange observed here, probably may be due to the increasing concentration of total organic content in the casts, as organic matter is known to posses rich cation exchange sites.

The chemical composition of the casts differ from that of their control. The casts had higher concentration of exchangeable cations, Ca^{+2} (ranging from 0.08-0.18%), Mg^{+2} (ranging from 0.029-0.042%), K^{+} (ranging from 0.15-0.21%) than their respective controls (Table-3.2). Similarly, available phosphorous (P) concentration increased from 3.8 ppm to 5.6 ppm in the casts compared to the control. De Vleeschauwer and Lal (1981) also reported higher concentration of exchangeable cations, Ca^{+2}, Mg^{+2}, K^{+} and available P, in the casts. Sharpley and Syers (1976) have reported that the amount of water extractable organic P in freshly deposited casts was twice that found in underlying soil. Similar results were also obtained by Mansell **et al.** (1981). This indicates that the P present in the plant litter became more plant available as a result of passage through the worm gut and excretion as surface casts. All these must have had an organic origin. Moreover, the waste itself was poor in exchangeable cations, this could be attributed to the lack or poor organic matter as seen in table-3.2.

The rate of mineralization of crop residue is significantly influenced by the activity of earthworms (Crossley and Hoglund, 1962; Edwards and Heath, 1963). Earthworms incorporate the crop residue in the soil and break it down for further mineralization by micro-organisms. The higher total nitrogen (ranging from 0.069-0.141%) observed in the present analysis of earthworm casts may have had an organic origin. The waste itself was deficient in nitrogen as can be seen in the control treatments (Table-3.2), which is the universal property of every mine spoil. Results of several studies have shown that earthworm casts are enriched in nitrogen relative to the surface horizon (Gupta and Sakal, 1967; Wantabe, 1975). The increase in N-content of casts has been attributed to the intimate mixing of plant matter with mineral soils in the digestive tract of earthworm (Lunt and Jacobson, 1944). Lindquist (1941), Barley and Jennings (1959) also suggested that the action of digestive secretions on this mixture enhanced the decomposition of organic matter. A part of the nitrogen present in the waste products of worms metabolism is in the form of ammonium ions, urea and possibly uric acid and allantoin (Needham, 1957) and these are either readily available for plant uptake or are rapidly ammonified. It is therefore, quite probable that similar conditions prevailed in the present investigation. Syers **et al.** (1978) claculated that 73 percent of the total nitrogen content of litter removed from the surface by earthworms was accumulated in casts.

The casts were richer in soluble salt content (ranging from 0.14-0.20 m mhos) compared to their corresponding controls (ranging from 0.14 - 0.17 m mhos), as seen from their higher electrical conductivity (Table -3.2). This is in accordance with the results obtained by Joshi and Kelkar (1952). They showed that the electrical conductivity of the casts was much higher (ranging from 0.11-0.29 m mhos) than their surface soils (ranging from 0.08-0.22 m mhos). The digestion of the plant litter along with soil as it moves down through the worm gut, where it disintegrates and gets triturated probably releases soluble mineral salts in the worm excreta.

Biological Properties.

Total bacterial population, as reflected in table-3.3, increased with increase in quantity of ground leaf matter added as amendments to the waste. The total bacterial population in controls ranged from 3.7×10^3 - 8.2×10^3/g of soil, whereas in the casts it varied from 3.9×10^3 - 5.9×10^4/g of casts. The increase in bacterial population can be possibly attributed to the greater organic content than the corresponding controls. It is a well established fact that the greater the organic matter the larger is the microbial population. It is also quite probable that the earthworms may influence microbial populations by transporting organisms due to its activity. The increase in bacterial count in worm casts have also been reported by other workers (Dawson, 1947; Joshi and Kelkar, 1952), communition of soil aggregates in gut may expose fresh surfaces to microbial attack (Rovira and Greacen, 1957). Barley and Jennings (1959) reported that earthworms in culture experiment promoted other decomposers. Stockli (1928) showed that virtually all the species and physiological groups sought were present in greater numbers in earthworm castings than in the soil. The work of these workers corroborate the present findings, in casts from iron ore mine waste.

3.4.3. Effect of Earthworm on Plant Growth.

The appearance of the experimental plants showed that they were better supplied with nitrogenous food than the controls, they were dark green in colour, broader in leaf and profusely branching.

The study showed that **Pheretima orientalis** can significantly improve yield of **Crotolaria retusa** in terms of height, dry weight and pods (Table-3.4). Similar results with casts and worms in pot culture have been obtained by Nijhawan and Kanwar (1952) in wheat, Joshi and Kelkar (1952) in wheat and jowar, using other species of **Pheretima**. Several other workers have reported higher crop yield using members of **Lumbricidae**. Ponomareva (1962) obtained 100 percent higher dry matter production than controls in pot culture experiments with earthworm casts. With grass, wheat and clover, Van Rhee (1965) obtained dry matter production on an average 287, 111 and 877 percent higher than the control pots. In the present investigation the percentage increase in dry matter production ranged from 2.7 - 10.2 percent over the controls. These observations in a way are in agreement with the results obtained by the above workers. But, however the lower dry matter obtained in **C. retusa** grown in iron ore mine waste may probably be due to the poor fertility of the waste, which is very much deficient in essential plant nutrients.

The gradual increase in root lenght, ranging from 12.93-23.14 cm in earthworm inoculated pots and nodulation ranging from 10-37 nodules/plant, could be attributed to the earthworm loosening and mulching the soil, facilitating aeration and drainage by their burrows. The profusely branching root system might have improved the chances for an efficient **Rhizobium**-root interaction. That earthworms increase the volume of root materials penetrating the soil has been demonstrated by Edwards and Lofty (1980) in the laboratory and by Stockdill and Cossens (1966), Ellis **et al.** (1977) and Edwards and Lofty (1980) in the field. Edwards and Lofty (1980) showed that the zone of maximum root growth of barley coincide with the zone of major activity of the earthworm species . This is in agreement with the results obtained in the present findings.

This conclusive result and substantial improvement in the growth and yield of **C. retusa**, resulting from the earthworm activity cannot be attributed to any single factor but is probably due to a combination of beneficial effects.

The physical and chemical changes alone could not have accounted for the recorded increase in yield, but the ingestion, mineralization and re-distribution of organic material and plant nutrients by the earthworms must have been beneficial. Moreover, the analysis revealed an increase or improvement in the availability of plant nutrients. The enrichment and improved soil reaction (pH) of the casts might have encouraged more vigorous root development and improved uptake of plant nutrients.

The influence of **Pheretima orientalis** in partly mineralising the plant litter and bringing about physical, chemical and microbiological changes in the place where they dwell can now be well established.

Earthworm introduction on iron ore mine waste with suitable amendments does appear quite feasible and can be recommeded as a means of enhancing diversity of these areas, with the goal of hastening their return to equilibrium.

CHAPTER FOUR

THE POTENTIAL OF IPOMOEA PES-CAPRAE (L) SWEET FOR IRON ORE MINE WASTE STABILIZATION

4.1. INTRODUCTION

Ipomoea has invaded substantial area of iron ore mine waste at Pale-Mines (Goa). This has occurred, since the construction of iron ore pelletization plant, way back in 1966, for which sea sand was utilized to build the super structure. It has successfully colonized areas with both varying pH and elevated metal levels, which are toxic to many plant species.

Metal ions are particularly important for healthy plant life. Excesses or deficiencies of metal ions has effect on plant growth and morphology which are well documented (Epstein, 1972; Hewitt and Smith, 1975).

Heavy metals have received considerable attention partly due to their natural occurrence. High concentration depress plant growth, although certain metals are required in very small amounts for healthy growth.

Metal contaminated wastes in various parts of the world usually contain more than one metal and these may occur at toxic concentration e.g. metalliferous mine spoils, smelter wastes, coal spoils, sewage sludge and refuse compost.

Studies on the effect of single toxic metal on plants or comparisons of toxicity of two metals have been frequently reported by various workers, the effect of zinc and copper on **Agrostis stolonifera** (Wu and Antonovics, 1975), effect of iron on growth in maize and radish (Agarwala **et al**. 1965). Investigation comparing the toxicity of a number of metals include, copper, nickel, cobalt, zinc, chromium and manganese in reducing fresh weight in mustard (**Sinapis alba**) by Dekok (1956), aluminium, cadmium, chromium, copper, iron, mercury, manganese, nickel, lead, zinc on radicle of rye grass (Wong and Bradshaw, 1982). Metal toxicity studies on plants have been reviewed by Woolhouse (1983), aluminium tolerance has been studied by Foy **et al**. (1969).

Available literature indicates that no much work has been done to understand the cumulative effect of iron, aluminium and manganese which occur in high concentrations in iron ore tailings.

In the present study the plant was grown in different composition of iron ore tailings. The plant was studied for its morphological, biochemical responses and chemical position.

4.2. MATERIALS AND METHODS

Mature and healthy **Ipomoea** cuttings of same size and having same number of leaves were collected from the colonized areas of Pale-mines. They were grown in clay pots (30 x 40 cm) size, filled with different percentage composition of iron ore tailings prepared with garden soil as given below.

Percentage of Iron Tailings	Percentage of Garden Soil
-	100
25	75
50	50
75	25
100	-

All the compositions were analysed for its physical and chemical constituents. The plants were maintained in a shady place till they showed signs of establishment. They were later exposed to normal sunlight and allowed to trail on aluminium trays filled with the same soil composition as in the clay pots. Each composition was replicated five times. Plants were watered whenever necessary. The experiment was carried out at Pale-Mines Research Nursery.

Plants were studied for their morphological characteristics viz.; plant height, leaf size, length of petiole and thickness of the 4th leaf. For biochemical responses, chlorophyll was extracted and estimated according to the method of Arnon (1949). Proteins and sugars were extracted by modifying slightly the methods given by Loomis and Shull (1937) and Osborne (1962). Proteins were estimated according to the method outlined by Miller (1959) and sugars by the method described by Dubois **et al.** (1951). Proline was extracted and estimated according to the method described by Bates **et al.** (1973).

At the end of the experiment, the plant was separated into shoots and roots, thoroughly washed in detergent to remove the earth and contaminants, then with several changes of distilled water. Shoot and root dry weight was determined after drying it to 80°C for 48 hours. The dry material was powdered on a pulveriser.

An amount (0.1g) of dried ground shoot and root were separately digested in an acid mixture (1 ml 60% $HClO_4$; 5 ml conc.HNO_3; 1 ml conc. H_2SO_4) as described by Allen (1974) and was made to suitable volume with double distilled water, and later analysed on Perkin-Elmer 3030 Atomic Absorption Spectrophotometer (USA). All chemicals used were of analytical grade.

4.3. OBSERVATIONS

4.3.1. Chemical Characteristics of Different Composition of Iron Ore Tailings.

Chemical characteristics of the different composition of iron ore tailings prepared with garden soil is depicted in table-4.1. All compositions varied in their pH, tending to be alkaline from 6.5 for garden soil (0% tailings) to 7.2 for 100 percent tailings. The organic carbon content decreased from 2.1 to 0.5 percent with increasing percentage of tailings from 0 to 100 percent. The soluble salt content (as reflected in the electrical conductivity) decreased from 0.18 to 0.09 m mhos/cm^2 with increase in the percentage of tailings in the soil composition. High iron content (51.0%) was observed in 100 percent tailings and lowest in garden soil (24.5%). There was a decrease in alumina in the different soil mixtures from 19.11 (garden soil) to 11.65 percent (100% tailings). Similar was the case with silica which decreased from 25.608 to 10.952 percent. There was an increase in manganese content with increase in tailings in the soil compositions from 0.2 to 1.2 percent (0 to 100% tailings). Calcium and magnesium decreased with increase in tailings in the soil mixtures and ranged from 0.2 to 0.04 percent and 0.04 to 0.01 percent (garden soil to 100% tailings) respectively. All the macro-nutrients viz; nitrogen, phosphorous and potassium decreased with increase in tailings in the different compositions. Nitrogen ranged from 51.0 to 5.0 kg/ha, posphorous 12-4 kg/ha and potassium 300-60 kg/ha (garden soil-100% tailings) respectively.

4.3.2. Morphological Characters of I. pes-caprae Grown in Different Composition of Iron Ore Tailings.

The morphological characters of **Ipomoea** grown in different composition of iron ore tailings is presented in table-4.2. There was an inverse co-relation between the height of plant, breadth of fourth leaf, length of petiole and percentage of tailings in the soil composition. The height of the plant decreased from 95.9 to 26.5cm. The breadth of fourth leaf decreased from 6.5 to 4.77cm and whereas the length of petiole decreased from 8.0 to 5.3cm with increasing percentage of tailings in the soil composition from 0 to 100 percent. However, a distinct co-relation was observed between the thickness of leaf and percentage of tailings in the soil composition. The thickness of the leaf increased from 0.38mm (0% tailings) to 0.61mm (100% tailings). As the percentage of tailings increased (from 0 to 100%) in the soil composition, the shoot dry weight and root dry weight decreased from 28.02 to 3.78g/plant and 5.87 to 1.93g/plant respectively.

4.3.3. Biochemical Responses of I. pes-caprae to Different Composition of Iron Ore Tailings.

Biochemical analysis of plants grown in different composition of iron ore tailings are embodied in table-4.3. It was observed that the chlorophyll 'a', 'b' and hence (a + b) were greatly affected with increase in iron ore tailings in different composition of soil mixtures. Both chlorophyll 'a' and 'b' decreased with increase in percentage of tailings in the soil composition (Fig. 4.1). Chlorophyll 'a' decreased from 78.76 to 39.3mg percent and chlorophyll 'b' from 115.57 to 57.78mg percent whereas, the total chlorophyll (a + b) decreased from 194.33 to 97.08mg percent. Similarly, a decrease in total soluble sugars and protein content was also observed with increasing percentage of

Table-4.1. Chemical characteristics of different composition of Iron Ore Tailings.

Percentage of Iron Ore Tailings	pH	OC %	EC m mohos/ cm^2	Fe %	Al_2O_3 %	SiO_2 %	Mn %	Ca %	Mg %	Avail. N kg/ha	Avail. P kg/ha	Avail. K kg/ha	LOI %
0 (Garden Soil)	6.5	2.1	0.18	24.5	19.11	25.60	0.2	0.12	0.040	51.0	12.0	300.00	17.1
25	6.6	1.1	0.15	31.0	17.84	20.95	0.5	0.07	0.039	40.3	10.0	200.00	11.2
50	6.8	1.0	0.11	38.5	13.45	15.92	0.6	0.057	0.025	21.7	7.0	179.00	9.55
75	6.9	0.7	0.10	43.0	12.78	14.08	0.8	0.05	0.016	9.5	5.0	100.00	8.41
100	7.2	0.5	0.09	51.0	11.65	10.95	1.2	0.04	0.010	5.0	4.0	60.00	7.40

pH -1: 2.5 soil to water ratio.

Electrical conductivity (EC) - 1: 2.5 soil to water ratio.

Organic carbon (OC) -chromic acid method.

Iron, Aluminium and Manganese - AAS.

Available Nitrogen - Kjeldhal's method.

Calcium, Magnesium and Potassium - Neutral normal ammonium acetate method.

Phosphorous - Bray's method.

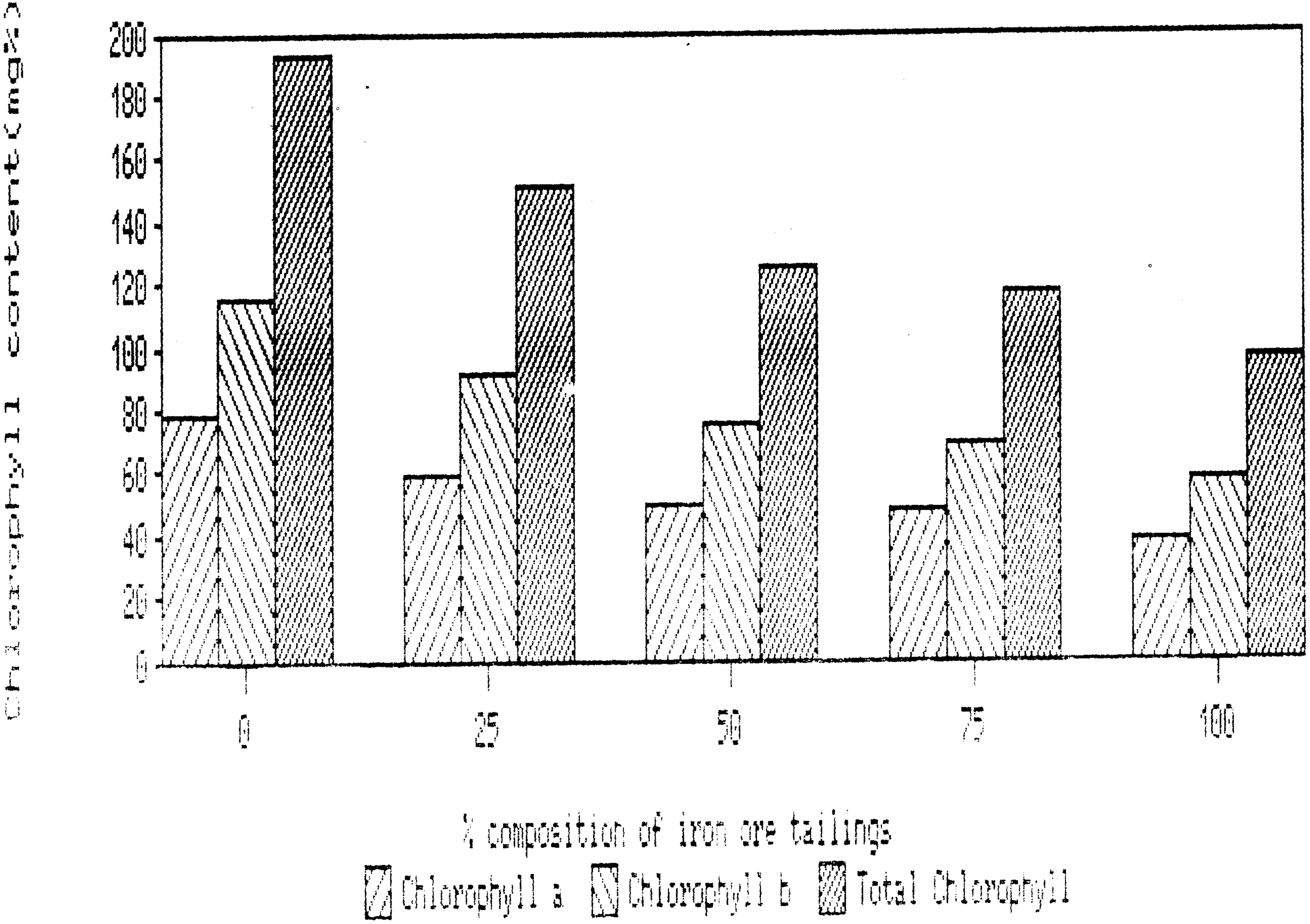


Fig. 4.1 - Effect of different composition of Iron Ore Tailings on the Chlorophyll content of **Ipomoea pes-caprae** (L) Sweet

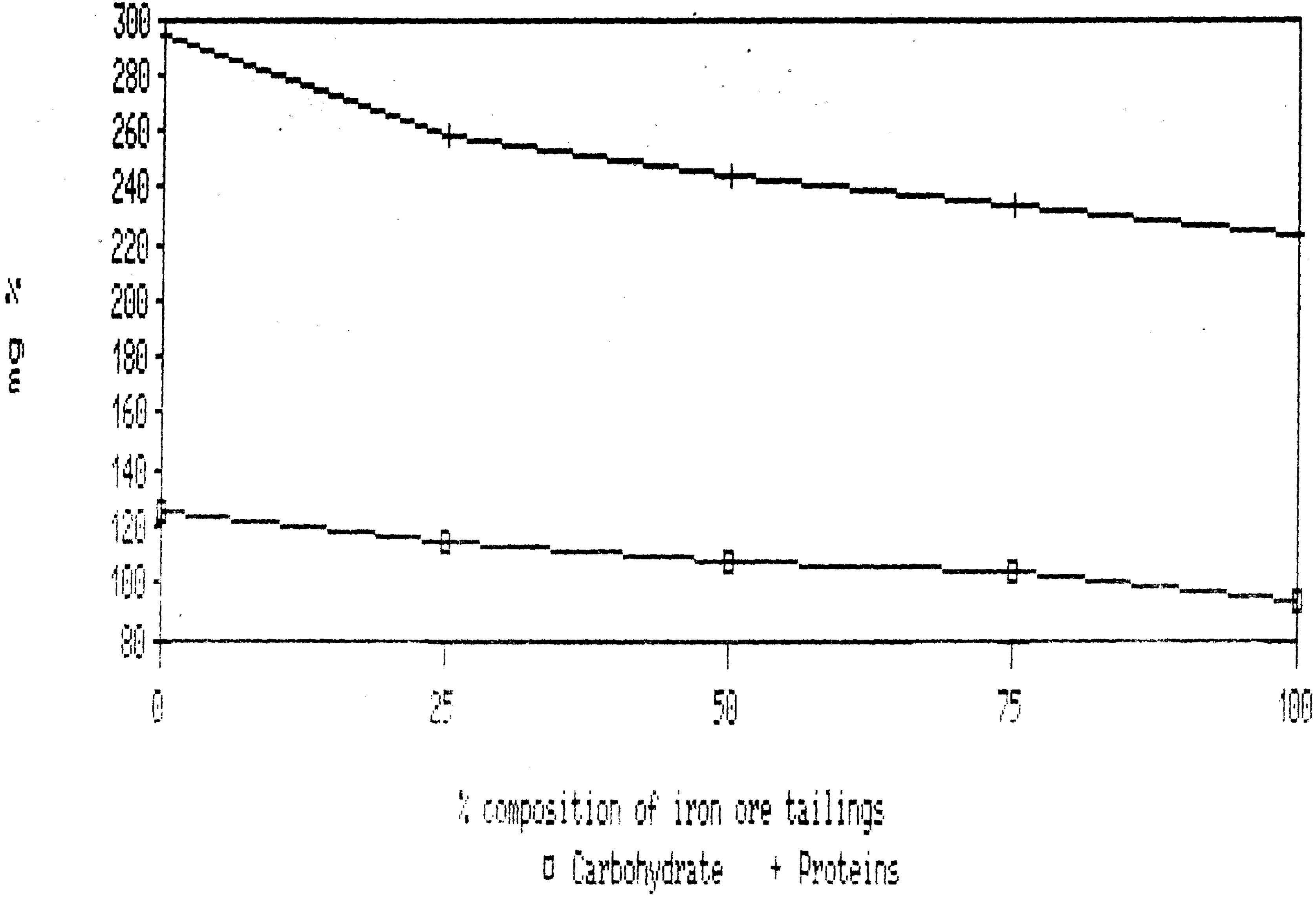


Fig. 4.2 - Effect of different composition of Iron Ore Tailings on the Carbohydrate (soluble sugars) and Protein content of **Ipomoea pes-caprae** (L.) Sweet.

