The Bioscan 7 (3) : 539-542, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

STATUS AND DIVERSITY OF VESICULAR ARBUSCULAR MYCORRHIZA IN DIFFERENT AGE SERIES SPONGE IRON SOLID WASTE DUMPS WITH RESPECT TO RECLAMATION

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KEY WORDS
VAM fungi
Solid waste dump
Reclamation

Received on : 28.06.2012

Accepted on : 19.08.2012

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ABSTRACT

In the present study status and diversity of VAM fungi in different age series sponge iron solid waste dumps were investigated. The study revealed that the freshly laid dump (D_o) was devoid of any VAM fungi. With increasing age of the waste dump VAM pore density gradual increased in 1, 3 and 5 years old dumps. A total of 35 VAM species belonging to 5 genera namely *Glomus, Gigaspora, Acaulospora, Scutellospora and Sclerocystis* were recorded, out of which 30 were noted in waste dumps. It was also found that *Glomus* and *Acaulospora* were present in high density. *Glomus mosseae* was found to be dominant species with highest relative spore density. Species richness and diversity index showed increasing trend with the dump age. It was concluded that VAM fungi adapted in waste dumps can be used for successful reclamation programme.

INTRODUCTION

India has emerged as the world's largest producer of sponge iron, accounting for 13% of the global production and the coal based sponge iron production contributes about 80% of the total capacity in the country (CSE, 2011). Huge amount of solid waste is generated in form of char, dust, accretion material and fly ash from coal based sponge iron industry (CPCB, 2007). Dumping of such industrial solid waste creates a large area of derelict land (Roy *et al.*, 2002). In developing country like India reclamation of derelict land is an urgent necessity and one of the effective strategies for the reclamation is through revegetation (Singh *et al.*, 2002). However, revegetation of the iron industry solid waste dump is a stupendous task in consideration to the hostile pedosphereic environment (Pandey and Maiti, 2008) and this can be achieved through restoration of soil microorganisms (Mukhopadhyay and Maiti, 2009). One of the most important group of microorganisms which can effectively support the vegetation development in such scenario is mycorrhiza.

Mycorrhiza is the symbiotic association between soil born fungi with the roots of higher plant (Sieverding, 1991). Endomycorrhiza forming vesicle and arbuscules in cortical cells of the root are known as vesicular arbuscular mycorrhizal (VAM) fungi. It has been well established that VAM fungi improves plant growth in terms of better plant nutrient uptake, water relations, ecosystem establishment, plant diversity, productivity, production of growth promoting substances, protection against root pathogens and toxic stresses (Allen and Allen, 1980; Hayman, 1982; Schreiner *et al.*, 1997; Smith and Read, 1997; Quilambo, 2003; Turk *et al.,* 2006). Importance of VAM fungi for reclamation iron ore mine (Jasper *et al.*, 1988), coal mine spoil (Lambert and Cole, 1980; Misra *et al.*, 1990; Mukhopadhyay and Maiti, 2009), lime stone mine spoil (Rao and Tak, 2002; Quoreshi, 2008) and semiarid degraded land (Pigott, 1982) are well emphasized. Distribution and diversity of VAM fungi in different degraded sites have been documented by many workers (Mehrotra, 1998; Singh *et al.*, 2003; Singh and Jamaluddin, 2011). However reports on the distribution and diversity of VAM fungi on sponge iron solid waste dumps are scanty in India, where large numbers of sponge iron industries are mushrooming. Therefore in the present study status and diversity of VAM fungi in different age series sponge iron solid waste dumps were analyzed with the aim to develop reclamatory strategy.

MATERIALS AND METHODS

Study site: Geographical location of the area is between 20º11' NL and 84º19' EL. Altitude of the area is about 245m above the mean sea level. Climate of the area is tropical monsoonal, having three distinct seasons *i.e.* summer (March-June), rainy (July-September) and winter (October- February). The mean annual rainfall in the area is 1422mm, 80% of which fall during rainy season. Mean air temperature of the area varies from a minimum of 10ºC during December to a maximum of 45ºC during May. The relative humidity fluctuates from minimum of 40% (May) to maximum of 83% (August).

In the sponge iron solid waste dumping site, accumulation of solid waste over years resulted in formation of different age series of dumps. Dump age is expressed as time since the establishment of dump in the site. For the present study freshly laid dump (D_0), 1 year (D_1), 3 year (D_3) and 5 year (D_5) old dumps were selected. During dumping of the solid waste, when the dump attains sufficient height, natural soil of the adjacent area is covered over the dump. However, the soil cover is not uniform all over the dump. Thus, D_1, D_3 and D_5 are the mixture of the solid waste and soil. There is also vegetation growth in D_1, D_3 and D_5. However in D_0 there is no soil cover and no vegetation growth.

