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Arbuscular Mycorrhizal (AM) fungal diversity of degraded iron ore mine wastelands of Goa.

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Abstract
Arbuscular mycorrhizal (AM) colonization and spore count was assessed in 50 plants species from Codli iron ore mine site. Most of the plant species assessed had low to high level of colonization and spore density. Mycorrhizal colonization in herbs ranged from 8% in *Biophytum sensitivum* to 99% in *Cassia tora*. Average root colonization in herbs, shrubs and trees was 50.25%, 50.25% and 47.43% respectively. Spore density exhibited great variations among various plant species ranging from 9 spores $100g^{-1}$ rhizosphere soil in *Canscora diffusa* to 396 spores $100g^{-1}$ rhizosphere soil in *Vernonia cinerea*. Average spore density recorded was higher in shrubs (122 spores $100g^{-1}$ soil) followed by herbs (102.5 spores $100g^{-1}$ soil) and was least in trees (98.87 spores $100g^{-1}$ soil). Arbuscular mycorrhizal fungal spore numbers were related to colonization ($r=0.534$; $P<0.01$). A total of 40 AM fungal species belonging to four genera *viz*., *Acaulospora, Gigaspora, Glomus* and *Scutellospora* were isolated during the study. *Glomus* was the most dominating genus followed by *Scutellospora*, *Acaulospora,* and *Gigaspora*. In the present investigation, spores of *Glomus microaggregatum* Koske & Gemma inhabited the spores of *Glomus macrocarpum* Tul. & Tul. and, spores and sporocarps of *Glomus sinuosum* with numbers varying from 1 to 10. The inhabited spores were found to be fresh, while the occupied spores were old and devoid of spore contents.

Key words: Arbuscular mycorrhizal (AM) fungi, spore density, iron ore mine.

Introduction
Mining is one of the most degrading actions of man on the earth as it physically tears up the earth's surface, producing gaping holes and barren heaps, changes the geomorphic pattern and contaminates the environment. Surface mining disrupts mycorrhizal population thus leaving minimal levels of endophyte inoculum (Reeves *et al*., 1979; Allen and Allen, 1980). Natural re-establishment of vegetation on dry, nutrient poor abandoned mined land is a slow process even when plant propagules are available. To facilitate natural succession and to reclaim the drastically disturbed sites that lack topsoil, low-cost techniques of establishing vegetation must be developed. A more economical and long lasting alternative is to reintroduce mycorrhizal fungi adapted to local natural vegetation on the sites identified for revegetation (Parkinson, 1978).

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Earlier studies have dealt with the role of AM fungi in reclaimed and disturbed soils (Aldon, 1978). The occurrence of AM fungi in mine spoils has been reported earlier (Ponder, 1979; Zak and Parkinson, 1982; Waaland and Allen, 1987). The occurrence of AM fungal association in herbaceous plants growing in mine spoils has been documented by Daft and Nicolson, (1974). Similarly, Allen and Allen (1980) reported mycorrhizal colonization in plants growing on reclaimed strip mine in Wyoming. These investigations stress the importance of AM association in allowing successful recolonization, establishment, and growth of herbaceous plant species on disturbed sites.

Arbuscular mycorrhizal fungi by virtue of their symbiotic associations with roots of most vascular plants are among the most significant microbes in terrestrial ecosystems. They offer good scope for their use in plant growth improvement because of their nutrient mobilization capacity and moisture retention capacity. Mycorrhizae are not only more efficient in utilizing available nutrients from the soil (Bowen and Smith, 1981), but also are involved in transfer of nutrients from components of soil minerals and organic residues to solution and in nutrient cycling in an ecosystem (Jeffries and Barea, 1994).

Arbuscular mycorrhizal fungi are sometime reported to be an important associate of many pioneer plants, which may require AM colonization in order to survive on disturbed lands (Jehne and Thompson, 1981). They are particularly useful in detoxifying heavy minerals by chelation (Khan *et al.,* 2000).

Studies related to the diversity of AM fungi from iron ore mines are very scarce (Rodrigues, 1999; Sastry and Johri, 1999). Hence, the present investigation was carried out with an aim to study the colonization and diversity of native AM fungal species in the rhizosphere soils from iron ore mine wastelands at Codli Goa.