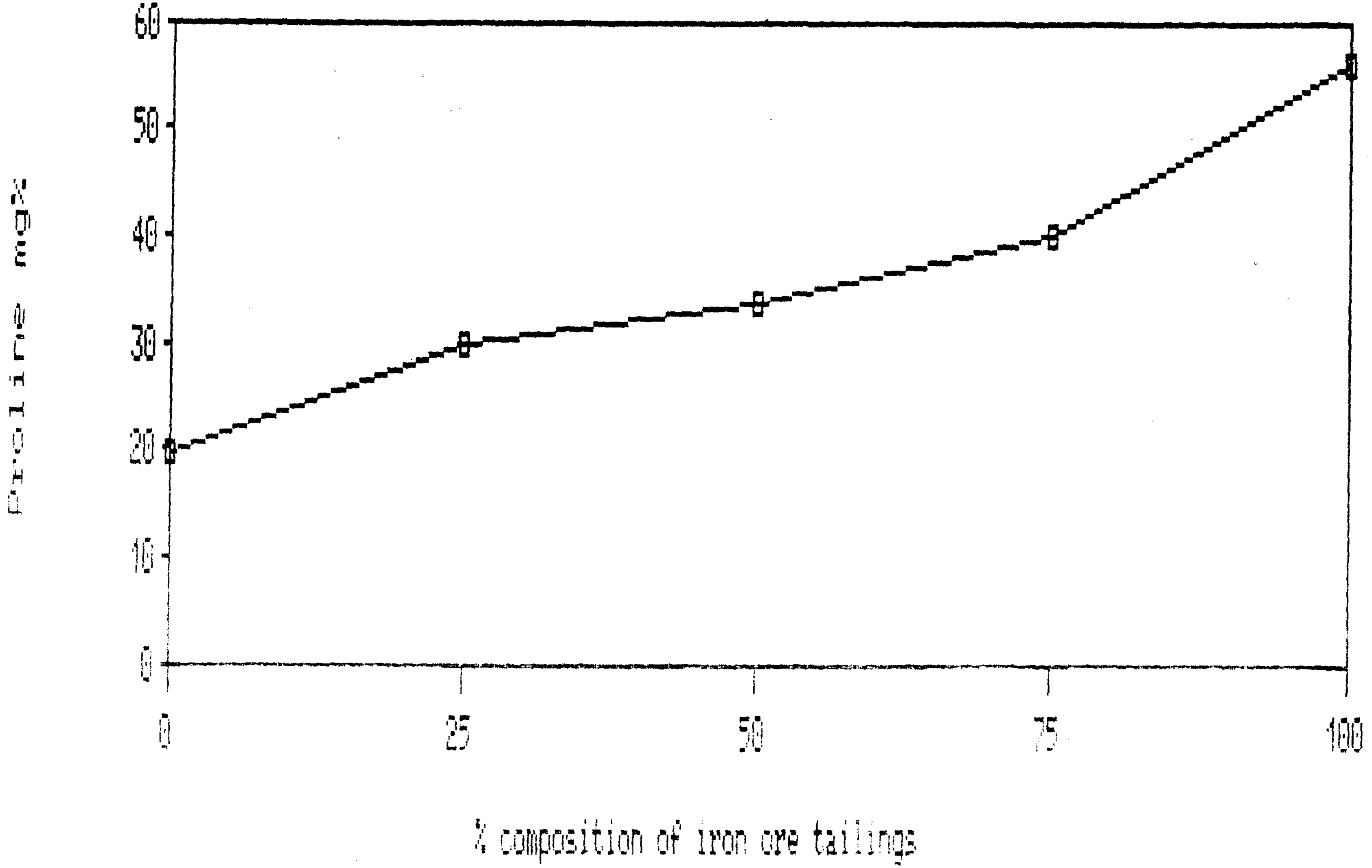


Fig. 4.3 - Effect of different composition of Iron Ore Tailings on the Proline content of **Ipomoea pes-caprae** (L) Sweet

tailings in the soil compositions (Fig. 4.2). Total soluble sugars decreased from 127 to 95mg percent and proteins from 295 to 224.5mg percent. However, there was a direct relationship between the percentage of tailings in the soil composition and proline content in fresh leaves of **Ipomoea**. The proline content increased with increasing percentage of tailings in the soil composition (Fig. 4.3)) and varied from 20-56mg percent.

4.3.4. Chemical Analysis of I. pes-caprae Grown in Different Composition of Iron Ore Tailings.

Concentration of three major elements viz; Fe, Al and Mn in **Ipomoea** is represented in table-4.4. There was a direct co-relation between Fe, Al and Mn level in the soil and accumulation in plant. The concentration of these elements in the plant tissue reflect their high concentration in the different compositions. All the three elements analysed indicated that their concentration was much higher in the roots than in the shoots. With increase in percentage of tailings in the soil compositions there was an increase in the concentration of Fe and Mn in the plant roots and shoots. However, Al concentration in the plant tissue decreased as the percentage of tailings in the soil compositions increased from 0 to 100 percent. Iron concentration in roots and shoots of plants grown in zero percent tailings was 1510 and 1420 ppm and in that of plant grown in hundred percent tailings it was observed to be 2840 ppm in roots and 2060 ppm in shoots respectively. Whereas, the concentration of Mn in roots and shoots of plant grown in zero percent tailings was 160 ppm and 140 ppm and in that of hundred percent tailings it was 295 and 325 ppm respectively. Maximum concentration of aluminium was observed in plants grown in garden soil with 745 ppm in roots and 410 ppm in shoots and minimum was observed in plants grown in hundred percent tailings, 500 ppm in roots and 225 ppm in shoots, which reflect the decrease in level of aluminium as the percentage of tailings increased in the soil compositions.

Table - 4.2. Morphological responses of *I. pescaprae* (L) Sweet to different composition of Iron Ore Tailings.

Plant responses		Percentage of Iron Ore Tailings.			
	Garden Soil	25	50	75	100
Height of plant (cm)	95.9 ± 10.1	80.1 ± 12.9	56.7 ± 11.2	46.3 ± 8.7	26.5 ± 6.9
Breadth of 4th leaf (cm)	6.5 ± 0.50	5.9 ± 0.51	5.6 ± 0.36	5.1 ± 0.29	4.77 ± 0.34
Length of petiole (cm)	8.0 ± 0.67	6.5 ± 0.43	6.1 ± 0.39	5.6 ± 0.48	5.30 ± 0.41
Thickness of leaf (mm)	0.38 ± 0.02	0.46 ± .035	0.469 ± 0.03	0.54 ± 0.03	0.61 ± 0.04
Shoot dry weight (g)	28.025 ± 2.56	15.04 ± 2.12	11.11 ± 1.95	8.730 ± 1.02	3.78 ± 0.67
Root dry weight (g)	5.87 ± 0.97	4.92 ± 0.91	3.01 ± 0.65	2.16 ± 0.48	1.93 ± 0.37

± Denotes Standard Deviation.

Table - 4.3. Biochemical responses of *Ipomoea pescaprae* to different composition of Iron Ore Tailings.

Percentage of Iron Ore Tailings	Chlorophyll (mg%) a	Chlorophyll (mg%) b	Chlorophyll (mg%) (a+b)	Carbohydrates (soluble sugars) mg%	Total Proteins mg%	Proline mg%
0(Garden Soil)	78.76	115.57	194.33	127.00	259.00	20.00
25	59.6	91.9	151.5	115.00	260.00	30.00
50	49.96	75.07	125.03	108.00	244.5	34.00
75	48.93	69.6	118.53	105.00	235.0	40.00
100	39.3	57.78	97.08	95.00	224.5	56.00

Results are mean of three determinations.

Table 4.4. Chemical analysis of _Ipomoea pescaprae_ grown in different composition of Iron Ore Tailings

Percentage of Iron Ore Tailings.	Plant part	Fe ppm	Mn ppm	Al ppm
0 (Garden Soil)	Shoot	1420	140	410
	Root	1510	160	745
25	Shoot	1530	135	376
	Root	1050	165	710
50	Shoot	1650	175	350
	Root	1975	180	659
75	Shoot	2000	240	351
	Root	2835	280	613
100	Shoot	2060	295	225
	Root	2840	325	517

Results are mean of three determinations.

4.4. DISCUSSIONS

4.4.1. Chemical Characteristics of Different Composition of Iron Ore Tailings.

The conditions under which **Ipomoea pes-caprae** was grown in different composition of iron tailings were not normal. The soil environment and plant metabolic factors may influence the mineral composition of **Ipomoea** plants. The nutrient status of the soil mixtures (Table-4.1) and high concentration of Fe (ranging from 24.5-51.0%), Al_2O_3 (ranging from 11.65-19.11%) and Mn (ranging from 0.2-1.2%), low organic content (ranging from 0.5-2.1%) and pH (6.5-7.2) may all collectively or individually affect the growth of **Ipomoea** plants. The pH (6.5) slightly acidic tending to be alkaline (pH 7.2) hinders the availability of most if not all micro-nutrients, which is aggravated by their deficiency. The high concentration of iron, aluminium and manganese may have deleterious effect on plant growth.

The lower content of organic carbon is an indication of poor availability of nutrients. Nitrogen, phosphorous, potassium, calcium and magnesium are nutrients required in relatively large amounts to sustain plant growth and development in which the different soil mixtures were practically deficient. Preparation of different compositions of soil, with iron ore tailings and garden soil did not improve the soil conditions, but increased the concentration of iron from 24.5 to 51.0 percent and manganese from 0.2 to 1.2 percent and decreased aluminium concentration from 19.11 to 11.65 percent (Table-4.1) in which the plants were grown to study their morphological, biochemical responses and chemical composition.

Mine tailings are extremely low in essential nutrients (Antonovics **et al.** 1971; Bradshaw and Chadwick, 1980; Williamson **et al.** 1982). The analysis of hundred percent iron ore tailings showed that they have low concentration of calcium (0.04%), magnesium (0.01%), available nitrogen (5.0 kg/ha), available phosphorous (4.0 kg/ha) and available potassium (60 kg/ha), table-4.1. The concentration of these essential nutrient elements in natural soil (garden soil) was calcium (0.12%), magnesium (0.04%), available nitrogen (51.0 kg/ha), available phosphorous (12.0 kg/ha) and available potassium (300 kg/ha) respectively. There is a wide range of chemical constraints that can prevent plant growth (1) toxicity (2) extreme acidity or alklinity (3) low nutrient status and (4) chemical concentration. Extremes of any of these can prove sufficient to prevent plant growth, but generally several constraints act together to produce particularly hostile environment (Williamson **et al.** 1982).

4.4.2.Morphological characters of Ipomoea pes-caprae Grown in Different Composition of Iron Ore Tailings.

The results obtained (Table-4.2) in the present investigation showed that with increasing percentage of tailings (from 0-100%) in the soil composition, there was a decrease (from 95.9-26.5 cm) in growth of plant, which corresponds to the increasing iron (from 24.5-51.0%) and maganese (from 0.2-1.2%) concentration in soil compositions (Table-4.4). Wheeler **et al.** (1985) showed that at high concentration of iron (50mg 1^{-1}) in the soil medium caused decreased growth in shoot of **Epilobium hirsutum**, similar observations were also made by Pegtel (1986) in the growth of **Succissa pratensis.** He found that with higher concentration of iron (above 5 ppm) in the culture solution, growth reduction of **S. pratensis** increased proportionately. The present findings with different composition of iron ore tailings and **Ipomoea pes-caprae** as a test plant is consistent with the results obtained by the above workers, in different plants in culture solutions and soil.

Depression in growth of **Ipomoea** plants in different composition of iron ore tailings correspond also to the increasing concentration of manganese in the different soil compositions. The growth decreased (from 95.9-26.5 cm) with increase in manganese concentration from 0.2-1.2 percent (Table-4.4). Reduction in plant growth due to increasing concentations of manganese in culture solutions and soil medium have been shown by various workers in different plants. Vlamis and Williams (1973) showed that with increasing concentration of manganese in culture solution from

0-10 ppm there was decrease in growth of Romaine lettuce. Similar observations in growth reduction were also made by Ohki (1976) in soyabeans, Kuo and Mikkelsen (1981) in sorghum. The results of these workers are in confirmity with the present findings.

The breadth of the leaf decreased from 6.5 - 4.77 cm (Table-4.2) with increase in percentage of tailings in the soil composition, which implies to the increase in iron and manganese in the substrates. Morris and Pierre (1949) have shown in certain legumes like lespedeza, soyabean, cowpeas and sweet clover that with increasing concentration of manganese in culture solution there was a decrease in the size of leaves, with slight chlorosis. Similar observations were also made by Lohnis (1951) in some vegetable crops. Sharma **et al.** (1988) observed a decrease in leaf area in pigeon pea at increasing level of manganese (above 0.55 mg Mn^{-1}) in the growth medium.

Thickening in the leaf (from 0.38-0.61mm) was observed with increase in iron and manganese in the soils. This may possibly be co-related to the enlargement of the mucoid cells, producing profuse mucous, to ward off the action of excess iron and manganese that may interfere with the cellular metabolic processes in the leaf. It seems likely therefore that this is an appparent edaphic physiological adaptation to the type of soil environment, conferring on it a metal tolerant characteristics, imperative for its survival under adverse conditions as found in tailings. The evidence presented here may suggest the evolution of a certain degree of tolerance to iron and manganese in **Ipomoea** plants, apparently related to the common presence of these two major polluting metals in the iron ore mine wastes. Thickening of leaf and decrease in its size have been also reported by Foy **et al.**(1978) due to aluminium excess in growth medium.

Shoot dry weight and root dry weight were adversely affected due to increasing percentage of iron tailings in the soil compositions. Shoot dry weight decreased from 28.025 to 3.785g/plant and root dry weight decreased from 5.87 - 1.93g/plant

(Table-4.2) with increase in iron (from 24.5 - 51.0%) and manganese (0.2-1.2%) concentration in the soil composition (Table-4.1). Effect of iron and manganese on shoot and root growth have been reported by several workers, Shetron and Spindler (1983) found that the roots of **Medicago sativa** growing in iron tailings had a lower dry weight than the plants grown in natural soils. Wheeler **et al.** (1985) in the case of **Epilobium hirsutum** and **Juncus subnodulosus**, and Pegtel (1986) in **Succisa pratensis** observed a reduction in shoot and root dry weight with increasing concentration of iron and manganese in the culture medium.

Vlamis and Williams (1973) showed in lettuce plant that with increasing concentration of manganese from 0.1 ppm to 10.0 ppm in culture solution, there was a decrease in dry weight of the root, Ohki (1976) also made similar observations in case of soyabeans. The report of these workers in different taxa corroborate the present result in **Ipomoea pes-caprae** grown in different composition of iron ore tailings with elevated level of iron and manganese.

Iron and manganese can greatly reduce yields without exhibiting clearly identifiable symptoms in plant tops. Except for suppression of growth, no visual symptoms of toxicity were observed in plants grown in increasing concentration of iron ore tailings in the soil compositions. Although no chlorosis was noticed, a variation in leaf colour was observed from dark green to pale green with increasing percentage of tailings in the soil compositions. Some cases of manganese toxicity may or may not be accompanied by chlorosis, apparently depending on the availability of iron (Kriel, 1941; Hopkins **et al.** 1944). A decrease in plant growth occurs, when plants become severely chlorotic but even when the chlorosis is corrected or is absent there are large decrease in growth, when plants are exposed to high concentration of manganese in culture solutions (Hopkins **et al.** 1944; Berger, 1948; Vlamis and Williams, 1962).

Dry weight production is the net result of the accumulatior of structural components derived from photosynthesis and metabolism. Factors that limit photosynthesis can limit growth. Manganese toxicity in plants has been associated with auxin destruction in cotton (Morgan **et al.** 1986; Morgan **et al.** 1976), reduced number of cells leaf^{-1} and cell volume in sugar beets (Terry **et al.** 1975) and reduced nitrate reductase activity in lettuce (Jones and Menary, 1974) and soyabean (Heenan and Campbell, 1980). These manganese toxicity effects may have adversely affected **Ipomoea** plant growth and development in iron ore tailings having high concentration of manganese.

4.4.3. Biochemical Responses of Ipomoea pes-caprae to Different Composition of Iron Ore Tailings.

Any morphological change seen in a plant grown under natural or stress conditions are due to the biochemical responses of the plant to the stress. Excess or deficiencies of the metal ions have effects on the metabolic processes of the plant leading to the biochemical changes in the plant systems. Epstein (1969) pointed out that an element in excess can interfere with metabolism through competition for uptake, inactivation of enzyme, displacement of essential elements from functional sites or alteration of the structure of water. The exact physiological mechanism of metal toxicity or tolerance are still debated, these may well be different in different plant species and varieties and be controlled through different biochemical pathways to ward off the metal toxic effect.

Chlorophyll 'a' and 'b' and hence total chlorophyll (a + b) were adversely affected due to the increasing percentage of tailings in the soil composition. Chlorophyll 'a' decreased from 78.76 to 39.3 mg percent and 'b' from 115.57 to 57.78 mg percent (Tabl-4.3) with increase in iron and manganese in the different compositions. Kelley as early as (1912) and Johnson (1924) have shown that excess manganese, at pH values above 5.5, deprived the availability of iron and thus failed to produce chlorophyll in pineapple, Sideris and Young (1949) also made similar observations in the same plant. Agarwala **et al.** (1964) have reported a decrease in chlorophyll content in barley leaves with excess manganese in the medium. White **et al.** (1974) hypothesized that manganese interfered with iron utilization in leaves for chlorophyll synthesis. The decrease in chlorophyll due to the presence of heavy metals in the growing medium may be due to the interference with the synthesis of proteins and structural components of chloroplast (Nag **et al.** 1981). Higher level of manganese (5.5 mg l^{-1}) are found to reduce concentration of chlorophyll considerably in pigeon pea (Sharma **et al.** 1988). Clairmont **et al.** (1986), Csatorday **et al.** (1985) have also reported the inhibition of chlorophyll synthesis by manganese toxicity. Ohki (1985) also observed a similar depression in chlorophyll content in wheat.

