Diversity of Arbuscular Mycorrhizal Fungi Associated with Plants Growing in Fly Ash Pond and Their Potential Role in Ecological Restoration

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Abstract Root colonization and diversity of arbuscular mycorrhizal fungi (AMF) were analyzed in plants growing in fly ash pond. Eight species could be separated morphologically, while phylogenetic analyses after PCR amplification of the ITS region followed by RFLP and sequencing revealed seven different AM fungal sequence types. Phylogenetic analysis showed that these sequences cluster into four discrete groups, belonging to the genus *Glomus* and *Archaeospora*. Inoculation of plants with spores of AM fungal consortia (*Glomus etunicatum*, *Glomus heterogama*, *Glomus maculosum*, *Glomus magnicaule*, *Glomus multicaule*, *Glomus rosea*, *Scutellospora heterogama*, and *Scutellospora nigra*) along with colonized root pieces increased the growth (84.9%), chlorophyll (54%), and total P content (44.3%) of *Eucalyptus tereticornis* seedlings grown on fly ash compared to non-inoculated seedlings. The growth improvement was the consequence of increased P nutrition and decreased Al, Fe, Zn, and Cu accumulations. These observations suggested that the inoculation of tree seedlings with stress adapted AM fungi aid in the reclamation of fly ash ponds.

Introduction

Fly ash disposal is a major environmental concern throughout the coal-based power generating countries. In India alone, the annual production of fly ash is about 120 MT produced by 82 power plants and is expected to rise to 150–170 MT per year by the end of 2012 [24].

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Disposal of fly ash has significant impact on terrestrial and aquatic ecosystems due to leaching of toxic substances from the ash into soil and ground water, as well as reduction in plant establishment and growth [18, 51, 52]. In many cases, the reclamation practices have been ineffective in reducing mortality of tree seedlings due to deficiency of essential nutrients (usually N and P), low soil microbial activity, high soluble salt concentrations of trace elements, and the presence of compacted and cement layers on ash disposal sites [41]. The lack of mycorrhizal symbionts, which enhance nutrient supply to the host plants and alleviate biotic stress, may be partly responsible for the problems in revegetation [43]. Mycorrhizal fungi are a ubiquitous group of soil microorganisms that form symbiotic association with roots of the majority of terrestrial plant species [42]. Arbuscular mycorrhizal fungi (AMF) are considered to be ecologically important as they play important roles in the restoration of contaminated ecosystems by improving plant nutrition and fertility of degraded land [7].

Spontaneous selection of infective and effective AM fungi can be a long process in fly ash ponds. Application of selected metal tolerant AMF strains may increase the rate of restoration, efficiency of phytoremediation and speeds up these processes [47]. Isolation and mass inoculum of AM fungi that are adapted to fly ash may have more beneficial effect as compared to other AM fungi. It has also been demonstrated that the use of adapted AM fungal strains can be more effective in restoration and bioremediation studies than the non-adapted strains [46]. Molecular and biochemical basis of well-adapted AM fungal strains for restoration and bioremediation correlated with their origin from polluted sites is still not clear. Hence, it is necessary to understand the ecological role of AM fungi associated with plants growing in fly ash. Selvam and Mahadevan [41] studied the distribution of AM fungal

species naturally associated with plants in an abandoned lignite fly ash pond. Amendment of farm yard manure to fly ash along with AM fungi improved the growth, physical properties of fly ash, and reduction of toxic metals [19]. Several researchers have reported the effects of selected isolates of AM fungi on plant growth, nutrient uptake, and aggregation of fly ash [11, 52]. The morphological methods may not reflect the actual diversity and functionally active AM fungi associated with plant roots [8]. It has hitherto been difficult to identify each individual fungal species that inhabits the root of a plant. Alternatively molecular approaches have been shown to hold great promise in estimating biodiversity and population structure of AM fungi [39]. Molecular identification of AM fungi based on the sequences of ITS and LSU of rDNA has been widely used to characterize AM fungal communities under native forests, grasslands, agriculture ecosystems, and contaminated soils [13, 21, 36, 37].

In this investigation, we have determined the colonization levels and the diversity of AM fungi associated with different plants growing on a fly ash pond using both spore morphology and molecular characterization. An attempt has also been made to understand the effect of these AM fungi on the growth of *Eucalyptus tereticornis* seedlings grown on fly ash.

