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Characterization of Physico-chemical Properties and their Impact on Enzyme Activities in a Chronosequence Coal Mine Overburden Spoil as Biomarker of Reclamation Process

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Abstract

Mining activities lead to land degradation and alter ecosystem functions. Monitoring land degradation status is essential to take appropriate and timely conservation measures. Soil genesis during early years of mine spoil reclamation is critical and may help to predict reclamation success. The microbial activity is significantly influenced by the physico-chemical properties, and hence, the assessment of these changes is essential for soil management practices. In the present investigation, the physico-chemical characterization and the activities of six different enzymes (amylase, invertase, protease, urease, phosphatase and dehydrogenase) were periodically analyzed with respect to different coal mine overburden spoil in chronosequence over a period of 10 yr, and compared with the native forest soil, in order to assess their effectiveness in reclaiming mine overburden spoil. Comparative analysis suggested that there was gradual increase in enzyme activities from a nutrient deficient situation (fresh mine spoil) to an enriched soil (native forest soil). Besides, the variation in enzyme activities was significantly attributable to differences in physico-chemical properties. Stepwise multiple regression analysis was performed in order to determine the contribution of different physico-chemical properties influencing the variability in enzyme activities. Further, principal component analysis was able to discriminate six coal mine overburden spoils and native forest soil into independent clusters on the basis of their physico-chemical properties and enzyme activities. The study clearly revealed that the change in microbial indices in terms of enzyme activities were more responsive and correlated very well with the extent of land degradation, and therefore, can serve as biomarker for reclamation studies.

Keywords: Mine overburden spoil; Physico-chemical properties; Enzyme activity; Reclamation

Introduction

Soil is a vitalizing system where the prolonged interaction between the microorganisms, organic matters and soil minerals influence the physico-chemical, biological properties of the terrestrial systems. Soil acts as a critical controlling component in an ecosystem. Mining activity, specifically open cast mining, often lead to land degradation with adverse changes in soil textural and structural attributes [1]. During surface mining, the overlying soil is removed, and the fragmented rock is heaped in the form of overburden. The mine overburden spoil constitutes a mixture of coal seam, coarse rocks, sands, dusts, shale, pebbles and other impurities [2,3]. The surface mining thus results in nutrient deficient condition with loss of soil organic C, leading to a long lasting drastic condition for both plants and soil microorganisms. Being deficient in plant nutrients, it represents a disequilibriated geomorphic system [4], and poses problem for the process of pedogenesis [5,6], revegetation [2,7], and restoration [8-11]. There have been reports about slow recovery of mine spoil restoration due to constraints in microbial growth [8,12-14] and vegetation secession [2,10].

Understanding microbial diversity has become an important field of research, as well as resource management. Soil microbial populations play an important role in decomposition and mineralization of organic matter by producing various enzymes [15]. The biochemical functions in soil subsystems are catalyzed by soil enzymes [16,17], and considered to be a bio-indicator of soil fertility because of their involvement in biogeochemical cycling of C, N and P [18,19]. Soil enzymes are derived primarily from microorganisms [20], either as extracellular secretions, and/or products from lysed cells [21,22], which provides an insight into microbial dynamics and activity [23]. The soil microbial activity is an important constituent for ecosystem functioning, in which the interpretation of biological and biochemical trait can be favorable for identifying the impacted ecosystem of coal mine overburden spoil [24]. The enzymatic study revealed important information about the origin, existing nature and catalytic properties of soil enzymes [25]. Soil enzyme activities have been related to soil physico-chemical properties [26,27]; microbial community structure [28,29], vegetation [28,30], disturbance [27,31] and succession [32,33]. Various factors such as pH [34,35], texture, hydrological regime [14,36-38], and plant nutrient status [39], regulate the soil enzyme activity. Relationship between soil enzymatic activities and the successional level of soil microorganisms has been substantiated by many workers [3,23,40-42].

Soil enzymatic studies can be potentially used to monitor and assess soil restoration process of perturbed ecosystem [23,34,42,43]. Bacterial population is the major source of amylase [37], which hydrolyzes starch mainly to dextrans and a small quantity of maltose. Soil invertase hydrolyzes sucrose into -D glucose and β-1 fructose, and serves as an important diagnostic clue to soil functioning [44,45]. Protease is a proteolytic enzyme that hydrolyzes the peptide bonds, which links the amino acids in the polypeptide chain to form a protein structure [46,47]. Proteases are particularly important in C and N cycling, and such activity tends to be regulated by the soil microorganisms. The net

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impact of nutritional regulation of soil protease activity can be observed at the overall microbial community [48-50]. Soil urease is secreted by urolytic microorganisms and root exudates [51-53], which hydrolyzes urea to ammonia and appears to be dependent on the metabolic state of soil microbial population [52,54,55]. Soil acid phosphatase (Orthophosphoric monoester phosphohydrolase), which hydrolyzes orthophosphoric monoester to alcohol and orthophosphate, acts as intermediary enzyme in the transformation of organic phosphate into inorganic forms [56-58]. Dehydrogenases are mainly linked to oxidation-reduction involved in microbial respiratory processes [18,56,59-61]. Being intracellular, the soil dehydrogenase activity is considered as an index of endogenous microbial activity [62,59,37].

In view of the increased mining activities and decreasing soil fertility, it is of utmost concern to monitor the coal mine spoil reclamation over time, which pave the way of greater understanding the direction of improving soil fertility. Since soil enzyme activity is linked with several ecosystem processes and exhibited rapid response to both natural and anthropogenic disturbances, it has been suggested as suitable indicator of soil quality assessment. The comparative assessment of enzyme activities represents the direct expression of soil microbial community to metabolic requirements, and hence will provide information about the linkage between resource availability, microbial community structure and function, and ecosystem processes. Since enzyme activity is linked with several ecosystem processes, it is important to quantify the contribution of different physico-chemical properties affecting the soil enzyme activities. Realizing this, the present study was designed to assess the impact of different soil physico-chemical properties on enzyme activities in a chronosequence coal mine overburden spoil over time, which can be used as indices for reclamation study.

Materials and Methods

Study site

The present study was carried out in the Basundhara (west) open cast colliery in the Ib valley of Mahanadi Coalfields Limited (MCL), Odisha, India (Geographical location: 22°03'58"-20°04'11" north latitude and 83°42'46"- 83°44'45" east longitude). The coal mine overburden spoil have been grouped into six different age series (fresh: OB_0, 2 yr: OB_2, 4 yr: OB_4, 6 yr: OB_6, 8 yr: OB_8 and 10 yr: OB_{10}) on the basis of their formation. Tropical dry deciduous forest was considered to be the natural vegetation of the study site, which experiences a semi-arid climate (1300 mm rainfall y^{-1}, annual average temperature 26°C, relative humidity 15%) with three distinct seasons, i.e. summer, rainy and winter. Table 1 provides the vegetational characteristics of six different mine overburden spoil and nearby forest soil (NF) of the region.

Mine spoil sampling

Sampling was done in accordance with the general methods for soil microbiological study [63]. Sampling was done three times, i.e. summer (April), rainy (July) and winter (January), representing three different seasons during the study period. Each coal mine spoil overburden was divided into 5 blocks, and from each block, five spoil samples were collected randomly from (0-15) cm soil depth by digging pits ($15\times15\times15$ cm^3), referred to as 'sub-samples'. The sub-samples collected from each block of an overburden were thoroughly mixed to form one 'composite sample'. Thus, from each overburden, five composite samples were collected. Similar strategy was followed for different coal mine overburden spoil (OB_0, OB_2, OB_4, OB_6, OB_8 and OB_{10}), as well as nearby native forest soil (NF). The composite samples were homogenized, sieved (0.2 mm) and stored at 4°C until analyzed.