Impaired chlorophyll development by heavy metals may be due to interference with the synthesis of proteins, the structural component of chloroplast. The effect of heavy metals presumably blocks either the synthesis or the activity of the enzyme-proteins responsible for chlorophyll biogenesis.

Agarwala **et al.** (1977) showed that in chlorotic plants of barley, the reduction of chlorophyll by excess manganese was counteracted by iron. This may probably be the case in the present experiment as the plants growing in elevated level of manganese and iron exhibited no visual chlorotic symptoms, which indicated that iron which is found in high concentration in the tailings may partially counteract and suppress the toxic effects of manganese in chlorophyll synthesis.

A reduction in total soluble sugars and proteins was observed with increasing concentration of iron and manganese in the soil compositions. The soluble sugar content decreased from 127 to 95 mg percent (Table-4.3). Sideris and Young (1949) have shown that the manganese content influenced the sugar content of pineapple plants. They found that with high manganese content there was a decrease in the total sugar content in the leaves of plant. Kelley (1912) has shown that soils containig a high percentage of manganese yielded poorly and had lower amounts of starch in pineapple leaves.

Total protein content in the leaves of **Ipomoea** plants decreased from 295 to 224.5 mg% (Table-4.3) with increasing concentration of iron and manganese in the soil composition. Sideris and Young (1949) have reported that excess manganese affected the protein content of pineapple plant. They also reported a positive co-relation between chlorophyll and protein. Similar observation is also made in the pesent experiment with **Ipomoea** plant. Del Rio **et al.** (1978) have shown that prolonged exposure to excess iron in nutrient cultures decreased total soluble proteins in pea plants. The results obtained by the above workers support and are in agreement with the present findings.

Moreover, it is not beyond the scope of this chapter, if it is argued that the carbohydrate synthesis, besides other several factors depend very much on the chlorophyll content of leaves. Since the chlorophyll is badly affected due to increasing concentration of iron and manganese with increasing percentage of iron ore tailings in the soil composition, it must be possibly due to this reduction in chlorophyll that has deprived the plant its normal carbohydrate synthesis and hence the depression in total soluble sugars and proteins with increase in percentage of tailings in the soil compositions. Decrease in sugar and protein have been reported by Foy (1974) due to aluminium toxicity in several plants and Sarkunan **et al.** (1984) in rice plants.

It is well known that there is an inter-relationship between sugars, proteins and chlorophyll biosynthesis in plants. Presence of excess heavy metals such as manganese in the growing medium inhibits photosynthesis and depression in the biosynthesis of chlorophyll-proteins (Ohki, 1985; Nable **et al.** 1988). Impaired chlorophyll development may be due to interference with the synthesis of proteins by heavy metals. It is probable that the excess of an element apart from other effects upsets the protein due to enzyme repression or inhibition. It may however, be inferred from the data presented in table-4.3, that low levels of chlorophyll, sugars and proteins in **Ipomoea** plants growing in different composition of soil may probably be attributed to the toxicity imposed by heavy metals involving an overall disruption in the synchronization of different metabolic processes occuring in the cells.

Proline accumulated in the leaves with increase in percentage of tailings in the soil compositions. Proline content increased from 22 to 56mg percent (Table-4.3) with increase in iron and mangannese in the soil. Plants have been known to accumulate high levels of proline, when subjected to water stress (Aspinall & Paley, 1981), nutrient deficiencies (Savitakaya, 1976; Ghildiyal **et al.** 1986), air pollutants (Godzik and Linsters, 1974; Soldatini, 1987) and pesticides (Deshpande and Swamy, 1987).

The biochemical mechanisms leading to the induction of proline accumulation and its physiological significance have not yet been elucidated. Goas, Goas and Larher (1970) reported that proline accumulation indicated a disturbance in nitrogen metabolism. Kudrev and Istakhov (1967) and Kudrev (1970), have suggested that proline accumulation is caused by decomposition of such products as proteins. Stewart (1973) showed that proline accumulation during wilting was caused by decreased protein synthesis and that carbohydrates prevent proline oxidation. It is probable that accumulation of proline in **Ipomoea** plants growing in different composition of tailings at increasing level of iron and manganese may be a stress response to these metals resulting from a decrease in rate of protein synthesis as observed in the present investigation. Also it can be added here that failure of cellular machinery or specific biochemical pathway as that of protein synthesis might result in accumulation of proline in the amino acid pool that remains unincorporated in proteins due to depression or inhibition of protein synthesis. Protein synthesis decreases in stressed plants (Hsiao, 1973), so that demand for amino acids slows down and they will accumulate if not end product regulated.

It may be suggested that proline accumulation is of adaptative value as part of plant's strategy for withstanding the hostile enviroment due to metal excess, nutrient deficiency, water scarcity and heat tolerance as existing in the iron ore tailings.

4.4.4. Chemical Analysis of Ipomoea pes-caprae Grown in Different Composition of Iron Ore Tailings.

With increasing percentage of tailings (from 0-100%) in soil compositions, there was an increase in the amount of iron, manganese and decrease in concentration of aluminium in the soil compositions as well as in the plant tissues, both in roots and shoots (Table-4.4). It is well known that these elements inhibit the growth of the plant when in excess (Foy **et al.** 1978). Micronutrients are essential for plant growth and development but in lesser amounts than macronutrients.

Th The increasing concentration of iron and manganese and decrea decreasing concentration of aluminium in plant shoots and roots reflect their concentration in different composition of tailings. Results of plant tissue analysis revealed that **Ipomoea** plants accumulated 2840 ppm of iron and 295 ppm of manganese in shoots of plants grown in hundred percent tailings. Accumulation of such high level indicates the tendency of this plant as a bio-accumulator.

Iron and manganese absorption, translocation and accumulation in **Ipomoea** plants is a function of iron and manganese present in the tailings. Iron and manganese content of **Ipomoea** plants shoots and roots increased as level of iron and manganese increased in the soil compositions. This is in agreement with the results obtained by Wheeler **et al.** (1985) in **E. hirsutum** and **J. subnodulosus.** They found that with increasing concentration of iron and manganese in the growing medium there was an increasing concentration of these metals in their plant tissue. Okhi (1985) also made similar observations in wheat.

Toxic levels of iron, aluminium and manganese are known to occur in many acid soils (Hewitt, 1953; Rorison, 1960). In the present investigation the pH of soil increased from 6.5 to 7.2, which is tending to be alkaline. The iron and manganese concentration in plant tissue however, increased with increasing soil pH. This unexpected response of plant iron and manganese concentration to pH has been reported in nutrient solution (Munns **et al.** 1963) over the range of pH 5 to 7. Similar observations were made by Jones (1957) in straw of vetch and rye in alkaline soil, he showed that concentration of manganese increased with increasing manganese in soil.

The increased iron and manganese concentration in **I. pes-caprae** grown in different compositions of iron ore tailings may probably be due to physiological effects of pH or may be due to other causes. Amphoterism could also contribute to greater iron and manganese availability at higher soil pH.

Jaffre and Heim (1977) reported in **Maytenus** species that, manganese accumulation depends on the total manganese in the soil and independent of pH. Gupta and Chipman (1976) reported manganese toxicity of carrots in a sphagnum peat at pH 7.8-8.1. The results of these workers confirms the present evidence in **Ipomoea** plants in different compositions.

The soil environment and plant metabolic factors may have influenced mineral composition of **Ipomoea** plants. It is interesting to note that none of the plants exhibited symptoms of chlorosis and toxicity in the presence of such large amounts of iron and manganese in the tissue, as has been reported by many workers in the past in different taxa.

Translocation of excess iron and manganese to their shoots, both strongly suggest some adverse effect of high iron and manganese on this plant. Although the growth was depressed without any visible signs and symptoms of injury, it is speculated that other factors acting singly or cumulatively with iron and manganese excess may have effected the plant growth.

It appears that **Ipomoea** has acquired physiological immunity to high iron and manganese, when present together in nutrient deficient soils. The resistance to iron and manganese toxicity is independent of geographical origin and hence the soil type on which this plant was originally found growing. This may have an interesting ecological significance for plants which naturally grow in soils under conditions of high concentration of both iron and manganese, where normal growth appears to be quite unaffected by this soil conditions as is shown to be true in **Ipomoea pes-caprae** in present investigation.

The ability of **Ipomoea** plants to tolerate iron and manganese may be part of an adaptive mechanism acquired by this plant over a period of time, that enables it to grow under environment as existing in iron ore mine wastes. It is quite probable that the high mucous and latex in this plant tissue may possibly act as buffer regulating the supply and flow of these two metals to the active sites in the cells.

Ipomoea plants growing in the tailings is subjected to a hostile environment especially the various adverse edaphic factors including the rather high content of iron, aluminium and manganese, it must have undergone physiological adaptation to combat the unfavourable influences. The limitation on plant growth may be due to combination of deficiencies of major plant nutrients, particularly nitrogen and phosphorous, and high concentration of metal ions which are demonstrably toxic to most plants. While the toxic effect of iron and manganese not only have adverse influence on plant growth but it is an important one, which occurs widely in iron ore mine wastes.

In conclusion, the evidence presented here suggests the evolution of a high degree of tolerance in **Ipomoea pes-caprae** to iron and manganese apparently related to the common presence of these two major polluting metals in iron ore mine wastes, perhaps associated with somewhat alkaline pH at the site. This in turn suggest some common physiological mechanisms of tolerance for both metals found together. Not only **Ipomoea** plant have to withstand high concentration of iron and manganese but also nutrient deficiency stress.

Metal tolerant species have been used in attempts to reclaim and recolonize metal contaminated wastelands (Bradshaw **et al.** 1965). It is envisaged that this metal tolerant population could be used for reclaiming the iron ore mine wastes for the purpose of primary stabilization of the disolated and degraded areas and mitigating the leaching of these metals into the surrounding productive areas.

Ipomoea pes-caprae plants which have wide medicinal uses could be potentially exploited for the manufacture of pharmaceutical products. It would not only cover the waste but contribute greatly to soil economy and fertility due to its dead foliage and above all help to restore organic matter and nurient cycling. Nevertherless, this could be the prime step in reconstruction of ecosystem.

CHAPTER FIVE

ESTABLISHING VEGETATION ON IRON ORE MINE WASTES

5.1. INTRODUCTION

Two classes of waste result from the mining of ores: 1) piles of surface overburden, waste rock and lean ore, and 2) a fine grained waste resulting from the ore beneficiation process and deposited in man-made basins. Both classes of waste occupy large segments of landscape in the vicinity of the mine and can detract from the aesthetic quality of natural landscape.

The existence of such areas of land is undesirable from several points of view; land contaminated by heavy metal waste represent a serious source of environmental pollution through erosion by wind and water. The leaching of these waste can adversely affect the flora and fauna of terrestrial and waterbased ecosystems.

All these waste materials are infertile, contain heavy metals like Fe and Mn, which creates an environment hostile to plant growth and they are instantly recognisable for their sparse or complete lack of vegetation. The absence of vegetation heightens the risk of pollution which can occur in several ways.

Physical stabilization by covering the toxic waste material with an innocuous amendment and chemical techniques which rely on the formation of an air and water resistant surface crust (Dean **et al.** 1971) require simple engineering, but are expensive and ineffective for permanent reinstatement. Such treatments do not improve the visual appearance of waste, yet the need for visual improvement is often important. Since mines are often conspicuously situated in areas of natural beauty.

The alternative is to cover the waste with vegetation (Street & Goodman, 1967; Smith and Bradshaw, 1972), this can effectively camouflage the wastes giving it an aesthetic appeal, provide stability, prevent wind and water erosion and control serious effets of pollution. It is economical, rapid and results in the most complete surface protection.

5.2. METHODOLOGY

5.2.1. Selection of Plant Species

The selection of plant species was based on a number of attributes. The most important being the ability of the species to colonize the new land surface, to survive drought and to tolerate infertile soils. Other attributes considered important, include longevity, timber quality and value to native fauna.

The iron ore mine soils are physically and chemically infertile. They become super saturated in wet season, but dry out rapidly when rain cease. Therefore plants would experience considerable long periods of moisture stress and only drought tolerant plants would survive.

Native or local plant species growing on similar soil materials and environment have evolved to survive and reproduce successfully under the prevailing extremes in temperature and soil moisture, while growing in soils of poor fertility.

Native flora can be permanently established, cheaply and efficiently since the seeds are available locally. Fertilizer requirement of native species are generally low. Also it would be expected that native flora will be self-sustaining and maintenance free.

5.2.2. Collection of Seed.

Seeds were collected in the dry season from healthy trees and shrubs from unmined habitats and native forests close to Pale-Iron Ore Mines, where species have already reached reproductive age. Seeds were either picked from the branch or ground. From larger shrubs and small trees, the branches carrying seeds were pruned and laid out on large tarpaulins or spread on specially prepared ground to dry until it is released from its pods or capsule. In certain cases the pods were beaten to release the seed. They were dried and stored at suitable temperature. Sufficient seed from about 21 species were collected to ensure that each species is adequately represented. It is expected that the resultant high diversity will favour the formation of stable ecological relationship in the revegetated flora. In a few cases young seedlings were collected from native species and nursed till planting on the wastes.

5.2.3. Raising of Seedlings in Nursery.

Sampling and germination tests were carried out in order to improve the efficiency of the collection of viable seed. Hard coated seeds were either treated by soaking in boiling water or dilute acids such as hydrochloric, prior to sowing. The treated seeds were put in seed bed or allowed to germinate in steel trays. Germinated seeds were potted in polybags (12 x 20 cm) size, filled with potting mixtures comprising 20% sand, 30% decomposed farmyard manure and 50% loamy soil. The poly pots were filled manually, and arranged in rows under a temporary palm leaves shady arcade. They remained in this shade till signs of their establishment were observed, and watered with a sprinkler. More than 40,000 seedlings representing 21 species were raised in Pale-Iron Ore Mines Nursery during 1986.

5.2.4. Planting on the Iron Ore Waste.

Direct seeding of the iron ore mine spoil at Pale would be uneffective for several reasons. Therefore planting stock was raised in Pale-Iron Ore Mines Nursery, which was more practical and economical, as most of the species were found to have a very poor field success rate.

Planting was carried out after good rain, when soil was very moist with the onset of monsoons, during June '86. Planting was done at 2 x 2m spacing pits. The soil was mixed with farmyard manure at the time of plantation. The transplanted species depended only on monsoon rains for their moisture requirement. They were not watered during dry periods.

The objective of this investigation was to identify adapted plant species for iron ore mine waste reclamation.

5.2.5 Experimentation Approach

The results obtained here are of 3 years period from June, 1986 to May 1989, to identify plant species for establishing vegetation on iron ore mine wastes.

Plantation was done first in June 1986 with 21 different native species on un-mined soil and iron ore mine spoils without fertilizer, their performances in terms of survival percentage and growth were observed during first and third year (Table-5.1).

According to their performance during first year, six species were selected for planting on the waste during 1987.

Three plots were maintained as follows:-

1)Unmined soil with and without amendments.
2)Iron ore mine waste without amendments(dumps and tailings)
3)Iron ore mine waste with amendments (dumps and tailings)

The amendments were made with crushed sea-weeds and commercially available fertilizer (NPK-19 : 19 : 19).

Sea-weeds are known to contain growth promoting hormones, trace elements and micro-nutrients (Challen and Hemingway, 1965). Beneficial effects from use of seaweed and seaweed extracts have been obtained on plants, crop yield, their seed germination, their resistance to fungal and insect attack (Brain **et al.** 1973). Two different species of sea-weeds were collected from the intertidal area of Anjuna, Chapora and Vagator beaches of North Goa District, during January and February '87. They were washed, sun dried and powdered on a steel mill and stored at suitable temperature.

Ten plants of each species were undertaken for their growth performance, each plant was given a basal dose of 100g of seaweeds and 50 gms of NPK. In all there were four treatments as follows:-

1)Treatment with **Padina** sp.
2)Treatment with **Sargassum** Sp.
3)Treatment with mixed sea-weeds (50g **Padina** + 50g **Sargassum**)
4) N.P.K.

Amendments were made during mid-August and their growth performance was monitored, and cost economics was worked out for the area under study maintaining 3 x 4m spacing at the time of plantation.

Table - 5.1. Percentage Survival and Growth of Trees and Shrubs after 1st and 3rd year of Plantation on Unmined Soil and Mine Wastes.

Name of plant species	Age of planting stock. months	Ht. at planting (cm)	Unmined Soil				Dump Waste				Iron Ore Tailings			
			Survival %		Growth (cm)		Survival %		Growth (cm)		Survival %		Growth (cm)	
			1 yr	3 yr	1 yr	3 yr	1 yr	3 yr	1 yr	3 yr	1 yr	3 yr	1 yr	3 yr
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acacia auriculiformis	4	46.6 ± 5.23	80	75	70.2 ± 7.38	101.44 ± 5.07	70	60	65.39 ± 6.77	90.25 ± 5.98	75	70	68.7 ± 7.32	95.23 ± 5.61
Acacia nilotica	3½	50.2 ± 7.42	85	80	71.6 ± 6.34	87.4 ± 7.14	80	80	68.1 ± 7.92	82.01 ± 6.87	85	80	70.2 ± 6.10	85.31 ± 7.36
Anacardium occidentale	3	38.3 ± 6.32	80	70	48.6 ± 5.93	65.0 ± 4.24	65	65	45.12 ± 6.81	60.05 ± 5.38	75	70	46.29 ± 6.10	62.7 ± 5.27
Azadirachta indica	6	53.5 ± 8.10	90	75	72.3 ± 6.18	102.31 ± 6.05	60	55	65.7 ± 5.97	93.01 ± 4.76	65	65	68.71 ± 3.97	98.3 ± 6.52
Alstonia scholaris	10	65.7 ± 7.90	95	85	88.7 ± 5.52	102.28 ± 8.24	85	80	84.0 ± 6.95	97.39 ± 7.92	85	85	87.1 ± 6.05	101.20 ± 6.77
Acacia pleniformis	4	51.2 ± 6.63	80	70	65.8 ± 6.71	88.66 ± 5.5	60	50	65.1 ± 7.81	85.9 ± 5.12	80	60	67.31 ± 5.9	85.07 ± 5.65
Bauhinia purpurea	3	70.6 ± 8.92	95	80	119.8 ± 6.80	162.57 ± 8.71	85	80	107.3 ± 9.32	125.46 ± 9.57	90	85	115.6 ± 7.98	130.30 ± 9.32

Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Casuarina equisetifolia	4	55.7 ± 6.53	90	80	85.7 ± 8.43	120.44 ± 7.87	80	75	80.5 ± 9.79	110.57 ± 7.98	85	85	78.16 ± 7.12	102.10 ± 8.82
Erythrina Variegata	10	60.3 ± 5.07	85	65	88.6 ± 6.15	95.0 ± 6.58	70	40	85.2 ± 6.23	90.36 ± 6.97	50	30	79.9 ± 6.21	89.02 ± 7.32
Garcinia indica	5	15.8 ± 4.98	95	80	26.9 ± 6.2	43.08 ± 9.39	80	75	24.3 ± 5.42	42.71 ± 5.38	75	70	23.71 ± 5.16	45.27 ± 6.10
Leucaena leucocephala	6	100.6 ± 7.95	90	85	164.7 ± 5.72	210.5 ± 15.16	60	60	150.3 ± 9.38	195.40 ± 10.71	65	60	158.5 ± 9.97	200.6 ± 9.34
Parkia biglandulosa	4	25.1 ± 6.20	75	70	46.1 ± 7.01	107.53 ± 9.8	70	70	40.1 ± 8.12	93.20 ± 6.45	75	70	42.2 ± 7.6	95.3 ± 7.62
Pterocarpus marsupium	6	45.6 ± 4.76	90	85	66.5 ± 8.03	89.0 ± 7.39	85	75	63.5 ± 6.93	85.7 ± 5.89	80	75	62.8 ± 5.10	86.00 ± 5.68
Pitecellobium dulce	10	46.9 ± 5.39	85	85	69.5 ± 6.13	96.7 ± 7.38	75	70	65.03 ± 6.93	91.7 ± 5.89	70	70	67.3 ± 7.19	90.21 ± 7.32

Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Syzygium cumini	4	60.8 ± 6.24	85	80	82.9 ± 6.73	103.10 ± 7.08	80	80	79.2 ± 7.98	98.5 ± 9.37	85	80	75.8 ± 8.67	93.30 ± 6.17
Syzygium zylenica	5	30.3 ± 5.13	90	85	43.6 ± 6.17	67.5 ± 5.5	85	80	41.1 ± 5.93	61.9 ± 4.8	90	80	40.7 ± 6.02	65.3 ± 5.13
Terminalia bellerica	10	35.6 ± 6.11	80	70	53.5 ± 7.4	79.87 ± 6.19	80	65	54.4 ± 6.23	70.63 ± 6.58	90	75	51.4 ± 6.90	69.82 ± 5.97
Terminalia tomentosa	10	25.9 ± 4.8	75	60	38.5 ± 7.9	49.7 ± 6.76	80	50	31.6 ± 5.37	43.61 ± 5.38	70	60	35.3 ± 4.9	45.73 ± 5.16
Thespesia populnea	10	40.7 ± 8.20	85	80	69.3 ± 6.1	89.7 ± 6.31	75	70	58.5 ± 7.32	85.0 ± 7.30	70	70	63.9 ± 7.0	87.32 ± 8.91
Terminalia arjuna	6	37.5 ± 5.9	90	80	55.68 ± 5.01	89.81 ± 6.6	85	75	50.79 ± 7.8	82.37 ± 6.52	85	80	51.1 ± 6.02	83.07 ± 4.39
Xylia xylocarpa	6	41.02 ± 6.8	95	80	53.71 ± 6.34	88.11 ± 7.14	80	75	50.9 ± 4.32	83.61 ± 5.17	75	75	53.5 ± 5.91	90.68 ± 7.91

± Denotes Standard Deviation.