Materials and Methods

Study Site

Fly ash pond located at National Aluminium Company Ltd. (NALCO), Damnjodi (18°46′22″N, 082°53′23″E), Orissa, India, was selected for this study. The fly ash chemical properties are represented in Table 1. The roots and the surrounding fly ash rhizosphere samples were collected from six dominant plant species, *Acacia pennata* (*Mimosaceae*), *Calotropis gigantea* (*Asclepiadaceae*), *Cassia occidentalis* (*Caesalpiniaceae*), *Cynodon dactylon* (*Poaceae*), *Lantana camara* (*Verbenaceae*), and *Jatropha gossypifolia* (*Euphorbiaceae*) growing in the pond in the month of April 2007 (summer season). Ten plants each of above mentioned species were randomly selected, and about 500 g of rhizosphere sample of each plant was collected at a depth of 0–30 cm. Root and soil samples representative for each plant species were pooled separately to yield one composite sample. The samples were placed in polythene bags and stored at 5°C until processed.

AM Colonization and Spore Morphological Assessment

The percent root length colonization of AM fungi was calculated by the gridline intersect method [15] after

Table 1 Chemical properties of fly ash collected from NALCO, Damanjodi, India

Parameters	Units
pH	7.35
EC (mS/cm)	0.30
Organic Carbon (%)	0.30
Olsen P (mg/kg)	2.30
N (mg/kg)	85.0
Elemental analysis (mg/kg)	
Al_2O_3	281,500
Fe_2O_3	51,500
P_2O_5	4,500
SO_3	13,500
CaO	8,900
MgO	3,800
Na_2O	3,700
MnO	500
K_2O	800
ZnO	100
CuO	150

EC electrical conductivity

staining with trypan blue [33]. AM fungal spores were isolated from rhizospheric fly ash samples by wet sieving and decanting technique [14]. The spores were identified following current taxonomic criteria [40] and also using information of INVAM (http://www.invam.caf.wdu.edu/).

DNA Extraction and PCR Amplification

Fine root samples collected from fly ash pond were pooled and ground to powder in liquid nitrogen. Five plants of each species colonized with AM fungi were used for DNA extraction. Genomic DNA was extracted using DNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen, Hilden, Germany). Nested PCR was performed for amplification of partial 18S-ITS1-5.8S-ITS2 rDNA region of AM fungi as described by Redecker [35], with minor modification. The first round of amplification was performed using NS5 and ITS4 primers [50] with an annealing temperature of 51°C. For the second amplification, PCR amplicons were diluted in TE buffer (1:10, 1:50, and 1:100), and PCR was performed using genus specific primers ARCH1311, ACAU1660, LETC1670, GLOM1310, PARA1313, in combination with ITS4i in separate reactions [36]. The reverse primer GLOM5.8 was used only in combination with NS5 primer [36]. The 25-μl reaction mixture consisted of 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 0.5 μM of each primer, and 0.15 units of *Taq* DNA polymerase (Larova, Teltow, Germany). The PCR conditions were as follows: initial denaturation at 95°C for 4 min, followed by 25 cycles of 1 min at 95°C, 1 min at 61°C and 1 min at 72°C, and a final extension of 72°C for 8 min.

Cloning and Sequence Analyses

PCR products were run on 1.5% agarose gel, and the target bands were excised and purified with QIAquick PCR purification Kit (Qiagen, USA) following the manufacture's recommendations. The purified products were cloned separately into pTZ57R/T vector of the InsTA-clone™ PCR Cloning Kit (Fermentas) and transformed into competent *Escherichia coli* DH5α. The plasmid DNA was isolated from 150 randomly selected transformants, and inserts were re-amplified with specific genus primers. RFLP analysis was performed using *Hinf*I and *Mbo*I enzymes as per manufacturer's recommendations and run on a 1.8% agarose gels. The clones of distinct RFLP patterns were sequenced using Applied Biosystems DNA sequencer. Sequence similarities were determined using the BLAST analysis of NCBI. Sequences of AM fungi were deposited at NCBI database under the accession numbers HM159456 to HM159462. The sequences were aligned using MAFFT v 6.240 [20] with other sequences obtained from GenBank. The phylogenetic analysis was carried out by neighbor-joining method using MEGA software [45] with Kimura 2-parameter model. Bootstrap values of 1000 were used to assess the relative support for each clade.