Materials and Methods

Study site: Codli a 30-year-old mine, situated in Sanguem, South Goa ($15^0$20'53"N Latitude and 74^0 8'33"E Longitude) is spread over an area of 300 ha. with an actual area of 290 ha. presently under mining.

Soil Analysis: For soil analysis, mine reject samples were collected from a depth of 0-25cm from 5 different locations of Codli mine and were brought to the laboratory in polyethylene bags. Samples were passed through 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, the composite sample was processed three times to get the mean value.

Soil pH was measured after dilutions with distilled water (1:1 w/v soil: water) soon after the samples were brought to the laboratory. Electrical Conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). Total nitrogen was determined by micro-Kjeldahl method (Jackson, 1971). Total phosphorus was determined

by molybdenum blue method (Jackson, 1971). Total potassium was determined by Flame photometric method (Jackson, 1971). Total calcium and magnesium were determined by titrimetric method. Organic carbon was analyzed by Walkley and Black's rapid titration method (Jackson, 1971).

Sampling: Fifty-five plant species, which includes ferns, herbs, shrubs and trees belonging to 29 families, were taken up in the present study. For shrubs and trees, the roots were dug and traced back to plant, which ensured that the roots belonged to the intended plant species. Root samples of herbs were collected by uprooting the entire plant. In case of bulbs, tubers and corms, the plants were uprooted along with the bulbous portion and the finer roots. Rhizosphere soils samples were collected from a depth of 15-20cm in polyethylene bags and were brought to the laboratory and were stored at 4^0C till further processing.

Assessment of Arbuscular Mycorrhizal (AM) colonization and spore density: The roots were freed from the adhering soil, gently washed and cut into 1cm segments. Later the root bits were cleared with 10% KOH, acidified with 1N HCl and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970) and were left overnight for staining. Percentage of root colonization was carried out by using slide method (Giovannetti and Mosse, 1980). In case of plants with storage organs, bulbs, corms and tubers, besides staining roots, freshly collected bulbs, corm, and tubers were also examined for arbuscular mycorrhizal colonization after staining. In case of ferns, the root bits were cleared with 2.5% KOH (Koske and Gemma, 1989), acidified with 5N HCl and stained with 0.05% trypan blue.

Hundred grams of rhizosphere soil sample was taken from each plant and assayed for spore count using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Estimation of spore density was carried out as per the procedure given by Gaur and Adholeya, (1994). Intact spores were picked up using a wet needle and were mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983) on a glass slide for identification.

Identification of Arbuscular mycorrhizal (AM) fungal species: Intact and crushed spores in polyvinyl alcohol lacto-glycerol (PVLG) with or without Melzer's reagent were examined under Leica compound microscope. Taxonomic identification of spores to species level was based on spore morphology, ornamentation, wall characteristics using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al*., 2000; Morton and Redecker, 2001; Schubler *et al*., 2001) by matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu). Spore colour was examined under Leica stereomicroscope using intact spores immersed in water.

Spores in the rhizosphere soil were multiplied using *Eleusine coracana* (L.) Gaertn*, Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus* sp. as host plants. The spores isolated from the trap cultures were later used for confirming the identified spores recovered during the study period.

Plant Identification: Plants collected in the present study were identified using floras (Rao, 1985 & 1986; Matthew, 1991; Mohanan and Henry, 1994; Naithani *et al*., 1997).

Statistical analysis: The data on AM colonization was arcsine square root transformed and spore numbers were log-transformed prior to statistical analysis. Pearson's correlation was used to understand the relationship between root colonization and spore density. Frequency of occurrence was calculated by using the formula given below.

$$\text{Frequency (\%)} = \frac{\text{Number of samples in which AM species occurred}}{\text{Total number of samples studied}} \times 100$$

Results and Dicussion

Soil analysis results revealed that mine rejects were slightly acidic with pH of 6.06 and Electrical Conductivity (EC) of 0.11 mmho cm^{-1}. The results revealed that the rejects were deficient in Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), and Organic carbon (Table 1). Electrical Conductivity (EC) was very low indicating that there is no likelihood of salinity problems. The pH of the reject was slightly acidic, thus posing no problems for plant growth. Similar observations have been recorded earlier (Rodrigues *et al*., 1997).

Table 1: Soil characteristics at Codli iron ore mine site.