The objective here was to look for cheap source of soil ameliorant for the establishment phase and study the plant growth response.

5.3 RE-VEGETATION COSTS.

Costs directly associated with establishing native vegetation per hectare of iron ore mine waste are listed in table-5.2, a breakdown of costs is shown. The total cost of revegetation includes, cost of seed collection and raising of seedlings, site preparation cost, cost of planting, transportation and fertilizer cost for the year 1987. The total cost of revegetation each year will vary significantly, depending upon the appreciation and inflation in each cost factor. The fertilizer cost can be reduced by a margin of 2%, if seaweeds are exploited and utilized as a source of manure. Only the transportation and collection will be the part of expenditure. However, it would serve dual purpose, as a slow release fertilizer, conserve moisture and add to the organic content of the waste. Current per hectare cost for tree planting and fertilized once, is ₨. 5082-00 (300-71 $).

Table-5.2. Breakdown of Revegetation cost/hectare area of Iron Ore Mine Waste.

3 x 4 m spacings 825 trees / ha.	**Cost/ha Rs. p**	**Cost/ha $**
Cost of seed collection & raising of seedlings (825) @ Rs.1.50/seedling	1237-50	73-20
Cost of site preparation (including contouring)	555-00	32-84
Cost of tree planting @ Rs.3/tree	2475-00	146-45
Transportation cost (from nursery to site)	200-00	11-83
Fertilizer cost (206.25 kgs) @ 200g/tree	425-00	24-56
Farm Yard Manure	200-00	11-83
	5082-50	300-71

Cost of Sea-weed collection and transportation is not included.

The objective of this study was to re-establish a complete ground cover using native flora after mining as quickly as possible, which in turn will support the native flora and fauna and gradually increase the inherent fertility of the substrate by building up organic matter while providing some protection against erosion during the establishment phase.

5.4. RESULTS AND DISCUSSIONS

The native plant species planted on three different sites (unmined soil, dump waste and tailings), their height and survival percentage is shown in table-5.1. Among the twenty one plant species planted, thirteen species showed extremely high survival percentage, viz; **Anacardium occidentale** (65-70%), **Alstonia scholaris** (80-85%), **Bauhinia purpurea** (80-85%), **Pterocarpus marsupium** (75-80%), **Syzygium cumini** (80%), **Xylia xylocarpa** (75-80%), **Casuarina equisetifolia** (75-80%), **Garcinia indica** (70-80%), **Parkia biglandulosa** (70%), **Pithecellobium dulce** (70-85%), **Syzygium zylenica** (80-85%), **Terminalia bellerica** (65-75%), and **Terminalia arjuna** (75-80%), and rapid growth, on all the three sites tested. Species performance differed significantly. There was a variability in the growth and survival percentage in all the species for all the different sites. Survival of all species declined with time. For most species the greatest decline appeared from the time of planting to the first year of establishment. However, between first and third year the mortality was some what stabilized in few cases, if not all. Mortality was higher on dumps than on unmined soil and tailings. Growth trends were similar to survival trends. It is quite probable that the differences in physical and chemical characteristics of the sites particularly low water retention may have contributed to the growth and survival percentage variability for the three sites.

The sandy nature of the dump increases permeability and reduces water retention capacity. In addition, the surface composition coupled with the steep undulating slopes and susceptibility to water erosion may have contributed to the poor performance on the dumps (Fig. 5.1). In contrast to this, the tailing basins represented more uniform sites, and the finer

EXPLANATION OF PLATE - VII

ESTABLISHING VEGETATION ON IRON ORE MINE WASTES

Fig. 5.1. Revegetated dump.
Fig. 5.2. Revegetated tailing pond.

Fig. 5.1

Fig. 5.2

textured materials of the tailings retained larger amounts of water. This might have accelerated the growth of plants during dry periods (Fig. 5.2).

Besides physical and chemical characteristics of waste, temperature and elevation would also seem to determine the performance of these species and their adaptability. Day and Ludeke (1980) have shown that native shrubs and trees can be successufully used for reclamation of copper mine wastes in U.S.A. They also suggested that revegetation especially in semi-arid regions, where the average rainfall is low and comes in violent bursts over a short period of time should include plant species adapted to the environment.

The selection of tree and shrub species planted on all the three sites were based on three criteria (1) performance of trees and shrubs on similar sites (2) local species growing on similar soil materials and (3) ease of obtaining planting material. All the species tested were locally collected around the mining sites.

Analysis of the soils from all the three sites selected for revegetation are discussed in chapter-I (Table-1.2 and 1.3). The pH (6.6) of dump waste was slightly acidic and higher than the unmined soil (pH-6.2), whereas the pH (7.2) of tailings was alkaline. This implies that plants that grow well in slight acidic to near alkaline environment would therefore be better adapted to these specific iron ore mine wastes, than plants that grow best under near acidic conditions.

It has been already shown in the soil analysis data (in chapter-I) that the nitrogen content of the waste is much lower (ranging from 8-15 kg/ha) than that of unmined soil (46 kg/ha). High doses of nitrogen would be needed by plants growing in iron ore mine wastes. The waste are also very deficient in available phosphorous (4-6 kg/ha), potassium (40-85 kg/ha) and organic matter (ranging from 0.22-0.48%). But however, it was observed that the selected species (Table-5.1) seems to be performing well in such nutrient deficient conditions.

Table-5.3. Concentration of Fe and Mn in Plant Tops grown in Unmined Soil and Iron Ore Mine Wastes.

Name of plant species	Unmined Soil		Dump Waste		Tailings.	
	Fe ppm	Mn ppm	Fe ppm	Mn ppm	Fe ppm	Mn ppm
1. Anacardium occidentale	200	27	235	38	256	46
2. Acacia nilotica	185	33	193	35	201	39
3. Acacia auriculiformis	179	30	181	43	210	50
4. Azadirachta indica	116	20	141	31	278	36
5. Alstonia scholaris	205	39	225	46	261	58
6. Acacia pleniformis	162	26	172	39	310	46
7. Bauhinia purpurea	191	43	216	62	253	72
8. Erythrina Variegata	150	21	156	34	201	46
9. Casuarina equisetifolia	198	25	200	49	265	57
10. Garcinia indica	105	33	127	56	198	66
11. Leucaena leucocephala	123	28	134	48	179	59
12. Parkia biglandulosa	187	49	190	56	216	74
13. Pterocarpus marsupium	176	44	188	68	211	86
14. Pitecellobium dulce	145	31	173	47	203	62
15. Syzygium cumini	193	52	217	63	238	85
16. Syzygium zylenica	200	49	222	60	243	81
17. Terminalia bellerica	167	34	183	48	219	76
18. Terminalia tomentosa	153	37	171	49	196	60
19. Thespesia populnea	131	32	153	51	183	70
20. Terminalia arjuna	164	41	179	61	206	76
21. Xylia xylocarpa	168	46	185	59	214	80

Fe and Mn concentrations are mean of three determinations.

Similarly, it has also been observed that the plants also accumulate high content of Fe and Mn in their tissue (Table-5.3) without showing any external abnormalities or characteristic visual symptoms, which means that plants are adapted to tolerate high concentration of these excess metal ions present in their growing medium. It is quite probable that plant species showing rapid growth absorb and accumulate higher concentration of these metals compared to the slow growers without any indication of serious symptoms. Content of Fe and Mn in the plant tops from tailings and dump was excessive in comparison to the ones that were grown in natural soils. These values from both sites are a reflection of their concentration in the wastes to which the native species are adapted.

A measure of success in establishing vegetation on these wastes could be the growth and survival percentage. Lack of success may presumed to be caused by a combination of low water holding capacity, undesirable mineralogy, nutrient imbalance and erodibility. Most success in establishing vegetation has been achieved in tailings. These also require the least amount of site preparation. In course of years, it would be the best soil forming materials.

The present study revealed a highly variable growth pattern for all the species, in all the three revegetated sites. It would require further study to determine whether the variable growth is related to the heavy metal concentrations and their effect on plant chemical composition, or the variations in the physical and chemical properties of the wastes.

Of the twenty one native species studied, thirteen plant species named earlier proved to be the most effective, even though the wastes represented an adverse site for plant growth. These would provide adequate vegetation for erosion control and reduce run-off velocity of the waste. Once this is achieved, annual grasses can be incorporated into the soil surface to improve the soil medium, so that adapted perennial species would provide enough top coverage for their proliferation, growth, and minimize the adverse climatological conditions. This long term stabilization would function as a plant community and supply organic matter. Organic amendments often useful in speeding up succession, play a minor role in building up a large stable organic matter fraction (Jurgensen, 1979). Plant species capable of high production, initially will lead to more rapid accretion of surface organic matter. This would assist growth of species of longer life and greater economic value. Deep-rooting plants will resist drought and high surface temperatures.

Such a mixed community will not only stabilize the wastes but provide a habitat for fauna and help blend the disturbed land area into the surrounding environment.

5.4.1. Response of Six Plant Species to Soil Amendments.

Growth and girth of six plant species amended with the four different treatments is represented in table-5.4. The foregoing parameters increased when sea weeds were added in all the three sites. Commercial fertilizer showed appreciable increase in height and girth over the controls, however it was lower than the ones treated with sea weeds. Maximum growth was observed in plants treated with mixed sea-weeds compared to the two added separately. In general, plant species produced significant desirable growth in the one grown in tailings. The growth therefore differed between soil materials and among plant species. It has been observed that with optimum soil moisture and fertilizer, tailings would help to provide rapid and attractive plant cover in these disturbed areas, at least in the initial stages of revegetation. This abandoned areas thus could be most effectively brought under long range vegetation and help ground cover.

It has been suggested by several workers, e.g. Bennett (1977) that almost any plant can be grown on strip-mined lands, if environmental and nutritional requirements are met. Nutritional requirements include heavy application of nitrogen and phosphorous fertilizers and organic enrichment of soil materials to improve soil texture and increase its resistance to surface crusting (Leullen, 1977). However, the present study has indicated that

Table-5.4. Response of Six Plant Species to Different Soil Amendments.

Name of plant species	Treatment	Age of plant stock. months	Ht. of plant (cm)	Girth at planting. (cm)	Unmined Soil				Dump Waste				Tailings.			
					Growth (cm)		Girth (cm)		Growth (cm)		Girth (cm)		Growth (cm)		Girth (cm)	
					1 yr	2 yr	1 yr	2 yr	1 yr	2 yr	1 yr	2 yr	1 yr	2 yr	1 yr	2 yr
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Anacardium occidentale	Control	3	35.42 ± 4.29	1.41 ± 0.091	49.9 ± 4.2	84.4 ± 5.6	2.56 ± 0.29	3.5 ± 0.31	45.2 ± 3.2	69.0 ± 3.1	2.47 ± 0.4	3.8 ± 0.3	44.10 ± 4.10	77.0 ± 3.17	2.52 ± 0.31	3.3 ± 0.20
	Padina sp.				55.6 ± 4.97	91.3 ± 5.7	2.72 ± 0.19	4.2 ± 0.26	49.2 ± 3.9	75.0 ± 4.27	2.75 ± 0.29	4.5 ± 0.17	46.1 ± 5.5	80.0 ± 4.5	2.63 ± 0.28	3.9 ± 0.30
	Sargassum sp.				57.1 ± 6.36	92.7 ± 5.2	2.69 ± 0.16	4.0 ± 0.22	51.6 ± 3.7	76.3 ± 4.21	2.73 ± 0.25	4.6 ± 0.27	46.9 ± 5.5	79.01 ± 4.5	2.90 ± 0.19	4.3 ± 0.27
	Mixed seaweed (**Padina** sp. + **Sargassum** sp.)				62.09 ± 6.68	94.63 ± 5.21	2.75 ± 0.26	4.5 ± 0.29	53.04 ± 4.2	79.02 ± 6.10	2.76 ± 0.18	4.91 ± 0.26	50.2 ± 5.17	83.3 ± 5.9	2.85 ± 0.26	4.37 ± 0.021
	N.P.K.				56.0 ± 5.1	90.3 ± 4.9	2.61 ± 0.24	3.8 ± 0.17	49.17 ± 3.9	72.2 ± 5.17	2.64 ± 0.23	4.0 ± 0.27	47.5 ± 4.15	80.9 ± 5.71	2.55 ± 0.21	3.5 ± 0.19

Contd.

Table-5.4. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Bauhinia purpurea	Control	3	69.55 ± 5.69	1.86 ± 0.21	105.12 ± 7.98	150.0 ± 8.1	3.09 ± 0.21	4.9 ± 0.25	100.5 ± 8.15	142.6 ± 9.5	3.0 ± 0.27	4.5 ± 0.23	109.6 ± 7.09	157.0 ± 8.2	3.85 ± 0.29	4.33 ± 0.17
	Padina sp.				110.7 ± 6.53	165.09 ± 7.3	3.86 ± 0.39	5.10 ± 0.31	108.3 ± 7.77	162.01 ± 6.9	3.8 ± 0.28	4.8 ± 0.27	116.9 ± 8.06	173.0 ± 7.98	3.91 ± 0.27	4.29 ± 0.22
	Sargassum sp.				112.5 ± 7.15	170.9 ± 6.8	3.96 ± 0.25	5.15 ± 0.23	109.9 ± 6.98	165.77 ± 8.3	3.85 ± 0.29	4.9 ± 0.26	123.6 ± 8.29	177.3 ± 7.25	4.17 ± 0.29	5.3 ± 0.19
	Mixed sea-weeds (**Padina** sp. + **Sargassum** sp.)				115.0 ± 8.91	190.61 ± 8.45	4.1 ± 0.29	5.3 ± 0.27	110.69 ± 9.1	185.5 ± 8.8	4.01 ± 0.25	5.15 ± 0.26	124.9 ± 0.29	195.0 ± 9.3	4.20 ± 0.27	5.47 ± 0.2
	N.P.K.				108.77 ± 7.15	159.68 ± 8.5	3.8 ± 0.22	5.09 ± 0.27	104.6 ± 8.2	154.35 ± 9.9	3.6 ± 0.23	4.8 ± 0.29	107.9 ± 8.07	163.0 ± 6.9	3.97 ± 0.29	4.4 ± 0.18

Table-5.4. contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Alstonia scholaris	Control	6	37.29 ± 5.05	1.75 ± 0.16	53.31 ± 6.35	85.37 ± 7.13	2.9 ± 0.27	3.5 ± 0.19	50.5 ± 5.21	82.07 ± 6.31	2.85 ± 0.29	3.45 ± 0.24	54.9 ± 5.37	87.5 ± 8.15	3.1 ± 0.17	3.61 ± 0.29
	Padina sp.				57.9 ± 5.97	90.0 ± 4.39	3.1 ± 0.23	3.7 ± 0.26	56.15 ± 4.91	89.17 ± 5.19	2.97 ± 0.26	3.68 ± 0.32	60.2 ± 4.39	93.41 ± 5.61	3.2 ± 0.23	4.0 ± 0.25
	Sargassum sp.				59.2 ± 5.32	95.9 ± 7.6	3.3 ± 0.29	4.0 ± 0.26	57.5 ± 5.17	94.01 ± 5.39	3.25 ± 0.27	3.91 ± 0.27	62.17 ± 5.16	98.43 ± 4.97	3.5 ± 0.14	4.4 ± 0.19
	Mixed sea-weeds (**Padina** sp. + **Sargassum** sp.)				65.07 ± 4.05	97.35 ± 5.6	3.74 ± 0.26	4.3 ± 0.25	63.0 ± 0.02	96.1 ± 4.91	3.65 ± 0.27	4.41 ± 0.21	70.4 ± 0.17	102.10 ± 5.91	3.7 ± 0.2	4.5 ± 0.11
	N.P.K.				55.67 ± 5.79	89.7 ± 5.15	3.0 ± 0.28	3.7 ± 0.19	53.52 ± 5.31	87.1 ± 4.9	3.07 ± 0.25	3.69 ± 0.31	56.09 ± 6.67	92.06 ± 5.38	3.4 ± 0.29	3.89 ± 0.23

Table-5.4. Contd.