Physico-chemical Characterization

The physico-chemical properties of different age series coal mine overburden spoil, as well as nearby NF soil were analyzed following standard protocols. Soil texture analysis included the estimation of the percentage clay percentage (<0.002 mm), silt (0.06 mm-0.002 mm), and sand (2 mm-0.06 mm). Bulk density of different mine overburden spoil as well as NF was calculated, following the method prescribed in TSBF Handbook [119]. The moisture content and water holding capacity was determined following the protocol proposed by Mishra [64]. Soil pH (1:2.5 ratio of soil: water) was measured with digital pH meter (Make: Systronics, Model: MK VI). Soil organic carbon (SOC) content in mine spoil, as well as NF soil was determined by partial oxidation method [65]. Total nitrogen (TN) was determined using Kjeldahl method [66]. The $NaHCO_3$ extractable phosphorous (EP) in different mine spoil and NF soil was estimated using chloro-stannous reduced molybdophosphoric blue colour method in HCL [67].

Enzyme activities

Amylase activity of different coal mine overburden spoil, as well as nearby NF samples were determined by spectrophotometric method (540 nm), in adaptation to the procedures described by Somogyi [68] and Roberge [69], by taking starch as substrate and incubated at 30°C for 24 hr. Invertase activity was estimated by using sucrose as substrate, incubated at 37°C for 24 hr, and determined by taking absorbance at 540 nm using spectrophotometer [70]. Protease activity was also determined by spectrophotometric method (700 nm), with sodium caseinate as a substrate [71]. Urease activity of different mine overburden soil samples, as well as NF, was determined by titration method using 0.005 NH_2SO_4 with boric acid indicator [72]. The phosphatase activities of different mine overburden spoil, as well as NF samples were determined by spectrophotometric method (400 nm), using *p*-nitrophenyl phosphate as substrate [73]. Dehydrogenase activity was measured by the following reduction of 2,3,5- triphenylotetrazolium chloride (TTC), as an artificial electron acceptor to red-coloured triphenyl formazon (TPF), which were determined spectrophotometrically [23,74].

Statistical analysis

The data obtained from the mine spoil analyses were subjected to simple correlation analysis to test the level of significance between physico-chemical properties and soil enzyme activities among six different age series coal mine overburden spoil, as well as nearby native forest soil samples using SPSS Statistics 17.0 software. Stepwise multiple regression analysis was employed to model the quantitative relationship between different soil enzyme activities and physico-chemical properties using Minitab 16 software. Principal component analysis (PCA) was performed using Statistrix PC DOS Version-2.0 (NH Analytical software).

Results and Discussion

The study of physico-chemical characterization has been emphasized in restoration studies, because soil is one of the primary agents in determining vegetation development [75]. The complex physico-chemical and biochemical changes that occur as a function of mining activities is difficult to monitor the ecological effects and restoration success of mined regions [76]. In this context, a chronosequence of coal mine overburden spoil provides the opportunity to evaluate changes in soil quality over time, representing variable time series of mine overburden spoil undergoing ecological succession under similar conditions. Besides, directional change in soil physico-chemical properties over time, following disturbance indicates soil development

Age of overburden	Vegetation (tree, shrub and herbaceous species)		
	Tree	Shrub	Herb
OB_0	No vegetation.		
OB_2		*Ocimum zyantia* L. *Argemone maxicana* L. *Tephrosia purpurea* L.Pers.	*Evolvulus alsinoides* L. *Cyperus rotundus* L.Pers.
OB_4	*Acacia leucophloea* Roxb. *Acacia catechu* (L.f.) Willd. *Acacia nilotica* (L.) Willd. *Cassia siamea* Lam. *Cassia spectabilis* L. *Emblica officinalis* Gaertn. *Dalbergia latifolia* Roxb.	*Ocimum zyantia* L. *Tephrosia purpurea* L.Pers.	*Eragrostis amabilis* L. *Calatropis procera* R.Br. *Aristida adscensicnis* L.
OB_6	*Acacia leucophloea* Roxb. *Acacia catechu* (L.f.) Willd. *Emblica officinalis* Gaertn. *Acacia nilotica* L. Willd. *Cassia siamea* Lam. *Cassia spectabilis* L. *Dalbergia latifolia* Roxb.	*Ocimum zyantia* L. *Tephrosia purpurea* L.Pers.	*Eragrostis amabilis* L. *Calatropis procera* R.Br. *Aristida adscensicnis* L. *Dactyloctenium acgyptium* L. *Atylosia scarabacoides* L. *Clitoria ternatea* L.
OB_8	*Acacia leucophloea* Roxb. *Cassia spectabilis* L. *Tamarindus indica* L. *Dalbergia latifolia* Roxb. *Acacia nilotica* (L.) Willd. *Cynodon dactylon* L.Pers. *Pongmia pinnata* L.	*Tephrosia purpurea* L.Pers.	*Tridax procumbens* L. *Calatropis procera* R.Br. *Aristida adscensicnis* L. *Dactyloctenium acgyptium* L. *Digitaria setigera* L. *Atylosia scarabacoides* L. *Clitoria ternatea* L. *Desmodium triflorum* L.
OB_{10}	*Acacia leucophloea* Roxb. *Caesalpinia pulcherrima* L. *Madhuca indica* Gmel. *Melia azedarach* L. *Tamarindus indica* L. *Cassia siamea* Lam. *Albizzia lebbeck* Benth. *Cassia spectabilis* L. *Dioscoria bulbifera* L.	*Tephrosia purpurea* L.Pers. *Asparagus racemosus* Willd. *Sida cordifolia* L.	*Eragrostis amabilis* L. *Tridax procumbens* L. *Cyperus rotundus* L. *Cynodon dactylon* L.Pers. *Calatropis procera* R.Br. *Aristida adscensicnis* L. *Dactyloctenium acgyptium* L. *Digitaria setigera* L. *Atylosia scarabacoides* L. *Clitoria ternatea* L. *Desmodium triflorum* L.
NF	*Shorea robusta* Gaertn. *Pterocarpus marsupium* Roxb. *Terminalia tomentosa* DC. *Terminalia belarica* Gaertn. *Terminalia chebula* Gaertn. *Butea monosperma* Lam. *Holarrhena antedysenterica* L. *Diospyros melanoxylon* Roxb.	*Diospyros melanoxylon* Roxb. *Holarrhena antedysenterica* L. *Woodfordia fructicosa* Kurz. *Zizyphus nummularis* Wt.&Arn. *Phoenix sylvestris* L. *Buchanania lanzan* Spreng. *Gardenia turgida* Roxb. *Ixora parviflora* Vahl. *Butea monosperma* Lam. *Phoenix humilis* Royle. *Madhuca indica* Gmel.	*Achyranthus aspera* L. *Andrographis peniculata* Burm. *Heteropogon contortus* L. *Atylosia scarabaeoides* Benth. *Asparagus racemosus* Willd. *Evolvulus numularis* L. *Hemidesmus indicus* R.Br. *Aristida setacea* Retz. *Cyperus rotundus* L.Pres. *Cynodon dactylon* L.Pers. *Tephrosia purpurea* L. Pers. *Indigofera tinctoria* L. *Tridax procumbens* L. *Sida cordifolia* L. *Phaseolus trilobus* L. *Eragrostis amabilis* L.

Table 1: Vegetation pattern in six different coal mine overburden in chronosequence as well as nearby native forest (NF) at the study site.

route [77]. The comparative assessment of physico-chemical properties, as well as soil enzyme activities of different mine overburden spoil would help to quantify and evaluate specific biological processes in due course of time. Soil enzyme diversity among different mine spoil over time was evaluated as differences in activity, which can provide insight into the microbial community response to the changing nutrient resources and the relative importance of different nutrients. As soil enzyme activity is closely associated with the living biomass, it can significantly improve the ability to link microbial function (enzyme activity) with microbial physiology (nutrient stress) and resource availability.

Physico-chemical characterization

The comparative account of different physico-chemical properties of six different coal mine overburden spoil in chronosequence ($OB_0 \rightarrow OB_{10}$), as well as nearby NF soil has been presented (Table 2).

