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Reclamation of Fluorspar Mining Waste Land Using *Trichoderma*

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Abstract Largest deposits of Fluorspar ore are available in the Ambadungar area of Kadipani in Vadodara district. Reclamation of Waste Mining land is a major problem. Plantation is the oldest technology available for the restoration of lands degraded by human activity. A study was conducted to find out the plant growth in nearby area and the size of leaves was compared with normal plants. The study included growing of maize and cowpea in soil containing different percentage of mining soil wastes. Biomass of seedlings and chlorophyll contents of raised plants will provide a clue whether plantation in mining land is possible or not. Physico-chemical properties and microbiological characteristics of soil were analyzed.

Keywords: *fluorspar, Zea mays L, Vigna radiata L, mining, reclamation, heavy metals and Chlorophyll Trichoderma*

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1. Introduction

Mining activities result in extensive soil damage, causing drastic disturbances in landscape, altering the ecological environment of soil microorganisms, thereby disrupting the functional stability of the microbial community. The ultimate goal of mine land reclamation means the reestablishment of productive healthy and sustainable ecosystem for post mining activity. Currently criteria for successful restoration have largely been restricted to soil erosion, physicochemical status and vegetation characteristics. Microbial community can proceed detectable changes in soil physicochemical properties, thereby providing early signs of environmental stress or ecological environment evolution in the mining area.

Microorganisms differ greatly in their tolerance for pH, oxidized versus reduced environment. The physical and chemical conditions become more restrictive, the diversity of microbial types that can maintain themselves. The ecosystem so developed are dominated by acidophilus sulphur and iron oxidizers. Some prokaryotic acidophilus heterotrophic organisms are present.

Fluorspar forms a sizeable and economically exploitable mineral deposit in Kadipani, Vadodara, India. The Gujarat Mineral Development Corporation (GMDC) is mining the mineral Flourspar by open cast mining method. Fluorite occurs in association with carbonatite suite of alkaline rocks belonging to Deccan- trap in sub province of lower Narmada Valley. The quality of fluorite is both acid-grade and metallurgical-grade (less than 96% CaF_2 content) [1].

Due to open cast mining a large area is disturbed and requires reclamation. To suggest suitable plantation a preliminary study was undertaken. Heavy metal stress, contributed by elements like Cadmium or Manganese is one of the crucial factors that limit the distribution and Productivity of major healthy crop plants poses severe health hazards to human beings.

One possible approach is to make use of the hyper-accumulators plants & the phytoremediation techniques. Furthermore, a general solution to this problem is chelation, which is generally understood as carbon binding to a compound resulting in a neutrally charged complex that can move more freely through a variety of substrates. Several chelators are known to perform this function in soil plants [2].

Restoration has been attempted on an experimental scale in various parts of the country and its implementation for the whole area has also started at some locations. In some cases the plantation of exotic and horticulture species has also been considered as restoration, while in other cases, *e.g*. Neyveli, the degraded land has been made productive and changed to agricultural land.

The farming land is declining gradually and the main reasons are, intensive use of agricultural practices, urbanization, biotic and abiotic stress etc. Among the abiotic stresses the salinity problem is increasing at an alarming rate throughout the world. Use of Cyanobacterial and other biofertilizer helps to reclaim the soil and reduce the effect of NaCl stress.

1.1. *Trichoderma*: A Unique Biocontrol Agent Enhancing Plant Growth

Trichoderma uses a variety of mechanisms to provide protection against several plant pathogens and plant diseases, and enhance plant growth and development of

the mechanisms for control of pathogen by mycoparasitism and antibiosis, adversely affect the growth and development of the pathogen by competing for the nutrients, oxygen or space, alter fitness of the pathogen. Enhance plant growth and its tolerance to stress. Synthesize cell wall degrading enzyme s(lytic enzymes) that degrade the cell wall of pathogen. *Trichoderma* is an antagonism, may directly kill the pathogen either by antibiosis or by mycoparasitism [3].