Sample collection: Waste samples were collected from different age series dump by random sampling method. The samples were collected randomly from 0-15cm depth by digging pits (15 x 15 x 15cm) from five sites for each dump. Samples were collected both from the rhizosphere of plants and from bulk soil and finally mixed to get a homogenous mixture. These sub-samples were brought to the laboratory in sterilized polythene bags and mixed thoroughly to form a composite sample. After sorting out larger pieces of materials and root fragments, the samples were subjected to sieving in 2mm mesh. Each of the samples was divided into three replicates for analysis. Soil sample from adjoining area of the waste dump was taken as control.

Isolation and identification VAM spore: The VAM fungal spores were isolated by wet sieving and decanting method of Gerdemann and Nicholson (1963) followed by sucrose density gradient centrifugation technique as described by Daniel and Skipper (1982). Isolated spores were counted using a dissection microscope at 40X magnification and the enumerated data was expressed as spore density (per 100g dry soil). The spores were mounted on PVLG (Polyvinyl alcohol lactoglycerol) with Meltzer's reagent and were identified up to species level with the help of VAM fungi identification manual of Schenck and Perez (1987) and based on morphological descriptions published by INVAM (http://invam.caf.wvu.edu). The primary identification of fungi genera was ensured under the dissecting microscope and was further confirmed under the compound microscope.VAM fungi species and spore density (per 100g dry soil) were measured and described. Using the data obtained, the following indices of species structure were assessed:

a) Species richness = No. of species present in a particular site

b) Relative spore density $= \dfrac{\text{Spore density of a particular species}}{\text{Spore density of all species}} \times 100$

c) Shannon Diversity Index ($\overline{H}$) as per Shannon and Wiener (1963):

$\overline{H} = -\Sigma\,(n_i / N \ln n_i / N)$

Where, n_i = Relative spore density of each species; N = Total Relative spore density of all species

d) Simpson Diversity index = 1-D

As per Simpson (1949), where D (Simpson Dominance Index) $= \Sigma(n_i / N)^2$;

Where, n_i = Relative spore density of each species

N = Total Relative density of all species

e) Evenness index (J) as per (Pielous, 1975)

$J = \overline{H}/\ln S$

Where, $\overline{H}$ = Shannon Diversity Index; S = Total no. of species

RESULTS AND DISCUSSION

First of all VAM species along with their relative spore density in different age series waste dumps and the adjacent natural soil has been presented in Table 1. As evident from the table, all the 35 different VAM species were isolated from the natural soil. However from the fresh dump (D_0), no mycorrhizal spores could be detected. From the subsequent age series dumps (D_1, D_3, and D_5) the numbers of species encountered were 14, 18 and 30 respectively (Table 2). Absence of vegetation along with improper pedological conditions (Kullu and Behera, 2011) may be the explanation for such observation in D_0 dump. With the establishment of vegetation due to the successional stages on subsequent age series dumps led to the detection of more qualitative range of mycorrhizal species.

All the 35 detected species of VAM fungi belong to 5genera (Table 2) and the genus *Glomus* was noted to be the taxonomically most diverse with 5 to 12 species. Wide adaptability of *Glomus* to varied soil condition has been reported by Pande and Tarafda (2004). Dominance of *Glomus* was followed by *Acaulospora*. The predominance of *Glomus* and *Acaulospora* might be due to their sporulating characteristics i.e. these two genera usually take shorter time to produce small sized spore in comparison to the large spore producer *Gigaspora* and *Scutellospora* species in same environment (Nandakwang *et al.,* 2008).