Textural analysis of different age series mine spoil over time revealed considerable variations in soil texture. The data indicated a decline trend in sand percentage from OB_0 (86.8%) to OB_{10} (75.9%). However, the clay fraction showed a reverse trend, i.e. maximum in OB_{10} (11.3%) and minimum in OB_0 (5.4%). Similar textural composition was also exhibited with respect to slit, which varies from 7.8% (OB_0) to 12.8% (OB_{10}). The slit (13.8%) and clay (12.1%) percentage exhibited by the NF soil is found to be higher, as compared to different mine spoil (Table 2). Soil texture affects other soil properties, which in turn determine microbial growth and activity, and hence, reported as a key determinant of microbial ecology. Progressive increase in clay% from OB_0 to OB_{10}

Parameters	Mine spoil collected from different age series coal mine overburdens						Native Forest soil (NF)
	OB_0	OB_2	OB_4	OB_6	OB_8	OB_{10}	
Sand (%)	86.8± 2.1	84.6 ± 1.5	81.4 ± 1.6	79.5 ± 1.1	77.8 ± 1.2	75.9 ± 1.8	74.1 ± 1.2
Silt (%)	7.8 ± 0.6	8.5 ± 0.6	9.9 ± 0.3	10.6 ± 0.8	11.5 ± 0.5	12.8 ± 1.2	13.8 ± 0.6
Clay (%)	5.4 ± 1.2	6.9 ± 0.7	8.7 ± 0.9	9.9 ± 0.6	10.7 ± 0.8	11.3 ± 1.3	12.1 ± 0.5
Bulk Density (g/cm^3)	1.752 ± 0.049	1.605 ± 0.021	1.364 ± 0.019	1.331 ± 0.028	1.294 ± 0.026	1.275 ± 0.014	1.252 ± 0.019
WHC (%)	27.5 ± 1.121	31.3 ± 1.005	36.1 ± 0.984	38.3 ± 0.833	41.2 ± 0.743	43.8 ± 1.413	46.348 ± 0.833
Moisture (%)	6.831 ± 0.103	7.138 ± 0.141	7.422 ± 0.097	7.541 ± 0.143	7.783 ± 0.121	7.955 ± 0.087	11.219 ± 0.132
Soil pH	6.11 ± 0.03	6.24 ± 0.04	6.38 ± 0.02	6.45 ± 0.05	6.62 ± 0.07	6.71 ± 0.06	6.87 ± 0.07
Organic C (mgC/g spoil)	nd	0.151± 0.024	0.779 ± 0.048	1.057 ± 0.127	1.533 ± 0.242	2.004 ± 0.249	3.625 ± 0.25
Total N (µgN/g spoil)	nd	8.514 ± 0.425	45.833 ± 2.248	72.896 ± 4.078	113.555± 3.901	169.830 ± 8.95	2510 ± 28.43
Extractable P (µgP/g spoil)	nd	nd	4.254 ± 1.246	8.063 ± 1.636	11.061 ± 0.764	12.581± 1.411	275 ± 9.97

nd: Not detectable. Values are mean ± SD, calculated from seasonal (summer, rainy, winter) mean values.

Table 2: Physico-chemical properties of mine spoil samples collected from different age series coal mine overburden ($OB_0 \rightarrow OB_{10}$) as well as native forest soil.

may be due to the gradual establishment of vegetation over time [2], or vegetation succession in reclaimed coal mine overburden spoil [78,79]. Root of the vegetational component specifically root exudates in the form of organic acids promotes disintegration of coarse particles to finer clay particles [80,81]. Clay being an important primary particle contributes to the soil structural stability, aggregation [80,82], and developed resistance to the soil erosion with age of overburden [2,83]. However, natural plant succession is very slow in coal mine spoil, but rising of plantations may accelerate this process, leading to a self sustained ecosystem in a relatively short period of time. The variation in vegetation pattern in age series mine overburden (Table 1), may be due to the variation in nutritional status of mine spoil that act as a major factor limiting plant growth [84,85].

The textural composition and particle size distribution is a major factor in governing successful revegetation on reclaimed mine spoil, as it influences BD, WHC, MC and nutrient availability [86]. BD exhibited a decline trend from OB_0 (1.752 g/cm^3) to OB_{10} (1.275 g/cm^3) with age of overburden. The BD in NF soil (1.252 g/cm^3) was observed to be less as compared to OB_{10} (Table 2). Importance of BD lies with the fact that it regulates space, air and water availability to soil microorganisms [87]. A decline in BD can be interpreted as a reduction in the soil compactness because of the development of soil micropore space [88]. According to Ohta and Effendi [88], it is the clay fraction which has an ultimate bearing on the soil BD. An increased level of clay fraction contributes to the development of soil micropore space that reduces the soil BD. In the light of this concept, the gradual accumulation of clay fraction and organic matter input because of the vegetation development led to the development of soil micropore space that ultimately reduced the soil BD. Compared to NF soil, mine spoil have low MC, high BD and low porosity.

However, the WHC showed the reverse trend over time, which varies from 27.5% (OB_0) to 43.8% (OB_{10}). The moisture content also showed the similar trend, i.e. minimum in OB_0 (6.831%) and maximum in OB_{10} (7.955%). The WHC and MC in nearby NF soil were found to be 46.348% and 11.219%, respectively (Table 2). MC exhibited a progressive improvement with age of mine spoil, which agreed with the findings of Dutta and Agrawal [84]. This can be due to the positive influence of the canopy cover on OB_{10}, which prevented the loss of soil water through evaporation by not allowing direct exposure of soil surface to incoming radiation. Across the sites, higher MC in NF soil as compared to different mine spoils is due to dense vegetation cover and gradual supplement of organic matter [75]. Several researchers have reported lower clay %, high soil BD, low WHC, and poor physical conditions of mine spoil [10,81,84,89-91].

The pH level up to 6.5 can lead to an increase in P availability; circumneutral pH can decrease the availability of some micronutrients and shifts microbial composition, especially microrrhizal relationships on which native trees depend. Higher pH can lead to herbaceous competition and often out-compete tree [92]. This suggested that pH should be site-specific, and could be added as an additional criterion for soil classification and mapping. Soil pH in case of all mine spoil samples was estimated to be in acidic range, which varies from 6.11 (OB_0) to 6.71 (OB_{10}). The pH of NF soil was found to be 6.87 (Table 2). Soil reaction 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 explained [1]. Acidification of mine spoil is due to different mineral deposits has also been reported [2,35,92]. Improvement of soil pH due to both passive and active reclamation either by natural succession or by plantation strategy on coal mine spoil has been reported [2,84]. Promotion of organic matter decomposition on degraded soil was reported to lower soil acidity [93].

A wide variation in OC was exhibited, which varies from 0.151 mgC/g spoil (OB_2) to 2.004 mgC/g spoil (OB_{10}). However, the OC in NF (3.625 mgC/g soil) was comparatively higher than different mine spoil (Table 2). Increase in OC was found to be correlated with the increase clay % in ecologically disturbed lands [94]. According to Marshman and Marshall [95], clay acts as an absorption sink for organic material. Increase in the soil organic fraction with the increase in clay can also be due to the fact that organic complexes being absorbed onto the clay surface are being physically protected against decomposition [96], which leads to an accumulation of OC in different mine spoil over time. OC in association with primary soil particles is reported to promote soil aggregate formation, soil structural stability and nutrient retention capacity [97], and hence considered to be the most reliable indicator for monitoring land degradation [98]. There have been reports about the increase in OC, along with the restoration of coal mine spoil [2,31,40,97]. The clay% and OC content was positively correlated ($r=0.894$; $p<0.01$) (Table 4). Establishment of vegetation and gradual input of litter from the vegetation compartment during the course of passive or active restoration have been reported to be the reason for such improvement in OC over time. An increased level of clay% contributes

to the development of soil micropore space from OB_0 to OB_{10}, which ultimately reduced the BD. The negative correlation between BD and OC (r=-0.780; *p<0.05.*) substantiated the concept [2,90].

The TN and EP exhibited improvement in different mine spoil over time. In the present study, the OC, TN and EP content in OB_0 was beyond the detectable limit. The TN ranged from 8.514 µgN/g spoil (OB_2) to 169.83 µgN/g spoil (OB_{10}). Similarly, the EP varies from 4.254 µgP/g spoil (OB_4) to 12.581 µgP/g spoil (OB_{10}). However, the TN and EP content in NF soil was found to be 2510 µgN/g soil and 175 µgP/g soil, respectively (Table 2). The variation on OC with respect to different mine spoil was positively correlated with TN (r=0.856; $p<0.05$) and EP (r=0.848; $p<0.05$) (Table 4). The gradual accumulation of soil nutrients from mine spoil to enriched NF soil may be attributed to the input from the plant species capable of nitrogen fixing potential, as well as development of mycorrhiza and other nutrient immobilizing microbial colonization.

