7

Prospects in Reclamation of Iron ore Mine Waste Lands: Role of Arbuscular Mycorrhizal (AM) Fungi and Inoculation Procedures

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INTRODUCTION

Mining is one of the most common activities of ancient and modern world. Mining is regarded as the second largest industry after agriculture and has played a vital role in the development of civilization from ancient days. Most valuable materials for man such as metals, chemicals, fuels for energy, rocks and stones for building comes from mining (Trivedy, 1990). Land surfaces are inevitably disturbed in seeking to win ores from the earth. Mechanization and improved technology has brought increasingly large tracts of land into state of disturbance. With increasing demands, land has been constantly exploited for raw materials from the natural environment. Land is not a resource, which automatically renews itself like rainfall and sunlight. It is a finite resource, being diminished by the spread of industry and urbanization (Coleman, 1979).

The State of Goa with an area of approximately 3702 sq. km lies on the West Coast of India between 15°48'00"N and 14° 53'54" N Latitude and 74°20'13"E and 73°40'33"E Longitude. In Goa, mining commenced in 1910. However, commercial exploitation began in 1947 when the erstwhile Portuguese Government granted over 700 concessions all over Goa. During those days the concept of pollution and conservation were not given due weightage and the people welcomed widespread mining activities as an additional source of income and employment. In a way, therefore the growth of mining has proceeded simultaneously with the growth of agriculture

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and population. Goa has been a prime exporter since 1950, as much as 300 million tons of iron ore has been exported. Present production of iron ore is of the order of 15 million tons per year which constitutes 40% of the total iron ore production in the country and 50% of its export. The estimated reserves of iron ore as on today is around 400 million tons and is expected to last for another 25-30 years at the present rate of mining.

The mining operations are such that, two classes of wastes are produced *viz.*, (i) piles of surface overburden waste rock and lean ore, which constitutes the reject dumps, and (ii) a fine grained waste resulting from the ore beneficiation process and deposited in large man made basins called tailing ponds. The latter kind of waste materials are termed as tailings.

It is true that in the process of mining mineral resources from the earth, disturbance to environment and ecosystem is unavoidable. In other words, mining is to some extent an unavoidable destructive process. Though, there are problems of mine wastes in terms of erosion, environmental pollution, damage to adjoining agricultural fields, forests, *etc.*, many a times they are exaggerated. These hazards are within measurable limits and can be easily ameliorated to a significant extent by extensive research and planning to control the impacts.

MINING AND ITS IMPACT ON ENVIRONMENT

In terms of employment and foreign exchange earnings, mining industry plays an important role in Goa's economy. On the other hand, however, the wastes produced by mining activities are likely to pose a serious threat to the environment if proper measures are not taken to re-establish vegetation at the mining sites.

The tailings occupy large segments of the landscape in the vicinity of the mine and diminish the aesthetic quality of the natural landscape. The tailing basins may occupy upto 40% of a mine site land area (Shetron and Duffek, 1970). Essentially open caste mining involves excavation and movements of large volumes of earth's crust. A ton of iron ore mined for instance produces 2 to 3 tons of waste. Dean and Havens (1971) estimated that the total tonnage of such wastes in United States covers about 200 million acres. The annual accumulation exceeds one billion tons, which are distributed over an area of approximately 2 million acres. In the Western States, nearly one half million tons are being produced daily (Neilson and Peterson, 1972). The excavation of iron ore exposes large chunks of earth's crust to the atmosphere that intrude upon the landscape.

Mining accounts for a substantial proportion of the loss of land of primary production. In India, 7,85,000 hectares of land is reported to be under mining operations (Baliga, 1985). Indiscriminate mining since 1961 has destroyed 50,000 hectares of forest in Goa and it is estimated that during all these years as much as 900 to 1000 million tons 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 other types of clays, slimes, *etc.*

With the present annual production of 15 million tons of iron ore, it is expected that 40-50 million tons of wastes have to be stored per year, and approximately 150 million cubic meters of water is to be discharged from pits to the drainage system. Mine waste dumps are biggest man-made hillocks, volume and height of such dumps increases every year. Most of the waste dumps rise up to 50-60 meters high with 50° -55° angle of repose. These being unconsolidated are prone to slumps and slides due to heavy monsoon rains.

Damage to environment my mining activity has been caused largely by reject dumps, pumping out of muddy waters from the working pits including those where the excavations have gone below the water level, and slimes from the beneficiation plant. The damage is more conspicuous during monsoon, when the rainwater carries out the washed out materials from the mine waste dumps to the adjoining agricultural fields and water streams. The slimes and silts, which enter the agricultural fields get hardened on drying, thus making aeration and root penetration difficult. Indiscriminate dumping of rejects has rendered over 10,000 hectares of agricultural land infertile, pollute the springs and wells, and also cause silting of waterways especially during monsoon. Such silting of waterways over the years have caused flooding of adjacent fields and inhabited areas during the dry seasons. The noise created due to the blasting operations, movement of heavy vehicles, operation of heavy machineries and dumping of iron ore poses a constant problem in the surrounding areas. Dust from blasting, crushing and transportation 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 diseases like asthma are frequently common in inhabitants of the area and the mineworkers. The dust has its effects on nearby communities, industrial machinery and demanding effects on vegetation by blocking plant pores and hampers photosynthesis.

