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QUANTIFYING THE CONTRIBUTION OF DIFFERENT SOIL PROPERTIES ON ENZYME ACTIVITIES IN DRY TROPICAL ECOSYSTEMS

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ABSTRACT

Microbial activity is significantly influenced by soil texture, hydrological regimes, flow dynamics, chemical pollutants, and an assessment of these changes is essential for soil management. In the present investigation, soil microbial activity (as an index of soil enzymes i.e. amylase, invertase, protease and dehydrogenase) and its relationship with different physico-chemical properties with respect to seven different soils has been addressed. The variation of soil enzyme activity in question was significantly attributable to differences in soil texture, C, N and P content, bulk density, water holding capacity, moisture content and soil pH. Comparative analysis of soil enzyme revealed that there was gradual increase in amylase, invertase, protaease and dehydrogeanse activity from a nutrient deficient situation (fresh mine spoil) to an enriched soil (forest soil). Stepwise multiple regression analysis was performed to determine the contribution of different factors influencing the variability in enzyme activity. Amylase and invertase activity in seven soil samples indicated positive direct correlations with OC, TN, clay content, and negative correlation with bulk density. On the contrary, protease activity showed poor correlation with clay content, WHC and MC, but significant positive correlation was noticed with OC, TN and AP content. The findings demonstrated that soil OC, TN, AP and clay content are the important determinants for dehydrogenase activity (indicative for organic matter transformation) and moderate correlation with soil pH, MC and WHC. The dehydrogenase activity showed a positive correlation with protease activity ($r = 0.994$; $p<0.01$), which explained 98.8% of the variability in protease activity. Principal component analysis was able to discriminate seven different soils into independent clusters on basis of their soil physico-chemical properties and enzyme activities. However, the change in soil enzyme activity correlated very well with the extent of land degradation and can serve as a useful indicator of soil status.

Keywords: amylase, invertase, protease, dehydrogenase, soil.

INTRODUCTION

Soil is an important component of all terrestrial ecosystems as well as a main source of production in agriculture and forestry. Soil is a dynamic system in which continuous interaction takes place between soil minerals, organic matter and organisms that influences the physico-chemical and biological properties of terrestrial systems. Its function is essential for the maintenance of biogeochemical cycles of all nutrients and affects other components of ecosystems, both biotic and abiotic. However, it would be difficult to establish a single biological or chemical measurement that could adequately reflect soil quality without taking into consideration the factors affecting the formation of a given soil (Doran and Parkin, 1994). Due to the complexity of soil structure and function, a good soil quality indicator to understand the soil functioning must be integrative combining a number of measurements into an easily understood and quantitative measure.

Enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in the soil. Soil enzymes are believed to be primarily of microbial origin but also originate from plants and animals (Tabatabai, 1994). They are usually associated with viable proliferating cells, but enzymes can be extracted from both living and dead cells (Tabatabai, 1994). Soil enzymes are considered to be indicative measures of soil fertility (Zahir *et al*., 2001) and bioremediation activities (Margesin *et al*., 2000) due to the fact that they participate in elemental cycling, decomposition of organic matters, rapid response to changes in soil management and hence are considered fundamentally good indicators for soil quality (Dick, 1994; Dick *et al*., 1996; Venkatesan and Senthurpandian, 2006; Kizilkaya *et al*., 2007; Abdalla and langer, 2009). Many studies have also suggested that soil enzymes can be used as indices of soil contamination, soil fertility and soil health (Masciandaro *et al*., 1998; Saviozzi *et al*., 2001). Soil enzyme activity is variable with substrate supply (Degens, 1998; Tateno, 1988); provide useful linkage between microbial community composition and carbon processing (Waldrop *et al*., 2000) and sensitive indicators to detect the changes occurring in soils (Gonzalez *et al*., 2007).

Criteria for choosing enzyme activities as biomarker to assess soil quality is based on their sensitivity to soil management practices, importance in nutrient cycling, organic matter decomposition and bioremediation activities. The starch hydrolyzing enzymes such as amylase and invertase were chosen for their critical role in releasing low molecular weight sugars that are important energy sources for microorganisms (Bandick and Dick, 1999; Shi *et al*., 2008; Rahmansyah and Sudiana, 2010). Soil proteases are extra-cellular enzymes produced mainly by bacteria, which degrade proteins,

