Soil ecophysiological and microbiological indices of soil health: a study of coal mining site in Sonbhadra, Uttar Pradesh

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Abstract

The present study involves the discovery of quality of soils associated with dump mines and native forest encompassing the Kakari mine, a coal mining site in Sonbhadra, to distinguish the effect of coal mining on associated soil ecosystem. Soil ecophysiological indicator of the site was analyzed in terms of floral diversity by method of quadrat analysis, which revealed that dumps were devoid of any floral community whereas the natural forest around the mines was enriched with a number of plant species. Importance value index (IVI) was calculated and with respect to their IVI index the tree species viz., *Acacia mangium* (23.17, 24.48 & 26.81) and *Acacia catechu* (20.34, 22.10 & 25.36) were found to be the most dominant species. Soil enzymatic and physico-chemical analysis were analyzed by standard method and the result emphasized that dump soils possess poor enzymatic activity and acidic pH due to the decomposition of waste material in dumps which eventually affects the microbial diversity of soil and its fertility. The plant growth promotion (PGP) and stress tolerance characteristics of bacterial isolates were further examined so that the potential isolates will be utilized further to reclaim and remediate soil property of degraded soils. The promising isolates were characterized by BIOLOG identification system and isolate NU36 and NU25 showed greatest similarity with *Pseudomonas* sp. and *Acinetobacter* sp. respectively. Based on the potential to produce plant growth promoting substances and withstand in stressed environment the most promising isolates will be used to remediate soil and reclaim soil characteristics.

Keywords: Coal mining, dump, floral diversity, soil enzyme activities, IVI, BIOLOG

1. Introduction

Coal is an important fossil fuel which is involved in the production of power and energy all over the world. Coal mining processes affect the surrounding environment by polluting the nearby water, soil and agriculture lands (Harun *et al.*, 2014). Mining activity generates an extensive amount of waste material which gets deposited on the surface and destroys soil nutritional and functional properties by disrupting soil microbial population, texture, organic matter and mineral components (Bell *et al.*, 2001). It leads to the degradation of existing natural vegetation, ecological communities, water quality, agricultural and forest lands. The opencast coal mining technique involves the deposition of overburden material and other pollutants which make the soil nutritionally and microbiologically deprived eventually leads to the removal of green cover, top soil and organic matter. The soil debris is heaped in the form of dumps and classified as mine spoil (Chaulya *et al.*, 2000). The continuous mining process cause the removal of plant species, make the soil unfertile and creates environmental pollution by degrading soil, water and air. Hence, it is a major concern to evaluate the soil quality and vegetation status of the soil surrounding the coal mines and this will be helpful in identifying the impact of coal mining on the surrounding environment. Floral diversity is greatly affected by the soil properties. Numerous researches proved the adverse effect of mining on plant life and soil quality (Wong, 2003; Borpujari, 2008; Hossain *et al.*, 2015). Soil characteristics are recovered by restoring the ecosystem conditions which can be done by revegetating the dump mines. It has been important to restore the land ecosystem by the revegetation of mine dumps. A number of approaches are available for the revegetation of coal mine dumps; most suitable is the use of native and indigenous plant species with the help of microbes found in the vicinity of plant roots. Several reports are available on the

natural succession of different plant species on the barren dumps after certain interval of time (Benidire *et al.*, 2016; Borpujari, 2008; Hazarika *et al.*, 2006) but the process of natural succession usually proceeds at slow rate.

The process of reclamation involves the returning of the degraded land to near about its original stage by restoring the nutritional property of soil through revegetating the land. For a successful revegetation the selection of appropriate plant species is very important. The bioremediation involves the use of native plant applied with the rhizospheric microbes which involves the establishment of stable biogeochemical cycle and improves soil quality. The restoration of vegetation cover on the overburden dumps of coal mines will be helpful for human beings by stabilizing soil structure and property and make it nutritionally rich so that it creates less harm to the environment (Wong, 2003). It will make the ecosystem healthy by creating proper vegetation and improving soil structure and quality so that it can supports plant growth and reduces pollution.

Ecoremediation technology based on the management of plant microbe soil interactions is useful for the reclamation of soil ecosystems degraded by either mining or industrial activities. Plant-soil interactions are important for understanding the role of biotic abiotic factors in the functioning the degraded ecosystem, but very few knowledge is available related to these interactions, in the context of ecosystem restoration and succession (Merino *et al.*, 2015). The natural habitat of the belonging site are utilized for the remediation of contaminated and dump soil. With this background, the present study is envisaged to examine soil characteristics and select the most dominant tree species with the potential microbes which will be helpful for the effective reclamation and remediation of the degraded soil.

Figure 1. Location of the study site: (a) Kakri mine in Sonbhadra district (b) plant diversity analysis by quadrat method; (c) coal dumps in the mining area

2. Materials and Methods

2.1. Study site and sampling

The study site is situated in the Sonbhadra district of Uttar Pradesh at 27.68°N latitude and 83.06°N longitude. A representative area with mine tailings and dumps in the southern part of the Sonbhadra was surveyed and suitable sites were selected. Kakari mines (Lat 24°16'55"- 24°18'90"N and Long 82°74'25"- 82°76'72"E), an open mine developed by the Northern Coalfields Limited located beside the Govind Ballabh Pant Sagar reservoir was selected for plant diversity analysis as well as for collection of soil samples (Figure 1). The native forest nearby the coal mine area and the mine dump sites were surveyed for analyzing floristic composition in the area. Three sampling sites representing various categories of natural forest were selected for investigating vegetation and sampling. The vegetation status of the selected site was investigated by quadrat method. At each site five different quadrat of size 10 m × 10 m, 3 m × 3 m and 1 m × 1 m were selected for trees, shrub and herb species

respectively (Figure 1). For each quadrat, individuals of trees, shrubs and herbs were counted and the information was collected for further identification. The data collected were analyzed to calculate frequency, density, abundance, and importance value index (IVI) for each quadrat as per method described by Curtis and McIntosh (1950) to select the predominant species in the area. Abundance to frequency (A/F) ratio indicated as Whitford's index was calculated to examine the species distribution pattern in the forest and classified into three different pattern i.e. into regular (< 0.025), random (0.025 - 0.05) and contagious (> 0.05) (Whitford, 1949). The species diversity was determined by Shannon Weiner diversity index (H') and Simpson's diversity Index (Munishi, 2008).

