Mycorrhizal association with the Selected dominant vegetation developing on an age series of coalmine over burdened dumps in Sonepur Bazari area, Raniganj, West Bengal

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Abstract

Mycorrhizal fungi are an important soil microbial community. Natural colonization of mycorrhizal fungi is ubiquitous in nature and plays important roles for autophytoremediation in abandoned areas like on coal mine dumps. The present work deals with the mycorrhizal association with the roots of plants collected from three different age series of coal mine over burdened dumps in the Sonepur Bazari area, West Bengal, India. An age series of three Over Burdened Dumps (OBDs) which were 4, 12, and 20 years old were selected and named accordingly. Among the grasses *Cynodon dactylon* and *Saccharum spontanium* show highest infection in all the OBDs. Some leading non-grass herbaceous members showing mycorrhizal infection are *Alternanthera sessilis, Chromolaena odorata, Croton bonplandianum, Calotropis gigantica, Lantana camara and Evolvulus nummularies, Bonplandianum, Calotropis gigantica, Lantana camara and Evolvulul nummularis, Cassia seamea* and *Azadirachta indica* are two leading members in respect of mycorrhizal root infection among trees in OBD-12 and OBD-20 and there is no representative of them in OBD-4. It is also observed that hyphal infection is very common and near cent percent in all cases studied which helps in transport of essential nutrients from the surrounding rhizosphere. So it can be concluded that mycorrhizal infection in the vegetation is essential for better survival of plants in the hostile environment like coalmine spoil.

Keywords : Mycorrhizal, Autophytoremediation, Plant communities, Overburdened dumps (OBD), Coalmine, Raniganj, Age-series,,

Introduction

Open cast coal mining, especially in the recent past, has been creating immense perturbations in the coal belts of West Bengal. In surface mining the coalmine debris is heaped in the form of dumps around the mining area and is called spoil. The spoil is a mixture of disintegrated rocks and rocky soils with coal residues. This mixture is hostile to the growth of both plants and microbes because of impoverished organic matter content, detrimental pH, and draught arising from coarse texture or oxygen deficiency caused by compaction (Agarwal *et al.* 1993). Due to the adverse physico-chemical and biological properties of mine spoil (Juwarkar *et al.* 2004), natural succession of plant species on these dumps is often prevented (Singh *et al*. 1996). However, it is desirable to develop vegetation on over burdened dumps for the purpose of reclamation and environment stability of the area (Roberts et al., 1981; Singh and Jha 1992). In this context micro-organisms are expected to play an important roles especially for the nutrient supply for the roots of the colonizing plants (Montesinos, 2003). The mycorrhizal fungi also helps in nutrient cycling for the development of self sustaining ecosystem on coal mine over burdened dumps (Mehrotra 1991). The role of Mycorrhizae in the development of re-vegetation in coalmine spoil has also been emphasized by many workers (Jasper *et al.,* 1988; Johnson and McGraw, 1988). It often causes root infection and extends the hyphae in rhizospheric environment to absorb essential nutrients (Benthlenfalvay and Linderman, 1992; Salvia and Williams, 1992; Marschner and Dell, 1994; Smith and Read, 1997). It is also reported by Leyval *et al*. (1997) and Smith and Read (1997) that roots uptake more amount of nutrients when they are infected by mycorrhizae. Significant Mycorrhizal association ~~on~~ in the roots of coalmine vegetation has been reported by Kumar *et al.,* (2003) and Ekka and Behera, (2010).

Considering the above facts in mind, we have endeavored to investigate the mycorrhizal association with the roots of dominant vegetation of the selected study area. This has been done particularly during the process of autophytoremediation on an age series basis. Such a kind of study is relevant in order to reclaim the largely devastated potential land of mining areas of West Bengal which also includes our study site which is Sonepur Bazari area,

Materials and methods:

This study was carried out at Sonepur Bazari area which is covered by the Surface Coal Mine Project with mountainous over burdened mining dumps. It is located in eastern part of Raniganj Coalfields, ECL, Burdwan, West Bengal, India**.** The geographical location of this site is at 23^0 48'' North Latitude and 87^0 47" East Longitude, the topography of which is slightly undulating and rolling marked by small ridges and valleys **(Fig. 1)**. The climate is tropical monsoon with very hot summer (average temperature of 42^oC) and a cold winter often experiencing temperature as low as 6^oC. The average rainfall amounts to 1450 mm/year. Total land acquired for this project is 2404.85 Ha including the land for over burdened dumps.

