

ASSESSMENT OF VAM SPORE DENSITY AND ROOT INFECTION FROM ALLUVIAL SOIL OF EASTERN PART OF RANIGANJ COALFIELD AREAS

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KEY WORDS

Mining
Vesicular-arbuscular
Mycorrhizal
Rhizospheres
Reclamation
Root colonisation
Nutrients

Received on :
07.03.2011

Accepted on :
15.06.2011

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ABSTRACT

The indices of occurrence of VAM are widespread in natural vegetation, but intensity of distribution and root infection varies among types of species, nature of edaphic factors of soil. In this study, we observed that the VAM spore density and their colonization also positively correlated with depth of soil profile ($p < 0.05$); available nitrogen, organic matter and available phosphorous. The most commonly genus of VAM spores found in the study area are *Glomus*, *Gigaspora*, *Acaulospora*, *Enterophospora* and *Sclerocystis*. *Glomus* and their size frequency distribution range from 50-75 μm and 75-100 μm . The highest number of VAM spore density/5 g of soil were found as 360.0 ± 12.5 and for VAM spore density/100g of soil were found as 702.0 ± 23.87 in mine 3. The highest number of VAM spore density was reported under the *Acacia auriculoliformis*. The percentage of root colonisation observed in seven native species are; *Dalbergia sissoo* (91%), followed by *Prosopis juliflora* (84%), *A. auriculiformis* (79%) and *A. scholaris* (64%), *Polyalthia longifolia* (58%), *Cassia seamea* (31%), and *Azardirachta indica* (21%). The studies revealed that vesicular-arbuscular mycorrhiza is essential to assess the self-sustaining ecosystem of soil quality and which should be used for amendments in mine degraded soils for reclamation and restoration process.

INTRODUCTION

Opencast coal mining generates a variety of mineral wastes that are brought to the surface and replace the original topsoil. Topsoil is an essential component for land reclamation in mining areas. It is seriously damaged if it is not mined out separately without being contaminated, eroded and protected. Systematic handling and storage practices can protect topsoil while in storage and after it has been redistributed onto the degraded area. Impact of surface coal mining on topsoil quality in Indian context has been described. Most of the opencast operations are working at 100 m depth, with some up to 160 m depth (Bose, 2003) which generate huge amount of overburden materials. Overburden materials are waste rocks removed by opencast mining operations and dump outside, which is known as OB dumps. The overburden dumps are unstable and will also become source of pollution. These mine degraded soils are a man-made habitat which also presents a wide range of problems for establishing and maintaining a vegetation cover (Maiti, 1997). The adverse physico-chemical properties tend inhabits soil forming process and plant growth. So, stabilization of mining waste through revegetation usually requires the use of topsoil management to ameliorate the physical and chemical properties of the waste and to provide a source of energy for the reestablishment of a microbial community. The various soil factors that affect the establishment of plants in mine degraded soils. Among these

the most significant are moisture availability, mineral composition, soil texture, the quantity and quality of SOM, microbial and enzyme activities, mineral nutrients and others such as polycations, etc. According to Tate *et al.* (1985) states that apart from soil physical and chemical properties, long term plant community stability on mine degraded soils relies upon the development of a functional soil microbial community. One group of soil microorganism important to the development of long-term plant community structure is mycorrhizal association (Barca *et al.*, 1992).

Mycorrhiza is formed by association between a plants root and a fungus and by far majority of vascular plants are involved in there association. Mycorrhizal fungi are known to affects growth of most plant species in mine degraded areas. Phosphorus, nitrogen, zinc, and copper are the most commonly reported elements whose uptake is enhanced by mycorrhiza in plants; however, acquisition of other mineral nutrients required for plant growth may also be enhanced. They increase phosphorous uptake, enhance uptake of other plants nutrients by root system and are beneficial in the biological nitrogen fixation of rhizobium, biological control of root pathogens and drought resistance. It is also emphasized by several scientists, the beneficial role of vesicular arbuscular mycorrhizae in mine spoils revegetation. Also, the mycorrhizal associations are essential to the colonization of nutrient-deficient soil heaps left after mining (Brundrett, 1991). There

