



# SUCCESSION OF CULTURABLE MICROBES ON RHIZOSPHERE SOIL OF IRON ORE MINED OVERBURDEN DUMP IN DALLI RAJHARA, DURG, CHHATTISGARH, INDIA

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## Abstract

The assessment of microbiological changes that occur during the maturation of overburden dump in iron ore mined. Dalli Rajhara overburden dump was located in Balod dist. of Chhattisgarh. 0, 3, 7, 8 and 9 year old OB dump was planted by different agencies. Rhizosphere soil was collected from different planted and natural growing planted species. Our results indicate that over time, the microbiologically disturbed overburden dump leads to the development of microbial communities that approximate those of undisturbed soil. When the age of dump were increase number of bacteria and fungi was increased in all age overburden dump. Different plant growth promoting rhizobacteria (PGPR) *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp. and fluorescent *Pseudomonas* sp. was isolated. In 0 year dump (fresh dump) these bacteria was completely absent and *Azotobacter* sp. was completely absent in all year OB dump.

**Key words :** Mining, microorganism, plantation, succession.

## Introduction

Iron ore is one of the most common minerals on the surface of the earth. India is recognized to be among the top fourth biggest exporters of iron ore. The ore was removed by mining activity. The mining are changes in soil stratification, reduced biotic diversity, alteration of structure and functioning of ecosystems; these changes ultimately influence water, nutrient dynamics and tropical interactions (Ghose, 2004; Sadhu *et al.*, 2012; Verma *et al.*, 2017). The other limiting factors for revegetation of mine spoil may be salinity, acidity, poor water holding capacity, inadequate supply of plant nutrients and accelerated rate of erosion (Jha and Singh, 1990). These spoils are not suifig. for both plant and microbial growth (Agrawal *et al.*, 1993). Plantations imparta favorable role in the biological reclamatiion of mine spoil due to modification of the soil characteristics.

The microbes are responsible for most biological transformations and drive the development of sfig. and labile pools of carbon (C), nitrogen (N) and other

nutrients, which facilitate the subsequent establishment of plant communities (Schulz *et al.*, 2013). Microbes play a pivotal role in the cycling of nitrogen; they exclusively mediate nitrogen fixation, denitrification and nitrification (Aislabie and Deslippe, 2013). Microbes exist throughout the soil profile in the rhizosphere of plants and around macropores (Bundt *et al.*, 2001; Fierer *et al.*, 2007). Plant-associated microorganisms play an important role by degrading, detoxifying or sequestering the pollutants and by promoting plant growth (Weyens *et al.*, 2015; Verma *et al.*, 2015).

In iron ore mined overburden dump in Dalli Rajhara, Durg, Chhatisgarh has been planted by different government and private organization. Name of species planted in different years old OB dump and plain plantations are mainly *Tectona grandis*, *Cassia siamea*, *Gmelina arborea*, *Peltophorum pterocarpum*, *Albizia lebbeck*, *Ficus religiosa*, *Ficus benghalensis*, *Gmelina robusta*, *Delonix regia*, *Pongamia pinnata*, *Cassia fistula*, *Dendro calamus* sp., *Leucaena leucocephala*, *Eugenia jambolana*, *Terminalia tomentosa*, *Terminalia arjuna*, *Psidium guajava*, *Dalbergia sissoo*,

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*Eucalyptus* hybrid, *Albizia procera*, *Alstonias cholaris*, *Bauhinia veriegata*, *Annona squamosa*, *Mangifera indica*, *Azadirachta indica*, *Butea monosperma* and *Emblca officinalis* (Banerjee *et al.*, 1997).

The main objective of the study is to understand the succession in culturable microorganism (bacteria and fungi), these are primary requirement for plant establishment and growth. The study not only helps to compare the culturable microorganism of the mine overburden spoil and native soil, but also helps in understanding the future scope of growth of vegetation in the region.

## Materials and Methods

### Study site

Dalli is located on a hill range bounded by 20°33'0" and 20°34'30" N latitude and 81°1' 0" and 81°4'30" E longitude and Rajhara mines are bounded by 20°33'0" and 20°35' 0" N latitude and 81°0'45" and 81°07'0" E longitude under Balod (District) in the state of Chhattisgarh, India. In Dalli-Rajhara 5 mines located in nearby area they are Dalli, Rajhara, Mahamaya, Dulky and Jharandalli. Jharannala is cutting across Dalli and Rajhara hills. The climate of Balod (district) is tropical. The year is divisible in to three well marked seasons viz., summer (March to June), monsoon (July to October) and winter (November to February). Temperature raises up to even 47° C in the month of may/ june and comes down to 6.5°C in December/January. The mean annual rainfall is 1400 mm, 75-80% of which occurs in the monsoon period. The vegetation of natural forest is dominated by teak (*Tectona grandis* Linn. F.). In the present study, following plantation of different age group raised on OB dump and plain areas (adjoining plantation outside the OB areas) of Dalli Rajhara were studied in detail for edaphic and microbial attributes.

1. Fresh OB dump, No plantation (0 year dump, D<sub>0</sub>)
2. 2011-12 OB dump plantation (3 year dump, D<sub>3</sub>)
3. 2005-06OB dump plantation (7 year dump, D<sub>7</sub>)
4. 2006-07OB dump plantation (8 year dump, D<sub>8</sub>)
5. 2007-08OB dump plantation (9 year dump, D<sub>9</sub>)
6. Plain plantation or undisturbed soil plantation (Natural soil).

### Soil sampling

Soil sample were collected from planted trees in iron ore mined OB dump of different age groups including 9, 8, 7, 3, 0 and natural vegetation. Sample uses for microbial quantification were taken from rhizosphere zone. In lab sample were homogenized and spread on paper to remove

plant material, they are air dried, sifted with 2mm mesh sieve and stored at 4°C used for experiment (Parkinson, 1979).