Within this area, a series of 3 Over Burdened Dumps of different ages, henceforth referred to as age-series, was selected and named suffixing the respective age as OBD-4, OBD-12, and OBD-20. Here we took selected dominant species for the present Mycorrhizal study. All the dominant species on all OBDs are randomly sampled out along with their fine roots and rhizospheric soil and separated carefully. Then all roots are brought to laboratory in plastic bags separately for mycorrhizal study. The root samples were washed under running water and then cut into 1 cm pieces. It was then treated with 2N KOH solution at 80^oC for 30 minutes and then washed in running tap water. The root samples were then treated with 3.5% HCl at 80^oC for 30 minutes (Kormanik and McGraw, 1982). Then it was washed in water repeatedly and stained with trypan blue (0.05%) for 30 minutes at 80^oC. The stained root samples were observed under light microscope to see the mycorrhizal infection and its types such as hyphae, arbuscule and vesicles. From the observation under microscopic field, percentage of mycorrhizal infection was estimated by the following equation.

$$\text{Mycorrhizal infection (\%)} = \frac{\text{No. of mycorrhizae infected roots}}{\text{Total No. of roots observed}}$$

Occurrence (%) of different categories such as hyphae, arbuscule and vesicle of infection was calculated as:

$$\text{Occurrence of types (\%)} = \frac{\text{No. of times, the category of infection detected in a sample}}{\text{Total No. of samples observed}}$$

Results:

All total 3, 5 and 6 grass species (Table. 1) are studied from OBD-4, OBD-12 and OBD-20 respectively and in all cases *Cynodon dactylon* showed highest percentage of mycorrhizal infection. It is 84%, 89% and 94% on OBD-4, OBD-12 and OBD-20 respectively. *Saccharum spontanium* gets the second position and scored 84%, 89% and 83% on OBD-4, OBD-12 and OBD-20 respectively. Other grass members have moderate level of root infection between 64-79%.

Similar to grass species 6, 36 and 41 non grass herbaceous plants (Table. 2) are studied from OBD-4, OBD-12 and OBD-20 respectively. Except *Solanum surattense* all the species studied from OBD-4 are characterized by very high percentage of mycorrhizal infection ranging from 79-96%. (Table-2). Out of 36 species collected from OBD-12, 4 namely *Cajanus scarabaeoides, Convolvulus prostrates, Sida rhombifolia* and *Urena lobata* did not possess any mycorrhizal infection. Among them 10 are highly susceptible to mycorrhizal fungi and contained more than 80% of mycorrhizal infection in *Alternanthera sessilis, Achyranthes aspera, Chromolaena odorata, Parthenium hysterophorus, Croton bonplandianum, Calotropis gigantica, Hyptis suaveolens, Lantana camera, Evolvulus alsinoides* and *Evolvulus nummularius.* Some resistant plants against mycorrhizal fungi on OBD-12 are *Sida acuta, Eclipta alba* and *Vernonia cinnera,* and *Solanum surattense* possess less than 30% mycorrhizal infection and others shows moderate level of infection between 31-79% root infection. Out of 40 collected species from OBD-20, five did not show any mycorrhizal infection namely *Solanum xanthocarpum* in addition of 4 studied from OBD-12, and eleven are highly infected by mycorrhizal fungi namely *Clerodendron viscosum* in addition to recorded 10 species from OBD-12. Some plants possess very low level of infection namely *Ocimum cannum, Ocimum basilicum, Tribulus terrestris, Phyllanthus niruri* in addition of four recorded from OBD-12. Rest seventeen have moderate level of infection between 31-79%.