Table 1: Mine details of Raniganj coalfield, India

Mining Properties	Sonepur Bazarai OCP	Khottadih OCP	Nakrakonda OCP
Geological Reserve (MT)	407.97	22.57	5.73
Mineable reserve (MT)	214.98	13.95	4.83
Rated capacity (mty)	3.4	1.0	0.30
Stripping ratio (m ³ /t)	4.72	4.8	5.4
Maximum depth of mine (m)	245	221	62
Minimum depth of mine (m)	61	58	21
Alluvial soil thickness (m)	13.90-17.20	3-16	3.50-12.2
OB removal (million m ³)	13.0	3.0	7.5

are evidences that VAM determine the rate of succession in mined land (Doerr *et al.*, 1984). Vesicular-arbuscular mycorrhizal (VAM) fungi are a major component of rhizospheric micro-flora in natural ecosystems, and play significant roles in the decomposition of soil organic matter, mineralization and cycling of plant nutrients (Beare *et al.*, 1997; Bagayoko *et al.*, 2000; Pare *et al.*, 2000). Vesicular-arbuscular mycorrhizal (VAM) fungi have been used as an amendment to alleviate stresses encountered by plants established on mine wastes. In highly disturbed areas such as the opencast mine waste sites, the absence of mycorrhizal fungi may account for the poor survival of plants used for stabilization. The occurrence of vesicular-arbuscular mycorrhizas is widespread in natural vegetation, but intensity varies among types of species, nature of edaphic factors (Maiti, 1997), and intensity of disturbance as in case of opencast mining.

The pattern of distribution and activity of VAM were varying both temporally and spatially (Vishnevetsky and Steinberger, 1997; Lorgio *et al.*, 1999). Temporal and spatial variability in the quantity and quality of available resources are generally thought to be responsible for variations in the distribution of soil micro-organisms. Edaphic factors that cause increases in the activity of soil micro-organisms include soil moisture (Braunberger *et al.*, 1996; Siguenza *et al.*, 1996), organic matter (Lorgio *et al.*, 1999) and nitrogen (Aziz and Habte, 1989; Lorgio *et al.*, 1999) and available phosphorous.

The aim of this study was to investigate the status of VAM spore density, size distribution frequency of VAM spores and root colonisation in different host plants species found in eastern part of Raniganj Coalfield. It is anticipated that this study will present a preliminary data on status of vesicular-arbuscular mycorrhiza is essential to assess the self-sustaining ecosystem of soil quality and which should be used for amendments in mine degraded soils restoration and for reclamation process.

Study area

Raniganj Coalfield constitutes an important coalfield of India where coal mining started as early as 1774 by British owned companies and by various other private coal-mining companies of Bengal. In 1974 all the working mines were regrouped for effective management and exploitation strategy and since then this region is known as Eastern Coalfield of Coal India Limited. Geographically this coalfield lies between latitudes 23°25' N and 23°50' N and longitudes 86°38' E and 87°20' E. However, major portion (79%) of this coalfield lies in the interfluvies of the Ajay and the Damodar rivers forming northern and southern boundary of the region, respectively. The northwestern margin is approximately marked by a north-

south flowing Barakar river; however, the Coalfield limit extends beyond this along the Khudiya nala upto Nirsra town. Similarly, the limit of the eastern margin of Raniganj Coalfield presently extends upto Durgapur town but according to a recent survey, as reported by Geological Survey of India, it extends upto Panagarh - Domra area *i.e.* 20 km east of present margin.