Enzyme activity

Soil enzyme activity indices appeared to be more informative and highly reliable, as it responded more clearly to parent material properties, and hence, considered to be an important indicator of mine spoil genesis over time [6,99]. Further, the relationships between soil organic matter, microbial biomass and activity have been proposed as indicators of soil maturity [100,101]. Comparative analysis on enzyme activities indicated minimal activity in OB_0, which may be due to the reduced microbial population caused by the toxic effects and oxidative stress of mine spoil metal impurities, there interference in osmotic balance and nutrient deficiency [102].

Amylase is complex enzyme belongs to glycoside hydrolase group enzymes [α-amylase (α-1, 4–glucan-4 glucanohydrolase; E.C. No. 3.2.1.1), β-amylase (β-1,4-glucanmaltohydrolase; E.C. 3.2.1.2), glucoamylase (α-1, 4–glucanglucohydrolase; E.C. 3.2.1.3)]. The amylase activity in different age series coal mine spoil showed a range of 1.253 to 4.571 µg glucose/g spoil/hr, with minimum in OB_2 and maximum in OB_{10}. The amylase activity is quite higher in NF soil (13.124 µg glucose/g soil/hr), as compared to different mine spoil (Table 3). Such variation in amylase activity in mine spoil may be due to the variation in available soil nutrients and diversity of microbiota [103,50,46], which is positively correlated with OC (r=0.963; $p<0.01$) (Table 4).

Similarly, the invertase activity (β-fructofuranosidase; E.C. 3.2.1.5) showed progressive increase from OB_2 (6.642 µg sucrose/g spoil/hr) to OB_{10} (348.331 µg glucose/g spoil/hr). The invertase activity in NF soil was estimated to be 849 µg sucrose/g soil/hr (Table 3). The variation in invertase activity in different mine spoil, as well as NF soil is positively correlated with OC (r=0.953; $p<0.01$) (Table 4). The decrease in amylase and invertase activity is attributable mainly to the declination of enzyme synthesis due to the accumulation of heavy metals and associated toxic effects on soil microbes [104,105]. The heavy metals may cause changes in the active center and structure of soil enzymes, thus making the amylase as well as invertase concentration decrease and inhibit the decomposition of starch and sucrose, respectively. Besides, the interaction of heavy metals inhibits the microbial growth [106,107], thus reducing the synthesis, secretion of enzymes, and finally leading to the decrease in amylase and invertase activity.

The protease activity depends on the distribution of proteolytic bacteria and the amount of proteinaceous substrate availability in soil organic matter. The protease activity was comparatively higher in NF soil (215.813 g glucose/g soil/hr), with respect to different mine spoil (Table 3), which may be due to vegetation and the associated difference in litter inputs and root exudation in NF soil ([108]. The progressive increase in protease activity from OB_2 (3.042 g tyrosine/g spoil/hr) to OB_{10} (39.226 µg glucose/g spoil/hr) was found to be closely related to the progressive improvement in OC (r=0.911; $p<0.01$) and TN (r=0.992; $p<0.01$) (Table 4), or NH_4-N accumulation facilitated by vegetation cover in course of time, and distribution of proteolyitc bacteria [109,46,47]. Further, the gradual N accumulation stimulates soil microbes for enhanced production of C-degrading enzymes.

Urease (Urea amidohydrolase; E.C. 3.5.1.5) is mostly an extracellular enzyme, representing up to 63% of the total activity in soil. The 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. Urease activity exhibited progressive increase from 3.354 µg NH_4^+/g soil/hr (OB_2) to 20.121 µg NH_4^+//g soil/hr (OB_{10}) (Table 3). Urease activity in OB_0 was beyond detectable limit, which may be due to the nutrient deficient situation in OB_0. Higher urease activity was exhibited by NF soil (58.541 µg NH_4^+//g soil/hr). The six mine spoil, as well as NF soil, undergo different pedogenic processes, and thus unlikely to have similar urease origin. The variation in urease activity is due to the variation in physico-chemical properties of soil [110], MC [109], organic matter, gradual N accumulation, which is considered as the substrate for soil urease [59,111,112], and synthesis of urease enzyme by increased microbial population [18]. Urease activity exhibited positive correlation with MC (r=0.993; $p<0.01$), OC (r=0.963; $p<0.01$), and TN (r=0.960; $p<0.01$), which indicated that this

Parameters	Mine spoil collected from different age series coal mine overburdens						Native Forest soil (NF)
	OB_0	OB_2	OB_4	OB_6	OB_8	OB_{10}	
Amylase activity (µg glucose/g/hr)	nd	1.253 ± 0. 124	2.034 ± 0.112	2.263± 0.171	3.655 ± 0.279	4.571 ± 0.205	13.124 ± 1.153
Invertase activity (µg sucrose/g/hr)	nd	6.642 ± 0.498	25.228 ± 5.211	83.331 ± 4.781	121.013 ± 7.372	348.331 ± 4.636	849.335 ± 6.389
Protease activity (µg tyrosine/g/hr)	nd	3.042 ± 0.058	8.801 ± 0.534	23.692 ± 1.428	28.437 ± 2.127	39.266 ± 2.574	215.813 ± 12.911
Urease activity (µg NH_4^+/g /hr)	nd	3.354 ± 0.027	5.299 ± 0.121	9.463 ± 0.261	14.317± 1.032	20.121 ± 1.576	58.451 ± 2.834
Phosphatase activity (µg PNP/g /hr)	nd	nd	10.108 ± 1.005	26.495 ± 1.554	35.407 ± 2.901	49.617 ± 2.250	92.118 ± 3.107
Dehydrogenase activity (µg TPF/g /hr)	0.056 ± 0.011	0.144 ± 0.039	0.291 ± 0.034	0.458 ± 0.052	0.948 ± 0.041	1.275 ± 0.043	4.006 ± 0.115

nd: Not detectable. Values are mean ± SD, calculated from seasonal (summer, rainy, winter) mean values.

Table 3: Enzyme activities of mine spoil samples collected from different age series coal mine overburden ($OB_0 \rightarrow OB_{10}$), as well as native forest soil.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16
X1	1															
X2	-0.988**	1														
X3	-0.994**	0.966**	1													
X4	0.938**	-0.880**	-0.965**	1												
X5	-0.999**	0.985**	0.995**	-0.944**	1											
X6	-0.760*	0.832*	0.711	-0.579	0.753	1										
X7	-0.991**	0.995**	0.975**	-0.895**	0.991**	0.813*	1									
X8	-0.930**	0.971**	0.894**	-0.780*	0.925**	0.937**	0.959**	1								
X9	-0.618	0.712	0.561	-0.414	0.610	0.980**	0.685	0.856*	1							
X10	-0.608	0.701	0.550	-0.405	0.599	0.978**	0.674	0.848*	1.000**	1						
X11	-0.810*	0.878**	0.763*	-0.630	0.805*	0.995**	0.862*	0.963**	0.959**	0.954**	1					
X12	-0.790*	0.871*	0.729	-0.571	0.780*	0.972**	0.843*	0.953**	0.943**	0.936**	0.982**	1				
X13	-0.708	0.791*	0.654	-0.507	0.698	0.995**	0.766*	0.911**	0.992**	0.990**	0.984**	0.970**	1			
X14	-0.805*	0.876**	0.755*	-0.610	0.797*	0.993**	0.857*	0.963**	0.960**	0.955**	0.998**	0.989**	0.986**	1		
X15	-0.915**	0.961**	0.875**	-0.742	0.906**	0.935**	0.945**	0.995**	0.860*	0.852*	0.959**	0.961**	0.917**	0.966**	1	
X16	-0.782*	0.859*	0.728	-0.578	0.775*	0.992**	0.840*	0.954**	0.966**	0.962**	0.996**	0.989**	0.989**	0.998**	0.958**	1

** Correlation is significant $p<0.01$ and * correlation is significant $p<0.05$.
Xi (i=1-16) stands for sand, slit, clay, bulk density, water holding capacity, moisture content, pH, OC, TN, EP, amylase, invertase, protease, urease, phosphatase and dehydrogenase activity.

