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Diversity of Arbuscular Mycorrhizal Fungi in the cement dust polluted sites of Ariyalur District, Tamil Nadu

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Abstract

Cement industry is considered as an essential alley in the modern world as pollution by cement dust cannot be avoided completely but can only be minimized by adopting suitable production strategies or exploiting adapted plants/microbes. Arbuscular mycorrhizal (AM) associations are integral, functioning parts of plant roots and are widely recognized as enhancing plant growth on severely disturbed sites, including those that are polluted with cement and other pollutants including heavy metals. The present study was carried out to assess the effect of cement on AM population at different sites in Ariyalur district. The study revealed that the physico-chemical characteristics of the soil varied and the AM diversity also varied within the sites. The AM fungal population reduced drastically in the sites near the industry. And even in these sites species of AM especially *Glomus* species were isolated. Isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems.

Keywords: Cement dust pollution, AM diversity, *Glomus.*

Introduction

Cement the most widely used building material throughout the world is one of the most basic industries involved in the development of a country. With the increase in demand for cement in India, the numbers of factories are increasing each year and both consumption and production of cement has increased greatly in recent years. The cement industry has been recognized to be playing a vital role in the imbalances of the environment and producing air pollution hazards The aerial discharge of cement factories consist of Particulate matter, Sulphur dioxide and Nitrogen oxides producing continuous visible clouds which ultimately settle on the vegetation, soil and affects whole biotic life around, as a result the whole ecosystem around the cement factory is subjected to extraordinary stress and abuse. Dust from cement factories leads to considerable change in pH and accumulation of emitted metals in soil which may affect both the composition and physiological processes of microorganisms leading to a reduction in microbial biomass and enzymatic activity (Kulandaivel *et al*., 2015). Soil microorganisms play an important role in the overall soil metabolism. Although there are many studies on the effect of dust particles on plants and animal life, very little work is carried out on soil microorganisms especially on Arbuscular Mycorrhizal (AM) fungi. Arbuscular mycorrhizal fungi form symbiotic associations with most economically important plants (Smith and Read, 2008). These fungi improve plant growth under low fertility conditions, confer tolerance to some plant pathogens, improve water balance of the plants, and contribute to the formation of soil structure and also help plants to become established in new areas

(Kavitha and Nelson, 2013). So, the present study was aimed at investigating natural AM fungal (AMF) spore diversity in the rhizosphere soil of cement polluted groundnut cultivated fields located in and around Tamil Nadu Cements Corporation Ltd, Ariyalur, Tamil Nadu, South India.

Materials and Methods

Soil samples were collected from three cultivable lands in and around the Tamil Nadu Cements Corporation Ltd., Ariyalur (Table - 1). Ten replicate samples were drawn from each site from a depth of 10 cm, kept in polythene bags, labelled, and stored at 4 °C till analysis. The physico-chemical characteristics of the soils were analysed as per standard procedures (Byju, 2001).

Collection of root and soil samples

The collection of roots and soil samples from groundnut cultivated field was done. Soil samples and fine roots were collected in polythene bags from each site. About 200-250 gm of soil samples from rhizosphere of each plant at a depth of 15-30 cm was collected in polythene bags. These samples were mixed to form a composite sample and then brought to laboratory and used for the isolation of AM fungal spores, mycorrhizal quantification, root colonization and stored at 5-10° C. The fine roots in the sample were removed, rinsed with tap water and fixed in formalin: acetic acid: alcohol (FAA), and used for the determination of root colonization. The soil samples were then air dried in the shade at laboratory temperature for AM spore counting.

