Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

| ISSN: 0972-6268 | Vol. 15 | No. 3 | pp. 853-858 | 2016 |
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Original Research Paper

# Biological Properties of Selected Overburdens of Singrauli Coalfields

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**Nat. Env. & Poll. Tech.**
**Website: www.neptjournal.com**

*Received:* 05-07-2015
*Accepted:* 18-05-2015

**Key Words:**
Enzymatic activity
Microbial population
Overburden
$CO_2$ flux

## ABSTRACT

Coalfield mine overburden or abandoned mine sites is a major environmental concern. Overburden materials are nutrient-poor, loosely adhered particles of shale, stones, boulders and cobbles and are devoid of true soil character. Eco-restoration or natural transformation of overburden materials to soil for cultivation is a time taking process. In the present study, we focused on how the OB materials are different from nearby natural soil and explored the characterization of physical, chemical and biological properties of different aged overburden undergoing the process of eco-restoration. Further, we have analysed various microbial population, enzymatic activities and microbial respiration process in different aged overburdens *vis-a-vis* nearby soil. Microbial population was more in 16 year old overburden among different aged OB but less than nearby soil. Dehydrogenase, Urease activity showed an increasing trend with maturity age of overburden representing higher microbial population, while alkaline phosphatase activity is not following any trend. Soil microbial respiration was found to be increased with age of overburden. Carbon mineralization rate constant in all overburden lied in a narrow range (0.020- 0.011) $day^{-1}$ and it did not show any significant variation as compared to native forest soil (0.03 $day^{-1}$). Mineralizable carbon was found more in native soil (7.95 mg C/kg of overburden) and 16 year old overburden (5.56 mg C/kg of overburden). Cumulative $CO_2$ evolved was more in native forest soil (8.67 mg C/kg), and was comparable with 16 year old overburden (5.4 mg C/ kg). Microbial population, enzymatic activity and carbon mineralization can act as an indicator for analysing changes in overburden spoil properties due to ecorestoration.

## INTRODUCTION

Opencast coal mining leads to generation of mine tailings and other reject materials (referred to as overburden, OB) is considered as a major contributor to the ecological and environmental degradation (Gogoi et al. 2007). Overburden materials are nutrient-poor, loosely adhered particles of shale, stones, boulders, cobbles, and so forth and are devoid of true soil character (Matson et al. 1997, Deka Boruah 2006). Ecological succession in a mine OB is a lengthy process. A minimum period of 50 years to a century is required to establish advanced specific plant species in denuded, mine OB-filled land (Dobson et al. 1997). Traditionally, mines are the sole mineral supply source, and exploration for coal is conducted without giving much regard to its serious impacts on the ecology and environment. Therefore, the coal mining industry is being placed under the red category i.e., it is in the top bracket of environmental degradation (Chaoji 2002). The chief environmental impacts due to mining are changes in soil stratification, reduced biotic diversity, and alteration of structure and functioning of ecosystems, these changes ultimately influence water and nutrient dynamics and trophic interactions (Matson et al. 1997, Ghosh 2004, and Almas 2004). Present study dealt with changes in microbial properties of different aged OB undergoing ecorestoration and compared with native forest soil. This could help in understanding variation in biological properties of different aged overburden spoil and scope of applying artificial interventions for improving properties of overburden for plant growth.

## MATERIALS AND METHODS

### Study Area

Overburden samples were collected from the Bina Extension Opencast Project (OCP) of Northern Coalfields Limited (NCL) located in Singrauli Coalfields, which was situated partly in Sonebhadra district of Uttar Pradesh and partly in Singrauli district of Madhya Pradesh (Fig. 1).

### Sample Collection and Preparation

Overburden samples were collected from 10 different sites

with spade for individual overburden. The samples were thoroughly mixed and a 500 g of composite sample from each site was collected. These soils were air dried and passed through 2 mm sieve. The collected overburden samples were from Forest area ($S_1$), 5 year old overburden ($S_2$), 12 year old overburden ($S_3$) and 16 year old overburden ($S_4$).

