

Exploration of Diversity Status of Beneficial Microbes in Iron Ore Mines in Bellary and Chitradurga Districts, Karnataka State

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Abstract

Attempt was made to investigate the diversity status of beneficial microbial populations in different iron ore mine lease (core zone) and outside (buffer zone) mine lease areas including agricultural fields of Sandur, Hospet and Bellary taluks in Bellary district and Chitradurga, Hiriyur, Holalkere and Hosadurga taluks in Chitradurga district, Karnataka state. It was observed that all the samples had Arbuscular Mycorrhizal (AM) fungal association and Plant Growth Promoting Rhizobacteria (PGPR), but exhibited variation in population density. It was also found that the samples collected from core zone (mined out) area had very less population of AM fungi and PGPR as compared to buffer zone and agriculture fields. A total of 26 different AM fungal species belonging to three genera such as *Acaulospora*, *Glomus* and *Gigaspora* were recorded in Bellary district. Among them, the genus *Glomus* was found to be the dominant with 19 species, followed by *Gigaspora* with 4 species and *Acaulospora* with 3 species. Apart from this, three types of PGPR viz., *Azotobacter*, *Azospirillum* and *Bacillus* were recorded from the study areas in Bellary district. A total of 23 different AM fungal species belonging to three genera such as *Acaulospora*, *Gigaspora* and *Glomus* were recorded in Chitradurga district. Among them, the genus *Glomus* was found to be the dominant with 17 species, followed by *Gigaspora* and *Acaulospora* with 3 species each. Similarly, four types of PGPR viz., *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* were recorded from different study locations but variation in population density in Chitradurga district. It was observed that the microorganisms occurring in the mined out areas (core zone) are removed along with the overburden (OB) wastes. However, the microbial organisms that have been recorded in the proposed mine lease areas (non-mined out core zone) are also occurring in the buffer zone of mine lease areas in different study locations during the period of investigation. The impacts, management and conservation measures of these beneficial microbes and their role in reclamation and rehabilitation of iron ore mines are discussed.

Keywords: Iron ore mine, arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, overburden dump.

Introduction

In India, about 6,83,671 hectares of land is undermining and mining activities on these lands have created wide spread environmental damage that severely affected not only the landscape but also the social well-being (Prasad, 1988). Moreover, ecological problems found at mining sites posing health hazards to nearby villages where the residents as recorded by high incidence of asthma and stomach ailments due to the mining dust and contamination of water. Mining also has led to discharge of millions of tons of waste rock and soils called 'mine spoils' are dumped over millions of hectares as wastelands. Reclamation of these mine spoils is very essential to overcome the health hazards and ecological problems. But, reclamation of mine spoils is difficult because the mine spoils has low water holding capacity, lacks organic matter, deficient in Nitrogen,

Phosphorus and other essential plant nutrients and useful microbes. Using conventional reclamation techniques of grading, re-soiling, and fertilizing of mine spoils are not economically feasible. However, planting of trees and other local vegetation directly on the mine spoils is an excellent alternative method for reclamation. This method will help for stabilization of mine spoils so that the mined out wastes will be sealed away from the environment. Microbes are ubiquitous in nature and they are found in air, water, soil and inside plants, animals and human beings. They can live in extreme conditions such as volcanic vents (350°C) to frigid waters of Antarctic lakes, a top high mountains, polar snow, deserts and forest ecosystems including mining areas. Soil organisms contribute to a wide range of essential services to the sustainable function of agriculture and forest ecosystems.

They are acting as the primary driving agents of nutrient cycling, degrading organic matters, modifying soil physical structure, increasing the amount of essential elements and enhancing plant growth and health. Beneficial microorganisms such as Plant Growth Promoting Rhizobacteria (PGPR) and mycorrhizal fungi are ubiquitous and constitute an integral component of terrestrial ecosystems, forming symbiotic associations with plant root systems. The term mycorrhiza is literally meaning “Fungus Root” to denote the association between certain non-parasitic soil fungi and host plant roots where the relationship is not pathogenic. There are two major types of mycorrhizas viz., Ecto- and Endomycorrhizas. The ectomycorrhizal (ECM) fungi are commonly associated with tree species belong to Gymnosperms and certain Angiosperms. Endomycorrhizal fungi especially Arbuscular Mycorrhizal (AM) Fungi are widely distributed in varied ecosystems and associated with all the plants. In lowland humid tropics, most of the vascular plant species form AM fungal associations. The mycorrhizal associations are so common under natural soil conditions while a non-mycorrhizal tree is usually an exception. They are extremely important as they help in transfer of phosphorus and other essential nutrients to the plant system from the soil. The mycorrhizal fungi improve seedling growth and survival by enhancing the uptake of nutrients and water and increasing root life span. The mycorrhizal fungi have a great influence on the survival of plants in new and reclaimed mining areas such as bauxite, coal, copper, iron and others. These symbiotic fungi help in improving plant growth, survival and its establishment in wastelands and degraded lands by improving site quality and they are the cornerstone for proper establishment of functional forest ecosystem.

