STUDIES ON PHYSICO-CHEMICAL CHARACTERISTICS AND MICROBIAL DIVERSITY OF TOP-SOIL COLLECTED FROM PROPOSED COAL MINING AREA OF LATEHAR DISTRICT, JHARKHAND

A Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Technology

In

Biotechnology

By

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on physico-chemical characteristics and microbial diversity of top-soil collected from proposed coal mining area of Latehar district, Jharkhand "** by **RAMARAJU KURAM (211BM2007)** submitted to the **National Institute of Technology, Rourkela** for the award of Master of Technology in Biotechnology during the session 2011-2013 is a record of bonafide research work carried out by him in the Department of Biotechnology and Medical Engineering under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

Place: Rourkela
Date:

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DECLARATION

The present study entitled "**Studies on physico-chemical characteristics and microbial diversity of top-soil collected from proposed coal mining area of Latehar district, Jharkhand"** is based on my original research work and no part of the thesis has so far been submitted for the award of degree in Master of Technology in Biotechnology or any other degree or diploma to the **NIT Rourkela,** Orissa, India or elsewhere.

Place: Rourkela, Odisha

Date: **(Ramaraju Kuram)**

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(Ramaraju Kuram)

ABSTRACT

Mining is the extraction of valuable minerals from earth. Mining creates pollution which affects the surrounding environment. Loss of many biological species and deterioration of soil quality were the ultimate consequences. During mining, companies should adopt mitigation measures for maintaining good environmental conditions. EIA (Environment Impact Assessment) is required to understand the status of the environment that will help in taking up appropriate strategy to combat any adverse effect on it. Soil status is one of the key factors in the EIA. Land rehabilitation and mine reclamation are very crucial after finishing of mining. For this purpose the present study was conducted on physico-chemical characteristics and microbial diversity of top soil from selected villages (Nawari, Tubed and Dhobiajharan) in the proposed coal mining area of Tubed Coal Mines Ltd., Latehar district, Jharkhand, India. The results showed that the pH of the soil was found to be slightly acidic in nature. The bulk density and the specific gravity of the soil samples were very low, that indicates that the soil samples contain higher organic matter which is suitable for the growth of plants. The Chloride content ranged between 8.62 – 12.69 mg/g which is far higher than normal range (0.3 -1 mg/g). Potassium content is extremely high in all the soil samples which ranged between 566- 597 Kg/ha except the soil samples from Dhobiajharan village which is found to be very low 63 Kg/ha (normal range is 198.5-254.1 Kg/ha). 5 bacterial species (*Bacillus* species, *Micrococcus* species, *Pseudomonas* species, *Staphylococcus* species and *Enterobacter* species) and one fungal species (*Aspergillus* sp.) were identified from the soil samples. From the result it is concluded that the soils is rich in macronutrients which is essential for the growth of plants. Hence the suitability of the soil should be maintained after completion of mining in the proposed area.

KEY WORDS: mining, physico-chemical characteristics, microbial diversity, fungal diversity, biochemical tests

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ABBREVIATIONS

cm	-	Centimeter
ºC	-	Degree centigrade
et al.	-	And others
g	-	Gram
ha.	-	Hectare
hrs.	-	Hours
HCl	-	Hydrochloric acid
H_2O_2	-	Hydrogen peroxide
m	-	Meter
µg	-	Microgram
µl	-	Microlitre
mg	-	Milligram
ml	-	Millilitre
mm	-	Millimeter
mM	-	Millimolar
M	-	Molar
NA	-	Nutrient agar
N	-	Normal
P_2O_5	-	Phosphorous pentoxide
K_2O	-	Potassium dioxide
NaCl	-	Sodium chloride
Km^2	-	Square kilometer
H_2SO_4	-	Sulphuric acid
UV	-	Ultra Violet
Wt.	-	Weight
w/w	-	Weight by weight

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CHAPTER 1

INTRODUCTION

CHAPTER 1

1. INTRODUCTION

Mining comes under the second group of the human's earliest endeavors, 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. It's discovering of various minerals or their derivatives parallel to the history of civilization: the Stone Age, the Steel Age, the Bronze Age, the Nuclear Age, and the Iron Age.

Formation of coal takes millions of years to complete by geological processes. It is typically found as 'seams' in deep underground. Coal was discovered by Illinois in 1673, although before that the Hopi Indians used it to bake pottery in 1000 AD. Coal 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 micro biological 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. Microbes in top soils are critical to any natural course. Microbial communities can be experiential on the basis of several parameters that imitate the behavior of soil microbes, such as enzyme action or grouping of fatty acids (Ibekwe and Kennedy, 1998). Dehydrogenase and β-Glucosidase are intracellular enzymes connected in microbial respiratory metabolism (Von Mersi and Schinner, 1991). Two biochemical methods, phospholipid fatty acids and Fatty acid methyl ester that both utilize fatty acid biomarkers to evaluate the mixture of microorganisms occur in soil.

Soil bacteria and fungi play essential roles in a variety of biogeochemical cycles (BGC) (Trevors, 1998b; Molin and Molin, 1997; Wall and Virginia, 1999). 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), plant physical condition (Smith and Goodman, 1999; Srivastava et al., 1996), soil organization (Dodd et al., 2000; Wright and Upadhyaya, 1998) and soil productiveness (O'Donnell et al., 2001; Yao

et al., 2000). Our awareness of soil microbial diversity is limited because inability to study soil microorganisms. Torsvik et al., (1990a, b) predicted that in 1 g of soil there are 4000 different bacterial ‘‘genomic units’’ basis on DNA–DNA reassociation. So far about 5000 bacterial species have been estimated and described (Pace, 1997, 1999).

As per our knowledge only about 1% of the soil bacterial population is able to culture by standard laboratory conditions. It is not confirmed that whether these 1% species belongs to bacterial population (Torsvik et al., 1998). Predicted that is around 1,500,000 species of fungi are present in the globe (Giller et al., 1997). By current standard laboratory methods we are unable to culture many fungi (Van Elsas et al., 2000; Thorn, 1997). Anyway to study bacterial population of soil by molecular methods have been used. Research on fungi is very little has been conducted (Van Elsas et al., 2000). 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; Van der Heijden et al., 1998; 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.

1.1 OBJECTIVE OF THE PRESENT STUDY

Keeping the above case into consideration, the present study was aimed with the following objectives

1. Scientific study on the physical and chemical characteristics of the top soil of three selected villages of the proposed coal mining area.
2. Study of the microbial diversity in the top soil of three selected villages of the proposed coal mining area and identification of the micro flora by various biochemical tests.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

2. REVIEW OF LITERATURE

Activities associated with mining may cause a variety of potential health problems to the vicinity communities. The mining is extensive in the sense of the environmental legacy as there are millions of active and abandoned mines in the world. Mineral extraction from earth creates many health risks which are very difficult to define. It is a complex task in such a way that gives variety of products, different types of potential exposure hazardous materials and routes of contact. Mineral related processes releases many types of toxicants such as metalloids, mineral dusts and metals which are cause indirect effects like potential of vector borne diseases. During process of mining, produces gas emissions like sulfur dioxide and carbon dioxide in large amounts. Conventional crude oil and coal extraction processes release polycyclic aromatic hydrocarbons (PAHs), oil sand wastes and tailing water which are suspected to be carcinogens. The geotechnical and structural features of mine sites must also be concerned, as release of tailing dam contents which are hazardous to human communities (Filion et al., 1999). Mineral and fuel extractions indirectly or directly contaminate the water supplies and food sources which are threat to community health. So the balance between the risks and benefits in the mining industry should be thoroughly regulated (Cook et al., 2011).

2.1 Mining

The mining means extraction of worthy minerals and other geological materials from the earth. It is mainly conducted for the economic interest. During extraction of minerals from earth and transport of minerals requires the removal of soil also in some cases in the mining process. The waste removal is a major considered factor in mining process and the complete information regarding the waste materials characterisation. The nature of mining course generates an

inevitable impact on the environment both during the mining operations and years after the mine is closed. During mining only mitigation measures should be adopted.

2.2 Coal mines

Coal has been a source of energy for almost as long as Homo sapiens have inhabited the planet. It has been used dates back more than 3000 years to China where it may have been helpful to smelt copper. In some areas coal seams can be available on the surface only which is ready to utilize for energy source but this surface coal requires chimney in indoors and it burns dirty. The difficulty is transportation of coal. So, overcome from this problem is coal beneficiation process conducts near the site. Due to this reason, the exploration of coal throughout history was reasonably irregular. In the early ages of Common Era there is confirmation for its exploit in Iron Age in Britain, in addition to in Rome. In the U.S. its use started since 1300's by Hopi Indians for cooking and pottery making in the desert south west.

The advances in mining of coal, ventilation and transportation in Medieval Europe its attractiveness increased as popularity. Nevertheless, after Industrial Revolution starting it took great importance as requirement of energy through coal was supplemented to conventional strategies to produce energy. For large scale industries of manufacturing and transportation needs great amounts of energy. Coke is used in manufacturing of steel it requires lots of energy which was supplied by coal. James Watt discovered coal powered steam engine by utilizing of coal. This technology leads to the invention of train and the steam–powered boat, which is eventually made possible that fast and consistent transport of the manufacturing goods to distant places (Wali, 1987).

2.3 Different types of coal resources

Many national and international countries proposed classification coals. The coal is widely traded and distributed around the world. The different classifications of coal by various nationals proved that these classification systems have been useful for sort out of coal resources as different ranks given to the coal. Rank has given by means of amount of coalification, or alteration. Coal is continuously subjected to many advances as is called evolution, beginning from lignite to sub-bituminous and bituminous to finally anthracite. Coal develops in the course of as raise in temperature and water content falls by pressure increases and the raise in carbon content. Black coal means the collective forms of bituminous coal, anthracite coal and Sub-bituminous coal.

Table 1: Classification of coals by rank, ASTM system

Class	**Fixed Carbon**		**Volatile**		**Matter Energy**
	Dry (%)	**Moist (%)**	**Dry (%)**	**Moist (%)**	**Moist (*MJ/kg*)**
I. Anthracite	> 98–86	> 92–81	< 2–14	< 2–15	35.5–31.4
II. Bituminous	86–54	81–45	14–57	13–40	35.8–24.4
III. Sub-bituminous	55–53	45-37	53–55	36–38	26.7–19.3
IV. Lignite (brown coal)	52	32–26	32–35	38–50	< 19.3

The different types of coal are given rank based on the different amounts of conversion result. According to the ASTM categorizing there are four main types of coal: anthracite, bituminous, lignite, and sub bituminous (ASTM, 1987). This classification is given based on carbon percentage present in coal and heating value of coal which is connected with the heat and pressure during processing of coal. The maximum heating value and carbon content is present in

Anthracite compared to other coals. It contains carbon percentage is 86-98% and heating value approximately 15,000 Btu's per pound. The single largest type of coal is Bituminous which can find in U.S. and carbon content is less than that of anthracite. The heating value range is 10,000-15,000 Btu's per pound. Subbituminous coal which after bituminous coal in the context of both heat value and carbon content. It consist nearly 35-45% carbon, and moisture content is controls the heating value to around 8,000 and 13,000 Btu's per pound. Lignite is having the lowest value of coal in this group. It contains carbon content about 25-35% and having a lot of moisture, which decrease the heat value about 4,000 and 8,000 Btu's per pound.

2.4 Coal mining technology

Coal is mainly mined divided into two methods, one is surface mining or opencast mining and other one is underground mining or deep mining. The geology of the coal deposits decides the choice of mining method. The mainly operated method is underground mining which accounts about 60% of coal production in the world. Several countries are practicing surface mining process which is considered to be main mining process.

2.4.1 Underground Mining

Again underground mining divides into two types, one is room- and- pillar and second one is long wall mining. Coal deposits are mined by the process of cutting a network of rooms converts into the coal seam by room and pillar method and some pillars of coal leaving for gives the support to the roof of mine. Up to 40% of the total coal is in the form of pillars; anyway left coal was mined at the last stage of mining which is called retreat mining. At the last stage of

mining the roof is allowed to collapse and the mine was The roof is then allowed to collapse and the mine is dumped (Han et al., 2006).

Long wall mining engages with help of mechanical shearers which are useful to coal extraction from seam or face. Thorough research work plan is essential to develop a careful planning on geology of the mining site in long wall technology. The length can change from 100-350mm of coal face. Hydraulically powered equipped are helps temporarily during extraction of coal. After completion of coal extraction the walls are allowed to collapse. About 75% of the coal can be extracted from panels of coal in deposits that can expand 3km along with the coal seam. Room and pillar mining is more advance than long wall mining as accesses easily to the production of coal by using machinery which cost around $5 million whereas long wall mining machinery cost $50 million. The preference of mining method is based on site specific and also economically concerned. Sometimes, both methods should be used for mining.

2.4.2 Surface Mining

Surface mining is also called as opencast or open cut mining. When coal seam is vicinity to the surface, then only this method is economically benefitted (Cavers et al., 1986). 90% or more of the coal extraction is possible under this method. It is recovers more coal from site than underground mining. It occupies many square kilometers of area and utilizes large machinery which includes drag lines power shovels bucket wheel excavators and conveyors. These equipment are essential to remove the over burden and transport of over burden. The explosives are used for breakdown of over burden rocks and soil. Then the over burden is removed by draglines or by truck and shovel. After exposing of coal mine, it is fractured and drilled then systematically mined by using strips (Choquet et al., 1993). The collected coal is then loaded on

the trucks or conveyors which are carrying this load to the site of use. Sometimes distant transportation is required to reach preparation plant of coal. Preparation coal plants should be near the site of mining.