Isolation of AM fungi from soil

'Wet-Sieving and Decanting technique' (Gerdemann and Nicolson, 1963) was used for isolation of AM fungi spores. For this, sieves of different sizes i.e. 150µm, 120µm, 90µm, 63µm, and 45µm were used. Soil sample (100gm) was thoroughly mixed in 500ml water in a beaker using magnetic stirrer and allowed to settle overnight. The sieves were placed in the following order 150µm, 120µm, 90µm, 63µm and 45µm from top to bottom. The water of the beaker was decanted on a series of sieves, on which spores were trapped and then they were washed with running tap water. The trapped spores were transferred to Whatman No. 1 filter paper by repeated washing with water. Spores were picked using a needle under stereo binocular microscope. The spores were mounted on glycerol for further observation.

Mycorrhizal quantification

Quantitative estimation of AM fungal spores was done by modified Grid line intersect method given by Adholeya and Gaur (1994). In this method, the filter paper was divided into many small compartments by a ball point pen and each compartment was numbered. The total numbers of spores were counted under stereo binocular microscope by using counter and the species richness was recorded.

Roots with AM-Fungi Sampling and Staining

Freshly collected root samples were washed gently to free from soil particles. Roots were treated with 10 % KOH solution for 30 min in a hot bath. Treated roots were washed with water and treated with 2 % HCl solution. Acidified root samples were stained with 0.05 % trypan blue (or acid fuchsin) in lactic acid for 10-15 min in a hot bath or for a few hours without heating. The roots were destained with lactic acid or lacto-glycerol and observed first under a dissecting microscope with transmitted illumination and then observed under a compound microscope. Fungal structures are stained and can be easily recognized (Phillips and Hayman, 1970). The mycorrhizal colonization was determined by using the following formula.

$$\text{Root colonization}(\%) = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

Identification of AM fungi

The main structure of AM fungi, the spores was used for identification. Following morphological criteria *viz*., colour, size, shape, wall structure, bulbous suspensor, the number and arrangement of spores in the sporocarps were used for AM fungi identification. These AM fungi spores were identified using the identification manual of Walker (1983), Shenck and Perez (1990) and Mukerji (1996).

Results and Discussion

Wide variations were found in the physico-chemical characteristics of the soils under study (Table - 1). The soils of sites 4 and 5 showed neutral tendency, while in other sites, they were alkaline. The alkalinity was found to be higher near the factory and declined as the distance increased. The soil water holding capacity was high in site 1 and 2. The soil of site 5 was rich in organic matter whereas, those near the factory sites had lesser content. Higher values for Nitrogen, Phosphorous, Calcium and Magnesium were recorded in sites 1 and 2 whereas, site 5 showed a high value for Potassium.

Table 1 Physico-chemical properties of cement dust polluted soil (Mean ± SD; n=10)

Properties of the Soil	Name of the site*				
	S1	S2	S3	S4	S5
Distance from the factory (km)	0.5	2	3	4	5
Nature of soil	Red sandy	Clayey	Clay with silt	Red sandy	Red sandy
Temperature (ºC)	29 ± 2.7	28 ± 1.6	27 ± 2.2	28 ± 1.8	26 ± 2.1
Soil density	2.34 ± 0.7	1.85 ± 0.8	2.37 ± 0.9	1.41± 0.4	2.02 ± 0.6
pH	8.5 ± 0.3	8.2 ± 0.4	8.0 ± 0.3	7.5 ± 0.3	7.5 ± 0.2
EC (dSM^{-1})	0.5 ± 0.1	0.45 ± 0.1	0.41 ± 0.08	0.39 ± 0.1	0.4 ± 0.09
Water Retention capacity %	37.5 ± 2.4	27.6 ± 3.0	17.62 ± 3.2	15.224 ± 2.5	13.0 ± 2.3
Organic matter %	1.04 ± 0.6	1.37± 0.3	1.85± 0.6	2.24± 0.7	4.47± 0.5
Nitrogen (kg/ha)	52.5± 2.4	54.3± 2.6	52.4± 3.7	53.1± 2.8	60.0± 2.6
Phosphorous (kg/ha)	33 ± 0.7	28 ± 0.8	28 ± 0.6	27 ± 0.7	21 ± 0.9
Potassium (kg/ha)	75 ± 2.4	85 ± 3.9	85.4 ± 2.8	92.4 ± 4.5	96 ± 5.1
Calcium (mg/100 gm soil)	334 ± 6.8	328 ± 4.7	210 ± 5.3	171 ± 4.6	145 ± 7.3
Magnesium (mg/100 gm soil)	321 ± 7.7	271 ± 9.8	269 ± 6.6	145 ± 3.7	146 ± 5.1