On OBD-4 there is no tree species observed so the study was restricted only to the members grown on OBD-12 and OBD-20. Total seven tree species (Table. 3) are studied from both the OBDs. In OBD-12 four species namely *Butea monosperma, Dalbergia sissoo*, *Azadirachta indica* and *Cassia seamea* show very high percentage of infection and ranging between 72-93%. Other three species shows low to moderate level of infection ranging between 28-56%. On the other hand in OBD-20 the percentages of infection become slightly reduced in all cases except *Leucaena leucocephala* and *Zizyphus mauritiana* where it is increased to 62 and 39% respectively.

Further, three types of mycorrhizal structures were observed in most of the cases studied i.e. hyphal, vesicular and arbuscular. The hyphal structure is more common and ranges between 85-100% of total infection. The arbuscular infection are very low in all the cases as it is short lived and extend only over a period of 4-15 days (Carlin and Brown 1982). On the other hand vesicular types of infection are comparatively more in the roots collected from OBD-12 and OBD-20 as it forms at later stage when the mycorrhiza is no longer engaged in active absorption. It is also observed that vesicular type infection is increased in older OBDs.

Discussion

Majority of the plant species studied in different OBDs possessed a good mycorrhizal association. This indicates that mycorrhizal fungi play a crucial role in plant succession in hostile coalmine spoil. Comparatively high percentage of mycorrhizal infection in the roots of grasses and non-grass herbaceous species was found in the present study which is similar to the study of Kumar *et al.* (2003), Javaid *et al*. (2007) and Leung *et al*. (2007). This indicates that grass and non-grass herbaceous species play important role in initiation of vegetation on hostile coalmine spoil. In *Cynodon dactylon* and *Saccharum spontanium* very high percentage of root infection are observed in this study. This type of trend has also been noticed by Javaid *et al.* (2007), Leung *et al.* (2007) and Ekka & Behera (2010). Absence of mycorrhizal infection in some members of non-grass herbaceous species like *Cajanus scarabaeoides, Solanum xanthocarpum, Convolvulus prostratus*, *Urena lobata, Sida rhombifolia* are may be due to susceptibility of these plants to heavy metal as Mycohhriza helps to accumulate heavy metals in plant body (Koomen *et. al.,* 1990). Among trees, very high percentage of root infections were observed in *Cassia seamea* and *Azadirachta indica* than other tree species indicative of its greater contribution in phytoremediation of coalmine spoil. This type of trends is also observed by McKell (1989) and Mehrotra (1998). Similar to earlier findings of some authors (Kumar *et. al.,* 2003, Pezzani *et. al.,* 2006), in the present study we also observed three types of mycorrhizal infection i.e. hyphal, Vesicular and arbuscular with very higher incidence of hyphal infection which helps in transport of essential nutrients from the surrounding rhizosphere (Newman *et. al.*, 1994, Fitter *et. al.,* 1998 and Nasim, 2005,). The presence of arbuscules is normally used to designate VAM association. Arbuscule are ephemeral structure, which may be absent in young roots, where as vesicles are considered as storage organ. So, in the present study vesicles are found to increase in OBD-20 than the others. Among the different plants the variation in root infection may be due to plant age, morphology and physiology of the roots. However, it has been reported that VAM association may be reduced due to different disturbances (Brundrett, 1991). So finally it can be concluded that, except few species, a moderate to high level of mycorrhizal infection are observed in all species studied in the present work which indicates the importance of mycorrhizal association in plants colonizing in the coalmine spoil.

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Table 1.

Name of the Species	Family	OBD-4				OBD-12				OBD-20			
		Infection (%)	Category			Infect-ion (%)	Category			Infection (%)	Category		
			H	V	A		H	V	A		H	V	A
Cyperus rotundus L	Cyperaceae	63±6	61	12	13	76±3	76	25	11	65±4	57	23	17
Cynodon dactylon L(Pers)	Poaceae	84±4	84	08	17	97±1	87	21	0	94±2	94	37	0
Andropogon aciculatus Retz	Poaceae	-	-	-	-	57±4	50	14	07	47±4	41	0	11
Dactylon aegyptiaca L	Poaceae	-	-	-	-	88±3	85	13	19	65±5	57	0	13
Pennisetum pedicellatum Trin.	Poaceae	-	-	-	-	-	-	-	-	89±2	85	13	9
Saccharum spontanium L.	Poaceae	87±2	87	6	12	93±2	93	9	16	81±3	81	14	15

