**BIODIVERSITAS**
Volume 12, Number 2, April 2011
Pages: 107-111

ISSN: 1412-033X (printed edition)
ISSN: 2085-4722 (electronic)
DOI: 10.13057/biodiv/d120208

# Status and diversity of arbuscular mycorrhizal fungi and its role in natural regeneration on limestone mined spoils

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Manuscript received: 28 September 2010. Revision accepted: 14 December 2010.

## ABSTRACT

*Singh AK, Jamaluddin (2011) Status and diversity of arbuscular mycorrhizal fungi and its role in natural regeneration on limestone mined spoils. Biodiversitas 12: 107-111.* Limestone mined spoils are devoid of adequate population of beneficial microbial flora. Arbuscular mycorrhizal fungi (AMF) are very important constituent of plant- soil-microbe system. In mined spoils the population of AMF is greatly reduced and hence the spoils become very inhospitable for establishment of vegetation. In the present investigation, status of AMF population and its effect on natural regeneration process is studied. It is well known fact that the arbuscular mycorrhizal fungi play very important role in establishment of vegetation in degraded lands. Plantation of seedlings inoculated with arbuscular mycorrhizal fungi provide favorable soil conditions for naturally growing vegetation in the mined overburden spoils. Physico-chemical properties of soil are converted suitable for planted species and thus it allows other species to grow and also provide shade to protect the herbaceous vegetation. Introduction of plant species attracts immigration of other species and if they established, may result into a very distinctive floral cover on disturbed lands. Thus, invasion of native plant species along with planted species may play a significant role in increasing the plant diversity on mined spoils.

**Key words:** Arbuscular mycorrhizal fungi, diversity, inoculation, lime stone, mine spoils, natural regeneration.

## INTRODUCTION

Limestone mine sopils are generally hostile to plant growth. as these spoils are devoid of essential soil nutrients and beneficial microbial flora particlarly arbuscular mycorrhzal fungi (AMF). AMF are very important for the development of long term plant community structure. The absence of AMF may account for poor survival of plants on disturbed lands like mined out spoils. AMF play a crucial role in plant nutrient uptake, water relations, ecosystem establishment, plant diversity, and productivity of plants. Mycorrhizas also protect plants against root pathogens and toxic stresses. The fundamental importance of the arbuscular mycorrhizal association in restoration and to improve revegetation of disturbed mined lands is well recognized (Mukhopadhyay and Maiti 2009). Arbuscular Mycorhizal fungi have great potential in establishment and survival of plants under natural as well as stressed condition of mined out lands (Quoreshi 2008). The limestone mined spoils also exhibit reduced level of infectivity of AMF due to soil disturbance while AMF have been advocated for successful restoration of different mine spoils (Misra et al. 1990; Parihar 2007). Poor AMF population and reduced infectivity inhibits nutrients mineralization and immobilization, consequently the establishment of plants and ultimately affects the process of ecological succession. In the present investigation, an effort is made to study the status and diversity of AMF on limestone mined out spoils of differnt age groups. Study also endeavours to reveal the importance of AMF inoculation in natural regeneration calcarious mined out spoil material.

## MATERIALS AND METHODS

### Physico-chemical analysis of soil

Soil samples of overburden materials were collected from limestone mined overburden dumps of different age groups including 10 years old, 5 years old and 0 year old (fresh spoil) unvegetated dumps. The soil samples were carefully collected in polyethylene bags and their openings were tied with rubber bands. The soil samples so collected were used for the study of status of AMF, nutrients status and physico-chemical characteristics of limestone mined spoils of different ages soil. For the purpose of estimation of the concentration of trace elements (Ni, Pb, Mn, Zn, Fe, Cu and Co) in the limestone mines spoil, DTPA extraction method prescribed by Lindsay and Norvell (1978) was applied. Soil physico-chemical analysis was made by using methods of Black (1965).

### Isolation and identification AMF

Soil samples were collected and processed for isolation of AMF spores. To extract AMF spores, wet - sieving and decanting technique of Gerdemann and Nicolson (1963) was applied. Genera and species of AMF were identified on the basis of morphology of their resting spores by consulting taxonomic manual of Schenck and Perez (1990). In order to study the natural regeneration on limestone mined overburden dumps, quadrates of size 2 m x 2 m were laid randomly. Vegetational data were then quantitatively processed for frequency, relative frequency and abundance according to the formulae given by Curtis and McIntosh (1950).