Glomus mosseae was found to be dominant in all the waste dumps having relative density of 13.79% in D_1, 14.14% in D_3 and 10.71% in D_5 (Table 1). *G. mosseae* was also noted to be the dominant in control soil (Relative spore density = 9.51%). VAM species like *Glomus mosseae, Glomus deserticola, Glomus monosporum, Glomus intraradices, Glomus reticulatum, Gigaspora gigantea, Gigaspora decipiens, Acaulospora denticulate, Acaulospora scrobiculata, Acaulospora spinosa, Scutellospora nigra, Scutellospora gregaria* and *Sclerocystis rubiformis* were detected in all the waste dumps. This indicated the adaptability of these species to the adverse condition of the waste dumps. Species like *Glomus fasciculatum, Glomus macrocarpum, Glomus clavisporum, Gigaspora albida, Acaulospora delicata, Acaulospora elegans, Acaulospora bireticulata, Acaulospora mellea, Scutellospora reticulata, Scutellospora minuta* and *Sclerocystis sinusa* were recorded in D_5 but not recorded in D_1 and D_3. Six no. of species such as *Glomus heterosporum, Gigaspora margarita, Gigaspora candida, Acaulospora laevis, Acaulospora foveata and Scutellospora heterogama* were found in D_3 and D_5 but were absent D_1. The occurrence of

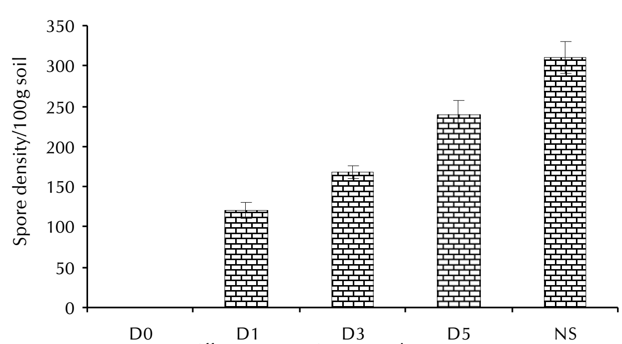

Figure 1: VAM spore density (No. of spore/100g soil) in different age series waste dumps and Control. (D_0: Freshly laid dump, D_1: 1 year old dump, D_3: 3 year old dump, D_5: 5 year old dump, NS: natural soil)

Table 1: Species composition and relative spore density of different VAM species in waste dumps and natural soil. (D_1: 1 year old dump, D_3: 3 year old dump, D_5: 5 year old dump, NS: natural soil)

S. No.	VAM species	Relative spore density				
		D_0	D_1	D_3	D_5	NS
1	*Glomus mosseae* Nicolson and Gerdemann	-	13.79	14.14	10.71	9.51
2	*Glomus deserticola* Bloss and Menge	-	12.41	13.13	7.14	5.16
3	*Glomus intraradices* Schenck and Smith	-	6.90	6.06	5.36	3.26
4	*Glomus fasciculatum* Gerdemann and Trappe	-	-	-	2.86	2.72
5	*Glomus monosporum* Gerdemann and Trappe	-	4.83	2.02	3.57	4.35
6	*Glomus geosporum* Nicolson and Gerdemann	-	-	-	-	3.26
7	*Glomus aggregatum* Schenck and Smith	-	-	-	2.14	2.17
8	*Glomus macrocarpum* Tulasane and Tulasane	-	-	-		3.26
9	*Glomus reticulatum* Bhattacharjee and Mukerjee	-	10.34	4.04	3.57	4.08
10	*Glomus heterosporum* Smith and Schenck	-	-	5.05	2.86	3.80
11	*Glomus clavisporum* Almeida and Schenck	-	-	-	-	2.72
12	*Glomus luteum* Kenn. Stutz and Morton	-	-	-	1.79	2.99
13	*Gigaspora margarita* Nicolson and Gredemann	-	-	2.53	3.57	2.45
14	*Gigaspora gigantean* Nicolson and Gredemann	-	6.90	3.03	2.86	1.63
15	*Gigaspora albida* Schenck and Smith	-	-	-	2.14	2.17
16	*Gigaspora candida* Bhattacharjee *et al.*	-	-	6.06	3.21	1.63
17	*Gigaspora decipiens* Hall and Abbott	-	6.21	5.56	3.57	1.90
18	*Acaulospora denticulata* Sicverding and Toro	-	12.41	10.10	4.29	2.72
19	*Acaulospora scrobiculata* Trappe	-	9.66	8.08	4.29	3.26
20	*Acaulospora delicata* Walker *et al.*	-	-	-	2.86	2.17
21	*Acaulospora elegans* Trappe and Gerd.	-	-	-	3.21	2.72
22	*Acaulospora spinosa* Walker and Trappe	-	3.45	4.04	1.79	2.17
23	*Acaulospora bireticulata* Rothwell and Trappe	-	-	-	1.43	1.36
24	*Acaulospora laevis* Gerdemann and Trappe	-	-	2.53	2.50	2.99
25	*Acaulospora mellea* Spain and Schenck	-	-	-	2.14	2.45
26	*Acaulospora foveata* Trappe and Janos	-	-	2.02	2.86	1.36
27	*Scutellospora heterogama* Walker and Sanders	-	-	2.02	3.21	5.16
28	*Scutellospora gregaria* Walker and Sanders	-	3.45	3.03	2.14	2.17
29	*Scutellospora nigra* Walker and Sanders	-	4.14	5.05	4.29	2.72
30	*Scutellospora persica* Walker and Sanders	-	-	-	-	1.90
31	*Scutellospora reticulata* Walker and Sanders	-	-	-	2.14	1.90
32	*Scutellospora minuta* Walker and Sanders	-	-	-	1.43	1.63
33	*Sclerocystis pellucida* Walker and Sanders	-	-	-	-	1.36
34	*Sclerocystis rubiformis* Gerdmann and Trappe	-	5.52	1.52	3.21	2.72
35	*Sclerocystis sinusa* Gerdmann and Bakshi	-	-	-	2.86	2.17