1.	2.	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pterocarpus marsupium	Control	6	51.36 ± 5.93	1.23 ± 0.27	79.87 ± 7.98	110.7 ± 7.3	3.98 ± 0.36	5.27 ± 0.29	72.12 ± 6.37	102.3 ± 7.8	3.81 ± 0.23	4.93 ± 0.29	80.3 ± 7.31	121.6 ± 6.9	4.01 ± 0.22	5.15 ± 0.29
	Padina sp.				82.15 ± 8.5	115.0 ± 6.9	4.18 ± 0.27	5.36 ± 0.92	76.31 ± 5.99	104.8 ± 6.95	4.6 ± 0.21	5.30 ± 0.22	81.6 ± 5.51	122.7 ± 7.6	4.10 ± 0.27	5.39 ± 0.33
	Sargassum sp.				85.28 ± 6.93	118.5 ± 8.2	4.33 ± 0.20	5.40 ± 0.26	78.2 ± 6.41	107.5 ± 6.91	4.07 ± 0.23	5.75 ± 0.27	85.7 ± 7.2	125.2 ± 6.56	4.32 ± 0.30	5.63 ± 0.27
	Mixed sea-weeds. (**Padina** sp. + **Sargassum** sp.)				86.14 ± 5.8	118.4 ± 5.32	4.41 ± 0.26	5.45 ± 0.19	80.3 ± 5.98	111.9 ± 6.81	4.32 ± 0.25	6.01 ± 0.29	90.3 ± 6.3	131.3 ± 5.9	4.37 ± 0.24	5.75 ± 0.22
	N.P.K.				86.59 ± 5.73	119.6 ± 7.30	4.04 ± 0.17	5.11 ± 0.29	77.69 ± 6.97	109.7 ± 7.3	4.12 ± 0.23	5.66 ± 0.21	86.4 ± 6.11	123.1 ± 6.6	4.22 ± 0.28	5.54 ± 0.20

Table-5.4. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Syzygium cumini	Control	3	35.33 ± 3.14	1.05 ± 0.09	56.5 ± 5.39	94.9 ± 6.68	3.01 ± 0.18	4.71 ± 0.21	50.1 ± 4.97	88.4 ± 6.13	2.85 ± 0.12	4.21 ± 0.25	57.2 ± 5.81	90.6 ± 5.17	3.78 ± 0.35	5.16 ± 0.29
	Padina sp.				59.1 ± 6.1	96.2 ± 7.17	3.36 ± 0.27	4.87 ± 0.23	54.7 ± 5.65	92.7 ± 6.92	3.07 ± 0.31	4.6 ± 0.28	60.5 ± 8.75	95.2 ± 5.57	4.0 ± 0.19	5.20 ± 0.2
	Sargassum sp.				62.05 ± 7.08	97.9 ± 5.9	3.52 ± 0.28	4.93 ± 0.26	58.37 ± 4.81	95.07 ± 4.96	3.33 ± 0.25	4.79 ± 0.24	63.8 ± 4.99	96.9 ± 5.12	4.2 ± 0.23	5.5 ± 0.16
	Mixed sea-weeds (**Padina** sp. + **Sargassum** sp.)				64.0 ± 5.17	99.3 ± 4.89	3.61 ± 0.25	4.98 ± 0.23	60.9 ± 5.35	97.5 ± 6.85	3.55 ± 0.32	4.86 ± 0.22	66.1 ± 4.93	98.8 ± 5.37	4.39 ± 0.28	5.61 ± 0.23
	N.P.K.				63.0 ± 5.29	98.16 ± 4.35	3.55 ± 0.26	4.96 ± 0.22	55.2 ± 5.18	94.1 ± 7.21	3.29 ± 0.26	4.71 ± 0.19	60.7 ± 6.6	96.6 ± 4.79	4.01 ± 0.24	5.4 ± 0.32

Table-5.4. Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Xylia xylocarpa	Control	6	45.69 ± 5.55	1.51 ± 0.24	63.27 ± 7.5	80.19 ± 5.9	3.27 ± 0.20	4.90 ± 0.27	62.01 ± 8.10	75.4 ± 6.18	3.07 ± 0.27	4.51 ± 0.20	65.31 ± 7.17	80.52 ± 8.5	3.5 ± 0.26	4.3 ± 0.29
	Padina sp.				65.1 ± 5.9	83.3 ± 5.85	3.39 ± 0.29	4.97 ± 0.23	63.2 ± 4.96	79.0 ± 5.27	3.11 ± 0.29	4.60 ± 0.23	67.5 ± 7.9	85.32 ± 7.9	3.56 ± 0.24	4.39 ± 0.23
	Sargassum sp.				67.9 ± 6.4	90.5 ± 8.12	3.40 ± 0.31	4.99 ± 0.27	69.9 ± 5.96	82.0 ± 5.15	3.15 ± 0.22	4.65 ± 0.25	69.21 ± 6.85	83.9 ± 6.67	3.69 ± 0.21	4.45 ± 0.27
	Mixed sea-weeds (**Padina** sp. + **Sargassum** sp.)				70.2 ± 5.6	92.1 ± 6.01	3.49 ± 0.23	4.98 ± 0.19	70.1 ± 6.31	82.7 ± 4.39	3.19 ± 0.21	4.69 ± 0.22	73.5 ± 5.5	84.6 ± 6.12	3.81 ± 0.23	4.69 ± 0.29
	N.P.K.				64.5 ± 6.83	87.3 ± 5.58	3.40 ± 0.26	4.59 ± 0.24	65.7 ± 7.7	78.66 ± 6.19	3.11 ± 0.22	4.55 ± 0.18	66.9 ± 5.3	82.6 ± 6.06	3.70 ± 0.35	4.47 ± 0.32

± Denotes Standard Deviation

addition of commercial fertilizers such as NPK does not help much in stimulating the growth of plants in iron ore mine wastes, this may be probably due to the leaching of these nutrients due to lack of organic matter. Secondly, it does not improve the structure and texture of the wastes. Natural fertilizers such as sea weeds would therefore help two folds, (1) help to restore organic matter of the waste, improve its structural and textural properties and contribute to conservation of moisture, and (2) it would help as a slow release fertilizer which are not the characteristics of the commercial fertilizer. This can be sufficiently added to the soil after developing substantial organic matter. Areas disturbed by mining have low fertility and organic matter content (Day **et al.** 1982). The addition of organic matter, commercial fertilizer and supplemental irrigation water increase vegetation establishment in disturbed sites (Ludeke **et al.** 1974).

Chemical evaluation of unmined soil and iron ore mine wastes suggest that plants that grow well in slight acidic to near alkaline environment may be better adapted to these specific sites. The nitrogen, phosphorous and potassium needs of plants growing in iron ore mine wastes may be less than the needs of the same fertilizer elements for plants growing in unmined soil, as the plants seem to have adapted to nutrient deficient soil like conditions. However, plants growing in iron ore mine waste dumps may have a greater requirement for water than plants growing in tailings and unmined soil. The possibility of water stress problems appears to be greater in iron ore mine waste dumps than in unmined soil and tailings which can be partially rectified by using sea weeds as soil ameliorants.

It is evident from the present study that the addition of liberal amounts of sea weeds to iron ore mine wastes and unmined soil would be beneficial for establishing vegetation, while preparing these soil materials for revegetation.

In the revegetation of these disturbed areas, it is important that plant species have a high survival percentage and densely spaced because this would greatly contribute to the production of dense root communities. Fine soil particles like those found in these wastes would be stabilized by forming aggregates around compact root system.

Plant species with dense foliage and producing thick top cover would reduce the direct impact of raindrops on soil particles and would reduce run-off velocity, prevent water erosion and arrest dust pollution. When run-off water is checked by vegetation it has more time to seep into the soil and replenish the soil moisture for plant growth. Once established the tree species would be effective producers of permanent vegetative cover on disturbed sites.

The fallen foliage due to the deciduous and semi-deciduous nature of trees would incorporate ample organic matter into the wastes and this would help soil development.

Native adapted species are cheap to regenerate. During 1986 wet season, the direct cost of establishing trees on iron ore mine waste averaged ₨ 5082-50/hectare ($300.71). By increasing the carrying capacity of the mined land and using plants suitable for nutrient deficient soils, the cost of revegetation could be markedly reduced, but however, these benefits are offset by the high costs of labour in the mining community. Rainfall is reliable and predictable although the average rainfall comes in violent burst over a short period of time. Therefore, the long term prospects for trees are good.

Detailed performance data and management expertise and cost statistics are still required before it is possible to fully assess the long term commercial viability of plantation for forestry on these wastes.

An early evaluation of the twenty one pioneer species trials showed that several tree species performed well and gave encouraging response at Pale-iron ore mines. The most promising were:- **Anacardium occidentale, Alstonia scholaris, Bauhinia purpurea, Pterocarpus marsupium, Syzygium cumini, Xylia xylocarpa, Casuarina equisetifolia, Garcinia indica, Parkia biglandulosa, Pithecellobium dulce, Syzygium Zylenica, Terminalia bellerica** and **Terminalia arjuna** because of their growth rate, drought tolerance, Table-5.5. Expected Yield of Volume per Hectare in Cubic Metres by Number of Stems and value after 10 years and using 3 x 4 m Spacings.

Sr.No.	Name of tree species	No. of trees/ha	Yield/tree (m^3)	Yield/ha (m^3)	Present value of timber/m^3	
					Rs.	$
1.	Alstonia scholaris	825	0.072	59.4	435	25.73
2.	Xylia xylocarpa	825	0.0504	41.62	1155	68.34
3.	Bauhinia purpurea	825	0.065	53.62	425	25.73
4.	Pterocarpus marsupium	825	0.0425	35.05	1800	106.50
5.	Anacardium occidentale	825	0.055	45.37	435	68.34
6.	Syzygium cumini	825	0.079	65.175	435	25.73

Calculations are based on data obtained from Forest Dept. Govt. of Goa.

resistant to nutrient deficiency stress and economic viability. Spacing can be increased by thining to encourage rapid diameter growth

It has been seen that a number of species grow quite well in iron ore mine wastes and have some timber potential. All the species tried are suitable climatically but cannot be expected to produce high volumes. If the timber keeps up its reputation and fetches a really high price, plantation in their own right should be encouraged. It is most important therefore, to test the timber quality of Pale grown trees, as soon as possible. Till to-date there are no such tests conducted on these species planted on the iron ore mine wastes, because no research has been carried out to test the viability and suitablity of these materials for commercial forestry.

The expected volume return presented in table-5.5, for six species is based on the data obtained from Forest Department, Govt. of Goa for plants grown in natural soils, at age ten years. Predicted, equivalent returns for iron ore mine waste sites would be similar but the quality of wood is required to be questioned. It is highly recommended that planting with these species continue on an experimental scale so that opportunity is given to answer these questions.

At this stage it seems to be sensible and it would be convincing and plausible to suggest the cultivators and mine owners, to encourage the plantation of these species, which will greatly cut the revegetation and maintenance costs, rather than depending on exotic species, which would incur additional expenditure and raise the cost of reclamation.

SYNOPSIS OF THE THESIS

India is endowed with a wide range of industrial resources, its mineral wealth includes surplus bauxite, mica, iron ore, etc. The rapid strides in exploration of mineral resources, to cope with the ever increasing demand of mankind intensified the mining activity in the country. Presently, there are more than 9,000 leases which cover an area of approximately 8,500 km^2.

Open cast mining presently contributes about thirty percent of the total mineral production in the country and by 2,000 A. D., it is expected to rise to more than fifty percent (Juwarkar **et al.** 1989). The mineral exploration and mining although is an age old practice in India, but since last two decades it has increase by many folds and has a social, economical and environmental impact.

In Goa, the open cast mining for rich iron and manganese ore deposits have caused major disturbance to the landscapes. And the wastes produced by mining are a serious threat to the environment. Yet, mining is an important dominant industry and forms back bone of Goa's economy while fishing and agriculture come only next.

The excavation for these minerals exposes large volumes of earth's crust to the atmosphere that intrude upon the landscape, the mining operation is such that, two classes of waste are produced (1) piles of surface overburden, waste rock and lean ore, and (2) a fine grained waste resulting from the ore beneficiation process and deposited in man-made basins called tailing ponds.

The annual production of iron ore in Goa is around 13.5 m tons. A tonne of iron ore mined for instance, produces almost 2-3 tons of waste, which means accumulation of 27-40 m tons of waste/year, as per the present mining rate. Since the operation of Pale Mines in 1954, the surface overburden, waste rock and lean ore has been piled into 9 dumps spread over 30 hectares and three tailing basins occupy an area of 10 hectares of land around the mines.

These wastes resulting from mining and processing operations have the potential for environmental pollution through erosion by wind and water. The " leaching " of these wastes may adversely effect the flora and fauna of terrestrial and water based ecosystems. Equally, these areas largely devoid of vegetation are visually unattractive and can detract from the aesthetic quality of the landscape.

The effective stabilization and partial restoration of vegetation on such degraded and disturbed area has become a priority and research workers have over the past two decades devoted their attention increasingly to viable solutions. Smith and Bradshaw (1972) have expressed the view that although stabilization by resin compounds and other physical means may offer a temporary solution, the only permanent solution lies in the reclamation of these wastes by covering them with vegetation. James (1966) stressed the importance of bringing into being a plant community that would be self-perpetuating without further attention or artificial aid.

In the present work, attempts were being made to establish vegetation on iron ore mine wastes, response of plant species to wastes were studied with and without soil amelioration, impact of earthworm, and mircobial effects on plant growth in iron ore mine wastes were investigated.

A review of the recorded literature reveals that, little work has been done in U.S.A. (Shetron **et al.** 1977) to establish vegetation on alkaline iron tailings, and on the role of micro-organisms in the reclamation of mine wastes (Jurgensen, 1978) Therefore, it was felt worthwhile to undertake the present study, to establish vegetation on iron ore mine wastes (dumps and tailing basins) at Pale-Goa.

I. DESCRIPTION OF STUDY AREA

Pale is situated in the Bicholim Taluka, North Goa District, of Goa State, between Eastings 04-07 and Northings 35-38.

The climate is tropical/monsoonal with hot wet summers and dry mild winters.

Average annual rainfall recorded over the three years (1987-1989) ranges from 2374.9 to 4119.5 mm.

The high relative humidity varies from 74-95 percent.

A survey of the natural vegetation around the mining sites indicated that, it is semi-deciduous and potentially evergreen, open scrub forest, with more or less similar floral diversity.

Physical analysis of the wastes showed that, the wastes had high bulk density (1.3-1.7g/cm^3), particle density (2.8-3.0g/cm^3) and porosity (44.4-53.5%), but poor water holding capacity.

Chemically, the wastes were rich in iron and manganese. But were poor in organic matter (0.22 - 0.48%) and deficient in essential plant nutrients.

II. MICRO-ORGANISMS AND THE RECLAMATION OF IRON ORE MINE WASTES

A. STUDIES ON AZOTOBACTER SPECIES IN IRON ORE MINE WASTE.

Ecological survey of **Azotobacter** species from iron ore mine waste dumps was carried out, the waste from different dumps were analysed for chemical and biological properties. Potential nitrogen fixing **Azotobacter** species from rhizosphere of **Cassia tora,** L. were isolated, identified and characterized and their nitrogen fixing ability was determined. The waste was amended with different rates of dried ground rice straw. The best strain was used in plant inoculation test.

The waste from dumps were slightly acidic to near neutral in pH (6.0-6.5). Concentration of iron ranged from 35-56 percent and manganese from 0.2-2.4 percent. All dumps irrespective of their age were poor in organic matter (0.32-0.48%) and other essential plant nutrients. Highest bacterial count recorded was 9.8 x 10^4/g of dry waste and lowest 3.7 x 10^4/g of dry waste. Similarly, **Azotobacter** population varied from 2.2 x 10^2 to 3.1 x 10^2/g of dry waste.

Based on the biochemical and cultural characteristics, isolates obtained from the rhizosphere of **Cassia tora** were identified and classified to be, **A. macrocytogenes, A. vinelandii** and **A. chrocoocum. Azotobacter chrocoocum** was found to have maximum nitrogen fixing ability (17 mg/g of sucrose).

Inoculation of **C. tora** with **A. chrocoocum** in iron ore waste significantly improved plant height, internodal length, leaf length, early flowering, increased the number of flowers, and the flowering occured at lower nodes. There was increase in length of pod and number of seed/pod. Nitrogen and dry matter yield increased substantially.

B. ESTABLISHMENT AND SELECTION OF SUCCESSFUL VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI FOR LEUCAENA LEUCOCEPHALA (LAM.) DE WIT IN IRON ORE TAILINGS.

An effective native rhizobial strain was isolated from pink healthy nodules of **Leucaena leucocephala** and it was confirmed by biochemical and plant inoculation tests.

In all there were twelve isolates. Isolates, viz; LL-1, LL-2, LL-3, LL-4, LL-5, LL-6, LL-7 and LL-8 were indentified to be **Rhizobium** by morphological and biochemical tests. The isolates LL-3 was found to have maximum nitrogen fixing capacity, that was screened by plant inoculation test. All strains were not equally effective.

Four endomycorrhizae were tried to screen the effective fungus for **Leucaena leucocephala** in iron ore tailings, viz; **Gigaspora margarita, Glomus mossae, Glomus fasciculatum, Glomus macrocarpum.** Dual inoculation test with these fungi and the isolated effective native rhizobial strain were undertaken.

The study indicated that, of all the four fungi tested, **G. fasciculatum** greatly accelerated plant growth, there was enlargement of the fourth leaf, increase in chlorophyll content (383 mg/100g), produced maximum spores in the root zone, improved nodulation, significantly increased plant dry weight and nitrogen content, and recorded maximum percentage colonization and phosphorous uptake.

These observations indicate that **G. fasciculatum** can be successfully establish in iron ore tailings and could be utilised as a plant inoculant for iron ore waste reclamation.

III. IMPACT OF EARTHWORM INTRODUCTION ON IRON ORE MINE WASTE AND PLANT GROWTH

Iron ore mine waste was mixed with dried ground leaves of **Mangifera indica**, L. at three different rates. Earthworm " **Pheretima orientalis** " were inoculated. Its survival percentage was recorded, casts were analysed for physical, chemical and biological properties. And effect of earthworm on growth of **Crotalaria retusa,** L. were studied.

Survival percentage increased with increase in dried ground leaves. Maximum survival (80%) was observed in pots with 300g of leaf matter. Inoculation greatly improved soil physical properties and availability of plant nutrients. Total bacterial count in casts was accelerated with increase in leaf matter. Plant growth was enhanced by 50 percent over the controls and there was a marked improvement in nodulation, plant dry matter and pod yield.

IV. THE POTENTIAL OF IPOMOEA PES-CAPRAE (L.) SWEET FOR IRON ORE MINE WASTE STABILISATION

Ipomoea pes-caprae was grown in different percentage composition of iron ore tailings, prepared with natural soil (% Tailing : % Natural soil; 0:100, 25:75, 50:50, 75:25 and 100:0). Thet soil compositions were analysed for its chemical properties. The plant was studied for its morphological and biochemical responses, and chemical composition.

The study revealed that iron and manganese percentage increased and the available plant nutrients decreased, with increase in percentage of tailings in the soil coposition. There was a co-relation between the percentage of tailings and the plant responses. Decrease in the height of plant, length of petiole and dry matter was observed, thickness of leaf increase from 0.38 to 0.61 mm with increase in percentage of tailings in the soil composition.

Similarly, there was a depression in the biochemical parameters like chlorophyll content from 194.3 to 97.08 mg%, carbohydrates 127 to 95 mg% and proteins from 295 to 224.5 mg%. Proline content increased from 20 to 56 mg% with increase in tailings in the soil composition.

Concentration of iron and manganese increased in roots and shoots proportionately, but a decrease in the uptake of aluminium in both shoots and roots was observed with increase in percentage of tailings in the soil composition. No visual toxicity symptoms were observed.

V. ESTABLISHING VEGETATION ON IRON ORE MINE WASTES

Twenty one native plant species adapted to the local environment were selected from the areas around the mining sites, for plantation on wastes and unmined soil. Six species were selected based on their performance, to study their growth response to sea weeds and commercial fertilizer (NPK - 19:19:19) treatments.

Of the twenty one species planted on the waste -; **Anacardium occidentale, Alstonia scholaris, Bauhinia purpurea, Pterocarpus marsupium, Syzygium cumini, Xylia xylocarpa, Casuarina equisetifolia, Garcinia indica, Pithecellobium dulce, Syzygium zylenica, Terminalia bellerica** and **Terminalia arjuna** showed high survival percentage and responded favourably to the waste.

The investigation revealed that, all the six plant species responded best to the sea-weed treatment than to commercial fetilizer and can potentially exploited as a cheap source of manure in iron ore mine waste reclamation, for improving soil properties and plant growth.

REFERENCES

JAMES, A. L. (1966). Stabilizing mine dumps with vegetation. **Endeavour**, 25, 154-157.

JURGENSEN, M. F. (1979). Micro-organisms and the reclamation of mine wastes. **In:** Forest soils and land use (G. T. Youngberg, Ed.), Fort Collins, Colorado State University, Department of Forestry and Wood Sciences, pp. 251-286.

JUWARKAR, A. S.; THERGAOKAR, V. P.; MALHOTRA, A. S. and ANIMESH KUMAR (1989). Reclamation of mined land and mine spoil using Sewage sludge and soil. **In:** National seminar on Protction of Environment and Ecology by Mining Industry. Papers - Vol. I, Feb., 3-4, 1989, Panjim (Goa).

SHETRON S. G.; HAMIL, B. M.; JURGENSEN, M. F.; SEAGALL, R. T.; JONES, L.; LENNOX, L. and PRATHER, J.(1977). Establishing Vegetation on Alkaline Iron and Copper tailings. **Geological Survey Division, Mich. Dept. of Nat. Res. Report of Investigation,** 17.

SMITH, R. A. H. and BRADSHAW, A. D. (1972). Stabilization of toxic mine wastes by the use of tolerant plant populations. **Trans. Instn. Min. Metall.,** 81:4, 230-237.

BIBLIOGRAPHY

BIBLIOGRAPHY

ABBOTT, L.K and ROBSON, A.D. (1978). Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. **New Phytology**, 81, 575-87.