The most common hazards of open cast mining of iron ore have been the defacing of landform by development of depression and elevation or sloppy terrain. It also leads to large-scale deforestation, destruction of wild life and natural resources resulting in a fragile ecosystem lacking in flora and fauna. In the process of mining the topsoil is removed, leaving bare rock, thereby making it hard for vegetation to become re-established. Normally, natural processes would gradually recolonise the mine sites and spoils heaps building up the soil and reclothing the landscape with vegetation. However this can take a long time. Meanwhile, the unprotected surface is subjected to erosion leading to the clogging rivers and lakes with silt. Thus the common approach towards stabilization i.e. establishment of a permanent cover of vegetation involves not merely growing plants. But it necessitates bringing into a plant community that will maintain itself indefinitely without further attention or artificial aid such as irrigation. Such a performance could be achieved most advantageously, by selecting species adapted to growth, spread and reproduction under the inimical conditions. Most of the plants, which are desirable for the revegetation of these lands, are dependent on " Mycorrhizal Fungi".

ECOSYSTEM, PLANT SUCCESSION AND MINING

The vegetation together with the soil in which it has its roots, the associated fauna, and the environment that surrounds them form a closely interrelated and interdependent to the ecosystem. Although ecosystems are sensitive to the outside influences, they are self-sustaining. Once properly established, they need no further support. This is because of natural cycling of accumulated materials, which maintain the vegetation and the other organisms with it. After a major disturbance, vegetation slowly and gradually develops over a period of time: a process termed plant succession.

If the above two properties (self-sustaining and capacity to develop) of the ecosystem are considered, then one may presume that after mining disturbance, there is no need of any revegetational efforts *i.e.*, a self-sustaining vegetation cover will develop naturally. But the process of natural succession will take many years.

The aim of revegetation of mining sites is to achieve vegetation cover within a few years, so that the subsequent succession may take place at a rapid pace. Hence, it is obvious that one must look for the appropriate treatments and management strategy so that useful vegetation can be established quickly economically leading to a self-sustaining ecosystem.

CHARACTERISTICS OF MINE WASTES

For 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 over the entire area is not easy, 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, rock waste and due to interaction of various factors.

Soil texture is used extensively as a guide to evaluate soil water storage, water availability, surface erosion, land stability and chemical properties (Shetron and Trettin, 1984). Natural soil consists of an inorganic framework of sand, silt and clay particles, intimately mixed with organic material. The physical analysis of iron ore wastes reveals that the tailings and rejects have high bulk and particle density which is normally the characteristic feature of metalliferous mine waste (Rodrigues and Bukhari, 1996 & 1997) (Table 1 & 2). The bulk density of natural soils fall within the range of 1.0 – 1.5 g cm^{-3} (Williamson *et al.*, 1982) and particle density 2.63 g cm^{-3} (Waddington, 1969). Bulk density is a useful measure of compaction to root penetration. Surface accumulation of fines in slim dams may give a bulk density as high as 7.5 g cm^{-3} with low infiltration (Ruschena *et al.*, 1974).

Cation exchange capacity (CEC) is important as it is a measure of total exchangeable cations (calcium, magnesium, potassium and sodium) in soil materials (Black, 1968). Low water holding capacity of the rejects and tailings can be attributed to the poor soil texture, structure and organic matter content which are known to be responsible for improving water holding capacity.

Maclean and Dekker, (1976) studied the pH of different wastes and reported large variations in acidity among different sites ranging from pH 1.5 to above 10. Varying soil pH changes the concentration of many nutrients and toxic ions in soil solutions as well as the concentrations of hydrogen ions (Russell, 1973). In solutions of acid soils, there are often higher concentrations of aluminium and manganese, and lower concentrations of calcium, magnesium and molybdenum as compared to that in alkaline soils (Porter *et al.*, 1987).

Nutrient deficiencies are widely reported as a major limitation, particularly in terms of a low or a complete lack of organic matter and nitrogen in mining wastes. Smith and Bradshaw, (1970) stated that micro-nutrient deficiencies are frequently encountered in the mine wastes. Wong *et al.*, (1983) showed that the tailings were alkaline, lacking in organic matter and nitrogen, but were rich in metals such a Fe, Zn, Cu, Mn, Mg and Ca.

Table—1 Some properties of iron ore mine rejects in Goa. (Rodrigues and Bukhari, 1997).

Properties	*Mean (S.D.)*
pH	6.02 (0.18)
EC (mS /cm)	0.051 (0.012)
Total N	93.2* (N.A)
Available N	3.8* (N.A.)
P	1.5 (N.A.)
SO_4^{-2}	<0.1 (N.A.)
Ca	1.76 (0.80)
Mg	0.92 (0.55)
K	0.76 (0.26)
Na	2.60 (0.54)
Cu	<0.05 (N.A.)
Fe	<0.1 (N.A.)

Concentrations in $\mu g.g^{-1}$ oven dry spoil.
N.A. = Not applicable.
S.D. = Standard deviation.
EC = Electrical conductivity.
* = Mean of two replicates taken from bulked samples.

Shetron (1983) reported that in iron ore tailings the organic matter and nitrogen are essentially non existent, phosphorus levels are low; Ca, Mg, K and metal range in availability; have alkaline pH and low cation exchange capacity.