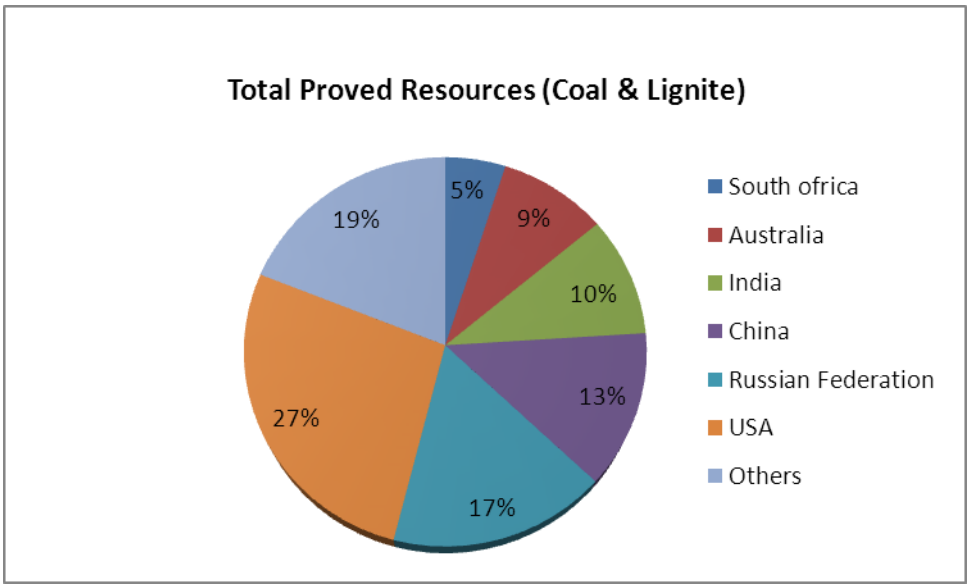

Figure 1: Coal resources of the World

2.5 Coal mining in India and its regulatory frame work

Coal is the most rich fossil fuel reserve present in India. Coal mining in India has been conducting dates back to the 18th century. Coal has a comparatively high significance for the economic development of a country. An anticipated 55% of India installed capacity of 124,287 MW of power production is due to coal based thermal power plants. India is the 3rd place in production of coal in the world and India has the 4th largest resources of coal in the world (approx 197 billion tones). There are 44 major coalfields presented in the India, in adding to 17 in the northeastern area. The geological resources of coal have been estimated coking coal is 24123 Mt and noncoking coal is 162 914 Mt, up to a deepness of 600 m. The usual stripping

ratio through the last three decades was 1.97m 3 /t (Chaulya et al., 2000). These overburden dumps alter the natural land geography, influence the drainage system and stop the natural succession of plant growth (Bradshaw and Chadwick 1980) consequential acute troubles of soil erosion and environmental pollution (Singh et al., 1994; Singh et al., 1996).

In India, there are two types of mining operated, one is opencast and second one is underground mining. Opencast mining is a developmental activity, which damages the natural ecosystem by numerous mining activities. In opencast mining, the upper soil is taken out and the fragmented rock is heaped in the shape of overburden dumps (Ghosh, 2002). Dump materials are left over the land in the mode of overburden dumps. These occupies huge amount of land, which loses its original use and generally deteriorates the soil qualities (Barpanda et al., 2001). These get spread over the neighboring fertile land and disturb their natural quality, and growth of fresh leaves. It has been found that overburden dump top materials are typically poor in major nutrients. Hence, most of the overburden dumps are do not use for plantation. Coal with a proven reserve of 860 billion tonnes is mined the most in the world. The major reasons are the soaring power demand in India and China, the growing worldwide steel production, and lastly, the increasingly stringent environment regulations.

Table 2: Key Players in Indian Coal Sector

Coal Producing Companies	Production (Mtes)
Coal India Ltd (CIL) (A Govt. of India Enterprise)	324(85%)
Singareni Collieries Co. Ltd. (SCCL) (AP St. Govt. & Govt. of India Jt. Venture)	36(9%)
Captive Producers (Steel & Power)	22(6%)

As a flourishing economy, India experiencing energy security as a growing challenge and the coal production is estimated to grow at a CAGR of about 7% during 2011-12 to 2013-14. The

Indian coal market is set to witness great boost in near future because of the rising government initiatives. Allocation of coal blocks and stake sales in PSU are some of the main steps that were taken by the government to improve the production and asset in the coal industry.

Typically, Indian coal is classified as following quality aspects:

- Lower to medium grade coal
- Low sulphur
- High ash
- Low moisture

There is not much important in sulphur content in the terms coal quality where India normally having low sulphur content which significant effect on the environment.

Coal Industry in India is regulated largely by the provisions of The Coal Mines (Nationalization) Act, 1973 - This act nationalizes the Coal Sector. Mines & Minerals (Development & Regulation) Act, 1957 – This act is regulating Exploration & Exploitation of Minerals. The Coal Bearing Areas (Acquisition & Development) Act, 1957 – This act is to facilitating acquisition of coal bearing land. Environmental Protection Act, 1986 - To conduct mining operation in an environmental friendly manner. Coking Coal mines in India were Nationalized in 1971 & Non Coking Mines in 1973.

2.6 Coal mining in Jharkhand state of India

The State of Jharkhand was created as 28th State of the Indian Union by the Bihar Re-organization Act on 15th November 2000, the birth anniversary of the legendary Bhagwan Birsa Munda. It comprises of the forest tracts of Chhotanagpur plateau and Santhal Paragna. It is bounded by Bihar on the North, Orissa on the South, Chhattisgarh on the west and West Bengal

on the East. The State covers 79.70 lakh ha area (2.42% of the geographical area of the country) with a population of 269 millions (as per Census 2001), the state accounts for 2.6% of the total population of the country. It has sizeable Tribal population (26.3%). Topography of the State is mostly undulating, hilly and sloping with mountains, forests, river basins and valleys. It has a rich endowment of forest and mineral resources. It has some of the richest deposits of coal and iron Ore in the world. It is the largest producer of coal, copper, kynite and mica in the country. It is blessed with rich fauna and flora **(**Jharkhand state website**).** The development of mining technology enabled the progressive substitution in the 1950s of the old methods based on underground exploitation by modern and profitable surface extraction technologies. That is the reason why the volumes of metal extracted were increased (Martinez et al., 1993). As a consequence of the long-lasting mining activities, the mountainous landscapes in this area are strongly transformed: numerous spoil piles and pits extend for many kilometers. These mining wastes contain high amounts of heavy metals such as lead, zinc, copper, or cadmium (Conesa, 2001; Garcia et al., 2001a). The preservation of most of these metals in the topsoil is explained by their immobility, related by their chemical behaviour and environment characteristics. Among the latter, the geological material and low precipitation combined with very high evapotranspiration are worth mentioning; the chemical composition of wastes is also important.

2.7 Mining and Biodiversity

Biodiversity is the degree of change of life forms within a given species, ecosystem and an entire planet. Biodiversity is evaluating the health of ecosystems. These comprise diversity within species, between species and within ecosystems. While the word “biodiversity” may not be well known or unspoken, the ecological services offered by biodiversity are very important to

everyday existence. Not a day, hour, or even second leaves by that we do not based on biodiversity for continued existence (Sengupta, 1980). The mixture of a diversity of life forms and their connections with each other and with the rest of the environment has made Earth a exclusively habitable place for humans. Biodiversity is essentially valuable as a mean of getting better our understanding of the structure and execution of ecological communities and environment as a whole. This disagreement focuses on the conservation of all species, even if they are ecologically equal species. Biodiversity carry out a number of ecological services for humankind that have economic, aesthetic or recreational worth (Sengupta, 1980).

2.8 Impact of mining on biodiversity

Mining projects through their process affects the biodiversity both directly and indirectly. Direct or main impacts from mining can result from any activity that engage land clearance (such as access road construction, exploration drilling, and overburden stripping or tailings impoundment construction) or direct discharges to water bodies (riverine tailings disposal, for instance, or tailings impoundment releases) or the air (such as dusts or smelter emissions). Direct impacts are usually readily identifiable (Wali, 1987). Indirect or secondary impacts can result from social or environmental changes induced by mining operations and are often harder to identify immediately. Cumulative impacts occur where mining projects are developed in environments that are influenced by other projects, both mining and non-mining.

The potential for significant impacts is greater when mining occurs in remote, environmentally or socially sensitive areas. Due to the continuing demand for minerals, the depletion of resources in readily accessible areas and changing technologies and economics in the mining sector, mining is increasingly being proposed in remote and biodiversity-rich

ecosystems that were previously unexplored and undeveloped for minerals. This has also been made possible by the implementation of mining sector fiscal and regulatory reforms to encourage foreign direct investment in many developing countries (Barapanda et al., 2001). This trend in opening up new prospective areas to mineral resources development provides an opportunity for the mining industry to demonstrate that practices have improved. It can also represent a threat, however, and poor performance could limit access to some highly prospective areas. Despite the significant potential for negative impacts on biodiversity from mining operations, there is a great deal that companies can do to minimize or prevent such impacts in areas identified as being appropriate for mining. There are also many opportunities for companies to enhance biodiversity conservation within their areas of operations. Being proactive in the assessment and management of biodiversity is important not only for new operations but also for those that have been operating for many years, usually under regulatory requirements that were less focused on the protection and enhancement of biodiversity (Claassens et al., 2005).

In the early stages of exploration, impacts on biodiversity are limited, although they can become more significant as exploration progresses. At a macro-level, however, assuming exploration efforts identify economically viable mineral deposits, the initial choice of exploration area can have a profound long-term influence on the impacts on biodiversity. Therefore even at this very early stage it is critically important to have some appreciation of likely long-term interfaces with biodiversity (Sengupta, 1980). The direct impacts on biodiversity are more extensive than for other exploration techniques, as drill sites must be cleared, and new access roads are often required for equipment. Drill pads sometimes need to be established within relatively undisturbed ecosystems, and it requires intensive management to limit the associated disturbance and subsequent rehabilitation of that disturbance. Simple management measures can

include minimizing the number of access roads, keeping tracks as small as possible and rehabilitating tracks as soon as practicable. In addition, biodiversity may be affected by water abstraction for drilling fluids or by spillage or leakage of fuels, oils and drilling fluids during exploration drilling. Where exploration camps are established, surface water pollution may result from wastewater discharges, sewage disposal, and small-scale waste rock dumps (and related heavy metal and sediment drainages), which may affect aquatic biodiversity or contaminate drinking water sources for wildlife (Ghose, 2004).

Different mining methods present different risks and opportunities for biodiversity. Underground mines typically have a small footprint associated with ore extraction and processing. Open pit mines progressively deepen and widen, increasing the areas disturbed each year and offering few opportunities for early rehabilitation. Open cast mines usually offer opportunities for progressive rehabilitation, as the mined areas may be recon toured behind the active mining areas (Cairney, 2000). The clearing of overburden and pit development are often the most dramatic visual impacts of mining, but even with large mines the areal extent of the pit can be quite limited. The primary impacts on biodiversity result from land clearance for the pit, access routes, and progressive expansion into new areas. Typically, large, long-life mines undergo many expansions in area and capacity, generating a sequence of events that can be the equivalent of new mines being started (Mohapatra and Goswami, 2012).

2.9 Impacts of mining on soil quality

Mining can pollute soils over a large area. Agricultural actions near mining project may be predominantly affected. According to a study commissioned by the European Union: *"*Mining process routinely alter the surrounding landscape by exposing before uninterrupted earthen

materials. Erosion of exposed soils, take out mineral ores, tailings, and fine material in waste rock piles can effect in considerable sediment loading to surface waters and drainage ways. In adding, spills and leaks of hazardous materials and the evidence of contaminated windblown dust can direct to soil contamination.

Human health and environmental danger from soils usually fall into two categories: (1) contaminated soil was consequential from windblown dust, and (2) soils contaminated from chemical spills and remains. Fugitive dust can cause important environmental problems at some mines. The innate toxicity of the dust depends upon the closeness of environmental receptors and sort of ore being mined. High levels of lead, arsenic and radionuclide in windblown dust typically create the maximum risk. Soils contaminated from chemical spills and residues at mine sites may cause a direct contact risk when these materials are misrepresented as fill materials, ornamental landscaping, or soil extension (MINEO Consortium, 2000).

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

3. MATERIALS AND METHODS

The study was carried out on top soil collected from proposed mining area, Tubed Coal Mines Limited, Latehar, Jharkhand, India. Soil physico-chemical characterization and microbial diversity were conducted. Soil physical properties like texture, moisture content, specific gravity, bulk density were determined. Chemical properties of soil like alkalinity, pH, organic matter content, organic carbon content, chloride content, phosphorus, potassium were also determined. Microbial diversity of the top soil 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.

3.1 Proposed study area

The proposed study area extends to an area of 460 HA. or 1136.66 acres and comprises of six villages (one fully covered namely Dhobiajharan (Thana No.-336) and five partially covered namely Ambajharan (Thana No.-335), Tubed (Thana No.-333), Nawari (Thana No.-337), Mangra (Thana No.-330) and Dihi (Thana No.-334). In the present study only three villages like Dhobiajharan, Tubed and Nawari were selected for soil and microbial analysis. The project area in each village is summarized in the table below. The study site extends between Latitudes (N): 23°50'09" and Longitudes (E): 84°34'09" & 84°35'45".

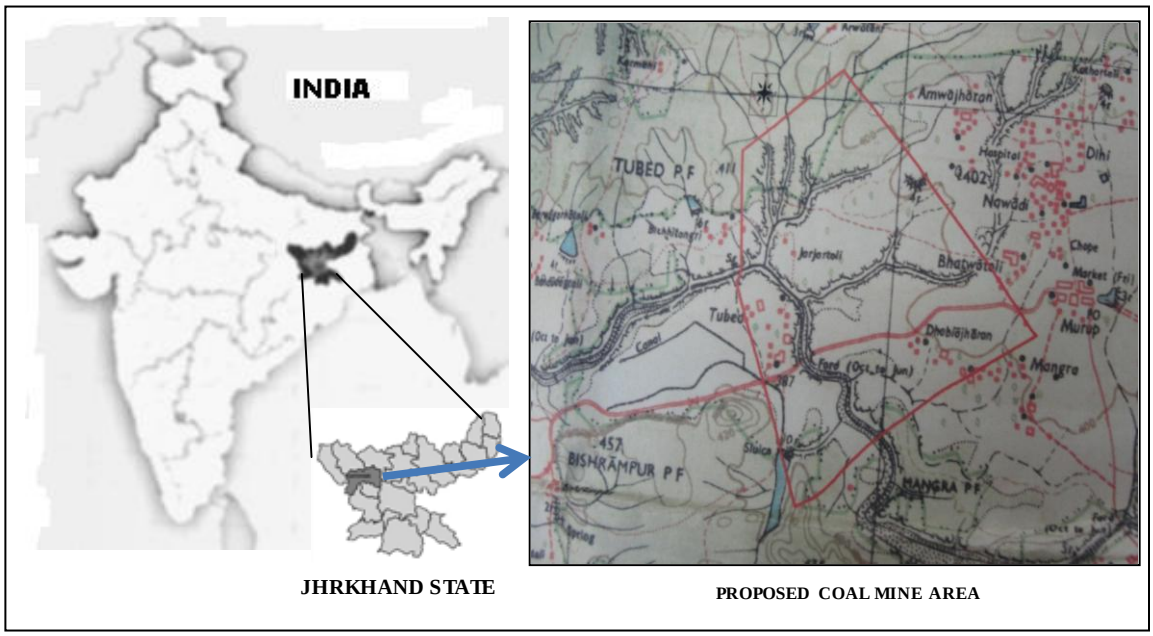

Figure 2: Proposed study site for coal mining in the Latehar district of Jharkhand

Table 3: Total sampling area in each village of the proposed study site

Sl. No.	Name of village	Project Area (In Acre)
1	Tubed	251.36
2	Dhobiajharan	312.29
3	Newari	86.15

3.2 Sample collection

Field survey was conducted in the three villages viz: Dhobiajharan, Tubed and Nawari and soil samples were collected from various locations in and around the proposed mining sites by random sampling design method (Tripathy et al., 1998).