ABBOTT, L.K. and ROBSON A.D. (1982). The role of Vesicular Arbuscular Mycorrhizal Fungi in Agriculture and the selection of Fungi for Inoculation. **Aust. J. Agric. Res.**, 33, 389-408.

ABD-EL-MALEK, Y.(1971). Free-living Nitrogen fixing bacteria in Egyptian soils and their possible contribution to soil fertility.**Plant and soil**, sp. vol., 423-442.

AGARWALA, S.C.; SHARMA, C.P. and KUMAR, A. (1964). Inter relationship of iron and manganese supply in growth, chlorophyll and iron porphyrin enzymes in barley plants. **Plant Physiology**, 39, 603-609.

AGARWALA S.C.; SHARMA, C.P. and FAROOQ,S. (1965). Effect of iron supply on growth, chlorophyll, tissue iron and activity of certain enzymes in maize and raddish. **Plant Physiology**, 40, 493-499.

AGARWALA, S.C.; BISHT, S.S. and SHARMA, C.P. (1977). Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley. **Can.Jour.Bot.**, 55, 1299-1307.

ALDON, EARL.F. (1978). Reclamation of coal mined land in the southwest. **J. Soil and Water Cons.**, 33(2), 7579.

ALLEN, E.B. and ALLEN, M.F (1980). Natural re-establishment of vesicular arbuscular mycorrhizae following strip mine reclamation in Wyoming. **Journal of Applied Ecology**, 17, 139-47.

ALLEN,M.F.; MOORE, T.S. and CHRISTENSEN, M. (1980). Phytohormone changes in **Boutelona gracilis** infected by Vesicular-arbuscular mycorrhizae, I. Cytokinin increases in the host plant. **Can. Jour. Bot.**, 58, 371-374.

ALLEN, S.E. (1974). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford, London.

ALEXANDER, M. (1965). Nitrification.**In:** Soil Nitrogen (W. V. Bartholomew and F.E. Clark, eds.) **Amer. Soc. Agron**, Madison. p. 307-343.

ALLISON F.E.; GADDY, V.L.; PINCK, L.A. and ARMIGER (1947) **Azotobacter** inoculation of crops: Effect on crops under greenhouse conditions.

ANONYMOUS (1957). Manual of Microbiological Methods. McGraw Hill Book Co. Inc., New York, U.S.A..

ANTONOVICS, T.; BRADSHAW, A.D. and TURNER R.G. (1971). Heavy metal tolerance in plants. **Adv. Ecol. Res.**, 7, 1-85.

ARNON D.I. (1949). **Plant Physiology**, 24,1.

ASPINALL, D. and PALEG, L.G. (1981). Proline accumulation. Physiological aspects. **In:** The Physiology and Biochemistry of Drought resistance in plants. (Paleg, L.G. and Aspinall, D. Eds.) pp. 205-441. Academic press, New York-Sydney-London.

AZCON-AGUILAR, C.; BAREA, J.M.; AZCON, R. and OLIVARES, J. (1982). Effectiveness of **Rhizobium** and VA mycorrhiza in the introduction of **Hedysarum coronarium** in a new habitat. **Agric. Environ.**, 7, 199-206.

AZIMI, S.; GIANINAZZI, P.V. and GIANINAZZI, S. (1980). Influence of increasing soil phosphorous levels on interactions between vesicular arbuscular mycorrhiza and Rhizobium in Soyabean. **Can. J. Bot.**, 58, 2200-2205.

BARBER, D.A. (1968). Microorganisms and the inorganic nutrition of higher plants. **Ann. Rev. Pl. Physiol.**, 19, 71-88.

BAREA, J. H. and BROWN, M.E. (1974). Effects on plant growth

Produced by **Azotobacter paspali** Related to synthesis of plant growth Regulating Substances. **J. App. Bact.**, 37.583-593.

BARLEY, K.P. and JENNINGS, A. C. (1959). Earthworms and Soil lertility - III. The influence of earthworms on the availability of nitrogen. **Aust. J. Agri. Res.**, 10, 364-370.

BARLEY, K. P. (1961). The abundance of earthworms in agricultural lands and their possible significance in agriculture. **Adv. Agron.**, 13, 249-268.

BASSALIK, K. (1913). (On silicate decomposition by soil bacteria) **Z. Garungsphysiol.** 2, 1-32 (G).

BATES, L. S.; WALDEN, R.P. and TEARE, I.D. (1973). Rapid determination of free proline for water stress studies. **Plant and Soil**, 39, 205-207.

BENNETT, O. L. (1977). Northern, southern, eastern strip mining: old problems, new solution. **Crops and Soils Magazine**, 29 (4), 19-20.

BERGER, K. C. and GERLOFF, G. C. (1948). Manganese toxicity of potatoes in relation to strong soil acidity. **Soil Sc. Soc. Am. Proc.**, 12, 310-314.

BJORKMAN, E. (1970). Forest tree mycorrhiza - The conditions for its formation and significance for tree growth and afforestation. **Plant and Soil,** 32, 589-610.

BLANCK, E. and GIESECKE, F. (1924). (The effect of earthworms on the physical and biological properties of soil). **Z. Pflanz, Dung.** 3 (B), 198-210 (G).

BOWEN, G. D. and ROVIRA, A. D. (1976). Microbial colonization of plant roots. **Ann. Rev. Phytopath.,** 14, 121-144.

BOWEN, J. E (1972). Manganese - Silicon interaction and its effect on growth of sudan grass. **Plant and Soil,** 37, 577-588.

BOX, T. W. (1979). Surface mine rehabilitation - some experiences in the United States. **In:** Managements of lands affected by mining. Workshop, Kalgoorlie, Western Australia, 1978 (R. A. Rummery and K. M. W. Howes, Eds.) Perth, CSIRO, pp. 55-67.

BRADSHAW, A. D.; McNEILLY, T.S. and GREGORY, R. P. G. (1965). Industrialization, evolution and the development of heavy metal tolerance in plants. **In:** Ecology and the Industrial Society. British Ecological Society Symposium, No. 5, pp. 327-343.

BRADSHAW, A. D. (1970). Pollution and plant evolution. **New Scientist,** 17, 497-500.

BRADSHAW, A. D. and CHADWICK, M. J. (1980). The Restoration of Land. Blackwell, Oxford.

BRAIN, K. R.; CHAPLOPIN, M. C.; TURNER, T. D.; BLUNDEN, G. and WILDGOOSE, P. B. (1973). **Plant Sci. Lett.**, 1, 241.

BREED, R.S.; MURRAY, E. G. D. and SMITH, N. R. (1957). Bergy's Manual of Determinative Bacteriology. London: Bailliere, Tindall & Cox.

BROUZES, R.; MAYFIELD, C. I. and KNOWLES, R. (1971). **Plant and Soil** sp. vol., 481-494.

BROWN, G.C. (1963). Interactions involving nutrient elements. **Ann. Rev. Plant Physiology,** 14, 93-106.

BROWN, N. E.; BURLINGHAM, S. K. and JACKSON, R. M. (1962). Studies on **Azotobacter** species in soil. I. Comparison of media and techniques for counting **Azotobacter** in soil. **Plant and Soil,** 17, 309-319

BROWN, M. E.; BURLINGHAM, S. K. and JACKSON, R. M. (1964). Studies on Azotobacter species in soil. III - Effects of artificial inoculation on crop yields. **Plant and Soil,** 20 (2), 194-214.

BROWN, M. E.; JACKSON, R. M. and BURLINGHAM, S. K. (1948). Effects produced on tomato plants, **Lycopersicum esculentum**, by seed or root treatment with gibberellic acid and indol-3 yl-acetic acid. **J. Exp. Bot.**, 19, 544.

BROWN, M. E. (1972). Plant growth Substances Produced by Micro-organisms of Soils and Rhizosphere. **J. App. Bact.**, 35, 443-451.

BROWN, M. E. (1976). Role of **Azotobacter paspali** in Association with **Paspalum notatum. J. App. Bact.**, 40, 341-348.

BROWN, R. W.; JOHNSTON, R. S.; RICHARDSON, B. Z. and FARMER, E. E. (1976). Rehabilitation of alpine disturbances: Beartooth Plateau, Montana. **In:** High-altitude Revegetation Workshop. No. 2 (R. H. Zuck and L. F. Brown, Eds.). Colo. St. Univ., Ft. Collins. pp. 58-73.

BROWN, M. E. (1982). **In:** Bacteria and Plants (M. E. Rhodes-Roberts and F. A. Skinner, Eds.) Academic Press, London. pp. 25-41.

CHALAL, V. P. S. and JOSHI, P. K. (1978). Effect of inoculation with different strains of **Rhizobium** on gram (**Cicer arielinum**, L.) **Ind. J. Microbiol.**, 18 (2), 101-102.

CHALLEN, S. B. and HEMINGWAY, J. C. (1965). Growth of higher plants in response to feeding with seaweed extracts. **Proc. Fifth. Intl. Seaw. Symp.**, 5, 359-367.

CLAIRMONT, K. B.; HAGAR, W. B. and DAVIS, E. A. (1986). Manganese toxicity to cholorophyll synthesis in tobacco callus. **Plant Physiology**, 80, 291-293.

CLARK, F. E. (1967). Bacteria in Soil. **In:** Soil Biology. (A. Burges and F. Raw, Eds.) Academic Press, New York. pp. 15-49.

COHEN, S. and LEWIS, H. B. (1949). Nitrogenous metabolism of the earthworm (**Lumbricus terrestris**). **Fed. Proc.**, 8, 191.

COOK, A.G.; CRITCHLEY, B. R.; CRITCHLEY, U.;PERFECT, T. J.; RUSSELL-SMITH, A. and YEADON, R. (1979). The effect of soil treatment with D.D.T. on the biology of a cultivated forest soil in the sub-humid tropics. **Pedobiologia**, 19, 279-292.

COPE, F. (1962). The development of a soil from an industrial waste ash. **Int. Soil Conf.**N . Z., 859-63.

CRESSWELL, C. F. (1973) Changes in vegetational composition, nutritional status, soils and microbial populations with the establishment of vegetation on gold-mine dumps on the Witwatersrand. **In:** Ecology and Reclamation of Devasted Lands. Vol. 2. (R. J. Hutnik and G. Davis, Eds.) Gordon Breach, New York. pp. 335-359.

CROSSLEY, D. A. and HOGLUND, H.P. (1962). A litter burial method for the study of micro-arthropods inhabiting leaf litter. **Ecology**, 43, 571-574.

CRUSH, J. R. (1974). Plant growth responses to vesicular-arbuscular mycorrhizas. VII - Growth and nodulation of some herbage legumes. **New Phytol.**, 73, 745-54.

CSATORDAY, K.; GOMBS, Z. and SZALONTAI, B. (1985). Manganese and Cobalt toxicity in Chlorophyll biosynthesis. **Proc. National Acad. Sci.** U.S.A. 81, 476-478.

CUNDELL, A. M. (1977). The role of micro-organisms in the revegetation of strip-mined land in the Western United States. **J. Range Mgt.**, 30, 299-305.

CZAPOWSKYJ, M. M. (1973). Establishing forest on surface mied land as related to fertility and fertilization. **In:** Forest Fertilization Symp. Proc. USDA For. Serv. Gen. Tech. Rep. NE-3. pp. 132-139.

DAFT, M. J. and NICOLSON, T. H. (1974) Arbuscular mycorrhizas in plants colonizing coal wastes in Scotland. **New Phytol.**, 73, 1129-1138.

DAFT, M. J. and EL-GIAHMI, A. A. (1974). Effect of Endogone mycorrhiza on plant growth. VII - Influence of infection on the growth and nodulation in French Beans (**Phaseolus vulgaris**). **New Phytology**, 73, 1139.

DAFT, M.J.; HACSKAYLO, E. and NICOLSON, T.H. (1975). Arbuscular mycorrhizas in plant colonising coal spoils in Scotland and Pennsylvania. **In:**Endomycorrhizas (F. E. Sanders, B. Mosse and P. B. Tinker, Eds.) Academic Press, London p. 561-580.

DANCER, W. S.; HANDLEY, J. F. and BRADSHAW, A. B. (1977a). Nitrogen accumulation in Kaolin mining wastes in Cornwall. II - Forage legumes. **Plant and soil,** 48, 303-314.

DANIELS, B. A. and TRAPPE, J. M. (1980). Factors affecting spore germination of the vesicular arbuscular mycorrhizal fungus, **Glomus epigaeus. Mycologia**, 72, 457-471.

DATE, R. A. (1976). Principles in **Rhizobium** strain selection. **In:** Symbiotic Nitrogen Fixation in Plants. (P. S. Nutman, Ed.) Cambridge Univ. Press, London-New York.pp. 137-150.

DAWSON, R. C. (1947). Earthworm microbiology and the formation of water stable aggregates. **Proc. Soil Sci. Soc. Amer.**, 12, 512-516.

DAY, A. D. and LUDUKE, K. L. (1973). Stabilizing Copper Mine Tailings Disposal Berms with Giant Bermuda Grass. **Jour. Env. Qual.** Vol. 2(2), pp. 314-315.

DAY, A. D. and LUDEKE, K. L. (1980). Reclamation of of Copper Mine Wastes with Shrubs in the Southwestern U.S.A. **Journal of Arid Environment,** 3(2), 107-112.

DAY, A. D.; TUCKER, T.C. and THAMES, J. L. (1982). Response of Plant species to Coal Mine Soil Materials and Fertilizers in a Semiarid Environment. **IM & EJ,** April, 1982. pp. 21-25.

DEAN, K. C.; HAVENS, R. and VALDEZ, E.G. (1969). Stabilization of Mineral Wastes. **Ind. Wat. Eng.**, 6(10). 30-33.

DEAN, K. C. and HAVENS, R. (1970). Stabilization of mill tailings. Paper presented at Natl. Western Min. Conf., Colorado Mng. Assoc., Denver, Colo.

DEAN, K. C.; HAVENS, R. and VALDEZ, E. G. (1971). USBM finds many routes to stabilizing mineral wastes. **Min. Engng.**, 23,61-63.

DEAN, K. C.; HAVENS, R.; HARPER, K. T. and ROSENBAUM (1973). Vegetative stabilization of mill mineral wastes. **In:** Ecology and Reclamation of Devasted Lands. Vol. 2. (R. J. Hutnik and G. Davis, Eds.). Gordon and Breach, New York. pp. 119-136.

DEKOCK, P. C. (1956). Heavy metal toxicity and iron chlorosis. **Ann. Bot.**, 20, 134-141.

DEL RIO, L. A.; GOMEZ, M.; YANEZ, J.; LEAL, A. and LOPEZ GEORGE, J. (1978). Iron deficiency in pea plants effect on catalase, peroxidase, chlorophyll and proteins of leaves. **Plant and Soil,** 49, 343-353.

DESHPANDE, A. A. and SWAMY, G. S. (1987). Induction of Proline accumulation by methyl parathion in Sorghum (**Sorghum bicolor,** L.) **Curr. Sci.**, 56, 1068-1070.

DE VLEESCHAUWER, D. and LAL, R. (1981). Properties of worm casts under secondary Tropical Forest Regrowth. **Soil Sci.**, 132 (2), 175-181.

DOWN, C. G. (1975). Problems in vegetating metal-toxic mining wastes. **In:** Minerals and the Environment. (M. J. Jones, Ed.) Institution of Mining and Metallurgy. pp. 395-408.

DRIFT, J. VAN DER (1955). (Influence of earthworms on humus formation.) **Tuinbouwk. Onderz. Jversl.**, 31-32 (Du).

DUBOIS, GILLES, M. K.; HAMILTON, J. K.; REBERS, P. A. and SMITH, F. (1951). A colorimetric method for the determination of sugars. **Nature**, 168, 167.

DUKE, H. (1981). Handbook of Legumes of World Economic Importance. Plenum Press, New York.

DUSSERE, C. (1902). (On the effect of earthworms on the chemical conditions of soils). **Landw. Jb. Schweiz.**, 16, 75-78 (G).

EDWARDS, C. A. and HEATH, G. W. (1963). The role of soil organisms in breakdown of leaf material. **In:** Soil Organisms. (J. Doekson and J. Van Der Drift, Eds.). North Holland, Amsterdam, pp. 76-84.

EDWARDS, C. A. and LOFTY, J. R. (1980). Effects of earthworm inoculation on the root growth of direct drilled cereals. **J. App. Ecol.**, 17, 533-544.

ELLIS, F. B.; ELLIOT, J. G.; BARNES, B. T. and HOWSE, K. R. (1977). Comparison of direct drilling reduced cultivation and ploughing on the growth of cereals. 2- Spring barley on a sandy loam soil : Soil physical conditions and root growth. **J. Agric. Sci.**, 891, 631-642.

EPSTEIN, E. (1969). Mineral metabolism of halophytes. **In:**Ecological aspects of Mineral Nutrition of plants. (I. H. Rorison, Ed.) Oxford, Edinburg :Blackwell. pp. 345-353.

EPSTEIN, E. E. (1972). Mineral Nutrition in Plants. Wiley, London.

ERNST, W. (1972). Foroschungberichte Land. **Nordrhein Westfalen,** 2251, 1-38 (From Ernst,1980).

ERNST, W. (1980). The impact of industrial pollution on life. A paper presented in the **Diamond Jubilee Symposium on Environment.** Nov. 17-19, at the Institute of Science, Bombay.

EVANS, A. C. (1948). Studies on the relationship between earthworms and soil fertility. II. Some effects of earthworms on soil structure. **Ann. App. Biol.**, 35 (1), 1-13.

FINK, A. (1952). (Ecological and pedological studies on the effects of earthworms on soil fertility.) **Z. PflErnahr. Dung.** 58 (103), 120-145. (G).

FOY, C. D.; FLEMING, A. L. and ARMIGER, W.H. (1969). Aluminium tolerance of soyabean varieties in relation to calcium nutrition. **Agron. J.**, 61, 505-511.

FOY, C. D. (1974). Effects of Aluminium on plant growth. **In:** The plant root and its environment (E. W. Carson, Ed.). Charlottesville. The University Press of Virginia, U.S.A. pp. 601-642.

FOY, C. D.; CHANEY, R. L. and WHITE, M. C. (1978). The Physiology of metal toxicity in plants. **Annual Review of Plant Physiology,** 29, 511-566.

GARRETT, S. D. (1970). Pathogenic Root-Infecting Fungi.(Cambridge University Press).

GERDEMANN, J. W. and NICOLSEN, T. H. (1963). Spores of mycorrhizal endogone species extracted by wet sieving and decanting. **Trans. Br. Mycol. Soc.**, 46, 235-244.

GHILDIYAL, M. C.; PANDEY, M. and SIROHI, G. S. (1986). Proline content in Linseed varieties as influenced by Zinc nutrition. **Ind. J. Pl. Physiol.**, 20, 368-374.

GIBBS, B. M. and SHAPTON, D. A. (1968). **Identification methods for microbiologists**, part B. The Society for applied Bacteriology technical series No.2 academic Press London, New York.

GOAS, M.; GOAS, G. and LARHER, F. (1970). Nitrogen metabolism of halophytes: Utilization of glutamic acid by young Aster tripolium plants. **C. R. H. Acad. Sci. Ser. D.** (Paris) 271 (20), 1763-66.

GODZIK, S. and LINSLEUS, H. F. (1974). **Environ. pollution,** 7, 25.

GOMMEL, F. P. (1975). Establishment of grass on Waste from iron smelting. **Environ. pollution,** 8 (1), 35-44.

GOODALL, D. W. and GREGORY, F. G. (1966). Chemical composition of plants as an Index of Nutritional Status. **Commonwealth Bur. Hortic. Tech. Commun.** No. 17, 1966.

GOODMAN, G. T. (1974). Ecology and the problems of rehabilitating wastes from mineral extraction. **Proc. Roy. Soc.**, Lond. A. 339, 373-387.

GOULD, A. D. and LIBERTA, A. E. (1981). Effect of top-soil storage during surface mining on the viability of vesicular arbuscular mycorrhiza. **Mycologia**, 73, 914-22.

GOVINDA RAO, V. S.; BAGYARAJ, D. J. and RAI, P. V. (1983). Selection of an efficient VA mycorrhizal fungus for finger millet. **Zentralbl. Microbiol.**, 13, 415-419.