Soil samples collected were collected in triplicate from both from the native and degraded soils of Kakri mine. Native soils (NF1-NF6) were belonged to the rhizospheric and non-rhizospheric regions of the dominant plant found in the natural forest contiguous to the mining sites in addition dump soils (DM1-DM4) belonged to the various dumps developed by the mining processes. Dump soils were collected from the

depth of 9-10 inches where as the rhizospheric soils were collected from the soil around the root up to a depth of 15-20 cm and all the soil samples were carefully stored in plastic bags at 4 °C for further analysis. The number of microorganism in the soil sample was determined by standard spread plate count method technique by the surface inoculation on appropriate culture media. The bacterial colonies from soil samples were isolated by serial dilution plating on nutrient agar medium (Harley and Prescott, 2002). The plates were then incubated at 28° for 24-48 h. The bacterial colonies were then observed and the distinct colonies were streaked to obtained fresh culture. For isolation of fungus, soil samples (1 g) were serially diluted and plated on rose bengal chloroamphenicol agar medium. The plates were then observed for growth after incubation period of 7 days at a temperature of 25 °C. Total viable bacterial and fungal count were calculated and expressed in CFU.g⁻¹. The bacterial isolates were further examined for their biochemical profiling by BIOLOG identification system (Biolog, Hayward, CA, USA). The 24 hr old bacterial culture growing on biolog universal agar (BUG) medium was inoculated into the inoculating fluid (IF) and further incubated for 18-22 hr into Biolog GEN III plate. After the complete incubation the plates were read automatically with the Biolog OmniLog® ID System.

2.2. Analysis of soil physico-chemical and enzymatic activity

Soil samples were analyzed for physiochemical parameters like soil texture, pH, electrical conductivity, organic carbon, available phosphorus and available potassium content. Soil texture analysis included the estimation of clay (<0.002 mm), silt (0.06 mm-0.002 mm) and sand (2 mm-0.06 mm) percentage. The texture, pH, electrical conductivity and organic carbon of the soil samples was determined by using the standard

procedure as described by Kalra and Maynard (1991) and the level of available phosphorus and available potassium concentration was determined using Bray method (Simonis & Setatou, 1996).

Soil dehydrogenase and phosphatase activities were determined by the procedure described by Tabatabai (1982). Soil dehydrogenase activity was calculated by using 2, 3, 5-triphenyl tetrazolium chloride (TTC) as a substrate. The substrate was reduced to triphenyl formazan (TPF) a red colored compound which was measured calorimetrically at a wavelength of 485 nm. The concentration of TPF produced was determined by using of standard curve and expressed as µg TPF.g⁻¹dry soil h⁻¹ over control. Soil phosphatase activity was measured using p-nitro phenyl phosphate as a substrate and was expressed as mg p-nitrophenol (pNP) per gram dry soil and incubation time. Urease activity was calculated by the procedure described by Kandeler & Gerber (1988) using urea as a substrate. The concentration of ammonia produced was determined by colorimetric method and expressed as mg NH₄⁺-N released/ (g dry soil. 24 hr). Catalase activity was measured by KMnO₄ titration method with H₂O₂ as substrate and expressed in 0.1 mol/L KMnO₄ solution titrated/ (g dry soil•20 min) (Zhan *et al.*, 2010).

2.3. Characterization of bacteria for plant growth promotion and stress tolerance

The isolated bacterial colonies were screened for various plant growth promotion characteristics as well as for their survival in harsh environmental conditions. IAA production was estimated both qualitatively and quantitatively according to the method described by Loper and Schroth (1986) by Salkowski method. The amount of IAA produced in culture medium was calculated by comparing it with standard curve in mg/ml. Bacterial isolates were screened for phosphate solubilization ability on Pikovskaya's (PVK) agar

medium containing tri-calcium phosphate (TCP) as a substrate (Premono *et al.*, 1996). Siderophore production was examined by CAS assay; chrome azurol S agar medium was prepared according to the composition described by Schwyn and Neilands (1987). Single colonies of bacterial isolates were spot inoculated and incubated at 28 °C for 2 weeks. The colonies with orange zones were considered as siderophore producing strains.

The isolates were screened for drought tolerance as per the method described by Sandhya *et al.*, (2009). Nutrient broth medium amended with different concentration of PEG 6000 having water potentials of -0.0573 MPa, -0.1573 MPa, -0.3073 MPa, -0.4973 MPa, -0.73 MPa and -1.2 MPa was inoculated with 1% of bacterial culture cultivated overnight in nutrient broth. The tubes were then incubated at 28-30 °C and OD was recorded at 600 nm after 24-48 h. The isolates were tested for temperature resistance by incubating the bacterial isolates at different temperature ranged between 10 °C to 50 °C for 24 hr. Salt tolerance potential of isolates was screened by growing the bacteria isolates in medium having salt concentration from 0.5% to 15%. Isolates were further screened for pH tolerance by growing the bacterial culture within a range of pH 4- pH 10 (Joshi and Modi, 2013). The selected bacterial isolates were tested for metal resistance. The 24 hour old grown bacterial cultures were incubated in nutrient broth amended with metal salt viz., cadmium chloride (CdCl₂), lead chloride (PbCl₂), and zinc chloride (ZnCl₂) at various concentration ranged between 1 mM to 10 mM, the inoculated broth was then incubated at 28 °C-30 °C for 24-48 hours and absorbance of the sample were taken at 600 nm. Bacterial isolates were considered stress tolerant if an OD of 0.1 or above was recorded.

2.4. Statistical analysis

All the data obtained were statistically analyzed and presented with the appropriate standard deviation and standard error method. The data obtained from the assessment of physicochemical and enzymatic activities for various soil samples were analyzed for correlation analysis to obtain the level of significance between them. The Pearson's correlation coefficient for all the variables was calculated to determine the linear correlations between them. The data analysis and preparation of graphs was done by using the software Origin-Lab- OriginPro 8. 3.