Table 2: VAM genera represented by different no. species recorded in sponge iron solid waste dumps and natural soil. (D_1: 1 year old dump, D_3: 3 year old dump, D_5: 5 year old dump, NS: natural soil)

VAM Genera	No. of Species (%)				
	D_0	D_1	D_3	D_5	NS
Glomus	-	5 (36%)	6 (33%)	9 (30%)	12 (34%)
Gigaspora	-	2 (14%)	3 (16%)	5 (17%)	5 (14%)
Acaulospora	-	4 (29%)	5 (28%)	9 (30%)	9 (26%)
Scutellospora	-	2 (14%)	3 (16%)	5 (17%)	6 (17%)
Sclerocystis	-	1 (7%)	1 (6%)	2 (7%)	3 (9%)

Table 3: Species richness and diversity of VAM fungi in sponge iron solid waste dumps and natural soil. (D_1: 1 year old dump, D_3: 3 year old dump, D_5: 5 year old dump, NS: natural soil)

Waste dumps	Species richness	Diversity index		Evenness index
		Shannon	Simpson	
D_0	-	-	-	-
D_1	14	2.463	0.907	0.933
D_3	18	2.538	0.924	0.878
D_5	30	3.286	0.957	0.966
NS	35	3.449	0.964	0.970

some VAM species in older dumps but their absence in younger dumps might be due to the fact that these species are able to colonize the waste dump once the younger dumps are ameliorated by vegetational input. Establishment of more number of host plants growing on the older waste dumps perhaps promoted to form symbiotic association. All the VAM species recorded in waste dumps were found in the natural soil taken as control from the adjacent area of the waste dumps. This is because, during the dumping of sponge iron solid waste, soil from the adjacent area of the waste dump is also dumped along with the waste to stabilize the waste dump. So, the soil acts as the source of VAM inoculum in the waste dump. Some of the VAM species such as *Glomus geosporum, Glomus macrocarpum, Glomus clavisporum, Scutellospora persica* and *Sclerocystis pellucida* though recorded in control soil, were not found in waste dumps. These species might not be able to adapt and establish themselves to the adverse condition of the waste dumps.

The spore density of VAM increased with the age of the waste dump, which was highest in oldest D_5 dump (Fig. 1). Natural soil from the adjacent area of the waste dump taken as control had relatively more spore density than that in waste dumps. Analysis of variance (ANOVA) indicated that there was significant difference in the spore density of different waste dumps and control at 0.05 level of probability.

Mycorrhizal species richness, Shannon and Simpson diversity and Evenness indices data have been represented in the Table 3. Species richness was found to be highest in D_5 (30), followed by D_3 (18) and least in D_1 (14). There is gradually increasing trend in species richness and also in diversity index (both Shannon and Simpson) with increasing age of the waste dumps. The value of evenness index indicated even distribution of the different VAM species in all the waste dumps. Diversity of the VAM fungal communities has been related the diversity of the plant communities (Rabatin and Stinner, 1989). Mycorrhizal fungus diversity increases in parallel with that of plants after severe disturbance (Helm *et al*., 1996; Brundrett and Abbott, 2002) As the age of the sponge iron waste dump increases, diversity of the plant also increases (Kullu and Behera, 2011), this can be correlated with the increasing diversity of the VAM fungi in different age series sponge iron solid waste dump.

ACKNOWLEDGEMENTS

Authors thank Head, School of Life Sciences, Sambalpur University for providing facilities for the work and one of the author (BK) acknowledges the financial support of CSIR in form of the NET-JRF for the work.

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