### Microbial assessment

The population of different types of microorganism e.g. fungi, bacteria and plant growth promoting bacteria (PGPB), for example bacteria of nitrogen cycle (*Rhizobium* sp., *Azotobacter* sp., *Azospirillum* sp.) and phosphate solubilising bacteria (PSB) were isolated by standard techniques using different selective media for different microorganism given below: *Rhizobium* sp. was isolated by using yeast extract mannitol agar (YEMA) media (Fred *et al.*, 1932; Vincent, 1970), *Azotobacter* sp. was isolated on Jensen's media (Jensen, 1954; Norris and Chapman, 1968), *Azospirillum* sp. was isolated on semi-solid N<sub>2</sub> free malate media (Okon *et al.*, 1977) and PSB in King's B media. Nutrient agar media (NA) and potato dextrose agar (PDA) media were used for estimating total bacteria and fungi population in soil sample.

### Preparation of serial dilution

Serial dilution of soil was prepared (Aneja, 2007). 1ml of the sample was placed in a sterile Petridish and 10 ml of sterile cooled (40°C) PDA, NA media, Jensen's media (*Azotobacter* sp.) and King's B media for PSB was added in different media (Warcup, 1950).

### *Azospirillum* sp.

*Azospirillum* sp. was isolated from the roots of planted tree at OB spoil of mined area. The feeder root was cut into 1 cm pieces. Surface sterilized with H<sub>2</sub>O<sub>2</sub> for 3 minutes washed with sterilized water and suspended in 15 ml culture tube containing 5 ml semisolid nitrogen free bromothymol blue medium followed by incubation at 27°C. After 3 days gray white ring of 2 mm diameter was observed on the surface of the root segment followed by change in the medium colour from greenish yellow to blue after one week indicating the growth of *Azospirillum* sp. (Subbarao and Dommergues, 2000; SubbaRao, 1983; Vincent, 1970).

### *Rhizobium* sp.

Root system of plant is washed in running water to remove adhering soil particles. They were immersed in 0.1% acidified HgCl<sub>2</sub> for 4-5 mintues. Nodules which were surface sterilized with HgCl<sub>2</sub> were washed repeatedly with sterile water and dipped in 70% ethyl alcohol followed by more washing with sterile water. The nodule was crushed in a small aliquot of sterile water with the help of glass rod. The serial dilutions were prepared from the nodules extracts and aliquot of

appropriate dilution is spread on yeast extract mannitol agar. The plates were incubated upto 10 days in an incubator at 26°C.

$$\text{cfu/g} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Weight of sample}}$$

#### Analysis after incubation

Incubate the plate at 37°C for 24 hour (for bacteria) and 27°C for 3 to 4 days (for fungi) in an BOD incubator. After completing the incubation period, counts the colonies as colony forming unit (cfu) obtained on NA media and PDA using the following formula (Rajavaram *et al*, 2010).

Jensen's medium plates were incubated at 28°C. After 3 days of incubation, flat, soft milky and mucoid colonies of *Azotobactersp.* developed on agar plate (Thompson, 1989; Vincent, 1970). After isolation beneficial bacterial isolates were grown in selective growth medium and culture were maintained for further experiments and application (Jenson, 1954).

## Results

### Microbial content (bacteria and fungi) of iron ore OB soil and NS

#### Status of culturable bacteria in OB and NS (natural, undisturbed soil)

Status of culturable bacteria in rhizosphere region of both kind of plants (planted and naturally growing) on 3, 7, 8 and 9 year old OB dump and NS was analysed (figs 1 and 2). Colony forming unit (CFU) of bacteria was counted in 10<sup>6</sup> dilutions on the NA plates after an incubation period of 24hr at 37°C.

#### Status of culturable bacteria in planted and naturally growing species in OB dump

In 3 year old OB dump planted species showed maximum number of culturable bacterial population in *Mangifera indica* followed by *Moringa pterygosperma*, *Psidium guajava* and minimum was observed in *Tamarindus indica* followed by *Artocarpus heterophyllus*. The mean CFU in the samples was 2.61 x 10<sup>6</sup>/g of spoil soil. Similarly in 7 year old OB dump planted tree species showed maximum number of bacteria in *Butea monosperma* followed by *Eucalyptus* hybrid, *Pongamia pinnata* and minimum was observed in *Ailanthus excelsa* followed by *Ficus religiosa*, *Psidium guajava*. The mean CFU in the samples was 3.48 x 10<sup>6</sup>/g of spoil soil. In 8 year old OB dump the planted tree species showed maximum number of bacteria in *Cassia fistula* followed by *Dalbergia sissoo*, *Pongamia pinnata* and minimum was observed in *Gmelina robusta* followed by *Butea monosperma*,