Results

3.1. Floral diversity

The natural forest around the coal mine as well as the mine spoil was surveyed for analyzing the existing natural vegetation. The survey revealed that no vegetation cover was observed on the mine spoil due to the adverse environmental conditions. A total of 25 plant species were identified in the forest around the mining site belonging to 23 genera and 15 families. Among the 25 plant species, 16 species were represented by trees (*Woodfordia fruticosa*, *Lagistomia parviflora*, *Acacia catechu*, *Acacia mangium*, *Dalbergia sissoo*, *Leucaena leucocephala*, *Acacia auriculiformis*, *Solanum nigrum*, *Boswellia sacra*, *Azadirachta indica*, *Diospyros melanoxylon*, *Milius tometosa*, *Manilkara hexandra*, *Murraya paniculata*, *Chloroxylon swietenia* and *Bambusa strictus*), 5 species by shrubs (*Lantana camara*, *Achyranthes aspera*, *Sida cordifolia*, *Bromus tectorum* and *Peristrophe paniculata*) and the remaining 4 species were represented by herbaceous species (*Plumbago zeylanica*, *Saccharum spontanium*, *Cynodon dactylon*

and *Eragrostis japonica*); majority of the plant species belonged to the family *Poaceae*, *Fabaceae* and *Rutaceae*. Tree species were selected for the further studies and the data obtained from the quantitative analysis of trees are presented in Table 1a-b1. Importance value index (IVI) value indicates that *Acacia mangium* is the most dominantly growing species having IVI value in site I, II & site III (23.17, 24.48 & 26.81) followed by *Acacia catechu* (20.34, 22.10 & 25.36). Maximum density (4.4/100 m², 2.4/100 m² & 2.2/100 m²) is recorded for *Acacia mangium* in each of the three study sites respectively among the other tree species. The respective IVI index value for different trees is given in Table 1a-1b. Importance value index provides the information of important and dominant plant species in a plant community. Shannon-Wiener Index (H') and Simpson's index was calculated for the different sites. Both the Shannon wiener and Simpson's diversity index was highest in site I (2.70, 0.93) with reference to site II (2.45, 0.92) and site III (2.36, 0.91) which indicates the presence of immense species diversity of trees in the site I. Simpson's Diversity Index calculate diversity by considering both richness and evenness of the species and the index value ranges between 0 to 1 which signifies that every species came across belonged to different species. The overall distribution pattern observed for site I, II and III are regular and random distribution pattern for all the encountered species and presented in Table 1a-1b.

3.2. Microbial diversity

Soil microorganism are primarily involved in maintaining the ecosystem functions and are responsible for the transformation of soil nutrients and organic matter (Merino *et al.*, 2015). The result obtained from the study is presented in Figure 2 (a) and it is evident that the highest bacterial diversity was observed in the

rhizospheric soils as compared to the non rhizospheric soils and to the dump soils. The cfu for bacteria obtained from the native soils ranged from 103.33×10^5 g⁻¹ in NF1 to 323.67×10^5 g⁻¹ in NF2 for rhizosphere, 42.67×10^5 g⁻¹ to 142.72×10^5 g⁻¹ for non- rhizospheric and for mined soil it ranged from 6.4×10^3 g⁻¹ to 9.22×10^3 g⁻¹. For soils collected from native forest, maximum bacterial count was recorded in soil sample NF2 indicating the presence of immense bacterial population in comparison with the other soils (Figure 2a). On comparing dump soils to native soils, high microbial diversity is observed in native soil. A total sixty four bacterial isolates were obtained which were further screened for different plant growth promotion and stress tolerant mechanism. The number of fungi obtained from rhizospheric soil is ranged from 2.66×10^2 g⁻¹ in NF3 to 35.67×10^2 g⁻¹ in NF1 (Figure 2b). For the rhizospheric soils and the non rhizospheric soils highest fungal count was recorded in the rhizospheric soil.

3.3. Physicochemical analysis and enzyme activities of soil

The data obtained from the particle size distribution, textural classification and physicochemical analysis of soil samples from dump and natural forest is presented in Table 2. Soil fertility is coupled with soil pH and an alkaline pH suggests a healthy soil suitable for plant growth (Maharana *et al.*, 2013). The growth of plant suffers much both under very low as well as high pH. To enumerate the current condition of soil, samples were analyzed for pH testing. In the present work, the dump soils retain an acidic pH (5.39-6.29) indicating a less fertile soil where as soils NF1 and NF4 possess an alkali pH (7.27, 7.06) which is advantageous for vegetation sustainability. The pH of soil is positively correlated with the clay content ($r = 0.662$;

Table 1a. Quantitative Structure of tree species in the study area for Site I, Site II and Site III

SITE I					
S. No.	Trees	Density	IVI	W (A/F ratio)	Distribution pattern
1	<i>Woodfordia fruticosa</i>	1.2	10.31	0.018	Regular
2	<i>Lagistomia parviflora</i>	1.2	10.31	0.018	Regular
3	<i>Acacia catechu</i>	3.6	20.34	0.036	Random
4	<i>Acacia mangium</i>	4.4	23.17	0.044	Random
5	<i>Dalbergia sissoo</i>	1.6	11.73	0.025	Random
6	<i>Leucaena leucocephala</i>	1	9.67	0.015	Regular
7	<i>Acacia auriculiformis</i>	1	8.09	0.027	Random
8	<i>Manikara hexandra</i>	1.2	10.31	0.018	Regular
9	<i>Murraya paniculata</i>	1.6	11.73	0.025	Random
10	<i>Chloroxylon swietenia</i>	3.2	18.92	0.032	Random
11	<i>Solanum nigrum</i>	1.2	10.31	0.018	Regular
12	<i>Azadirachta indica</i>	1.4	11.02	0.021	Regular
13	<i>Boswellia sacra</i>	0.8	7.38	0.022	Regular
14	<i>Diospyros melanexylon</i>	2.8	17.5	0.028	Random
15	<i>Milius tometosa</i>	1.4	12.54	0.014	Regular
16	<i>Bambusa strictus</i>	0.6	6.67	0.016	Regular

Table 1b. Quantitative Structure of tree species in the study area for Site I, Site II and Site III

SITE II					
1.	<i>Woodfordia fruticosa</i>	1	14.11	0.015	Regular
2.	<i>Acacia catechu</i>	2	22.10	0.02	Regular
3.	<i>Acacia mangium</i>	2.4	24.48	0.024	Regular
4.	<i>Dalbergia sissoo</i>	0.8	10.88	0.022	Regular
5.	<i>Leucaena leucocephala</i>	1.4	16.49	0.021	Regular
6.	<i>Manikara hexandra</i>	1.4	16.49	0.021	Regular
7.	<i>Murraya paniculata</i>	1.2	13.26	0.033	Random
8.	<i>Chloroxylon swietenia</i>	1.6	19.72	0.016	Regular
9.	<i>Solanum nigrum</i>	1.2	15.30	0.018	Regular
10.	<i>Diospyros melanexylon</i>	1.8	20.91	0.018	Regular
11.	<i>Milius tometosa</i>	1.2	15.30	0.0187	Regular
12.	<i>Bambusa strictus</i>	0.8	10.88	0.022	Regular
SITE III					
1.	<i>Woodfordia fruticosa</i>	1	15.94	0.0156	Regular
2.	<i>Lagistomia parviflora</i>	1.2	10.31	0.018	Regular
3.	<i>Acacia catechu</i>	2	25.36	0.02	Regular
4.	<i>Acacia mangium</i>	2.2	26.81	0.022	Regular
5.	<i>Dalbergia sissoo</i>	0.8	14.49	0.0125	Regular
6.	<i>Leucaena leucocephala</i>	1	13.76	0.0277	Random
7.	<i>Murraya paniculata</i>	1.2	19.56	0.012	Regular
8.	<i>Chloroxylon swietenia</i>	1.4	21.01	0.014	Regular
9.	<i>Solanum nigrum</i>	0.6	10.86	0.016	Regular
10.	<i>Diospyros melanexylon</i>	1.6	20.28	0.025	Random
11.	<i>Milius tometosa</i>	1	15.94	0.015	Regular
12.	<i>Bambusa strictus</i>	1	15.94	0.015	Regular

