International Journal of Environmental Science and Toxicology Research (ISSN: 2408-7262) Vol. 2(3) pp. 43-54, March, 2014
Available online http://www.internationalinventjournals.org/journals/IJESTR

Full Length Research Paper

Sustainable reclamation of coal mine spoil dump using microbe assisted phytoremediation technology

Sanjeev Kumar Singh, Prashant R. Thawale and *Asha Ashok Juwarkar

Eco-System Division, CSIR-National Environmental Engineering Research Institute (NEERI), Nehru Marg, Nagpur – 440020 (INDIA)

Abstract

Mine spoil dump generated due to mining activities poses drastic physico-chemical and biological constraints for sustainable vegetation. The various environmental problems that are associated with mining activities are deforestation, removal of fertile topsoil, unstable slopes prone to sliding and erosion, siltation of water bodies due to wash off of mineral overburden dumps, air pollution due to discharge of dust and noise pollution. Microbe Assisted Phytoremediation Technology (MAPT) has been developed for sustainable restoration of coal mine spoil dump. Results indicated that the application of MAPT had improved up to 3-5 folds the physico-chemical and microbiological properties of the coal mine soil dump. The inoculation of biofertlizers significantly increased the different microbial groups such as *Azotobacter, Rhizobium* and vesicular arbuscular mycorrhizal (VAM) spores, which were practically absent in the coal mine spoil dump. The toxicity of the heavy metals present in the spoil was also reduced to a large extent. Thus, MAPT is an excellent tool for fast restoration of coal mine spoil dump that provides better supportive material for anchorage and growth of the plants leading to the development of sustainable eco-system, carbon sinks, and mitigating the various environmental hazards and improving the environmental health of the nearby contaminated sites.

Keywords: Coal mine spoil, *Azotobacter*, *VAM,* Environmental restoration.

INTRODUCTION

Land is one of the most important resources on which human beings depend. The mining disrupts the aesthetics of the landscape and soil components such as soil horizons and structure, soil microbe populations, and nutrient cycles which are otherwise crucial for sustaining a healthy ecosystem (Kundu and Ghose, 1997). The overburden of dumps includes adverse factors such as elevated bioavailability of metals, elevated sand content, lack of moisture, increased compaction, and relatively low organic matter content. The effects of mine wastes can be multiple, such as soil erosion, air and water pollution, toxicity, geo-environmental disasters, loss of biodiversity, and ultimately loss of economic wealth (Wong, 2003; Sheoran et al., 2008).

In India, 5,510 mining leases are spread all over, covering an area of 800,000 hectares. In 2001–2002, about 1870 million tonnes of overburden was being removed to obtain 623 million tonnes of minerals in India (IBM, 2001). This overburden often poses a major management problem due to its volume and the presence of pollutants, particularly heavy metals like Cu, Zn, Pb and Cd. Mining activity results in the production of about 250 million tonnes of waste material, namely mine spoil and mine tailing, disposal of which constitutes one of the most serious environmental challenges to the mining industry (Juwarkar et al., 1989, 1992, 2001, 2004, 2007). Several useful processes in rhizosphere, namely humification, soil aggregation, nitrogen and carbon mineralisation are practically inoperative and may take several decades to establish in mine spoils (Mills, 1985). In 2001-2002, about 1870 million tones of overburden were being removed to obtain 623 million tones of minerals in India (IBM, 2001). It is reported that $4m^3$ of overburden produced per tone of mined coal and the expected production of 250 M tones of coal through open cast mining resulted in 1000 M^3 overburdens. For producing this much amount of coal it is estimated that

*Corresponding Author Email: aa_juwarkar@neeri.res.in; Tel: +91-0712-2249764

land at the rate of about 60 Km^2 is degraded in direct mining and approximately 75 km^2 per year for external overburden and spoil dumps. (Kundu and Ghose, 1997). The gradual build-up of such material due to extensive mining activity may endanger not only the agroforest productivity but also the ecosystem and ecological balance.

The removal of topsoil from an adjacent unmined site and covering mine waste is a commonly accepted reclamation practice (Williamson et al., 1991), but it has proven to be an expensive approach. Alternate materials, many of which are considered solid wastes, need to be evaluated for their utilization as topsoil replacements for mine waste reclamation (Bradshaw and Chadwick, 1980). It has been stated that the approaches to revegetation can be described in terms of three different basic philosophies: (1) ameliorative, (2) adaptive, and (3) agricultural. The ameliorative approach relies on achieving optimum conditions for plant growth by improving the physical and chemical nature of mine wastes using organic matter, lime and other amendments. Alternatively, mixing or cover materials may be deployed. This approach is commonly used in preference to the adaptive approach because it is quicker, requires less forward planning, and is less labour-intensive (Johnson et al., 1994).

Soil amendment is a major requirement for the successful establishment of vegetation in metal contaminated soils. The rejuvenation of mine spoil dump and mined land productivity and fertility through amendment of organic material such as effluent treatment plant sludge, a waste product of the paper mill industry, biofertilizers and endomycorrhizal fungi will enable restoration of the degraded land ecosystem and helps to create luxuriant forests in the country, providing a substantial carbon dioxide sink vital for biosphere integrity, build up of nutritive top soil and enhanced ground water recharge. The use of paper mill sludge on agricultural land has been assessed by Bellamy et al. (1995). Organic matter in the form of waste materials offers great advantage in redevelopment of drastically disturbed soils. Direct stimulation of plant growth on degraded land results from mineralization of plant nutrients contained in the organic matter through soil microorganisms. The level of organic matter in the soil is a result of the balance between biomass synthesis and mineralization. Stroo and Jencks (1982) found that recovery of microbial activity in degraded land was directly related to the organic matter and nitrogen accumulation. Traditional ways to increase the organic matter in cultivated areas include the use of animal manures, composts or green manures. Maintenance of soil organic matter is achieved through the use of surface mulches, such as straw, hay or industrial composts. If enough organic matter is not present, then the use of fertilizers and mulches should be considered as major management practices. Many observations and experiments carried out under both field and laboratory conditions have confirmed the importance of soil organic matter in the formation and stabilization of soil aggregates. The two components involved in the aggregation processes are the soil polysaccharides and soil humic substances. The wastes are valuable resources and exploitation of their nutrient potential is today's need to conserve the depleting natural resources. Further, the organic wastes play a vital role in improvement in physico-chemical properties of spoil besides providing organic substrate for proliferation of soil micro flora.