3.3 Analysis of soil samples

A soil auger was used to obtain volume samples with a minimum of 0.5 kg of soil per sampling area. The top 0-10 cm of the soil cover layer was sampled. Samples were obtained using aseptic techniques. Soil samples were placed in tightly sealed plastic bags and kept at 4°C to keep them field moist and to preserve biological properties. Soil moisture content was determined gravimetrically after drying soil samples at 105°C. The chemical and physical analyses for the characterization of soil cover layers of the respective sites were done. The air dried topsoil samples were ground and pass through 2mm sieve. The collected topsoil samples after coning and quartering then sieving (2mm) were used for analysis of different soil quality parameters. The following methods are briefly mentioned underlined.

3.3.1 *pH*

The pH value which is a measure of the hydrogen or hydroxyl ion activity of the soil water system indicates whether the soil is acidic, neutral or alkaline in reaction. Crop growth suffers much both under very low as well as high pH. The instrument for pH measurement commonly used is a digital pH meters have single electrode assembly. The instrument being a potentiometer, the pH scale has to be calibrated before use with buffer solutions of known pH values. 20 gm of soil is taken in a 100ml beaker to which 40 ml of distilled water is added (Ghosh et al., 1983). The suspension is stirred at regular intervals for 30 minutes and the pH is recorded. The suspension is stirred well just before the electrode are immersed and readings are taken.

3.3.2 *Moisture content*

The standard method for determining moisture content of soil is the oven- drying method. This is the procedure recommended for soil. Moisture content measured by gravimetric method and expressed as percentage. Loss of weight of the samples was calculated to determine the moisture content.

3.3.3. *Bulk Density*

About 10 g of soil sample was dried in an oven at 105°C until a constant weight is attained. Then a little dried soil was transferred to a measuring cylinder and the volume was recorded. Then the weight of the volume was again measured using a weighing balance (Saxena, 1989).

$$\textbf{Bulk density (g/cm}^3\textbf{) = Weight of soil (g) / Volume of soil (cm}^3\textbf{)}$$

Where 1 ml = 1 cm^3

3.3.4 *Specific gravity*

About 10 g of soil sample was dried in an oven at 105°C until a constant weight is attained. Then a pre weighed glass bottle of known volume was filled with the dried soil samples and its weight was recorded in a weighing balance. Another pre weighed glass bottle of the same volume was filled with distilled water and its weight was recorded (Saxena, 1989).

$$\textbf{Specific gravity} = (A_2 - A_1) / (B_2 - B_1)$$

Where A_2- weight of the bottle with soil; A_1- weight of empty bottle used for soil; B_2- weight of bottle with distilled water and B_1- weight of empty bottle used for water

3.3.5 *Organic carbon content*

The soil is grounded and completely passed through 0.2 mm sieve (80mesh) and 1gm is placed at the bottom of a dry 500 ml conical flask. Add 10 ml of potassium dichromate (1N) in the 500 ml conical flask, swirled and conical flask gently to disperse the soil in the dichromate solution. Then 20 ml of sulphuric acid is run in run in and swirled again two or three times. The flask is allowed to stand for 30 minutes and there after 200 ml of distilled water along with 10 ml of or tho– phosphoric acid is added and 1ml of diphenylamine indicator. The whole contents are titrated with ferrous ammonium sulphate solution till the color flashes from blue – violet to green. For a final calculation, a blank is run without soil (Saxena, 1989).

3.3.6 *Alkalinity content*

About 10 gm of air-dry soil was taken and to it 100 ml of distilled water was added to make up a suspension of 1: 100 w/v. Out of it about 50 ml of the suspension was taken in a flask and 2-3 drops of phenolphthalein indicator was added. Then the solution was titrated against sulphuric acid until solution becomes colourless (end point) (Saxena, 1989).

Total alkalinity (mg/L) = t X 1000 / S

Where t= volume of titrate used in ml; S= volume of sample in ml

3.3.7 *Chloride content*

About 10 gm of air-dry soil was taken and to it 100 ml of distilled water was added to make up a suspension of 1: 100 w/v. About 10 ml of sample was taken in a flask and 5-6 drops of potassium chromate indicator was added to it. The colour of the sample became yellow. It was

titrated against silver nitrate until a persistant brick red colour appears (end point) (Saxena, 1989).

Chloride (mg/l) = (V X N X 35.475 X 1000) / S

Where V= volume of titrate (ml), N= normality of titrant (0.02) and S = volume of sample (ml)

3.3.8 Available phosphorus

In a 25 ml volumetric flask, 5 ml of the soil is taken and adding 5 ml of dickman and bray reagent. Then neck of the volumetric is washed down and the contents are diluted to about 22 ml, then1 ml of dilute stannous chloride solution is added and volume is made up to the mark. The intensity of the blue color is measured (using 660 nm) just after 10 minutes and the concentrations phosphorus is determined from the standard curve (Olsen, 1954).

3.3.9 Potassium content

Potassium content in soil was firm by using by flame photometry. Prepare 0.5M aqueous solution of ammonium acetate/acetic acid by taking 38.55g ammonium acetate and suspend it in 29mls of glacial acetic acid and diluting to 1 litre with distilled water. Precisely weigh 10g of soil and transport to a plastic bottle together with 50ml of acetate/acetic acid solution. Stopper the bottle and shake with an automatic shaker for 30 minutes. Take out from the shaker; permit standing for several minutes. Filter the solution through a Whatman No.30 filter paper. Arrange standard potassium solutions to cover the range 0-l00 ppm as follows: Aspirate the 100 ppm standard and set the exhibit to read 100. Without touching the controls aspirate the other standards and verify that they give a linear response. Decide the potassium content of the soil

take out by spraying the solution without additional dilution into the flame photometer and reading the display.

Concentration potassium (mg/100gms soil) = displayed value (ppm) x vol extractant solution x100 / weight sample x 1000

3.4 Microbial diversity

3.4.1 *Microbial populations*

Microbial populations such as bacteria and fungi were carried out for different soil and water samples following standard dilution plate technique (Megharaj et al., 2003). In this method, 1mL water sample was taken and volume was madeup 100mL with sterile water which was further serially diluted to get 10−4 dilution. From these diluted samples, 1 mL water sample was dispensed over each of three replicates and then media for growth of different microorganisms were added nutrient agar used for isolation of bacteria while potato dextrose agar and ammonium chloride-starch agar medium were used for fungi and actinomycetes respectively, the petriplates were incubated at 35 °C for 48 h for bacteria and 25 °C for 72 h for fungi. The microbial populations were enumerated as colony-forming units (CFU) from a serial dilution of soil suspensions. The microbial colonies were counted in the three replicaplates and the average values were calculated. The populations of microorganisms were considered from the number of microbes multiplied by the dilution factor for each sample.

3.4.2 *Isolation of Bacteria*

The media used in this research was nutrient of agar medium. 28g of nutrient agar powder was weighed and dissolved in 1000ml and of distilled water. It was stirred vigorously

and dissolved using hot plate after which was sterilized in autoclave for 15 minutes at 121°C. It was then allowed to cool after which it was dispensed in Petri dishes and allowed to solidify. Portions of the suspension were inoculated on the nutrient agar by streaking and were incubated at 37°C for 24hours. After which colonies with a clear zone of inhibition were observed.

3.4.3 Gram's staining

Colonies that were grow on nutrient agar where gram stained in accordance with standard gram staining procedure described by Todar *et al*. (2005). To study the Gram's stain (crystal violet) i.e. Gram (+ve) or Gram (-ve) characters of the isolates, took culture and diluted suspensions of the bacteria (8-12hr old) were smeared on the clean slides, air dried. Heat fixed by passing over a flame for 2-3 times. The slides were flooded with crystal violet solution for 1 minute, washed with water and flooded with Gram's Iodine for 1minute. The slides were washed with water and decolorized with 95% ethyl alcohol dropped from a dropping bottle unit no violet color was from drain off alcohol. The slides were washed with water and counter stained with safranin stain for about 30 seconds and washed with water. The slides were air dried and examined under a microscope using 100x objective using daylight filter.

Composition of Gram's stain**:**

A. Crystal violet solution:

Solution I: Crystal violet (85%) dye 2 gm dissolved in 20ml of 95% ethyl alcohol.

Solution II: Ammonium oxalate monohydrate 0.2 gm dissolved in 20ml distilled water.

Equal parts of solution I with solution II were mixed and used for staining.

B. Gram's iodine: Iodine 1 gm and KI 2 gm dissolved in 300 ml of distilled water, stored in a brown bottle.

C. Safranin solution: Safranin 2.5 gm in 100 ml of 95 % of ethyl alcohol. Add 10 ml of alcoholic solution with 100 ml water.

3.4.3.1 Sub Culturing

Bacteria isolates having shown a cleared zone of inhibition on nutrient agar plates were sub cultured into nutrient agar slants for short time preservation and to purify the isolates. The bacteria were inoculated in nutrient agar slant using a sterile wire loop and inculcated at 37°C for 24hours. The slant bottles containing the bacteria were kept in refrigerator at 4°C for short time storage before biochemical tests were ran on the isolates for identification.

3.4.4 Biochemical tests for bacterial identification

Biochemical tests were performed according to standard procedure of Cappuccino and Sherman, (2002).

3.4.4.1 Indole Test:

This test is used to check ability of the organisms to form indole from tryptophan or to detect the presence of enzyme tryptophanase which converts tryptophan to indole. One percent tryptophan broth in a test tube was inoculated with bacteria colony. After incubation period of 37°c for 48hours, then one militre (1ml) of chloroform was added to the broth. The test tube was shaken gently, then 2.1 of Kovac's reagent were added and this was also shaken gently and allowed to stand for twenty (20) minutes. The formation of red coloration at the top layer indicated positive and yellow coloration indicates negative.

Composition of as follows:

Table 4: Tryptophan broth composition

Ingredients	**grams/litre**
Peptone (containing sufficient tryptophan)	20
NaCl	5
pH	7.4
Reagent: Kovac's reagent:	
Ingredients	**content**
Amyl or isoamyl alcohol	150 ml
Dimethyl aminobenzaldehyde	10 gm
Conc.HCl	50 ml

3.4.4.2 Catalase Test:

Presence of enzyme catalase which catalyses breakdown of hydrogen peroxide into water and oxygen was studied on culture plates of nutrient agar flooded with hydrogen peroxide solution. This was carried out by putting a drop of hydrogen peroxide on a clean slide. With the edge of another slide, a colony of the organism was picked and allowed to be in contact with the hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction.

3.4.4.3 Citrate Utilization Test:

Ability of the bacteria to grow in a medium containing citrate as sole source of carbon and energy source is detected. Citrate utilization is monitored by appearance of growth and increase of pH from 6.8 which is indicated by the change in color of bromothymol blue indicator

of the medium. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was inoculated for 24 hours to 72 hours. The development of deep blue color after incubation indicates a positive result. No growth and yellowish-green color of the slant indicated negative result.

Medium composition:

Table 5: Simon's citrate medium composition

Ingredients	grams/litre
NaCl	5
$MgSO_4.7H_2O$	0.2
$NH_4H_2PO_4$	1
KH_2PO_4	1
Sodium citrate	5
pH	6.8
Bromothymol blue	40 ml (0.2% solution)
Agar	20

3.4.4.4 MR Test:

The test is used to detect acid production from glucose. Production of acid lowers the pH of the medium below 4.2 which is detected by the pH indicator methyl red. Bacteria were inoculated into tubes containing methyl red- voges proskauer (MRVP) broth and incubated at 30 ±0.1°C for 72 hours. Some small quantity (2-3 drops) of methyl red test was added. A red colour

on the addition of the indicator signified a positive methyl red test while yellow colour signified a negative test.

Table 6: MRVP broth composition

Ingredients	grams/litre
Peptone	5
NaCl	5
Glucose	10
pH	7

3.4.4.5 Oxidase Test:

To detect presence of the enzyme oxidase in the bacteria was performed. It catalyses transport of electrons between bacteria and the redox dye (methylene blue). Few drops of methylene blue were added to 72 hour culture in nutrient broth media. Positive reaction was indicated by change in colour of the broth to colourless within few seconds.

Reagent: 0.2% solution of methylene blue in distilled water

3.4.4.6 Urease test:

Presence of enzyme urease which splits urea into ammonia and CO_2 was detected by inoculating bacterial cultures into tubes containing urease broth with filter sterilized urea, incubated at 30±0.1°C for 72 hours. Purplish pink coloration of the medium indicated positive reaction.

Table 7: Urea medium composition

Ingredients	grams/litre
Peptone	1
NaCl	5
K_2HPO_4	2
Glucose	1
Urea	2
pH	6.8
Phenol red	6ml

3.4.4.7 Voges-proskauer (acetoin production) test

Ability of many bacteria to ferment carbohydrates (especially glucose) with production of acetyl methyl carbinol reduction product into neutral products and carbon dioxide instead of organic acid is assessed. Bacteria were inoculated into tubes containing MRVP broth and incubated at 30±0.1°C for 72 hours. After incubation a mixed solution α-napthol and potassium hydroxide were added to 2.5 to 5 ml of culture. Development of crimson red colour of the medium indicated positive result.

Reagent: 3ml of 5% α-napthol in absolute ethanol mixed with 1ml of 40% KOH.

3.4.4.8 Nitrate reduction test

The ability of the microorganisms to reduce nitrate to nitrite is detected through the test. Bacteria were inoculated into nitrate broth incubation at 30±0.1°C for 96 hours. After inoculation sulphanillic acid and α-naphthyl amine mixture (1:1) was added. Appearance of deep colour

indicated positive result. If colour does not appear the culture was diluted 2-5 fold and toasted again. Composition of the medium:

Table 8: Nitrate broth

Ingredients	grams/litre
KNO_3	0.2
Peptone	5
pH	7.2

Reagent:
Solution –A: sulphanic acid 8gm dissolved in 1litre 5N acetic acid
Solution –B: α- naphthylamine 5gm dissolved in 1 litre 5N acetic acid.
Equal volume of solution A and B was mixed just before use.

3.4.4.9 Hydrogen sulphide (H_2S) production test

Hydrogen sulfide can be produced at least in small amounts from sulfur containing amino acids by a large number of bacteria. Methods showing hydrogen sulfide production by suspending strips of paper impregnated with lead acetate above culture are of variable sensitivity and are of limited value. Precise test most be poised at definite level of sensitivity. Hydrogen sulfide is demonstrated by its ability to form black insoluble ferrous sulfide on the test strip. The test strip should be prepared by cutting white filter paper into strips approximately 5 by 50 mm, soaking them in a saturated solution of lead acetate, sterilizing in a plugged test tube and drying in an oven at 120°C. One of these strips should be replaced in the mouth of the culture before incubation in a position that one quarter to one half of the strip projects bellow the cotton plug.
The tubes were incubated at 20°C for at least 7 days and the blackening of the strips were observed day to day.