Geology, climate and vegetation

This Coalfield has a roughly rectangular geographical shape and is surrounded by Archaean rocks on all sides except in the eastpart of coalfield where the boundary is obscured due to alluvial cover. The general strike of the Lower Gondwana is nearly E-W in the western as well as in the eastern part but varies to NW-SE or even NNW-SSE in the central part. General dip of the formation varies between 30-20° southerly. Therefore, the oldest rocks are exposed along the northern margin and younger rocks outcrop successively as we proceed towards southern margin. Geologically the area constituted a part of the Indo-Gangetic alluvial plains and belongs to Eocene ages. Initially the soils of the study site were moderately alkaline at the lower depth with low permeability. Climatically this area is sub humid and tropical which has three seasons viz. summer, rainy and winter in succession. The annual rainfall is 1206 mm (2008) and means annual temperature is around 25.4°C. The maximum temperature often goes over 40°C in summer (May-June, 2008) and minimum temperature goes down below 10°C. The rainfall takes place during monsoon and may exceed 300 mm per month during the wettest months (June- September, 2008).

The native vegetation in and around the mining or non-mining areas is typically mixed dry deciduous forest with *shorea robusta*, *Terminalia tomentosa*, *Butea monosperma*, *Dalbergia sisso*, *Madhuca indica*, *Terminalia arjuna* and *Azadirachata indica*. During the rainy seasons, herbaceous vegetation rapidly covers the adjacent area of mine spoils and biomass peaks occur in late September or October.

Mine details

The three mines from where the samples were collected are mechanized opencast coal mines. The locations of the mines have been shown in (Fig. 1). The brief details of the mines are given in Table 1 and their latitude and longitude are given in Table 2.

Table 2: Latitude and longitude of different mines

Mine site	OCP Mines	Latitude	Longitude
Mine 1	Sonepur Bazarai	23° 41' 0.3" N	87° 13' 6" E
Mine 2	Nakrakonda	23° 43' 0" N	87° 17' 7" E
Mine 3	Khottadih	23° 43' 2" N	87° 13' 7" E

MATERIALS AND METHODS

Soil sampling and analysis

Soil samples were collected from freshly exposed alluvium benches in the aforesaid opencast mines of Raniganj Coalfield. Soil samples were collected from channels dug in the alluvium benches from different points at different depths. The soil samples were packed in pre-cleaned, acid treated and air-tight plastic bags and transferred to the laboratory for further processing and analysis.

From the rhizospheric of each tree species, one composite sample was collected by mixing five sub-samples and reduced the weight approximately to 0.5 kg by conning-quartering method. Five replicates were done for each tree species. For VAM infection study, fine feeder roots were carefully removed from each tree species, washed with water and fixed in FAA (13mL formalin, 5mL glacial acetic acid and 200mL 50% ethyl alcohol) immediately after collection (Reeves *et al.*, 1979). After collection, the samples were air dried at room temperature (30–35°C) and lightly crushed with a mortar and pestle and passed through a 200-micron mesh. One part was used for the physicochemical analysis of OB materials and other part used for enumeration of VAM spores.

Physical analysis

In the laboratory, soil sub-samples were air-dried at the room temperature (25°-30°C) for one week. The sub-samples were homogenized/gently crushed using an agitate mortar and