Establishment of a mat of vegetation that is both self-sustaining in terms of long-term management is an important element in rejuvenation of mined lands. However, the work is restricted to plantation of few forestry species and no emphasis is given to restoration of total forest ecosystem lost in the process of mining operation. The rejuvenation of mine spoil dump and mined land productivity and fertility through amendment of organic materials such as press mud, farm yard manure and dairy waste as waste products of different industries, biofertilizers and endomycorrhizal fungi will enable restoration of the degraded land ecosystem and helps to create luxuriant forests in the country providing substantial carbon dioxide sink vital for biosphere integrity, build up of nutritive top soil and enhanced ground water recharge. This study describes the application and outcomes of the Microbe Assisted Phytoremediation Technology (MAPT) for sustainable reclamation of coal mine spoil dump using economical and ecological important plant species. MAPT involves the use of effluent treatment plant (ETP) sludge as an organic amendment, biofertilizer and a source of mycorrhizal fungi and suitable plant species.

MATERIAL AND METHODS

Study area

The site selected for the study was 10 hectares (ha) in area of overburden dump at Padmapur coal mine under Western Coal fields Ltd. in Chandrapur district located 165 km south of Nagpur, India. The Padmapur coal mine is near to Tadoba National Park and Tiger Reserve. It is an open cast mine with total lease hold area of about 557 ha. and the mining quarry area of 143 ha. The area falls between $20^0$11'31" N (north of equator) and $79^0$33'77" E (east of prime Greenwich meridian). The topography of the study area is undulating and climate is semi arid eco-region with high summer average temperatures of 49°C and cold winter temperatures of 10°C. The average rainfall is 600-900 mm per year. The location of the experimental site is shown in the Figure 1. The over burden dump selected for the study is more than 4 years old. The status of the dump is depicted in Figure 2. The

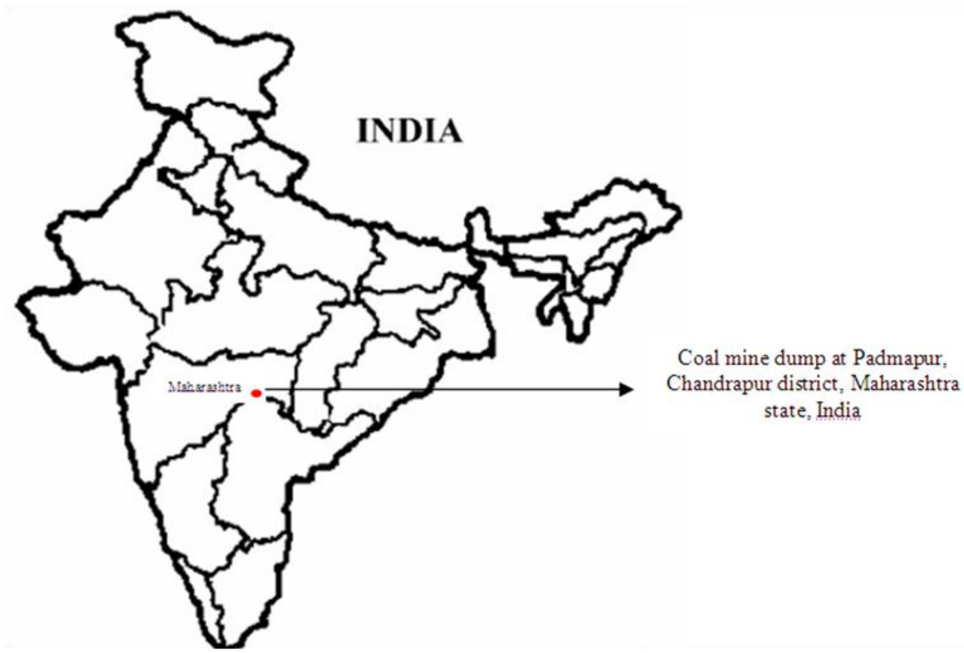

Figure 1. Location of barren coal mine dump at Padmapur, Chandrapur District, Maharashtra, India

Figure 2. Barren coal mine dump at Padmapur, Chandrapur District, Maharashtra, India

dump height is about 35 m with slope angle of 50-55^{0}C.

Sampling technique adopted for coal mine spoil

Composite soil was collected following the zigzag pattern of sampling. Based on the slope and depth, each portion was divided into two parts and a zigzag line having about 8–10 corners on both the sides was drawn on the middle of the field so that it covered the whole area. Auger tools were used for drawing the sample. Before taking the sample, surface litter or any stone etc. was scraped away and samples were collected from 10–20 spots in the fields at each corner of the zigzag line at a depth of 15–20 cm. The samples were collected by hand and placed on a clean piece of paper or cloth and mixed thoroughly. Excess soil was discarded by quartering and approximately one kg of soil was collected. The soil was quickly dried at room temperature or in the shade and stored in plastic bags. The information sheet was filled out completely and placed inside the sample bag and one was kept outside the bag and the sample sent to the laboratory immediately for analysis.

Coal mine spoil characterization

Weight of the coal mine spoil was taken for the coarser fraction (42 mm) material remaining after sieving through a 2 mm sieve. The mine spoil samples collected from the study area were analyzed for physico-chemical parameters as per the standard methods (Piper, 1994; Black et al., 1965). Microbes such as bacteria, fungi, actinomycetes and nitrogen fixing strains of *Rhizobium* and *Azotobacter* were analyzed by following standard methods for soil microbial populations using plate count method and were expressed in terms of colony forming units, CFU/g (Black et al., 1965; Page et al., 1982).