Inoculating nursery seedlings with selective bio-inoculants (bio-fertilizer) holds promise for improving seedling quality, reducing transplanting period, out planting performances, increased resistance to root or soil borne diseases, parasitic nematodes and climatic stress. Also use of these beneficial microbes as bio-inoculants (bio-fertilizers) would reduce the cost of chemical fertilizers involved in plantation programmes. The effective utilization of bio-inoculants for trees will not only provide economic benefits but also improve and maintains the soil fertility. To afforest mined out areas by planting local grasses, herbs, shrubs and trees, the topsoil is very much essential material because the topsoil is always having humus material, seeds of local grasses, herbs, shrubs and trees and also having native beneficial microbes as compared to subsoil. The topsoil is enriched with organic matter, minerals and has characteristics of nutrient supply and water uptake to the plants. So, it is more hospitable to plant growth. To deliver its full potential for improved plant growth it must retain the advantageous chemical, physical and biological

properties (Visser *et al.*, 1984). In general during mining activities, the topsoil is stripped off and stored as stockpiles until the site is ready for restoration, which may take many years. Studies on reclaimed bauxite mine spoil surface with topsoil in Australia also suggest that recovery of biological and microbial activity as a good indicator of reclamation success (Jasper *et al.*, 1998). But in stored mine spoils and OB wastes, the microbes like bacteria, fungi, actinomycetes and algae are reduced when compared to undisturbed sites (Miller and Cameron, 1976). Further, carbon content of mine spoils is significantly lower due to lack of microbes (Kareem and McRae, 1984). All these characters lead to reduced soil quality, nutrient cycling and lower availability of nutrients in mine spoils and OB wastes. Moreover, the process of opencast iron, manganese, bauxite mining scars the landscape and destroys different microbial communities. Apart from unsightly impacts, the degraded environments created in the aftermath of open cast mining often cannot support biomass development. In other words, extensively mined out lands usually do not possess sufficient surface soil for plant growth. Therefore, it is essential to introduce the beneficial microbes such as Mycorrhizal (ECM and AM) fungi, PGPR such *Azotobacter*, *Azospirillum*, *Rhizobium* and *Phosphobacterium* in mine spoils along with the suitable local grasses, herbs, shrubs and tree species for the better reclamation. Very limited reports are available on the status of beneficial microbes in coal and iron ore mines in different parts of India (Ganesan *et al.*, 1991; Mohan *et al.*, 2016). Hence, it is important to know the diversity and abundance status of different beneficial microorganisms in any given area before subjecting the areas to activities like mining. Therefore, an attempt was made to investigate the status of microbial populations such as PGPR and AM fungi in roots and soil samples collected from different sample plots of iron ore mine lease (core zone) and outside mine lease (buffer zone) areas at 3 different taluks viz., Bellary, Hospet and Sandur in Bellary district and at 4 different taluks such as Chitradurga, Hiriyur, Holalkere and Hosadurga taluks in Chitradurga district, Karnataka state.

Materials and methods

Collection of soil samples: Roots and soil samples were collected from 36 different sample plots and 40 different sample plots in core zone and buffer zone areas of different mine leases in Bellary and Chitradurga districts respectively. All the samples were brought to the laboratory for processing and estimation of soil microbial population by adopting standard procedures and techniques. The samples were kept in refrigerator at 4°C until further use.

Enumeration of soil microbial population: The soil microbial load viz., PGPR and AM fungi was determined from different soil samples collected from various sample plots.

Serial dilution and plating techniques as described by Parkinson *et al.* (1971) was adopted for enumerating the population of bacteria, fungi and actinomycetes. The AM fungal spore population was estimated by using wet sieving and decanting technique (Gerdemann and Nicholson, 1963). AM fungal root colonization was done by using root clearing and staining techniques (Phillips and Hayman, 1970; Koske and Gemma, 1989) and data on percent root colonization of AM fungi was also estimated by using gridline intersect method (McGonigle *et al.*, 1990) by using following formula:

$$\text{Percent root colonization} = \frac{\text{No. of root segments colonized}}{\text{Total No. of root segments examined}} \times 100$$

Estimation of soil bacteria and fungal population: A known quantity of soil sample was weighed (1 g) and suspended in 100 mL sterile water blank. The flask was placed over a gyrator shaker allowed to agitate for 10 minutes. After 10 minutes, using a sterile pipette 10 mL solution was withdrawn from the flask and transferred to another water blank with 90 mL of sterile water. Similarly, dilutions of the samples from 10^{-2} to 10^{-7} were prepared by serial dilution method (Parkinson *et al.*, 1971). For enumerating bacteria, 1 mL diluent from 10^{-7} was transferred aseptically to a petri dish containing 15-20 mL of molten cooled Nutrient Agar medium. The plates were then incubated at room temperature for 2-3 d. After incubation, counts of the bacterial colonies were made visually.

