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Research Article

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Microbiology of Soil Collected from Bankola and Sankerpur Kenda of Raniganj Coalfield

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Abstract:

The present study was conducted on microbial diversity of soil from selected sites (Bankola and Sankerpur Kenda) in the proposed coal mining area of Raniganj coalfield, West Bengal India. Geographic location, way point, nearest town, mining area, total mining area, total overburden area, status of mining operations, and number of overburden, habitation, agricultural field, barren land, and forest/plantation was considered as basis of the soil sample selection. Soil physicochemical properties were also analyzed. Soil samples were processed according to standard microbiological techniques for microbiological study. Identification of the species of microorganisms was performed by Gram staining as well as by biochemical and antibiotic tests as per standard method. The results of the present study revealed that Bacillus sp. found in Bankola region whereas Pseudomonas sp. present in Sankerpur kenda area.

Key words: Mining, Physico-chemical characteristics. Microbiology, Gram staining, Biochemical tests.

I. INTRODUCTION

Mining comes under the second group of the human's earliest endeavours, after agriculture, which is ranked as the first one. These two industries are basic industries of early civilization. Little has been changed in the importance of industries. Fishing and lumbering consider as branch of agriculture and oil and gas production consider as mining. Since then these two industries became basic producers to the modern civilization. From prehistoric time to present, mining is very important to human existence. Mining means any naturally occurring substance from earth for useful purposes (Thoms, 1974). The history of mining is attractive.

Coal was discovered by Illinois in 1673, it mainly has been used for a source of energy. As technology sophisticated, nevertheless, the use of coal for the source of energy became very important (Toy et al., 1987). A number of discoveries played a significant role in making way for the industrial revolution. Mineral extraction process affects the land, water and air which are proximity to the mining site. This process makes sure the return in productivity of the affected land. Every million tonne of coal excavated by surface mining process damages a surface area of about 4 ha in India.

During this mining process, several changes take place in the physical, chemical, and microbiological characteristics of soil as a result of storage. Soil is a system, in which constant interface between soil minerals and microorganisms control the physico-chemical and biological properties of global ecosystem. Anthropogenic actions such as mining activities, especially, open cast mining, have effected in radical alternations in their geochemical cycles and often lead to land degradation. It changes the soil textural and structural characteristics (Pederson et al., 1980; Silburn and Crow, 1984). Soil characteristics are important factors controlling natural processes. For example, bacterial interaction to soil can be influenced by clay content (Ling et al., 2002; Guber et al., 2005). A further vigorous characterization of soil characteristics must be studied for better understanding of the bacterial fate and transport in the environment. Chemical characteristics of a soil need to study as well. Parameters such as pH and specific ion content and organic matter are critical for shaping the kind of microbial communities that can present in a particular soil. Microbial communities can be experiential on the basis of several parameters that imitate the behaviour of soil microbes, such as enzyme action or grouping of fatty acids (Ibekwe and Kennedy, 1998). Soil bacteria play essential roles in a variety of biogeochemical cycles (BGC) (Molin and Molin, 1997). These are accountable for the cycling of organic compounds. Soil microorganisms as well control above-ground ecosystems by providing nutrients to plant (Timonen et al., 1996; George et al., 1995). As per our knowledge only about 1% of the soil bacterial population is able to culture by standard laboratory conditions. Anyway to study bacterial population of soil by molecular methods have been used. The whole organisms in the world depend on activities of microbial communities (Pace, 1997). Soil microorganisms are very important in which to maintaining the cycling of nutrients and for motivating above-ground ecological unit (Klironomos et al., 2000; Ovreas, 2000). At the same time as many anthropogenic actions such as mining, urbanization, agriculture, pesticides utilize and pollution by industrialization can possibly affect soil microbial diversity. Our knowledge is limited to study the bacterial diversity changes can influence the below-ground and above-ground ecosystems.

II. MATERIALS AND METHODS

The soil samples were collected from two representative sites of Bankola and Sankerpur kenda at Raniganj Coal Field. Top soil was removed and about 500 gm of soil samples were collected from those areas and mixed them thoroughly put in a clean sterile polythene bags, sealed with rubber bands and the samples were analyzed in the

laboratory. After completion of assessment of the spoil physicochemical properties like pH, organic carbon, nitrogen, potassium, and phosphorus, following standard methods (Hajek et al. 1972, Issac et al. 1984, APHA, 1998), soil samples were transported to the microbiological laboratory. By using media and serial dilution method microbiological study were performed. Microbial colonies were enumerated as colony forming units (cfu). Microbial diversity was carried out by various assays such as gram's staining, indole test, catalase test, MR-VP test, citrate utilization test, oxidase test, urease test, hydrogen sulfide gas production test etc.