Nursery Experiment

The inoculum of AM fungi was prepared in earthen pots by isolating the spores from the rhizosphere of fly ash and root pieces. The inoculum was propagated in fly ash using maize, wheat, and sorghum as trap plants. No fertilizers were applied while multiplying the AM fungi. The pots were watered with tap water and maintained under green house conditions. The inoculum was prepared with density of 450–500 spores per 10 g of fly ash along with AM-infected root pieces. The majority of the spores found in the inoculum were *Glomus rosea*, *Glomus etunicatum*, *Glomus heterogama*, *Glomus maculosum*, *Glomus magnicaule*, *Glomus multicaule*, *Scutellospora heterogama*, and *Scutellospora nigra*. Seeds of *E. tereticornis* were surface sterilized and sown in trays containing heat sterilized (121°C for 3 h) sand:soil (1:1 w/w) mixture. Two-week-old seedlings with similar height (5 cm) were used for plantation. The nursery experiment consisted of two treatments: control (fly ash alone) and inoculated treatments (fly ash + AM fungi) with 15 replicates each. Fly ash was filled in polythene bags (1.5 kg capacity). The inoculum (10 g) was mixed with fly ash and transplanted one seedling per bag. Seedlings were watered regularly and allowed to grow for 6 months without adding any fertilizer. Plants were harvested after 6 months and analyzed for various parameters.

Plant Growth Parameters and AM Fungal Colonization

The height of the plants and dry biomass of shoots and roots were determined. Chlorophyll content was estimated after extraction in 80% chilled acetone according to Arnon [2]. The percent root length colonization of AM fungi was assessed. The dried and ground shoot material of *E. tereticornis* seedlings were digested with the mixture of concentrated $HNO_3/HClO_4$ (3:1 v/v) until the contents become colorless and only white dense fumes appeared. It was cooled, and diluted HCl was added [31]. The concentration of different metals (Al, Fe, Zn, and Cu) was measured in the filtrate by Inductively Coupled Plasma Mass Spectroscopy.

Physico-chemical and Biochemical Analysis of Fly Ash

The pH and electric conductivity (EC) of fly ash was determined. Organic carbon was estimated according to the method described by Walkley and Black [49]. The total N content was determined by total organic carbon (TOC) analyzer (Thermo-Hyper TOC, Thermo scientific, USA) coupled with total nitrogen module. Available P was estimated by Bray and Kurtiz [6] method. Urease activity was determined by the method of McGarity and Myers [23], and the assay of acid phosphatase activity was determined by measuring the p-nitrophenol released by phosphatase activity [44].

Statistical Analysis

The data were statistically analyzed, and the means were compared using *t* test at $P < 0.05$. The statistical analyses were performed using GraphPad prism software v.4.03.

Results

Arbuscular Mycorrhizal fungal colonization was observed in root samples of all the plants growing in fly ash pond. Average percent colonization of AM fungi varied from 14 to 33%. Maximum colonization was observed in *J. gossypifolia*, while the minimum in *C. dactylon* (Table 2). Spores of AM fungi were very low in rhizosphere soil samples of each plant species and varied from 8 to 40 spores/kg (Table 2). The higher number of spores was counted in the rhizosphere soil of *A. pennata* (Table 2). Spores isolated from fly ash pond were identified based on their morphology. Eight different AM fungal morphotypes were observed, and among these, six were identified as *Glomus* (*G. etunicatum*, *G. heterogama*, *G. maculosum*, *G. magnicaule*, *G. multicaule*, and *G. rosea*) and two as *Scutellospora* (*S. heterogama* and *S. nigra*). *G. rosea* was

Table 2 Arbuscular mycorrhizal (AM) fungal colonization of different plant species growing in fly ash pond and number of spores present in the rhizosphere soils of plants collected from fly ash pond, NALCO, Damanjodi, Orissa