MYCORRHIZAL FUNGI

In 1842, Vittadini proposed that tree rootlets are nourished by certain fungal mycelia, which mantle them, as observed by him more than a decade earlier. This hypothesis was elaborated to a theory of mutalistic symbiosis by Bernhard Frank (1885) who coined the term "mycorrhiza" to denote the symbiotic association formed by fungal mycelia with plant roots (Gr. myces = fungus; rhizo = roots). The concept of fungus-root symbiosis has since been a subject of extensive research. Though the word was introduced in 1885, mycorrhizae itself, of course are millions of years older. It is generally believed that Arbuscular mycorrhizal (AM) fungi evolved early in the history of vascular plants (Trappe, 1987, Morton, 1990). Despite of their geological age (Birch, 1986; Pirozynsky and Dalpe, 1989) and their crucial role in origin of plants very little is known about their phylogenetic origin (Sancholle and Dalpe, 1993). The members of Glomales are believed to have been present as early as the Cambrian period (Pirozynsky and Dalpe, 1989). They played a pivotal role in the origin of the terrestrial flora. Early Devonian plants showed the presence of the endosymbionts represented by non-septate mycelia, coiled hyphae, irregularly shaped thin walled spherical structures resembling the vesicles. Arbuscule like structures are recently been reported from plants preserved in Rhynie Chart (Remy *et al.*, 1995). In their symbiotic habit, the mycorrhizal fungi constitute a special group among root inhabiting fungi.

Table—2. **Some properties of iron ore mine tailings in Goa. (Rodrigues and Bukhari, 1996).**

Properties	*Mean (S.D.)*
pH	6.48 (0.07)
EC (mS /cm)	0.065 (0.018)
Total N	60.3* (N.A)
Available N	1.7* (N.A.)
P	1.9 (N.A.)
SO_4^{-s}	<0.1 (N.A.)
Ca	2.34 (0.57)
Mg	0.75 (0.17)
K	0.71 (0.30)
Na	4.85 (2.94)
Cu	<0.05 (N.A.)
Fe	<0.1 (N.A.)

Concentrations in $\mu g.g^{-1}$ oven dry spoil.

N.A. = Not applicable.

S.D. = Standard deviation.

EC = Electrical conductivity.

* = Mean of two replicates taken from bulked samples.

ADVANTAGES OF MYCORRHIZAL FUNGI

Mycorrhizal association is a universal phenomenon throughout the plant kingdom and is beneficial and even indispensable for the life and healthy growth of the host plants. Because almost all plant species of natural vegetation and the agricultural crop plants of the tropics live in association with fungi, it should be possible to increase productivity through manipulation of mycorrhizal systems.

Absence of suitable mycorrhizal fungi is one of the main reasons for the failure of afforestation programmes. Tree species, especially exotics, fail to establish on afforestation sites in the absence of their fungal symbionts. Repeated attempts for the last twenty years to raise pine plantation in Puerto Rico failed due to lack of ectomycorrhizae on their roots. The pine seedlings would grow to 5-30 cm in height, then become chlorotic, and die. Transfer of ectomycorrhizal soil from successful plantations in the mainland to Puerto Rico nurseries helped in the successful establishment of pine plantations on this island. The inoculated plants of slash pine grew healthy and reached a height of 2.0- 2.5 m in three years. Similarly, success in raising pine plantations in South Africa was achieved only when ectomycorrhizal inoculum from Netherlands was introduced in South African nurseries. Soil from South African pine nurseries was then used as soil inoculum in Kenya and this resulted in large scale successful pine plantations in that country.

Normal growth occurs only when mycorrhizal fungi present in the site colonize and establish mycorrhiza on the roots. It may be possible to grow mycotrophs without its fungal symbiont if nursery plants are given a high dose of fertilizers. However, such seedlings when transplanted in the field where mycorrhiza is absent and nutrient is discontinued for economic reasons, the growth of plants is retarded appreciably in many cases.

Mycorrhizal plants are more efficient to draw nutrient from soils, particularly soils poor in available phosphorus as compared to non-mycorrhizal plants. Mycorrhizae benefit their hosts in a variety of ways. They increase the absorptive surface of colonized roots through inducing profuse branching. The extrametrical hyphal growth of these fungi can explore large soil areas for acquisition of nutrients from the soil. The hyphae of mycorrhizal fungi are able to mobilize nutrients from the substrates where such nutrients are in unavailable form for absorption by the plant roots. The fungal hyphae assimilate nutrients from such substrates through their active metabolic process and these assimilated nutrients are then transported to the plant roots.

Besides, direct nutritional advantages, mycorrhizae have also been accredited with other benefits to the host plants such as ability of arbuscular mycorrhizal roots to overcome water stress by stomatal regulation in *Citrus* (Levy and Krukum, 1980). Mycorrhizal inoculation also stimulates rooting (Barrow and Roncadri, 1977) growth and transplant survival (Bryan and Kormanik, 1977) of cutting and seedlings raised in sterilized nursery media. It also increases disease resistance by depressing root penetration and larval development of nematodes (Sikora, 1978). In addition to this, mycorrhizal plants have shown to have greater tolerance to toxic heavy metals, to drought, to high soil temperature, to saline soil, to adverse soil pH than the non mycorrhizal plants (Schenck, 1984). Arbuscular mycorrhizal fungi also bind soil into semi stable aggregates, thus improving the structure of the soil. Because of these attributes, mycorrhizae are now considered important in the establishment of plants in inhospitable sites like mine wastelands.