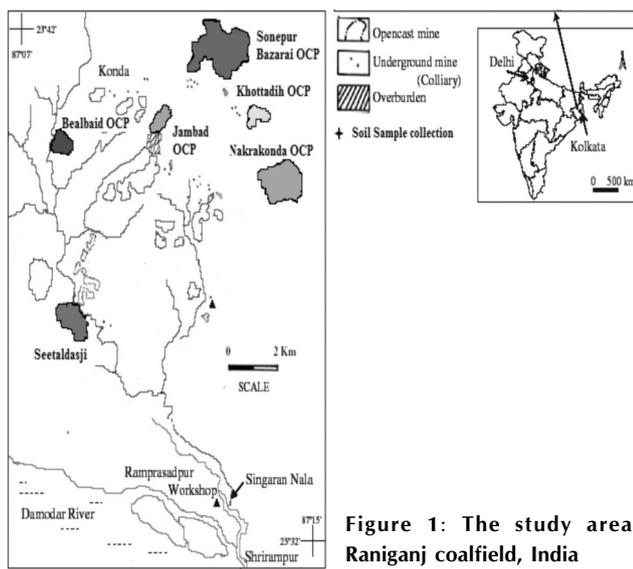


Figure 1: The study area, Raniganj coalfield, India

pestle and sieved through a standard sieve of 2 mm mesh size. The soil sub-samples with particle size of < 2 mm were divided in parts for further characterization. Samples were analyzed for particle size by sieving method and moisture content by gravimetric method.

Chemical analysis

The pH and electrical conductivity (EC) was determined in soil/water (1:2.5; w/v) suspension with a pH meter and Conductivity meter respectively. Organic carbon was estimated by rapid dichromate oxidation technique (Walkley

and Black, 1934), Available nitrogen was determined by the alkaline potassium permanganate method (Subbaih and Asija, 1956), available phosphorus by Bray's method (Bray and Kurtz, 1966), exchangeable K, was extracted by neutral 1(N) ammonium acetate solution (soil-to-extractant ratio of 1:10) and determined by Flame photometer (Jackson, 1973).

Microorganism evaluation

Study of VAM Spores

VAM spores occurring in the rhizospheric soil samples were extracted by Wet-sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). Approximately 50g of soil was taken in a 1000 mL measuring cylinder and tap water was added, mixed the content, and 30 minutes were given for settlement of heavy particles at the bottom. The suspension was decanted by using two sieves: 30-mesh (500 micron) to arrest debris and 400-mesh (38 micron) to retain all VAM spores. The suspension retained on 500- μ m sieve was directly examined under microscope, if any large sporocarp was present. The material retained on the 38- μ m sieve was centrifuged in a 20-60% (w/v) sucrose gradient and centrifuged at 3000 rpm for 5 min. Spores were collected from first interface and the sucrose solution was washed off. These spores will be free of nematodes cysts and other contaminants found in the other interface. The supernatant was filtered in a marked-filter paper as suggested by Gaur and Adholeya (1994). The filter paper was observed under Stereozoom research microscope at a magnification of up to 60 to 90X (LEICA, EZ4), and spores were counted and expressed per g of soil.

Thereafter, 50 to 70% of them were mounted on slides with polyvinyl-lactic acid-glycerol. Only the healthy-looking spores were mounted. The spore morphology was studied under compound microscope (OLYMPUS BX 60 Japan; transmitted light intensity - 100W halogen; at a magnification of 210x-840x) and the image was captured by digital camera and transferred on-line to a computer and measurement was done with the help of Microlite image plus software (version - 4.0). Only 60 to 85% of the spores mounted on slides could be identified to the species level or attributed to a specific morphospecies; the rest consisted mostly of old and decaying spores with missing clear features. The spores were identified following the key of Schenck and Perez (1988), which is based on spore wall morphology and thickness.

Root infection

Root bits (size 1cm) were boiled in 10 % KOH for 15-20 minutes in water bath (sometimes even in 60 minutes for hard roots, like *Tectona* and *Azardirachta*), washed in tap-water, and stained in lacto-phenol following Phillips and Hayman (1970) method. For confirmation of infection, the presence of intercellular infection vesicles, arbuscular or both characters of VAM infection were recorded and percent root colonization was obtained by grid line method.