release NH_4-N and are very important in nitrogen cycle (Sardans *et al*., 2008). Initial breakdown of proteins from the soil organic matter is virtually mediated by soil proteases (Anjaneyulu *et al*., 2011; Subrahmanyan *et al*., 2011). Thus, the estimation of proteases provides information on nitrogen mediated biochemical processes in soil (Sardans and Penuelas, 2005; Tischer, 2005). Dehydrogenase activity is directly linked with living cells associated with microbial oxido-reduction processes (Alef and Nannipieri, 1995; Stepniewska *et al*., 2007), important for organic matter degradation and transformation. Since, dehydrogenase activity is not active as extracellular enzymes in soil, it is considered to be a good indicator of overall microbial activity (Garcia *et al*., 1997; Pascual *et al*., 1998; Taylor *et al*., 2002; Quichano and Maranon, 2002).

In view of the increased mining activities, urbanization, environmental pollution, decreasing soil fertility and adverse effects on soil microorganisms, it is of utmost concern to study the drastically altered soil properties and function, which pave the way for greater understanding the direction of improving the soil fertility. An attempt was made in the study with an aim to provide a comparative account on the variations in different soil physico-chemical as well as activities of different soil enzymes such as amylase, invertase, protease and dehydrogenase in seven different soil samples exposed to different anthropogenic disturbances. Since enzyme activity is linked with several ecosystem processes including soil formation, organic matter transformation and bioremediation activities, it is important to understand the different physico-chemical factors affecting the enzyme activities. Realizing this, the present study was initiated to assess the impact of different soil parameters on enzyme activity, and to illustrate if soil enzyme activities can be used as indices for soil fertility and health.

MATERIALS AND METHODS

Study site

The study was carried out in sponge iron mines in Noamundi (85° 30' 33.61" east longitude and 22° 9' 49.96" north latitude), maintained by Tata Iron Steel Corporation limited (TISCO), which is located in the revenue district of West Singhbhum, Jharkhand, India. The study site is situated away from the mean sea level i.e. about 540m altitude. The study site is surrounded by a number of new, old and abandoned mines of iron ore overburden. Tropical dry deciduous forest is considered to be the natural vegetation of the area, but rapid development of transportation network and industrialization led to the decline of forest cover mainly due to the felling and biotic interferences. Mean annual temperature and humidity is around 19.67°C and 20%, respectively.

Sampling and analysis

Sampling was done in accordance with the general methods for soil microbiological study (Parkinson *et al*., 1971). Seven different sites [fresh mine spoil (FMS); 6yr old mine spoil (MS); degraded waste land soil (DWS); grassland soil (GS); pesticide-treated soil (PAS); agricultural soil (AS) and forest soil (FS)] were selected for sampling near Noamundi. Each site was divided into 3 blocks, and during each sampling five soil samples were collected randomly from 0-15 cm soil depth by digging pits of (15 x 15 x 15) cm^3 size in each block. These samples collected from each block were referred to as sub-samples, which were brought to the laboratory in sterilized polythene packets and thoroughly mixed to form one composite sample. Thus, during each sampling, three composite samples were obtained from each site. Similar strategy has been followed for soil sampling from different study sites in the month of August. The composite samples were subjected to sieving (2mm mesh size) for characterization.

Soil physico-chemical properties were analyzed following standard protocols. Soil texture analysis included the estimation of the percentage of clay (< 0.002mm), silt (0.06mm - 0.002mm) and sand (2mm - 0.06mm). Bulk density of the mine spoil was calculated following the method prescribed in TSBF Handbook (Anderson and Ingram, 1992). The moisture content and water holding capacity was determined following the protocol proposed by Mishra (1968). Soil pH (1:2.5 ratio of soil: water) was measured with digital pH meter (Make: Systronics, Model: MK VI). Soil organic carbon (SOC) was determined by partial oxidation method (Walkley and Black, 1934). Total nitrogen content determined using of the Kjeldahl methods (Jackson, 1958). The available phosphorous content in soil sample was estimated using chloro-stannous reduced molybdophosphoric blue colour method in HCL (Olsen and Sommers, 1982).

Amylase activity of the different soil samples were determined in adaptation to the procedures described by Somogyi (1952) and Roberge (1978) by taking starch as substrate and incubated at 30°C for 24hr. Invertase activity was determined by spectrophotometric method (Ross, 1983) by using sucrose as substrate and incubated at 37°C for 24hr. Protease activity was determined by spectrophotometric method (Ladd and Butler, 1972) with sodium caseinate as a substrate. 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 (Nannipieri *et al*., 1990; Alef and Nannipieri, 1995).