III. RESULTS AND DISCUSSION

Microorganisms in soil are critical for the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation; decomposition of organic matter, the cycling of carbon, nitrogen, phosphorus and toxin removal. The analysis of soil samples collected from Bankola and Sankerpur Kenda area of Raniganj coalfield (RCF), revealed that in Bankola region soil pH is strongly acidic where as soil pH is strongly alkaline in Sankerpur Kenda area (Table: III) . Texture of soil samples are coarse and color of the soil samples are brownish to black (Table: III).The nitrogen content is high in Bankola where as it is low in Sankerpur kenda. Potassium content of all the soil samples is found to be same in range. Organic carbon content is varied from 0.36 to 0.45 in these two different areas of RCF. Phosphorus content is medium in range in Bankola region whereas phosphorus content is low in range at Sankerpur kenda region (Table: III).

In this present study, Serial dilutions of soil were performed and by using nutrient agar medium plates were prepared for producing colonies in petriplate. Microbial culture of soil samples of Bankola region show $20X10^4$ bacterial colonies whereas Sankerpur kenda shows $85X10^4$ bacterial colonies. These colonies suggest the presence of particular microbial population on those sites of Raniganj coalfield. Identification of microorganisms by morphological characterization of bacterial samples (Table: V), Gram's staining as well as by biochemical tests (Table: VI) reflected that *Bacillus sp.* found in Bankola region where as *Pseudomonas sp.* found in Sankerpur kenda area of Raniganj coalfield.

Table I: Distribution of Selected Two Study Sites of Raniganj Coal Field (RCF), West Bengal, India, under Eastern Coalfields Mining Authority, Government of India.

Name of the areas	**Geographic location**			**Way point**	**Nearest town**	**Mining area**	**Total mining area (m^2)**	**Total overburden area (m^2)**
	Latitude	**Longitude**	**Elevation**					
Bankola	23°39'52.9"N	87°14'32"E	109	376	Ukhra	Bankola	54000	540
Sankerpur (Kenda)	23°40'19.3"N	87°13'30"E	138	370	Ukhra	Kenda	542800	314400

Table II: Present Status of Selected Two Study Sites in Raniganj Coal Field (RCF), West Bengal, India.

Name of the areas	**Status of mining operations**	**No. of overburden**	**Habitation Present**	**Agricultural field**	**Barren Land**	**Forest / Plantation**
Bankola	Closed for six years	1	ECL Staff Quarters	Present	Absent	Absent
Sankerpur (Kenda)	Running for six years	3	ECL Staff Quarters	Present	Present	Absent

Table III: Determination of Physico-Chemical Properties of The Soil Samples

Area	**Color**	**Texture**	**pH**	**Nitrogen (kg/Acre)**	**Potassium (kg/Acre)**	**% of Organic Carbon**	**Phosphorus**
Bankola	Brownish	Coarse	5.0	300	20	0.36	11
Sankerpur (Kenda)	Black	Coarse	8.5	25	20	0.45	8

Table IV: Determination of Microbial Load by Using Nutrient Agar Medium in The Soil Samples

Name of the areas	**10-4(no. of colonies)**	**Organisms per gram of soil (CFU/gm)**
Bankola	20	$20X10^4$
Sankerpur (Kenda)	85	$85X10^4$

Table V: Morphological Characterization of Bacterial Samples Isolated from The Soil Samples

Bacterial Isolate	**Bacterial code**	**Size**	**Shape**	**Margin**	**Color**	**Elevation**	**Texture**
Bankola	B1	0.1 cm	Smooth	Entire	White	Raised	Moist
Sankerpur (Kenda)	S1	0.2 cm	Circular	Entire	Yellow	Raised	Mucoid

Table VI: Identification of Bacterial Species Isolated from Soil Samples

Biochemical tests	B1	S1
Gram's staining	+ ve	- ve
Indole Test	- ve	+ ve
Catalase Test	+ ve	+ ve
Citrate Utilization Test	+ ve	- ve
MR Test	+ ve	+ ve
Oxidase Test	+ ve	+ ve
Urease test	+ ve	- ve
VP Test	- ve	- ve
Nitrate reduction test	- ve	- ve
H2S production test	- ve	- ve
Starch hydrolysis test	- ve	- ve
Antibiotic sensitivity test	+ ve	- ve
Bacterial species identified	*Bacillus sp.*	*Pseudomonas sp.*

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