Apart from nutrient benefits to plants derived from mycorrhizal symbiosis, it is known to impart disease resistance in plants. In *Pinus echinatus*, mycorrhizal roots resist infection due to *Phytopthora cinnamomi*, where as uninfected roots are highly infected. This is based on the hypothesis that the rhizosphere and the sheath surface of mycorrhizal roots possess microflora different from this in non-mycorrhizal roots. In order to derive maximum benefits from mycorrhizae, it is necessary that nursery raised seedlings have optimum level of mycorrhization on their roots for which introduction of suitable mycorrhizal species at nursery stage should form an integral part of nursery management practices.

BROAD CLASSIFICATION OF MYCORRHIZAE

Based on colonization anatomy, two major groups of mycorrhizae have been recognized. *Viz.*, Ectomycorrhizae and Endomycorrhizae.

Characteristics of Ectomycorrhizae

1. Prevalent in the temperate regions, in forest and ornamental tree species.

2. Characterized by the presence of a mantle of fungal tissue around the host rootlet, and intercellular penetration of the rootlet cortex (Hartig net), and not intracellular.

3. Fungi belong to Basidiomycetes, which form sporophores and release air-borne spores. The inoculum thus is easily spread by wind from one location to another.
4. Host plant roots with ectomycorrhizal colonization are short, swollen, dichotomously branched with distinctive colours *viz.*, white, black, orange, yellow or olive green.
5. It is fairly easy to isolate a number of ectomycorrhizal fungi in pure culture by using routine microbiological techniques and grow them saprophytically. Thus, it is possible to produce inoculum on a large scale.

Characteristics of Endomycorrhizae

1. These fungi are prevalent in the tropics.
2. The colonization is both intercellular and intracellular. In this case, there is no 'Hartig net' and no 'mentle'.
3. Endomycorrhizal fungi cannot be cultured in vitro conditions. The production of endomycorrhizal inoculum requires the growth of a susceptible host plant. The inoculum has to be produced under sterile glasshouse conditions.

Classification of Endomycorrhizae

The Endomycorrhizae are again classified into 4 major groups viz., i) Arbuscular mycorrhizae (AM); ii) Arbutoid mycorrhizae; iii) Ericoid mycorrhizae, and iv) Orchid mycorrhizae.

Arbuscular Mycorrhizae (AM) Fungi:

The arbuscular mycorrhizal fungi are non-septate and ubiquitous. Despite their near omnipresence, the AM fungi have, until recently, received very little attention, because the AM fungi can neither be cultured in the absence of a living root nor isolated on agar plates by standard microbiological techniques. It is now well established that many plants cannot grow adequately without AM fungi, especially in phosphate-deficient soils. Arbuscular mycorrhizal fungi are characterized by the presence of arbuscules and vesicles.

Arbuscules-are similar to haustoria, developed by repeated dichotomous branching of hyphae that enter in the cortical cells. Each arbuscular tip sometimes appears to be surrounded by a cloud of granular material. They remain viable or active only for a short period *i.e.* 4-15 days. Their main function is nutrient transfer between symbionts. The cause of their destruction is the digestion of the fungal cell wall by host chitinase activity.

Vesicles-develop as terminal or intercalary swellings of the inter- or intra-cellular hyphae. They may be spherical or oval or lobed. Vesicle size and shape usually depends on the host nutrient conditions. They are known to contain oil droplets. When young, they have thin walls and contain homogenous protoplasm. They remain thin walled and function as storage organs for food or develop into thick walled chlamydospores functioning as reproductive structures.

Arbutoid Mycorrhizae

This type of colonization is suggested to be a transition between ectomycorrhizae and endomycorrhizae. It is hence, sometimes called as ectendomycorrhiza. This type of colonization is characterized by intracellular penetration. There is also a Hartig net and occasionally a fungal sheath. It is structurally intermediate between ectomycorrhizae and endomycorrhizae. Due to this type of colonization, root dimorphism might occur, with colonized roots remaining shorter. Only a few fungal species are known to form arbutoid colonization *viz., Amanita, Cortinarius* and *Boletus.*

Ericoid Mycorrhizae

This type of colonization is prevalent in the members of the family Ericaceae and hence the term Ericoid mycorrhizae. In this type of colonization, the fungal hyphae penetrate the epidermal and cortical cells and the fungus ramifies within each cell to form a coil or knot of filaments occupying much of the volume within the cells. The stele is not invaded. Hyphal knots can be readily detected at 200 to 400x magnification when observed under a microscope after staining with 0.05% trypan blue stain. Presently only one fungal species *viz., Pezizella ericae* is known to cause this type of colonization.