Statistical analysis

The data from soil analyses were subjected to simple correlation analysis to test the statistical significance of soil physico-chemical properties and soil enzyme activities between seven soil samples using SPSS Statistics 17.0 software. Stepwise multiple regression analysis was employed to model the quantitative relationship between an enzyme activity and soil physico-chemical properties using Minitab 16 software. Principal

components analysis (PCA) was performed using Statistrix PC DOS Version-2.0 (NH Analytical software).

RESULTS AND DISCUSSIONS

Physico - chemical characterization

A comparative account of soil textural characteristic of soil samples collected from different study sites (FMS, MS, DWS, GS, PTS, AS and FS) has been represented in Table-1. Textural analysis of soil samples indicated a decline trend in sand percentage from FMS (87.8%) to FS (72.5%). However, the clay fraction showed the reverse trend i.e. maximum in FS (13.3%) and minimum in FS (4.4%). The clay percentage in MS (6.7%) is higher as compared to FMS. The GS (9.7%) also exhibited higher clay percentage as compared to DWS (8.9%). Similar textural property was also exhibited with respect to slit content i.e., maximum in FS (14.2%) and minimum in FMS (7.8%).

Data relating to the bulk density of different spoil samples suggested a gradual decline trend from FMS (1.852 g/cm^3) to FS (1.259 g/cm^3). However, the water holding capacity (WHC) showed the reverse trend as compared to bulk density, which ranges from 24.501% (FMS) to 46.648% (FS). It is evident from the data that there is gradual improvement in water holding capacity from a degraded (FMS) to an enriched ecosystem (FS). The moisture content also showed the similar trend i.e., minimum in FMS (6.643%) and maximum in FS (11.329%). Soil pH of all the sites was observed to be in the slightly acidic range (6.1 to 6.8). The general observation on soil pH indicated the gradual progress of soil pH towards the neutral range i.e. from FMS to FS (Table-1).

A wide variation in organic carbon (OC) content was exhibited with respect to different soil types, which varies from 0.174% (FMS) to 2.469% (FS) i.e., minimum in FMS and maximum in FS (Table-1). The OC content in MS (0.307%) was higher as compared to FMS (0.174%). It was further observed that there was gradual increase in organic carbon content from a nutrient deficient situation (FMS) to an enriched soil (FS).

The total nitrogen (TN) content also showed the similar trend i.e. progressive increase from FMS (0.004%) to FS (0.202%) (Table-1). It was further evident from the data that the TN content in FS (0.202%) was comparatively higher as compared to GS (0.061%), PTS (0.087%) and AS (0.116%).

The available phosphorous (AP) content in different soil types ranges from 70.445 µg/g soil (FMS) to 1091.509 µg/g soil (FS). Further, the data suggested that the available phosphorous content in FS was expectedly higher as compared to FMS (Table-1).

The present finding demonstrated considerable variations in soil texture with respect to seven different soils. Soil texture affects other soil properties, which in turn determine microbial growth and activity (Ladd *et al*., 1996), and hence reported as a key determinant of microbial ecology. The clay percentage showed progressive increase from FMS to FS (Table-1). Gradual establishment of the vegetation cover can be one of the reasons for the increase in clay formation (Jha and Singh, 1991). Clay being an important primary particle contributes to the soil structural stability and aggregation (Van Veen *et al*., 1985; Gregorich *et al*., 1991; Vimmerstedt *et al*., 1989).

Importance of bulk density lies with the fact that it regulates space, air and water availability to soil organisms. A decline in bulk density from FMS to FS can be interpreted as a reduction in soil compactness because of the development of soil micropore space (Ohta and Effendi, 1992). The water holding capacity and moisture content showed an increasing trend from FMS to FS. Reduced soil water loss by the canopy shading due to vegetation has also been reported by Lal (1989). Several researchers also reported lower clay percentage, high bulk density, low water holding capacity, low moisture content and poor physical conditions of FMS (Jha and Singh, 1991; Singh and Singh, 2006; Juwarkar *et al*., 2009). In general, the fine-textured soils have more micropores as compared to sandy soils. It is evident from the data that there is gradual improvement in clay percentage, water holding capacity, moisture content from a degraded to an enriched ecosystem. Across the study sites, the higher moisture content in FS as compared to remaining sites. This situation arises because of dense vegetation cover in FS and addition of more organic matter resulting in higher moisture content retained (Singh *et al*., 2010). The reason of decreased moisture level in DWS and GS may be due to decreased in organic mater and exposed surface soil, which promote drying.