Parameter	Values
pH	6.06 ± 0.054
Electrical Conductivity (EC)	0.11 ± 0.01
Nitrogen (mg 100 g^{-1})	52.6 ± 3.22
Phosphorus (mg 100 g^{-1})	124 ± 5.47
Potassium (mg 100 g^{-1})	48.8 ± 1.30
Calcium (mg 100 g^{-1})	6.17 ± 0.54
Magnesium (mg 100 g^{-1})	1.596 ± 0.57
Organic carbon (%)	0.22 ± 0.01

Values are mean of five readings.
± - Indicates Standard dviation.

Arbuscular mycorrhizal colonization was recorded in all the plant species examined in the study. However, the extent of colonization exhibited variations (Table 2).

The mycorrhizal colonization was characterized by intraradical and extramatrical hyphae, intracellular hyphal coils, inter- or intra-cellular vesicles and/or arbuscules. In ferns, root colonization ranged from 15% (*Lygodium flexuosum*) to 30% (*Adiantum philippense*). Mycorrhizal colonization in herbs ranged from 8% (*Biophytum sensitivum*) to 99% (*Cassia tora*). Among the shrubs, percent root colonization ranged from 20% (*Lantana camara*) to 75% (*Calotropis gigantea*). Among tree species root colonization ranged from 15% (*Acacia mangium*) to 75% (*Samanea saman*). Average root colonization in herbs, shrubs and trees was 50.25%, 50.25% and 47.43% respectively.

Spore density exhibited great variations among various plant groups (Table 2). Spore density in herbs ranged from 9 spores $100g^{-1}$ in *Canscora diffusa* to 396 spores 100 g^{-1} rhizosphere soil in *Vernonia cinerea*. Among the shrubs studied, the spore density ranged from 20 spores $100g^{-1}$ rhizosphere soils in *Lantana camara* to 168 spores $100g^{-1}$ rhizosphere soil in *Ricinus communis*. Among the 15 tree species studied, the spore density ranged from 20 spores $100g^{-1}$ soil in *Leucaena leucocephala* to 200 spores $100g^{-1}$ rhizosphere soil in *Anacardium occidentale*. Average spore density recorded was higher in shrubs (122) followed by herbs (102.5) and was least in trees (98.87) with spore number given in parenthesis.

Variations in spore number have been reported earlier by Kruckelmann, (1975) who found significant differences in spore number in six different plant species growing in monoculture for sixteen years. The influence of host plant on incidence of AM fungi has also been observed by Schenck and Kinloch, (1980) on a woodland site newly planted with six agronomic crops and grown in monoculture for seven years. Hayman (1975) and Iqbal *et al*., (1975) recorded difference in spore numbers between plant species.

The variations in extent of mycorrhizal colonization among different plant species observed confirm earlier findings of Manjunath and Bagyaraj, (1982), who stated that the extent to which plants respond to AM colonization varies with plant species. Gerdemann, (1965) has shown that the colonization pattern of AM fungal species can be distinctly different in various plant species. According to Tommerup (1992) the fungi vary in their colonization patterns due to differences in rate of intra-radical growth, amount of hyphae per entry point, and growth of external mycelium along roots before entry points is formed. Similarly, Muthukumar and Udaiyan (2000) in their studies on arbuscular mycorrhizas of plants growing in Western Ghats region of Southern India reported variation in colonization levels in various plant species.

In ferns, the morphology of the colonization process is very much similar to that found in angiosperms. However, the colonization is not ubiquitous and seems to be dependent on the systematic position of the fern species (Zhi-Wei, 2000). Boullard (1959) suggested that a close correlation exists between AM fungal colonization and fern evolution.

The present study indicated a positive ($r=0.534$, $P<0.01$) correlation between spore number and root colonization. Forty arbuscular mycorrhizal fungal species were recovered from the rhizosphere of 55 plant species from iron ore mine wastelands at Codli. The arbuscular mycorrhizal fungal species recorded belonged to four genera *viz.*, *Acaulospora, Gigaspora, Glomus* and *Scutellospora* [Fig. 1(a-e)].

Among the ferns examined, maximum AM fungal species were found in the rhizosphere of *Lygodium flexuosum* (6) followed by *Adiantum philippense* (4) and *Selaginella tenera* (3) with the number of AM species given in parenthesis. Arbuscular mycorrhizal fungal species richness in other herbs ranged from 2-9 per plant. Maximum AM fungal species were recorded in *Chromolaena odoratum* (9) and *Mimosa pudica* (9) while minimum were observed in *Neanotis foetida* (2) with the number of AM fungal species given in parenthesis (Table 2).