Table 4: Simple correlation coefficient of different soil properties.

enzyme can be used to make some inferences about nitrification (Table 4).

Phosphatase activity (Orthophosphoric monoester phosphohydrolase; E.C. 3.1.3.2) appeared to be more dependent on the metabolic state of soil, biological activity of microbial population, and hence their activity level can be used as an index for microbial activity in soil [57]. Wide variation in phosphatase activity was exhibited with respect to different mine spoil, which ranged from 10.108 g PNP/g spoil/hr (OB_4) to 49.617 µg PNP/g spoil/hr (OB_{10}). The phosphates activity in OB_0 and OB_2 were beyond detectable limit (Table 3). Phosphatase activity showed positive correlation with EP (r=0.852; $p<0.05$) (Table 4).

Estimation of dehydrogenase activity is attractive due to the fact that they are an integral part of soil microorganisms and are involved electron transport system of oxygen metabolism, and requires an intracellular environment (viable cells) to express its activity [113]. Dehydrogenase is considered to be an index of microbial activity [74,114], and metabolic status of soil microorganisms [37,61,103,115]. The data showed consistent increase in dehydrogenase activity from OB_0 (0.056 µg TPF/g spoil/hr) to OB_{10} (1.275 µg TPF/g spoil/hr). Highest dehydrogenase activity was observed in NF soil (4.006 µg TPF/g soil/hr), which may be due to higher organic matter that support increased microbial activity and microbial biomass, and consequently the concentration of dehydrogenase [116]. Dehydrogenase activity exhibited positive correlation with OC (r=0.954; $p<0.01$) (Table 4). Further, the variation in dehydrogenase activity may be attributed to the change in soil microbial community composition with a change in the community of dehydrogenase [117].

The dehydogenase and protease enzymes are independent to each other, indicating soil organic matter transformation and initial breakdown of proteins are self-regulated process. The dehydrogenase activity was positively correlated with protease activity (r=0.989; $p<0.01$), which explained 97.8% variability (Table 4). Initial organic matter transformation by dehydrogenase during the microbial respiration made available substrate to protease, and subsequently higher protease activity was achieved in NF soil. In contrast, the mine spoil ($OB_0 \rightarrow OB_{10}$) may be lacking proteinaceous substrates in its integral part of soil organic matter [47]. It can, therefore, be concluded that the microbial metabolic status of NF soil is comparatively higher as compared to different mine overburden spoil.

Stepwise multiple regression analysis

The stepwise multiple regression analysis was performed to determine the contribution of different soil physico-chemical properties on enzyme activity in different mine spoil over time. The OC explained about 92.7% of the variability in amylase activity. Additional 7% variability in was explained by MC as 2nd variable (Table 5). The TN as 1st variable explained 91.8% of the variability in amylase activity in different mine spoil, as well as NF soil and 2nd variable of importance in explaining the 8% variability was pH ($p<0.001$). Besides, clay fraction explained about 58.2% of the variability, and additional 41.3% by MC as 2nd variable of importance (Table 5). Stepwise multiple regression analysis revealed the relationship between invertase activity in different mine spoil and OC, which explained 90.9% of the variability and an additional 7.4% was accounted by BD as 2nd variable (Table 5). Besides, the TN explained about 88.8% of the variability ($p<0.001$) in invertase activity. Further, 53.1% of the variability was explained by clay fraction, and an additional 45.3% ($p<0.001$) was explained by OC as 2nd variable (Table 5).

Stepwise multiple regression analysis revealed the relationship between protease activity and OC, which explained 83% variability, and an additional 16.8% variability was accounted by EP as 2nd variable (Table 5). Besides, TN explained about 98.4% of the variability ($p<0.001$) in protease activity, and an additional 1.4% by OC as 2nd variable. Further, the clay fraction explained about 42.7% of the variability in protease activity. The 2nd and 3rd variables of importance in explaining the variability were TN and BD ($R^2=0.999$; $p<0.001$) (Table 5).

About 92.7% of the variability in urease activity was explained by OC, and an additional 6.9% ($p<0.001$) was explained by TN as 2nd variable. The TN explained about 92.1% of the variability in urease activity (Table 5). The 2nd and 3rd variable of importance in explaining the variability were slit fraction and BD ($R^2=0.998$; $p<0.001$). Besides, the clay fraction explained about 56.9% of the variability in urease activity, an additional 42.1% was explained by MC as 2nd variable ($p<0.001$), and a marginal effect was contributed by BD as 3rd variable. Further, about 73.4% of the variability in urease activity was explained by pH, an additional 26.2% of variability was explained by TN as 2nd variable, and a marginal effect by BD as 3rd variable. The enzyme activity can be influenced directly by alternation in pH value.

Enzyme activity	Equation(s)	R^2*
Amylase activity	= -0.5643 + 3.37 OC	0.927
	= -15.1584 + 0.89 OC + 2.23 MC	0.997
	= 1.958 + 0.00452 TN	0.918
	= -38.37 + 0.00327 TN + 6.3 pH	0.998
	= -8.853 + 1.367 Clay	0.582
	= -19.598 + 0.201 Clay + 2.702 MC	0.995
Invertase activity	= -104.1 – 236 OC	0.909
	= -1215.7 + 321 OC + 709 BD	0.985
	= 73.609 + 0.315 TN	0.888
	= -654.3 + 93 Clay	0.531
	= 441.3 – 78 Clay + 372 OC	0.984
Protease activity	= -27.5281 + 55.9 OC	0.830
	= -0.6948 + 15.7 OC + 0.581 EP	0.998
	= 11.3733 + 0.082 TN	0.984
	= -0.4019 + 0.0656 TN + 14.2 OC	0.998
	= -145.12 + 20.54 Clay	0.427
	= -27.35 + 4.47 Clay + 0.0754 TN	0.998
	= -181.99 + 10.45 Clay + 0.0727 TN + 71 BD	0.999
Urease activity	= -4.3476 + 15.46 OC	0.927
	= 0.1819 + 8.5 OC + 0.0109 TN	0.996
	= 7.205 + 0.02074 TN	0.921
	= -27.131 + 0.01473 TN + 3.43 Slit + 14.9 BD	0.998
	= -41.7 + 6.2 Clay	0.569
	= -91.41 + 0.8 Clay + 12.5 MC	0.990
	= -181.16 + 5.75 Clay + 11.24 MC + 44.8 BD	0.998
	= -398.68 + 63.94 pH	0.734
	= -172.3 + 28.05 pH + 0.01518 TN	0.996
	= -271.48 + 40.21 pH + 0.01404 TN + 14.772 BD	0.998
Phosphatase activity	= -73.644 + 11.4 Clay	0.757
	= 7.126 – 1.2 Clay + 27.5 OC	0.990
	= -149.561 + 5.9 Clay + 23.1 OC + 68 BD	0.996
	= 19.8 + 0.2692 EP	0.746
	= -77.1 + 0.1181 EP + 9.63 Slit	0.990
	= -155.86 + 0.0927 EP + 12.82 Slit + 32 BD	0.996
	= -252.88 + 0.1065 EP + 6.72 Slit + 86 BD + 9.3 Clay	0.999
	= -693.25 + 112 pH	0.891
	= 87.93 – 14 pH + 28.3 OC	0.990
Dehydrogenase activity	= -0.36384 + 1.0629 OC	0.910
	= -0.01734 + 0.5305 OC + 0.00084 TN	0.994
	= -1.95461 + 0.8176 OC + 0.00061 TN + 1.1754 BD	0.998
	= -15.52356 + 0.2734 OC + 0.00085 TN + 1.5362 BD + 2.1088 pH	0.999
	= -2.829 + 0.514 Clay	0.530
	= 2.123 – 0.355 Clay + 1.68 OC	0.988
	= -1.797 – 0.192 Clay + 0.93 OC + 0.426 MC	0.997
	= -7.26 + 0.053 Clay + 0.61 OC + 0.531 MC + 1.95 BD	0.999

*All R^2- values are significant at $p<0.001$. BD: Bulk density; MC: Moisture content; OC: Organic Carbon; TN: Total Nitrogen; EP: Extractable Phosphorous.