**Statistical analysis**

The observation data were subjected to analysis of variance (ANOVA). One way analysis of variance was carried out to compare the means of different treatments. Means separation tests were performed using least significant difference (LSD) at the P<0.05 level after significant F values were obtained.

## RESULTS AND DISCUSSION

**Soil characteristics**

Table 1 reveals that there was a significant variation (p<0.05) in the available nitrogen content among the mined spoils of different age groups. Available nitrogen increased significantly with the age of spoils. Vimmersted et al. (1989) also reported an increase in nitrogen concentration in calcareous mined spoils. Several others workers such as Wali (1987) in North Dakota, Russel and La Roi (1986) in Alberta also recorded an increase in N with the ageing of the mined spoils. Jencks et al. (1982) also found increase in nitrogen concentration with age in coal mine spoils. Available phosphorus ($P_2O_5$) concentration also followed the same trend with maximum concentration in 10 years old spoils, which was followed by 5 years old spoils. The concentration of available phosphorus was minimum in 0 years old spoils. Available phosphorus concentration increased significantly (P<0.05) with the age of spoils. Available potassium ($K_2O$) estimated maximum in 10 years spoils while it showed its minimum concentration in 0 year old spoils. Banerjee et al. (2001) have also reported an increase in available phosphorus ($P_2O_5$) and potassium ($K_2O$) with the age of mined spoils. This increase was possibly due to increase in the vegetational cover due to natural succession of herbs, shrubs and a few tree species. The microbes in the rhizosphere of these plants enhanced these nutrients possibly through rock weathering and made available to the growing vegetation. The physico-chemical status of limestone mined spoils is presented in Table 2. The spoils of all age groups showed alkaline soil reaction. The pH was highest (8.5) in 0 year old over burden material and lowest (8.2) in 10 years old mined spoil. Similarly the electric conductivity (EC) also followed more or less the same trend. Organic matter was maximum in 10 years old mined overburdens and minimum in 0 year old (fresh) overburdens. Organic carbon content was also maximum in 10 years old spoil and minimum in 0 year old (fresh) spoil dump. It is notaable that this trend in increase of organic carbon was due to natural regeneration, which contributed litter thereby, raising the organic matter and organic carbon content in 10 years old dumps. Thus from the physicochemical characteristics it is evident that the spoil were alkaline in reaction, highly calcareous and nutritionally very poor. Percent $CaCO_3$ decreased with the age of mine spoils. It was highest (18.8%) in 0 year old spoil and lowest (7.3%) in 10 years old spoil. The plant roots and microbes in the rhizosphere may secrete certain secondary metabolites, which possibly dissolve the $CaCO_3$ in the older dumps. The concentration of different heavy metals in spoil of different age groups is presented in Table 3. The concentration of Ni, Pb, Fe and Cu decreased with the aging of the spoils while the concentration of Mn, Zn and Co increased with the age of spoil. All such variations may be due to interaction between plant roots of growing vegetation and the microbial succession occurring in the mined spoils. The increasing concentrations of heavy metals in mine spoils are considered to pose potentially serious hazards in the soil-plant system. Toxic level of concentration of heavy metals in mine spoil constitute an important threat for establishment and further growth, survival and development of plants on mine spoils.

**Table 1**. Status of N, P and K in limestone mined spoils of different ages.

| Age of spoils (year) | Available (kg/ha) | | |
|---|---|---|---|
| | N | P | K |
| 10 | $64.78^c$ | $14.92^c$ | $239.7^c$ |
| 5 | $62.12^b$ | $4.96^b$ | $188.0^b$ |
| 0 (Fresh spoil) | $32.80^a$ | $3.46^a$ | $173.9^a$ |
| CD (0.05) | 3.0968 | 1.0202 | 7.9767 |
| SE ± | 1.3429 | 0.44242 | 3.4591 |