Soil reaction as recorded through the measurement of pH was noted to be in acidic range (6.14 to 6.83). Acidification in FMS and MS due to different mineral deposits has also been reported (Jha and Singh, 1991). Promotion of organic matter decomposition in degraded soil also has been reported to lower soil acidity (Sahani and Behera, 2001).

According to Marshman and Marshall (1981), clay acts as an absorption sink for organic matter. Increase in organic carbon in FS as compared to FMS with the increase in clay can be due to the fact that organic complexes being absorbed onto clay surface are being physically protected against decomposition (Vanveen and Kuikman, 1990), which led to an accumulation of organic carbon level in FS. Soil organic matter influences on soil aggregate formation, soil structural stability and nutrient retention capacity (Garcia *et al*., 1996). The relationship between the clay fraction and organic carbon content was positively correlated ($r = 0.963$, $p<0.01$) (Table-2). The study illustrated that the capacity of the forest soil to preserve soil organic carbon in clay and slit-sized particles was greater than that of agricultural soils (Matus *et al.*, 2008). An increased in clay fraction and organic matter input because of vegetational development contributes to development of soil micropore space from FMS to FS, which ultimately reduced the soil bulk density. The negative correlation between bulk density and soil organic

carbon (r = -0.926; p<0.01) substantiated the concept (Table-2) and is in agreement with the findings of Jha and Singh (1991) and Juwarkar *et al.* (2009). The AS showed higher level of organic carbon than PTS, because the increased use of pesticides has led to contamination of soil with toxic chemicals (Perucci and Scorponi, 1994; Jayamadhuri *et al*., 2005). The total nitrogen and available phosphorus content in different soil samples also exhibited similar trend. The variation in organic carbon with respect to different soils were positively correlated with total nitrogen (r = 0.955; p<0.01) and available phosphorous (r = 0.991; p<0.01) (Table-2). In the present study, the significantly lower organic C, N and P at FMS than at remaining sites may result from highly disturbed surface area (Singh *et al.,* 2009). The data suggested the gradual accumulation of soil nutrients from degraded soil to enriched soil, which may be attributed to the input from the growing plant species capable of nitrogen fixing potential as well as development of mycorrhiza and other nutrient immobilizing microbial colonization.

Table-1. Physico-chemical properties as well as enzyme activities of soil samples collected from fresh mine spoil (FMS), 6yr old mine spoil (MS), degraded wasteland soil (DWS), grassland soil (GS); pesticide treated soil (PTS), agricultural soil (AS) and forest soil (FS). (Values are mean ± SD; n = 3).

Parameters	**FMS**	**MS**	**DWS**	**GS**	**PTS**	**AS**	**FS**
Sand (%)	87.8 ± 2.1	83.4 ± 1.5	80.5 ± 1.4	78.8 ± 2.2	74.5 ± 1.1	73.8 ± 1.2	72.5 ± 1.2
Slit (%)	7.8 ± 0.5	9.9 ± 0.4	10.6 ± 0.5	11.5 ± 0.6	13.2 ± 0.3	13.5 ± 0.4	14.2 ± 0.5
Clay (%)	4.4 ± 0.6	6.7 ± 0.5	8.9 ± 0.7	9.7 ± 0.3	12.3 ± 0.6	12.7 ± 0.5	13.3 ± 0.8
Bulk density (g/cm^3)	1.852 ± 0.019	1.664 ± 0.021	1.531 ± 0.022	1.354 ± 0.018	1.285 ± 0.016	1.278 ± 0.017	1.259 ± 0.024
Water holding capacity (%)	24.501 ± 1.015	32.311 ± 1.336	35.325 ± 1.234	40.986 ± 1.141	43.928 ± 1.352	44.785 ± 1.095	46.648 ± 1.087
Moisture content (%)	6.643 ± 0.103	7.422 ± 0.154	7.541 ± 0.191	8.675 ± 0.097	10.398 ± 0.158	10.509 ± 0.146	11.329 ± 0.183
pH	6.14 ± 0.03	6.39 ± 0.04	6.47 ± 0.02	6.62 ± 0.05	6.71 ± 0.04	6.77 ± 0.05	6.83 ± 0.04
Organic Carbon (%)	0.174 ± 0.009	0.307 ± 0.011	0.779 ± 0.042	1.256 ± 0.093	1.667 ± 0.074	2.064 ± 0.081	2.469 ± 0.111
Total Nitrogen (%)	0.004 ± 0.001	0.015 ± 0.001	0.041 ± 0.004	0.061 ± 0.002	0.087 ± 0.006	0.116 ± 0.004	0.202 ± 0.008
Available phosphorous (µgP/ g soil)	70.445 ±0.043	91.707 ± 0.222	422.009 ± 11.978	507.785 ± 43.367	823.289 ± 49.682	908.041 ± 29.235	1091.509 ± 60.138
Amylase activity (µg glucose/g soil/hr)	1.732 ± 0.026	6.132 ± 0.341	16.249 ± 0.751	33.222 ± 0.527	42.281 ± 0.556	44.976 ± 0.935	55.394 ± 0.843
Invertase activity (µg sucrose/g soil/hr)	5.071 ± 0.895	35.532 ± 5.616	196.726 ± 11.932	264.088 ± 9.847	565.514 ± 10.095	735.414 ± 8.197	1209.305 ± 22.581
Protease activity (µg tyrosine/g soil/hr)	2.109 ± 0.855	8.189 ± 1.185	26.565 ± 1.803	61.186 ± 4.774	98.864 ± 4.601	122.717 ± 5.484	315.275 ± 17.911
Dehydrogenase activity (µg TPF/g soil/hr)	0.036 ± 0.004	0.269 ± 0.013	0.585 ± 0.061	0.893 ± 0.065	1.181 ± 0.058	2.036 ± 0.073	4.436 ± 0.073