Figure 2. Comparative analysis of microbial diversity and enzyme activity for both the native and dump soils (a) Total viable bacterial count and (b) fungal count for native (rhizospheric & non rhizospheric) (c) DHA and Catalase activity (d) Phosphatase and Urease activity

Table 2. Soil sample analysis with respect to soil texture and physico-chemical parameters

Soil sample	% Sand	% Silt	% Clay	Soil texture	pH	Electrical conductivity	Organic carbon	Available phosphors (Kg/ha)	Available potassium (Kg/ha)
NF1	46.33±3.05	24.73±1.16	28.93±3.78	Clay loam	7.27±0.06	0.22±0.02	0.43±0.015	33.02±1.06	123.78±1.68
NF2	44.23±2.04	26.33±2.25	29.43±3.97	Clay loam	5.58±0.47	0.14±0.01	0.94±0.04	32.31±1.94	172.28±1.85
NF3	46.8±2.52	28.23±1.34	24.96±2.01	Loam	5.93±0.20	0.48±0.01	0.60±0.01	86.11±1.4	135.22±3.5
NF4	45.4±3.245	27.06±1.30	27.53±3.76	Sandy clay loam	7.06±0.20	0.23±0.02	0.81±0.011	36.03±0.36	156.71±2.02
NF5	45±4.04	27±4.58	28±2.64	Loam	6.93±0.45	0.27±.01	0.86±.01	56.66±2.08	128.33±3.05
NF6	39.33±2.51	29±2.00	31.66±2.08	Clay loam	6.33±0.37	0.24±.02	0.64±.01	44.66±2.51	132.66±1.52
DM1	67.67±3.21	17.76±1.32	14.56±4.39	Sandy loam	5.4±0.18	0.55±0.01	0.59±0.01	31.71±1.94	93.48±1.43
DM2	52.76±1.96	20.56±0.68	26.66±1.36	Sandy clay loam	6.29±0.19	0.54±0.02	0.65±0.01	26.81±1.00	87.97±0.84
DM3	55.42±2.10	23.69±1.18	20.79±2.05	Sandy clay loam	5.39±0.21	0.51±0.02	0.61±0.02	29.15±1.17	89.24±0.91
DM4	59.09±2.21	21.40±2.22	19.50±1.90	Sandy clay loam	5.45±0.20	0.52±0.01	0.54±0.01	28.05±1.07	96.46±1.32

The maximum EC value was found in dump soils as compared to other soil samples which can be toxic to plants and may prevent them from obtaining water from the soil. It was observed from the data that electrical conductivity was positively correlated with the sand content and negatively correlated with silt and clay content (Table 3). Organic carbon is referred to the amount of carbon recovered from microorganism and plant found in the soil and according to Ghosh *et al.*, (1983) soil having 0.8% organic carbon is referred to as healthy soil and for soil having percentage of organic carbon 0.4 or below that referred as poor quality of soil *i.e.* deficient in proper nutrient and microbes. The percentage of organic carbon for the given soil samples was found to be in the range of 0.43% (NF1) to 0.94 % (NF2). The result shows that dump soils have pH in the range of 0.54% to 0.65%, which

indicates an average quality of soil. Sample NF4 and NF2 has percentage of organic carbon above 0.8 signifies a healthy soil which support plant and microbial growth. It was reported that soil particles influence organic carbon (OC) in soil and in coordination they promote formation of soil aggregates maintain soil stability and nutrient holding ability, thus considered as an important indicator of soil fertility (Maharana *et al.*, 2013 ; Rajan *et al.*, 2010). The available phosphorus content ranged from 26.81 Kg/ha to 86.11 Kg/ha. The available phosphorus was highest in NF3; whereas the lowest concentration of available phosphorus was found in the DM2. The available K concentration ranged from 87.97 Kg/ha (DM2) to 172.28 Kg/ha (NF2). The available potassium content was positively correlated with the organic carbon content (0.677, $p < 0.05$) (Table 3)

Table 3. Pearson correlation coefficient between different soil properties

	CFU	DHA	Phosphatase	Urease	Catalase	pH	EC	Sand	Silt	Clay	OC ⁺	AP ⁺	AK ⁺
CFU	1												
DHA	0.558	1											
Phosphatase	0.864**	0.749**	1										
Urease	0.83**	0.496	0.864**	1									
Catalase	-0.652*	-0.766**	-0.881**	-0.778**	1								
pH	0.185	0.217	0.446	0.458	-0.484	1							
EC	-0.799**	-0.287	-0.735**	-0.818**	0.496	-0.577*	1						
Sand	-0.726**	-0.55	-0.889**	-0.805**	0.766**	-0.603*	0.792**	1					
Silt	0.715**	0.750**	0.907**	0.744**	-0.826**	0.448	-0.688*	-0.921**	1				
Clay	0.668*	0.361	0.795**	0.772**	-0.654*	0.656*	-0.791**	-0.962**	0.779**	1			
OC	0.63*	0.239	0.424	0.174	-0.156	0.055	-0.502	-0.393	0.377	0.369	1		
AP ⁺	0.363	0.837**	0.658*	0.367	-0.794**	0.146	-0.032	-0.386	0.594*	0.207	0.076	1	
AK ⁺	0.933**	0.642*	0.833**	0.771**	-0.618*	0.356	-0.852**	-0.735**	0.748**	0.662*	0.635*	0.323	1

**Correlation is significant $p < 0.01$ and * correlation is significant $p < 0.05$

OC⁺ - Organic carbon, AP⁺ - Available phosphorus, AK⁺ - Available potassium

To enumerate soil biological activity, soil dehydrogenase, phosphatase, urease and catalase activity was calculated and compared for native soil and for the dump soils (Figure 2c & 2d).