**Soil Biological Analysis**

**Microbial population counting:** Microbial populations of bacteria, fungi and actinomycetes were analysed by standard plate count method. Suitable dilutions of overburden were poured on selective media, Nutrient agar for enumeration of total bacteria (Chonkar et al. 2007), Potato dextrose agar for fungi (Chonkar et al. 2007), Ken Knight and Munaier's medium for actinomycetes, (Chonkar et al. 2007). Results were expressed in terms of colony forming units (CFU) per gram soil. The procedure involved weighing 10 g of the test OB sample under aseptic conditions in sterile paper, and transferring the sample to 90 mL of distilled water, shaking thoroughly for uniform mixing and from this suspension, transferring 1mL to a test tube containing 9 mL sterile distilled water. This is $10^{-1}$ dilution. From this further dilutions can be made by transferring 1mL to 9 mL of sterile distilled water aseptically to any desired level say $10^{-4}$, $10^{-5}$, $10^{-6}$ and so on. 1mL of required dilution ($10^{-4}$ for fungi and actinomycetes, $10^{-6}$ for bacteria) was transferred into sterile Petri plates. Plates were incubated in the inverted position at 28°C in incubator for 2 days for fungi, 4 days for bacteria and 1 week for actinomycetes.

**Enzymatic activities:** Dehydrogenase activity in different mine spoil and NF samples was measured by spectrophotometric method following the reduction of 2,3,5-triphenylotetrazolium chloride (TTC) as an artificial electron acceptor to red-coloured triphenyl formazon (TPF) (Chonkar et al. 2007).

Urease activity was determined by spectrophotometric method using DAM (diacetylmonoxime) in presence of thiosemicarbazide, $H_3PO_4$, and $H_2SO_4$ (Pal & Chonkar 1981).

Phosphatase activity of different mine overburden spoil as well as NF samples was determined by spectrophotometric method (400nm) using *p*-nitro phenyl phosphate as substrate (Chonkar et al. 2007).

**Microbial basal respiration:** It was determined following the alkali absorption technique (Chonkar et al. 2007). Fig. 2 shows set up for trapping $CO_2$.

A nonlinear least-square regression analysis was used to calculate parameters from cumulative data of C mineralization. The first-order kinetics equation was used to calculate the potentially mineralizable C ($C_0$):

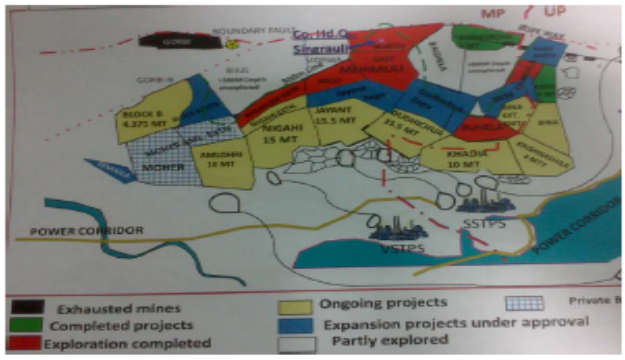


Fig. 1: Location of Bina open cast mines, Singrauli, NCL.

$$C_m = C_0 (1\text{-}e^{-kt})$$

Where, $C_m$ is the organic C mineralized at any specific time $t$, and $k$ is the first-order rate constant. The coefficients of correlation (r) were used to evaluate the goodness of fit.

## RESULTS AND DISCUSSION

**Soil Microbial Population:** Microbial population was counted in nearby forest soil and different aged overburden of Singrauli coal field. Bacterial population was found maximum in $S_1$ as bacteria prosper in neutral to alkaline range, so its population was found maximum in $S_1$ as native forest soil provides congenial environment for microflora, followed by $S_3$, $S_4$ and $S_2$ OB samples (Table 1). Similar results were observed by Juwarkar & Singh (2007). Environmental stresses brought about by mining activities and contamination by heavy metals in $S_2$ as compared to $S_4$ could be adducted for the reduction in microbial population and diversity (Monson et al. 2006). Plantation in overburden results in increase in bacterial activity in soil, so microbial population increases with age in overburden with plantation up to 16 years.

Fungal population was found to be maximum in $S_1$ ($23\times10^4$) and $S_4$ ($30\times10^4$), it may be due to prevailing favourable moisture, sufficient organic matter, and least in $S_2$ ($3\times10^4$) and $S_3$ ($12\times10^4$) per g of OB. Fungal species which were identified in this overburden were *Alternaria*, *Helminthosporium*, and *Aspergillus*. These fungi were commonly airborne fungi and thus first to colonize on mine spoils. Similar result was found by Visser et al. 1979. Actinomycetes population was found very low in overburden, $S_4$ overburden sample had more colonies which may be due to high pH of spoil due to plantation of trees and nature of Actinomycetes to survive in minimal nutrient condition, but Actinomycetes population is less as compared to native forest soil (Table 1). Fig. 3 showed microbial community in different aged overburden.