Isolation and identification of AM fungal spores from soil samples: Soil samples were collected from the rhizosphere of various host plants in different sample plots in the study locations of both Bellary and Chitradurga districts. A small amount from the rhizosphere soil was dug out to a depth of 10-25 cm, after scraping away the top 1-2 cm soil. The soil samples were collected in neat and clean zip-lock polythene bags and stored at 5°C until the samples were processed. 100g rhizosphere soil was thoroughly mixed and dispersed in 1 L water and the suspension was left undisturbed for 5-10 minutes to allow the heavier particles to settle. Then the suspension was decanted through 710, 250, 125, 75 and 45 μm sieves and the remains on the sieves were washed into beakers (Gerdemann and Nicholson, 1963). After settlement of heavier particles, the supernatant was filtered through girded filter papers. Each filter paper was spread on the petri dish and observed under a dissecting microscope. The intact AM fungal spores were counted and picked up with a wet needle and mounted in polyvinyl glycerol-lactophenol with or without Melzer's reagent on a micro slide for identification. The intact and the crushed spores were examined under a compound microscope and identified according to Trappe (1982) and Schenck and Perez (1987).

Results and discussion

Root colonization and soil spore population of AM fungi:

Data on percent root colonization and soil spore population of AM fungi were recorded from buffer zone (outside mine lease), core zone (mine lease), agriculture fields and OB dump plantation areas in 3 different taluks of Bellary district is presented in Table 1. This study revealed that Hospet and Sandur taluks recorded high and moderate level of percent root colonization and spore population of AM fungi from samples collected from agriculture fields and OB dump plantation sites. In contrast, very high percent root colonization and spore population was recorded from buffer zone (outside mine lease) area. Hospet taluk recorded high percent root colonization and soil spore population of AM fungi from samples of buffer zone (outside mine lease) areas, followed by samples collected from buffer zone of Sandur taluk. The samples collected from Bellary taluk revealed moderate and low percent root colonization and soil spore population in different study locations.

Data on percent root colonization and soil spore population of AM fungi recorded from the mine lease (core zone), outside mine lease (buffer zone), OB dump plantation areas and agriculture fields in 4 different taluks of Chitradurga district is presented in Table 2. The study revealed that Chitradurga taluk recorded moderate and low level of percent root colonization and spore population of AM fungi from agriculture fields and OB dump plantation sites. In contrast, very high percent root colonization and spore population was recorded from outside mine lease areas. Hiriur taluk recorded high percent root colonization and soil spore population of AM fungi from mine lease and outside mine lease areas. The agricultural fields had moderate percent root colonization and soil spore population. The OB dump plantation samples had less percent colonization and soil spore population of AM fungi. The similar trend was found in the samples collected from different mine sites of Holalkere and Hosadurga taluks.

Population status of soil beneficial bacteria: Data on bacterial population from mine lease (core zone), outside mine lease (buffer zone) areas; agriculture fields and OB dump plantations of Bellary, Hospet and Sandur mine sites of Bellary district is presented in Table 3. It was found that maximum population of PGPR was recorded from the samples collected from buffer zone (outside mine lease area) in all the study locations. This is followed by the samples collected from agriculture fields and OB dump plantation sites during the period of investigation. It was also observed that three types of beneficial bacteria *viz.*, *Azotobacter*, *Azospirillum* and *Bacillus* were recorded from different samples screened.

Table 1. Mean percent root colonization and soil spore population of AM fungi from Bellary, Hospet and Sandur taluks in Bellary district.

Sample plots	Bellary		Hospet		Sandur	
	% colonization	Spore population	% colonization	Spore population	% colonization	Spore population
Buffer zone	41-50	166-264	68-83	411-566	68-79	359-493
Core zone	12-14	41-49	19-22	53-76	22-18	57-70
Agri. field	43-48	201-263	53-59	236-298	51-56	206-264
OB dump	22-24	104-131	30-36	125-144	31-36	139-150

Mean of 3 replications.

Table 2. Mean percent root colonization and soil spore population of AM fungi from Chitradurga, Hiriyyur, Holalkere and Hosadurga taluks in Chitradurga district.

Sample plots	Chitradurga		Hiriyyur		Holalkere		Hosadurga	
	% colonization	Spore population	% colonization	Spore population	% colonization	Spore population	% colonization	Spore population
Buffer zone	71-79	343-482	63-69	308-396	74-81	403-488	69-73	366-377
Core zone	55-58	273-297	51-55	242-291	60-69	367-386	51-57	203-252
Agri. field	45-50	196-201	42-50	167-193	41-45	166-197	31-37	133-166
OB dump	22-28	104-124	20-24	97-112	24-30	108-129	19-23	92-102

Table 3. Status of beneficial bacterial population recorded from various sample plots of Bellary, Hospet and Sandur taluks in Bellary district.