**Table 2.** Physico-chemical properties of lime stone mined spoils of different ages.

| Properties | Spoil age | | |
|---|---|---|---|
| | 10 years | 5 years | 0 year |
| Organic matter (%) | 0.22 | 0.20 | 0.10 |
| Organic carbon (%) | 0.13 | 0.11 | 0.05 |
| $CaCo_3$ equivalence (%) | 7.3 | 9.2 | 18.8 |
| Exchangeable $Mg^{++}$ (me/100g) | 11.8 | 10.0 | 9.4 |
| Exchangeable $Ca^{++}$ (me/100 g) | 27.8 | 26.4 | 37.6 |
| pH Soil: $H_2O$ (1:5) | 8.2 | 8.4 | 8.5 |
| EC (mmhos/cm) | 0.12 | 0.15 | 0.19 |

**Status of arbuscular mycorrhizal fungi (AMF)**

The efforts for biological rejuvenation of mined spoils need the evaluation of the arbuscular mycorrhizal status of the spoil. Thus it becomes imperative to study the arbuscular mycorrhizal status of the mined spoil, so that further rehabilitation strategy can be formulated and thus the technology may be applied in the field. It investigates the occurrence and status of AMF occurring in limestone mined areas. It is evident from the Table 4 that the population of AMF spores increased with the age of overburden dumps which was in high frequency in the older dumps. It was observed that there was a considerable difference in the population of AMF spores in the soil of limestone mined overburden dumps of different age groups with a maximum spore density in 10 years old overburden dump. Chandra and Jamaluddin (1999) reported increase in spore densiy and diversity of AMF with increase in the age of mine spoil. They also explained relative spore density and distribution of different AMF species.They found the genera *Glomus* and *Acaulospora* dominant on spoil of all age groups.

**Relative AMF spore density and diversity**

The extent of indigenous AMF in lime stone mined spoil was determined. The distribution of AMF provides a generalization of the occurrence of AMF in the lime stone mined spoils of different age groups. The relative spore density provided a general idea about the AMF species, which reflect its tolerance and adaptability towards the calcareous soil and other edaphic conditions of mined spoil. AMF species belonged to the genera *Glomus* and *Acaulospora* were dominant as compared to others. This fact must be related to their sporogenous characteristics, i.e. *Glomus* and *Acaulospora* species usually take a short time to produce small spores, compared with the large spores of *Gigaspora* and *Scutellospora* species in the same environment (Nandakwang et al. 2008)

Relative spore density of different AMF isolated from lime stone mined overburden of different age group is presented in the Table 5. Ten years old spoil was represented by *Glomus mosseae* with maximum relative spore density of 11.2% of the total isolate of 10 years old spoil. *Glomus deserticola* scored second with 9.9% relative density. *Acaulospora scrobiculata* was found third with 8.5% relative spore density, which was followed, by *Acaulospora denticulata* (7.9%), *Gigaspora gigantea* (7.9%), *Glomus intraradices* (7.2%), *Scutellospora heterogama* (7.1%), *Scutellospora persica* (6.6%), *Acaulospora delicata* (6.6%), *Glomus arborense* (5.9%), *Gigaspora margarita* (5.9%), *Gigaspora rosea* (5.5%), *Glomus fasciculatum* (5.3%) and *Scutellospora verrucosa* (4.5%). *Acaulospora denticulata* and *Gigaspora gigantea* showed the same status of occurrence with 7.9% relative spore density. Likewise *Acaulospora*

**Table 3.** Status of trace elements in limestone mined spoils of different age group dumps

| Age of spoil (Year) | Concentration of trace elements (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ni | Pb | Mn | Zn | Fe | Cu | Co |
| 10 | 0.028 | 0.36 | 0.75 | 0.41 | 0.43 | 0.21 | 0.24 |
| 5 | 0.11 | 0.57 | 0.41 | 0.24 | 0.48 | 0.38 | 0.17 |
| 0 | 0.13 | 1.3 | 0.17 | 0.20 | 0.57 | 0.45 | 0.02 |
| CD (0.05) | 0.082363 | 0.037019 | 0.0065932 | 0.0087675 | 0.018891 | 0.0087367 | 0.029970 |
| SE ± | 0.029665 | 0.013333 | 0.0023747 | 0.0031578 | 0.0068041 | 0.0031467 | 0.010794 |