Table 5: Stepwise multiple regression analysis of soil enzymes (amylase, invertase, protease, urease, phosphatase and dehydrogenase) on soil physico-chemical characteristics.

Stepwise multiple regression analysis revealed that the clay fraction explained about 75.7% of the variability in phosphatase activity. The 2nd and 3rd variables of importance in explaining the variability were OC and BD (R^2=0.996; $p<0.001$). Besides, EP explained about 74.6% of the variability in phosphatase activity (Table 5). The 2nd, 3rd and 4th variables of importance in explaining the variability were slit, BD and clay fraction (R^2=0.999; $p<0.001$). Further, pH contributed 89.1% of the variability in phosphatase activity, and an additional 9.9% was contributed by OC as 2nd variable (Table 5).

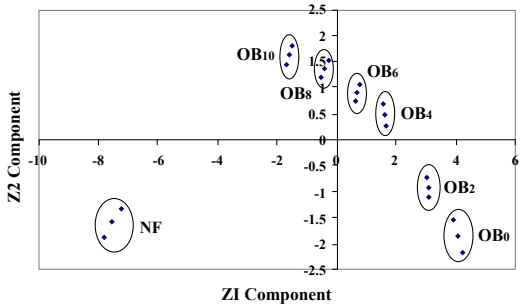

Figure 1: Principal component analysis based on physico-chemical properties and enzyme activities in different mine spoil ($OB_0 \rightarrow OB_{10}$) as well as nearby NF soil.

Stepwise multiple regression analysis suggested that 91% of the variability was explained by OC ($p<0.001$). The 2nd, 3rd and 4th variables of importance in explaining the variability in dehydrogenase activity were TN, BD and a marginal effect by pH respectively (R^2=0.999; $p<0.001$). Further, about 53% of the variability in dehydrogenase activity was explained by clay faction (Table 5). The 2nd, 3rd and 4th variables explaining the variability in dehydrogenase activity were OC, MC and a marginal effect by BD respectively (R^2=0.999; $p<0.001$).

Considering the tropical dry deciduous forest as natural vegetation of the study site, attempt was made to compare the spoil features of different mine overburden spoil in chronosequence ($OB_0 \rightarrow OB_{10}$) over time, and nearby NF soil using principal component analysis [118-120], on the basis of their physico-chemical properties and enzyme activities, in which the Z_1 and Z_2 components accounts for 99% cumulative variance (Figure 1).

Conclusion

The assessment of physico-chemical indices appeared to be more informative to characterize soil fertility, and could be used to guide the selection of appropriate additional reclamation strategies. Comparative study of physico-chemical properties and enzyme activities would provide greater insight into the pathways by which the energy and nutrient flow through the soil food web. Assessment of enzyme activity could significantly increase our understanding of the linkages between resources availability, microbial community structure and function, and ecosystem processes. Stepwise multiple regression analysis was used to quantify the contribution of physico-chemical properties on enzyme activities, which can provide insight how the enzyme activity contributed by soil microorganisms is responding physiologically to the fluctuations in available nutrients in different age series coal mine overburden spoil in course of time.

The goal of ecological rehabilitation is to accelerate natural successional processes to increase productivity, soil fertility and biotic control over biogeochemical fluxes within the recovering ecosystem. In order to increase fertility status of mine spoil, there is the need to improvise OC by use of agricultural wastes, compost and bio-fertilizers. Appropriate amount of both phosphate and potassium may be applied for better soil quality. Besides, a healthy population of soil microorganisms can stabilize the ecological system in soil due to their microbial activity and ability to regenerate nutrients to support plant growth. Any change in their population and activity may affect nutrient cycling as well as

availability of nutrients, which indirectly affect productivity and other soil functions. On the basis of the soil analysis of the study site, the following tree species such as *Acacia auriculiformis*, *Acacia arabica, Eucalyptus species, Cassia siamea, Dalbergia sissoo, Prosopis species, Bamboo* may be planted. Further, a new approach called 'Microbe Assisted Green Technology' (MAGT) is an integrated biotechnological approach serves as model for mine spoil reclamation and development of lush green vegetation on mine overburden spoil. The process is eco-friendly and cost-effective with numerous environmental benefits.

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References

1. Johnson CD, Skousen JG (1995) Mine soil properties of 15 abandoned mine land sites in West Virginia. J Environ Qual 24: 635-643.
2. Jha AK, Singh JS (1991) Spoil characteristics and vegetation development of an age series of mine spoils in a dry tropical environment. Vegetatio 97: 63-76.
3. Mummey DL, Stahl PD, Buyer JS (2002) Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of reclaimed and undisturbed sites. Soil Biol Biochem 34: 1717-1725.
4. Agrawal M, Singh J, Jha AK, Singh JS (1993) Coal-based environmental problems in a low rainfall tropical region. Lewis Publishers, Boca Raton, USA 27-57.
5. Ciolkosz EJ, Cronce RC, Cunningham RL, Peterson GW (1985) Characteristics, genesis, and classification of Pennsylvania mine soils. Soil Science 139: 232-238.
6. Roberts JA, Daniels WL, Bell JC, Burger JA (1988) Early stages of mine soil genesis as affected by top soiling and organic amendments. Soil Sci Soc Am J 52: 730-738.
7. Mc Sweeney K, Jansen IJ (1984) Soil structure and associated rooting behavior in mine soils. Soil Sci Soc Am J 48: 607–612.
8. Visser S, Zak J, Parkinson D (1979) Effects of surface mining on soil microbial communities and processes. Pregamon Press, NewYork, USA.
9. Schafer WM (1984) Mine-soil restoration and maturity: a guide for managing mine-soil development. Proc Symp Surface Coal Mining and Reclamation. Great Plains 172-185.
10. Nath A (2004) Ecosystem approach for rehabilitation of coal mine areas. Proceedings of the National Seminar on Environmental Engineering with special emphasis on Mining Environment, NSEEME-2004, India.
11. Juwarkar AA, Jambulkar HP, Singh SK (2004) Appropriate strategies for reclamation and revegetation of coal mine spoil dumps. Proceedings of the National Seminar on Environmental Engineering with special emphasis on Mining Environment, NSEEME-2004, India.
12. Visser S, Griffiths CL, Parkinson GD (1983) Effects of surface mining on the microbiology of a prairie site in Alberta, Canada. Can J Soil Sci 63: 177-189.
13. Lindemann WC, Lindsey DL, Fresquez PR (1984) Amendment of mine spoil to increase the number and activity of microorganisms. Soil Sci Soc Am J 48: 574-578.
14. Gildon A, Rimmer DL (1993) Soil respiration on reclaimed coil-mine spoil. Biol Fertil Soils 16: 41-44.
15. Burns RG (1982) Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol Biochem 14: 423-427.
16. Burns RG (1983) Extracellular enzyme-substrate interactions in soil. In: Microbes in their natural environment, Cambridge University Press, Cambridge, UK 249-298.
17. Sinsabaugh RL, Antibus RK, Linkins AE (1991) An enzyme approach to the analysis of microbial activity during plant litter decomposition. Agric Ecosyst Environ 34: 43-54.
18. Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31: 1471-1479.
19. Schoenholtz SH, Van Miegroet H, Burger JA (2000) A review of chemical and physical properties as indicators of forest soil quality: Challenges and opportunities. For Ecol Manag 138: 335-356.
20. Ladd JN (1978) Origin and range of enzymes in soil. Academic Press, New York, USA 51-96.
21. Tabatabai MA (1994) Soil enzymes. In: Methods of soil analysis. Soil Sci Am, Medison, USA 775-826.
22. Gianfreda L, Bollag JM (1996) Influence of natural and anthropogenic factors on enzyme activities. Soil Biochem 9: 123-193.
23. Nannipieri P, Greco S, Ceccanti B (1990) Ecological significance of the biological activity in soil. Soil Biochem. 6: 293-355.
24. Harris JA (2003) Measurments of the soil microbial community for estimating the success of restoration. Eur J Soil Sci 54: 801-808.
25. Perez Mateos M, Gonzales Carcedo J (1987) Effect of fractionation on the enzymatic state and behavior of enzyme activities in different structural soil units. Biol Fertil Soils 4: 151-154.
26. Amador JA, Glucksman AM, Lyons JB, Gorres JH (1997) Spatial distribution of soil phosphatase activity within a riparian forest. Soil Science 162: 808-825.
27. Kujur M, Gartia SK, Patel AK (2012) Quantifying the contribution of different soil properties on enzyme activities in dry tropical ecosystems. ARPN Journal of Agriculture and Biological Science 7: 763-772.
28. Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32: 1837-1846.
29. Kourtev PS, Ehrenfeld JG, Häggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83: 3152-3166.
30. Sinsabaugh RL, Carreiro MM, Repert DA (2002) Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60: 1-24.
31. García C, Hernández T (1997) Biological and biochemical indicators in derelict soils subject to erosion. Soil Biol Biochem 29: 171-177.
32. Tscherko D, Rustemeier J, Richter A, Wanek W, Kandeler E (2003) Functional diversity of the soil microflora in the primary succession across two glacier forelands in the Central Alps. Eur J Soil Sci 54: 685-696.
33. Baldrian P, Trogl J, Frouz J, Snajdr J, Valaskova V, et al. (2008) Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal mining. Soil Biol Biochem 40: 2107-2115.
34. McCarty GW, Siddaramappa R, Wright RJ, Codling EE, Gao G (1994) Evaluation of coal combustion byproducts as soil limiting materials: their influence on soil pH and enzyme activities. Biol Fertil Soils 17: 147-172.
35. Schinner F, Sonnleitner R (1997) Bodenokologie: Mikrobiologie and Bodenenzymatik, Teil IV Anorganische Schadstoffe. Springer Verlag, Berlin, Germany.
36. Tscherko D, Kandeler E (1999) Classification and monitoring of soil microbial biomass, N-mineralization and enzyme activities to indicate environmental changes. Die Bodeakultur 50: 215-226.
37. Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers and enzymatic activities in surface soils and sub-soils using various techniques. Soil Biol Biochem 34: 387-401.
38. Ebersberger D, Niklaus PA, Kandeler E (2003) Long term CO2 enrichment stimulates N-mineralization and enzyme activities in calcareous grassland. Soil Biol Biochem 35: 965-972.
39. Adriano DC, Page AL, Chang EAC, Straughn I (1980) Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: A review. J Environ Qual 9: 333-344.
40. Srivastava SC, Jha AK, Singh JS (1989) Changes with time in soil biomass C, N and P of mine spoils in a dry tropical environment. Can J Soil Sci 69: 849-855.
41. Dick RP (1994) Soil enzyme activities as indicators of soil quality. Soil Sci Soc Am Inc, Madison, WI.
42. Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Biological indicator if soil health 121-156.