Orchid Mycorrhizae

This type of mycorrhiza has been proposed as one of the most complex of the symbiotic interaction. Members of the genera *Neottia, Limodoron, Epipogon* and *Vanilla* are dependent on mycorrhizae for growth. The entry of the fungal hyphae is a must for further growth of the seedlings. The fungal members which exhibit this type of colonization include *Fomes, Corticum* and *Rhizoctonia.*

STAGES OF DEVELOPMENT OF AM FUNGI

Arbuscular mycorrhizal fungal spores occur in physiologically inactive stages in soil. The spore germinate, grows and multiplies in the presence of actively growing roots of plants. The development of AM fungi in roots can be divided into four stages (Tommerup and Briggs, 1988):

- Spore germination and hyphal growth from infective propagules of AM fungi.
- Growth of hyphae from soil to host roots. The mycelial systems surrounding the roots are dimorphic (Mosse, 1959; Nicolson, 1967).
- Penetration and successful initiation of colonization in roots. Hyphae penetrates mechanically and enzymatically into cortical cells (Kinden and Brown, 1975). At the point, penetrating hyphae may or may not form appresoria (Abbott, 1982).
- Spread of colonization and development of internal hyphal system, arbuscules, which bifurcate inside a cell and bring about nutritional transfer between two symbionts and vesicles which, develop as terminal or intercalary swellings in inter- or intra-cellular hyphae. They are responsible for storage and vegetative reproduction.

CURRENT STATUS OF AM FUNGI

Endomycorrhizae produced by the nonseptate fungi are commonly called as "Arbuscular Mycorrhizal (AM) Fungi". These fungi belong to the Family Endogonaceae of the Order Endogonales and Class Zygomycetes (Trappe and Schenck, 1982). It includes six genera viz., *Acaulospora, Gigaspora Entrophospora, Glomus, Sclerocystis* and *Scutellospora*.

Benjamin (1979) placed eight genera of endogonaceous fungi under Endogonaceae in a single family Endogonaceae. Morton and Benny (1990) divided this order in two independent Orders *viz.*, Endogonales and Glomales on the basis of their spore structure and mycorrhiza development (Table 1).

Table—3: Classification of AM fungi.

Old Classification (Gerdemann & Trappe, 1974; Benjamin, 1979; Warcup, 1990)		*Present Classification (Morton & Benny, 1990)*		
Order:	Endogonales	1.	Order:	Endogonales
Family:	Endogonaceae		Family:	Endogonaceae
Genera:	*Endogone*		Genera:	*Endogone*
	Sclerogone			Sclerogone
	Glomus			
	Sclerocystis	2.	Order:	Glomales
	Acaulospora		Sub-order:	Glomineae
	Entrophospora	*(i)*	Family:	Glomaceae
	Gigaspora		Genera:	*Glomus*
	Scutellospora			***Sclerocystis***
		(ii)	Family:	Acaulosporaceae
			Genera:	***Acaulospora***
				Entrophospora
			Sub-order:	Gigasporineae
			Family:	Gigasporaceae
			Genera:	***Gigaspora***
				Scutellospora

Taxonomy of Glomales is based on the structure of their spores/sporocarps (Morton, 1990a). Some authors lay more emphasis on the wall structure, which also is a valid criterion (Walker, 1983, 1992). Glomales are divided into sub-orders. Glomineae and Gigasporineae (Table 3). The six genera included in this order are placed under three families (Morton and Benny, 1990). Till present about 150 species of fungi are genuine and validly published. Schenck and Perez (1990) have scientifically done the accumulation of the data on identification of AM fungi.

TYPES OF ECTO- AND ENDO-MYCORRHIZAL INOCULA FOR NURSERIES

The following types of inocula being employed for introduction of ecto- and endo-mycorrhizal fungi in nurseries are described below:

I. SOIL INOCULUM

Soil inoculum consists of mycorrhizal roots, spores, chlamydospores, hyphae, hyphae strands, rhizomorphs and other propagules of mycorrhizal fungi. This method has the disadvantage of transferring inadvertently pathogenic fungi to the nursery and the planting site. This is particularly objectionable if the transport is made between countries and it is not permissible under quarantine regulations. Also the soil inoculum is bulky, messy, and it is difficult to transport to the nursery and involves cost of transport. Finally, the same soil inoculum introduced into the same host species may induce different growth responses due to different fungal symbionts.

The endomycorrhizal inoculum can be chopped finely using hard tools. This inoculum is usually air dried to about 5-20% moisture and resembles granular fertilizer in its consistency.

II. INOCULATION WITH SPORES

The spores of ectomycorrhizal fungi are easy to collect and store, cheap to transport and can be taken to long distances. Spore inoculum is commonly used in case of ectomycorrhizal fungi belonging to class Gasteromycetes, the members of which produce fruiting bodies in the form of puff balls and truffles. At maturity, these fruiting bodies are full of powdery mass of spores. These fruiting bodies can be stored at 4-6 ^{0}C for 6-12 months without losing their viability. The following methods are being used for spore inoculation of nursery soils.

(a) Spore Soil Mix

In this, approximately 2 μg of spore powder (containing approximately 1×10^{12} spores) is thoroughly mixed with one kg of sterilized or fumigated soil and this spore mix is used as inoculum. For inoculation of nursery beds or potting mixture, 1 kg of soil spore mix is evenly spread over one square meter of nursery bed soil and the soil is then thoroughly racked up to uniformly distribute the spore inoculum in the soil.

(b) Seed Encapsulation with Spores

Slurry is made by suspending spores in water and the sticker is added to the slurry. The seeds are then dipped in the slurry and kept overnight before sowing in nursery beds.