Table-2. Simple correlation coefficients of soil properties.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1.000													
X2	-0.998**	1.000												
X3	-0.999**	0.994**	1.000											
X4	0.975**	-0.970**	-0.976**	1.000										
X5	-0.987**	0.987**	0.984**	-0.994**	1.000									
X6	-0.967**	0.973**	0.961**	-0.918**	0.942**	1.000								
X7	-0.990**	0.992**	0.986**	-0.986**	0.998**	0.948**	1.000							
X8	-0.965**	0.964**	0.963**	-0.926**	0.944**	0.979**	0.952**	1.000						
X9	-0.885**	0.893**	0.877**	-0.810*	0.852*	0.926**	0.871*	0.955**	1.000					
X10	-0.971**	0.964**	0.974**	-0.926**	0.939**	0.974**	0.944**	0.991**	0.938**	1.000				
X11	-0.971**	0.969**	0.969**	-0.956**	0.965**	0.979**	0.965**	0.990**	0.926**	0.983**	1.000			
X12	-0.892**	0.899**	0.885**	-0.804*	0.846*	0.943**	0.866*	0.960**	0.994**	0.950**	0.927**	1.000		
X13	-0.791*	0.806*	0.779*	-0.703	0.755*	0.861*	0.777*	0.886**	0.981**	0.864*	0.853*	0.970**	1.000	
X14	-0.792*	0.807*	0.780*	-0.700	0.755*	0.851*	0.782*	0.887**	0.983**	0.861*	0.844*	0.970**	0.994**	1.000

****** Correlation is significant $p < 0.01$ and * correlation is significant $p < 0.05$.
Xi (i = 1-14) stands for soil sand, slit, clay, bulk density, water holding capacity, moisture content, pH, organic carbon, total nitrogen, available phosphorous, amylase activity, invertase activity, protease activity and dehydrogenase activity.

Enzyme activity

The amylase activity in different soil samples showed a range of 1.732 to 55.394 µg glucose/g soil/hr, with minimum in FMS and maximum in FS (Table-1). The data suggested that amylase activity is quite higher in FS and slightly decreased in AS, PTS and much lower in GS, DWS. The amylase activity in MS (6.123 µg glucose/g soil/hr) was found to be higher as compared to FMS (1.732 µg glucose/g soil/hr).

Comparisons of invertase activity showed similar trend like that of amylase activity i.e., progressive increase from 5.071 (FMS) to 1209.305 µg glucose/g soil/hr (FS). The AS exhibited higher invertase activity as compared to PTS. Similarly, the invertase activity was found to be higher in MS (35.532 µg glucose/g soil/hr) as compared to FMS (Table-1).

The protease activity in different soil samples showed a range of 2.109 to 315.275 µg tyrosine/g soil/hr, with minimum in FMS and the maximum in FS. The protease activity was found to be higher in MS (8.819 µg tyrosine/gsoil/hr) as compared to FMS. Similarly, the protease activity in AS, GS and DWS was found to be 122.717, 61.186 and 26.565 µg tyrosine/g soil/hr, respectively (Table-1).