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments colonised}}{\text{Total number of root segments examined}} \times 100$$

RESULTS

Physicochemical properties

Table 3: Mean values and standard deviations of the metals and physico-chemical properties as determined in the soils of Sonepur Bazari, Khottadih and Nakrakonda OCP

Properties	Mine 1	Mine 2	Mine 3
Fe	0.72	5.07	3.96
Zn	0.53	0.40	0.30
Mn	8.79	8.16	3.30
Cu	0.46	0.28	0.18
CEC	13.97	20.03	21.11
CLAY	6.24	10.90	13.59
pH	8.34	7.65	7.52
OC %	0.38	0.23	0.39
EC	0.15	0.11	0.11
N	27.50	24.44	42.38
K	4.23	06.12	6.72
P	10.58	19.65	16.70
OM%	0.67	0.68	0.68

The physicochemical and nutritional characteristics for the three different mines are presented in Table 3. The pH of soils in different profile layers were varying from upper surface soil profile to lower depth profile. The pH value ranges from 6.4 ± 0.04 – 8.7 ± 0.05 ; 6.4 ± 0.01 – 8.5 ± 0.01 ; 6.3 ± 0.01 – 8.8 ± 0.1 in mines 1, 2 and 3. The variation of soil pH was neutral to basic. The alkaline nature of lower soil profile is due to occurrence of carbonates such as (CO_3^{2-}) and bicarbonate (HCO_3^-) which comes from the geological parent materials.

The electrical conductivity of soil of three mines was ranged from 0.08 ± 0.001 ds m^{-1} – 0.21 ± 0.008 ds m^{-1} ; 0.03 ± 0.01 – 0.21 ± 0.004 ; and 0.04 ± 0.004 – 0.20 ± 0.003 ; in mines 1, 2 and 3 and were grouped under class A (< 0.5 ds m^{-1}) *i.e.*, major soils were within safe limit of salinity. These values indicate that the presence of soluble salts is very small amounts

in soil profile and there is very negligible salinity affects to plants growth. The EC value of 0.2 d Sm^{-1} to 0.8 d Sm^{-1} is optimal for growth of most plants. The particle size analysis shows that clay percentage is 1.8 ± 0.03 % to 18.5 ± 0.4 %; 12.1 ± 0.1 % to 25.9 ± 0.4 % and 11.2 ± 0.4 % to 31.3 ± 1.3 % at mine 1, 2 and 3. The overall soil texture is loamy sand followed by sandy loam in the lower part.

Nutrient value

The available nutrients in different profile layers as shown in Table 3; for available nitrogen were varied from 21 ± 0.9 kg/ha to 253 ± 0.8 kg/ha; the organic carbon percentage was in the range of 2.3 ± 0.8 to 0.5 ± 0.1 ; the available phosphorous in the study area ranges from 9.1 ± 0.3 (3.5 %) kg/ha to 12.7 ± 0.0 (0.2 %) kg/ha; and the depth wise distribution of exchangeable potassium ranged from 28.7 ± 0.02 (1.2 %) kg/ha to 135.5 ± 0.06 (0.9 %) kg/ha. The extractable DTPA-Zn in the studied profile varied from 0.3 – 6.2 mg kg^{-1} . Considering the critical limit for Zn as suggested by (Lindsay and Norvell, 1978) is 0.6 mg kg^{-1} . The Mn in the studied profiles varied from 2.14 – 142.80 mg kg^{-1} ; the Cu concentration varied from 0.2 – 1.8 mg kg^{-1} . All the values were well above the critical limit of 0.20 mg kg^{-1} suggested by Lindsay and Norvell (1978). Considering 1.0 mg kg^{-1} as critical limit for Mn deficiency (Lindsay and Norvell, 1978); all the soil had sufficient amounts of available Mn. The Fe concentration in soil profile layers ranged from 3.70 – 0.80 mg kg^{-1} . The critical limit of Fe is 4.5 mg kg^{-1} as suggested by Lindsay and Norvell (1978).