Trichoderma longibrachiatum is a soil fungus which is found all over the world but mainly in warmer climates [4]. This species was first characterized by Rifai [5]. It is an exclusively anamorphic species complex allied with the sexual species, it belong to Hypocrea schweinitzii. Evolutionary *T. longibrachiatum* is the youngest clad of *Trichoderma*.

T. longibrachiatum is a fast growing fungus. It typically produces off white colonies that change to grayish green with age. This species is able to grow over a wide range of temperature. *Trichoderma* reproduces through 1- celled, smooth walled conidia. *T. longibrachiatum* occurs commonly on decaying plant material. Efficacy of *Trichoderma* as biofertilizer and antioxidant and minerals was used for ripe tomatoes and soil health improvement in terms of nutrient availability and microbial population

Trichoderma uses cellulases to digest the cellulose from decaying plant biomass, and chitinases to digest the chitinous walls of other fungi.

Microbial degradation of insoluble macromolecules lignin, cellulose and chitin depends on the production of extracellular enzymes. Cellulases are produced by several microorganisms such as bacteria, yeast and fungi. However, the most extensively studied cellulases are those produced by efficient lignocellulose degrading fungi, particularly *Trichoderma*. Also, chitinases can hydrolyze the cell walls of many fungi. The microorganisms that can produce these enzymes are able to destroy the cell wall of many pathogenic fungi for nutrition purpose. Some antagonistic fungi such as *Trichoderma* can attack several plant pathogenic fungi by mycoparasitism as a result of chitinase production .

2. Methodology

A survey has been conducted at the mining site of Kadipani area, of District Vadodara at an interval of a month to collect soil samples. Soil samples were collected from mined, nearby unmined and dumping areas. Random samplings from different mining areas have been conducted. The samples were brought to the laboratory in polyethylene bags, and then study of soil samples was conducted.

2.1. Physico- Chemical Properties of the Mining Soil

Various parameters of the mining soil sample were checked and analyzed in the laboratory. The pH, Electrical conductivity, Moisture Content, Water Holding Capacity, Metallic content (EDXRF-Spectrometer), Organic Matter content, Organic Carbon, Available Phosphorus, Available Nitrogen, Exchangeable Sodium and Potassium.

All analysis was carried out according to the procedures given by Maiti and Banerjee [6].

2.2. Growing of Maize and Moong in Mining Soil Sample

In this method, 3 replicates of maize and moong were planted in four treatments:

1. Normal garden soil
2. 50: 50 % Normal + mining soil
3. 75: 25 % Normal + mining soil+ *Trichoderma*
4. 100 % mining soil.

10 seeds of maize and moong were sown in each replicates. After that it was kept in botanical garden where proper light, temperature and water was provided. The seed germination of 2, 4, 6, 10, 20, 30 days and 40 days in maize was recorded. The plant biomass and plant height was recorded in the period of 7, 14, 21, 28, 56 days. Concern over disturbance to the microbial diversity and consequently soil fertility (as these microbes are involved in biogeochemical processes), as well as economic constraints, have prompted fun- damental and applied research to look for new agro-biotechnologies that can ensure competitive yield increase [12].

2.3. The Percentage Germination rate, Chlorophyll Content, Biomass, Leaf Size, Plant Height Were Measured

Chlorophyll a, b and total chlorophyll were determined by following the method [7]. Biomass is regarded as the natural mass of organisms (fresh weight) in situ, just as they are or it can be measured in terms of the dried organic mass (dry weight). Ascorbic acid content was estimated following the method [8].

3. Results & Discussions

Percentage germination of *Zea mays* was maximum 95% in soil sample 2 after 40 days (Table 1). Percentage Germination in *Vigna radiata* L was also more in soil sample 2 (Table 2).