Table 9: peptone medium

Ingredients	gram/ litre
Beef extract	7.5
Peptone	25
NaCl	5
Ferrous chloride	10%
Agar	20

3.4.4.10 Carbohydrate metabolism (acid- gas production) test

The ability of the organisms to ferment carbohydrates and related compounds such as arabinose glucose, sucrose, raffinose, glycogen, glycerol, salicin, and citrate etc….and resultant acid and gas production is assessed by the process. The bacteria were inoculated in tubes containing nutrient broth and 1% sugar, bromothymol blue indicator and an inverted Durham's tube filled with the media to trapping evolved gas and incubated at 30±0.1°C for 72 hours. Acid production was indicated by change of the colour of the medium to yellow and gas production from bubble accumulation in the Durham's tubes.

Indicator: Bromothymol blue 10mg dissolved in 0.25ml of NaOH and added with 4.75ml of water.

3.4.4.11 Starch hydrolysis test

The starch hydrolyzing capacity of the microorganism is determined the formation of simple substances like glucose, dextrin, maltose etc. the amylase enzyme is useful to hydrolyze starch. The bacterial culture was inoculated on the nutrient agar medium with 1% starch solution. The bacterial culture was inoculated on the nutrient agar media and it is incubated at 30±0.1°C for 24hours. After culture formation the iodine solution was flooded over the medium for five minutes. The excess solution was decanted and the hydrolysis of starch was identified as

formation of clear zone around the bacterial colonies. The hydrolysis of starch is indicated by formation of reddish brown area. The clear zone diameter was measured the help of antibiotic inhibition zone scale and then the ratio was calculated from diameter of clear zone which gives the activity level.

Medium: Nutrient agar + 1% soluble starch

Iodine solution: Iodine 1gm, potassium Iodide (KI) 2gm and water 300ml. Initially KI was dissolved in water and then I was added.

3.4.4.12 Antibiotic sensitivity test for the organisms

Different antibiotics give the sensitivity to the growth of microbial culture. It was tested by the use of nutrient agar medium. After preparation of nutrient agar plants the test bacterial culture suspension was inoculated. Antibiotics were added as disc form. Different concentrations were used for the testing of sensitivity. The antibiotic sensitivity was identified as the formation of clear zones around the discs which means the inoculated bacteria was sensitive towards the antibiotic. If growth was observed that indicates the particular bacteria resistant to the antibiotic. The inhibition zone was measured by using antibiotic zone scale. The activity level described as the ratio of the inhibition zone.

3.4.4.13 Triple Sugar Iron Test (TSI)

The medium contains three sugars namely: glucose, lactose and sucrose. Phenol red was used as indicator and the detection hydrogen sulfide was done by using filter paper strips which were dipped in the lead acetate. If hydrogen sulfide was released by the culture of bacteria could become black. Agar slants were prepared for culturing of bacteria and inoculation of culture was done by the method of stabbing media with the help of sterilized straight wire loop. Streaking of culture was done by loop. After inoculation the culture was kept in incubator at 37^{o} C for 24 hrs.

After 24 hrs observation was done. The production of gas leads to the cracking of the medium. The production of gas was expected by blackening of buffer at the slant butt junction. The production of glucose fermentation was decided by the butt should become yellow. The lactose and sucrose fermentation was identified by the yellowing of the butts of slant media.

3.4.5 Identification of fungus by Lactophenol cotton blue staining

Lactophenol Blue Solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue. Place a drop of Lactophenol Blue Solution on a slide. Using an inoculating needle carefully spread the fungal culture into a thin preparation. Place a coverslip edge on the drop and slowly lower it. Avoid trapping air bubbles under the coverslip. Wait for about 5 minutes. If desired, seal the edges of the coverslip with nail polish or Paramount to preserve the mount as a reference slide. Observe under a microscope with low power for screening in low intensity.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4

4. RESULTS AND DISCUSSIONS

4.1 Sampling area

Soil samples were collected from the three villages in and around the proposed mining site. The geographical locations of different sites as recorded by the global positioning system (GPS) are presented in the Table-10.

Table 10: Geographical location of different sampling sites as recorded by GPS

SL. No.	Name of the Village	Longitude	Latitude
1	Tubed village	84°34’466”E	23°48’918”N
2	Dhobiajharan	84°35’168”E	23°49’206”N
3	Nawari	84°35’280”E	23°49’206”N

Soil fertility is an aspect of the soil-plant relationship. Fertility status of the soils is primarily and importantly dependent upon both the macro and micronutrient reserve of that soil. Continued removal of nutrients by crops, with little or no replacement will increase the nutrient stress in plants and ultimately lowers the productivity. The fertility status of the soils mainly depends on the nature of vegetation, climate and topography, texture of soil and decomposition rate of organic matter. Optimum productivity of any cropping systems depends on adequate supply of plant nutrients.

Figure 3: Collection of soil samples from different locations

4.2 Analysis of soil

Different soil samples were collected from three villages as mentioned earlier during January and March 2013 and were analyzed for various physical and chemical properties. The physical property of the soil include, color, texture, size, soli bulk density, specific gravity etc. and the chemical properties includes, pH, organic carbon content, organic matter content, total soluble sulfates, alkalinity and total calcium content.

4.2.1 Study of physical properties of soil samples

4.2.1.1 Color and texture:

Soil samples were observed to identify the Color and Texture. The results were shown in table5.

Table 11: color and texture of the soil samples

Sl. No.	Name of the Village	Color	Texture
1	Nawari	brown	Clayey loam
2	Tubed – 1	black sandy	Sandy
3	Tubed - 2	yellowish	Clayey loam
4	Dhobiajharan	yellowish	Clayey loam

4.2.1.2 pH

The pH of soil is very important because soil solution seize in it nutrients such as nitrogen, potassium, and phosphorus which are vital for growth of plants and endure various diseases. The pH of the sample under 7 were to be weakly acidic in nature (Brady, 2002). Brady, (2002), recognized that a pH range of 6.5 to 7.5 is most encouraging for plant nutrient

accessibility. If the soil solution is more acidic, plants cannot utilize N, P, K and other nutrients they required. In acidic soils, plants are more likely to absorb toxic metals and some plants eventually die of toxicity. Studies have discovered that among various environmental features, pH is important in affecting the surface charge of soils and the ease of use plant nutrient and microorganisms (Escobar and Hue, 2008). The pH of the top soil samples from three selected villages was determined and the results are presented in Table 12 and figure 4.

Table 12: pH of the soil samples

Sl. No.	**Name of the village**				**pH (mean)**
1	Nawari	7.12	7.16	7.14	7.14±0.02
2	Tubed -1	6.95	6.92	6.95	6.94±0.01
3	Tubed -2	6.7	6.72	6.63	6.68±0.04
4	Dhobiajharan	7.15	7.13	7.16	7.14±0.01

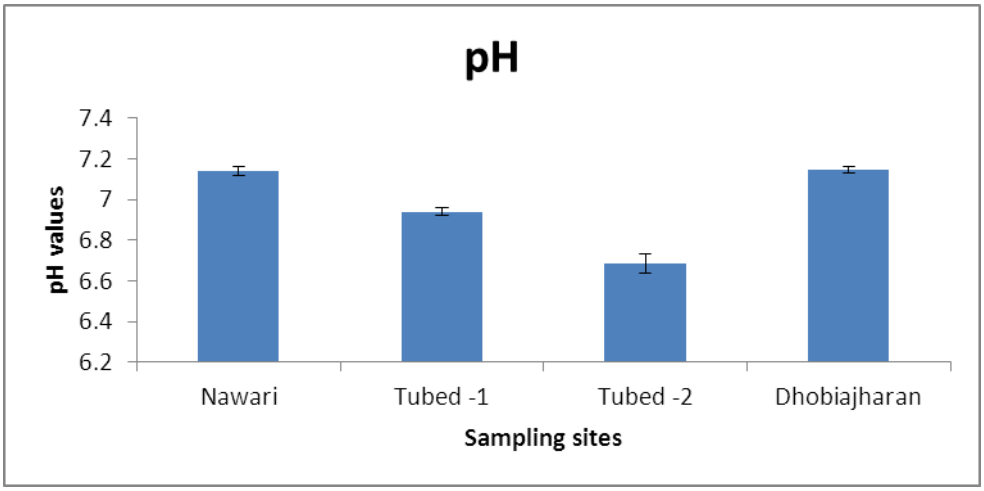

Figure 4: pH of the soil samples

The pH of the Tubed -1 site possesses 6.94 which is minimum compare with others and maximum pH 7.14 was found in Newari and Dhobiajharan. The pH range required for plant

growth was 6.5 to 7.5. So all these samples having within required pH range which are suitable for growth of plants.

4.2.1.3 Moisture

The amount of water connected with a given volume or mass of soil is a highly variable character. It can modify on time scales of minutes to years. However, most soil properties are more constant, and should be referenced to dry soil weight. Water content in soil is called soil moisture. The water is holding within the soil pores. Soil water is the key constituent of the soil in relative to plant growth. If the moisture content of a soil is optimum for plant growth, plants can quickly absorb soil water. All the water is not absorbed by the plants. Water is present in the soil in the form of thin film. Salts are dissolved in soil water, which is important as medium for provider of nutrients to growing plants. The moisture of the soil samples was shown in Table 13 and figure 5.

Table 13: Moisture content of the soil samples

Sl. No.	**Name of the Village**				**Moisture content (%)**
1	Nawari	9.6	9.2	9.4	9.4±0.2
2	Tubed - 1	9.6	10	9.7	9.76±02
3	Tubed - 2	9.4	9.2	9.5	9.36±0.15
4	Dhobiajharan	11.6	12	12.5	12.03±0.45

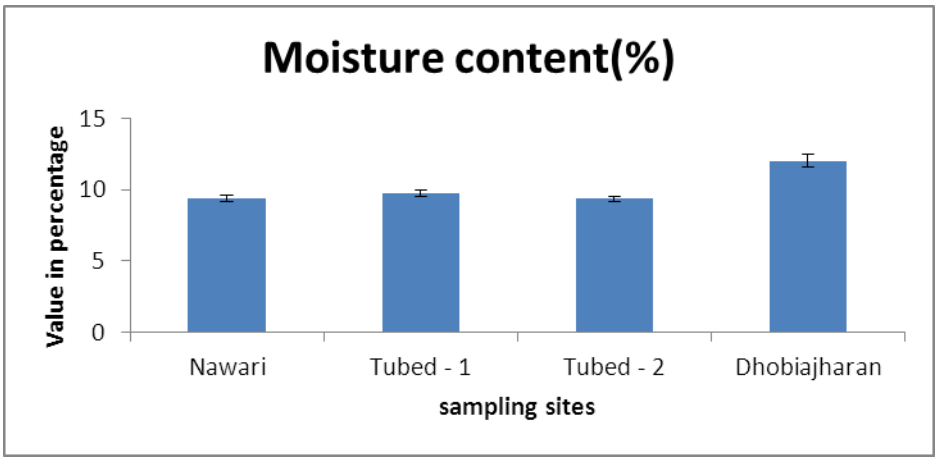

Figure 5: Moisture content of the soil samples

The identified moisture content was ranging from 9.36% to 12.03%. The maximum moisture content 12.03% was found in Dhobiajharan and the minimum moisture content 9.36% was found in Tubed-2. Moisture content requirement for the growth of plants is varies from one plant another. These results indicated that all soil samples having less soil water (moisture).

4.2.1.4 Bulk density

Bulk density is dependent on soil texture and the densities of soil mineral and organic matter elements, as well as their packing composition. Most rocks contain a bulk density of 2.65 g/cm3 so ideally, a medium textured soil with about 50 percent pore space will contain a bulk density of 1.33 g/cm3. Usually, loose soils rich in organic matter contain lower bulk density. Sandy soils have comparatively elevated bulk density because total pore space in sands is less than that of silt. Fine-textured soils like silt and clay loams which are haveing good structure contain higher pore space and lower bulk density evaluated to sandy soils. Bulk density characteristically raise with soil deepness where subsurface layers have less organic matter and root penetration contrast to surface layers. Therefore, it contains less pore space. Subsurface

layers are also matter to the packed together weight of the soil. The Bulk densities of soil sample were determined. The results were listed in Table 14 and figure 6.

Table 14: Bulk density of soil samples

Sl. No.	Name of the Village				Bulk Density(g/cm3)
1	Nawari	0.833	0.78	0.82	0.81±0.02
2	Tubed - 1	0.909	0.93	0.91	0.91±0.11
3	Tubed - 2	0.909	0.93	0.92	0.91±0.01
4	Dhobiajharan	0.909	0.95	0.91	0.92±0.02

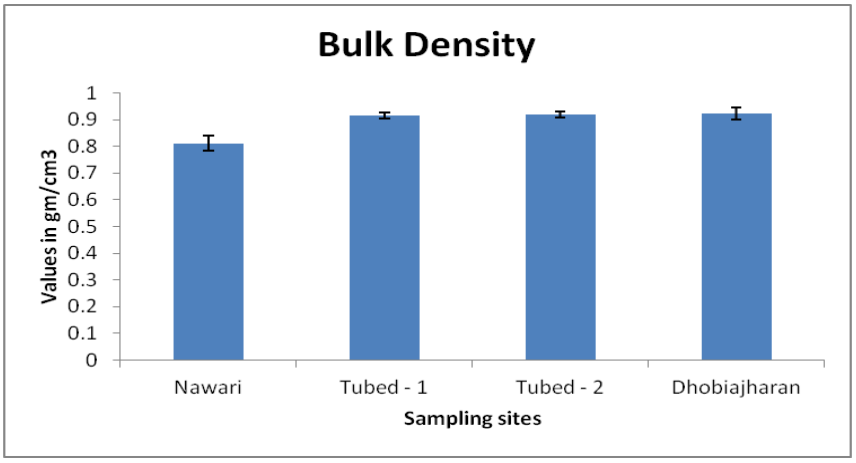

Figure 6: Bulk density of soil samples

The bulk density range is found to be 0.81 – 0.92 g/cm3. Nawari soil sample is having the minimum bulk density (0.81 g/cm3) and Dobiajharan having the maximum bulk density (0.92 g/cm3). The ideal bulk density is required for plant growth is 1.33 g/cm3. Sample containing high bulk density value cannot be used for vegetation and plantation growth. Tubed-1 site soil texture is sandy and bulk density was 0.91 g/cm3 which are suitable to growth of plants. Tubed-

2, Nawari and Dhobiajharan sites soil texture was clayey and bulk densities ware useful for crops growth. All soils having low bulk density which indicates presence of higher organic content.