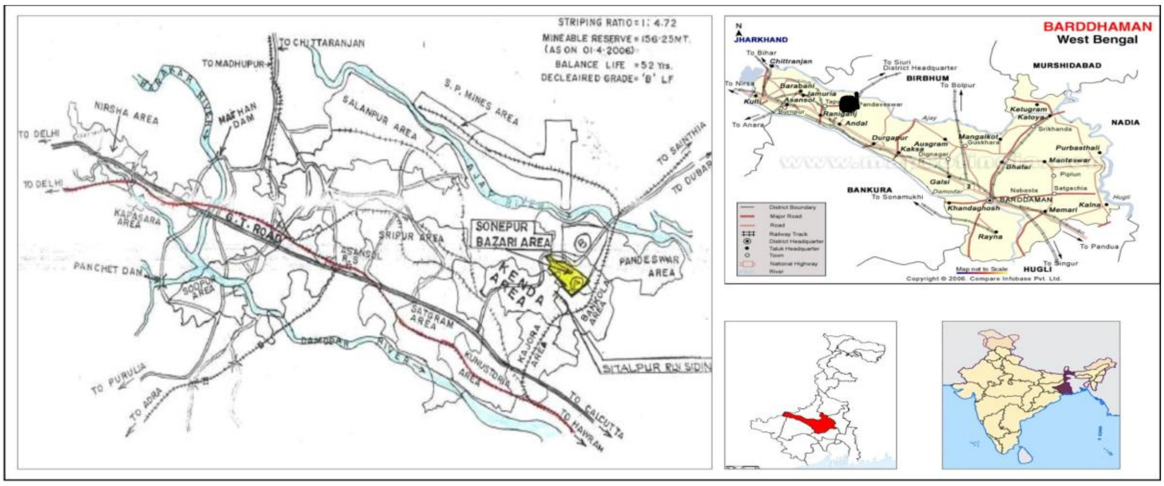

Fig. 1

Source: Mine area map : ECL. Burdwan district map taken from mapmyindia website

Map showing geographical location of study area.