Plate count method

Ten gram of spoil was serially diluted and 1 mL of respective dilution was used for pour plating using the following specific media:

- Nutrient agar medium for total bacterial count : Prepared by dissolving 3 gm beef extract and 5 g peptone in 1000 ml of distilled water. The pH of the medium was adjusted to 6.8 – 7.0 using 0.1 N NaOH. Then 15.0 g agar-agar was added and sterilizes the content at 121^o C for 30 mins.
- Rose Bengal agar medium for fungi : Prepared by dissolving 10 g dextrose, 5 gm peptone, 1.0 g dipotassium sulphate, 0.05 g rose Bengal, 0.10 g chloramphenicol, 15.0 g agar and 0.5 g magnesium sulphate in 1000 ml distilled water and the pH was adjusted to 7.2 ± 0.2 using 0.1 N HCl and sterilize the medium at 121 ^{o} C for 30 minutes.
- Ken Knights agar medium for actinomycetes: Prepared by dissolving 1.0 g dextrose, 0.1 g monopotassium dihydrogen phosphate, 0.1 g sodium nitrate, 0.1 g KCl, 0.1 g magnesium sulphate and 15.0 g agar- agar in 1000 ml distilled water and the pH was adjusted to 7.2 using 0.1 HCl / 0.1 N NaOH. Then sterilize the medium at 121 ^{o} C for 30 minutes.
- Jensen’s medium for *Azotobacter:* Prepared by dissolving 200 g sucrose, 1.0 g K_2HPO_4, 0.05 g $MgSO_4$ 7 H_2O, 0.5 g NaCl, 0.01g $FeSO_4$, 0.005 g Na_2MoO_4 and 2.0 g $CaCO_3$ in 1000 ml distilled water and the pH was adjusted to 7.0 – 7.2 by 0.1 HCl / 0.1 N NaOH. Then 15 g agar-agar was added and sterilizes the medium at 121 ^{o} C for 30 minutes.
- Yeast extract mannitol agar medium for *Rhizobium:* Prepared by dissolve 10 g mannitol, 0.5 g K_2HPO_4 0.2 g $MgSO_4$ $7H_2O$, g NaCl and 0.1 g yeast extract in 1000 ml distilled water and the medium was adjusted at a pH of 6.8 – 7.0 and 15.0 g agar- agar was added and sterilize the medium at 121 ^{o} C for 30 minutes.

The plates were incubated at 30^0 C for 4 days and the number of microorganisms expressed as colony forming unit/gram of spoils (CFU/gm).

Selection of plant species

A total of 10 native plant species such as *Tectona grandis, Dalbergia sissoo*, *Azadiracta indica, Cassia saimea, Acacia nilotica, Ficus religosa, Eugenia jambolina, Pongamia pinnata, Gmelina arborea* and *Dendrocalamus strictus* were collected from forest nursery, Nagpur and selected based on their such as climatological requirements, translocation and uptake capabilities, tolerance levels with respect to chemicals known to exist at the site, tolerance to drought-prone or poorly drained conditions, tolerance to pH and salinity of the soil and of groundwater, depth of the root zone, growth rate, transpiration rate or water use, whether it is deciduous or evergreen (affects the period of effectiveness), maintenance requirements, native vs. non-native species and commercial availability.

Isolation, identification and mass propagation of site-specific biofertiliser strains

For isolation of site specific biofertilizer strains, rhizospheric soil samples were collected from plants growing nearby mine spoil dumpsites. These soil samples were screened for vesicular arbuscular mycorrhizal (VAM) fungi by wet sieving and decanting method (Gerdman, 1963). Two genus of the VAM fungi were isolated and identified as *Glomus* and *Gigaspora* sp. Site-specific non-symbiotic nitrogen fixing strain was isolated from the rhizospheric soil samples and were collected from the vicinity of the roots of *Tectona grandis*, a non-leguminous plant growing nearby the site using nitrogen free Jensen's medium. The isolated strain was identified as A*zotobacter* species as per the Bergey's Manual of determinative Bacteriology (Bergey and Holt, 1984).

Symbiotic nitrogen fixing strain was isolated from the healthy root nodule of *Delbergia sissoo* a leguminous plant and was identified as *Rhizobium* species. Site-specific *Azotobactor* and *Rhizobium* strains were separately propagated in 2 litre capacity fermentor (BioflolI, New Brunswick, U.S.A) using yeast extract mannitol broth and Jensen's medium respectively. The fermentor was operated at pH - 7.0, temperature at 30^0 C, agitation at 400 rpm and aeration at 2 SLPM and one and half VVP (volume of air / volume of liquid). The titer value of biofertilizer inocula developed in the fermentor was estimated by dilution and plate count technique using above specified media. *Glomus* and *Gigaspora* were separately propagated in green house in polypropylene packets filled with sterilized soil and sand (1:1) using *Bryophyllum* species as the host plant.

Characteristics of ETP sludge used as organic amendment

ETP sludge a waste material collected from sugar industry closed to the study area was analyzed for physico-chemical parameters as per the standard methods (Piper 1994 and Black *et al.,* 1965). Microbes such as bacteria, fungi, actinomycetes and nitrogen fixing strains of *Rhizobium* and *Azotobacter* were also analyzed by following standard methods for soil microbial populations and were expressed in terms of colony forming units (CFU/g) (Black et al., 1965 and Page et al., 1982). The ETP sludge was applied @ 50T/ha in the month of June 1997. Based on its favorable characteristics such as pH, water holding capacity and organic matter it was selected to ameliorate the coal mine spoil. Further coalmine spoil had low pH and ETP sludge has alkaline pH. This helped to neutralize the acidic pH of spoil and create more favorable conditions for plant growth and microorganisms.