4.2.1.5 Specific gravity

The soil specific gravity is helpful in the phase association of air, water, and solids in a known volume of the soil. The awareness of specific gravity is necessary in calculation of soil characteristics like void ratio, degree of saturation and weight-volume relationship. The specific gravity of soil samples was calculated and result was presented in Table 9 and figure 7. The result was compared with the standard chart of normal range of specific gravity (Table 15).

Table 15: Specific gravity of the soil samples

Sl. No.	Name of the Village				Specific gravity
1	Nawari	1.16	1.21	1.26	1.21±0.04
2	Tubed - 1	1.11	1.3	1.32	1.24±0.11
3	Tubed - 2	0.96	1.4	1.38	1.24±0.24
4	Dhobiajharan	1.30	1.7	1.68	1.56±0.21

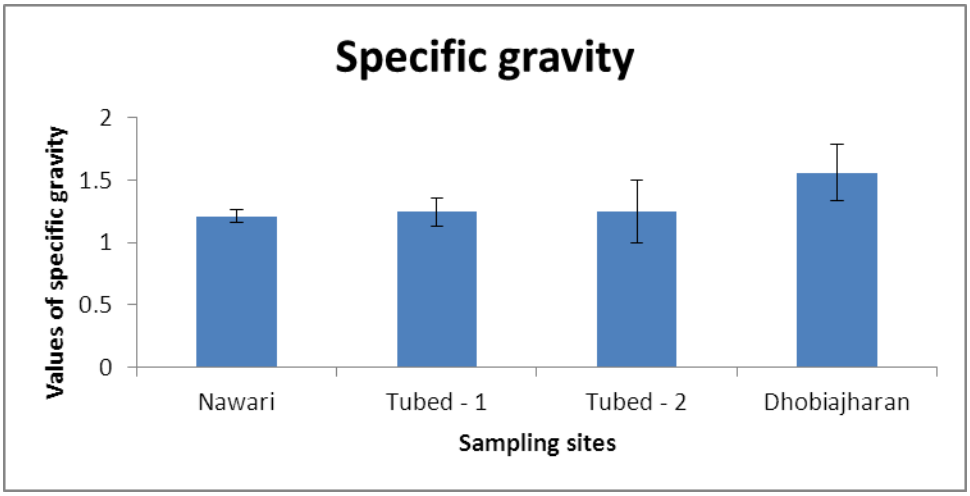

Figure 7: Specific gravity of the soil samples

The specific gravity range is found to be 1.21 to 1.56. The minimum specific gravity 1.21 is found in Nawari and maximum 1.56 is found in Dhobiajharan. This specific gravity value is below 2 which mean all soil samples are containing high organic matter. Organic matter in soils is very essential for growth of plants.

Table 16: Normal ranges of specific gravity

Sl. No.	Soil Type	Specific Gravity
01	Gravel	2.65-2.68
02	Sand	2.65-2.68
03	Silty sand	2.66-2.70
04	Silt	2.66-2.70
05	In organic clay	2.68-2.80
06	Organic Soils	Variable, may fall below 2

4.2.2 Study of chemical properties of soil samples

4.2.2.1 Organic matter

Organic matter is a pool of nutrients that can be into to the soil. The soil releases 4.5 to 6.6 pounds of P_2O_5, 20 to 30 pounds of nitrogen, and 2 to 3 pounds of sulfur each year by each percent of organic matter.. Organic matter performs somewhat like a sponge which can to absorb the water and grasp up to 90 percent of its weight in water. A huge benefit of the water-holding capacity in organic matter is that the total water can absorbed by the plants (Barber, 1984). Organic matter of soil samples was calculated and the result was presented in Table 17 and figure 8.

Table 17: Organic matter of the soil samples

Sl. No.	Name of the Village				Organic matter (%)
1	Nawari	1.96	2.22	1.94	2.04±0.15
2	Tubed - 1	3.56	3.79	3.88	3.74±0.16
3	Tubed - 2	7.73	7.69	7.76	7.72±0.03
4	Dhobiajharan	9.3	10.2	9.7	9.73±0.45

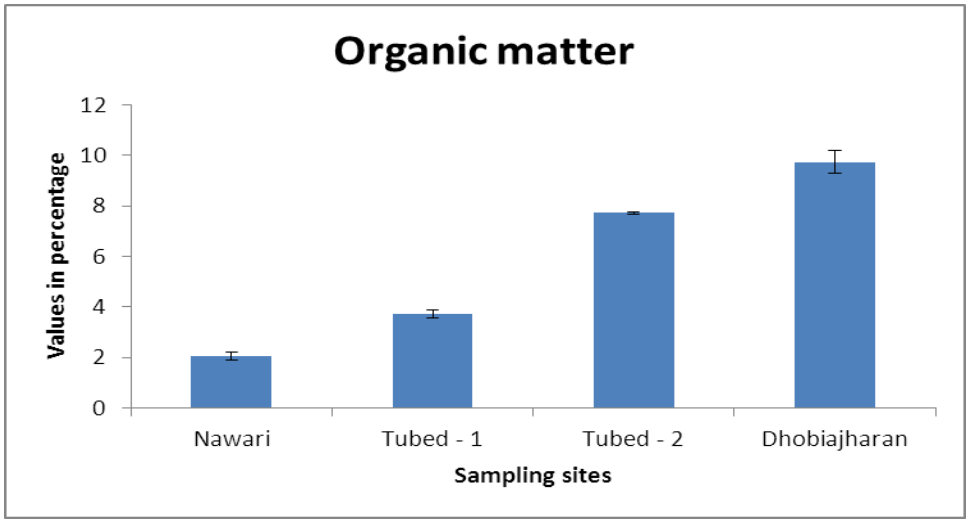

Figure 8: Organic matter of the soil samples

The Organic matter percent range was found to be 2.04 – 9.73%. High percentage of organic matter is found in Dhobiajharan (9.73%) and minimum percentage was found in Nawari (2.04). Coal mining area usually contains high organic matter. As the amount of organic matter present in a soil increase, the number of constant aggregates also increases. If the organic content high in soil the permeability increases and improved infiltration. It also holds up with waste humous matter which protects from erosion. Soil organic matter contains an accumulation of partially decomposed plant and animal materials. Organic matter is also produced by the decay of soil

microbes. In brief study, estimated that percentage of organic matter between 2.4 % to 14.75 % in all the samples, representing good accumulation of decomposed material (Brady, 1990).

4.2.2.2 Organic carbon

Soil organic carbon is important for soil fertility by which namely chemical, physical and biological fertility. Soil organic matter decomposition produces variety of minerals like nitrogen, phosphorus and other nutrients for growth of plant. SOC encourages as holding soil particles closely and improves the fertility. SOC increases the water holding capacity, water infiltration, root growth and gaseous exchange. As a food source for soil fauna and flora, Soil organic matter plays a vital role as to provide various nutrients to soil microorganisms by means of helps controls the soil food web. Organic carbon is formed from broken down of various plants and animals which is stored in the soil (Dekka et al., 2008). Organic carbon is very important to growth of plants. Soil carbon is the common name for carbon present in the soil in the form of organic content. Soil carbon is the largest terrestrial group of carbon (Batjes, 1996).

Organic carbon was determined in soil samples (Table 18 and figure 9).

Table 18: Organic carbon of the soil samples

Sl. No.	**Name of the Village**				**Organic carbon (%)**
1	Nawari	1.114	1.25	1.31	1.22±0.1
2	Tubed - 1	2.227	2.32	2.19	2.24±0.06
3	Tubed - 2	4.454	4.5	4.7	4.55±0.13
4	Dhobiajharan	5.568	5.6	5.45	5.53±0.07

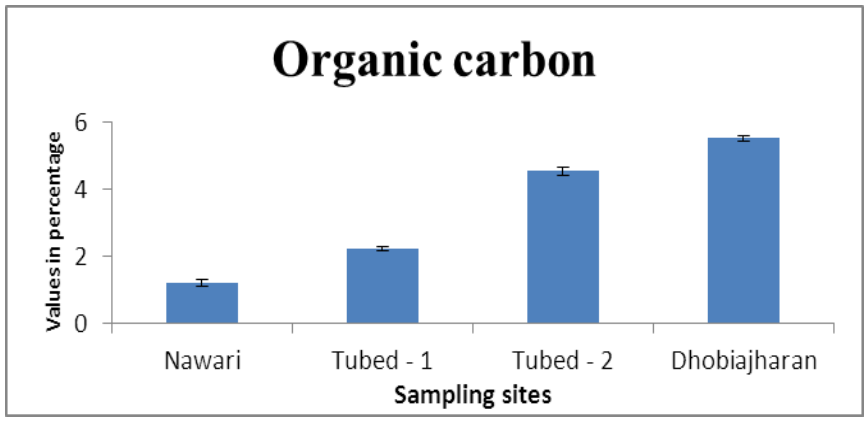

Figure 9: Organic carbon of the soil samples

The Organic carbon percent range was found to be 1.22 – 5.53 %. High percentage of organic carbon is found in Dhobiajharan (5.53%) and minimum percentage was found in Nawari (1.22%). Organic carbon levels greater than 0.8% is ranked as good quality of soil and less than 0.4% is ranked as low quality of soil (Ghosh et al., 1983). So all the soil samples are having organic content is more than 0.8% which indicates good quality of soil.

4.2.2.3 Alkalinity

Alkaline soils are having of high pH (> 9) and have a poor soil structure and a low infiltration capacity. Alkaline soils are difficult to take into agricultural production. Rainwater stagnates on the soil easily and, in dry periods, irrigation is hardly possible. Agriculture is limited to crops tolerant to surface water logging and the productivity is low. Soil alkalinity is connected with the existence of sodium carbonates (Na_2CO_3) in the soil. The alkalinity of soil samples from three different villages are shown in table 19 and figure 10.

Table 19: Alkalinity of the samples

Sl. No.	Name of the Village				Alkalinity (mcq/100)
1	Nawari	1.25	1.23	1.26	1.24±0.01
2	Tubed - 1	0.5	1	1.3	0.93±0.4
3	Tubed - 2	1.25	1.3	1.4	1.31±0.07
4	Dhobiajharan	2.5	2.46	2.47	2.47±0.02

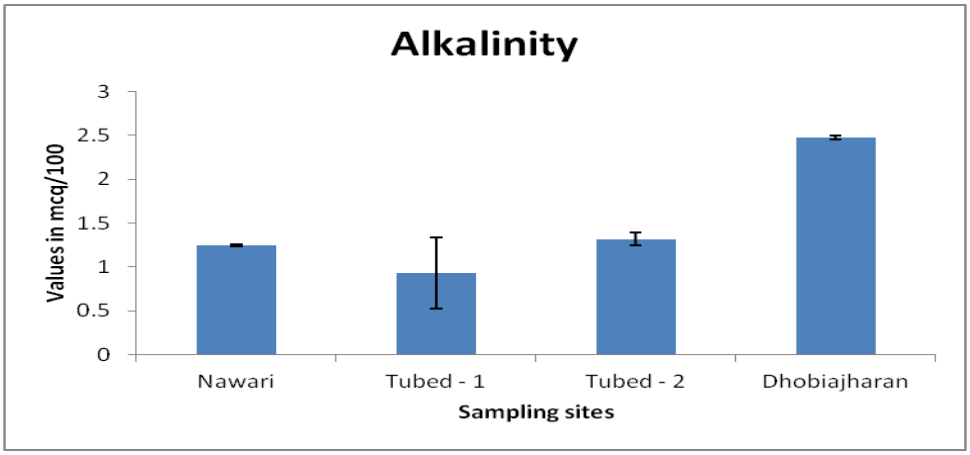

Figure 10: Alkalinity of the samples

The alkalinity of soil samples range is found to be 0.93 – 2.47 mcq/100. High alkalinity is found in Dhobiajharan (2.47 mcq/100) and minimum alkalinity was found in Tubed-1. So these soils are possessing low alkalinity which are useful for vegetation. These soils are not alkaline soils.

4.2.2.4 Chloride content

Weathering processes leads the formation of Chloride (Cl) which is one of the first elements from soils are formed. Cl mainly found in either in oceans or salt containing water pools. The irrigation water supplies chloride to the field depends on farm actions. The chloride content of soil samples is presented in table 20 and figure 11.

Table 20: Chloride content of the soil samples

Sl. No.	Name of the Village				Chloride content (mg/g)
1	Nawari	11.35	11.85	11.62	11.60±0.25
2	Tubed - 1	8.51	8.61	8.75	8.62±0.12
3	Tubed - 2	12.771	12.85	12.45	12.69±0.21
4	Dhobiajharan	8.51	8.651	8.71	8.62±0.1

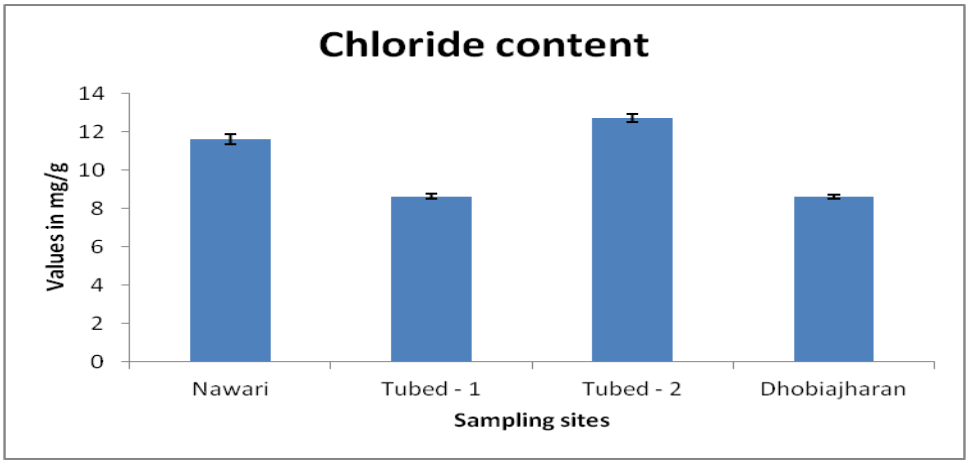

Figure 11: Chloride content of the soil samples

The chloride content range is found to be 8.62 to 12.69 mg/g. Chloride content maximum was present in Tubed -2 and minimum was present in both Tubed -1 and Dhobiajharan. Chloride is necessary as a micronutrient for optimal plant growth, at a range of only 0.3 – 1 mg/g dry

matter almost all plants (Marschner, 1986). In lithosphere the chloride content is nearly 500 mg/ kg.

4.2.2.5 Phosphorus content

Phosphorus is determined in the form of phosphorus pentaoxide. The normal potassium content soil are 136 to 337.5 kg/ha. Phosphorus (P) is an necessary element which is under macronutrient because the requirement of phosphorus is large amount for the growth of plants. The main role of phosphorus in the soil is to transfer energy in the microorganisms. Phosphorus in the organic compounds will help to transfer of energy within the cells as reactions required energy. In fresh water source the content of phosphorus is very low. If lakes and rivers are polluted with phosphorus a large number of algae will grow. The alga decreases the availability of dissolved oxygen and it becomes dirty. The average range phosphorus content in soils is 6.7-10.6 kg/ha. The phosphorus content of soil samples was presented in table 21 and figure 12.