Name of the plant	AM fungal colonization (%)	Total number of spores/kg	Identified AM fungal spores and occurrence times in parenthesis
A. pennata	27	40	*G. etunicatum* (6)
			G. heterogama (3)
			G. maculosum (5)
			G. rosea (9)
			S. nigra (4)
C. gigantea	19	9	*G. etunicatum* (2)
			G. heterogama (1)
			G. rosea (4)
C. occidentalis	26	30	*G. heterogama* (4)
			G. maculosum (3)
			S. heterogama (6)
			S. nigra (8)
C. dactylon	14	8	*G. heterogama* (2)
			G. rosea (4)
J. gossypifolia	33	30	*G. heterogama* (4)
			G. maculosum (3)
			G. rosea (6)
			S. nigra (3)
L. camara	31	30	*G. etunicatum* (2)
			G. heterogama (3)
			G. maculosum (1)
			G. multicaule (2)
			G. rosea (4)
			S. heterogama (2)
			S. nigra (1)

the dominant fungus from these soils. Among the six plant species, *Lantana camara* displayed all identified AM fungal species except *G. magnicaule* compared to other plants. AM fungal species and their occurrence for different plant species growing in ash pond are listed in Table 2.

Nested PCR results revealed that only GLOM1310/ITS4i and ARCH1311/ITS4i primers amplified the target DNA. After cloning of amplified products, a total of 44 RFLP types were detected. BLAST results of 18S-ITS1-5.8S-ITS2 rDNA region sequences indicated that among 44 cloned sequences, 7 clones (FA8, FA14, FA19, FA23, FA29, FA31, and FArc1) showed similarity to Glomeromycota and the other sequences either Ascomycetes or Basidiomycetes. Of these seven sequences, five sequences showed homology with the genus *Glomus* (FA8, FA19, FA 23, FA29, and FA31) and one to *Archaeospora* (FArc1). The FA14 sequence did not show homology with known sequences. Phylogenetic analysis revealed that these sequences were clustered into four groups; two groups