Planting technique adopted for field studies

The pitting technique was adopted for planting at the top surface and on slopes of mine dumps. Pits at the slopes of the dump were 0.6 m x 0.6 m x 0.6 m in size, and at elevated level were 1 m x 1 m x 1 m. Each pit was filled with 4 parts of spoil + 1 part of soil. The most responsive treatment screened under pot culture studies for sustainable was 4 part of spoil + 1 part of soil + ETP sludge at 50 t/ha. The VAM spores (10g) having approximately 30 spores were applied to each pit by mixing with the bedding material to enhance the nitrogen fixation, development of profuse root system in plants, solubilization and mobilization of nutrients. The saplings of selected plant species i.e. leguminous plants viz. *Dalbergia sisso, Cassia sp.,* etc. were treated with respective site specific cultures of *Bradyrhizobium japonicum* while the non-leguminous plants viz. *Tectona grandis, Gmelina arborea*, etc. were treated with *Azotobacter* sp. by root inoculation method before planting in pits at the rate of 10^5 cells per sapling for leguminous and nonleguminous plant species.

Calculation of tree biomass

Above ground biomass (AGB): Tree biomass has been calculated using algometric equation of Brown, 1997.

Biomass = $\pi D^2 h \times$ sp. gravity $\times 0.025$

where, h = height of plant species in meter

D = average tree diameter in cm and specific gravity is in g/cm^3 (and varies from 0.3-0.8g/ cm^3).

Below ground biomass (BGB): A default conversion factor of 0.26 of aboveground biomass was used to calculate the below ground biomass (IPCC, 2003).

Total biomass: Biomass is calculated by using the following formula:

Biomass = Above Ground Biomass + Below Ground Biomass

Total carbon: Carbon is estimated from plant by using the following formula:

Carbon, C = Biomass × 0.5

Statistical analysis

Statistical data on changes in physico-chemical characteristics of mine spoil, immobilization of heavy metals, effect of ETP sludge and biofertilizer inoculation on above ground and below ground biomass were analyzed using SPSS version 7.5 statistical software package. Results indicated that there were significant differences with 5% confidence level.

RESULTS AND DISCUSSION

Physico-chemical and microbiological characteristics of coalmine spoil dump

The coal mine spoil dump selected for sustainable restoration at Padmapur was devoid of vegetation because of poor physic-chemical properties. The results presented in Table 1 indicates that the bulk density of the coal mine spoil was high (1.48 ± 0.20 g cc^{-1}. The maximum water holding capacity and porosity of the spoil are 32.55 ± 4.24% and 36.24 ± 3.28% respectively. The pH of the mine spoil was slightly acidic (6.48 ± 0.42) and contained low soluble salts (1.20 ± 0.25 dS cm^{-1}).The spoil has low organic carbon content (0.40 ± 0.04%). The total nutrients content with respect to nitrogen, phosphorous and potassium were 12.60 ± 1.20 mg/100g, 9.25 ± 1.30 mg/100g and 6.80 ± 0.80 mg/100g respectively. However, available nutrients content with respect to nitrogen, phosphorous and potassium were 1.60 ± 0.24 mg/100g, 1.32 ± 0.18 mg/100g and 0.65 ± 0.20 mg/100g respectively. These results indicated that the nutrients status required for sustainable reclamation were very low. The results presented in Table 2 shows that the levels of different heavy metals present in the coal mine spoil dump. The concentrations of different heavy metals such as chromium, zinc, copper, iron, manganese, lead, nickel and cadmium clearly indicate that coal mine spoil had many constraints to inhibit the plant growth.

Similarly, the results presented in Table 3 indicates

Table 1. Physico-chemical characteristics of coal mine spoil

Parameters	Coal mine spoil
Physical	
Bulk Density g/cc	1.48 ± 0.20
MWHC, %	32.55 ± 4.24
Porosity, %	36.24 ± 3.28
Textural	
Sand, %	70.70 ± 3.12
Silt, %	21.80 ± 1.18
Clay, %	7.50 ± 0.80
Chemical	
pH	6.48 ± 0.42
ECe (dS/cm)	1.20 ± 0.25
Na (meq/l)	0.80 ± 0.16
K (meq/l)	0.24 ± 0.18
Ca (meq/l)	6.83 ± 0.25
Mg (meq/l)	2.00 ± 0.24
HCO_3 (meq/l)	2.90 ± 0.60
Cl (meq/l)	3.20 ± 0.98
Organic Carbon, %	0.40 ± 0.04
Total Nutrients (mg/100g)	
Nitrogen	12.60 ± 1.20
Phosphorous	9.25 ± 1.30
Potash	6.80 ± 0.80
Available Nutrients (mg/100g)	
Nitrogen	1.60 ± 0.24
Phosphorous	1.32 ± 0.18
Potash	0.65 ± 0.20

Mean ± SD, n=4

Table 2. Concentrations of different heavy metals present in coal mine spoil

Total Heavy Metals	Concentration, mg Kg^{-1}
Chromium, Cr	20.6 ± 1.12
Zinc, Zn	18.2 ± 0.55
Copper, Cu	21.3 ± 0.68
Iron, Fe	8214.2 ± 30.26
Manganese, Mn	48.2 ± 0.90
Lead, Pb	26.8 ± 0.87
Nickel, Ni	24.5 ± 0.80
Cadmium, Cd	12.3 ± 0.72

Mean ± SD, n=4

Table 3. Microbiological characteristics of coal mine spoil

Microbial Groups	CFU/g of soil
Bacteria	48
Fungi	19
Actinomycetes	4
Rhizobium	Nil
Azotobacter	Nil
VAM	Nil

that the microbial groups such as bacteria, fungi and actinimycete in the coal mine spoil were 48 CFU/g, 19 CFU/g and 4 CFU/g respectively. The mine spoil was devoid of any populations of inoculated strains of nitrogen fixers viz. *Azotobacter* and *Rhizobium,* while the VAM spores were totally absent in the coal mine spoil dump. These results depicted that the mine spoil was devoid of essential group of microorganisms for productive rhizosphere development.

