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Assessment of Microbial Community and Soil Enzyme Activity of Coal Mine Dumps of Sonbhadra Uttar Pradesh, India.

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***Abstract*— Mining activities alter the normal soil properties and negatively affect the ecosystem and environment by many ways. Assessing the damage done to the soil is very important to know the actual status of soil health. Soil health and fertility can be measured by the amount of microorganisms present and activity of different soil enzymes. Number of microorganisms shows amount of stress and unfavorable conditions. In the present study, colony forming units of microorganisms were found relatively higher in natural forest soils in comparison to soil collected from dump sites. Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes so they can express the changes that may have happened in the soil due to mining. Soil phosphatase and dehydrogenase activity in natural forest have been found higher as compared to dump soil. In contrast, higher catalase activity was observed in dump soil and lower in Natural Forest soil.**

***Keywords*—Mining, Soil Health, microorganisms, soil enzyme activities.**

I. Introduction

Soil provides a medium where a long-term interaction among microorganisms, soil minerals and organic matter can influence the physio-chemical and biological characteristics of terrestrial system. Mining activity, specifically open cast mining, often lead to land degradation with adverse changes in soil textural and structural attributes **(1)**. Open cast coal mining leads to the removal of overlaying soil and formation of heaps of rocks, coal seam, sand, minerals and soil with very low nutrient quantity **(2, 3)**. The Open cast mining thus leads to a nutrient deficient condition for soil microorganisms and plants, and poses problem for the process of revegetation **(2, 4)**, restoration **(5, 6)** and pedogenesis **(7, 8)**. Heavy metals are dangerous group of pollutants, which cannot be naturally degraded like organic pollutants and they accumulate in different parts of the food chain **(9)**. Soil Enzymes are produced by microorganisms, either as a extracellular secretion or intracellular product released by lysis of cell, which provides an insight into microbial activity and dynamics **(10)**. Biogeochemical cycles are regulated by the microbial activity in the soil or by the enzymes they produce **(11)**. These enzymes are constantly synthesized, accumulated, inactivate/decomposed in the soil, and during this they stabilize the soil structure and function by nutrient mineralization and cycling, transformation and energy metabolism, decomposition and formation of organic matter **(12)**, and decomposition of xenobiotics. Dumps of mining are also a threat to the surrounding environment as it contains several trace elements in high amounts **(13, 14)**. The problem of coal mine dumps and overburden generated by opencast mining is enormous in India. Presently, the north eastern coalfields of Coal India Limited (NECF-CIL), Margherita, Assam, have produced more than 1000 ha of mine OB wasteland. Reports are also available on the prospects and environmental issues related to the north east (NE) collieries **(15, 16).**

The present study aims to find out the effect of mining over the environment and ecosystem utilizing microorganisms and soil enzyme activity as possible indicators.

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II. Materials and Method

A. *Study site and Soil Sampling*

Sonbhadra is the second largest district of Uttar Pradesh India, with the area of 6788 km^2. Sonbhadra is located in the south-eastern ranges of Vindhyanchal mountains which are having treasures of minerals, metals and most importantly Coal. There are various coal mines in Sonbhadra out of which Kakari mine was selected for soil sample collection. The Kakari mine is located (Lat $24^o16'55"$- $24^o18'90"$N and Long $82^o74'25"$- $82^o76'72"$E) beside Govind Ballabh Pant Sagar**.** Kakri mines are developed under kakri project of Northern Coal fields limited a subsidiary of Coal India Limited.

Soil samples were collected from various dumps of the mine as well as from the nearby forest. Three samples in triplicate were collected from different spots at dump site (D1, D2 & D3) and same number of samples was taken from the nearby natural forest (NF1, NF2& NF3).

B. *Soil pH estimation*

For estimation of soil pH solution of soil and water was prepared in the ratio of 1:2 and incubated for half an hour

at 30°C and the pH was measured with the help of electrode of pH meter.

C. *Microbial Enumeration*

For counting the colony forming units, samples were serially diluted with 0.85% saline solution and plated on Nutrient agar (Hi-media) plates. After incubation of about 30hrs. colony forming units of different plates were counted with the help of colony counter, calculated and expressed in CFU/g.**(17).**

D. *Soil Enzyme Activities*

As soil enzyme activities are the indirect measures of the microbial activity in the soil, different soil enzymes named catalase, dehydrogenase and phosphatase were tested.

Catalase enzyme activity was assessed by titrimetric method using $KMnO_4$ **(18)**. 2 g of soil mixed with 40 ml of water and 5 ml of 0.3% H_2O_2 added to it and shaken for 20 minutes at 25^{0}C. Now solution was filtered and added 5ml of 1.5 mol/l H_2SO_4 to 10 ml of the filtrate, remaining H_2O_2 was measured by titrating it by 0.1 mol./l $KMnO_4$ solution. The activity was expressed as ml 0.1 mol/l $KMnO_4$ sol. titrated/(g dry soil x 20 min.)