VAM spore density

The VAM spore density was studied from different host plants species of rhizospheric soil and presented in Table 4. A variety

Table 4: Number density of VAM spore in Rhizospheric Alluvial Soil (0.0-0.15m) of opencast mining area

Mines	Depth of soil (m)	Mycorrhizal spore density / 5g of soil	Mycorrhizal spore density/100 g soil	Total No. of spore/5g soil	Total No. of spore /100g soil
1	0.0 - 0.15	174	519	174.0	519.0
2	0.0 - 0.15	193	516	193.0	516.0
3	0.0 - 0.15	360	702	360.0	702.0

Table 5: Average spore density in different rhizosphere of host plant in alluvial soil from three different OCP mines for 5g of soil

Name of host plant	Spore density /5g soil
<i>Acacia auriculoliformis</i>	367
<i>Alstonia scholaris</i>	266
<i>Azadirachta indica</i>	245
<i>Cassia seamea</i>	138
<i>Dalbergia sissoo</i>	356
<i>Polyalthia longifolia</i>	189
<i>Prosopis juliflora</i>	193

Table 6: Average spore density in different rhizosphere of host plant in alluvial soil from three different OCP mines for 100g of soil

Name of host plant	Spore density /100g soil
<i>Acacia auriculoliformis</i>	702
<i>Alstonia scholaris</i>	456
<i>Azadirachta indica</i>	402
<i>Cassia seamea</i>	316
<i>Dalbergia sissoo</i>	519
<i>Polyalthia longifolia</i>	337
<i>Prosopis juliflora</i>	389

Table 7: Size frequency distribution of VAM spore in alluvial soil of Mine 1

Range of spore size (μm)	No. of spores/ 5g of soil	Percentage (%)	Total no. of spores
30-50	8	4.5	174
> 50-75	42	24	
> 75-100	56	32	
> 100-150	32	18	
> 150-200	19	10	
> 200-250	9	5	
> 250-300	6	3.4	
> 300-400	2	1.1	
Range of spore size (μm)	No. of spores/ 100g of soil	Percentage (%)	Total no. of spores
30-50	12	2.3	519
> 50-75	168	31	
> 75-100	139	26	
> 100-150	85	16	
> 150-200	71	13	
> 200-250	24	5	
> 250-300	17	3.2	
> 300-400	3	0.6	

Table 8: Size frequency distribution of VAM spore in alluvial soil of Mine 2

Range of spore size (μm)	No. of spores/ 5g of soil	Percentage (%)	Total no. of spores
30-50	6	3.1	193
> 50-75	72	37	
> 75-100	53	27	
> 100-150	21	10	
> 150-200	19	9.8	
> 200-250	12	6.2	
> 250-300	8	4.1	
> 300-400	2	1.1	
Range of spore size (μm)	No. of spores/ 100g of soil	Percentage (%)	Total no. of spores
30-50	11	2.1	516
> 50-75	165	32	
> 75-100	118	22	
> 100-150	75	14	
> 150-200	62	12	
> 200-250	56	10	
> 250-300	24	4.6	
> 300-400	5	0.9	

Table 9: Size frequency distribution of VAM spore in alluvial soil of Mine 3

Range of spore size (μm)	No. of spores/ 5g of soil	Percentage (%)	Total no. of spores
30-50	12	3.3	360
> 50-75	172	47.7	
> 75-100	69	19	
> 100-150	51	14	
> 150-200	34	10	
> 200-250	16	4.4	
> 250-300	4	1.1	
> 300-400	2	0.5	
Range of spore size (μm)	No. of spores/ 100g of soil	Percentage (%)	Total no. of spores
30-50	20	3	702
> 50-75	182	26	
> 75-100	169	24	
> 100-150	95	13	
> 150-200	87	12.4	
> 200-250	28	4	
> 250-300	12	1.7	
> 300-400	9	1.3	