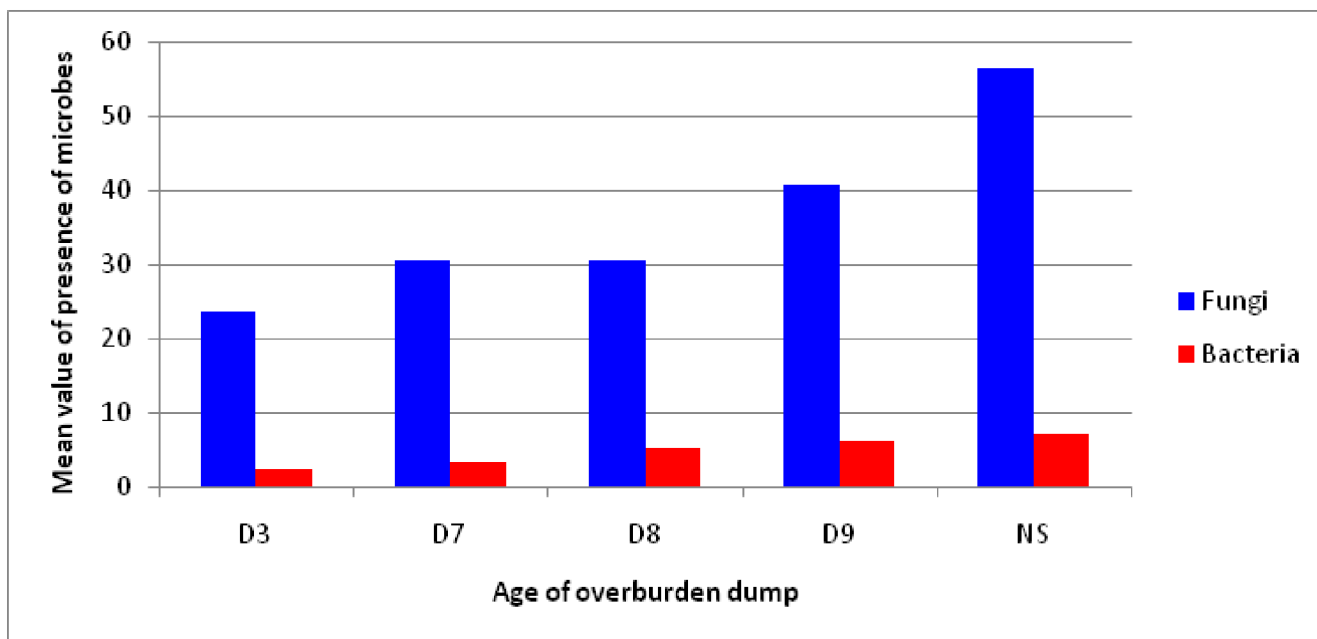
*Eucalyptus* hybrid. The mean CFU in the samples was 5.55 x 10<sup>6</sup>/g of spoil soil. In 9 year old OB dump the planted tree species showed maximum number of bacteria in *Delonix regia* followed by *Gmelina arborea*, *Albizia lebbeck*, *Azadirachta indica* and minimum was observed in *Cassia fistula* followed by *Bamboo sp.*, *Tectona grandis*. The mean CFU in the samples was 6.51 x 10<sup>6</sup>/g of spoil soil. Data was statistically significant at *P* = 0.05 (fig. 1). Bacterial status was observed in rhizosphere of naturally growing plant species on 3 year old OB dump. Maximum numbers of bacteria were observed in *Albizia lebbeck* and *Alternanthera sessilis* followed by *Dalbergia sissoo* and minimum was observed in *Hyptis suaveolens* followed by *Blumea alata*, *Tridax procumbens*. The mean CFU in the samples was 2.29 x 10<sup>6</sup>/g of spoil soil. Similarly in 7 year old OB dump maximum number of bacteria was observed in *Acacia nilotica* followed by *Ziziphus jujuba*, *Albizia odoratissima* and minimum in *Anogeissus latifolia* followed by *Calotropis procera*, *Hyptis suaveolens*. The mean CFU in the samples was 3.64 x 10<sup>6</sup>/g of spoil soil. In 8 year old OB dump maximum number of bacteria was observed in *Albizia lebbeck* followed by *Azadirachta indica*, *Woodfordia fruticosa* and minimum in *Ailanthus excelsa* followed by *Argemone maxicana*, *Lantana camara*. The mean CFU in the samples was 10.16 x 10<sup>6</sup>/g of spoil soil. In 9 year old OB dump maximum was observed in *Tecomas stans* followed by *Acacia auriculiformis*, *Nerium indicum* and *Woodfordia fruticosa* and in *Lantana camara* followed by *Cassia alata*. The mean CFU in the samples was 7.68 x 10<sup>6</sup>/g of spoil soil. Data was statistically significant at *P* = 0.05 (fig. 2).

#### Status of culturable bacteria in NS (natural, undisturbed soil)

In NS maximum bacterial population was observed in *Delonix regia*, *Tamarindus indica* followed by *Gmelina arborea*, *Dalbergia sissoo* and minimum was observed in *Psidium guajava* followed by *Ficus religiosa*, *Annona squamosa*. The mean CFU in the samples was 7.41 x 10<sup>6</sup>/g of spoil soil (fig. 1). Similarly maximum population in *Tridax procumbens* followed by *Ziziphus jujuba*, *Anogeissus latifolia* and minimum in *Argemone maxicana* followed by *Blumea alata* and *Nerium indicum* were recorded. The mean CFU in these samples was 9.98 x 10<sup>6</sup>/g of spoil soil. Data was statistically significant at *P* = 0.05 (fig. 2).

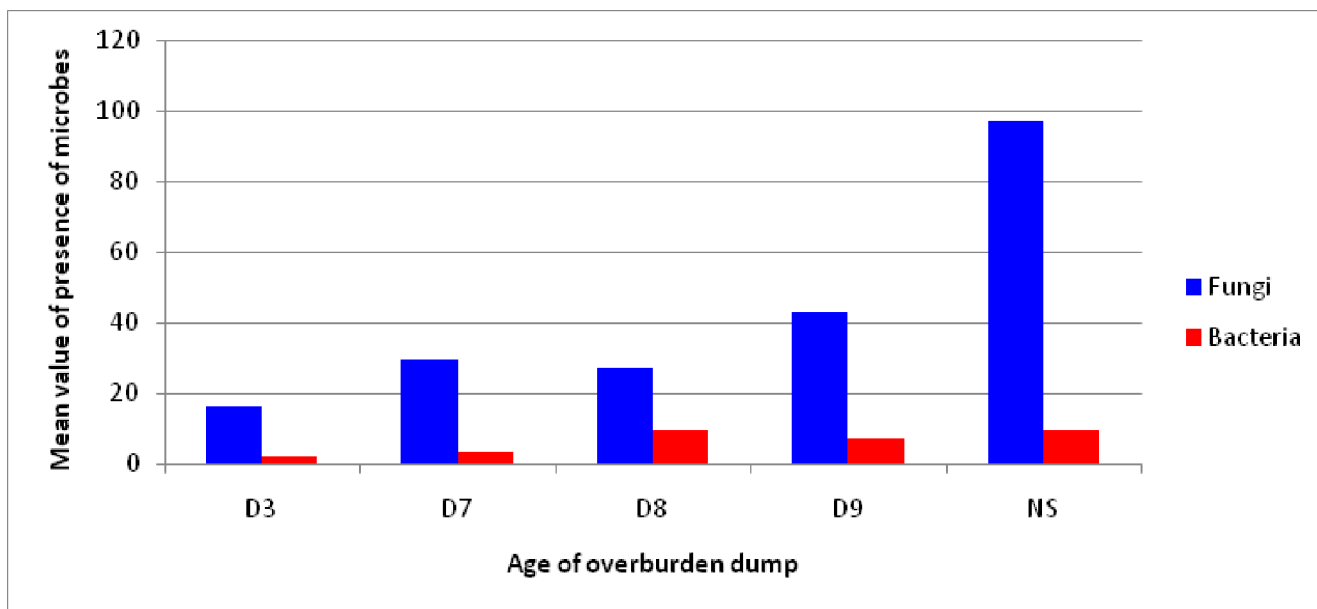
#### Status of culturable fungi in OB and NS (natural, undisturbed soil)

Status of culturable fungi in rhizosphere region of



Bacteria and fungi were measured in per g soil; D3-D9 represent age overburden dump.