The dehydrogenase activity ranged between 0.22 $\mu\text{g TPF.g}^{-1}\text{dry soil h}^{-1}$ to 3.79 $\mu\text{g TPF.g}^{-1}\text{dry soil h}^{-1}$ for different soil samples. The maximum dehydrogenase activity was found in the native soil sample of NF4 *i.e.* 3.79 $\mu\text{g TPF.g}^{-1}\text{dry soil h}^{-1}$ (Figure 2c). In the present study dehydrogenase activity is positively correlated with the phosphatase activity ($r=0.790$, $p<0.05$) and soil silt percentage (Table 3). Dehydrogenase activity was found to be correlated with the bacterial diversity of soil whereas the current study shows that DHA activity is not significantly correlated with bacterial diversity which reflects that the mining activities alter the environmental conditions such as presence of metal contamination which may inhibit the dehydrogenase activity. The result draws support from the finding Oliveira and Maria, (2006) which stated that DHA activity is inhibited by the presence of heavy metal contamination in soils.

Catalase is an intracellular enzyme exists in the soil and organisms widely and responsible for the decomposition of plant litter and for the decomposition of H_2O_2 . In soil, catalase activity has been greatly associated with the organic matter content. Maximum activity was found in dump soils and in rhizospheric soils of NF2. The catalase activity for various soil samples is shown in Figure 2c. Catalase activity recorded in dump soils was 3.54 ml of 0.1 M $\text{KMnO}_4/\text{g soil/h}$ and 3 ml of 0.1 M $\text{KMnO}_4/\text{g soil/h}$ for DM1 and DM2 respectively (Figure 2c). Catalase activity ranged between 1.46 ml of 0.1 M $\text{KMnO}_4/\text{g soil/h}$ to 3.54 ml of 0.1 M $\text{KMnO}_4/\text{g soil/h}$. The catalase activity is negatively correlated with the dehydrogenase activity and was found to be highest in dump soils which

differ from the recent studies (Mitrovica *et al.*, 2013). In comparison with phosphatase and urease, catalase activity is negatively correlated which indicate the presence of stress condition in soils of coal mine.

Urease enzyme involved in the hydrolysis of urea into ammonia and carbon dioxide and plays an important role in nitrogen cycling. Production of ammonia tends to increase soil pH and make soil alkaline in nature. Urease activity was found to be highest in the native soils in comparison with the degraded soils of dump. The maximum activity was found in the soil NF2 *i.e.* 0.583 $\mu\text{g}/\text{NH}_4^+/\text{g}/24\text{ h}$ (Figure 2d). On comparing urease activity between different soil samples; least activity was recorded in dump. The current findings indicate that for these soils urease activity was not correlated with the organic carbon content which contradicts the findings of Tabatabai, (1977) who reported that urease activity was significantly correlated with organic carbon. The urease activity was significantly correlated with the phosphatase activity which showed the soil is in biochemically active conditions (Table 3).

Phosphatase enzyme plays an important role in phosphorus cycle and is correlated with plant growth. It was observed that dump soil has the minimum phosphatase activity. Maximum activities was recorded in the rhizospheric soil sample NF4 *i.e.* 1.30 $\mu\text{M pNP/g}/24\text{ hr}$ indicating highest microbial activity in the soil. The Phosphatase activity ranged from 0.29 $\mu\text{M pNP/g}/24\text{ hr}$ to 1.30 $\mu\text{M pNP/g}/24\text{ hr}$ and presented in Figure 2d.

The findings of the present study suggested that urease and phosphatase activity were found to be positively correlated with bacterial diversity and maximum activity was recorded for natural soils due to the presence of high bacterial diversity.

3.4. Screening of bacterial isolates for plant growth promotion and stress resistance

The total bacterial isolates isolated from the dump soil as well as from the native soils of the dominant plant were screened for various plant growth promotion activity and stress tolerance potential. The native soil collected from rhizospheric and non rhizospheric regions of the dominantly growing plant viz., *Acacia mangium* & *Acacia catechu* which has high bacterial population these bacterial isolates possessed maximum plant growth promotion activities. The range and percentage of positive isolates varied greatly for each plant growth promotion activities. A total of sixty four bacterial isolates screened for their plant growth promotion activities viz., Indole acetic acid (IAA) production, phosphate solubilization and siderophore production. Phosphate solubilization activity was screened qualitatively on the Pikovskaya's agar plates and bacterial isolates were selected for phosphate solubilization by the presence of a clear halo around the bacterial colony. In the current work 28 isolates were found to solubilize phosphate with a solubilization zone ranged between 2 mm to 13 mm. It was observed that maximum phosphate solubilizing bacteria were associated with soil NF4. Isolates NU1, NU6, NU25, NU27, NU36 and NU47 were selected as the most efficient phosphate solubilizing bacteria having the solubilization zone between 6 mm-13 mm. The highest solubilization index was observed by NU36 with 13 mm of clear zone indicating phosphate solubilization. Bacterial isolates were further screened for the production of growth regulator i.e. IAA and it was found that majority of the isolates were positive for IAA production. The range of IAA produced by different bacterial isolates lies between 0.8 µg/ml to 77 µg/ml with an average concentration of 24.9 µg/ml. Isolate NU25 was selected as the highest producer of IAA i.e. 77 µg/ml. Very few isolates from

dump soils were found to produce a significant concentration of IAA, isolate NU51 from the dump soil has produced a significant concentration of IAA. Bacterial isolates able to form an orange halo zone were selected as siderophore producer. A total of 3 bacterial isolates (NU36, NU27, and NU25) were found to produce siderophore. Within the 24 h of incubation significantly an average siderophore production was observed and it was increased within the incubation period of 4-5 days. An orange halo zone was observed around the bacterial colony which indicates siderophore production. The most promising isolates out of the total sixty four bacterial isolates having several plant growth promotion activities are presented in Table 4.

The bacterial isolates were further screened for stress tolerance potential viz., drought, high temperature, salinity, pH and heavy metal. A total sixty four isolates were isolated and screened for drought resistance, out of which 51 isolates were grown at minimal water potential (-0.0573 MPa) i.e. 5% PEG 6000. Bacterial isolates showing luxuriant growth at water potential -0.73 MPa (25% PEG 6000) and -1.2 MPa (32.6 % PEG 6000) were selected as drought resistant bacteria (Figure 3a). It was observed that with the increasing concentration of PEG 6000 in the medium the concentration of bacterial cells got decreased. Bacterial isolates further screened for a temperature range of 10-50 °C. A wide range of isolates were found to be grown at a temperature of 20-30 °C. Least number of bacterial isolates was survived at a temperature of 10 °C. Only three bacterial isolates NU3, NU19 and NU24 were able to tolerate the maximum temperature of 50 °C. In the present study bacterial isolates growing at 40 °C were selected as temperature resistant. Further increase in temperature results in the decrease of bacterial growth. Furthermore, bacterial isolates screened for salt resistance within a salt concentration of 0.5 %-15%. A maximum number of bacterial isolates were grown within the range of 0.5%-4% salt concentration.