**Table 4.** Status of AM fungi in limestone mine spoils

| Age of spoil | AMF spore density ($100g^{-1}$ soil) | Genera of AMF |
|---|---|---|
| 10 years old | 760 | *Glomus, Acaulospora, Gigaspora, Scutellospora* |
| 5 years old | 540 | *Glomus, Acaulospora, Gigaspora* |
| 0 year old (fresh) | 240 | *Glomus, Acaulospora,* |
| Un-sterilized nursery soil | 850 | *Glomus, Acaulospora, Gigaspora, Scutellospora* |

**Table 5.** Relative spore density of different AMF species in different aged spoils

| AMF species | Relative AMF spore density (%) | | | |
|---|---|---|---|---|
| | 10 years old spoil | 5 years old spoil | 0 year old spoil (fresh) | Nursery soil |
| *Glomus* | | | | |
| *Glomus mosseae* | 11.2 | 12.0 | 10.0 | 7.1 |
| *Glomus deserticola* | 9.9 | 10.2 | 11.5 | 4.7 |
| *Glomus intraradices* | 7.2 | 9.2 | 8.3 | 8.2 |
| *Glomus fasciculatum* | 5.3 | 7.4 | 7.9 | 6.8 |
| *Glomus arborense* | 5.9 | 7.4 | 10.4 | 5.9 |
| *Acaulospora* | | | | |
| *Acaulospora denticulata* | 7.9 | 9.2 | 14.6 | 7.3 |
| *Acaulospora scrobiculata* | 8.5 | 10.4 | 20.0 | 5.9 |
| *Acaulospora delicata* | 6.6 | 9.2 | 17.5 | 7.6 |
| *Gigaspora* | | | | |
| *Gigaspora rosea* | 5.5 | 9.2 | ND | 8.2 |
| *Gigaspora margarita* | 5.9 | 8.5 | ND | 8.8 |
| *Gigaspora gigantea* | 7.9 | 7.4 | ND | 8.2 |
| *Scutellospora* | | | | |
| *Scutellospora persica* | 6.6 | ND | ND | 7.1 |
| *Scutellospora heterogama* | 7.1 | ND | ND | 7.6 |
| *Scutellospora verrucosa* | 4.5 | ND | ND | 6.5 |

**Table 6.** Natural regeneration in planted and unplanted area of limestone mined spoil

| Species | Planted area | | | | Unplanted area | | | |
|---|---|---|---|---|---|---|---|---|
| | Frequency (%) | Relative freq. (%) | Abundance | A/F | Frequency (%) | Relative freq. (%) | Abundance | A/F |
| *Phyllanthus niruri* | 60 | 19.35 | 1.8 | 0.0305 | 30 | 15.8 | 1.7 | 0.055 |
| *Tridax procumbens* | 50 | 16.12 | 1.8 | 0.036 | 40 | 15.8 | 1.5 | 0.037 |
| *Ocimum gratissimum* | 50 | 16.12 | 1.3 | 0.025 | 30 | 15.8 | 1.0 | 0.033 |
| *Argemone mexicana* | 60 | 19.35 | 2.2 | 0.037 | 40 | 21.09 | 1.5 | 0.037 |
| *Zizyphus mauritiana* | 20 | 6.45 | 1.0 | .05 | 20 | 10.5 | 1.0 | 0.05 |
| *Acacia nilotica* | 30 | 9.7 | 1.7 | 0.057 | 20 | 10.5 | 1.0 | 0.05 |
| *Parthenium hysterophorus* | 40 | 12.9 | 1.0 | 0.025 | 20 | 10.5 | 1.5 | 0.05 |

*delicata* and *Scutellospora persica* shared equal spore density of 6.6%.

Five-year old spoil was represented by *Glomus mosseae* which occurred with 12.0% of relative spore density followed by *Acaulospora scrobiculata* (10.4%), *Glomus deserticola* (10.2%), *Glomus intraradices, Acaulospora denticulata*, *Gigaspora rosea* (9.2% each), *Gigaspora margarita* (8.5%) while *Gigaspora gigantea*, *Glomus fasciculatum* and *Glomus arborense* occurred with minimum relative spore density of 7.4% (each). The occurrence of Genus S*cutellospora* was not detected.