(c) Spore Pellets

In Philippines, inoculum of ectomycorrhizal fungi in the form of tablets have been prepared under the name "MYCOGROE". These tablets consist of basidiospores of ectomycorrhizal fungi combined with soil as carrier and palletized in a tabletting machine. Pellets containing arbuscular mycorrhizal inoculum have been successfully used to inoculate plants. These pellets can be prepared by mixing 20 parts mycorrhizal inoculum (finely ground roots, soil and spores, pot culture), one part sedimentary-loess clay (mean particle size 16 μ) and one part tertiary sedimantary clay (mean particle size 2-6 μ). Add water until malleable and then roll into pellets.

(d) Mycorrhizal Beads

In Philippines, scientists have produced ecto-mycorrhizal beads, under the trade name "MYCORRHIZAL BEADS" by entrapping mycelia of *Pisolithus tinctorius* grown in liquid fermenters in calcium alginate beads. The entrapped mycelial inoculum has many advantages over solid medium inoculum. With this technique, the mycelium is produced in 1-2 weeks while in solid technique, several months are required to produce the inoculum.

(e) Pure Culture Inoculum

It is easier to produce ectomycorrhizal inoculum on a large scale using routine microbiological techniques. However, for arbuscular mycorrhizal (AM) fungi, large scale production of pure inoculum has been a major drawback as these fungi are obligate symbionts and hence requires the presence of a living host plant root and cannot be cultured using routine microbiological techniques. This process is time consuming and labour expensive. An extensive research is required to make pure cultures at reasonable time and cost.

INOCULATION PROCEDURES FOR NURSERIES

I. Broadcast Inoculations

This involves spreading a known quantity of mycorrhizal inoculum over a given area of soil surface and then mixing the inoculum into the soil to a depth of 10-20 cm before seeding. Several inocula *viz.* duff (consists of mycorrhizal tree roots and fungal propagules), sporocarps and spores, and pure culture vegetative mycelium have been applied in this manner to obtain mycorrhizal seedling in nurseries.

II. Banding of Inoculum Below Seeds

This involves placing the inoculum below the seed in a layer or band. This facilitates concentration of inoculum near developing roots. The major advantage of this method is that it requires only one third as much inoculum as the broadcast method. In addition, it saves time and labour. The only disadvantage being the need of an additional machine.

III. Slurry Dips

Slurries of mycorrhizal inoculum can be prepared by mixing the mycorrhizal inoculum with water, and a carrier such as clay or soil. The seedlings are inoculated by dipping them into the slurry prior to planting.

MYCORRHIZAL INOCULUM TECHNOLOGY

Successful production of mycorrhizal seedlings is dependent upon type and age of inoculum used, time of inoculation, inoculum density, inoculum placement and a number of host and fungus interactions. The mycorrhizal inocula consist of soil inoculum, mycorrhizal seedlings and roots, sporocarps and spores, and pure cultures of mycorrhizal fungi.

Seedlings can be inoculated at three different stages *viz.*, i. before seeds are sown, ii. When seeds are sown and, iii. After seedlings emerge. The most efficient stage would be to inoculate the seedlings before or when the seeds are sown. An efficient time to inoculate cuttings is at the time of propagation. It also depends upon the economic considerations and on the ecology of mycorrhizal fungi. As regards to economics, the stage when the seeds are sown or cuttings are propagated requires least amount of inoculum per volume of growing medium. Again, the newly developed rootlets of seedlings or cuttings are receptive to mycorrhizal colonizations, as they are non-lignified. The mycorrhizal fungi are also known to increase the rooting in cuttings and increase the root developments during propagation. It is also cost efficient to develop a mechanical inoculation system for use when seeds are sown or cuttings are propagated than any other later stages. Inoculation at the time of transplantation is time consuming, requires more inoculum, and the introduced fungi must be compatible with the native microorganisms and climatic conditions of the planting site.

ROLE OF AM FUNGI IN RECOVERY OF MINE WASTELANDS

Nicolson (1967) suggested that plant growth in industrial waste could be improved by incorporating AM fungi. Khan (1978) reported similar results for Australian coal spoils, noting that some members of Proteaceae were successful non-mycorrhizal invaders. However, species vary in their degree of dependency on mycorrhizal endophytes. Janos (1980) has explained that during succession, three main types characterize a range of ecological dependency : non-mycotrophs, facultative mycotrophs and obligate mycotrophs. In this case, obligate mycotrophs could fail to become established in sites of vary low inoculum density and may only become established after endophytes have colonized the area. If this is so, then these organisms are determinants of community composition during early succession and they may in part, control the progress of succession (Reeves *et al.*, 1979).

It is seen that iron ore mine rejects are poor in nutrients as indicated in Table 1. In a survey conducted at Sanquelim iron ore mines belonging to M/s Sesa Goa Limited, all the herbaceous plants growing on a 12 year old reject dump showed AM fungal colonization (Rodrigues & Bukhari, 1997) (Table 4). In all, a total of 27 species of AM fungi belonging to five genera were recorded from the iron ore mines (Rodrigues, 2000) (Table 5) (Plate I). Glasshouse studies conducted to evaluate effect of two AM fungal species (*Glomus mosseae* Nicolson and Gerdemann and *Glomus fasciculatum* (Thaxter *sensu* Gerdemann) Gerdemann & Trappe) on biomass of nine tree species grown on mine

Table—4: Degree of root colonisation (%) in some naturally occurring herbaceous plant species of iron ore mine wastelands of Goa. (Rodrigues and Bukhari,1997a)

Sr. No.	*Plant species*	*Family*	*Degree of root colonization %)*	*Type of colonization*
1.	*Lygodium flexuosum* (L.)Swartz.	Schizaeaceae	72	H A
2.	*Polygala elongata* Klein ex Willd.	Polygalaceae	10	H A
3.	*Impatiens Kleinii* W.& A.	Balsaminaceae	81	H V
4.	*Hydrocotyle asiatica* L.	Apiaceae	42	H A V

(Contd...)

