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Rehabilitation of Bauxite Mine Spoils With Beneficial Microbes and Fast Growing Tree Species

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Abstract

Bauxite mine spoils in Yercaud hills, India, was rehabilitated using beneficial microbes and suitable fast growing tree species in this study. Normally to rehabilitate the mine spoils, topsoil is spread over the mine spoils before planting trees. The topsoil has good structure, water holding capacity and beneficial microbes like arbuscular mycorrhizal (AM) fungi, which are very essential for plant growth. These qualities are lacking in the mine spoils as topsoil is stripped off during mining. In this study, instead of using top soils, *Glomus aggregatum* and other beneficial microbes *Rhizobium sp.*, *Azospirilum sp.* and Phosphobacterium (PSB) were inoculated to suitable tree species *Acacia auriculiformis*, *Casuarina equisetifolia,* and *Eucalyptus camaldulensis*. The seedlings were grown in sieved bauxite mine spoils as potting media under nursery conditions in for three months. From the nursery experiments, it was found that the AM fungal inoculation and other beneficial microbes not only improved the seedling quality in terms of biomass and growth but also ameliorate the bauxite mine spoils in soil aggregation. Thus, these seedlings with AM fungi and other beneficial microbes were directly transplanted at bauxite mine spoils without any site preparation, and the growth and survival rate were monitored for two years. The AM fungi and other beneficial microbes inoculated seedlings showed 90-95% survival over the control seedlings. Their growth was also significantly higher than the control seedlings. The *Rhizobiu*m sp. and AM fungi inoculated seedlings of *A.auriculiformis* were also showed good performance significantly in height and survival. In *E. camaldulensis,* the inoculation of AM fungi and PSB resulted in better growth than other treatments. In *C. equisetifolia,* the inoculation of AM fungi and *Frankia* was found more suitable. The soil analysis showed that the contents of N, P and K had improved in the rhizosphere of those tree seedlings. AM fungi were also recovered from the rhizosphere of *E. camaldulensis, C. equisetifolia* and *A. auriculiformis* planted at bauxite mine spoils.

Introduction

Mining activities have created wide spread environmental damage that severely affecting not only the landscape but also the social well-being of surrounding communities. Mining also has led to discharge of millions of tonnes of waste rocks called 'mine spoils' that became wastelands. During mining activities the topsoil, having good structure and beneficial microbes, is stripped off and dumped with mine spoils. The topsoil, enriched with organic matter, minerals and has characteristics assisting in nutrient supply and water uptake to the plants, are therefore more hospitable to plant growth. Furthermore, carbon content of mine spoils is low due to lack of microbes. All these characters lead to reduced soil quality, nutrient cycling and lower availability of nutrients in mine spoils that causes difficulties in rehabilitation. Introducing beneficial microbes such as arbuscular mycorrhizal (AM) and nitrogen fixing bacteria like *Rhizobium*, *Azospirillum*, and Phosphobacterium (PSB) in mine spoils along with the suitable trees for the better rehabilitation. Studies on restored bauxite mine spoil in Australia also suggest that recovery of biological and microbial activity could improve rehabilitation success (Jasper *et al.,* 1998). In this study, the bauxite mine spoils at Yercaud Hills, India, was selected for rehabilitation with fast growing tree species and beneficial microbes.

Materials and Methods

Study Site and selected tree species

The study proposed for reclaiming the bauxite mine spoils is at Yercaud Hill, Salem District, Tamil Nadu (11˚ 48' to 11˚ 50' and 78 ˚13' to 78 ˚14 E') at an elevation of 1,640 m a.s.l. where the Madras Aluminium Company (MALCo) has acquired about 190 ha of land for open cast mining. The site receives 1500 mm average rainfall annually and has a wide diversity of plant species. The bauxite mine spoils were collected from the mining area and used for nursery experiments. The selected tree species for this study are *Acacia auriculiformis*, *Casuarina equisetifolia,* and *Eucalyptus camaldulensis.*

Isolation and Culture of Beneficial microbes

AM fungi *Glomus aggregatum* Schenck and Smith was isolated from the rhizosphere of *Syzygium cumini* from the adjacent areas of bauxite mines by the method of Gerdemann and Nicolson (1963) and identified with Schenck and Perez manual (1990). The freshly collected *G. aggregatum* spores were multiplied and maintained in sterile soil media (alfisoil:sand) with *Sorghum bicolor* (L.) Moench (as a host) under laboratory conditions for six months in clay pots. *Rhizobium* sp. was isolated from the crushed nodules of *A. auriculiformis* and cultured in yeast extract mannitol medium under sterile conditions. The colonies of *Rhizobium* sp were again pure cultured in yeast extract mannitol broth and maintained in laboratory for further use. The cultures of *Azospirillum* and PSB were made in Rodriquez Caceres medium and Pikovaskya medium respectively. *Frankia* was isolated from the nodules of *C.equisetifolia* and cultured in P media for mass production.