Table 21: Phosphorus content of soil samples

Sl. No.	**Name of the Village**				**P_2O_5 (Kg/ha)**
1	Newari	17	15	12	14.66±2.51
2	Tubed - 1	11	13	17	13.66±3.05
3	Tubed - 2	6	9	13	9.33±3.51
4	Dhobiajharan	40	35	32	35.6±4.04

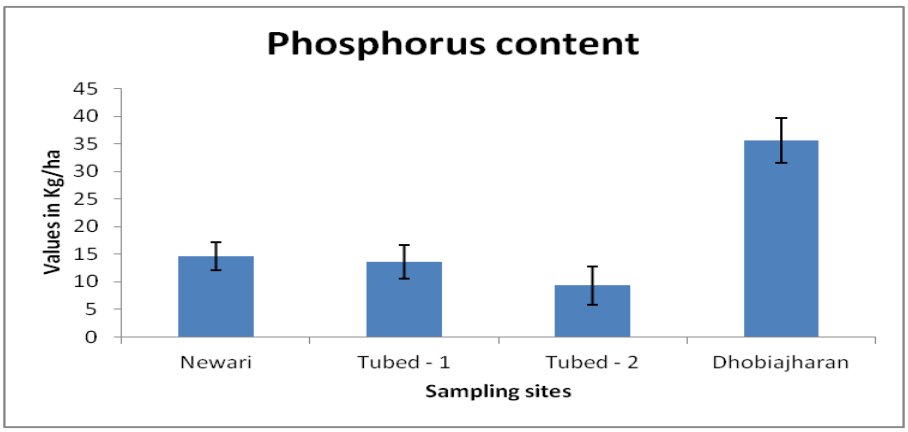

Figure 12: Phosphorus content of soil samples

Phosphorus content in soil samples was very high compare to normal ranges. Maximum 35.6 Kg/ha was found in Dhobiajharan and minimum 9.33 Kg/ha was present. Both the soils contain more than normal range of phosphorus. Adequate Phosphorus availability for plants was there which can stimulate early plant growth and hastens maturity. Although Phosphorus is essential for plant growth, mismanagement of soil P can pose a threat to water quality.

4.2.2.6 Potassium content

Potassium is present in sedimentary and metamorphic rocks. It contains nearly 25 g/ kg (Sheldrick, 1985). Potassium usually presents in mineral soils about generally ranges about 0.4 and 30 g /kg (Sparks, 1987). The agricultural soils are containing about 10 and 20 g/ kg (Jackson, 1964; Xie and Hasegawa, 1985). Total potassium contents present in soils generally range about 10,000 and 50,000 kg /ha in the upper 0.2 m of the soil profile. About 98% of potassium content is present in the form of mineral. Potassium content in soils about 2% is present in soil solution. It is in exchangeable phases (Sparks, 1987).

Table 22: Potassium content in the soil samples

Sl. No.	Name of the Village				K_20 (Kg/ha)
1	Newari	600	570	621	597±25.63
2	Tubed - 1	600	585	515	566.6±45.36
3	Tubed - 2	590	546	593	576.3±26.31
4	Dhobiajharan	60	70	59	63±6.08

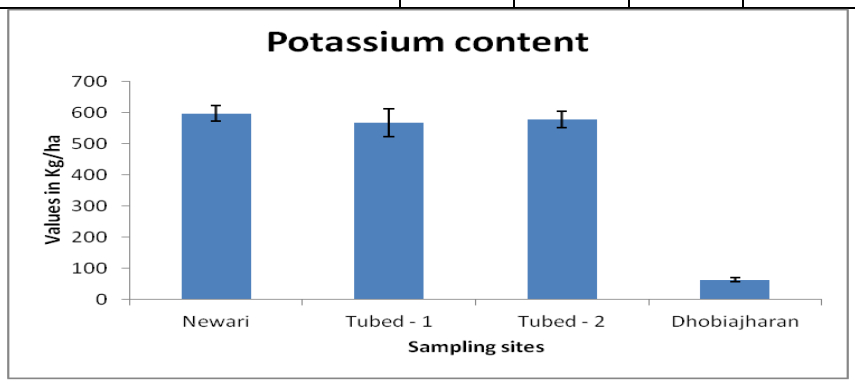

Figure 13: Potassium content in the soil samples

Potassium content high 597 Kg/ha was present in Newari and low 63 Kg/ha was present in Dhobiajharan. If the potassium levels are too high, plants may suffer due antagonistic effects (Table 16 and figure 13). The normal range of potassium content in soils is 198.5-254.1 kg/ha. The potassium content in the soil is means of original content, which is weathering of degree of potassium.

4.3 Microbial diversity

Both soil samples were screened for the microbial load in terms of bacterial load present in them. The total number of bacteria load in colony forming unit was also recorded by the standard plate count method. Determination of fungi and actinomycetes load in the soil and water samples. These bacteria presence indicates all types of nutrients are present which regulate

the growth. Soil fertility also depends on soil microbial diversity. Some nitrogen fixation bacteria provide nitrogen to leguminous plants. This symbiotic association is required each other as plant gives shelter to microbes in turn microbes provide nutrients to plants. Determination of bacterial load was done by using Nutrient Agar medium and the result was presented in Table 23.

Table 23: Determination of bacterial load in the soil samples

Name of the Village	**10^{-6}(no. of colonies)**	**Organisms per gram of soil (CFU/gm)**
Tubed-1	66	66X 10^6
Tubed-2	42	42X 10^6
Dhobiajharan	56	56 X 10^6
Nawari	12	12 X 10^6

Apart from this attempt was also made to determine the fungal diversity in the soil and water samples collected from the proposed mining sites. Potato dextrose agar media was used for fungal growth and the result was presented in table 18. The morphological characterizations of the isolated bacteria were done by standard procedure and the resulted are shown in table 19 and figure 14. Similarly the morphological characterizations of the fungal samples were shown in table 24.

Table 24: Determination of fungal load in the soil samples

Name of the Village	**10^{-6}(no. of colonies)**	**Organisms per gram of soil (CFU/gm)**
Tubed-1	1	1X 10^6
Tubed - 2	0	0 X 10^6
Dhobiajharan	2	2 X 10^6
Nawari	4	1 X 10^6

Table 25: Morphological characterization of bacterial samples isolated from the soil samples

		Morphological characteristics							
Name of the villages	**Bacteria code**	**Colour**	**Odour**	**Shape/form**	**Margin**	**Elevation**	**Size**	**Surface**	**Texture**
Newari	N1	Light green	Nil	Irregular	Undulated	Umbonate	2cm	Wrinkled	Moist
	N3	Creamy	Nil	Circular	Entire	Flat	0.6cm	Veined	Moist
Tubed 2	TT1	Yellow	Nil	Circular	Entire	Raised	0.2cm	Veined	Mucoid
	TT2	Creamy	Nil	Circular	Entire	Raised	0.4cm	Veined	Mucoid
Dhobiajharan	D1	Creamy	Nil	Circular	Entire	Raised	0.3cm	Veined	Mucoid
	D2	White	Nil	Circular	Entire	Raised	0.5cm	Veined	Dry
Tubed -1	No growth								

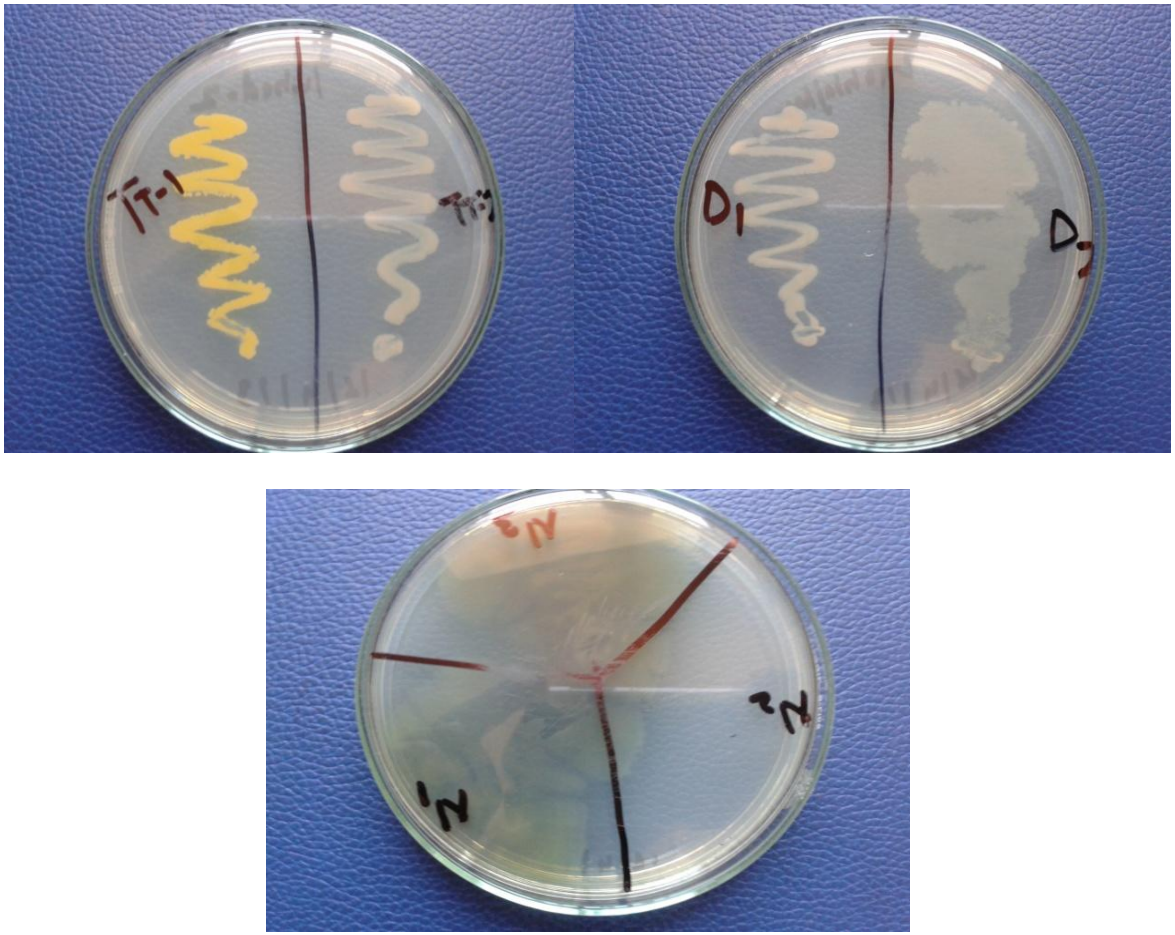

Figure 14: Morphological characteristics of isolated bacteria from soil samples

Table 26: Morphological characterization of fungal samples isolated from the soil samples

	Fungal code	Colour	Odour	Shape/form	Margin	Elevation	Size	Surface	Texture
Tubed-1	TF1	Yellowish	Nil	Irregular	Undulate	Raised	1.5 cm	Rough	Dry
Newari	NF1	White	Nil	Irregular	Filiform	Pulvinate	1.4cm	Rough	Dry
	NF2	Greenish	Nil	Irregular	Undulate	Pulvinate	2cm	Rough	Dry
Dobiajaran	DF1	Yellowish	Nil	Irregular	Undulate	Raised	1.5 cm	Rough	Dry

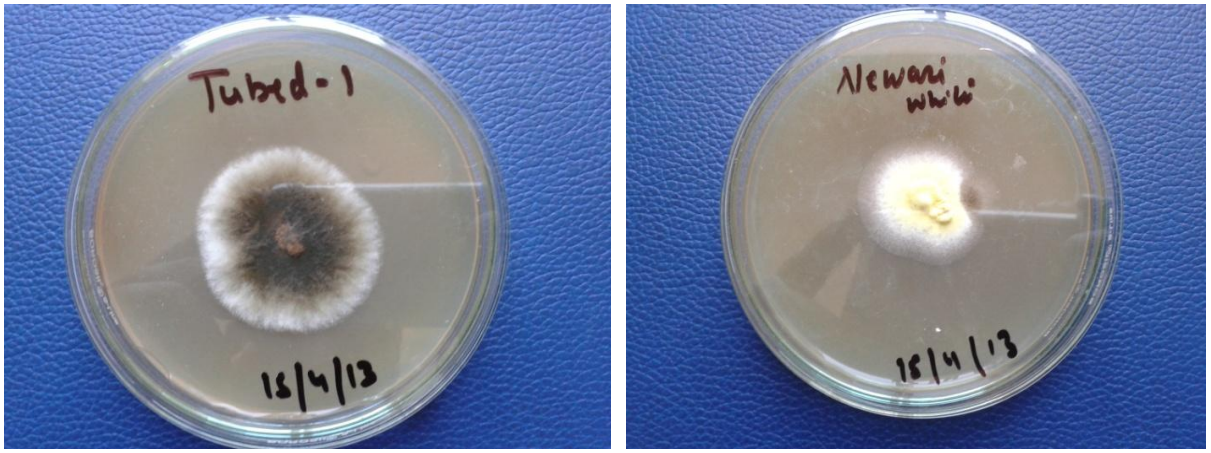

Figure 15: Morphological characteristics of isolated fungus from soil sample

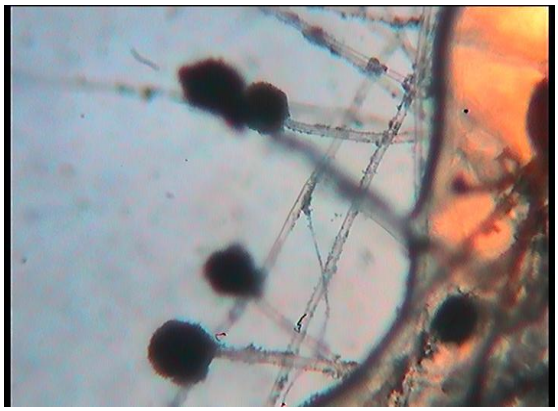

Figure 16: Lactophenol cotton blue staining of isolated fungus from soil samples

The fungus samples isolated from the soil samples of Tubed and Nawari villages are identified as *Aspergillus sp.* Features of *Aspergillus*: Hyphae are septate and hyaline. The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex. Vesicle is the typical formation for the genus *Aspergillus*. The morphology and color of the conidiophore vary from one species to another. Covering the surface of the vesicle entirely ("radiate" head) or partially only at the upper surface ("columnar" head) are the flask-shaped phialides which are both uniseriate and attached to the vesicle directly or are biseriate and attached to the vesicle via a supporting cell, metula. Over the phialides are the round conidia (2-5 µm in diameter) forming radial chains.