43. Bentham H, Harris JA, Birch P, Shorl KC (1992) Habitat classification and soil restoration assessment using analysis of soil microbiological and physico-chemical characteristics. J Appl Ecol 29: 711-718.

44. Tabatabai MA (1982) Soil enzymes. In: Methods of Soil Analysis, Part 2, Chemical, Microbiological Properties, Soil Sci Soc Am Inc, Madison, WI 903-948.

45. Shi JZ, Lu Y, Xu ZG, Fu SL (2008) Enzyme activities of urban soils under different land use in the Shenzhen city, china. Plant Soil Environ 54: 341-346.

46. Anjaneyulu E, Ramgopal M, Narasimha G, Balaji M (2011) Effect of pig iron slag particles on soil physico–chemical, biological and enzyme activities. Iranica Journal of Energy and Environment 2: 161-165.

47. Subrahmanyam G, Archana G, Chamyal LS (2011) Soil microbial activity and its relation to soil indigenous properties in semi–arid alluvial and estuarine soils of Mahi river basin, Western India. Int J Soil Sci 6: 224-237.

48. Sims GK, Wander MM (2002) Proteolytic activity under nitrogen or sulfur limitation. Appl Soil Ecol 19: 217-221.

49. Sims GK (2006) Nitrogen starvation promotes biodegradation of N-heterocyclic compounds in soil. Soil Biol Biochem 38: 2478-2480.

50. Rahmansyah M, Sudiana IM (2010) Soil microbial enzymatic activity relate to role of methanotrophic bacteria in tropical forest soil, Gunug Salak National Park. ARPN Journal of Agriculture and Biological Science 5: 51-57.

51. Dkhar MS, Mishra RR (1983) Dehydrogenase and urease activities in maize (Zea mays L.) field soils. Plant Soil 70: 327-333.

52. Palma RM, Conti ME (1990) Urease activity in Argentina soils: Field studies and influence of sample treatment. Soil Biol Biochem 22: 105-108.

53. Holland MA, Polacco JC (1992) Urease-null and hydrogenase–Null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. Plant Physiol 98: 942-948.

54. Xiaobin W, Jingfeng X, Grant CA, Bailey LD (1995) Effects of placement of urea with a urease inhibitor on seedling emergence, N uptake and dry matter yield of wheat. Can J Plant Sci75: 449-452.

55. Klose S, Tabatabai MA (1999) Urease activity of microbial biomass in soils. Soil Biol Biochem 31: 205-211.

56. Harris JA, Birch P (1989) Soil microbial activity in opencast coal mine restoration. Soil Use and Management 5: 155-160.

57. Kramer S, Green DM (2000) Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semi-arid woodland. Soil Biol Biochem 32: 179-188.

58. Drouillon M, Merckx R (2005) Performance of para-nitrophenyl phosphate and 4– methylumbelliferyl phosphate as substrate analogues for phosphomonoesterase in soil with different organic matter content. Soil Biol Biochem 37: 1527-1534.

59. Garcia C, Hernandez T, Costa F, Ceccanti B, Masciandaro G (1993) The dehydrogenase activity of soil as an ecological marker in processes of perturbed system regeneration. In: Proceedings of the XI International Symposium of Environmental Biogechemistry, Salamanca, Spain.

60. Garcia C, Hernandez T, Costa F, Ceccenti B, Masciandaro G (1993) Kinetics of phosphatase activity in organic wastes. Soil Biol Biochem 25: 561-565.

61. Caldwell BA (2005) Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49: 637-644.

62. Stroo HF, Jencks EM (1982) Enzyme activity and soil respiration in mine soils. Soil Sci Soc Am J 46: 548-553.

63. Parkinson D, Gray TRG, Williams ST (1971) Methods to study ecology of soil microorganisms. IBP Handbook No. 19, Blackwell Scientific Publ. Oxford, UK.

64. Mishra R (1968) Ecology Work Book. Oxford IBH, New Delhi, India.

65. Walkly A, Black LA (1934) An examination of the Degtjareff method for determining organic carbon in soil: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci 63: 251-263.

66. Jackson ML (1958) Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, NJ, USA 485.

67. Olsen SR, Sommers LE (1982) Phosphorous. In: Methods of soil analysis, Part 2, Miller RH, Keeney DR (Eds), American Soc of Agro Inc, Madison, WI.

68. Somogyi M (1952) Notes on sugar determination. J Biol Chem 195: 19-23.

69. Roberge MR (1978) Methodology of soil enzyme measurement and extraction. In: Soil Enzymes, Burns RG (Eds.), Academic Press, London, UK.

70. Ross DJ (1983) Invertase and amylase activities as influenced by clay minerals, soil clay fractions and topsoil under grassland. Soil Biol Biochem 15: 287-293.

71. Ladd JN, Butler JHA (1972) Short term assays of soil proteolytic enzymes activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem 4: 19-30.

72. Tabatabai MA, Bermner JM (1972) Assay of urease activity in soils. Soil Biol Biochem 4: 479-487.

73. Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1: 301-307.

74. Alef K, Nannipieri P (1995) Methods in applied soil microbiology and biochemistry. Academic Press, London, UK.

75. Singh AN, Raghubanshi AS, Singh JS (2004) Impact of native tree plantations on mine spoil in a dry tropical environment. Forest Ecol Manag 187: 49-60.