Among the shrubs, maximum AM fungal species were recorded in *Calotropis gigantea* (8) and minimum was recorded in *Lantana camara* (4) and *Ricinus communis* (4). In tree species, maximum AM fungal species were recovered from *Terminalia paniculata* (8) and minimum AM fungal species were recovered from *Casuarina equisetifolia* (4)*, Leucaena leucocephala* (4) and *Trema orientalis* (4) with the number of AM fungal species given in parenthesis. In the present study *Glomus* was the most dominating genus followed by *Scutellospora*, *Acaulospora,* and *Gigaspora* (Fig. 2). The existence of a positive correlation between spore number and root colonization suggests that factors that influence root colonization also influences sporulation (Brundrett, 1991).

The occurrence of AM fungal colonization and AM fungal spores in non-root underground parts of some plant species suggest that the arbuscular mycorrhizal fungi besides colonizing the plant roots can also colonize bulbs, corms and tubers. The results are in agreement with Rama Bhat and Kaveriappa (1997) who described the association of arbuscular mycorrhizal fungi in the tubers of *Colocasia esculenta* (L.) Schott as 'Mycotuber'. Similar observations were made in bulbs of *Allium sativum* L. (Kunwar *et al*., 1999) and in tubers of *Puereria tuberosa* (Wild) (Rodrigues, 1996).

In the present study, the absence of arbuscules in some plant species suggests that the hyphal coils may serve the function of arbuscules. The results are in agreement with Mago *et al.,* (1992) who have reported absence of arbuscules in Bryophytes. Among the various families, highest root colonization and spore density was reported from members of Leguminosae followed by Asteraceae as compared to other families. Legumes are generally known to be highly dependent on AM association, which are mainly implicated to the higher phosphorus demand for nodulation and nitrogen fixation (Smith and Daft, 1977; Carling *et al.,* 1978).

Leguminous plants by virtue of their dual symbiotic association that usually results in the fixation of nitrogen and uptake of available phosphorus are successful as pioneer colonizers, due to their ability to compensate for the infertility of the habitat

Table 2: Status of arbuscular mycorrhizal fungi from iron ore mine wastelands at Codli.

Family and Scientific name	*Spore density 100 g^{-1} soil	* Root colonization (%)	Identified AM fungal species
Acanthaceae			
Justicia procumbens L.	38 ± 4.58	48 ± 11.79	*Gi. margarita*, *G. intraradices*, *G . macrocarpum*.
Andrographis paniculata	55 ± 7.0	34 ± 3.61	*G. etunicatum*, *G. globiferum*, *G. taiwanensis*, *S. weresubiae*.
Amaryllidaceae			
Crynum vivipara var *viviparum*	90 ± 2.0	40 ± 7.94	*A. spinosa*, *A. undulata*, *G. fasciculatum*, *G. reticulatum*, *G. macrocarpum*, *G. sinousum*, *S. gregaria*.
Anacardiaceae			
Anacardium occidentale	200 ± 12	30 ± 2.66	*A. spinosa*, *G. constrictum*, *S. gregaria*, *S. pellucida*, *S. reticulata*
Araceae			
Amorphophallus commutatus	34 ± 3.61	20 ± 2.00	*G. geosporum*, *G. macrocarpum*, *G. monosporum*, *G. taiwanensis*.
Asclepidiaceae			
Calotropis gigantea	80 ± 6.24	75 ± 7.0	*A. laevis*, *A. scrobiculata*, *Gi. rosea*, *G. constrictum*, *G. fasciculatum*, *S. gregaria*, *S. pellucida*, *S. reticulata*.
Hemidesmus indicus	29 ± 4.36	34 ± 5.57	*Gi. margarita*, *G. etunicatum*, *G. fasciculatum*, *S. pellucida*.
Asteraceae			
Ageratum conyzoides	110 ± 5.57	39 ± 3.46	*Gi. margarita*, *A. scrobiculata*, *G. constrictum*, *G. rubiforme*, *G. sinuosum*, *G. taiwanensis*, *S. gregaria*.
Chromolaena odoratum	200 ± 7.0	53 ± 8.89	*A. scrobiculata*, *Gi. margarita*, *G. etunicatum*, *G. geosporum*, *G . macrocarpum*, *G. rubiforme*, *G. sinuosum*, *G. taiwanensis*, *S. gregaria*.