4.3.1 Biochemical characterization of bacterial sample

To identify the bacteria up to genus level, various biochemical tests were performed and the results were presented in table 22-25 and figure 17-30.

Table 27: Biochemical tests results for isolated bacteria form top soil samples of proposed study site of Latehar district, Jharkhand

		Biochemical characteristics									
Name of Village	**Bacteria code**	**Gram Staining**	**Catalase test**	**Urease test**	**Oxidase test**	**Citrate Utilization test**	**MR test**	**VP text**	**Nitrate reductase**	**Indole Production test**	**Starch hydrolysis**
Newari	N1	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve	- ve	+ve
	N3	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve	- ve	+ve
Tubed 2	TT1	- ve	+ ve	-ve	+ ve	- ve	+ ve	-ve	-ve	+ ve	+ve
	TT2	- ve	- ve	- ve	+ ve	+ ve	+ ve	-ve	-ve	+ ve	+ve
Dhobiajaran	D1	+ ve	- ve	+ ve	+ ve	+ ve	+ ve	-ve	+ve	- ve	-ve
	D2	- ve	+ ve	- ve	+ ve	+ ve	+ ve	-ve	-ve	- ve	+ve

Table 28: Triple sugar iron test for isolated bacteria form top soil samples of proposed study site of Latehar district, Jharkhand

Bacteria Code	Triple sugar iron test								
	Media supplemented with Sucrose			Media supplemented with Glucose			Media supplemented with Lactose		
	Gas	H_2S	Fermentation	Gas	H_2S	Fermentation	Gas	H_2S	Fermentation
N1	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
N3	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
TT1	-Ve	-Ve	-Ve	-Ve	-Ve	+Ve	-Ve	-Ve	-Ve
TT2	-Ve	-Ve	-Ve	-Ve	-Ve	+Ve	-Ve	-Ve	-Ve
D1	-Ve	-Ve	-Ve	-Ve	-Ve	+Ve	-Ve	-Ve	-Ve
D2	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve

Table 29: Carbohydrate metabolism test for isolated bacteria form top soil samples of proposed study site of Latehar district, Jharkhand

	Carbohydrate source			
Bacteria code	**Dextrose**		**Sucrose**	
	Acid	**Gas**	**Acid**	**Gas**
N1	+ve	-ve	ve-	-ve
N3	+ve	-ve	ve-	-ve
TT1	-ve-	-ve	ve+	-ve
TT2	+ve	-ve	ve+	-ve
D1	+ve	-ve	ve-	+ve
D2	+ve	+ve	ve-	-ve

Table 30: Antibiotic sensitivity test for isolated bacteria form top soil samples of proposed study site of Latehar district, Jharkhand

	Standard antibiotics (5 mg/ml)		
Bacteria code	**Ampicillin**	**Azithromycin**	**Ciprofloxacin**
	Inhibition zone in mm		
N1	23	38	43
N3	20	31	51
TT1	-	-	-
TT2	19	49	58
D1	25	34	41
D2	20	32	50