76. Lewis DE, White JR, Wafula D, Athar R, Tamar DT, et al. (2010) Soil functional diversity analysis of a Bauxite mined restoration chronosequence. Microbial Ecol 59: 710-723.

77. Aarde Van RJ, Smit AM, Claassens AS (1998) Soil characteristics of rehabilitating and unmined coastal Dunes at Richard Bay, KwaZulu-Natal, South Africa. Restoration Ecology 6: 102-110.

78. Heras MM, Nicolau JM, Espigares T (2008) Vegetation succession in reclaimed coal-mining slopes in a Mediterranean-dry environment. Environmental Engg 34: 168-178.

79. Mukhopadhyay S, Maiti SK (2011) Minespoil reclamation due to tree plantation: A chronosequence study. African J Basic Appl Sci 3: 210-218.

80. Vimmerstedt JP, House MC, Larson MM, Kasile JD, Bishop BL (1989) Nitrogen and carbon accretion on Ohio coal mine soils: influence of soil forming factors. Landscape Urban Plan 17: 99-111.

81. Banerjee SK, Das PK, Mishra TK (2000) Microbial and nutritional characteristics of coal mine overburden spoils in relation to vegetation development. J Indian Soc Soil Sci 48: 63-6.

82. Gregorich EG, Voroney RP, Kachanoski RG (1991) Turnover of carbon through microbial biomass in soils with different textures. Soil Biol Biochem 23: 799-805.

83. Parr JF, Papendick RI (1997) Soil quality: Relationship and strategies for sustainable dryland farming systems. Annals of Arid Zones 36: 181-191.

84. Dutta RK, Agarwal M (2002) Effect of tree plantations on the soil characteristics and microbial activity of coal mine spoil land. Trop Ecol 43: 315-324.

85. Ekka NJ, Behera N (2011) Species composition and diversity of vegetation developing on an age series coal mine spoil in an open cast coalfield in Orissa, India. Trop Ecol 52: 337-343.

86. Brady NC, Weil RR (2007) The nature and properties of soils. (13th Ed.) Pearson education Inc, USA.

87. Foissner W (1992) Comparative studies on the soil life in eco-farmed and conventionally farmed fields and grassland of Austria. Agril Ecosys and Environ, Elsevier Science Publishers, Amsterdam, the Netherlands 40: 207-218.

88. Ohta S, Effendi S (1992) Ultisol of lowland Dipterocarp forest in east Kalimantan, Indonesia. Soil Sci Pl Nutri 38: 197-206.

89. Srivastava SC (1999) Effect of coal mining on microbial biomass and nutrient availability in dry tropical forest of Vindhyan hill region. J Trop Forestry 15: 15-23.

90. Juwarkar AA, Yadav SK, Thawale PR, Kumar P, Singh SK, et al. (2009) Developmental strategies for sustainable ecosystem on mine spoil dumps: a case of study. Environ Monit Assess 157: 471-481.

91. Gudadhe SK, Ramteke DS (2012) Impact of plantation on coal mine spoil characteristic. International J Life Sc Biotech Pharma Res 1: 84-92.

92. Burger JA, Zipper CE (2002) How to restore forests on surface mined land. Powell river project, Virginia cooperative extension, USA 1-21.

93. Sahani U, Behera N (2001) Impact of deforestration on soil physico-chemical

characteristics, microbial biomass and microbial activity on tropical soil. Land Degrad Develop 12: 93-105.

94. Roberts RD, Marrs RH, Skeffington RA, Bradshaw AD (1981) Ecosystem development on naturally colonized china clay wastes. I. vegetation changes and overall accumulation of organic matter and nutrient. J Ecol 69: 153-161.

95. Marshman NA, Marshall KC (1981) Bacterial growth on proteins in the presence of clay materials. Soil Biol Biochem 13: 127-134.

96. Vanveen JA, Kuikman PJ (1990) Soil structural aspects of decomposition of organic matter by microorganisms. Biogeochem 11: 213-233.

97. Garcia C, Hernandez T, Costa F, Barahona A (1996) Organic matter characteristics and nutrient content in eroded soils. Environ Manag 20: 131-141.

98. Rajan K, Natarajan A, Anil Kumar KS, Badrinath MS, Gowda RC (2010) Soil organic carbon- the most reliable indicator for monitoring land degradation by soil erosion. Curr Sci 99: 823-827.

99. Machulla G, Burns MA, Scow KM (2005) Microbial properties of mine spoil materials in the initial stages of soil development. Soil Sci Soc Am J 69: 1069-1077.

100. Insam H, Domsch KH (1988) Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microbial Ecol 15: 177-188.

101. Anderson TH, Domsch KH (1990) Application of ecophysiological Quotients (qCO2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biol Biochem 22: 251-255.

102. Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fertil Soils 19: 269-279.

103. Pascule JA, Hernanzed T, Garcia C, Ayuso M (1998) Enzymatic activities in an arid soil amended with urban organic wastes: laboratory experiment. Bioresource Tech 64: 131-138.

104. Speir TV, Kettles HA, Parshotam A, Searle PL, Vlaar LNC (1995) A simple kinetic approach to derive the ecological dose value ED 50, for the assessment of Cr (VI) toxicity to soil biological properties. Soil Biol Biochem 27: 801-811.

105. Lee IS, Kim OK, Chang YY, Bae B, Kim HH, et al. (2002) Heavy metal concentrations and enzyme activities in soil from a contaminated Korean shooting range. J Biosci Bioengg 94: 406-411.

106. Zhi-xin Y, Shu-qing L, Da-wei Z, Sheng-dong F (2006) Effects of cadmium zinc and lead on soil enzyme activities. J Environ Sci 18: 1135-1141.

107. Gao Y, Zhou P, Mao L, Zhi Y, Zhang C, et al. (2009) Effect of plant species coexistence on soil enzyme activities and soil microbial community structure under Cd and Pb combined pollution. J Environ Sci (China) 22: 1040-1048.

108. Stone MM, Weiss MS, Goodale CL, Adams MB, Fernandez IJ, et al. (2012) Temperature sensitivity of soil enzyme kinetics under N- fertilization in two temperate forests. Global Change Biol 18: 1173-1184.

109. Sardans J, Peñuelas J, Estiarte M (2008) Changes in soil enzymes related to C and N cycle in soil C and N content under prolonged warming and drought in a Mediterranean Shrubland. Appl Soil Ecol 39: 223-235.

110. Dick WA, Tabatabai MA (1992) Potential uses of soil enzymes. In: Soil microbial ecology: Application in agricultural and environmental management. Marcel Dekker, New York, USA.

111. Kizilkaya R, Bayrakli B (2005) Effect of N enriched sewage sludge on soil enzyme activities. Appl Soil Ecol 30: 192-202.

112. Kizilkaya R, Ekberli I (2008) Determination of the effects of hazelnut husk and tea waste treatments on urease enzyme activity and its kinetics in soil. Turk J Agric For 32: 299-310.

113. Kandeler E, Dick R (2007) Soil enzymes: Spatial distribution and function in Agroecosystems. Taylor and Francis group, Boca Raton, usa.

114. Dick RP, Sandor JA, Eash NS (1994) Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agric Ecosyst Environ 50: 123-131.

115. Beyer L, Wackendorf C, Balzen FM, Balzer-Graf VR (1992) The effect of soil texture and soil management on microbial biomass and soil enzyme activities in arable soils of Northwest Germany. Agrobiol Res 45: 276.

116. Cooper JM, Warman PR (1997) Effects of three fertility amendments on soil dehydrogenase activity, organic C and pH. Can J Soil Sci 77: 281-283.

117. Masciandaro G, Ceccanti B, Ronchi V, Bauer C (2000) Kinetics parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilizers. Biol Fertil Soils 32: 479-483.

118. Ludwig JA (1988) Statistical Ecology: A primer in method and computing. John Wiley and Sons, USA.

119. Anderson JM, Ingram JSI (1992) Tropical soil biology and fertility. A handbook of methods. (2nd Edn), Oxford University Press, USA.

120. Appiah MR, Thomas RL (1982) Inositol phosphate and organic phosphorous content and phosphatase activity of some Canadian and Ghanain soils. Can J Soil Sci 62: 31-38.