Table 10: Intensity of root infection from different plant host species

Name of host plant	Type of infection			% Root
	Vesicles	Arbuscular	Hyphae	colonisation
<i>Acacia auriculiformis</i>	-	-	+	79
<i>Alstonia scholaris</i>	-	-	+	64
<i>Azadirachta indica</i>	-	-	+	24
<i>Cassia seamea</i>	-	-	+	31
<i>Dalbergia sissoo</i>	+	-	+	91
<i>Polyalthia longifolia</i>	+	-	+	58
<i>Prosopis juliflora</i>	-	-	+	84

of VAM spores were recorded from rhizospheric soils of different mining areas. They mainly belong to genus of *Glomus*, *Gigaspora*, *Acaulospora*, *Enterophospora* and *Sclerocystis*. *Glomus* is the most predominant genus found in the rhizospheric soil of mine area. The sizes of the VAM spores were found between 87-125 μ . As the VAM specificity is different, the density of the spore also differs from species to species. The highest number of VAM spore density / 5g of soil

were found as 360.0 \pm 12.5 in mine 3; followed by 193.0 \pm 9.45 mine 2 and 174.0 \pm 11.67 mine1 in the topsoil horizon of three opencast mines and then there is a decreasing trend beyond this. Same profile data was obtained for the VAM spore density / 100g of soil were found as 702.0 \pm 23.87 in the topsoil of same horizon. This show that the VAM spore density was most of all reside on the rhizospheric topsoil. VAM spore density is other most important parameters to assess the value of fertility index of soil. The colonization of VAM fungi could be easily quantified by studying the density of spores in the rhizosphere of host plant. As the VAM specificity is different, the density of the spores was also found to different from species to species Table 5.

Number of spore density

The size classification of VAM spores were carried out for the rhizospheric soils. The range of spore size varied from 30 to 400 μm . The maximum number of VAM spores were found in 50-75 μm (24%; 37% and 47.7% for 5g of soils and 31%; 32%; and 26% for 100g of soils); followed by 75-100 μm (32%; 27% & 19% for 5g of soils and 26%; 22% and 24% for 100g of soils); followed by 150-200 μm as shown in Table 7, 8 and 9. Most of the VAM spores were of *Glomus* species.

Root infection

Out of seven species studied in all three mines, *Dalbergia sissoo* found to be containing root infection (91%), followed by *Prosopis juliflora* (84%), *A. auriculiformis* (79%) and *A. scholaris* (64%), *Polyalthia longifolia* (58%), *Cassia seamea* (31%), and *Azadirachta indica* (21%). The percentage root infection in different plants species are given in Table 10.

DISCUSSION

Several environmental variables are known to affect the distribution and viability of VAM spore density in different soil profile and their root infection status. Soil profiles are often many meters deep, but with the majority of studies in soil microbiology focusing exclusively on the soil surface, we know very little about the nature of the microbial communities inhabiting the deeper soil horizons. So, the present study deal with the characterization of alluvial soil cover around the mining areas which have different soil characteristics variation in depth-wise profile significantly based on the different land use pattern and mining activities. Soil moisture, pH and available nutrients (N and P) and micronutrients (Fe, Cu, Mn and Zn) had varied influences on VAM fungi infection and spore number. The soil texture analysis showed that the soil is loamy in the upper part to sandy loam in lower depth of soil profile for increasing the soil sand and silt proportion respectively. It has been also recorded by Sahu *et al.* (2001) there is drastic change in the texture composition of soil downward along the soil profile. Vesicular-Arbuscular Mycorrhizal (VAM) fungi colonizing plant roots and ramify into the surrounding bulk soil extending the root depletion zone around the root system depends on soil texture (Turk *et al.*, 2006). In dune ecosystem, a negative correlation was observed between sand grain size and spore production (Koske and Halvorson, 1981). In all three mines sudden decrease in VAM spore counts with depth is due to the loamy sand texture, which regrets the occurrence of spore's density.