Analysis of mine spoils

Sieved bauxite mine spoils were analyzed for nutrient status by the method of Jackson (1971) and the physico and chemical properties of the bauxite mine spoils are given in Table 1.

Table 1 Physico chemical properties of sieved bauxite mine spoils (mean of 3 replicates)

pH	E.C mS	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
6.0 (± 1.24)	0.08 (± 0.02)	0.30 (± 0.025)	1.30 (± 0.68)	4.0 (± 1.56)

Inoculation of beneficial microbes in the selected tree species

The seedlings of selected tree species were raised in nursery and maintained with collected bauxite mine spoils as potting media. Inoculation of *Rhizobium* sp was achieved by applying 20 ml of rhizobial suspension to 7-day-old *A. auriculiformis* seedlings; whereas 10 ml of *Azospirillum* and PSB were applied to *E. camaldulensis* seedlings and 5 ml of *Frankia* was applied in *C. equisetifolia* seedlings. The seedlings were also inoculated with AM fungi along with these with other beneficial microbes (*PSB, Azospirilllum, Rhizobium, Frankia*) and maintained in the nursery for three months.

Harvest and assessment of seedlings

Ninety days after emergence the seedlings were harvested with their entire root system intact. The roots were washed free of soil and nodule numbers of *A. auriculiformis* and *C. equisetifolia* were counted. The root length, shoot length, number of branches and collar diameter of each seedling were measured.

Transplanting of seedlings

The beneficial microbes inoculated seedlings of *A. auriculiformis, C. equisetifolia,* and *E. camaldulensis,* were transplanted in the bauxite mine spoils in 1-foot deep pits at an espacement of 1.5 x 1.5 m. The growth and survival of all the seedlings were monitored at regular monthly intervals for two years.

Statistical analyses

The measured variables in the nursery and field experiments were analyzed by Analyses of Variance using Duncan's Multiple Range Test.

Results and Discussion

The inoculation of AM fungi and *Rhizobium* sp. significantly increased the growth of *A. auriculiformis*. The AM fungi caused large changes in the root system morphology of *A. auriculiformis*. AM fungi and *Rhizobium* sp. inoculated seedlings recorded significantly ($P<0.05$) increased in root length, shoot length, collar diameter and biomass. However, root nodules increased significantly in the seedlings inoculated with *Rhizobium* sp alone. Single and combined bio fertilizers (AM fungi, PSB, *Frankia*) inoculated seedlings were significantly ($P<0.05$) increased in their growth and bio mass over un inoculated control seedlings in *C. equisetifolia*. The triple inoculants (AM fungi + PSB + *Frankia*) treated seedlings showed significantly increased growth and biomass, nodule number and collar diameter than control and other treatments. In *E. camaldulensis* the triple bio fertilizers inoculated (AM fungi+ PSB + *Azospirillum*) seedlings showed significantly improved growth and bio mass than control and dual bio fertilizers inoculated seedlings. AM fungi inoculated seedlings showed increased root and shoot length over control and dual bio fertilizers inoculated *E. camaldulensis* seedlings (Tables 2 to 4).

After transplantation of seedlings at bauxite mine spoils their growth and survival were monitored at regular monthly intervals up to two years. Three months after planting at bauxite mine spoils the seedlings inoculated with AM fungi and *Rhizobium* sp showed increased growth over control. Two years after planting the seedlings showed significantly ($P<0.05$) increased growth, collar diameter and branches. *Frankia* and AM fungi + PSB + *Frankia* inoculated *C. equisetifolia* seedlings increased in their growth and collar diameter significantly ($P<0.05$) at the age of three months after planting. Whereas at the age of two years after planting, the triple bio fertilizers inoculated seedlings significantly increased ($P<0.05$) almost two folds over control.

In *E.camaldulensis* the bio fertilizers inoculated seedlings (AM fungi + PSB + *Azospirillum* sp.) showed improved growth and collar diameter at the age of three months after planting. At the age of two years after planting, the AM + PSB + *Azospirillum* sp inoculated seedlings increased the growth in five folds over control and also increase in the collar diameter. The survival of all the seedlings inoculated with beneficial microbes was recorded as 90-95%. In the present study, the growth and survival performance of beneficial microbes inoculated seedlings was significantly higher than uninoculated controls. Most field studies of AM fungi inoculated seedlings are restricted to disturbed sites according to literature. Field inoculation of *Prosopis juliflora* with AM and *Rhizobium* sp. in a semi arid wasteland significantly increased plant biomass and soil nutrient after six years of growth (Bhatia *et al.* 1998).