(Group I and II) belonging to genus *Glomus*, group III showing similarity with *Archaeospora* and group IV clustered without any reference sequences from the database. The sequences of FA19, FA23, and FA29 were clustered with *G. irregular*, *G. intraradices*, and some unidentified species of *Glomus* in group I. The FA31 clustered with *G. indicum* and FA8 with some unidentified species of *Glomus* in group II. Group III included sequence of FArc1 and was distantly associated with genus *Archaeospora*. The sequence of FA14 formed separate group (group IV) without any known sequences in the database (Fig. 1). The observations suggested that a limited number of AM fungi are associated with the plants growing in fly ash pond.
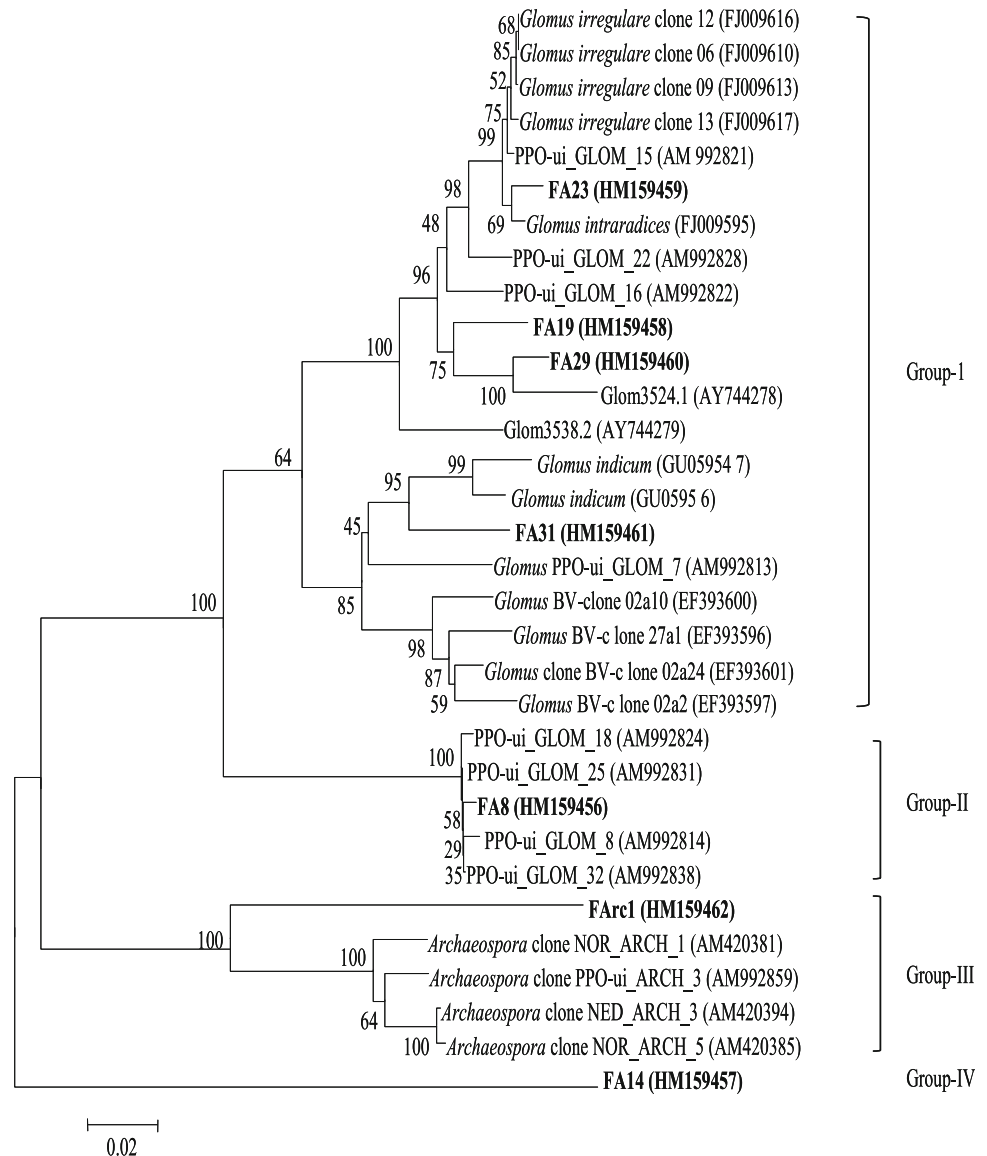
After 6 months, AM fungal colonization was observed in both inoculated and non-inoculated treatments. However, colonization of AM fungi in the inoculated treatments was four times more than the control treatment. AM colonization in control treatment may be due to the presence of some of the indigenous spores present in fly ash. The nature of AM fungal spores in control treatment was similar to some of the species (mostly *Glomus* spp.) occurring in fly ash. AM fungal colonization significantly increased the growth of *E. tereticornis* compared to the control treatment (Table 3). Inoculation of AM fungi increased the chlorophyll content in seedlings (55.5%) as compared to the control plants (Table 3). The AM fungal colonization significantly increased P concentration in the shoot of *E. tereticornis* in comparison with the non-inoculated treatment (Table 3). Uptake of Al, Zn, and Cu was significantly reduced in plants inoculated with AM fungi, but no significant difference in Fe uptake was observed (Table 3).

Chemical and biochemical properties of fly ash were positively affected by inoculum of AM fungi compared to initial or non-inoculated fly ash. Inoculation of AM fungi significantly increased the organic carbon (%), total N, and enzyme activities such as acid phosphatase and urease in fly ash compared to control treatment (Table 3). EC of fly ash was significantly high in both inoculated and non-inoculated treatments compared to initial value, and the maximum was recorded in AM inoculated fly ash (Table 3).

Discussion

The low rate of AM fungal colonization and the presence of very few spores in rhizosphere of plants grown on fly ash were attributed to adverse conditions. The soil structure and composition not only affect the spore population but also the biological activity of endophytes [26]. However, the presence of colonization and spores reflects the mycotrophic nature of the plant species studied and the ability

Fig. 1 Neighbor-joining phylogenetic analysis of AM fungal partial 18S-ITS1-5.8S-ITS2 rDNA region sequences obtained from plant root samples of fly ash pond. Sequences obtained in the study are shown in *boldface*. Numerical values on branches are the bootstrap values as percentage of bootstrap replication from 1000 replicate analysis. The scale represents substitution per site

of AM fungi to associate a wide range of host species in fly ash pond. In this study, it was reported that *G. rosea* had widest host range as it colonized most of the plant species. Variation in spore density/diversity and colonization of AM fungi associated with different host plant species may depend on a variety of mechanisms, including variation in host species and their phenology, mycorrhizal dependency, host plant-mediated alteration of the soil micro environment, or other unknown host plant traits [12, 22].