Table 4. Details of potential isolates with respect to PGP and stress tolerance potential and similarity index with neighboring bacterial species in biolog database

S.No.	Plant growth promotion activities				Stress tolerance potential			Biolog	
	Isolate Code	IAA ($\mu\text{g/ml}$)	Phosphate (mm)	Siderophore	Temperature tolerant (40°C)	Salt tolerant (10%)	pH tolerant (6-8)	Neighboring species in biolog database	% Similarity
1.	NU25	77 $\pm$ 3.60	9	++	++	++	+++	<i>Acinetobacter</i> spp.	56.9
2.	NU27	53 $\pm$ 2.24	6	+++	++	++	+++	NA	-
3.	NU36	54.6 $\pm$ 2.42	8	+	+++	++	+++	<i>Pseudomonas</i> spp.	57.5
4.	NU44	33.13 $\pm$ 2.16	13	-	++	+	+++	NA	-

-No growth, + Moderate growth/ Weak producer; ++ Good growth/Medium producer; +++ Excellent growth/Strong producer.

Out of 64 bacterial isolates nineteen isolates growing at a salt concentration of 10% were selected as salt tolerant and very few isolates were grown at maximum salt concentration 15% NaCl. pH tolerant bacterial isolates were then screened by growing bacteria within the range of pH 4-10. Out of sixty four only four bacterial isolates (NU7, NU3, NU4, and NU24) were grown at pH 4 and 18 isolates (NU2, NU34, NU55, NU21, NU7, NU30, NU3, NU19, NU23, NU27, NU46) were tolerant to pH 10. Isolates grown within the optimum range of pH 6-8 were selected. It was reported by several authors that rhizobacteria within the range of 6.5-7.0 were shown plant growth promotion activity such as phosphate solubilization (Maheshwar *et al.*, 2012). Bacterial isolates were further screened for heavy metal tolerance using three salts of cadmium, lead and zinc. The tolerance level of different bacterial isolates varied greatly. The maximum level of metal tolerance (1 mM- 10 mM) shown by the potential isolates is presented in Figure 3b, which is in agreement with

previous studies reported that rhizobacterial isolates showing metal tolerance activity in the range of 2 mM-8 mM were possess plant growth promotion potential as well as suited for the bioremediation studies (Belimov *et al.*, 2005; Ganesan, 2008). A total of four isolates NU25, NU27, NU36 and NU44 were selected as the most promising potential isolates on the basis of having multiple plant growth promoting characteristics and stress tolerance potential. These potential isolates were further characterized by BIOLOG and metabolic profile of the potential isolates is shown in Table 5a-5b. The neighboring species provided by the database with the maximum similarity index is shown in Table 4. Isolate NU25 and NU36 showed maximum similarity with *Acinetobacter* sp. and *Pseudomonas* sp. respectively whereas isolates NU27 and NU44 showed less than 50 % similarity with any of the bacterial strain from the BIOLOG database therefore not identified.

Figure 3 (a) Absorbance of bacterial isolates in at two different PEG6000 concentrations (b) Threshold level of metal tolerance by potential isolates

Table 5a. Substrate utilization pattern of selected isolates as determined by GEN III Biolog.

No.	Nutrient source	Bacterial Isolates				No.	Nutrient Source	Bacterial Isolates			
		36	25	27	44			36	25	27	44
A1	Negative Control					C1	α -D-Glucose	+		+	
A2	Dextrin			+		C2	D-Mannose			+	
A3	d-Maltose			+		C3	D-Fructose			+	
A4	d-trehalose	+		+		C4	D-Galactose				
A5	d-cellobiose			+	+/-	C5	3-Methyl Glucose				
A6	Gentibiose			+		C6	D-Fucose				
A7	Sucrose			+		C7	L-Fucose				
A8	D-Turanose			+		C8	L-Rhamnose				+/-
A9	Stachyose					C9	Inosine				
A10	Positive Control	+	+	+	+	C10	1% Sodium Lactate	+	+	+	
A11	pH 6	+	+	+	+	C11	Fusidic acid				+
A12	pH 5					C12	D-Serine	+/-	+		+
B1	D-Raffinose			+		D1	D-Sorbitol			+	
B2	α -D-Lactose					D2	D-Mannitol				
B3	D-Melibiose					D3	D-Arabitol				
B4	β -Methyl-D- Glucoside			+		D4	Myo-Inositol	+			
B5	D-Salicin			+		D5	Glycerol				
B6	N-acetyl-d-glucosamine			+		D6	D-Glucose-6-PO ₄				
B7	N-acetyl- β -d-mannosamine					D7	D-Fructose-6-PO ₄				

Table 5b. Substrate utilization pattern of selected isolates as determined by GEN III Biolog.

B8	N-acetyl-d-galactosamine					D8	D-Aspartic acid					
B9	Neuraminic acid					D9	D-Serine	+/-				
B10	1% NaCl	+	+/-	+	+	D10	Troleandomycin	+				+
B11	4% NaCl		+	+		D11	Rifamycin SV	+	+			+
B12	8% NaCl	+	+	+		D12	Minoocycline					
E1	Gelatin		+/-			G1	p-Hydroxy-Phenylacetic Acid	+				
E2	Glycine-L-Proline			+	+	G2	Methyl Pyruvate					
E3	L-Alanine	+	+			G3	D-Lactic acid methyl ester					
E4	L-Arginine	+	+			G4	L-lactic acid	+		+		
E5	L-Aspartic acid	+		+	+	G5	Citric acid	+		+		
E6	L-Glutamic acid	+		+	+	G6	α -Keto-Glutaric acid	+	+			+/-
E7	L-histidine				+	G7	D-malic acid					
E8	L-Pyrogutamic acid	+				G8	L-malic acid	+	+	+	+	
E9	L-Serine	+				G9	Bromo-Succinic acid					
E10	Lincomycin	+	+		+	G10	Nalidixic acid					+
E11	Guanidine Hel				+	G11	Lithium chloride				+	
E12	Niaproof 4	+				G12	Potassium Tellurite				+	
F1	Pectin					H1	Tween 40		+			
F2	D-Galactouronic acid					H2	γ -Amino-Butyric Acid	+				
F3	L-Galactonic acid Lactone					H3	α -Hydroxy- Butyric Acid			+		
F4	D-Gluconic acid	+		+		H4	β -Hydroxy-D,L- Butyric Acid	+				
F5	D-Glucuronic acid		+/-			H5	α -Keto-Butyric Acid		+			
F6	Glucuronamide	+				H6	Acetoacetic Acid					
F7	Mucic acid	+				H7	Propionic Acid	+				
F8	Quinic acid	+				H8	Acetic Acid	+	+	+	+	+
F9	D-Saccharic acid					H9	Formic Acid					
F10	Vancomycin	+	+			H10	Aztreonam		+			+
F11	Tetrazolium violet	+	+		+	H11	Sodium Butyrate		+			
F12	Tetrazolium blue	+	+		+	H12	Sodium Bromate					