Plantation is the oldest technology available to restore the degraded lands. The physico-chemical analysis of the soil was done. The pH of soil is alkaline 8.0. The elemental analysis of mined soil and the soil containing maize and moong seedlings was done. It was revealed that sample form mined soil shows higher calcium content.

Pot experiments were carried out in different percentages of mining soils and garden soils. The survey revealed that total chlorophyll content was poor in maize as compared to moong leaves. The maximum chlorophyll content was recorded in the 50-50% (normal garden soil + mining soil). In case of moong, the fresh weight and dry weight was less upto 20 days. The fresh weight of maize was higher in 25%-75% (Mining soil+ Normal soil). Thus, the biomass (Table 4)and chlorophyll content of maize and moong revealed that the mixing of mining soil with normal soil, plantation and healthy crops can be grown in the waste mined land (Table 3, Figure 1).

Table 1. Percentage Germination in Corn

Sr. No	% Germination in Zea mays L.							Soil sample
	2 days	4 days	6 days	10 days	20 days	30 days	40 days	
1.	5	6	10	14	22	65	92	Soil 1
2.	9	10	12	15	24	38	95	Soil 2
3.	7	9	11	14	21	68	84	Soil 3
4.	4	6	9	13	27	35	80	Soil 4

Table 2. Percentage germination in *Vigna* (moong)

Sr. No	% Germination in *Vigna radiata* L.						Soil sample
	2 days	4 days	6 days	10 days	20 days	30 days	
1.	0	52	60	70	92	92	Soil 1
2.	52	52	60	72	100	100	Soil 2
3.	50	50	70	70	90	90	Soil 3
4.	40	46	60	60	80	80	Soil 4

Table 3. Chlorophyll content of *Zea mays* L. and *Vigna radiata*

Sl No	Types of soil sample	Plant	Chlorophyll content mg/g								
			10 days			20 days			30 days		
			Chl a	Chl b	Tot. chl	Chl a	Chl b	Tot. chl	Chl a	Chl b	Tot. chl
1.	**Soil 1**	Zea mays	0.997	1.947	2.178	0.993	1.078	1.880	1.070	1.166	1.930
2.	**Soil 2**	Z. mays	1.264	1.082	1.376	1.064	0.574	1.147	0.786	0.940	1.605
3.	**Soil 3 Trichoderma**	Z. mays	1.055	1.148	1.356	1.032	1.74	2.38	0.917	0.979	1.902
4.	**Soil 4**	Z. mays	1.096	0.985	1.656	1.198	1.95	2.065	0.731	0.926	1.659
5.	**Soil 1**	Vigna radiata	1.122	1.310	2.358	1.349	1.041	2.072	1.124	1.539	2.665
6.	**Soil 2**	V.radiata	0.837	0.561	0.926	1.379	1.119	2.520	1.360	1.170	2.559
7.	**Soil 3Trichoderma**	V.radiata	1.357	1.119	2.520	1.192	1.043	2.265	1.085	1.452	2.540
8.	**Soil 4**	V. radiata	1.192	1.065	2.268	1.280	0.584	1.769	1.204	1.109	2.318

Table 4. Biomass of *Zea mays* L. and *Vigna radiata* L.