*S1- Kayarlabath; S2 - Pallakaveri; S3 – Ravuthampatti; S4 – Kallankuruchi; S5- Manaleri

The cleared root segments of groundnut plants collected from the cement dust polluted site showed the presence of vesicles and arbuscules (Fig. 1a & b). There was variation in the percentage of infection of roots within the different sites. The percentage of infection was lesser in sites near to the factory and increased as the distance increased. Spore population of AMF in soils polluted with cement dust varied with sites. The spore load was minimum in the soil polluted with cement and maximum was observed in soil collected from a distance of 5 km. (Table - 2). Altogether 14 species representing four genera *viz. Glomus*, *Acaulopora*, *Scutellospora* and *Gigaspora* were isolated from the soils polluted with cement dust. Of the different sites maximum number of AM species were encountered in sites 4, and 5. The predominant fungal species in all the polluted sites was *Glomus* species. It has been found *Glomus* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment (Bever *et al*., 1996). Because of their smaller spore size, *Glomus* species require less time to sporulate (Hepper, 1984) than *Gigaspora* and *Scutellospora* species and are therefore more adaptive in adjustment of sporulation pattern in varied environmental conditions (Stutz and Morton, 1996). *Glomus* and *Acaulospora* spp. have been reported from cement polluted sites of Kerala (Bindu and Harikumar, 2008). The reports on effect of cement dust on AM spores vary, several authors have observed an inhibitory effect while few studies have reported no or positive effect of the microbial/AM population. In the present study the diversity of AM was found to be inhibited by the cement dust. Although there was a reduction in the AM spore population some species of AM especially those of *Glomus* was dominant in cement polluted soils.

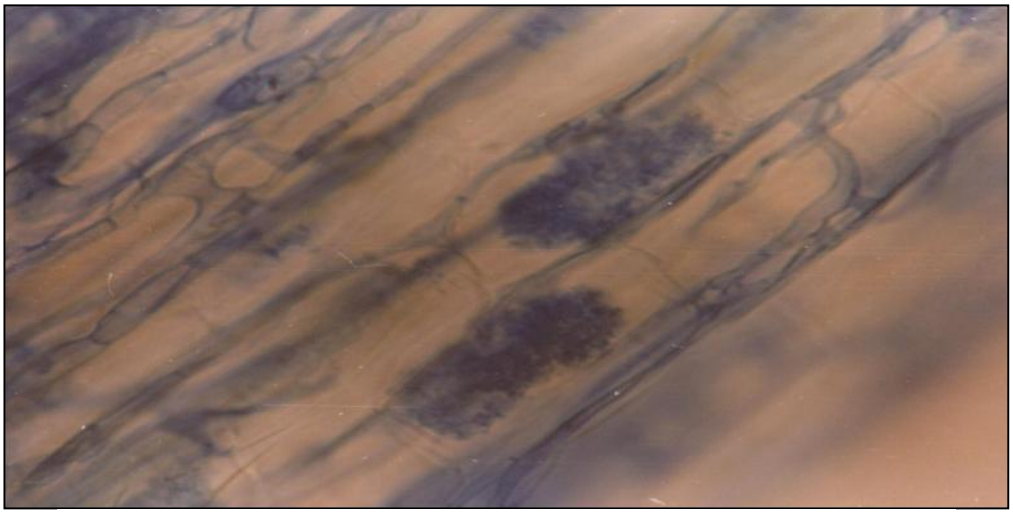

Fig. 1a. Cleared and Tryphan blue stained roots of groundnut collected from the cement dust polluted site showing arbuscules x 400