Sample plots	Total Bacterial colonies (cfu g ⁻¹)		
	Bellary	Hospet	Sandur
Buffer zone	35-49	113-159	107-135
Core zone	10-11	11-22	14-15
Agri. field	26-38	71-85	58-73
OB dump	22-23	38-47	25-32

Table 4. Status of beneficial bacterial population recorded from various sample plots of Chitradurga, Hiriyyur, Holalkere and Hosadurga taluks in Chitradurga district.

Sample plots	Total Bacterial colonies (cfu g ⁻¹)			
	Chitradurga	Hiriyyur	Holalkere	Hosadurga
Buffer zone	78-82	58-62	110-143	108-128
Core zone	57-76	44-53	91-108	81-93
Agri. field	45-47	32-34	55-62	42-45
OB dump	21-23	21-28	40-45	27-31

Data on bacterial population from different study areas viz., mine lease (core zone), outside mine lease (buffer zone) areas; agriculture fields and OB dump plantations of Chitradurga, Hiriyyur, Holalkere and Hosadurga mine sites of Chitradurga district is presented in Table 4. It was observed that three types of beneficial bacteria viz., *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* were recorded from mine sector in Hiriyyur taluk. The sampling site of buffer zone areas have recorded more bacterial colonies of different kinds of beneficial bacteria, followed by the samples collected from core zone areas. The samples collected from agriculture fields have recorded moderate bacterial population whereas the OB dump plantation had less bacterial population. Four types of beneficial bacteria viz., *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* were recorded from mine sectors in Chitradurga, Holalkere and Hosadurga taluks.

Among the different sampling plots screened, the buffer zone areas have recorded more bacterial colonies as compared to other sample plots in mine sectors of all the four taluks. The use of mycorrhizal bio-fertilizers particularly the AM fungi to enhance the uptake of nutrients (Phosphorus and Zinc) and water for establishment of seedlings on degraded lands including mined out areas and OB dumps. The mycorrhizal fungi profoundly influence the development of vegetation in reclamation and rehabilitation of mine spoils. Many earlier reports show that the mycorrhizal fungi bring an improvement on the growth of plants through an increased P uptake and better survival and establishment potential in mine wastes.

Impacts on microbes: The importance of AM fungi in regeneration of mining sites has been investigated by many earlier researchers in different parts of the world (Daft and

Nicolson, 1974; Daft *et al.*, 1975; Daft and Hacskaylo, 1976; Danielson *et al.*, 1979; Reeves *et al.*, 1979; Allen, 1984; Sylvia, 1988). Extensive colonization of AM fungi in plants growing in coastal soil was recorded by Daft and Hacskaylo (1976) who hypothesized that application of AM fungi may ensure successful reclamation and rehabilitation of coal mine areas. Ganesan *et al.* (1991) reported the distribution of different AM fungi in revegetated coal, lignite and calcite mine spoils of India. Their findings indicate that in the reclamation and rehabilitation of mine waste spoil, mycorrhizal fungi profoundly influence the development of vegetation. Caproni *et al.* (2003) observed that addition of top soil with the planting of mycorrhizal bio-fertilizers inoculated seedlings were efficient to colonize areas with AM fungi after open cast bauxite mining. Mohan *et al.* (2011) studied AM fungal interaction with important tree species in quartz sand mine spoil, Tamil Nadu and they found AM fungal association in rhizosphere soils of all the plants grown in the study area but variation in percent root colonization and soil spore population. Recently, Mohan *et al.* (2016) studied the diversity status of beneficial microbes in iron ore mines of Chitradurga district, Karnataka and they found that all the samples collected from different study sites had AM fungi and PGPR population but variation in population density. It was also observed by them that the core zone of mined out areas had less population of AM fungi and PGPR as compared to buffer zone area of iron ore mines.

Conclusion

The use of mycorrhizal bio-fertilizers particularly the AM fungi play an important role to enhance the uptake of nutrients (Phosphorus and Zinc) and water for establishment of seedlings/saplings on degraded lands including mined out areas and OB dumps. The AM fungi along with PGPR bio-inoculants have profoundly influence the development of vegetation in reclamation and rehabilitation of mine spoils. Hence, the mined overburden areas should be reclaimed and rehabilitated with vigour and suitable native plant species inoculated with potential mycorrhizal and PGPR bio-fertilizers (*Rhizobium*, *Azospirillum*, *Azotobacter*, *Phosphobacterium*) irrespective of other economic utility.

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