No	Type of soil sample	Plant	Biomass (ing)											
			10 days				20 days				30 days			
			Fresh wt		Dry wt		Fresh wt		Dry wt		Fresh wt		Dry wt	
			S wt.	R wt.	S wt.	R wt.	Swt.	R wt.	S wt.	Rwt.	Swt	R wt	S wt	Rwt
1.	**Soil 1**	Corn	0.784	0.714	0.090	0.150	0.892	0.635	0.102	0.115	1.802	0.280	0.426	0.104
2.	**Soil 2**	Corn	0.849	0.294	0.093	0.042	1.620	0.488	0.395	0.132	1.837	0.560	0.228	0.084
3.	**Soil *Trichoderma***	Corn	1.614	0.430	0.471	0.087	0.894	0.347	0.160	0.098	1.173	0.247	0.209	0.109
4.	**Soil 4**	Corn	0.710	1.230	0.65	0.140	0.996	0.207	0.223	0.052	0.724	0.307	0.415	0.192
5.	**Soil 1**	Moong	0.332	0.047	0.048	0.006	0.548	0.085	0.070	0.039	1.710	0.60	1.025	0.26
6.	**Soil 2**	Moong	0.429	0.066	0.062	0.005	0.968	0.115	0.342	0.047	1.970	0.66	0.790	0.34
7.	**Soil 3 *Tricho***	Moong	0.680	0.274	0.096	0.062	0.840	0.138	0.198	0.040	1.053	0.305	0.980	0.47
8.	**Soil 4**	Moong	0.309	0.040	0.055	0.009	0.801	0.062	0.194	0.022	1.621	0.562	0.887	0.32

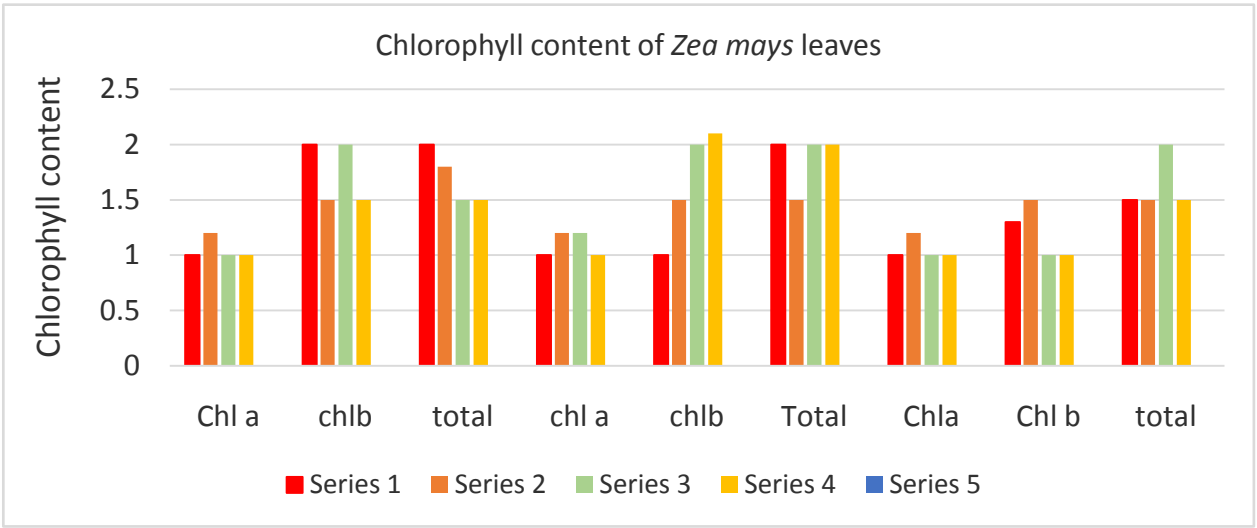

Figure 1. Shows the Chlorophyll content of *Zea mays* L.