Characteristics of ETP sludge used as an ameliorative material

The ETP sludge sample collected from Ballarpur Paper Mill, Chandrapur and was analyzed for various physic-chemical parameters. The results presented in Table 4 shows that the sludge has high organic carbon content (38.20 ± 2.41%) and water holding capacity (84.68 ± 3.26%) respectively. The high organic matter in sludge provides as substrate for microorganisms besides increasing the spoil organic matter levels. The high moisture water holding capacity (MWHC) helped in improving the mine spoil physical properties. The C:N ratio of the sludge was 6:1 and hence it is necessary to provide nitrogen through other sources. The ETP sludge was slightly alkaline in reaction (8.02 ± 0.64). The soluble salt concentration was in moderate range (2.62 ± 0.32 dS cm^{-1}).The concentrations of different heavy metals such as chromium were within the tolerance limit so as to pose severe toxicity to plants (Minoslov, 1999). The above results indicated that addition of alkaline ETP sludge to acidic coal mine helped to neutralize the pH of the coal mine spoil and create more favourable conditions or plant growth and microorganisms.

Effect of biofertlizers and ETP sludge on physico-chemical properties of the coalmine spoil dump

The physico-chemical and nutrient characteristics of the coal mine spoil were evaluated at periodic intervals, which indicated significant improvements in properties of the mine spoil due to amendments with biofertilizers and ETP sludge. After three years of plantation, the improvements in physico-chemical and nutrients status of the mine spoil were analyzed and the results are depicted in Table 5. The bulk density of the mine spoil decreased from 1.48 ± 0.20 g cc^{-1} to 1.08 ± 0.22 g cc^{-1}. The decrease in bulk density will reduce the compaction; facilitate the aeration and better penetration and spreading of plant roots thereby making the rhizosphere favourable for massive root development. The addition of amendments (i.e. ETP sludge and biofertilizers) increased the MWHC and porosity of the mine spoil from 32.55 ± 4.24 % and 36.24 ± 3.28 % to 52.12 ± 2.34 % and 56.24 ± 2.12 % respectively. This is because ETP sludge having good percentage of MWHC and porosity. This is well supported by the findings of Juwarkar et al. (2004). The slightly acidic nature of the mine spoil was due to maximum leaching of basic cations (Kundu and Ghose, 1994). Such acidic nature of the dump renders the medium hostile for plant growth because microbial activity and nutrient availability are optimum at neutral pH (Braddy, 1977).

In order to improve the pH of the mine spoil, suitable amendment materials is required. Thus, addition of ETP sludge and biofertilizers as an organic amendment improve the pH of the mine spoil from 6.48 ± 0.42 to 7.80 ± 0.20. Similarly, electrical conductivity improved from 1.20 ± 0.25 dS cm^{-1} to 1.86 ± 0.42 dS cm^{-1}. Amendment of coal mine spoil with ETP sludge resulted in the improvement of cations and anions status which are required for plant growth. The organic carbon content also increased from 0.40 ± 0.04 % to 1.28 ± 0.06 % due to high organic carbon content in ETP sludge. Since the mine spoil characterized was deficit in macronutrients viz. nitrogen, phosphorous and potassium. The poor availability of nitrogen in the mine spoil was due to the lack of mineralizable organic nitrogen and lower mineralization rates (Redder and Berg, 1997). Thus, the plants were inoculated with nitrogen fixing strains of microorganisms to fulfill the nitrogen requirement of the plant. These nitrogen fixers also secrete growth promoting substances that support and enhance the plant growth. The result presented in Table 5 indicates the improvements in nutrients status of the rhizosphere of the plants, which was due to the addition of ETP sludge as an organic amendment and the application of biofertilizers that fix the nitrogen from the atmosphere. This helped the plant to establish them on the mine spoil dump effectively.

Effect of biofertlizers and ETP sludge on microbial diversity status of the coalmine spoil dump

Successful reclamation of mine spoil dump relies upon the development of an active indigenous microbial diversity, which is responsible for the development of soil structure conducive to plant growth and production of plant nutrients, through a number of biogeochemical cycles. Although use of organic amendment improves the physico-chemical properties and structure of spoil, the long-term stability of the system depends upon the development of an indigenous microbial community that can contribute to different biogeochemical processes. The results presented in Table 6 indicates that after three years of plantation, there were improvements in the microbial populations of the coalmine spoil due to amendment with ETP sludge and biofertilizers. The microbiological analysis of rhizospheric samples collected from different plant species planted on coal mine spoil dump revealed that there were significant improvements

Table 4. Physico-chemical characteristics of ETP sludge

Parameters	ETP sludge
Physical	
Bulk Density g/cc	0.30 ± 0.02
MWHC, %	84.68 ± 3.26
Porosity, %	68.62 ± 2.74
Chemical	
pH	8.02 ± 0.64
ECe (dS/cm)	2.62 ± 0.32
Organic Carbon, %	38.20 ± 2.41
Total Nutrients (mg/100g)	
Nitrogen	110 ± 1.16
Phosphorous	410 ± 1.54
Potash	640 ± 4.29
Total Heavy Metals, mg/Kg	
Chromium, Cr	34.2 ± 2.13
Zinc, Zn	253.0 ± 2.58
Copper, Cu	44.8 ± 0.65
Iron, Fe	6240 ± 20.13
Manganese, Mn	640.0 ± 1.20
Lead, Pb	65.0 ± 1.10
Nickel, Ni	20.6 ± 0.45
Cadmium, Cd	8.2 ± 0.22

Mean ± SD, n=4

Table 5. Effect of biofertlizers and ETP sludge on physico-chemical properties of coal mine spoil dump after three years of plantation