GRAFF, O. (1970). Phosphorous content of earthworm casts. **Landbauforschung Volkenrode,** 20, 33-36.

GRANDT, A. F. (1978). Mined land reclamation in the interior coal province. **J. Soil Water Cons.**, 33, 62-68.

GRIFFITH, J. J. (1919). Influence of mines upon land and livestock in Cardiganshire. **Journal of Agricultural Science,** Cambridge, 9, 366-395.

GUPTA, M. L. and SAKAL, R.(1967). The role of earthworms on availability of nutrients in garden and cultivated soils. **J. Ind. Soc. Soil Sc.**, 15, 149-151.

GUPTA, U.and CHIPMAN, E. W. (1976). Influence of iron and pH on the yield and iron, manganese, zinc and sulphur concentrations of carrots grown on sphagnum peat soil. **Plant and Soil**, 44, 559-566

HACSKAYLO, E. (1972). Mycorrhizae : The ultimate in reciprocal parasitism ? **Bio. Science**, 22, 577-582.

HARLEY, J. L. (1969). The Biology of Mycorrhiza. Leonard Hill, London. p. 334.

HARRIS, M. M. and JURGENSEN, M. F. (1977). Development of **Salix** and **Populus** mycorrhizae in metallic mine tailings. **Plant and Soil**, 47, 509-517.

HAYMAN, D. S. (1978). **In** : Interactions between Non-pathogenic Micro-organisms and Plants(Y. R. Dommergues and S. V. Krupa, Eds.) Amsterdam, Elsevier. pp. 401-442.

HAYMAN, D. S. (1983). The Physiology of vesicular-arbuscular endomycorrhizal symbiosis. **Can. J. Bot.**, 61, 944-963.

HAYMAN, D. S.; MORRIS, B.J. and PAGE, R. J. (1981). Methods for inoculating field crops with mycorrhizal fungi. **Ann. Appl. Biol.**, 99, 247-253.

HEENAN, D. P. and CAMPBELL, L. C. (1980). Soyabean nitrate reductase activity influenced by manganese nutrition. **Plant cell Physiology**. 21, 731-736.

HOPKINS, E. F.; PAGAN, VICTOR and RAMIREZ SILVA, F. J. (1944). Iron and manganese in relation to plant growth and its importance in Puerto Rico. **J. Agr. Univ. Puerto Rico**, 28, 43-101.

HOPP,H. and HOPKINS, H. T. (1946). Earthworms as a factor in the formation of water stable aggregates. **J. Soil Water Conserv.**, 1, 11-13.

HOPP, H. and SLATER, C. S. (1948). Influence of earthworms and soil productivity. **Soil Sci.**, 66, 421-428.

HELYAR, K. R. and GODDEN, D. P. (1976). Soil phosphate as a capital asset. **In** : Reviews in Rural Science, 3 : Improving the efficiency of Phosphorous Utilization.(G. J. Blair, Ed.) Univ. New England Press, Armidale, N. S. W.

HELYAR, K. R. and GODDEN, D. P. (1977). The biology and modelling of fertilizer response. **J. Aust. Inst. Agri. Sci.**, 43, 22-30

HEWITT, E. J. (1948). Experiments on iron metabolism in plants. I. Some effects of induced deficiency. **Ann. Rept. Long Ashton Res. Sta.**, 66-80.

HEWITT, E. J. (1953). A biological approach to the problems of soil acidity. **Intern. Soc. Soil Sci. Trans** (Dublin), 1, 107.

HEWITT, E. J. and SMITH, E. A. (1975). Plant Mineral Nutrition. English Universities, London.

HSIAO, T. C. (1973). **Ann. Rev. Pl. Physiol.**, 24, 519-570.

HUTNIK, R. J. and DAVIS, G. (Eds.) (1973). Ecology and Reclamation of Devasted Land. Vol. 1 and 2. Gorden and Breach, New York.

ISLAM, R. and AYANABA, A. (1981). Effect of seed inoculation and pre-infecting cow-pea (**Vigna unguiculata**) with **Glomus mossae** on growth and seed yield of the plants under field conditions. **Plant and Soil**, 61, 341-350.

JACKSON, M. L. (1973). Soil Chemical Analysis. Prentice-Hall of India (Pvt.), New Delhi.

JACKSON, R. M.; BROWN, M. E.; BURLINGHAM, S. K. and BARFORD (1960). **Azotobacter** and plant growth. **Rep. Soil Microbiol. Dep. Rothamsted Exp. Sta.**, 91-93.

JACKSON, R. M.; BROWN, M. E. and BURLINGHAM, S. K. (1964). Similar effects on tomato plants of **Azotobacter** inoculation. **Nature**, 203, No. 4947, 851-852.

JAFFRE, T. and HEIM, R. (1977). Accumulation du manganese par des especes associees aux terrain ultrabasiques de Nouvelle-Caledonie. **CR Acad. Sci. Ser. D.** 284, 1573-1575.

JAMES, A. L. and MROST, M. (1965). Control of acidity of tailing dams and dumps as a precursor to stabilization by vegetation. **Int. Min. Metall.**, 65, 488-495.

JAMES, A. D. (1966). Stabilising mine dumps with vegetation. **Endeavour**, 25, 154-57.

JAMES, N. and SUTHERLAND, M. (1939). **Can. J. Res.**, 17, 97.

JANOS, D. P. (1983). Tropical mycorrhizas. **In:** Tropical Rain Forest Ecology and Management. (S. L. Sutton, T. C. Whitemore and A. C. Chadwick, Eds.) Blackwell, Oxford, pp. 327-345.

JEFFREY, D. W.; MAYBURY, M. and LEVINGE, D. (1975). Ecological approach to mining waste vegetation. **In:** Minerals and the Environment. (M. J. Jones, Ed.). **Inst. of Mining and Metallurgy**, London. pp. 371-386.

JEFFRIES, P. (1987). Use of mycorrhizae in agriculture. **CRC Crit. Rev. Biotechnol.**, 5, 319-357.

JEHNE, W. and THOMPSON, C. H. (1981). Endomycorhizae in plant colonization on coastal sand dunes at Cooloola, Queensland. **Australian Journal of Ecology**, 6 (3), 221-230.

JENSEN, H. L. (1965). **In:** Soil Nitrogen (W. W. Bartholomew and F. E. Clark, Eds.) **Amer. Soc. Agron. Monogr.**, 10, 436-480.

JENSEN, V. (1968). **In:** The Ecology of Soil Bacteria. (T. R. Gray and D. Parkinson, Eds.) Liverpool, Liverpool University Press.

JOHNSON, M. O. (1924). Manganese chlorosis of pineapples: its cause and control. **Hawaiian Agr. Expt. Sta. Bull.** No. 52.

JOI M. B. and SHINDE, P. A. (1976). Response of Onion Crop to Azotobacterization. **Journal of Maharashtra Agricultural Universities**, 1, (2-6), 161-162.

JONAS, F. (1973). Reclamation of areas damaged by mining activity in Czechoslovakia. **In:** Ecology and Reclamation of Devasted Lands. Vol; 2 (R. J. Hutnik and G. Davis, eds.) Gordon and Breach, New york. p. 379-394.

JONES, A. N. and HOWELLS, W. R. (1975). The partial recovery of metal polluted River Rheidol. **In:** Ecology of Resource Degradation and Renewal. (M. J. Chadwick and G. T. Goodman, Eds.)pp. 443-459. Symposium British Ecological society, 15, Blackwell Scientific Publications, Oxford.

JONES, L. H. P. (1957). The Relative Content of Manganese in Plants. **Plant and Soil**, 7 (4), 328-335.

JONES, L. M. (1972). Vegetation Establishment on Iron Mine Tailings, Dam Berms and Waste Rock Dumps. Masters Thesis, Mich, Tech. Univ.

JONES, R. H. and MENARY, R. C. (1974). The toxic effect of manganese on nitrate reductase. **In:** Mechanism of regulation of plant growth. (R. L. Bieleski, A. R. Ferguson and M. M. Creswell, Eds.) pp. 67-70. **Bull. 12. R. Soc. N. Z.** Wellington.

JOSHI, N. V. and KELKAR, B. V. (1952). The role of earthworm in soil fertility. **Ind. J. Agric. Sci.**, 22 (2), 189-196.

JURGENSEN, M. F. (1979). Micro-organisms and the reclamation of mine wastes. **In:** Forest soils and land use (C. T. Youngberg, Ed.). Fort Collins, Colorado State University, Dept. of Forestry and Wood Sciences, pp. 251-286.

KAPULNIK, Y.; OKON, Y.; KIGEL, J.; NUR, I. and HENNIS, Y. (1981). Effects of temperature, nitrogen fertilization and plant age on nitrogen fixation by **Setaria italica** inoculated with **Azospirillum brasilense** (strain cd). **Plant Physiology**, 68, 340-343.

KAPULNIK, Y.; SARIG, S.; NUR, I.; OKON, Y.; KIGEL, J. and HENIS, Y. (1981). Yield increases in summer cereal crops of Israeli fields inoculated with **Azospirillum Exp. Agric.,** 17, 179-187.

KAPULNIK, Y.; SARIG, S.; NUR, I. and OKON, Y. (1983). Effects of Azospirillum inoculation on yield of field grown wheat, **Can. J. Microbiol.**, 29, 895-944.

KATZNELSON, M. (1940). **Soil Sci.**, 49, 21.

KELLEY, W. P. (1912). The function and distribution of manganese in plants and soils. **Hawaiian Agr. Exp. Sta. Bull.** No. 26.

KLEEZKOUSKA, J.; NUTMAN, P. S.; SKINNER, F. A. and VINCENT, J. M. The identification and classification of **Rhizobium. In:** Identification methods for Microbilogist- Part B. Eds. Gibbs, B. M. and Shapton D. A. (1968). **The Soc. for Applied Bacteriology Technical Series,** No. 2. Academic Press, London, New York.

KNOWLES, R. (1974). **In:** Biologocal Nitrogen Fixation. (R. W. F. Hardy, Ed.) John Wiley and Sons Inc., New York.

KORMANIK, P. P.; BRYAN, W. C. and SCHULTZ, R. (1976). Endomycorrihizae, their importance in nursery production of hardwood seedlings. **In:** SE. Area Nurserymen's Conf. Eastern Session, Charleston, SC, 3-5 Aug. 1976, pp. 16-21.

KRIEL, H. T. (1941). A study of the effect of excess manganese on the iron and chlorophyll content and growth of tomato plants. Ph. D. Thesis, University of California, Berkeley.

KRISHNAMURTHI, B. G. (1962). Indian sugar, 11, 765.

KROL, M.; MALISZEWSKA, W. and SIUTA, J. (1972). Biological activity of soils strongly polluted with Sulphur. **Pol. J. Soil Sci.**, 5, 25-33.

KUDREV, T. and ISTATKOV, S. (1967). On the accumulation of free proline in the leaves of wheat under the influence of drought. **C. R. Acad. Bulg. Sci.**, 20 (7), 711-713.

KUDREV, T. (1970). On the problem of the formation of proline under the effect of drought. **C. R. Acad. Sci. Agri. Bulg.**, 3 (3), 261-264.

KUO, S. and MIKKELSEN, D. S. (1981). Effect of P and Mn on growth response and uptake of Fe, Mn and P by sorghum. **Plant and Soil**, 62, 15-22.

LAKSHMI KUMARI, M.; VIJAYALAKSHMI, K. and SUBBA RAO, N. S. (1975). Interaction between **Azotobacter** sp. and fungi. 1. In vitro studies with **Fusarium moniliforme** Sheld. **Phytopath, Z.**, 75, 27-30.

LAL, R. and AKINREMI, O. O. (1983). Physical properties of earthworm casts and surface soil as influenced by management. **Soil Sci.**, 135 (2), 114-122.

LAL, R. and DE VLEESCHAUWER, D. (1982). Influence of tillage methods and fertilizer application on chemical properties of worm castings in a tropical soil. **Soil Tillage Res.**, 2. 37-52.

LE ROY, J. C. and KELLER, H. (1972). How to reclaim mined areas, tailing ponds and dumps into valuable land. **World Min.**, 8(1), 34-41.

LIBERTA, A. E. (1981). Effects of top soil storage duration on inoculum potential of vesicular-arbuscular mycorrhizae. **Symposium on Surface Mining Hydrology, Sedimentology and Reclamation,** University of Kentucky, Lexingon, Ky 40506, Dec. 7-11.

LIM, L. L. and COLE, A. L. J. (1984). Growth response of white clover to vesicular arbuscular mycorrhizal infection with different levels of applied phosphorous. **N. Z. J. Agric. Res.**, 27, 587-592.

LIN, W.; OKON, Y. and HARDY, R. W. F. (1983). Enhanced mineral uptake by **Zea mays** and **Sorghum bicolor** roots inoculated with **Azospirillum brasilense. App. Environ. Microbiol.**, 45, 1775-1779.

LINDQUIST, B. (1941). Investigations on the significance of some Scandinavian earthworms in decomposition of leaf litter and structure of soil. **Svensk. Skags. U. Foren. Tidsler**, 39, 179-242.

LOFS-HOLMIN, A. (1981). Influence of different crop rotations on fluctuations of earthworm populations. **Darwin Centenary Earthworm Ecology Symposium.** 1981.

LOHNIS, M. P. (1951). Manganese toxicity in field and market garden crops. **Plant and Soil**, 3, 193-222.

LOOMIS, W. E. and SHULL, C.A. (1937). Methods in plant physiology. McGraw Hill, New York.

LOWRY, G. L. (1961). Some physico-chemical properties of weathering coal spoils and their influence on the growth of pine seedlings. **Diss. Abstr.**, 22, 1774-5.

LUDEKE, K. L.; DAY, A. D.; STITH, L. S. and STROEHLEIM, J. L. (1974). Pima studies tailing soil make-up as a prelude to successful revegetation. **Engineering Mining Journal,** 175, 72-74.

LUELLEN, W. R. (Ed.)(1977). Proper handling restores mined land to agriculture. **Crops and Soils Magazine**, 29 (7), 26.

LUNT, H. A. and JACOBSON, G. M. (1944). The chemical composition of earthworm casts. **Soil Sci.**, 58, 367-375.

MACLEAN, A. L. and DEKKER, A. J. (1976). Lime requirement and availability of nutrients and toxic metals to plants grown in acid mine tailings. **Can. J. Soil. Sci.**, 56, 27-36.

MALAJEZUK, N.; LINDERMAN, R. G.; KOUGH, J. and TRAPPE, J. M. (1981). Presence of vesicular-arbuscular mycorrhizae in **Eucalyptus** spp. and **Acacia** sp., and their absence in **Banksia** sp., after inoculation with **Glomus fasciculatum. The New Phytologist**, 87, 567-72.

MANJUNATH, A.; BAGYARAJ, D. J. and GOPALAGOWDA H. S. (1984). Dual inoculation with VA mycorrhiza and **Rhizobium** is beneficial to **Leucaena. Plant and Soil**, 78, 445-448.

MANSELL, G. P.; SYERS, J. K. and GREGG P. E. H. (1981). Plant availability of phosphorous in dead herbage ingested by surface casting earthworms. **Soil Biol. Biochem.** Vol. 13, 163-167.

Manual of Procedures for Chemical and Instrumental Analysis of Ores, Minerals and Ore Dressing Products (1979). Issued by Controller, Indian Bureau of Mines, Nagpur.

MARRS, R. H.; ROBERTS, R. D. and BRADSHAW, A. D. (1981). Ecosystem development in reclaimed China Clay Wastes. I. Assessment of vegetation and recapture of nutrients. **Journal of Applied Ecology**, 17, 709-717.

MARX, D. H. (1977). The role of mycorrhizae in forest production. **TAPPI Conf. Pap. Ann. Meet.** 1977, p. 151-161.

MEDVE, R. J.; HOFFMAN, F. M. and GAILHER, T. W. (1977). The effects of mycorrhizal-forming amendments on the revegetation of bituminous stripmine spoils. **Bull. Torrey. Bot. Club**, 104, 218-225.

MEHROTRA, C. L. and LEHRI, L. K. (1971). Effect of **Azotobacter** Inoculation on Crop Yields. **J. Indian Soc. Sci.** Vol., 19 (3), 243-248.

MEYER, L. (1943). (Experimental study of macrobiological effects on humus and soil formation.). **Bodevk- u. PflErnahr.** 29 (74), 119-140 (G).

MIKOLA, P. (1973). Mycorrhizal Symbiosis in Forestry practice. **In:** Ectomycorrhizae- Their Ecology and Physiology. (G. C. Marks and T.T. Kozlowski, Eds.) Academic Press, New York and London, p. 444.

MILLER, G. L. (1959). Protein determination for large number of samples. **Anal. Chem.**, 31, 964.

MILLIKAN, C. R. (1949). Effects on flax of a toxic concentration of boron, iron, molybdenum, aluminium, copper, zinc, manganese, cobalt and nickel in the nutrient solution. **R. Soc. Victoria Proc.**, 61, 25-42.

MISHUSTIN, E. N. and SHILNIKOVA, V. K. (1969). Free living Nitrogen Fixing Bacteria of the genus **Azotobacter. In:** Soil Biology Reviews of Research, UNESCO., pp. 72-109.

MISHUSTIN, E. N. and SHILNIKOVA, V. K. (1971). Biological Fixation of Atmospheric Nitrogen. MacMillan, London.

MITCHELL, B. A. (1959). The ecology of tin mine spoil heaps. **Malay. Forester**, 22, 11-132.

MOORE, P. D. and CHAPMAN, S. B. (1976). Methods in Plant Ecology. Blackwell Scientific Publications, Oxford, London.

MORGAN, P. W.; JOHAM, H. E. and AMIN, J. V. (1966). Effect of manganese toxicity on the indole-acetic acid oxidase system of cotton. **Plant Physiol.**, 41, 713-724.

MORGAN, P. W.; TAYLOR, D. M. and JOHAM, H. E. (1976). Manipulations of IAA-Oxidase activity and auxin deficiency symptoms in intact cotton plants with manganese nutrition. **Physiol. Plant.**, 37, 149-156.

MORRIS, H. D. and PIERRE, W. H. (1949). Minimum concentrations of Manganese necessary for injury to various legumes in culture solutions. **Agron. J.**, 41, 107-112.

MOSSE, B. (1972). The influence of soil type and Endogone strain on the growth of mycorrhizal plants in phosphate deficient soils. **Revue Ecologie Biologie Sol.**, 3, 529-37.

MOSSE, B. (1973). Advances in the study of vesicular-arbuscular mycorrhiza. **Ann. Rev. Phytopath.**, 11, 171-196.

MOSSE, B. (1975). Specificity of vesicular-arbuscular mycorrhizas. **In**:Endomycorrhizas. (R. E. Sanders, B. Mosse and P. B. Tinker, Eds. Academic Press, London. p. 469-484.

MOSSE, B. and TINKER, P. B. (Ed.) (1975). Endomycorrhizas. Academic Press, Lond.

MOSSE, B.; POWELL, C. L. and HAYMAN, D. S. (1976). Plant growth responses to vesicular-arbuscular mycorrhiza. IX - Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. **New Phytology**, 76, 331-342.

MULLER, K. (1975). The microflora of dumped soils in two opencast brown coal mining regions of Poland. **In**: Ecology and Reclamation of Devasted Lands. Vol. 1. (R. J. Hutnik and G. Davis, Eds.). Gordon and Breach, New York, p. 325-334.

MUNNS, D. N.; JACOBSON, L. and JOHNSON, C. M. (1963). Uptake and distribution of mananese in oat plants. **Plant and Soil**, 19, 185-195.

MUNNS, D. N. and MOSSE, B. (1980). **In:** Advances in Legume Science, pp. 115-125. (R. J. Summerfield and A. H. Bunting, Eds.) Univ. of Reading Press. England.

MURRAY, D. R. (1973). Vegetation of Mine Waste Embankments in Canada. Information Circular Mines Branch, Canada. IC 301, Ottawa.

MURRAY, D. R. (1977). Pit Slope Manual. Supplement, 10-1. Reclamation by Vegetation. CANHET. Report, 77-31. Canada Centre for Mineral and Technology.

NABLE, R. O.; ROBERT, L. HOUTZ and GEORGE, M. CHENIAE (1988). Early Inhibition of Photosynthesis during development of Manganese Toxicity in Tobacco. **Plant Physiology**, 86, 1136-1142.