In 0 year old (fresh spoil) spores of Genus *Gigaspora* and *Scutellospora* were not detected. *Acaulospora scrobiculata* exhibited maximum relative spore density, i.e. 20% which was followed by *Acaulospora delicata* (17.5%), *Acaulospora denticulata* (14.6%), *Glomus deserticola* (11.5%), *Glomus arborense* (10.4%), *Glomus mosseae* (10.0%), *Glomus intraradices* (8.3%) and *Glomus fasciculatum* with minimum relative density of 7.9%. 0 year old spoil was represented by Genus *Acaulospora.* The nursery soil harboured different species of genera *Glomus, Acaulospora, Gigaspora* and *Scutellospora,* which were fairly distributed.

**Natural regeneration in planted and unplanted area of mined spoil**

Plantation of suitable species accelerated the invasion of native herbaceous flora on mined spoils. In the present study experimental plantation of *Jatropha curcas*, *Pongamia pinnata*, *Ailanthus excelsa* and *Withania somnifera* was carried out. The planted seedlings were boosted up by inoculating a consortium of AMF. It was observed that the plantation of inoculated seedlings has remarkably accelerated the natural regeneration process in the planted area of the mined spoil. The pioneering species which occurred in the planted area of spoil were *Phyllanthus niruri, Tridax procumbens, Ocimum gratissimum, Argemone mexicana, Zizyphus mauritiana, Acacia nilotica* and *Parthenium hysterophorus* which established through successional process in mined spoil (Table 6). In planted area, the regeneration of *Phyllanthus niruri* and *Argemone mexicana* was recorded highest with frequency (60% each) and abundance value of 1.8 and 2.2 respectively which was followed by *Tridax procumbens, Ocimum gratissimum, Parthenium hysterophorus, Acacia nilotica* and *Zizyphus mauritiana.* In planted area the abundance value was recorded highest (2.2) for *Argemone mexicana.*The results in same trend were also recorded by Singh (2004). He observed higher density of herbaceous vegetation on naturally revegetated mine spoils as compared to unvegetated one.

In unplanted area, the occurrence of colonizing species was less frequent. *Tridax procumbens* and *Argemone mexicana* represented in higher frequency (40%) followed by *Phyllanthus niruri, Ocimum gratissimum, Zizyphus mauritiana, Parthenium hysterophorus* and *Acacia nilotica.* The abundance was recorded maximum (1.7) for *Phyllanthus niruri* which was followed by *A. mexicana, P. hysterophorus, T. procumbens, O. gratissimum, Z. mauritiana,* and *A. nilotica*. Though all the species occurred in both planted and unplanted area but frequency of occurrence and abundance of particular species were higher in the planted one. This is quite obvious that plantation played a catalytic role on immigration of surrounding species on mined out spoils. The plants which were pre-inoculated with arbuscular mycorrhizal consortia established well in the mined spoils and modified the soil characteristic, created favorable conditions for plants growth which led to start and accelerate natural regeneration process on spoils.

## CONCLUSION

Arbuscular mycorrhizal fungi due to their widespread occurrence and distribution constitute a significant part of every natural and cultivated ecosystem and play a major role in plant species diversity and survival. Distribution of AMF is related to soil and environmental condition and density of AMF species may vary from site to site. AMF play a pivotal role in the establishment and growth of plants under natural as well as stress conditions, particularly in nutrient deficient soils. The occurrence of different AMF species was expressed in terms of relative spore density. The occurrence and distribution of AMF varied with physico-chemical properties of soil. The density of spores also varied accordingly. In the present investigation although each species occurred in both planted and unplanted area but the frequency of their occurrence and abundance was greater in planted area as compared to unplanted area of the overburden dump. This is due to effect of plantation and inoculation of AMF to the planted species which promoted the colonizers for establishment. The AMF population in rhizosphere possibly contributed in the availability of nutrients needed by the growing vegetation. Thus, incoulation of AMF boosted up the growing seedlings and also altered the soil conditions which resulted into accelerated natural regeneration process on limestone mined out spoil. This also indicates that merely plantation on mine spoil does not guarantee the development of a well structured plant community but it needs a biological balance between above ground flora and below ground microbial flora for sustainability of vegetation on degraded lands.

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