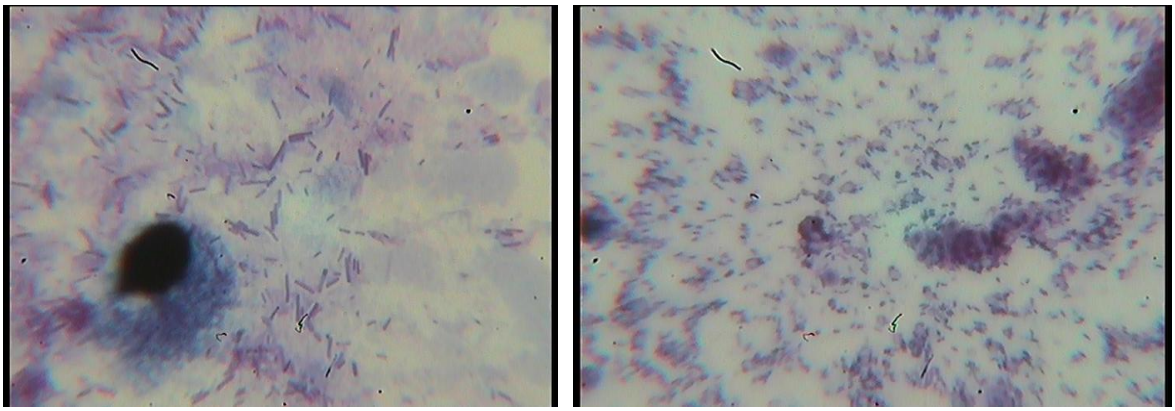

Figure 17: Gram staining of isolated bacteria from soil samples

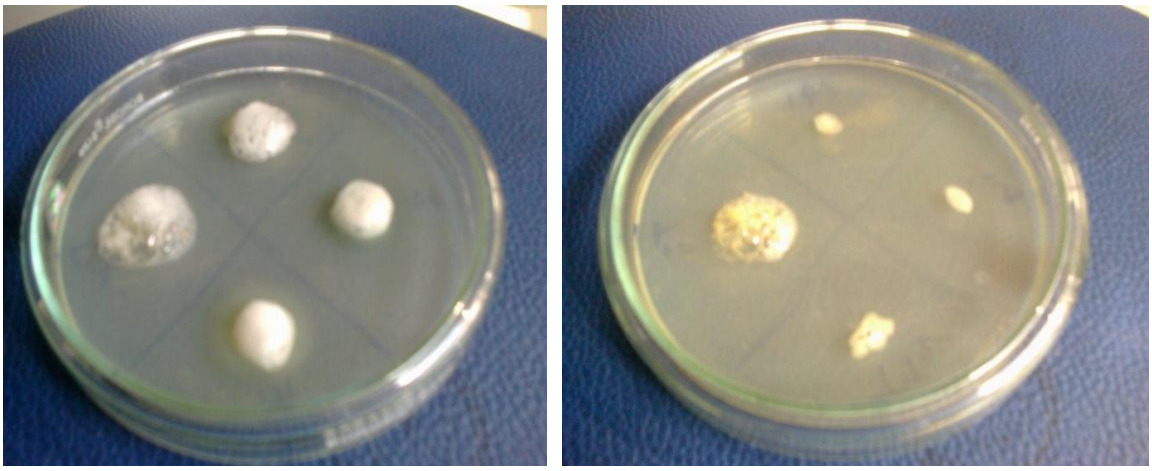

Figure 18: Catalase test of isolated bacteria from soil samples

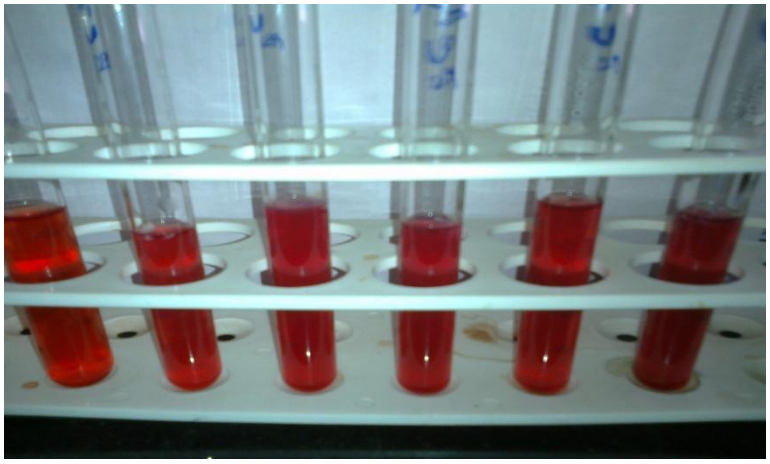

Figure 19: Urease test of isolated bacteria from soil samples

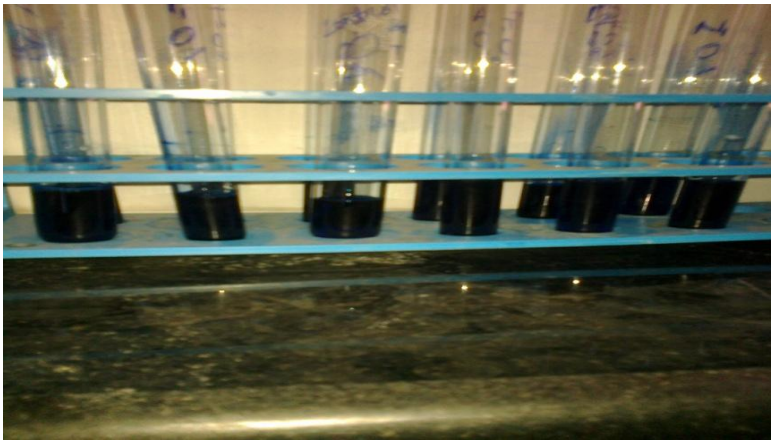

Figure 20: Oxidase test of isolated bacteria from soil samples

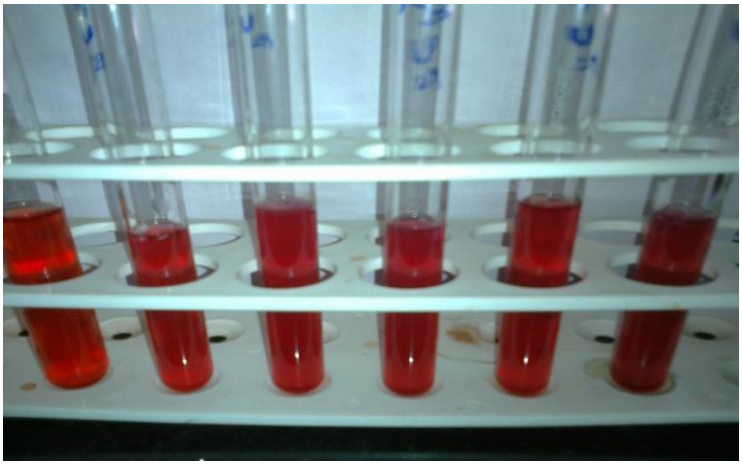

Figure 21: MR test of isolated bacteria from soil samples

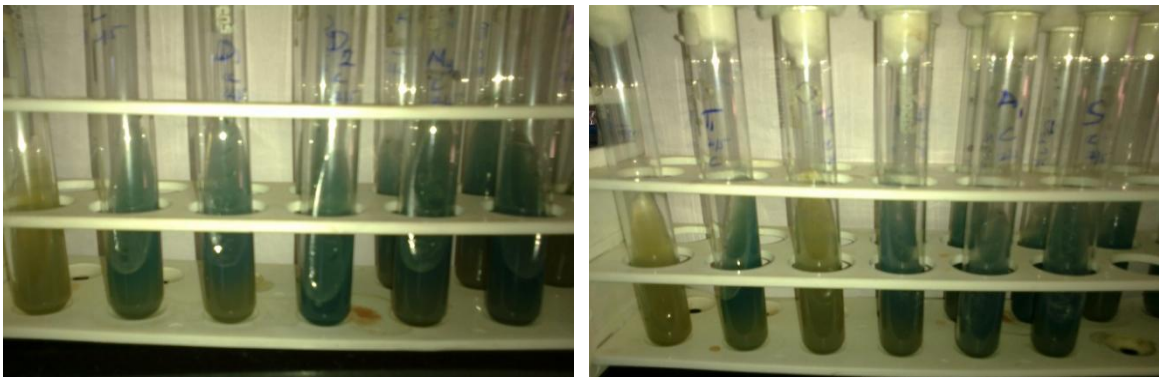

Figure 22: Citrate utilization test of isolated bacteria from soil samples

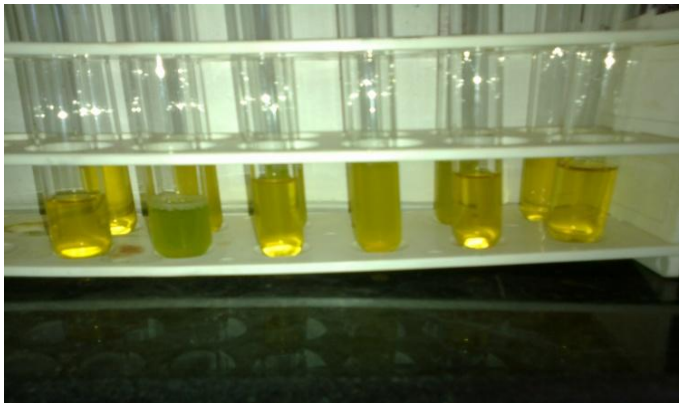

Figure 23: Indole test of isolated bacteria from soil samples

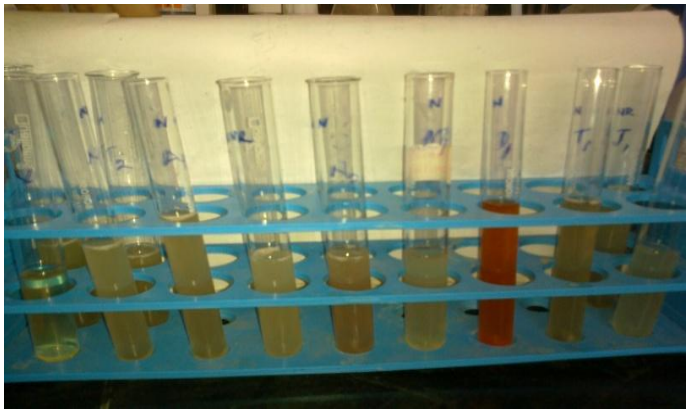

Figure 24: Nitrate reduction test of isolated bacteria from soil samples

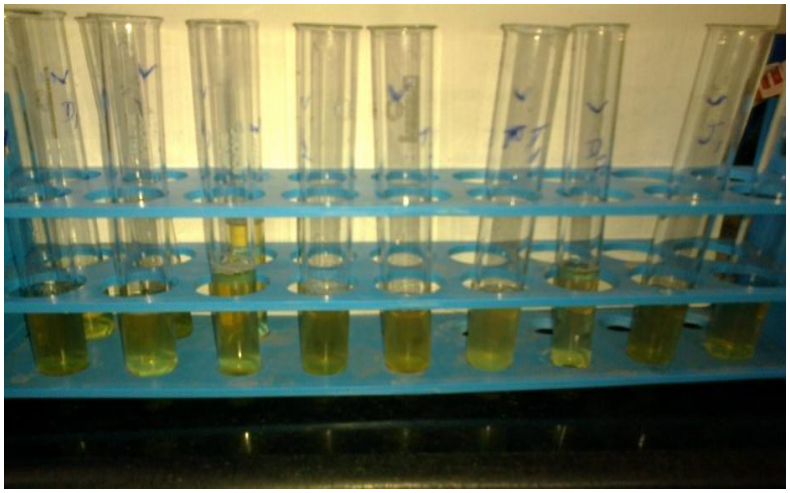

Figure 25: VP test of isolated bacteria from soil samples

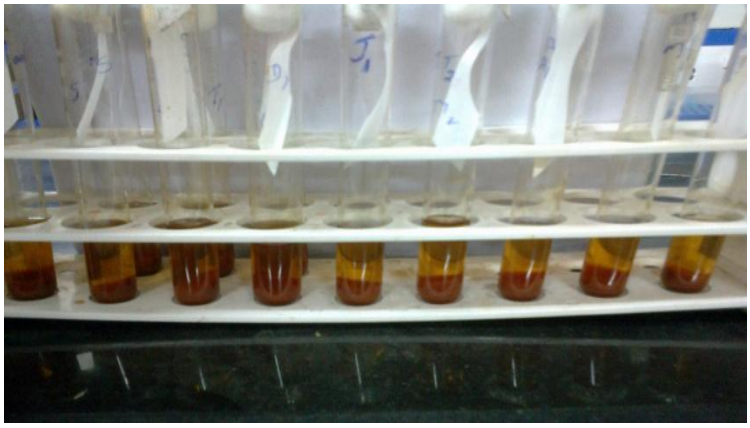

Figure 26: H_2S production of isolated bacteria from soil samples

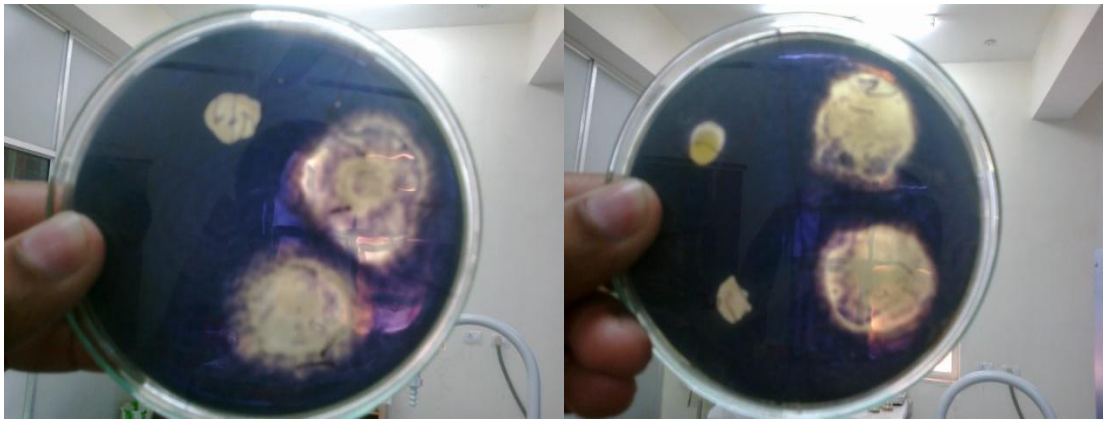

Figure 27: Starch hydrolysis of isolated bacteria from soil samples

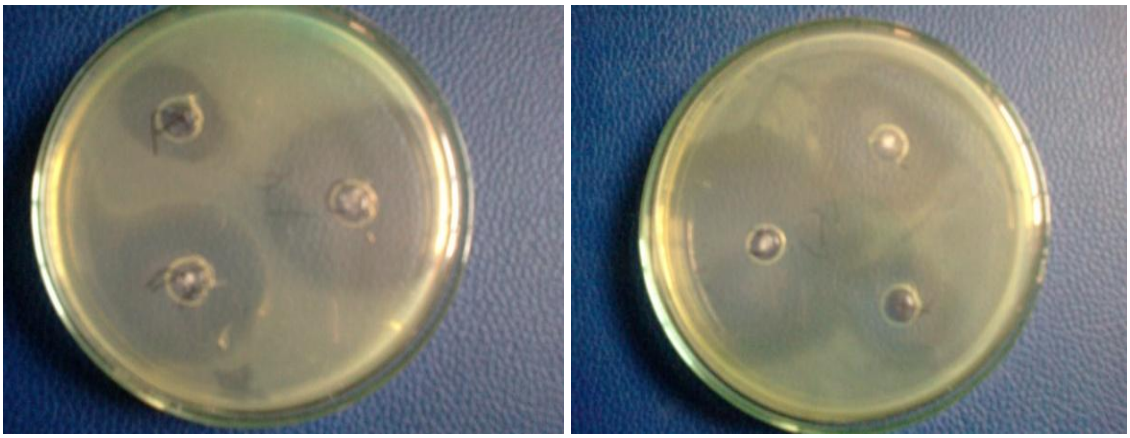

Figure 28: Antibiotic sensitivity test of isolated bacteria from soil samples

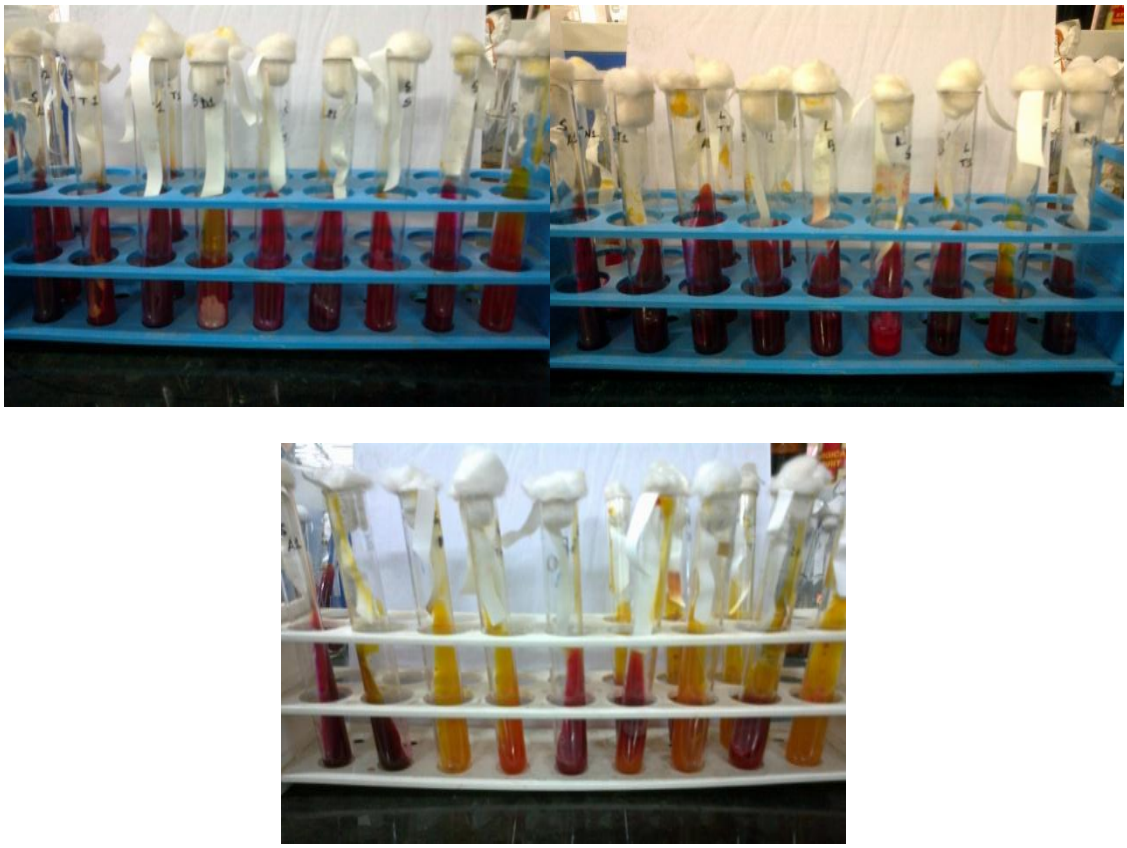

Figure 29: Triple sugar iron test of isolated bacteria from soil samples

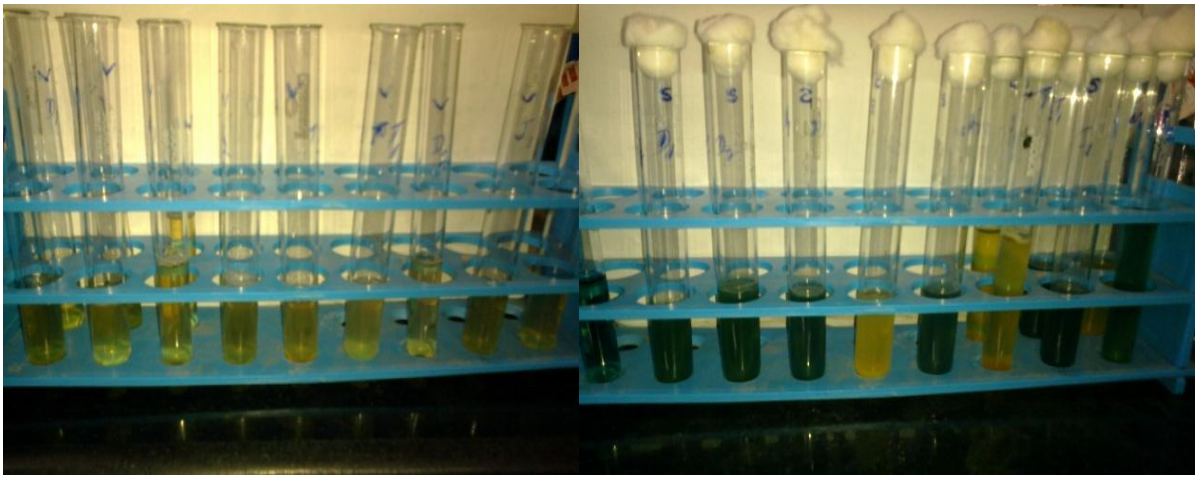

Figure 30: carbohydrate metabolism test of isolated bacteria from soil samples

4.3.2 Identification of unknown bacterial species

Identification of bacteria was done according to Bergey's Manual of Determinative Bacteriology by using various biochemical test results. The bacteria identified are presented in the table 26.

Table 31: Identification of bacterial species isolated from top soil samples

	Nawari		**Tubed-2**		**Dobiajharan**	
Biochemical tests	**N1**	**N3**	**TT1**	**TT2**	**D1**	**D2**
Gram's staining	+ ve	+ ve	- ve	+ ve	+ ve	- ve
Indole Test	- ve	- ve	+ ve	+ ve	- ve	- ve
Catalase Test	+ ve	+ ve	+ ve	- ve	- ve	+ ve
Citrate Utilization Test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
MR Test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Oxidase Test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Urease test	+ ve	+ ve	- ve	- ve	+ ve	- ve
VP Test	- ve	- ve	- ve	- ve	- ve	- ve
Nitrate reduction test	- ve	- ve	- ve	- ve	+ ve	- ve
H2S production test	- ve	- ve	- ve	- ve	- ve	- ve
Starch hydrolysis test	- ve	- ve	- ve	- ve	+ ve	- ve
Antibiotic sensitivity test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
Bacteria species identified	*Bacillus sp.*	*Micrococcus sp.*	*Pseudomonas sp.*	*Staphylococcus sp.*	*Staphylococcus sp.*	*Enteroacter sp.*

Identified bacteria are: **N1**- *Bacillus* species, **N3**- *Micrococcus* species, **TT1**- *Pseudomonas* species, **TT2-** *Staphylococcus* species, **D1**- *Staphylococcus* species, **and D2**- *Enterobacter* species

Bacillus is a genus of rod-shaped bacteria, Gram-positive. It is a member of the phylum Firmicutes. *Bacillus* species are catalase positive. *Bacillus* can found everywhere, and in the form of both free-living and pathogenic species. It can be obligate aerobes or facultative anaerobes. Under stressful environmental situations, the cells produce oval endospores. These endospores can stay dormant for extended time. These properties are originally distinct the genus. All such species are not related to each other and some may have been moved to other genera.

Micrococcus can find in a wide range of environments like dust, water, and soil. Micrococci are Gram-positive and spherical cells in shape ranging from about 0.5 to 3 micrometers in diameter. These characteristically appear in tetrads. Catalase test and oxidase test are positive where as indole test and citrate test are negative. *Micrococcus* contains a large cell wall, which might include in so far as 50% of the cell mass. *Micrococcus* genome is rich in guanine and cytosine (GC). It typically shows 65 to 75% GC-content. *Micrococcus* often contains plasmid (range from 1 to 100 MDa in size) that gives the organism with helpful characters.

Pseudomonas is a Gram-negative aerobic gammaproteobacteria. It is belonging to the family Pseudomonadaceae having 191 validly explained species. Culturing of bacteria in *in vitro* is very easy. An increasing number of *Pseudomonas* strain genome sequences availability has made the genus an outstanding focus for scientific research. Among all species the best studied species are *P. aeruginosa* and its role as an opportunistic human pathogen, *P. putida* a soil bacterium *P. syringae*, which is plant pathogen and *P. fluorescens* which is the plant growth promoting species. The associates of the genus display a immense deal of metabolic diversity, and therefore they are able to colonise a broad range of niches.

Staphylococcus is a genus. It is Gram-positive bacteria. They appear round (cocci), and appearance in grape-like clusters under the microscope. The *Staphylococcus* genus comprises no less than 40 species. Among these, nine are having two subspecies and one contains three subspecies. The majority species are harmless; exist in usually on the skin and mucous membranes of humans and other organisms. These are small amounts of soil microbial flora around the world.

Enterobacter is a genus of Gram-negative and facultative anaerobic. These are rod-shaped and non-spore-forming family Enterobacteriaceae. Some strains belongs to this genus are pathogenic and cause opportunistic diseases in immune compromised hosts, where as some are having onmechanical ventilation. The common infection sites are respiratory and urinary tracts. The genus Enterobacter includes in member of the coliform cluster of bacteria.

CHAPTER 5

SUMMARY AND CONCLUSION

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The analysis of top soil samples collected from the proposed mining area of Latehar district, Jharkhand revealed that pH of the soil is slightly acidic in nature. The soil is rich in high organic matter content mainly in Tubed-2, Dhobiajharan and very low in Newari village. The chloride content is high in Tubed-2 village where as it is low in Tubed -1 and Dhobiajharan village. Alkalinity is quite low for all the areas under study. However the moisture content in these soils is very low and thus is dry in nature. Phosphorus content of all the soil samples was found to be in the normal range. The bulk density and specific gravity were observed to be very low, that indicates soil samples contain higher organic matter which is favorable for the growth of plants in the soil. The presence of a diverse group of bacteria indicates that the soil is rich in all type of macro and micro nutrients suitable for their growth.

From the result it is concluded that the soils is rich in macronutrients which is essential for the growth of plants as well as microorganisms. Hence the suitability of the soil should be maintained like land rehabilitation or mine reclamation after completion of mining in the proposed area. Some mitigation measures must be adopted by the mining company to conserve and preserve the natural property of the soil.

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