NAG, P.; PAUL, A. K. and MUKHERJI, S. (1981). Heavy Metal Effects in Plant Tissues Involving Chlorophyll, Chlorophyllase, Hill Reaction Activity and Gel Electro-Phoretic Patterns of Soluble Proteins. **Ind. Journal of Exp. Biol.**, 19, 702-706.

NEEDHAM, A. E. (1957). Components of nitrogenous excreta in the earthworms **Lumbricus terrestris**, L. and **Eisenia foetida** (Savigny) **J. Exp. Biol.**, 34, 425-445.

NEILSON, R. L.(1965). Presence of Plant Growth Substances in Earthworms demonstrated by Paper Chromatography and Went Pea Test. **Nature, 208, 5015, 1113-1114.**

NEWTON, L.(1944). Pollution of the rivers of the West Wales by lead and zinc effluent. **Annals of App. Biology**, 31, 1-11.

NICHOLAS, A. K. and HUTNICK, R. J. (1971). Ectomycorrhizal establishment and seedling response of variously treated deep-mine coal refuse. **Penn. Dept. Envir. Res. Spec. Res. Rep. SR - 89, p.** 121.

NICHOLAS, D. T. D. (1965). Influence of the rhizosphere on the mineral nutrition of the plant. **In:** Ecology of Soil-Borne plant Pathogens (Baker, K. F. and W. C. Symder, Eds.) Univ. Calif. Press, Berkeley, p. 210-217.

NEILSON, R. F. and PETERSON, H. B.(1972). Treatment of Mine Tailings to Promote Vegetative Stabilization. Utah. St. Ag. Exp. Sta. Bull. pp. 22.

NIJHAWAN, S. D. and KANWAR, J. S. (1952). Physiochemical **properties of e**arthworm castings and their effect on the productivity of soil. **Ind. J. Agric. Sci.**, 22(iv), 357-373.

NORRIS, J. R. (1959). The isolation and identification of **Azotobacters. Lab. Pract.**, 8, 239.

NYE, P. H. (1955). Some soil forming processes in the humid tropics. 4. The Action of soil fauna, **J. Soil Sci.**, 22, 357-373.

OKHI, K. (1976). Manganese deficiency and toxicity levels for 'Bragg ' Soyabeans. **Agron. J.**, 68, 681-684.

OHKI, K. (1964). Manganese Deficiency and Toxicity on Growth, Development and Nutrient Composition in Wheat. **Agron. J.** Vol., 76, pp. 213-218.

OHKI, K. (1985). Manganese Deficiency and Toxicity Effects on Photosynthesis, Chlorophyll and Transpiration in Wheat. **Crop Sci.** Vol., 25, 187-191.

OSBORNE, D. J. (1962). Effect of kinetin on protein and nucleic acid metabolism in Xanthium leaves during senescence. **Plant Physiology**, 37, 595-602.

PEGTEL, D. M. (1986). Responses of plants to Al, Mn and Fe with particular reference to **Succisa pratensis**, Moench. **Plant and Soil,** 93, 43-55.

PETERS, T. H. (1970). Using Vegetation to Stabilize Mine Tailings. **Jour. Soil and Water Conservation**, 25, 65-66.

PETERSON, R. L.; ASHFORD, A. E. and ALLAWAY, W. G. (1985). Vesicular-arbuscular mycorrhizal associations of vascular plants on Heron Island, a Great Barrier reef coral Cay. **Aust. J. of Botany**, 33, 669-676.

PHILLIPS, J. H. and HAYMAN, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. **Trans. Br. Mycol. Soc.,** 55, 158-161.

PIPER, C. S. Soil and Plant Analysis. Hans Publishers, Bombay 1966.

PONOMAREVA, S. I. (1950). (The role earthworms in the creation of stable structure in lay rotations.). **Pochvovedenie**, 727-732 (R.)

PONOMAREVA, S. I. (1962). The importance of biological factors in increasing the fertility of sod-podzolic soils. **Z. PflErnahr Dung.** 97, 205-215.

PORTER, W. M.; ROBSON, A. D. and ABBOTT, L. K. (1987). Factors controlling the distribution of Vesicular-Arbuscular Mycorrhizal fungi in relation to soil pH. **Journal of Applied Ecology,** 24, 663-672.

POWERS, W. L. and BOLLEN, W. B. (1935). The chemical and biological

nature of certain forest soils. **Soil Sci.**, 40, 321-29.
PRAMANIK, B. N. and MISRA, A. N. **Ind. Journal Agric. Sci.**, 25,1.

PRATHER, JOEL G. (1973). Vegetative Stabilization of Reclaimed Copper Stamp-Sands. Masters Thesis, Mich. Tech. Univ.

PUH, P. C. (1941). Beneficial influence of earthworms on some chemical properties of the soil. **Sci. Soc. China, Biol. Lab. Contrib. Zool.**, 15, 147-155.

QUISPEL, A. (1974) (Editor). The Biology of Nitrogen fixation. North Holland Publishing Company, Amsterdam-Oxford. American Elsevier Publishing Company, Inc., N. York.

RANGASWAMI, G. AND SADASIVAM, K. V. (1964). Studies on the occurence of **Azotobacter** in some soil types. **J. Indian Soc. Soil Science**, 1, 12 (1), 43-49.

REDENTE, E. F. and REEVES, F. B. (1981). Interactions between Vesicular-arbuscular mycorrhiza and **Rhizobium** and their effect on sweet vetch growth. **Soil Sci.**, 132, 410-415.

ROBERTS, R. D.; MARRS, R. H.; SKEFFINGTON, R. A. and BRADSHAW, A. D. (1981). Ecosystem development in natural colonised china clay wastes. I. Vegetation changes and overall accumulation of biomass and nutrients. **Journal of Ecology**, 60, 153-161.

RORISON, I. H. (1960). **J. Ecol.**, 48, 678.

ROUGHLEY, R. J. (1976). **In:** Symbiotic Nitrogen Fixation in plants. (P.S. Nutman, Ed.) Cambridge Univ. Press, London, New York. pp.

125-136

ROVIRA, A. D. (1963). Microbial Inoculation of Plants. 1 - Establishment of free nitrogen fixing bacteria in the Rhizosphere and their effects on Maize, Tomato and Wheat. **Plant and Soil**, 19 (3), 304-314.

ROVIRA, A. and GREACEN, E. L. (1957). **Australian J. Agr. Res.**, 8, 659-673.

RUSSELL, E. J. (1910). The effect of earthworms on soil productiveness. **J. Agric. Sci.**, 3, 246-257

RUSSELL, E. W. (1973) (Ed.). Soil conditions and Plant Growth. The English Language Book Society and Longman (Lond.).

SALISBURY, E. J. (1923). The influence of earthworms on soil reaction and the stratification of undisturbed soils. **J. Linn. Soc.** (Bot.) 46, 415-425.

SARIG, S.; KAPULNIK, Y.; NUR, I. and OKON, Y. (1984). Response of non-irrigated **Sorghum bicolor** to **Azospirillum** Inoculation. **Exp. Agric.**, 20, 59-66.

SARKUNAN, V.; BIDDAPA, C. C. and NAYAK, S. K. (1984). Physiology of aluminium toxicity in rice. **Curr. Sci.**, 53, 822-824.

SAUCHELLI, V. (1969). Trace elements in Agriculture. Van Nostrand, New York, 1969.

SAVITSKAYA, N. N. (1976). **Biol. Naukmi.**, 19, 49.

SCHRAMM. J. R. (1966). Plant Colonization studies on black wastes from anthracite mining in Pennsylvania. **In:** Trans. Amer. Philosoph. Soc. New Series, Vol. 56 Part I (1966).

SEN, A. N. (1966). Inoculation of legumes as influenced by soil **atic conditions**. **Ind. J. Agri. Sci.**, 36, 1-7.

SEQUEIRA, J. O.; HUBBELL, D. H. and MAHMUD, A. W. (1984). Effect of liming on spore germination, germ tube growth and root colonization by vesicular-arbuscular mycorrhizal fungi. **Plant and Soil**, 76, 115-124.

SHARMA, C. P.; NEENA KHURANA.; CHATTERJEE, C. and AGARWALA, S. C. (1988). Response of pigeon pea to variable levels of manganese. **Proc. Ind. Acad. Sci.** (Pl. Sc.). Vol., 98, No. 4, pp. 283-290.

SHARPLEY, A.N. and SYERS, J. K. (1976). Potential role of earthworm casts for the phosphorous enrichment of runoff waters. **Soil Biol. Biochem.**, 8, 341-346.

SHARPLEY A. N. and SYERS, J. K. (1977). Seasonal variation in casting activity and in the amounts and release to solution of phosphorous forms in eathworm casts. **Soil Biology and Biochemistry**, 9, 227-231.

SHARPLEY, A. N.; SYERS. J. K. and SPRINGETT, J. A. (1979). Effect of surface-casting earthworms on the trasnport of phosphorous and nitrogen in surface runoff from pasture. **Soil Biology and Biochemistry**, 11, 459-462.

SHELDON, J. C. (1974). The growth of amenity trees in difficult conditions. **In:** Tree growth in the landscape. Kent, Wye College, pp. 69-77.

SHETRON, S. G. and CARROLL, D. A. (1977). Performance of trees and shrubs on metallic mine mill wastes. **J. Soil Water Cons.**, 32, 5, 222-225.

SHETRON, S. G. and DUFFEK, R. (1970). Establishing vegetation on iron ore mine tailings. **J. Soil Water Cons.**, 25, 227-230.

SHETRON, S. G.; HAMIL, B. M.; JURGENSEN, M. F.; SEGALL, R. T.; JONES, L.; LENNOX, L. and PRATHER, J. (1977). Establishing vegetation on alkaline iron and copper tailings. **Mich Dept. Nat. Resour. Geol. Surv. Div. Rept.**, 17. 14p

SHETRON, S. G. (1983). Alfalfa, **Medicago sativa**, L., establishment in mine mill tailings. I. Plant analysis of alfalfa grown on iron and copper tailings. **Plant and Soil**, 73, 227-237.

SHETRON, S. G. and SPINDLER, J. J. (1983). Alfalfa, **Medicago sativa**, L., establishment in mine mill tailings. II. Root patterns of alfalfa in iron ore tailings and natural soils. **Plant and Soil**, 73, 239-246.

SHRIKHANDE, J. G. and PATHAK, A. N. (1948). Earthworms and insects in relation to soil fertility. **Curr. Sci.**, 17, 327-8.

SHRIKHANDE, J. G. and PATHAK, A. N. (1951). A comparative study of the physio-chemical characters of the castings of different insects. **Indian J. Agric. Sci.**, 21, 401-407.

SIDERIS, C. P. and YOUNG, H. Y. (1949). Growth and chemical composition of **Ananas comosus** (L), Merr., in solution cultures with different iron-manganese ratios. **Plant Physiol.**, 24, 416-440.

SMITH, R. A. H. and BRADSHAW, A. D. (1970). Reclamation of toxic metalliferous wastes using tolerant populations of grass. **Nature** (Lond.), 227, 376-7.

SMITH, R. A. H. and BRADSHAW, A. D. (1972). Stabilization of toxic mine wastes by the use of tolerant plant populations. **Trans. Instn. Min. Metall.**, 81, 4, 230-237.

SMITH. R. M.; TYRON, E. H. and TYNER, E. H. (1971). Soil development on mine spoil. **W. Va. Agric. Exp. Sta. Bull.**, 604T.47p.

SMITH, S. E. and DAFT, M. J. (1977). Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non mycorrhizal **Medicago Sativa. Aust. J. Physiol.**, 4, 403-413.

SOLDATINI, G. E.; ZIEGLER, I. and ZIEGLER, H. (1978). **Planta**, 143, 225.

STARKEY, R. L. and DE, P. K. (1939). **Soil Sci.**, 47, 329.

STEWART, C. R. (1973). The effect of wilting on proline metabolism in excised bean leaves in the dark. **Plant Physiol.**, 51, 508-511.

STREET, H. E. and GOODMAN, G. T. (1967). Revegetation techniques in the Lower Swansea Valley. The Lower Swansea Valley Project (K. J. Hilton, Ed.), pp. 71-110. Longman, London.

STOCKDILL, S. M. J. and COSSENS, G. G. (1966). The role of earthworms in pasture production and moisture conservation. **Proc.**

STOCKLI, A. (1928). (Studies on the influence of earthworm on the soil condition.) **Landw. Jb. Schweiz**, 42, 5-121.

STOCKLI, A. (1949). (The influence of microflora and fauna on soil conditions.) **Z. PflErnahr. Dung.** 45, 41-53 (G)

STRZELEZYK, E. (1958). The effect of various cultivated plants on the number of **Azotobacter** and **Clostridium** in their rhizosphere. **Acta Microbiol. Pol.**

SUBHA RAO, N. S. (1977). Soil Micro-organisms and plant Growth. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.

SUBHA RAO N. S. (1988). Biofertilizers in Agriculture. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Bombay, Calcutta.

SUBRAMONEY, N. and ABRAHAM, A. (1962). **Sci. and Culture**, 28. 339.

SWARD, R. J. (1978). Infection of Australian heathland plants by **Gigaspora margarita** (a vesicular-arbuscular mycorrhizal fungi.). **Aust. J. Bot.**, 26, 253-64.

SYERS, J. K.; SHARPLEY, A. N. and KEENEY, D. R. (1978). Cycling of nitrogen by surface-casting earthworms in a pasture ecosystem. **Soil Biol. Biochem.**, 11, 181-185.

SYERS, J. K. and SPRINGETT, J. A. (1984). Earthowrms and soil fertility. **Plant and Soil**, 76, 93-104.

TEOTIA, S. P.; DULEY, F. L. and McCALLA, T. M. (1950). Effect of stubble mulching on number and activity of earthworms. **Neb. Agric. Exp. Sta. Res. Bull.**, 165, 1-20.

TERRY, N.; EVANS, P. S. and THOMAS, D. E. (1975). Manganese toxicity effects on leaf cell multipilication and expansion and on dry matter yield of sugar beets. **Crop Sci.**, 15, 205-208

THORNTON, I. (1974). Applied geochemistry in relation to mining and the environment. **In:** Minerals and the Environment. (M. T. Jones, Ed.), pp. 87-102. **Institution of Mining and Metallurgy**, London.

TINKER, P. B. H.(1975). Effects of vesicular-arbuscular mycorrhizas on higher plants. 29th **Symp. Soc. Exp. Biol.**, (D. G. Jennings and D. L. Lee, Eds.). pp. 325-349. Cambridge Univ. Press, London.

TITZE, J. F.; CRAIZ, G. and LAMONT, B. B. (1979). Vesicular-arbuscular mycorrhizas in Jarrah forest - a preliminary note. **Mulga Research Centre Annual Report** No. 3, 29-33.

UPPAL, B. N. **ET AL. (1939). Indian J. Agric. Sci.**, 9, 689.

VAISHYA, U. K. and GAJENDRAGADKAR, G. R. (1982). Effect of rhizobial inoculation on nodulation and yield of different genotypes of urid (**Vigna mungo**, (L) Wilzek) **Ind. J. Microbiol.**, 22 (2), 132-134.

VAN RHEE, J. A. (1965). Earthworm activity and plant growth in artificial cultures. **Plant and Soil**, 22 (1), 45-49.

VIMMERSTEDT, J. P. and FINNEY, J. H. (1973). Impact of earthworm introduction on litter burial and nutrient distribution in Ohio strip-mine spoil banks. **Soil Sc. Soc. of Am. Proc.**, 37 (3), 388-391.

VINCENT, J. M. (1956). **In:** Seventh International Grassland Congress. Palmeston, North. New Zealand, pp. 179-189.

VINCENT, J. M. (1970). A manual for the practical study of root-nodule bacteria. Blackwell Scientific Publications. Oxford and Edinburgh.

VLAMIS, J. and WILLIAMS, D. E. (1962). Ion competition in manganese uptake by barley plants. **Plant Physiology**, 37, 650-655.

VLAMIS, J. and WILLIAMS, D. E. (1964). Iron and manganese relations in rice and barley. **Plant and Soil**, 20, 221-231.

VLAMIS, J. and WILLIAMS, D. E. (1973). Manganese toxicity and marginal chlorosis of lettuce. **Plant and Soil**, 39, 245-251.

VOZZO, J. A. and HACSKAYLO, E. (1971). Inoculation of **Pinus caribaea** with ectomycorrhizal fungi in Puerto Rico. **For. Sci.**, 17, 239-245.

WADDINGTON, D. V. (1969). Soil and soil related problems. **In:** Turf grass Science (A. A. Hanson and F. V. Juska, Eds.), 80-129, Agronomy Series No. 14, American Society of Agronomy, Madison, Wisconsin, U. S. A.

WAKSMAN, S. A. (1952). Soil Microbiology. John Wiley & Sons, New York, U. S. A.

WARCUP, J. H. and McGEE, P. A. (1983). The mycorrhizal associations of some Australian Asteraceae. **The New Phytologist**, 95, 657-672.

WANTANABI, M. (1975). On amounts of cast production by the Megascolecial earthworm **Pheretima lupeinsis**. **Pedobiologia**, 15, 20-28.

WHEELER, B. D.; AL-FARRAJ, M. M. and COOK, R. E. D. (1985). Iron toxicity to plants in base rich wetlands : Comparative effects on the distribution and growth of **Epilobium hirsutum**, L. and **Juncus subnodulosus**, Schrank. **New Phytology**, 100, 653-667.

WHITE, M. C.; CHANEY, R. L. and DECKER, A. M. (1974).Differential varietal tolerance in soyabean to toxic levels of zinc in Sassafras sandy loam. **Agron. Abst.** 1974, 144-1445.

WILD, H. and WILTSHIRE, G. H. (1971). The problem of vegetating Rhodesian mine dumps examined. **Rhod. Cham. Mines J.** No. 11, 26-30.

WILSON, H. A. (1965). The microbiology of strip mine spoil. **West. Virginia Univ. Agr. Exp. Sta. Bull.**, 505 T, pp. 44.

WILLIAMS, R. O. (1949). Useful and ornamental plants of Zanzibar and Pemba. (Govt. Press, Zanzibar).

WILLIAMSON, N. A.; JOHNSON, M. S. and BRADSHAW, A. D. (1982). Mine Waste Reclamation. Published by : Mining Journal Books Ltd., London, England.

WITTICH, W. (1952). Our present knowledge about humus and the new approach to solving the raw-humus problem in forests.) **SchrReihe forstl. Fak. Gottingen**, 4, 1-106 (G).

WOLLNY, E. (1890). (Investigation on the course of decomposition of litter in ground with great earthworm activity). **SchrReike forstl. Fak. Gottingen**, 9, 7-33 (G).

WONG, M. H. and BRADSHAW, A. D. (1982). A comparison of the toxicity of heavy metals, using root elongation of Rye-grass, **Lolium perenne**. **New Phytol.**, 91, 255-261.

WONG, M. H.; LAU, W. M.; LI, S. W. and TANG, C. K. (1983). Root Growth of Two Grass Species on Iron Ore Tailings at Elevated Levels of Manganese, Iron and Copper. **Environ. Res.**, 30, 26-33.

WOOLHOUSE, H. W. (1983). Toxicity and tolerance in the response of plants to metals. **In:** Encyclopedia of plant ecology. New Series, Vol. 12C (O. L. Lange, P. S. Nobel, C. B. Osmond and H. Zeigler, Eds.) Springer- Verlag, Berlin.

WOOLHOUSE, H. W. (1983). Toxicity and tolerance in the responses of plants to metals. **In**: Encyclopedia of Plant Physiology. New Series, Vol. 12C, pp. 245-300. Springer-Verlag, Berlin.

WU, L. and ANTONOVICS, J. (1975). Zinc and Copper uptake by **Agrostis stolonifera** tolerant to both zinc and copper. **New Phytol.**, 75, 231-237.

WYSS, O. and WYSS, M. B. (1950). **N. J. Backt.**, 59, 287.

YAHALOM, E.; KAPULNIK, Y. and OKON, Y. (1984). Response of **Setaria italica** to inoculation with **Azospirillum brasilense** as compared to **Azotobacter chroococum**. **Plant and Soil**, 82, 77-85.