5.	*Nanotis foetida* Benth. & Hook.	Rubiaceae	79	H	V	
6.	*Spermacoce hispida* L.	Rubiaceae	37	H	V	
7.	*Blumea mollis* (D. Don) Merr.	Asteraceae	87	H	A	
8.	*Parthenium hysterophorus* L.	Asteraceae	81	H	V	
9.	*Vernonia cinerea* (L.) Less.	Asteraceae	11	H	V	
10.	*Canscora diffusa* (Vahl.) R.Br.	Gentianaceae	40	H	V	
11.	*Merremia tridentata* (L.) Hallier f .	Convolvulaceae	96	H	A	
12.	*Lindernia crustacea* (L.). F.Muell	Scrophulariaceae	90	H	A	
13.	*Lindernia parviflora* (Roxo.)Haines	Scrophulariaceae	60	H	A	
14.	*Ramphicarpa longiflora* Benth.	Scrophulariaceae	36	H	V	
15.	*Striga asiatica* (L.) Kuntze	Scrophulariaceae	93	H	V	
16.	*Centranthera hispida* R.Br.	Scrophulariaceae	56	H	V	
17.	*Justicia procumbens* L.	Acanthaceae	23	H	A	V
18.	*Gomphrena celosioides* C.Martius	Amaranthaceae	62	H	A	
19.	*Amorphophallus commutatus* Engler	Araceae	74	H	V	
20.	*Eriocaulon cinereum* R. Br.	Eriocaulaceae		29	H	V
21.	*Eragrostis amabilis* W & A.	Poaceae	29	H	V	
22.	*Heteropogon contortus* (L.) P. Beauv. Ex Roeme & Schultes	Poaceae	30	H	A	
23.	*Ischaemum semisagittatum* Roxb.	Poaceae	67	H	V	

Legend: H = Hyphae; A = Arbuscules; V = Vesicles.