The identification of AM fungi using spore morphology and molecular approaches revealed that most species belong to the genus *Glomus*. This is consistent with the results of Selvam and Mahadevan [41] and Bedini et al. [5], who had reported *Glomus* as the most common species in fly ash. In this study, the species identified based on molecular characterization were not similar to those identified by spore morphology. This can be attributed to

the fact that spore production might not have occurred in AM fungi associated with roots or some of these species may rely on vegetative strategies for colonization and dispersal [8]. On other hand, fungal spore diversity differs seasonally, with some fungi sporulating in late spring and others sporulating at the end of summer [34, 38]. Contrary to this, Guadarrama and Alvarez-Sanchez [17] reported that seasonality does not affect the abundance and richness of mycorrhizal spores. In this investigation, sampling was conducted in April (early summer) which may explain the presence of more AM fungal species detected by spore morphology versus molecular tools from roots. As spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts [38]. This factor can also affect spore number estimation as well as species diversity. In general, AM fungal identification is usually

Table 3 Influence of arbuscular mycorrhizal (AM) fungi on the growth of *E. tereticornis* seedlings and physico-chemical and bio-chemical properties of fly ash

Parameters	Control	AM fungi
Plant height (cm)	20.38 ± 1.3	37.6 ± 1.0*
Shoot dry weight (g)	0.83 ± 0.1	1.5 ± 0.1*
Root dry weight (g)	0.10 ± 0.0	0.2 ± 0.0*
AM fungal colonization (%)	18.4 ± 0.6	76.1 ± 2.3*
Chlorophyll *a* + *b* (mg/g fw)	0.91 ± 0.0	1.4 ± 0.1*
Plant total P (mg/kg)	243.4 ± 3.9	351.2 ± 5.8*
Fly ash chemical and bio chemical properties		
pH (1:2.5)	6.96 ± 0.2*	6.36 ± 0.3
EC (mS/cm)	0.45 ± 0.0	0.65 ± 0.0*
Organic carbon (%)	0.21 ± 0.0	0.29 ± 0.0*
Available P (mg/kg)	2.59 ± 0.2*	2.24 ± 0.1
Total N (mg/kg)	167.8 ± 4.6	208.9 ± 5.8*
Acid phosphatase (μM/g/h)	117.6 ± 4.3	321.7 ± 7.9*
Urease (μM/g/h)	17.18 ± 1.0	27.4 ± 1.1*
Elemental analysis (mg/kg)		
Al	1994 ± 75.6*	1455 ± 17.6
Fe	1240 ± 36.4	1185 ± 48.6
Zn	60.67 ± 2.5*	44.0 ± 2.2
Cu	38.33 ± 1.2*	23.33 ± 2.1

fw fresh weight, *EC* electrical conductivity

* Significant at *P* < 0.05

performed on the basis of specific spore morphological characteristics. However, the task of identifying spores based on their morphology is notoriously difficult due to alterations of morphological features during spore ontogeny or by parasitism [25, 48]. Therefore, combining molecular techniques with AM fungal morphotype assessment has enabled us to make detailed identifications of root-associated AM fungi in fly ash pond.

To the best of our knowledge, this is the first report on AM fungal molecular diversity in fly ash pond of electrostatic precipitator, although the molecular diversity of AM fungi had been reported from the bottom ash dump by Bedini et al. [5]. The primers GLOM1310 and ARCH1311 used in this study also amplified the sequences of Ascomycota and to a small extent Basidiomycota-related species (data not shown) due to nonspecific annealing of these primers. A similar observation was also reported by others [1, 35]. Phylogenetic analysis revealed that the sequences clustered into four discrete clades, and only a small portion of molecular clade was assigned to the known species including *G. intraradices* and *G. irregular*, leaving other sequences unidentified. Pennisi [32] emphasized on the growing number of unidentified fungal sequences without corresponding morphospecies records in the International Nucleotide Sequence Database Collaboration (INSDC)

stating that the fungal diversity is higher than its current estimate. Our phylogenetic results support the hypothesis of Öpik et al. [29] that AM fungi may exhibit different distribution patterns, and a number of AM fungi related to *G. intraradices* may have global distribution. The AM fungi identified through spore morphology such as *G. etunicatum*, *S. heterogama*, and *G. intraradices* reported in this investigation have also been found in heavy metal contaminated soils [16, 53], suggesting their adaptability to different stress conditions.