4. Discussion

Soil health is maintained by balancing soil biological and physico-chemical properties in a unique concentration. Assessment of soil health hence requires indicators of all these factors. Healthy soils are foremost for the integrity of terrestrial ecosystems to remain intact or to get better from disturbances (Das and Varma, 2011). Deterioration of soil, and thereby soil health, is of concern for human, animal, and plant health. Our study aims to examine the impacts of coal mining on soils of mining sites and associated environment. A survey was performed for the selected mining sites and surrounding environment for analyzing plant diversity and collection of soil samples. Two species viz., *Acacia mangium* and *Acacia catechu* were observed as the most dominant species of the tree community at all the sites (Table 1a-1b). *Acacia mangium* is not native to India but introduced in India in the mid 1960s and became prevalent at mining locations (Krisnawati *et al.* 2011). High IVI of *A. mangium* and *A. catechu* suggested its growth under a wide range of environmental variables. This result suggests that a species having maximum diversity have potential to acclimatize to stress conditions originated due to coal mining activities. As variations in environmental factors the adaptive abilities of organisms, only those species which are adapted to new conditions or those which can become accustomed to the new conditions, participate in the community formation (Agrawal and Agarwal 2000). The species vulnerable to the altered soil properties became diminished and more tolerant species become widespread (Buma and Wessman 2013). Altered soil properties in the area due to coal mining activities modified the response of the vegetation either by acting synergistically or antagonistically. In comparison to the surrounding sites of mining region, dump sites were deprived of any plant species. The Shannon's Diversity Index represents the species

richness and diversity in a structure and higher value of H' signifies greater diversity in the area. It was previously reported that any ecosystem having Shannon diversity greater than 2 ($H' > 2$) has been regarded as medium to high diverse regarding to species (Barbour *et al.*, 1999). The stability of the community is related to the species diversity, the higher the value of the diversity index, the greater will be the stability of community structure and function (McCann, 2000). Coal mining activities had a negative influence on species composition and diversity as it clearly shown that the no plant species were observed at dump sites which eventually affects the soil characteristics of the nearby surroundings. From the results it is clearly demonstrated that soil characteristics vary greatly between the natural soils and the dump soils. Dump soils are characterized as having less bacterial diversity in comparison to native soils which is in accordance with other authors (Mitrovica *et al.*, 2013). Maximum numbers of bacterial colonies were observed in comparison with the fungal population which is in agreement with the previous studies (Osazee, 2013). Dump soils were found to be associated with high fungal count than the rhizospheric and non rhizospheric soils because of the acidic nature of dump soils. The abundance of microbes in soil indicates a healthy and nutritive soil and suitable for plant growth. The quantity and activity of soil microbes reflect the soil conditions and level of soil development.

The textural analysis of soil samples exhibit that the dump soils contains considerably an extended amount of sand particles and majority of dump soils were characterized as sandy clay loam. The plant root exudates have the potential to disintegrate the coarse particles into smaller particles such as clay (Banerjee, 2000) and the percentage of clay is important for maintaining soil aggregation and structure (Gregorich *et al.*, 1991; Nyles and Ray, 1999). Least percentage of clay was recorded in soil DM1 which is of sandy

loam texture that is less suitable for plant growth. It was reported earlier that soil texture was used as an indicator of soil microbial characteristics in ecosystem as it influences soil microbial diversity and activity (Jha and Singh, 2011). The native soils collected from natural forest have maximum percentage of clay particles than the dump soils which is due to the presence of vegetation in natural forest. Nyles and Ray (1999) stated that soil samples having high sand content with lower clay content may contain leaching pollutant. It was observed that dump soils were less stable than the natural soils. The bacterial diversity of soils is influenced by the particle size distribution and it was reported that clay fraction has more diverse bacterial population than sand and silt fractions (Faoro *et al.*, 2010; Sessitsch *et al.*, 2001). The present study suggests that dump soils has a relatively low pH as compare to native soils. The pH of soils varied from alkaline to acidic. The alkaline pH indicates optimal range for plant growth but a lower pH cause problem for normal growth of the plants and it has been proved that soil pH was improved by the revegetation process (Jha and Singh, 2011; Yang *et al.*, 2015). It was reported previously that due to deposition of excessive mineral during mining, soil turn out to be acidic (Jha and Singh, 2011; Harun *et al.*, 2014). The low pH indicates that majority of the soils turns to be acidic due to pyrite oxidation found in coal that controls the lowering of pH which may be the reason of that restricts the growth of vegetation cover in dump mining soils. On comparing the physicochemical properties of dump and native soils, dump soils are characterized as degraded soils due to excessive pH and less nutritive content. Therefore, it necessary to reclaim the mining soils with the help of stress tolerant and dominant plant species.

Soil enzymes are a part of soil microorganism and plant involved in the functioning of soil by maintaining nutrient cycle, decomposition of organic matter

other pollutant and appeared to be more informative as they play an important role in maintaining soil health. The dehydrogenase activity is used as an indicator of microbial activity in soils as they participate in the electron transport chain and its activity is dependent on the utilization of the intracellular environment of microbes (Kandler *et al.*, 2007). It was observed that in the dump soils DHA activity is found to be low as compared to the soils of natural forest, which indicates the absence of microbial populations in dump soils. The presence of high DHA activity in natural soils rather than dump soils indicates the presence of high microbial diversity in native soils which is also in agreement with the of Reddy *et al.*, (1987) which suggested that dehydrogenase activity is high in native soil due to the presence of microbial diversity in rhizosphere of plant. Urease activity is highly associated with the vegetation of the soil which is also proven by the current study where high activity was observed in vegetative soils (Reddy *et al.*, (1987). In the dump soils urease activity was found to be less due to the absence of plant cover in dump sites. Speier *et al.*, (1980) concluded that urease activity is tending to be constant for soils with vegetation but it is decreased consistently in follow soils. Most often highest catalase activity is found in litter and humus rich surface and therefore maximum catalase activity was found in dump soils. Phosphatase activity indicates the metabolic state of soil thus relates with the biological activity of microbes and used as a good indicator of soil fertility. Maximum activity was observed in native soil which indicates the presence of dynamic microbial activity in the native soil of plants in comparison to dump soils which is found in agreement with the previous studies (Mitrovica *et al.*, 2013).