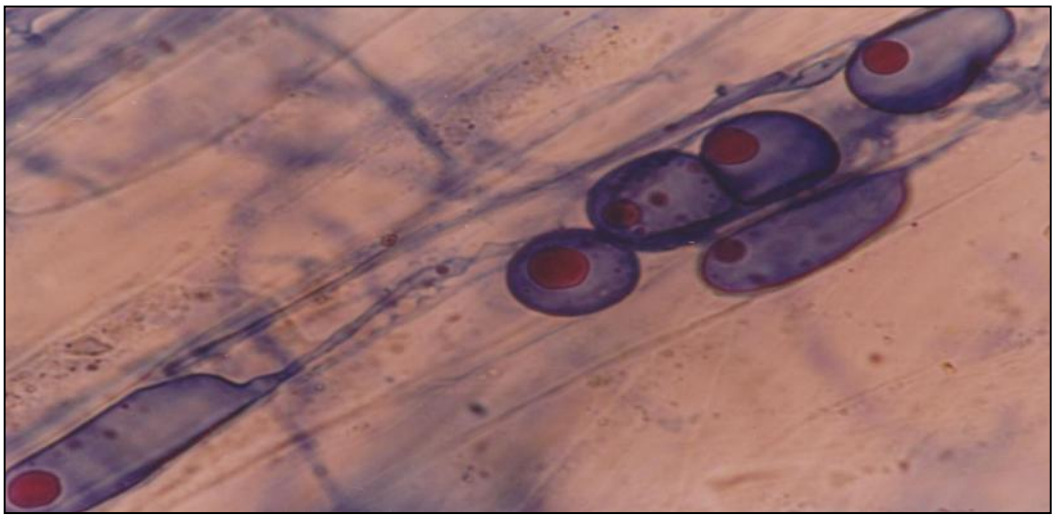

Fig. 1b. Cleared and Tryphan blue stained roots of groundnut collected from the cement dust polluted site showing Vesicles x 400

Table 2 Arbuscular Mycorrhizal diversity in the cement polluted sites in Ariyalur district, Tamil Nadu, South India

Name of the site	Spore density /100 gm soil	Species Richness	Arbuscular Mycorrhizal fungi identified
Kayarlabath	110	5	*Glomus aggregatum; G. fasciculatum; G. mosseae; Acaulospora spinosa; Scutellospora calospora;*
Pallakaveri	284	7	*Glomus aggregatum; G. fasciculatum; G. fulvum; G. reticulate; Acaulospora elegans; Scutellospora calospora; Gigaspora margarita*
Ravuthampatti	350	10	*Glomus aggregatum; G. fasciculatum; G. geosporum; G. mosseae; G. fulvum; Acaulospora spinosa; A. elegans; Scutellospora calospora; S. nigra; Gigaspora margarita*
Kallankuruchi	470	12	*Glomus aggregatum; G.fasciculatum; G.geosporum; G.mosseae; G.fulvum; G.reticulate; Acaulospora spinosa; A. elegans; Scutellospora calospora; S. nigra; Gigaspora margarita*
Manaleri	530	14	*Glomus aggregatum; G.fasciculatum; G.mosseae; G.pulvinatum; G.geosporum; G.reticulata; Acaulospora spinosa; A. elegans; A. biretculata; Scutellospora calospora; S. nigra; Gigaspora margarita; Gi. candida; Gi. decipiens*

The study reveals that AMF can survive and sustain in various polluted soils, though their distribution and composition vary. The study warrants the need for identifying and propagating AM species/strains, which can endure soil pollution so that they can be effectively, utilized in the reclamation of degraded land especially those that are polluted with cement dust. Further studies on the mass multiplication of the tolerant strain of *Glomus* sp. and their influence on the growth of selected legume crops are underway.

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