Dehydrogenase activity was measured by the reduction of 2,3,5-triphenylotetrazolium chloride (TTC) **(19, 20)**. For this 1.0g of soil was weighed and kept in a clean test tube and added 1.0 ml of TTC then incubated for 96 hours at 27^0 C, after which 10 ml of methanol was added to extract the triphenyl formazon(TPF) formed during the reaction. Amount of TPF released was determined by spectrophotometric analysis at 485nm.

For measuring phosphatase activity, 1.0g of soil sample was taken in a test tube and 5ml of 100.0mM phosphate buffer was included. 10.0mM of p-nitro phenyl phosphate (p-NPP) in 1.0ml solution was used as substrate. The final volume of the reaction mixture was adjusted to 10.0ml with the addition of requisite amount of distilled water. The tube was vortexed (2.0min.) at room temperature. It was then incubated at 37°C for 60min in shaker (100 rpm). After the incubation period, the sample was centrifuged at 10000 rpm for 5.0min. The clear supernatant was taken in a clean test tube and 10.0ml of 1.0M NaOH was added. The yellow colored filtrate was analyzed using a colorimeter at λ=430nm **(21).**

III. Results and Discussion

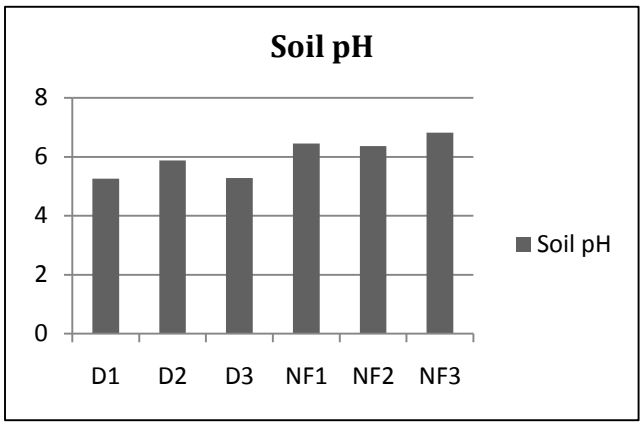
As the soil samples were subjected for the pH testing and it was found that the Dump 1(D1) is most acidic (pH 5.26) among all and NF 3 is most basic (pH 6.82). All the dump soil samples showed lower pH values which indicated towards higher acidity while all natural forest soils showed higher pH value than dump soils (Graph 1). The pH value of soil is often modeled as a positive liner relationship with soil fertility and productivity, where a high pH indicates a better soil. Improving soil chemical condition by the reduction of soil acidity has been well studied **(1)**Different studies have proved that due to excessive mineral deposition during mining soil turns acidic **(2, 22)**. Improvement of soil pH due to natural succession or by plantation strategy on coal mine spoil has been reported **(2, 23).**

The colony forming units when calculated revealed that all natural forest soil samples(NF1,NF2 & NF3) have much more colony forming units of microbes that the dump soil of Kakari mines, which proved that number of microorganisms substantially decrease in the dump soil.(Graph 2)

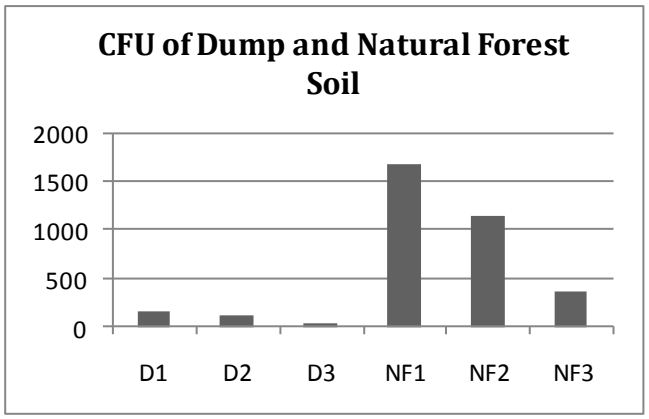
Soil catalase activity was found to be higher in the dump soil than in natural forest soils, and the highest catalase activity was measured in D2 that is 0.0075 ml of .1 M $KMnO_4$ g^{-1} of soil $min.^{-1}$, while minimum 0.00375 ml of .1 M $KMnO_4$ g^{-1} of soil $min.^{-1}$ showed by NF1. The high activity of catalase in dump soils shows high stress condition for microorganisms **(18)**.

Results of soil dehydrogenase activity assay showed the highest dehydrogenase activity in natural forest soil sample (NF 2) i.e. 1.02 µg.g^{-1}dry soil.h^{-1} and lowest activity in dump soil sample (D2) 0.1625 µg.g^{-1}dry soil.h^{-1}. Over all natural forest soil showed much higher dehydrogenase activity than dump soils. Estimation of dehydrogenase activity is very essential due to the fact that they are an integral part of soil microorganisms and are involved electron transport system, and requires viable cells to express its activity **(24)**. Dehydrogenase is considered to be an index of microbial activity and metabolic status of soil microorganisms **(25, 26, 27, 28)**. The results proved that microbial activity is higher in natural forest soil.