Vernonia cinerea	396 ± 4.25	66 ± 8.89	*A. spinosa*, *Gi. margarita*, *Gi. decipiens*, *G. constrictum, G. taiwanensis*.
Tricholepis glaberrima	350 ± 2.12	80 ± 5.57	*G. constrictum*, *G. dimorphicum*, *G. dominikii*, *G. fasciculatum*, *G. geosporum*.
Emilia sonchifolia	65 ± 2.65	94 ± 2.0	*G. constrictum*, *G . macrocarpum*, *G. sinuosum*, *S. gregaria*, *S. pellucida*, *S. werresubiae*.
Balsaminaceae			
Impatiens kleinii	98 ± 7.6	9 ± 1	*A. spinosa*, *G. fasciculatum*, *G. claroideum*, *G. monosporum.*
Caesalpiniaceae			
Delonix regia	96 ± 5.57	60 ± 8.0	*A. bireticulata*, *A. scrobiculata*, *A. spinosa*, *G. geosporum*, *G. sinuosum*, *S. weresubiae.*
Tamarindus indica	108 ± 5.57	50 ± 6.56	*A. spinosa*, *G. constrictum*, *G. etunicatum*, *G. globiferum*, *G. claroideum*, *G. taiwanensis*, *S. reticulata*.
Peltophorum pterocarpum	96 ± 3.0	38 ± 3.61	*A. spinosa*, *A. undulata*, *Gi. albida*, *G . macrocarpum*, *G. sinuosum*, *S. pellucida*.
Cassia tora	86 ± 2.0	99 ± 1.0	*A. spinosa*, *Gi. margarita*, *G. fasciculatum*, *G. macrocarpum*, *G. taiwanensis*, *S. weresubiae*.
Casuarinaceae			
Casuarina equisetifolia	72 ± 9.64	18 ± 4.36	*A. laevis*, *A. scrobiculata*, *A. spinosa*, *G. sinuosum.*
Combretaceae			
Calycopteris floribunda	164 ± 7.81	57 ± 6.0	*G. fasciculatum*, *G. claroideum*, *S. gregaria*, *S. reticulata*, *S. pellucida*.
Terminalia paniculata	192 ± 9.85	67 ± 5.57	*G. geosporum*, *G. lacteum*, *G. microaggregatum*, *G. monosporum,G. multicaule*, *G. clavisporum*, *G. coremioides*, *S. pellucida*.
Terminalia crenulata	72 ± 2.65	65 ± 4.0	*A. scrobiculata*, *Gi. margarita*, *G. fasciculatum*, *G . macrocarpum*, *S. gregaria*.
Euphorbiaceae			
Euphorbia hirta	144 ± 5.29	90 ± 4.0	*A. spinosa* , *G. constrictum*, *G.*

			etunicatum, *G. globiferum*, *G. macrocarpum*, *G. sinuosum*, *G. taiwanensis*.
Phyllanthus simplex	70 ± 2.65	70 ± 3.61	*A. scrobiculata*, *G. geosporum*, *S. pellucida*, *S. weresubiae*.
Euphorbia thymifolia	96 ± 4.36	90 ± 5.0	*A. scrobiculata*, *A. spinosa*, *G. fasciculatum*, *G. mosseae*, *G. taiwanensis*,*S. reticulata*.
Ricinus communis	168 ± 6.24	46 ± 7.55	*A. delicata*, *G. geosporum*, *G. taiwanensis*, *S. pellucida*.
Fabaceae			
Crotalaria prostrata	24 ± 4.58	68 ± 2.65	*Gi. margarita*, *G. globiferum*, *S. pellucida*.
Pueraria tuberosa	30 ± 7.0	35 ± 3.47	*Gi. margarita*, *G. geosporum*, *G. intraradices*.
Smithia salsuginea	290 ± 6.24	88 ± 3.61	*Gi. margarita*, *G. fasciculatum*, *G. claroideum*, *G. clavisporum*, *G. taiwanensis*, *S. gregaria*, *G. sp.*
Gentianaceae			
Canscora diffusa	9.0 ± 1.7	10 ± 4.58	*A. scrobiculata*, *Gi. albida*, *G. etunicatum*, *S. reticulata*.
Lecythidaceae			
Careya arborea	132 ± 6.24	60 ± 4.36	*A. laevis*, *G. constrictum*, *G. formosanum*, *G. globiferum*, *G . macrocarpum*, *G. rubiforme*.
Lamiaceae			
Leucas aspera	15 ± 2.64	20 ± 3.0	*G . macrocarpum*, *S. gregaria*, *S. weresubiae*.
Ocimum tenuiflorum	184 ± 5.57	61 ± 4.36	*G . macrocarpum*, *G. geosporum*, *S. reticulata*, *S. pellucida*.
Malvaceae			
Sida acuta	128 ± 6.56	83 ± 5.0	*A. scrobiculata*, *G. constrictum*, *S. reticulata*, *G. taiwanensis*.
Sida cordifolia	38 ± 4.58	25 ± 5.29	*G . macrocarpum*, *G. constrictum*, *G. globiferum*, *G. geosporum*, *S. pellucida*.
Sida rhombifolia	280 ± 7.55	54 ± 3.61	*Gi. margarita*, *G. fasciculatum*, *G. mosseae*, *G. taiwanensis*.
Mimosaceae			
Acacia auriculiformis	148 ± 5.29	45 ± 3.61	*A. scrobiculata*, *A. spinosa*, *G . macrocarpum*, *S. weresubiae*.
Acacia mangium	102 ± 2.65	15 ± 5.0	*A. scrobiculata*, *A. spinosa*, *G.*