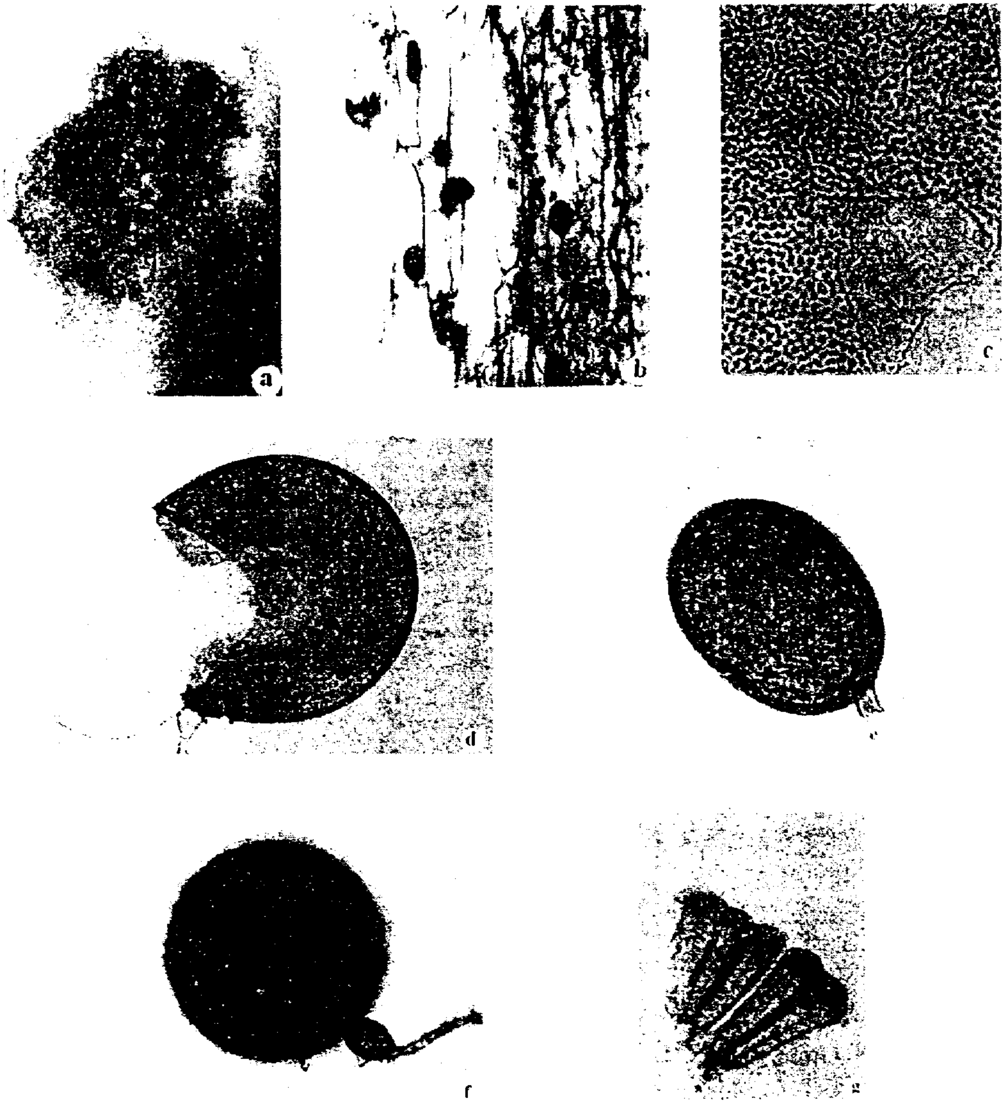

Plate I: a. Arbuscular Colonization (400x); b. Vesicular and hyphal Colonization (100x)
c. Surface ornamentation in *Acaulospora spinosa* spore (400x);
d. Spore of *Gigaspora margarita* (100x);
e. Spore of Glomus macrocarpum (400x);
f. Spore of *Scutellospora gregaria* (100x), and
g. Chlamydospores of *Sclerocystis taiwanensis* (400x)

rejects, revealed that the inoculated plants performed better than the uninoculated controls (Rodrigues, 1997). It was also noted that out of the nine tree species, *Glomus mosseae* Nicolson and Gerdemann appeared to be the best mycorrhizal inoculum for eight tree species (Table 6).

Table—5: Diversity of AM species found on iron ore mine reject dumps of Goa. (Rodrigues, 2000).

Sr. No.	*AM species*	*Sr. No.*	*AM species*
1.	*Glomus geosporum*	16.	*Glomus deserticola*
2.	*Glomus mosseae*	17.	*Gigaspora albida*
3.	*Glomus fasciculatum*	18.	*Gigaspora margarita*
4.	*Glomus hoi*	19.	*Gigaspora candida*
5.	*Glomus aggregatum*	20.	*Acaulospora nicolsonii*
6.	*Glomus australe*	21.	*Acaulospora spinosa*
7.	*Glomus reticulatum*	22.	*Acaulospora bireticulata*
8.	*Glomus clarum*	23.	*Acaulospora laevis*
9.	*Glomus constrictum*	24.	*Acaulospora morrawae*
10.	*Glomus caledonium*	25.	*Acaulospora foveata*
11.	*Glomus radiatum*	26.	*Acaulospora mellea*
12.	*Glomus etunicatum*	27.	*Scutellospora gilmorei*
13.	*Glomus albidum*		
14.	*Glomus monosporum*		
15.	*Glomus globiferum*		

Table—6: Effect of AM fungal species on dry weight (g) on various plant species grown on iron ore mine rejects. (Rodrigues, 1997).

Plant species	*Total dry wt. (g)*		
	Control	*G. F.*	*G. M.*
Delonix regia	1.48 (0.10)	2.28(0.14)	4.05(0.31)
Tamarindus indica	1.68 (0.06)	1.93(0.43)	2.54(0.25)
Acacia farnesiana	0.22 (0.03)	0.64(0.06)	1.00(0.13)
Acacia mangium	0.12 (0.00)	0.35(0.03)	0.38(0.03)
Adenanthera pavonina	0.82 (0.09)	1.37(0.10)	1.56(0.06)
Albizzia lebbeck	0.24 (0.02)	0.84(0.17)	0.86(0.12)
Leucaena leucocephala	0.31 (0.03)	0.74(0.05)	0.75(0.09)
Samanea saman	0.44 (0.07)	0.89(0.10)	2.36(0.26)
Ziziphus jujuba	0.35 (0.04)	0.78(0.07)	0.43(0.07)

Legend:

Control = Pure reject; G.F. = *Glomus fasciculatum* & G.M. = *Glomus mosseae* Mean of 5 replicates. Figures in the brackets denote Standard deviation values.

CONCLUSION

"Mine land is a fascinating challenge because the pre-existing ecosystems are extinguished". It is challenge to the biologists and engineers to replace them as they were. It is also a challenge to the soil scientists, ecologists and to the agriculturists to reconstruct an ecosystem from nothing at minimal cost.

Mine rejects are not true soils but are derived mostly from crushed bedrock and /or glacial deposits hence they are low in nutrients. In this relation, the role of microorganism in rehabilitation has received little attention than correction of nutritional deficiencies and imbalances, toxicity, moisture deficits and wind erosion.

Future revegetational research has to be oriented towards:

- Developing methods of maintaining inoculum level in soil.
- Developing techniques for introducing the endophytes in the soil.
- Naturally colonizing plant species should be given preference while considering revegetation strategies. Seedlings of such plant species should be inoculated with AM fungi in nursery stages and than transplanted to the target site. This would enhance plant growth and survival in the inhospitable sites.

In addition to this, alternative strategies such as reducing the angle of slope of reject dumps through terracing in order to improve water holding capacity, addition of organic materials like sewage sludge, sea weeds, green manure *etc.*, would help to elevate the soil status and enhance mycorrhization in spoils. Removal and storage of topsoil for reuse would make reestablishment of vegetation relatively easier as topsoil contains organic matter, plant nutrients, seed propagules and useful microbes. This would also lead to increase the inoculum potential of AM fungi thereby helping in plant growth and survival.

Thus mining industry need not lead to degradation of environment if those who are involved in such programmes apply a combination of imagination, care and scientific skill.

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