**Enzymatic activity:** Enzymes (dehydrogenase, urease, and

phosphatase) indicated minimal activity in $S_2$, which may be due to the reduced microbial population caused by the toxic effects and oxidative stress of mine spoil metal impurities, and their interference in osmotic balance and nutrient deficiency (Brooks 1995). The highest enzymatic activity was observed in NF soil as compared to different mine spoils (Table 2), which may be due to higher organic matter that support increased microbial activity and microbial biomass. Dehydrogenase is an intracellular oxido-reductase group of enzymes involved in electron transport system of oxygen metabolism, regulating the metabolic reactions in soil, and is considered to be an index of overall microbial activity (Dick 1997) and metabolic status of soil microbial community (Cladwell 2005). The dehydrogenase activity showed a consistent increase from $S_2$ (0.64 µg TPF/g spoil/hr) to $S_4$ (1.78 µg TPF/g spoil/hr). The highest dehydrogenase activity was observed in NF soil (3.28 µg TPF/g soil/hr) as compared to different mine spoils (Table 2), which may be due to higher organic matter that support increased microbial activity and microbial biomass (Mukhopadhyay & Maiti 2011). Further, the variation in dehydrogenase activity with respect to different mine overburden spoils in chronosequence may be attributed to the change in microbial community composition (Masciandaro et al. 2000).

Urease belongs to soil hydrolases, which is mostly an extracellular enzyme involved in urea hydrolysis (hydrolysis of soil amide N). Urease acts as intermediary enzyme in the transformation of organic nitrogen into inorganic forms (Dkhar & Mishra 1985). Hence, emphasis on urease activity has been given in order to evaluate N supply to plants, because large N losses to atmosphere by volatilization mediated by these enzymes. Higher urease activity was exhibited by nearby NF soil (11.3 µg $NH_4^+$/g soil/hr). The urease activity showed an increasing trend from $S_2$ (2.78 µg $NH_4^+$/g soil/hr) to $S_4$ (8.9 µg NH4 +/g soil/hr), which may be due to the variation in physico-chemical properties of soil, moisture content, organic matter, gradual accumulation of N over time (Gracia et al. 1993). Plant grown in overburden helps in supporting microbial population which are considered to be source of enzymes in soil. Phosphatase acts as intermediary enzyme in the transformation of organic phosphate into inorganic forms (Dkhar & Mishra 1985), and has a role in P cycling (Kramer & Green 2000). Wide variation in phosphatase activity was exhibited, which ranged from 9.74 µg PNP/g spoil/hr ($S_2$) to 12.65 µg PNP/g spoil/hr ($S_4$). Phosphatase activity appeared to be more dependent on the metabolic state of soil, biological activity of microbial population, and hence can be used as an index for microbial activity (Kramer & Green 2000).

**Overburden carbon di-oxide flux:** Soil $CO_2$ flux has been used as an early indicator of stress or soil restoration to determine the reclamation status of coalmine degraded sites (Sourkova et al. 2005). Soil $CO_2$ flux describes the level of microbial activity, soil organic matter content and its decomposition (Insam et al. 1991). Basal spoil respiration is considered as the reflection of the availability of slow flowing carbon for microbial maintenance and is a measure of basic turnover rate in soil. Carbon dioxide evolved initially declined up to 24hr. After 48 hr $CO_2$ evolution again increases up to 120 hr, the decline in $CO_2$ gas evolution may be due to priming effect of freshly added organic matter in forest area. In overburden samples ($S_2$ and $S_4$) decline in respiration rate follows a steep slope up to 24 hr. It may be due to lack of carbonaceous compounds. In $S_3$ overburden samples respiration rate slowly increases up to 24 hr, no change was observed till 48 hr then respiration rate increases sharply at 120 hr. Slow rate of carbon dioxide evolution may be due to presence of slowly degradable carbon compounds, as soon as they got mineralized respiration rate rapidly increases. Soil respiration ($CO_2$) includes respiration of soil microflora and fauna, respiration of plant roots, and some chemical processes. The rate of $CO_2$ production was found to be increased with age of overburden. Cumulative carbon mineralization in 168 hour is shown in Fig. 4.