			geosporum, *G . macrocarpum*, *S. pellucida*.
Mimosa pudica	154 ± 8.54	76 ± 6.24	*Gi. margarita*, *G. globiferum*, *G . macrocarpum*, *G. rubiforme*, *G. taiwanensis*, *S. gregaria*, *S. reticulata*, *S. sp*., *G. sp*.
Leucaena leucocephala	20 ± 5.0	40 ± 3.0	*A. scrobiculata*, *Gi. margarita*, *S. gregaria*, *S. weresubiae*.
Samanea saman	116 ± 5.57	75 ± 7.81	*A. scrobiculata*, *G. fasciculatum*, *G. globiferum*, *G . macrocarpum*, *S. gregaria*, *S. pellucida*.
Moraceae			
Artocarpus heterophyllus	85 ± 3.61	46 ± 6.56	*Gi. margarita*, *A. scrobiculata*, *G. constrictum*, *G. rubiforme*, *G. taiwanensis*, *S. pellucida*.
Onagraceae			
Ludwigia parviflora	68 ± 6.08	70 ± 6.24	*A. scrobiculata*, *Gi. margarita*, *S. pellucida*, *S. weresubiae*.
Oxalidaceae			
Biopytum sensitivum	19 ± 3.0	8 ± 2.65	*A. scrobiculata*, *G. fasciculatum*, *G. globiferum*.
Poaceae			
Cynodon dactylon	64 ± 5.57	48 ± 6.24	*A. spinosa*, *G. claroideum*, *S. gregaria*, *S. reticulata*, *S. pellucida*.
Dactyloctenium aegyptium	140 ± 0.54	65 ± 5.29	*Gi. rosea*, *S. gregaria*, *S. reticulata*, *S. pellucida*, *S. weresubiae*.
Ischaemum semisagittatum	102 ± 5.0	86 ± 6.08	*Gi. decipiens*, *Gi. margarita*, *G. fasciculatum*, *S. nigra*.
Pteridaceae			
Adiantum philippense	15 ± 4.36	30 ± 5.57	*A. spinosa*, *G. mosseae*, *G. sinuosum*, *G. taiwanensis*.
Rubiaceae			
Neanotis foetida	112 ± 7.0	15 ± 2.65	*G . macrocarpum*, *A. undulata*
Solanaceae			
Physalis minima	24 ± 3.0	16 ± 5.29	*A. scrobiculata*, *A. spinosa* , *Gi. albida*, *S. gregaria*
Tiliaceae			
Microcos paniculata	160 ± 3.0	60 ± 6.0	*A. spinosa*, *G . macrocarpum*, *G. taiwanensis*, *S. pellucida*, *S. weresubiae*.
Ulmaceae			
Trema orientalis	44 ± 4.0	55 ± 3.61	*Gi. margarita*, *G. flavisporum*, *G.*