Similar results were observed in this study in *A. auriculiformis*. In earlier studies growth of *Alnus cordata* inoculated with AM + *Frankia* was significantly improved in their growth in mine spoils one year after planting (Lumini *et al.*, 1994). In the present study, inoculation with AM + *Frankia* increased the growth and survival rate of *C. equisetifolia* at bauxite mine spoils due to the nutrient uptake of N and P through the beneficial microbes. An earlier study showed that colonization by AM fungi enhances plant survival and growth by decreasing phosphorus deficiency and water stress, improving membrane infectivity or by stimulation of oxidative enzyme produce (Salzer *et al.*, 1999). This is the reason the AM inoculated seedlings either singly, or with other beneficial microorganism, produced such high average survival rate of 90%. The significant growth enhancement of *Eucalyptus* spp with AM + *Azospirillum* or AM Fungi + PSB is may be due to increased population of nitrogen fixation, phosphorus mineralization and AM fungal colonization. These effects improve nutrient utilization efficiency of planted seedlings contributing to better plant survival at bauxite mine spoils.

Conclusions

The results of this study support a general conclusion that introduction of trees with beneficial microbes is a suitable and low cost rehabilitation technique to recover the degraded eco systems like mine spoils. The beneficial microbes are eco friendly that help to increase forest productivity in mine spoils.

Table 2 Growth response of *Acacia auriculiformis* to inoculations of arbuscular mycorrhizal fungi and *Rhizobium* sp. under nursery conditions (mean of 5 replicates)

Sl. No.	Treatments	Root length (cm)	Shoot length (cm)	Collar diameter (mm)	Nodules/ Plant	Branches	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
1.	T_1	11.6 a	8.3 b	0.95 a	-	4.3 a	0.017 a	0.116 a
2.	T_2	13.0 a	8.6 a	0.90 a	-	5.0 a	0.025 a	0.104 a
3.	T_3	13.0 a	6.0 a	0.91 b	21.6 b	4.3 a	0.020 b	0.099 b
4.	T_4	15.3 b	10.6 b	1.29 b	12 a	6.33 b	0.030 b	0.180 b

T_1: Control; T_2: AM; T_3: Rhizobium sp.; T_4: AM + *Rhizobium* sp.

Table 3 Growth response of *Casuarina equisetifolia* to inoculations of arbuscular mycorrhizal fungi and other beneficial microbes under nursery conditions (mean of 5 replicates)

Sl. No.	Treatments	Root length (cm)	Shoot length (cm)	Collar diameter (mm)	Nodules/ Plant	Branches / Plant	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
1.	T_1	5.7 a	4.2 a	0.34 a		-	0.0026 a	0.0076 a
2.	T_2	6.8 b	4.1 a	0.46 a	-		0.008 a	0.013 b
3.	T_3	7.8 b	6.3 a	0.46 a	1 a	-	0.0056 a	0.014 b
4.	T_4	8.7 bc	7.45 b	0.60 b	-	-	0.007a	0.010 b
5.	T_5	11.76 c	8.5 b	0.70 b	2 a	-	0.011 b	0.016 b
6.	T6	16.16 d	11.2 c	0.83 c	1 a	1	0.012 b	0.023 c

T_1: Control; T_2 AM; T3: *Frankia*; T_4: PSB; T_5: AM + *Frankia*; T_6: AM + *Frankia* + PSB

Table 4 Growth response of *Eucalyptus camaldulensis* to inoculations of arbuscular mycorrhizal fungi and other beneficial microbes under nursery conditions (mean of five replicates)

Sl. No.	Treatments	Root length (cm)	Shoot length (cm)	Collar diameter (mm)	Branches/ Plant	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
1.	T_1	5.6 a	5.0 a	0.35 a	1 a	0.007 a	0.012 a
2.	T_2	7.3 b	8.2 b	0.38 a	1 a	0.010 ab	0.018 ab
3.	T_3	5.9 a	6.2 a	0.35 a	1 a	0.0096 a	0.010 a
4.	T_4	5.3 a	6.1 a	0.35 a	1 a	0.0076 a	0.011 a
5	T_5	7.8 b	9.6 b	0.55 b	2 a	0.017 b	0.020 b

T_1: Control; T_2 AM; T_3 :PSB; T_4:*Azospirillum*; T_5: AM +PSB + *Azospirillum*
Means followed by same letter are not significantly different at p<0.05 of Duncan's Multiple Range Test.

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