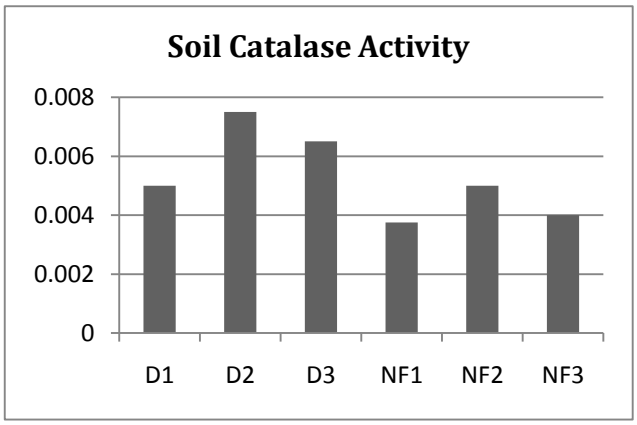
Phosphatase activity appears to be more affected by the metabolic state of soil, biological activities of microbial population, so their activity level can be used as an index for microbial activity in soil **(29)**. Highest amount of phosphatase activity was exhibited by NF1 0.00825 µM PNP g^{-1}soil $min.^{-1}$and lowest by D1 0.00219 µM PNP g^{-1}soil $min.^{-1}$. All natural forest soil samples showed higher phosphatase activity than dump, which can be positively correlated with the dehydrogenase activity and negatively with catalase activity.



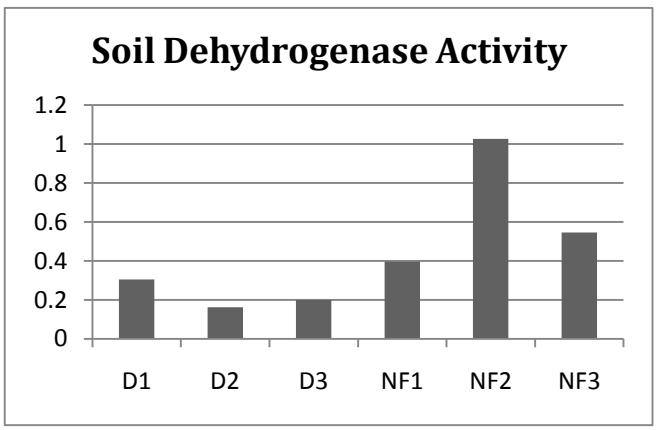
Graph 1: pH of different soil samples of Dump and Natural forest.



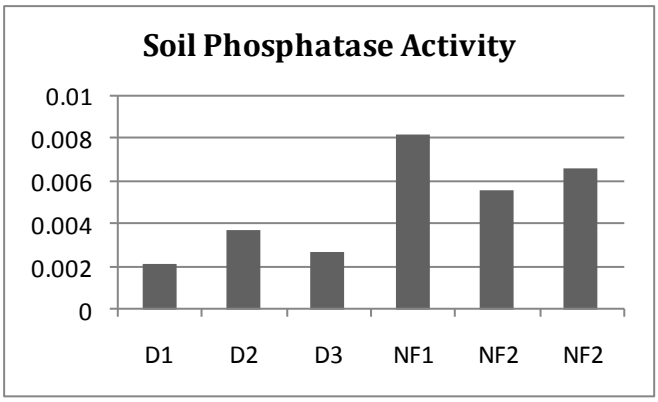
Graph 2: Comparison between colony forming units of natural forest and dump soil (all values in x10^4 CFU/g)



Graph 3: Comparison between soil Catalase activity of Dump and Natural Forest Soil (all values in ml of .1 M $KMnO_4$ g^{-1} of soil $min.^{-1}$).



Graph 4: Comparison of Soil Dehydrogenase activity between different samples of Dump and Natural forest (values expressed in µg TPF g^{-1}dry soil.h^{-1}).



Graph 5: Soil phosphatase activity of different Dump and Natural Forest soil sample (values in µM PNP g^{-1}soil $min.^{-1}$).

IV. Conclusion

This study was done to find the ill effects of mining over the environment. The microbial community and soil enzyme activity were taken as an indicator of environment, because these two parameters represent the health of the environment in different ways viz., microbes produce the soil enzymes and these enzymes regulate the biogeochemical cycles and these cycles play a vital role in maintaining the environment and ecosystem. The low number of microorganisms in the dump soil indicated towards contamination of heavy metals and stress conditions. The soil enzyme activity also co-related with the results as soil dehydrogenase and phosphatase activity has shown very low activity in the dump soil when compared to the Natural forest soil. The catalase activity confirmed the stress conditions in dump soils.

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In India, it has been estimated that about 10,000 hectares of land is affected by stockpiling of coal mine spoil. [30]

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