**Fig. 1 :** Mean value of presence of microbes in rhizosphere of planted tree species at different age overburden dump in iron ore mine spoil soil.



Bacteria and fungi were measured in per g soil; D3-D9 represent age overburden dump.

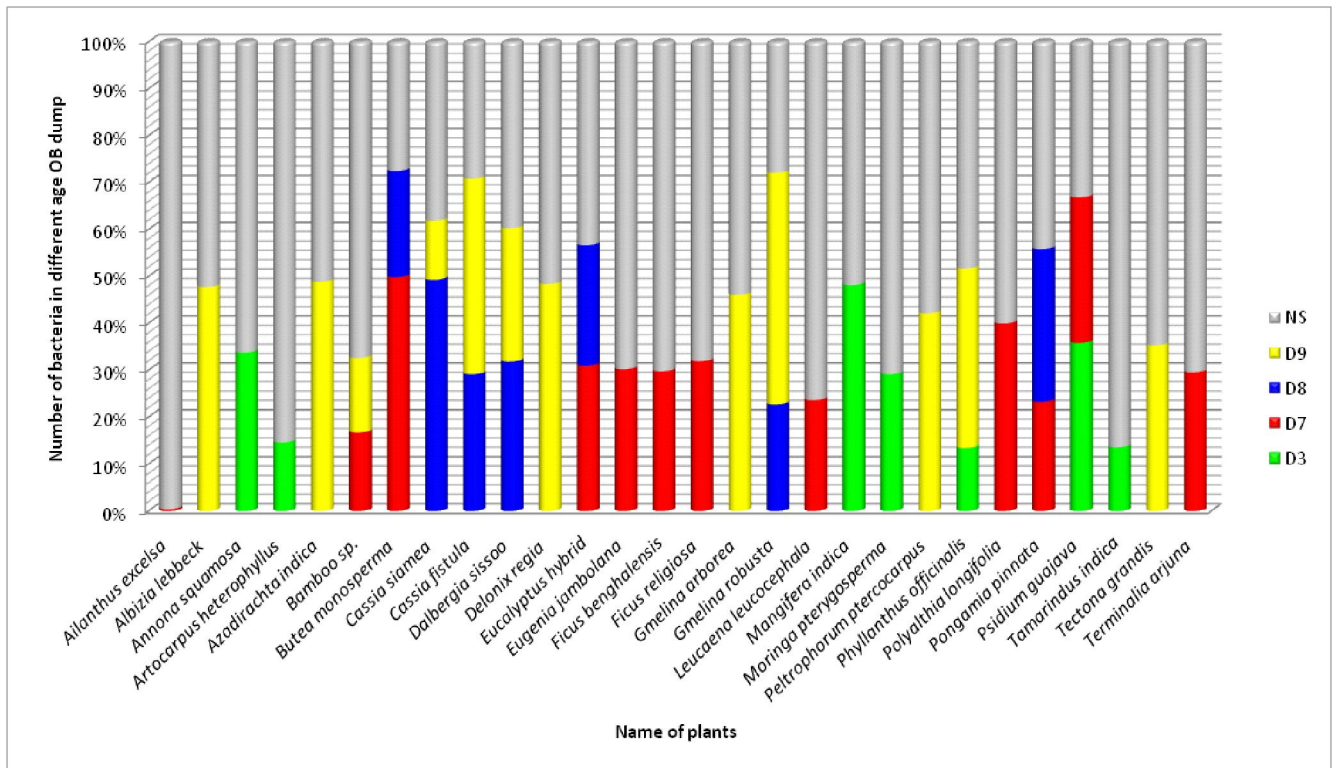
**Fig. 2 :** Mean value of presence of microbes in rhizosphere of naturally growing tree species at different age overburden dump in iron ore mine spoil soil.

both kind of plants (planted and naturally growing) on 3, 7, 8 and 9 year old OB dump and NS was analysed (figs 3 and 4). CFU of fungi was counted in  $10^4$  dilutions on PDA plates after incubation of 5-7 days at  $27^\circ\text{C}$ .