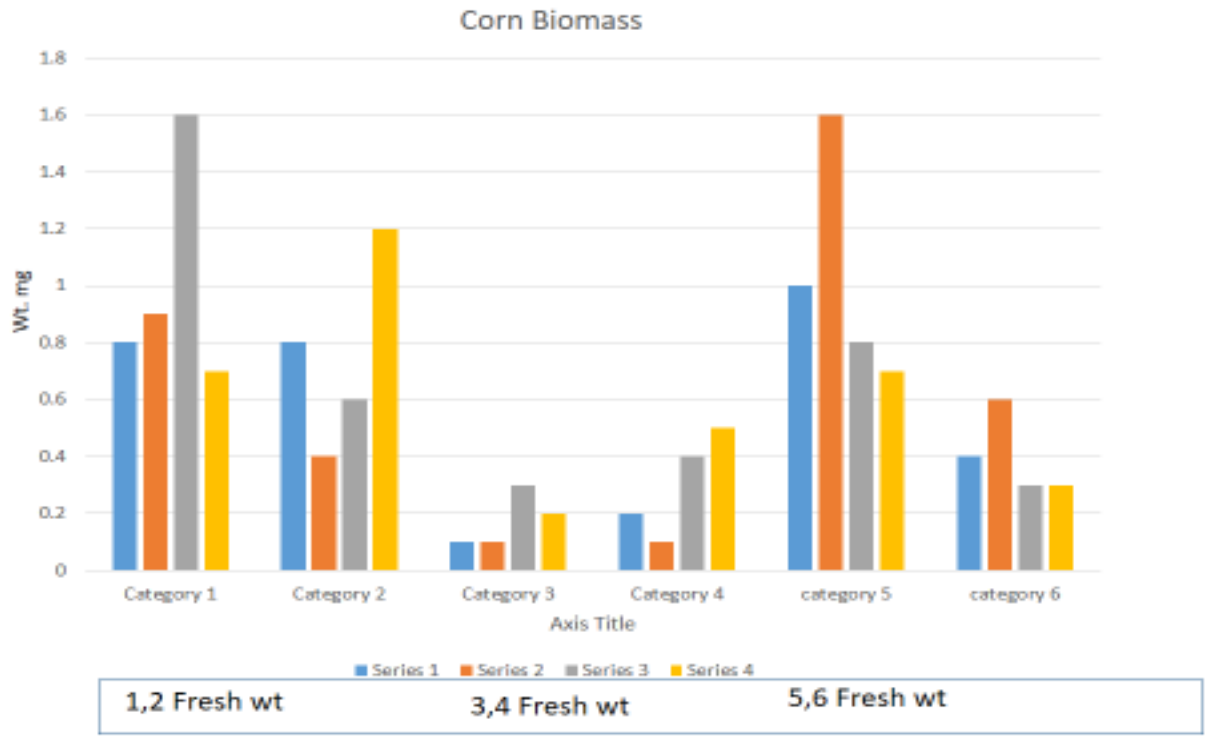

Figure 2. Shows the Biomass of *Zea mays* L

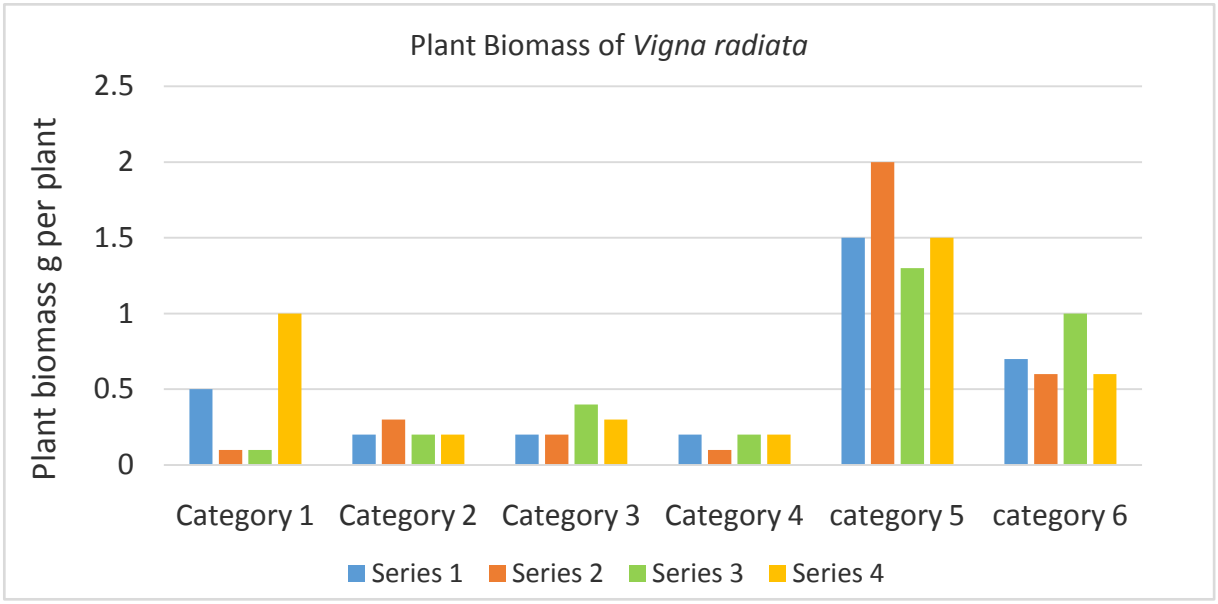

Figure 3. Shows the Biomass of *Vigna radiata*

The results are indicative of more growth in both the cases *i.e.* maize and moong (Figure 2, Figure 3). After one month it is observed that the biomass and chlorophyll content in maize and moong plants were healthy and fully grown in 50-50% (normal garden soil + mining soil).

By plantation, the degraded mined area can be restored aesthetically and hence the spoiled area can be recovered by growing maize, moong and such other crops by mixing with normal garden soil.

Trichoderma is very well known for its ability to colonize roots and enhancing plant growth and productivity. *Trichoderma* can colonize only local sites on roots. It helps in increased plant biomass. *Trichoderma* shows remarkable growth performance due to biocontrol nature and enhanced growth factors produced by this unique fungus.

Trichoderma induces changes in host plants and these changes are directly linked to stress related genes and proteins. Up regulation of sod (Mn) and sod (Cu) genes by *Trichoderma* have been reported in cucumber under NaCl stress. *Trichoderma harzianum* increased the GR (Glutathione reductase) activities under salt stress condition as compared to control. *Trichoderma* alleviate abiotic stress. *Trichoderma* spp. Have been known as biocontrol agents for the control of plant diseases for decades [9]. There are enhanced plant growth as a result of association of *Trichoderma* strains with plants but the effects as with other plant growth promoting microbes [10].