Table 2

Name of the Species	Family	OBD-4				OBD-12				OBD-20			
		Infection	Category			Infection	Category			Infection	Category		
		%	H	V	A	%	H	V	A	%	H	V	A
Amaranthes spinosus L.	Amaranthaceae	-	-	-	-	63±4	63	11	13	61±4	58	11	09
Amaranthes virides L.	Amaranthaceae	-	-	-	-	68±3	65	07	12	63±2	54	13	07
Alternenthera Sessiles R. Br.	Amaranthaceae	-	-	-	-	86±1	83	11	17	84±2	78	17	09
Achyranthes aspera L.	Amaranthaceae	85±2	85	09	10	81±2	81	0	11	80±2	75	06	18
Chromolaena odorata (L)	Compositae	-	-	-	-	96±1	90	11	17	91±2	85	17	21
Mikenia scandens Willd	Compositae	-	-	-	-	58±3	55	0	05	60±3	60	0	14
Tridex procumbens L.	Compositae	-	-	-	-	51±4	50	02	07	48±4	49	06	15
Vernonia cinnera Less.	Compositae	-	-	-	-	27±5	26	01	04	23±5	23	12	01
Eclipta alba Hessk	Compositae	-	-	-	-	23±3	23	0	0	21±5	21	05	02
Parthenium hysterophorus L.	Compositae	79±2	79	06	00	80±2	80	12	13	81±4	75	16	08
Phyllanthus niruri L.	Euphorbiaceae	-	-	-	-	-	-	-	-	28±4	28	0	09
Euphorbia hirta L.	Euphorbiaceae	-	-	-	-	79±2	79	0	14	75±4	70	0	17
Euphorbia microphylla Heyne	Euphorbiaceae	81±3	77	00	17	78±3	78	0	16	74±3	70	04	06
Croton bonplandianum Baill.	Euphorbiaceae	96±3	89	06	11	86±3	86	0	06	84±3	76	0	09
Tephrosia perpurea Pers	Fabaceae	-	-	-	-	41±6	36	03	06	37±5	35	11	0
Cajanus scarabaeoides (L.) Th.	Fabaceae	-	-	-	-	0	0	0	0	0	0	0	0
Desmodium trifolium DC.	Fabaceae	79±4	75	13	19	71±6	68	03	13	72±3	67	12	09
Solanum sisymbrifolium Lamk.	Solanaceae	-	-	-	-	46±4	46	0	08	51±4	51	0	13
Solanum xanthocarpum Schrad	Solanaceae	-	-	-	-	-	-	-	-	0	0	0	0
Solanum surattense Burm. L.	Solanaceae	33±7	30	6	7	26±8	26	0	03	28±6	27	12	0
Calotropis gigantica R. Br.	Asclepediaceae	-	-	-	-	87±1	84	05	16	83±3	78	12	08
Cassia tora L.	Fabaceae	-	-	-	-	69±4	63	04	09	71±3	70	16	01
Hyptis suaveolens L..	Labiatae	-	-	-	-	85±3	80	06	12	81±2	78	08	12
Ocimum basilicum L.	Labiatae	-	-	-	-	-	-	-	-	24±6	24	0	0
Ocimum cannum Sims.	Labiatae	-	-	-	-	-	-	-	-	28±6	28	0	04
Leucas aspera Spreng.	Labiatae	-	-	-	-	-	-	-	-	65±5	60	04	07
Anisomelis ovata R. Br.	Labiatae	-	-	-	-	74±3	71	02	07	70±2	70	22	12
Lantana camara L.	Verbenaceae	-	-	-	-	89±2	89	0	14	80±2	75	15	11
Convolvulus prostratus Forssk.	Convolvulaceae	-	-	-	-	0	0	0	0	0	0	0	0
Evolvulus alsinoides Wall	Convolvulaceae	-	-	-	-	82±1	80	0	21	80±1	74	09	12
Evolvulus nummularius L.	Convolvulaceae	-	-	-	-	90±1	87	0	17	86±1	80	11	09

Sida rhombifolia L.	Malvaceae	-	-	-	-	0	0	0	0	0	0	0	0
Sida acuta Burm.	Malvaceae	-	-	-	-	28±4	28	0	03	25±5	25	0	0
Urena lobata L.	Malvaceae	-	-	-	-	0	0	0	0	0	0	0	0
Boerhaavia repens L	Nyctaginaceae	-	-	-	-	71±2	67	04	13	67±3	65	12	08
Argemone maxicana L.	Papaveraceae	-	-	-	-	58±2	58	09	14	55±4	55	13	18
Hygrophyla spinosa T. And.	Acanthaceae	-	-	-	-	49±1	46	07	11	48±5	43	11	02
Oldenlandia corymbosa L.	Rubiaceae	-	-	-	-	-	-	-	-	55±5	51	03	12
Tribulus terrestris L.	Zygophyllaceae	-	-	-	-	-	-	-	-	26±6	26	0	0
Clerodendron viscosum Vent	Verbenaceae	-	-	-	-	-	-	-	-	86±3	81	21	16

Table3

Name of the Species	Family	OBD-4				OBD-12				OBD-20			
		Infection	Category			Infection	Category			Infection	Category		
		%	H	V	A	%	H	V	A	%	H	V	A
Butea monosperma Taub.	Fabaceae	-	-	-	-	72±3	62	0	19	70±3	52	11	21
Dalbergia sissoo Roxb.	Fabaceae	-	-	-	-	75±3	70	11	23	71±3	70	15	13
Leucaena leucocephala Dwit.	Fabaceae	-	-	-	-	56±3	51	09	21	62±2	51	16	16
Cassia seamea L.	Fabaceae	-	-	-	-	93±3	88	21	18	88±1	88	20	17
Cassia fistula L.	Fabaceae	-	-	-	-	46±2	41	0	09	39±5	41	10	06
Zizyphus mauritiana Lamk.	Rhamnaceae	-	-	-	-	28±6	27	0	12	39±4	27	09	11
Azadirachta indica L.	Meliaceae	-	-	-	-	78±4	75	21	16	72±3	75	25	09