#### **Status of culturable fungi in planted and naturally growing species in OB**

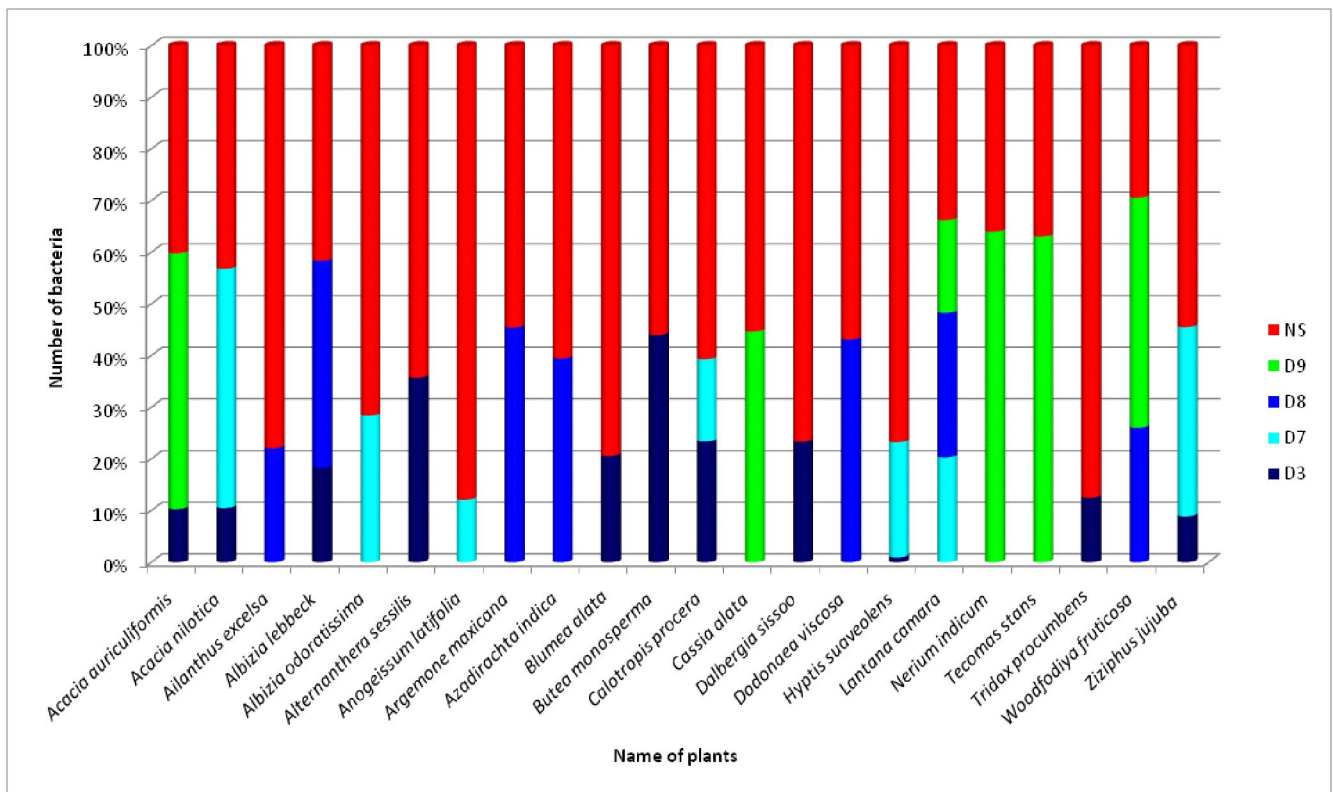
The population of fungi was observed in rhizosphere of planted tree species on different age of OB soil. In 3

year old planted dump maximum number of fungi was observed in *Mangifera indica* followed by *Moringa pterygosperma*, *Artocarpus heterophyllus* and however, minimum number was observed in *Tamarindus indica* followed by *Annona squamosa*, *Phyllanthus officinalis*. The mean CFU in the samples was  $23.93 \times 10^4/\text{g}$  of spoil soil. Similarly in 7 year old planted OB dump maximum number of fungi was observed in *Leucaena leucocephala* followed by *Psidium guajava*,



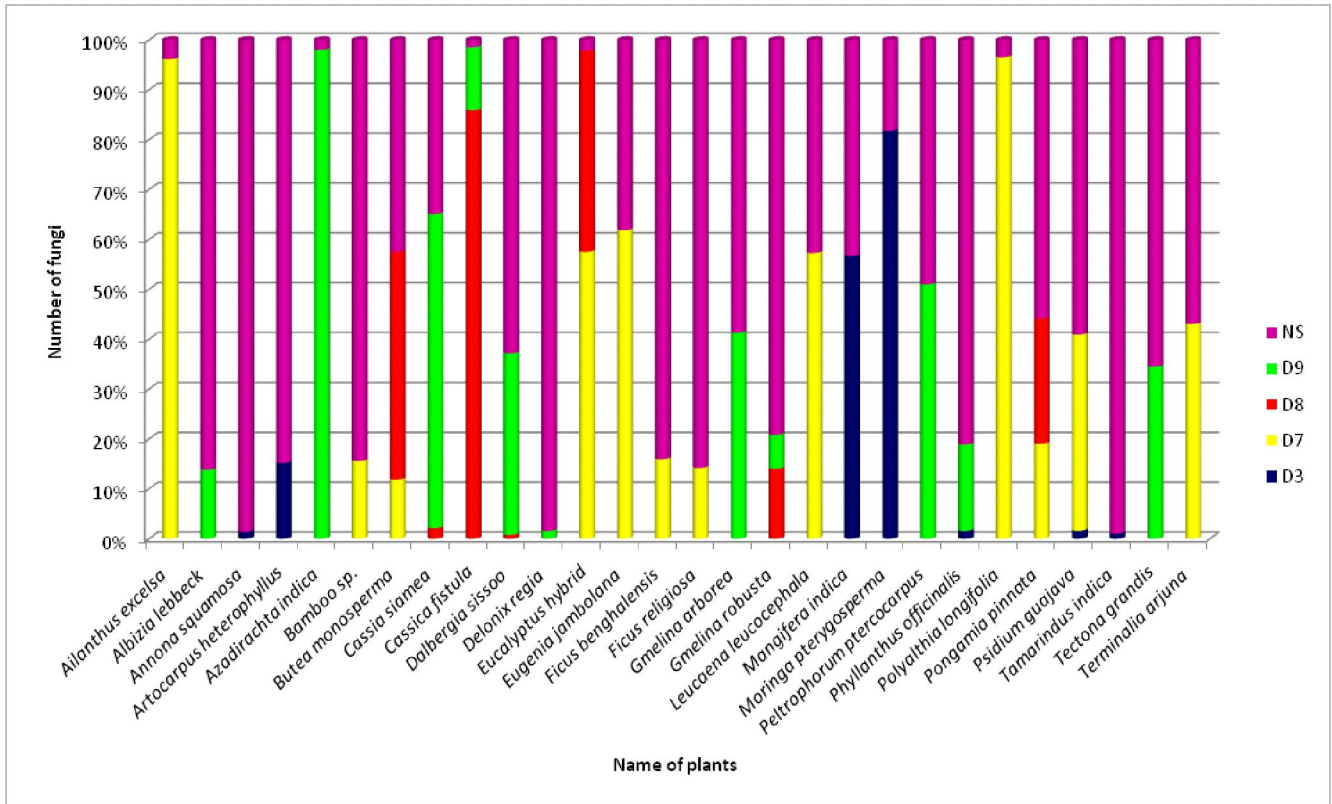
D<sub>0</sub> = fresh dump, D<sub>3</sub>, D<sub>7</sub>, D<sub>8</sub>, and D<sub>9</sub> = 3-9 year old dumps respectively, NS= natural soil

**Fig. 3 :** Status of bacteria in rhizosphere of planted tree species on different age OB and NS (per g soil; data presented in CFU at 10<sup>6</sup> times dilution).