Parameters	Coal mine spoil
Physical	
Bulk Density g/cc	1.08 ± 0.22
MWHC, %	52.12 ± 2.34
Porosity, %	56.24 ± 2.12
Textural	
Sand, %	50.00 ± 2.34
Silt, %	30.50 ± 0.42
Clay, %	19.50 ± 0.08
Chemical	
pH	7.80 ± 0.20
ECe (dS/cm)	1.86 ± 0.42
Na (meq/l)	0.42 ± 0.008
K (meq/l)	0.08 ± 0.03
Ca (meq/l)	3.20 ± 0.24
Mg (meq/l)	1.86 ± 0.04
HCO_3 (meq/l)	1.60 ± 0.08
Cl (meq/l)	1.82 ± 0.09
Organic Carbon, %	1.28 ± 0.06
Total Nutrients (mg/100g)	
Nitrogen	36.50 ± 2.34
Phosphorous	18.20 ± 1.86
Potash	12.30 ± 0.84
Available Nutrients (mg/100g)	
Nitrogen	3.92 ± 0.23
Phosphorous	2.70 ± 0.06
Potash	1.60 ± 0.36

Mean ± SD, n=4

Table 6. Effect of biofertlizers and ETP sludge on microbiological characteristics of coal mine spoil dump after three years of plantation

Microbial Groups	CFU/g of soil
Bacteria	85×10^5
Fungi	60×10^4
Actinomycetes	90×10^3
Rhizobium	23×10^4
Azotobacter	68×10^4
VAM	45

in the counts of bacteria, fungi and actinomycetes, along with improvements in the growth of nitrogen fixing bacteria. The counts of bacteria, fungi and actinomycete improved from 48 CFU/g, 19 CFU/g and 4 CFU/g to 85 x 10^5 CFU/g, 60 x 10^4 CFU/g and 90x 10^3 CFU/g respectively, which were almost similar to that of found in good productive soil. The populations of bacteria, fungi and actinomycete in good productive soil are found in the range of 1-36 × 10^6 CFU/g, 1-10 × 10^4 CFU/g and 1-30 × 10^4 CFU/g respectively. The populations of inoculated strains of nitrogen fixers viz. *Azotobacter* and *Rhizobium* also showed significant increase in the counts. The counts of *Azotobacter* and *Rhizobium* which were totally absent in the coal mine spoil increased to 68x 10^4 CFU/g and 23 × 10^4 CFU/g due to amendments with ETP sludge and biofertilizers. Similarly, the VAM spores also increased to 45 / g of spoil. Similar results were observed by Juwarkar and Singh (2007), where they have used municipal solid waste as an organic amendment to reclaim the coal mine spoil dump. These results indicated that ETP sludge being cellulosic in nature, provides good substrate for proliferation of microbial development, promoted the development of good rhizosphere and amelioration of adverse chemical and physical limitation on microbial community development (NEERI Report, 2000).

Effect of biofertlizers and ETP sludge on immobilization of different heavy metals present in the coalmine spoil dump

The heavy metals are retained in the soil in three ways; (1) Adsorption onto the surface of mineral particles, (2) Complexation by humic substances in organic particles and (3) Precipitation reactions. The result presented in Table 7 shows that at periodic intervals, the amendment of mine spoil with ETP sludge and biofertilizers inoculation significantly reduced the concentrations of different heavy metals such as chromium, zinc, copper, iron, manganese, lead, nickel and cadmium in the range of 50-60% after three years of plantation respectively. The decrease in heavy metal was due to amendment of ETP sludge, which contains an appreciable amount of organic carbon. Organic carbon has a strong positive influence on the adsorption of heavy metals (Strawn and Sparks, 2000, McBride et al., 2000). Organic amendments containing substantial amount of organic matter helps in decreasing the toxicity of heavy metals in soil, thus permitting the re-establishment of vegetation on metal contaminated sites (Kumar et al., 2007). Also the alkaline pH of ETP sludge plays a significant role in reducing the toxicity of metals because at alkaline pH, metals gets immobilized and translocation of metals is arrested (NEERI Report, 2000). Mycorrhizae also play an important role protecting plant roots from heavy metals (Smith and Reed, 1997). Mycorrhizae evolved heavy metal tolerance and may play significant role in the phytoremediation of the site (Chaudhury, 1998). These mycorrhizal associations increase the absorptive surface area of the plant due to extra metrical fungal hyphae exploring rhizospheres beyond the root hair zone, which in turn enhance water and mineral uptake. Thus, enhanced capability of high uptake of minerals results in more biomass production, which is a prerequisite for successful remediation.

Effect of biofertlizers and ETP sludge on the growth performance of the different plant species planted on coalmine spoil dump

Plant growth of any plant species is influenced greatly by its associated microflora and microbial biodiversity in the rhizosphere of the plant. Therefore, stress was laid on isolation of site specific and species-specific microflora from the rhizospheric soil samples collected from the nearby areas of the mine spoil dump. These isolated strains were introduced in the rhizosphere of saplings of different plant species at the time of plantation on the dump. This approach will facilitate the growth of the plant species under adverse conditions. The amendment of coal mine spoil with ETP sludge and biofertilizers improved the moisture availability for plant growth (NEERI Report, 2000). The site-specific, stress tolerant biofertilizers and endomycorrhizae, which were isolated from coal mine spoil dump, increased the biomass of the plants to a great extent (Table 8). Increase in biomass

Table 7. Effect of biofertlizers and ETP sludge on the levels of different heavy metals present in coal mine spoil dump after three years of plantation

Total Heavy Metals	Concentration, mg kg^{-1}
Chromium, Cr	10.8 ± 1.02
Zinc, Zn	12.3 ± 0.55
Copper, Cu	11.6 ± 0.08
Iron, Fe	4216.4 ± 16.24
Manganese, Mn	22.4 ± 0.86
Lead, Pb	10.2 ± 0.42
Nickel, Ni	9.8 ± 0.04
Cadmium, Cd	4.1 ± 0.02