The trends of $CO_2$ evolution from NF and overburden samples conformed well to the exponential first-order model $C_m = C_0 (1 - e^{-kt})$. Parameters calculated according to the model are given in Table 3. As can be seen, the $CO_2$ values ranged from a minimum of 4.26 for $S_2$ OB to maximum of 5.86 mg C $100g^{-1}$ for NF OB sample. The results confirm the pattern of the cumulative amount of $CO_2$ released from soil. Mineralizable carbon falls within a range of 4.26 to 5.86 mg C/kg of soil. Rate constant in different years overburden falls within a narrow range of 0.011 to 0.020/day as compared to NF (0.03/day) indicating that soil microbial respiration did not differ significantly in different aged overburden. The

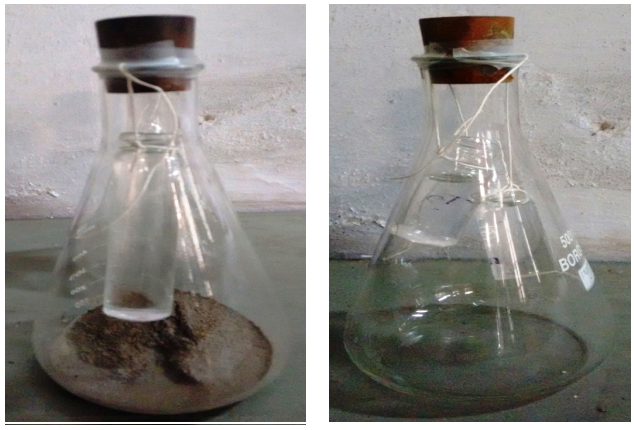

Fig. 2: Set up for analyzing microbial respiration.

relatively narrow range of k values among the spoil suggests that microbial respiration metabolized organic compounds were similar or had the same degree of availability for samples tested (Cooper & Warman 1997). Native forest soil did not show any significant variation in carbon mineralization pattern as compared to overburden. It may be due to addition of high C:N ratio plant residue in forest soil which decreases rate of organic matter mineralization.

Soil enzymes play a significant role in the degradation of organic matter and recycling of nutrients. The extracellular enzyme hydrolysis of organic matter is considered to be the rate limiting step in overall decomposition process (Sinsabaugh et al. 1993). Therefore, enzymatic activity may be used as an indicator of mineralization rate of carbon. Correlation data are provided in Table 4. Mineralized C is positively and significantly correlated with dehydrogenase activity (0.99** at 0.01 % level of significance). Potentially mineralizable C also shows positive correlation with dehydrogenase activity (r =0.99**). High dehydrogenase activity is a representative of good microbial population in soil. Microbial activity promotes carbon mineralization so it was significantly correlated. Rate constant and $C_0k$ had positive and significant correlation with dehydrogenase activity (r = 0.97*) and actinomycetes population (0.98*). The total carbon mineralization was strongly correlated to enzyme activities, but a little weakly correlated to soil organic carbon, which suggests that the effect of enzyme on carbon mineralization should be significant and direct. High enzyme activity should lead to high carbon mineralization, but the high soil organic carbon content could lead high carbon mineralization is not necessary, which means that enzymes and

Table 1: Microbial population in overburden.

| S.N | Bacteria (cfu/g of soil)×$10^4$ | Fungus (cfu/g of soil)×$10^4$ | Actinomycetes (cfu/g of soil)×$10^4$ |
|---|---|---|---|
| S1 | 900 | 23 | 7 |
| S2 | 90 | 3 | 0 |
| S3 | 200 | 12 | 0 |
| S4 | 550 | 30 | 2 |

Table 2: Enzymatic activity of different aged overburden.

| Enzymatic activity | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| Dehydrogenase (µg TPF/g/hr) | 3.28 | 0.64 | 1.34 | 1.78 |
| Urease (µg $NH_4^+$/g/hr) | 11.3 | 2.78 | 7.63 | 8.9 |
| Alkaline Phosphatase (µg PNP/g /hr) | 15.6 | 9.74 | 14.6 | 12.65 |

Table 3: Carbon mineralization parameter.

| Overburden | Co | k | $C_0k$ | r |
|---|---|---|---|---|
| S1 | 7.95 | 0.036 | 0.29 | 0.96 |
| S2 | 4.26 | 0.011 | 0.05 | 0.99 |
| S3 | 5.50 | 0.015 | 0.08 | 0.88 |
| S4 | 5.63 | 0.017 | 0.1 | 0.98 |

Table 4: Significant relation between carbon mineralization parameter and biological properties.

| | DHG | U | AP | Bacteria | Fungi | Actinomycetes |
|---|---|---|---|---|---|---|
| $C_i$ | 0.99** | 0.90 | 0.83 | 0.94 | 0.60 | 0.95 |
| $C_o$ | 0.99** | 0.91 | 0.85 | 0.93 | 0.61 | 0.94 |
| k | 0.97* | 0.82 | 0.75 | 0.93 | 0.52 | 0.98* |
| $C_ok$ | 0.97* | 0.80 | 0.72 | 0.92 | 0.50 | 0.98* |