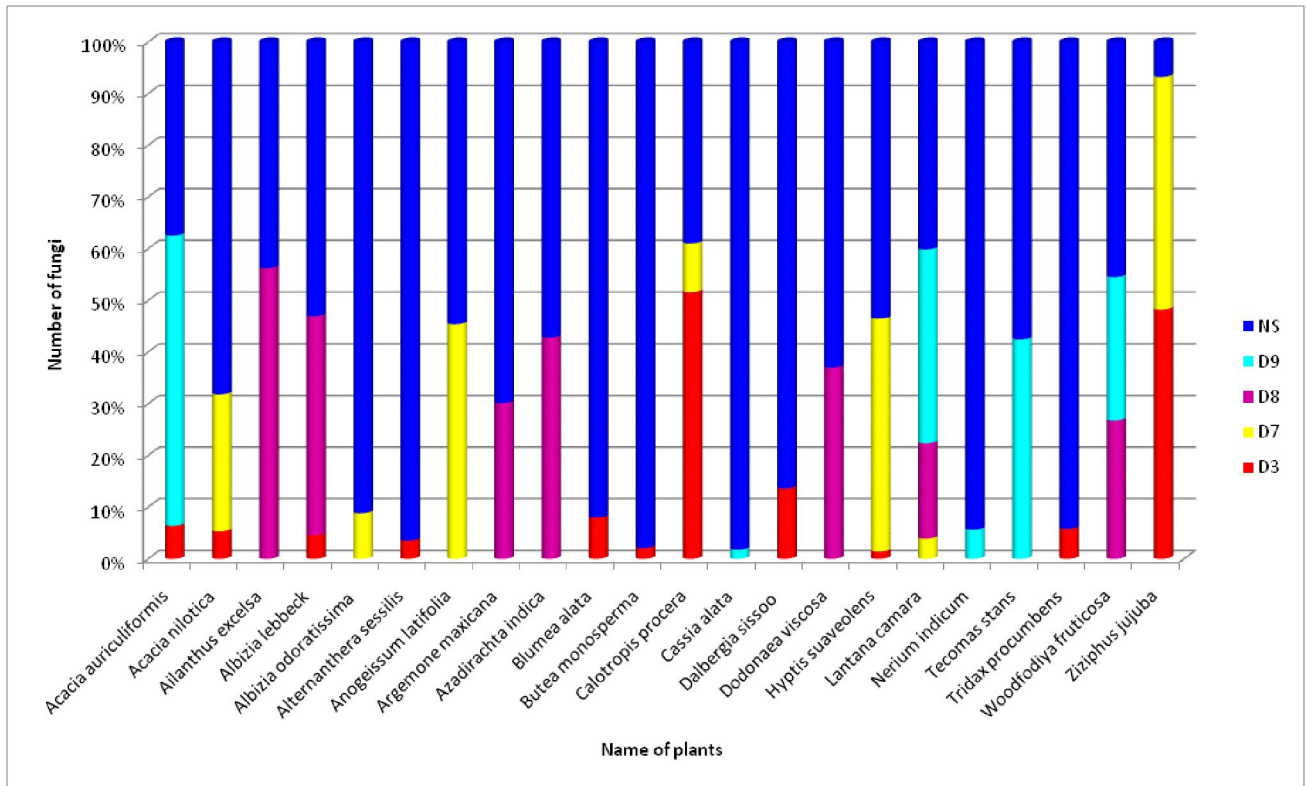
D<sub>0</sub> = fresh dump, D<sub>3</sub>, D<sub>7</sub>, D<sub>8</sub>, and D<sub>9</sub> = 3-9 year old dumps respectively, NS= natural soil.

**Fig. 4 :** Status of bacteria in rhizosphere of naturally growing species on different age OB and NS (per g soil; data presented in CFU at 10<sup>6</sup> times dilution).



D<sub>3</sub>, D<sub>7</sub>, D<sub>8</sub> and D<sub>9</sub> = 3-9 year old dumps respectively, NS= natural soil.

**Fig. 5 :** Status of fungi in rhizosphere of planted tree species on different age OB and NS (per g soil; data was presented in CFU in 10<sup>4</sup> times dilution).



D<sub>3</sub>, D<sub>7</sub>, D<sub>8</sub> and D<sub>9</sub> = 3-9 year old dumps respectively, NS= natural soil.

**Fig. 6 :** Status of fungi in rhizosphere of naturally growing tree species on different age OB and NS (per g soil; data was presented in CFU at 10<sup>4</sup> times dilution).

*Polyalthia longifolia* and *Ailanthus excelsa* and minimum was observed in *Ficus benghalensis* followed by *Terminalia arjuna* and *Ficus religiosa*. The mean CFU in the samples was  $30.62 \times 10^4$ /g of spoil soil. In 8 year old planted dump maximum number of fungi was observed in *Cassia fistula* followed by *Pongamia pinnata*, *Butea monosperma* and minimum was observed in *Dalbergia sissoo* followed by *Cassia siamea*, *Gmelina robusta*. The mean CFU in the samples was  $30.69 \times 10^4$ /g of spoil soil. In 9 year old planted OB dump maximum number of fungi was observed in *Peltophorum pterocarpum* followed by *Cassia siamea*, *Azadirachta indica* and minimum was observed in *Delonix regia* followed by *Gmelina robusta*, *Albizia lebbek*. The mean CFU in the samples was  $40.90 \times 10^4$ /g of spoil soil. Data was statistically significant at  $P = 0.05$  (fig. 3).