Mean ± SD, n=4

Table 8. Total biomass and carbon stock of the different plant species after three years of plantation on coal mine spoil dump

PLANTS SPECIES	Biomass (T ha^{-1})			
	AGB	BGB	TB	TC
Ficus religosa	10.42	2.12	12.54	6.27
Eugenia jambolina	8.64	1.86	10.50	5.25
Pongamia pinnata	23.84	5.02	28.86	14.43
Gmelina arborea	18.50	3.12	21.62	10.81
Acacia nilotica	54.23	10.12	64.35	32.18
Dendrocalamus strictus	85.90	16.20	102.10	51.05
Azadiracta indica	68.24	13.25	81.49	40.74
Cassia saimea	120.26	23.30	143.56	71.78
Tectona grandis	230.58	50.26	280.84	140.42
Dalbergia sissoo	108.88	25.20	134.38	67.19

AGB = Above ground biomass,
BGB = Below ground biomass,
TB = Total biomass,
TC = Total carbon

resulted increase in carbon sequestration by plants (IPCC, 2003). The isolated site-specific biofertilisers enhance the plants survival rates up to 95–99%, under the toxic physico-chemical conditions prevailing in the specific mine spoil dump to facilitate rapid establishment of natural biogeochemical cycles and regeneration of topsoil. Figure 3 showed the ecological restoration on coal mine spoil dump at Padmapur, Chandrapur District, Maharashtra, India.

The different plant species showed a good response towards application of ETP sludge as an organic amendment and the site-specific biofertiliser strains as shown in Figure 4. The plant height increased 10-15 times as compared to initial height due to amendment of coal mine spoil with soil, organic waste and biofertilizers. The maximum growth performance of the plant species in terms of height was shown by *Pongamia pinnata, Dendrocalamuc strictus, Dalbergia sisso and Tectona grandis* followed by other plant species.Thus, after three years of plantation, the different plant species showed a good response towards metal accumulation, biomass and carbon sequestration resulting sustainable reclamation on barren coal mine spoil dump.

Monetary benefits of MAPT (ha^{-1} yr^{-1})

The benefits of the technology were calculated in terms of ecology and economy. Table 9 represents the multiple benefits in US $. The reclamation of one hectare of mine land require a capital investment of US $ 1250 with financial return of US $ 36,562 after 20 years of plantation and a sort payback period of 30 months. The economic benefits in terms of forest products like timber and industrial wood, raw material for cottage industry, fuel wood and recreation accounts to be US $ 4687, whereas the ecological benefits in terms of oxygen production, soil conservation, development of top soil etc. accounts to be US $ 31,875. Thus, MAPT is not a cost effective process but also provides several ecological and economical benefits to the society.

1. Oxygen production of open forest – 3 T/ha/day,

Figure 3. Ecological restoration on coal mine dump after three years of plantation at Padmapur, Chandrapur District, Maharashtra, India

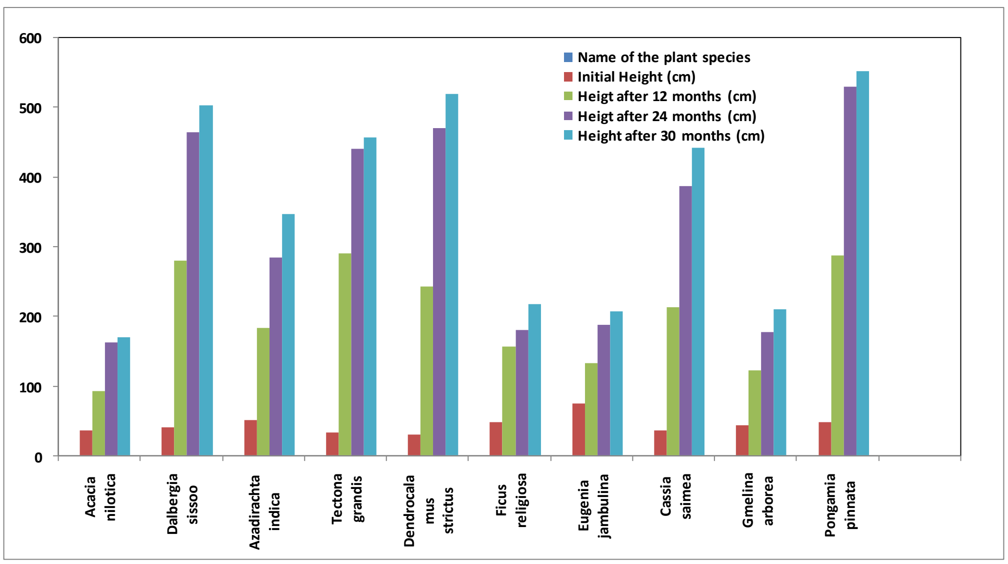

Figure 4. Growth performance of different plant species planted at coal mine spoil dump at Padmapur, *Chandrapur District, Maharashtra, India*

Table 9. Monetary benefits of MAPT (ha^{-1} yr^{-1})

Particulars	Amount (US $) ($ha^{-1}$ yr^{-1})
Ecologic Benefits	
Oxygen production[1]	273222.22
Control of soil erosion[2]	3644.44
Humus/ top soil build up[3]	5466.66
Control of air pollution[4] (CO_2, CO, SO_2 sink potential)	1822.22
Economic Benefits	
Timber and industrial wood[5] (potential cost)	555.55
Raw material for cottage industry[6] (bamboo, gum, fodder, non-edible oil seeds)	40266.66
Fuel wood[7]	444.44
Recreation[8]	377.77
TOTAL	**325799.96**

cost of oxygen in US $0.3125 per kg (Source: Pandit, (1997) Ecological Economics: Towards a Synthesis of Two Distinct Disciplines, *Current Science*, 72(2)