The establishment of vegetation on ash pond using suitable microorganisms is a useful strategy in environmental management. Till date, rehabilitation performance has been found to be variable in many studies, and very little information is available on the effect of microorganisms on the vegetation cover on fly ash pond. In this investigation, the growth of *E. tereticornis* seedlings was significantly increased in AM inoculated plants as compared to control seedlings. It is also evident that AM fungi promote plant growth and increase soil aggregation [11, 52]. The AM fungi quickly lose their symbiotic efficacy when cultivated without edaphic stresses of the environment from where they have been originally isolated [10, 28]. It is also known that the inoculum should be produced with original edaphic stresses especially for AM isolates from extreme environments [28]. The AM fungal colonization increased in the inoculated treatment due to higher amount of inoculum and their adaptation to fly ash stress, and these were able to form mycorrhizae with *E. tereticornis* seedlings. The presence of AM fungal colonization in non-inoculated plants may be due to low number of propagules in fly ash and limited AM fungal diversity that results in low rate of colonization. The nature of spores present in the control treatment was similar to some of the species especially *Glomus* spp. present in the fly ash, because the fly ash used for nursery experiment was collected from the same site where we studied the diversity of AM fungi.

Although, fly ash contains many essential elements for plant uptake, non-essential elements such as Al, Fe, Zn, and Cu and many other elements might impair with various metabolic processes and so either delay or inhibit the process. It has also been reported that heavy metals decreased P concentration in shoots of *E. globulus* in absence of AM fungi [3]. Although plants of *E. tereticornis* accumulated various amounts of Al, Fe, Zn, and Cu in both treatments, the level of these metals was less when inoculated with AM fungi. The AM fungi increased the tolerance of plants by enhancing plant growth with a resulting dilution effect of metal in host plant or binding of the metal to the fungal cell wall through chitin or glomalin and immobilization in the rhizosphere or the roots [7, 16]. It has also been found that AM fungi alleviate metal toxicity

especially Al, Fe, and Mn and enhance plant growth [27]. The production of chlorophyll was significantly reduced when plants were grown in contaminated soil [30]. However, total chlorophyll production increased in *E. tereticornis* plants significantly in the presence of AM fungal inoculum. The increased chlorophyll content of AM plants may results in increased photosynthesis efficiency of plants.

The chemical and biochemical properties of fly ash were improved by various degrees in inoculated treatments as compared to initial values. The pH of fly ash significantly reduced from slight alkaline to moderate acidic range in both treatments. The reduction of pH in fly ash may be due to the presence of sulfur content in fly ash while leaching of base ions [19]. Further, reduced pH levels in AM fungal inoculated fly ash samples may be attributed to the fact that AM fungi influenced organic acid exudation by plants resulting in reduced pH levels [9]. The AM fungi interact with other soil rhizosphere microorganisms and affect rhizodeposition, and thus the quality and quantity of organic C delivered to soil via fungal hyphae [4]. In general, enzymes are bio-chemical indicators of microbes in heavy metal contaminated soil. High enzymatic activity indicates improvement of contaminated soil properties as well as microbial community development. Among soil enzymes, acid phosphatase and urease play an important role in P, N, and C mineralization, respectively. In this study, acid phosphatase and urease enzyme activities increased in the inoculated treatments compared to the control.

Conclusions

The AM fungal diversity in the plant rhizospheres was variable among the different plant species in fly ash pond. *G. rosea* was the dominant fungus from these soils. Among the six plant species, *L. camara* was associated to all identified AM fungal species except *G. magnicaule* compared to other plants. The occurrence of AM fungi in low numbers in rhizosphere soil and in roots of plants growing in fly ash is an indication of their adaptation to fly ash. Inoculation of AM fungi improved growth and nutrient uptake of *E. tereticornis* seedlings and reduced translocation of metals into shoot portion. The application of fly ash adapted AM fungal species may aid in the environmental management and greening of fly ash ponds.

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