Fungal status in rhizosphere region of naturally growing tree species was observed and in 3 year old OB dump maximum number of fungi was observed in *Ziziphus jujuba* followed by *Calotropis procera*, *Dalbergia sissoo* and minimum was in *Alternanthera sessilis* followed by *Butea monosperma*, *Hyptis suaveolens*. The mean CFU in these samples was  $16.7 \times 10^4$ /g of spoil soil. Similarly in 7 year old OB dump maximum number of fungi was observed in *Ziziphus jujuba* followed by *Hyptis suaveolens* and *Anogeissus latifolia* and minimum in *Albizia odoratissima* followed by *Lantana camara*, *Calotropis procera*. The mean CFU in the samples was  $39.21 \times 10^4$ /g of spoil soil. In 8 year old OB dump maximum number of fungi was observed in *Acacia nilotica* followed by *Woodfordia fruticosa*, *Lantana camara* and minimum was in *Azadirachta indica* followed by *Ailanthus excelsa*, *Dodonaea viscosa*. The mean CFU in the samples was  $27.84 \times 10^4$ /g of spoil soil. In 9 year old dump maximum number of fungi was observed in *Lantana camara* followed by *Woodfordia fruticosa* and minimum was in *Nerium indicum* followed by *Cassia alata*. The mean CFU in the samples was  $43.11 \times 10^4$ /g of spoil soil. Data was statistically significant at  $P = 0.05$  (fig. 4).

#### **Status of culturable fungi in NS (natural, undisturbed soil)**

In NS maximum fungal population was observed in *Gmelina robusta* followed by *Tamarindus indica*, *Tectona grandis* and *Dalbergia sissoo* and minimum was in *Eucalyptus* hybrid followed by *Polyalthia longifolia* and *Cassia fistula*. The mean CFU in the samples was  $56.63 \times 10^4$ /g of spoil soil (fig. 3). Similarly number of fungi in *Lantana camara* followed by

*Dalbergia sissoo*, *Blumea alata* and minimum was observed in *Azadirachta indica* and *Ailanthus excelsa* followed by *Ziziphus jujuba*, *Nerium indicum*. The mean CFU in the samples was  $97.5 \times 10^4$ /g of spoil soil. Data was statistically significant at  $P = 0.05$  (fig. 4).

#### **Status of PGPR in different age OB dump**

Different plant growth promoting rhizobacteria (PGPR) were observed in rhizosphere region of different age plantation in study area. *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp. and fluorescent *Pseudomonas* sp. was isolated, by using different specific medium. In 0 year dump (fresh dump) these bacteria was completely absent. *Azotobacter* sp. was completely absent in all year OB dump. In 9 year old plantation *Rhizobium* sp. (5.79), *Azospirillum* sp. (6.56) and fluorescent *Pseudomonas* sp. (6.91) was increased in mine OB dump. *Azospirillum* sp., was present in higher number (13.06) followed by fluorescent PSB sp. (13.01) and *Rhizobium* sp. (9.99) (fig. 5). Data was statistically significant at  $P = 0.05$ .

## **Discussion**

### **Status of microorganisms (bacteria, fungus and PGPR)**

#### **Status of culturable bacteria and fungi**

Relation between plant and the soil biota is the 'biological engine of the earth' and it is responsible for driving many fundamental processes such as establishment of ecological niche, nutrient cycling and the maintenance of soil structure (Juwarkar *et al.*, 2009). Investigations were carried out on the microbial diversity of iron ore mine soil. In these studies culturable bacteria and fungi were observed, because currently culturing techniques are unable to grow all bacterium and fungus in the laboratory. That all organisms must be growing in their natural environment is axiomatic (Sharma and Pandey, 2010; Vartoukian *et al.*, 2010; Stewart, 2012). So in present investigation only culturable bacteria and fungi were observed. In mine OB land culturable bacteria and fungi was very low. It had been observed by Stroo and Jencks (1982) and Insam and Domsch (1988) that microbial growth and activity decreases as a result of mining. These statements were similar with the findings of present study.

In the present investigation, the bacterial populations were recorded in rhizosphere of different planted tree species in OB dump of different ages. The bacterial population was maximum  $6.51 \times 10^6$  in 9 year old dump while the population was lowest  $2.61 \times 10^6$  in 3 year old OB (fig. 1) and bacterial population was also recorded in

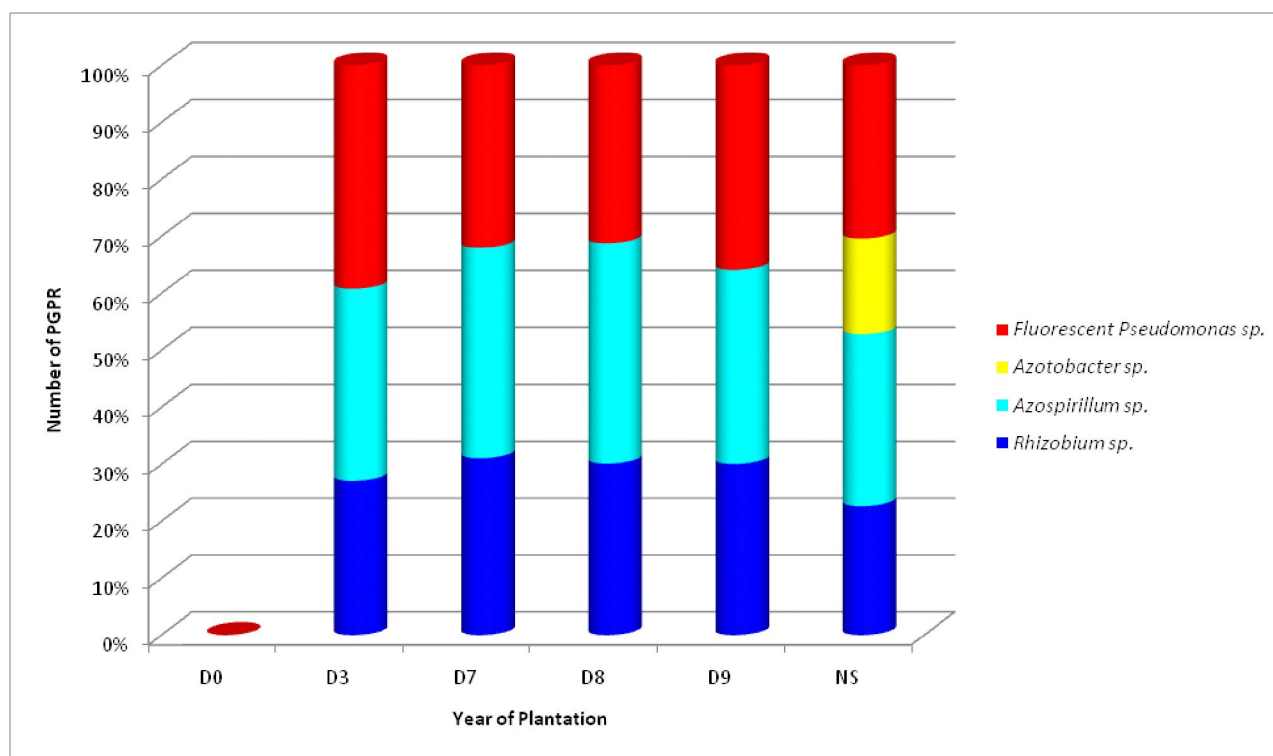


Fig. 7 : Status of PGPR in different age overburden dump (per g soil; Data presented in CFU at  $10^2$  times dilution).