So, soil texture is an important characteristic for the occurrence and colonization of VAM fungi spore density. The maximum number spore density were found in the top-most fertile soil which situated at 0-15 cm depth, while the lower profile of soil show negligible density of VAM spore counts. The topmost soil profile only show spore density because of major portion of soil nutrients are reside at the topmost layers within the 0-15 cm depth. As we go beyond the 0-0.15 m depth, the aeration status of soil decreases, due to which soil become anaerobic, which is not suitable for the viable VAM spore germination and growth. This decline was attributed to a reduction in oxygen availability with depth as evidenced by decreasing redox potential. The possible factors behind depleted propagule density may be, the low soil fertility, organic matter, soil texture, soil moisture and severe soil compaction. VAM (vesicular arbuscular mycorrhiza) show a wide range of tolerance to soil pH. The optimum pH for spore germination is slightly acidic (5.5-6.5). The results showed that in all three mines; the pH ranged in neutral to slightly alkaline which the suitable distribution of VA mycorrhizas spore density and root colonisation. It has been reported by Porter and Robson, (1987) also that soil pH is a major determinant of the distribution of VAM spores. The varying soil pH may affect the development and functioning of VA mycorrhizas by altering the concentration of many nutrients and toxic ions in soil solutions as well as hydrogen ions (Muthukumar *et al.*, 1994). VAM plant association may be stimulated by substance produced by organic matter and the properties of organic matter. McAfee and Fortin (1989) found that the amount of soil organic matter significantly affected mycorrhizal formation in plants. It has been also reported by Purnomo (2000) that the net N mineralization decreased with depth to 20 cm. The decrease in N mineralisation with soil depth was highly correlated with decreases in the organic carbon and total nitrogen. According to Jansa *et al.*, (2005), soil N concentrations did not correlated with the level of root colonization in the bioassay, although soil N concentrations correlated positively with plants biomass. It has been also mentioned by Marschner, (1995), this may be due to the only marginal importance of soil N in regulating mycorrhizal functioning, or to the fact that soil N availability is regulated by relatively complex mechanism compared to phosphorous, including organics matter turnover, N₂ fixation and N volatilization, as well as changes in chemical forms. Phosphorous (P) in the plant has often been considered another factors also controlling mycorrhizal infection (Menge *et al.*, 1978). Under low phosphorus nutrition, low P content of plants could correlate with a decrease in phospholipids levels; and increase in root member permeability would favors mycorrhizal infection (Graham *et al.*, 1981; Ratnayake *et al.*, 1978). High concentrations of heavy metals have been shown to adversely affect the size, diversity, and activity of microbial populations in soil. Heavy metals can delay, reduce, and even completely eliminate AM colonization and AMF spore germination in the field (Barea and Aguilar, 1999). However, the results of soil micronutrients (Cu, Zn, Mn, and Fe) were found within their critical limits except Cu. So, presences of trace elements are not affecting the distribution and colonisation of VA mycorrhizas. Other researchers found no correlation between the concentration of heavy metals (Zn,

Cu, Cd, Ni, etc.) and AMF populations suggested that beside this, mycorrhizae can play a crucial role in protecting plant roots from heavy metals (Khan, 2001). From the above study, AMF belonging to the genus *Glomus* is dominant in the rhizospheric soil profile of different host species growing in alluvial soil Raniganj coal mines, since 93% of the encountered AMF are within these two genera. We have found that VA mycorrhiza has different host specificity, the density of spores also differ from host to host. Maximum spore density was found in rhizospheric soils of *A. auriculiformis*, *Dalbergia sissoo*, *Alstonia scholaris* and *Azadirachta indica* were reported in 5g of soils. Same trend were also found in 100g of soil. Study of root infection showed that maximum root infection were recorded in *Dalbergia sissoo* found to be containing root infection (91 %), followed by *Prosopis juliflora* (84 %), *A. auriculiformis* (79%) and *A. scholaris* (64%), *Polyalthia longifolia* (58 %), *Cassia seamea* (31 %), and *Azadirachta indica* (21 %). The study concluded that these host plants having higher root infections are suitable for the biological reclamation of OB dumps.

ACKNOWLEDGEMENT

The authors like to thanks Eastern Coalfield Limited (ECL) for permission to carry out the study at Eastern Coalfield, Raniganj. They are also grateful to ISM, Dhanbad for providing research fellowship and necessary support during the study. The authors also thank to HOD, ESE and other technical staff for providing necessary laboratory support.

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