2. Based on expenditure required for prevention of contamination of land and water due to mine spoil dump erosion
3. Based on nutrient status of spoil compared with market price of chemical fertilizers
4. 6,7 Source: The Price of Forest, Proceedings of the Conference of Economics of the Sustainable use of Forest Resources, Delhi, April 2-4, 1990
5. On the basis of prevailing market prices in India
6. Pandit, (1997) Ecological Economics: Towards a Synthesis of Two Distinct Disciplines, *Current Science*, 72(2)

CONCLUSIONS

MAPT in combination with ETP sludge as an organic amendment is an ecosystem approach for sustainable reclamation of coal mine spoil dump, which led to the development of a supportive and nutritive rhizosphere and accelerated the biogeochemical cycles in a short span. Also, the fast recovery of degraded ecosystems through MAP provides carbon sinks, biomass as potential fuel, fibres, food, fodder and medicinal plants. The results indicate that the MAPT restored the productivity, fertility and stability of the mine spoil dump within two and a half years, leading to the development of a self–sustained ecosystem and turned the barren mine spoil dump into luxuriant green areas, thereby mitigating the environmental hazards and improving the environmental health of the surrounding areas.

ACKNOWLEDGEMENTS

Authors acknowledge the kind support and encouragement extended by Dr. Satish R. Wate (Director, CSIR-NEERI, Nagpur). The authors acknowledge the financial support provided by Department of Biotechnology, New Delhi, to carry out the work presented in this paper. The authors also acknowledge the support of Western Coalfield Limited, Nagpur, in providing the barren mine spoil dump for conducting the research.

REFERENCES

Bellamy KLC, Chongiand RA (1995). Paper sludge utilization in agriculture and container nursery culture. *J. Environ. Qual.,* 24, p. 1074-1082.

Bergey DH, Holt JG (1984). *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Williams and Wilkins, USA.

Black CA, Evans DD, White JL, Ensminger LE, Clark FE (1965). In Methods of soil Analysis: *Chemical and Microbiological Properties*, Agronomy 9, Part II, Medison, Wisconsin, USA.

Braddy NC (1988). Nature and Properties of soils, 9th edn. McMillan, New York.

Bradshaw AD, Chadwick MJ (1980). *The Restoration of Land*, Blackwell, Oxford.

Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998). Phytoremediation- focusing on accumulator plants that remediate metal contaminated soils. *Australian. J. Ecotoxicol*. 4; 37-51.

Gerdmann JW, Nicolson TH (1963). 'Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting', *Trans. Br. Mycol. Soc.*, Vol. 46, p.235.

Indian Bureau of Mines (IBM) (2001). *Annual Report*, Ministry of Steel and Mines, Govt. of India.

Juwarkar AA, Dubey K, Khobragade R, Nimje M, Singh SK (2001). Integrated biotechnological approach for phytoremediation of copper mine spoil dumps and tailing. Paper published in Proceeding of International Conference on *Industrial Pollution and Control Technologies (ICIPACT- 2001)* held during 7-10th December at JNTU, Hyderabad, India.

Juwarkar AA, Jambhulkar HP, Singh SK (2004). Appropriate strategies for reclamation and revegetation of coalmine spoil dumps. *In proceedings of the National Seminar on Environmental Engineering with special emphasis on Mining Environment,* NSEEME-19-20 March 2004, pp.1-9.

Juwarkar AA, Singh SK (2007). Utilization of municipal solid waste as an amendment for reclamation of coal mine spoil dump. *Int. J. Environmental Technology and Management*, Vol. 7, Nos. 3/4,407-420.

Juwarkar AS, Thawale PR, Mowade S, Moghe M, Juwarkar A (1994). 'Manganese mine spoil dump reclamation using pressmud and biofertilizer', *National Seminar on Minerals and Ecology*, Dhanbad.

Kumar GP, Yadav SK, Thawale PR, Singh SK, Juwarkar AA (2007). Growth of *Jatropha curcas* on heavy metal contaminated soil amended with industrial wastes and *Azotobacter*- A greenhouse study. *Bioresource Technology,* 99: 2078–2082.

Kundu NK, Ghose MK (1997). Shelf life of stockpiled topsoil of an opencast coal mine. *Environmental Conservation*, 24 (1): 24-30.

Mills AL (1985). 'Acid mine waste drainage: microbial impact on the recovery of soil and water ecosystems', in Tate, R.L. and Klein, D. (Eds.): *Soil Reclamation Processes*, Marcel Dekker, Inc., New York, pp 35–81.

NEERI Report (2000). Demonstration project on restoration of manganese and coalmine spoil and fly ash dumps through an Integrated Biotechnological Approach, sponsored by Department of Biotechnology, New Delhi.

Page AL, Miller RH, Keeney DR (1982). *Method of Soil Analysis: Chemical and Microbiological Properties*, (Agronomy 9), Part II, ASA, SSSA, Medison, Wiscosin, USA.

Radojevic M, Vladimir NB (1999). Practical Environmental Analysis, Royal Society of Chemistry, UK.

Reeder JD, Berg WA (1997). Nitrogen mineralization and nitrification in mine spoils. *J. Am. Soc. Soil. Sci.* vol .41.pp. 922-927.

Sheoran AS, Sheoran V, Poonia P (2008). Rehabilitation of mine degraded land by metallophytes. *Mining Engineers Journal* 10 (3), 11-16.

Stroo HF, Jencks EM (1982). Enzyme activity and respiration in minesoils. *Soil Science Society of America Journal*, *46*(3), 548-553.

Williamson JC, Johnson DB (1991). Microbiology of soils at opencast sites: II. Population transformations occurring following land restoration and the influence of rye grass/ fertilizer amendments. *Journal of Soil Science* 42, 9-16.

Wong MH (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*. 50,775–780.

How to cite this article: Singh KS, Juwarkar AA (2014). Sustainable reclamation of coal mine spoil dump using microbe assisted phytoremediation technology. Int. J. Environ. Sci. Toxic. Res. Vol. 2(3):43-54