Antifungal activity of *T. harzianum* against *A. alternata in vitro* The activity of T. *harzianum* in inhibiting the postharvest pathogen, *A. alternata* was studied using a modified dual culture method adopted by Jamdar *et al*. [11]. PDA plate was inoculated with 8mm disc of *A. alternata*, 10 mm from the edge of the plate. Then, 8mm disc of T. *harzianum* was cut by sterile cork borer, and then placed in the same plate 60mm far from *A. alternata* disc. Three replicates were performed. The inoculated plates were incubated in dark at 27±2°C for 4 days in a static incubator. Control plates were inoculated with *A. alternata* only. After the incubation period

4. Conclusions

The soil from Kadipani mines was brought to Botanical gardens and experiments were conducted in different combinations and with *Trichoderma*. The results indicated that presence of microbes helped in increasing the soil fertility, which is evidenced by increased plant growth.

Plantation is the oldest technology available to restore the degraded lands. The physico-chemical analysis of the soil was done. The pH of soil is alkaline. The elemental analysis of mined soil and the soil containing maize and moong seedlings was done. It was revealed that sample form mined soil shows higher calcium content. Four different soils were taken and in one *Trichoderma* was added. The effect was analyzed to know the better growth of two plants.

Pot experiments were carried out in different percentages of mining soils and garden soils. The survey revealed that total chlorophyll content was poor in maize as compared.

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Picture 1. Maize and Moong cultivation in the botanical garden

Picture 2. Maize and Moong cultivation in the botanical garden

Picture 3. Open cast Mining Site in, Kadipani Gujarat

Picture 4. Open cast Mining Site in, Kadipani Gujarat