Plant growth promoting rhizobacteria has gained worldwide recognition and an acceptance for soil remediation as well as for sustainable agriculture (Saharan and Nehra, 2011). It was quite evident from

the study that coal mining deteriorates soil quality, destroys surface vegetation and eventually creates a stressful environment for the survival of plants and microbes. Therefore, it is necessary to reclaim the soil characteristics of degraded dump soil with the help of eco-remediation approach. Study proposed by Yang *et al.*, 2015 proved that restoration and revegetation of mining soils results in the enhancement of soil properties, such as soil organic carbon, bulk density and water content (Yang *et al.*, 2015). Therefore, it is important to select effective PGPR isolates with stress tolerant potential which will enhance plant growth modifies soil characteristics. In addition the PGPR application also reduces the deleterious effect of chemical fertilizers and pesticides. Survival of an inoculated bacterial strain within the rhizosphere is affected by a number of abiotic factors such as high salt, high pH, and high temperature (Johri *et al.*, 1999). Therefore, an attempt was made to screen promising bacterial isolates of with abiotic stress tolerance and PGPR activity for better plant growth promotion.

For the screening of effective PGPR strain, three methods were simultaneously employed. These approaches included screening of rhizobacteria for *in vitro* IAA production siderophore production and the potential to solubilize inorganic phosphate. In the present study, out sixty four isolates, 28 isolates were found to be positive for phosphate solubilization and approximately 80% of the isolates were positive for IAA production. Maximum IAA production was exhibited by isolates NU20, NU25, NU27, NU36 and NU47. Isolate NU51 isolated from dump has produced IAA with a concentration of 34.699 µg/ml. It was reported earlier that several bacterial species belonging to mine tailing region were produced IAA in the range of 2.2-83.05 µg/ml (Yu *et al.*, 2014). Our findings of IAA production in soil isolates are in agreement with other workers (Mitrovica *et al.*, 2013; Benidire *et al.*, 2016). The result showed that only three isolates have the potential

to produce siderophore in CAS agar medium. It was reported previously that PGPR strains having siderophore production fulfill the iron availability to plants (Saharan, 2011). The result indicates that the out of 64 tested isolates; some isolates exhibit more than two or three PGP activities which may promote plant growth directly, indirectly, or synergistically. The results are similar to previous findings where multifarious plant growth promoting activities among PGPR have been reported (Dey *et al.*, 2004; Singh *et al.*, 2014).

The successful deployment of bacterial isolates in degraded as well as stressed ecosystem depends on their potential to tolerate and propagate under the adverse environmental condition such as high temperatures, drought, salt stress, pH, heavy metal toxicity, and so forth. Out of 64 isolates, twenty two isolates were found to tolerate a water potential of -0.73 MPa (25% PEG 6000) and 19 isolates grown at -1.2 MPa water potential. The maximum tolerance was shown by the isolates NU47. In the present study bacterial isolates growing at a temperature ranged between 30-40 °C were selected for further studies. A number of studies reported that the optimum temperature range for the growth of plant growth promoting rhizobacteria is 25-35 °C (Ali *et al.*, 2009). The salt and pH tolerance potential of bacterial isolates was also screened to select the efficient bacterial isolates. Salinity is an important environmental stress that decreases the plant growth. Tolerance to salt stress represents the ability of bacteria to respond and adapt the environmental changes. Out of 64 bacterial isolates nineteen isolates growing at a salt concentration of 10% were selected as salt tolerant bacterial isolates. Some of the researcher observed that bacterial isolates promote plant growth at a salt concentration of 5-6% (Sandhya *et al.*, 2009). Mining processes generates metal pollutants in soil and results in the establishment of metal tolerant microbial population. In the present study majority of the isolates were registered as cadmium and lead tolerant whereas only

few isolates were found to be zinc tolerant. Recent studies shows that metal tolerance activities were coupled with plant growth promotion activities primarily with siderophore production and they are proved as a potential rhizoremediators (Khan *et al.*, 2009; Tak *et al.*, 2013). It has also been reported that plant growth promoting rhizobacteria have potential to increase plant tolerance towards drought (Vurukonda *et al.*, 2016; Kaushal and Wani, 2016) as well as to ameliorate heavy metal stress (Khan *et al.*, 2009; Tak *et al.*, 2013). Drought and salinity are the most common abiotic stress that adversely affects the plant growth and development. Therefore, bacterial isolates selected on the basis of their potential to survive in stress as well as having plant growth promotion activity for further reclamation and restoration studies. Out of sixty four bacterial isolates, four isolates (NU25, NU27, NU36 and NU44) were selected on the basis of their stress tolerance and plant growth promotion potential. Bacterial isolates were characterized biochemically through BIOLOG and it was observed that all the potential isolates differed in the utilization of carbon and nitrogen sources and are functionally divergent.

5. Conclusion

The study presented here emphasize on the comparison of various soil samples collected from both the mining sites and from the native forest to evaluate the impact of coal mining on soil quality. The study revealed that the dump soils that belong to mining regions were characterized as having less nutritional content and devoid of any vegetation, as a result soil has acidic pH, less enzymatic activities and low bacterial communities which makes the soil less fertile, whereas all the native soils collected from the surrounding forest are much fertile due to its high nutritional status. Therefore, it is difficult to revegetate the dumps because of extremely poor conditions of the

soil and the biological reclamation does not take place within the shelf life of soil as the nutrient cycle gets destroyed and the soil eventually becomes unproductive. In our findings, *Acacia mangium* was found to be the dominant species and a high number of bacterial communities were recorded in the native soils associated with the plant, out of which a significant percentage of the bacterial isolates were characterized having plant growth promoting potential and stress tolerance properties. Therefore, it is evident from the study that the plant and microbial community has considerably influenced the soil fertility and may be effective in reclaiming soil properties. The present study leads to the delivery of promising bacterial isolates *i.e.* NU25, NU27 and NU36 which belonged to the natural habitat of mining having maximum potential to release plant growth promoting substances and survive in various stress conditions. Furthermore, the isolates along with dominantly growing plant species will go a long way to reclaim and remediate the soil properties of the mine dump soils of Sonbhadra, Uttar Pradesh.

Acknowledgement

The authors are thankful to Department of Biotechnology, Govt. of India for providing financial assistance and Director, Motilal Nehru National Institute of Technology, Allahabad for providing research facilities.

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