rhizosphere of naturally growing species in OB dump of different ages. These was maximum  $7.68 \times 10^6$  in 9 year old dump while minimum in  $2.29 \times 10^6$  in 3 year old OB (fig. 2). The fungal population was maximum  $40.90 \times 10^4$  in 9 year old dump while it was minimum  $23.92 \times 10^4$  in 3 year old OB (fig. 3) and similarly fungal population was recorded in rhizosphere of naturally growing species in OB dump of different ages. Maximum fungal population was recorded  $43.11 \times 10^4$  in 9 year old dump while minimum  $16.7 \times 10^4$  in 3 year old OB (fig. 4). A continuous increase in microbial number with age in our study indicates continuous soil redevelopment on mine spoils. Microbial processes are so important to ecosystem recovery that the activity of microorganisms may be used as an index of the progress of soil genesis in mine spoils (Schafer *et al.*, 1980; Segal and Mancinelli, 1987; Ross *et al.*, 1990; Banerjee *et al.*, 1999; Banerjee *et al.*, 2000; Maharana and Patel, 2015). Natarajan (1998; 2008) reported several bacterial from waste dump of mines from Bangalore (Karnataka) mine soil. Similarly, the bacterial species were isolated from waste dump of mines in India (Ray and Ray, 2009) and the effective conservation and utilization of microbial diversity in mine soil was reported by Kalia and Gupta (2005). More recently three bacterial groups, namely chemolithotrophic, acidophilic and thermophilic were isolated from coal mine soil at north India (Patel and Behera, 2011). Pinaki *et al.* (2007) investigated the microbial diversity from uranium

mine dump at Jaduguda, India. Plant and microbial diversities in coal mine polluted land in Dhanbad were reported (Kundu and Ghose, 1997; Dutta and Agrawal, 2002). Wilson (1965) recorded increased number of bacterial populations in mine spoils after the establishment of vegetation. All of the above studies give similar result, that number of microbes was less in initial year of dump and gradually increase with age.

Without the cycling of elements, continuation of life on earth would be impossible, since essential nutrients would rapidly be taken up by organism and locked in a form that cannot be used by others (Westover *et al.*, 1997). The reaction involved in elemental cycling are often chemical in nature, but biochemical reaction also play an important role (Lupway *et al.*, 1998). Microbial and biochemical characteristic are used as potential indicators of soil quality because of their central role in cycling of carbon and nitrogen and their sensitivity to change (Horwath *et al.*, 1998). It is evident from the above discussion that bacterial population raises with age of OB dump, which is an indication of soil development process and microbial succession along with vegetation establishment. Srivastava *et al.* (1989) noticed a direct relationship between age of spoil and microbial population and suggested that microbial status is a critical factor in the recovery of mine spoil and can be taken as a functional index of soil re-development. Microbial population and



activity are important factors in the cycling of plant nutrients, soil formation and rate of mineralization of soil organic matter are dependent on the size of microbial population (Ross *et al.*, 1990). Thus soil microbial population is directly involved in the nutrient transformation, maintenance of soil organic matter and nutrient cycling in the soil. Values of beneficial microbial population thus may prove one of the most satisfactory estimates of the soil restoration process (Smith and Paul, 1990). Prakasham and Banerjee (2001) emphasized higher bacterial and fungal populations in naturally vegetated area as compared with the bacterial population in copper mine OB dump.

Verma *et al.* (2017) observed that organic content of the mine spoil soil was significantly lower than the organic carbon content of the native soils. The main sources of organic carbon in the native soil are plant debris dead roots, rhizomes and the surface litter or dead leaves. The soil organic carbon plays a major role in biological activity and fertility of the soil higher organic carbon increase the soil porosity that supports the growth of the soil microorganism. The lower level of organic carbon in mine spoils causes disruption of the ecosystem functioning (Stark, 1977) and depletion of organic pool (Parkinson, 1979). When age of spoil was increased organic carbon and other physico-chemical parameters was also increased.

Verma *et al.* (2017) also observed that when age of spoil was increased concentration of heavy metal was decreased and these condition was also responsible for improve number of microbes and growth of planted and naturally growing plant species. Verma *et al.* (2016) observed that fungal isolates of iron ore mine OB dump have capability to observed heavy metal in laboratory condition. In present investigation, age of spoil was increased so number of culturable bacteria and fungi was also increased. Root exudates stimulate the growth of bacterial and fungal populations in the vicinity of the roots (Rovira, 1965). Several studies have indicated that the structural and functional diversity of rhizosphere populations is affected by the plant species due to differences in root exudation and rhizo-deposition in different root zones (Smalla, 2001). Rhizosphere microorganisms exert strong effects on plant growth and health by nutrient solubilization, N<sub>2</sub> fixation or the production of plant hormones (Smalla, 2001). Manoharachary (1977) reported a direct correlation of moisture and fungal members of various soils. Presence of fungi in the rhizosphere of plants is useful to plants due to their significance as phosphorus solubilizers and producer of plant growth promoting hormones (Garrett,

1956).

### Presence of PGPR in mine land

In the present study, number of PGPR in iron ore mine OB was very low and *Azotobactersp.* was completely absent in OB (fig. 5). Mishra *et al.* (1990) was also observed ammonifer, (*Nitrobacter sp.*, *Nitrosomonas sp.*) and denitrifier were absent in ferogenous soil of Daitary mine. Fungi, *Nitrobacter sp.* and *Nitrosomonas sp.* were absent in laminated iron ore soil of Daitary mine. However, only *Rhizobium sp.* was present in hematite iron ore soil of Daitary mine.

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