			multicaule, *S. reticulata*.
Verbenaceae			
Lantana camara	20 ± 1.0	20 ± 3.0	*A. spinosa*, *A. scrobiculata*, *G. geosporum*, *G. taiwanensis*.
Clerodendron viscosum	140 ± 4.0	52 ± 6.56	*G . macrocarpum*, *G. claroideum*, *G. clavisporum*, *G. taiwanensis*, *S. reticulata*.
Schizaceae			
Lygodium flexuosum	20 ± 5.29	15 ± 1.73	*A. spinosa*, *G. dimorphicum*, *G. etunicatum*, *G. lacteum*, *G. rubiforme*, *S. sp*.
Selaginellaceae			
Selaginella tenera	14 ± 4.36	20 ± 6.0	*Gi. margarita*, *G. geosporum*, *G. taiwanensis*.

* - Mean of three samples. ± Indicates Standard deviation.
Fungal Genera are abbreviated as: *A. - Acaulospora*, *Gi. - Gigaspora*, *G. - Glomus*, *S. - Scutellospora.*

Figure 2: Dominance of arbuscular mycorrhizal fungal genera from Codli iron ore mine site.

(Harley, 1970). A good number of legume species at Codli mine site is an indication of gradual improvement of the fertility status of the spoil because majority of them are found to be potential nitrogen fixers.

Present study revealed the occurrence of four arbuscular mycorrhizal fungal genera *viz.*, *Glomus, Acaulospora, Gigaspora* and *Scutellospora.* *Glomus* was the most dominant AM fungal genera associated with plants growing on nutrient deficient mined soils. Earlier Raman *et al*., (1993), identified *Glomus* and *Gigaspora spp*. in the mycorrhizospheres of 14 plant species colonizing a magnesite mine spoil in India. Whereas, Weissenhorn and Leyval (1995) isolated only *Glomus mosseae* and Duek *et al.,* (1986) isolated *Glomus fasciculatum* alone from the heavy metal polluted soils and, Pawlowska *et al*., (1996) surveyed a calamine spoil mound rich in Cd, Pb, and Zn in Poland and recovered spores of *Glomus aggregatum, Glomus fasciculatum* and *Enterophospora.* Predominance of the genus *Glomus* in the rhizosphere of plants growing on mine wastelands has also been reported by Lakshman, (1997), Sastry and Johri, (1999) and Uniyal, (2001).

Figure 3: Frequency of occurrence of some dominant AM fungal species from Codli iron ore mine site.

In the present investigation, spores of *Glomus microaggregatum* Koske & Gemma inhabited the spores of *Glomus macrocarpum* Tul. & Tul. and, spores and sporocarps of *Glomus sinuosum* with numbers varying from 1 to 10 [Fig. 1(f)]. The inhabited spores were found to be fresh, while the occupied spores were old and devoid of spore contents. Most frequently occurring AM fungal species recorded from Codli iron ore mine site are depicted in Fig. 3. Similar results have been reported by Koske *et al*., (1986) who reported the frequent occurrence of *Glomus microaggregatum* spores within the spores of other AM fungi.

Later, Almeida and Schenck (1990), and Muthukumar *et al*., (1993) have reported the occurrence of *Glomus microaggregatum* and *Glomus aggregatum* in the spores of *Glomus sinuosum*. Similarly, presence of *Glomus*-like spores within spores of *Glomus sinuosum* has been reported from Taiwan (Wu and Chen, 1993). Muthukumar and Udaiyan (1999) reported the occurrence of *Acaulospora*, *Glomus* and *Scutellospora* species within AM fungal species of *Gigaspora*, *Glomus* and *Scutellospora* in the Western Ghat regions, in Southern India.

Presence of AM fungal spores inside the dead spores of other AM fugal species suggests that spores of AM fungi act as a microhabitat when they are dead, apart from their normal role as propagules. This also suggests the ability of different AM fungal species to sporulate in close proximity with each other (Muthukumar and Udayan, 1999).

Recovery of large AM fungal diversity in the study site which accounts for nearly 30% of the total known AM fungi in the study site not only reveals the rich wealth of AM diversity sheltered in such stressful habitats but also indicates that extreme environments are the centers for evolution and conservation of biodiverse gene pool. These native isolates with the capacity to survive under stress conditions are instrumental in reclamation of disturbed sites. Thus, identification of the dominant native AM fungal species thriving on mine wastelands, their multiplication and proper utilization would make the re-establishment and regeneration attempts ecologically and economically viable in such constrained ecosystems.

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