** Significant at 0.01 level; * Significant at 0.05 level

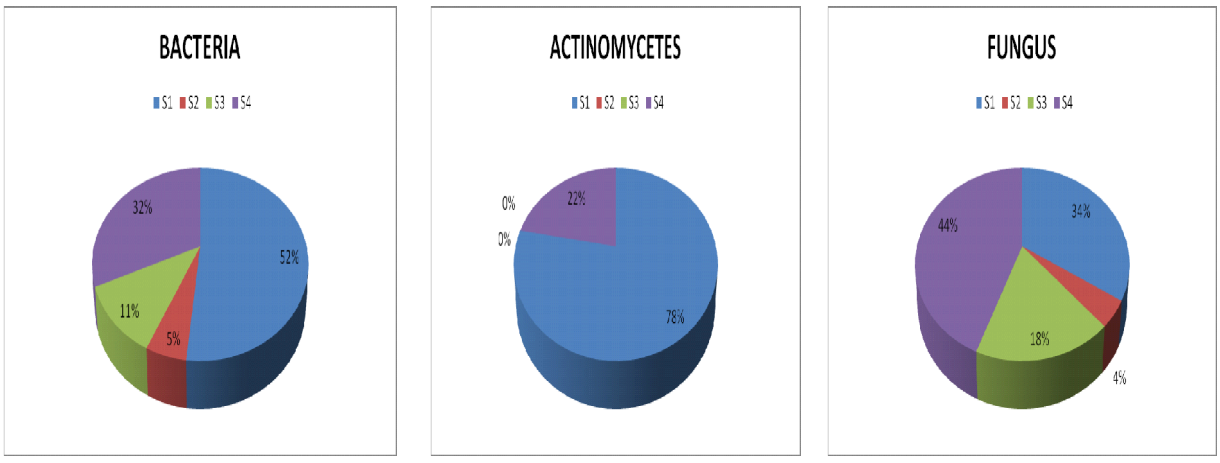


Fig. 3: Microbial population in different aged overburden.

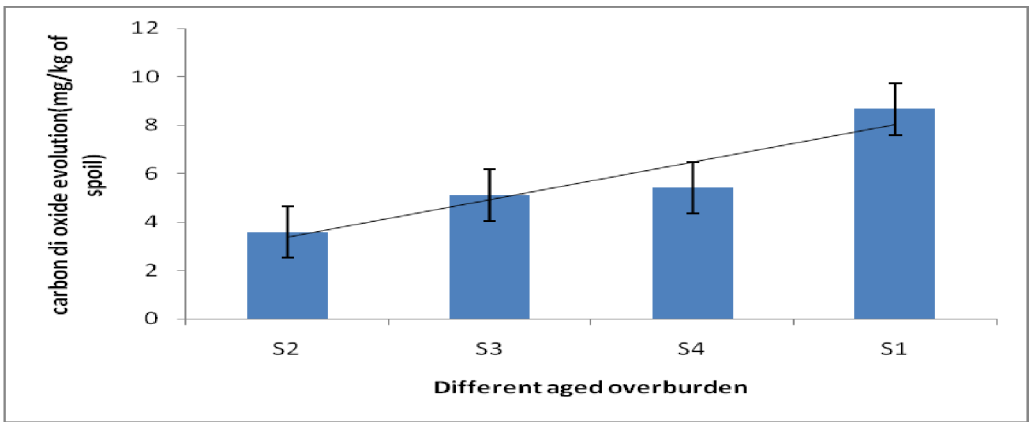


Fig. 4: Cumulative $CO_2$ evolution after 168 hr from the overburden samples.

microorganism were real catalysts for carbon mineralization, with soil organic carbon being substrates for the process (Feng et al. 2004).

## CONCLUSION

The study of biological properties of coal mine overburden helps us to know about scope of remediation of anthropogenic disaster caused by coal mining. Microbial population study reveals that the population of fungi, bacteria and actinomycetes were found more than the minimum number decided by soil quality standard for reclamation of overburdens i.e (CFU/g) $5\times10^3$, CFU/g 60 ,CFU/g $10^2$) for bacteria, fungi, and actinomycetes respectively. Thus, there is scope of remediation of overburden through various technologies as reforestation, using VAM propagules, biotechnological approach. Slow flowing carbon for microbial maintenance was found more in forest soil and was increasing with age of reclaimed overburden. Dehydrogenase activity and urease activity can be used as indicator of coal mine OB reclamation.

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