The dehydrogenase activity (µg TPF/g soil/hr) and its variation in seven different soil types showed a range of 0.036 to 4.436 µg TPF/g soil/hr, with minimum in FMS and the maximum in FS. The MS (0.269 µg TPF/g soil/hr) exhibited level of dehydrogenase activity as compared to FMS. Further, it is evident from the data that the dehydrogenase activity is comparatively higher in FS as compared to AS (2.036 µg TPF/g soil/hr) and PTS (1.181 µg TPF/g soil/hr), but much lower in GS and DWS (Table-1).

Enzyme activity that influence functional processes occurring in a given soil, play an important role in soil profiling and biological activity (Dick, 1994; Amejkalove *et al*., 2003). A comparative analysis on the activities of different soil enzymes (amylase, invertase, protease and dehydrogenase) revealed minimal activity in FMS as compared to remaining sites due to reduced microbial population caused by the toxic effects and oxidative stress of iron mine spoil, their interference in osmotic balance and poor nutrients (Brookes, 1995).

Amylase is complex enzymes that hydrolyze starch to reducing sugar. The amylase activity is quite higher in well conserved FS and slightly decreased in below forest and much lower in degraded land, which is closely related to increase in organic carbon and diversity of soil microbiota (Rahmansyah and Sudiana, 2010; Anjaneyulu *et al*., 2011). The variation in amylase activity was positively correlated with organic carbon content (r = 0.990; p<0.01) (Table-2). Besides, the increase in amylase activity in AS as compared to PTS can be explained due to the pesticide induced changes in starch degrading enzyme (Achuba, 2006), and unavailability of nutrients thus inducing stress (Perucci and Scarponi, 1994; Anigboro and Tonukari, 2008). The stepwise multiple regression analysis suggested that TN explained about 88.5% of the variability in amylase activity. Additional 10% variability in amylase activity was explained by taking OC as a second variable (Table-3). Further, the clay fraction as

first variable explained about 93.9% of the variability in amylase activity with respect to seven different soil samples and the second variable of importance in explaining the 5% variability was OC (p<0.001) (Table-3).

The increase in invertase activity and N-mineralization from FMS to FS was directly correlated with soil organic carbon (Shi *et al*., 2008). The cellular destruction caused by toxic substances in pesticides decreased the activity of soil invertase and glucose level (Atuanya, 1987; Perucci and Scarponi, 1994) and have been accompanied by reduction in nutrient mobilization (Anigboro and Tonukari, 2008). Stepwise multiple regression analysis revealed the relationship between invertase activity and OC, which explained 92.1% variability, and an additional 6.8% of the variability was accounted by TN as second variable (Table-3). Besides, clay fraction explained about 78.3% of the variability (p<0.001) in invertase activity. The second, third and fourth variable of importance in explaining the variability were total nitrogen, bulk density and moisture content (R^2 = 0.999; p<0.001) (Table-3).

The protease activity depends on the distribution of proteolytic bacteria and the amount of proteinaceous substrate availability in the soil organic matter. The increase in protease activity from FMS to FS is closely related to the progressive improvement in organic carbon, NH_4-N accumulation (Sardans and Penuelas, 2005; Tischer, 2005) and the distribution of proteolyitc bacteria (Sardans *et al*., 2008; Anjaneyulu *et al*., 2011; Subrahmanyan *et al*., 2011). About 78.4% of the variability in protease activity was explained by OC and additional 20.9% variability (p<0.001) was explained by TN as second variable. Further, the available phosphorous explained 74.6% of the variability in protease activity, and an additional 24.4% of the variability (p<0.001) were explained by TN as second variable (Table-3).

Dehydrogenase is an intracellular oxido-reductase group of enzymes regulating the metabolic reactions in soil (Smith *et al*., 1983; Schloter *et al*., 2003), and is considered to be an index of microbial activity (Dick, 1994, Alef and Nannipieri, 1995; Stepniewska *et al*., 2007) and metabolic status of soil microorganisms (Beyer *et al*., 1992, Pascual *et al*., 1998; Taylor *et al*., 2002). The data showed consistent increase in dehydrogenase activity from FMS to FS. Besides, the difference in dehydrogenase activity under pesticide treatments may be described by differences in the decomposition rates of pesticides, or to differential effects of different pesticides concentrations on the soil microbial community (Monkiedje and Spiteller, 2002; Nannipieri and Bollag, 1991). The stepwise multiple regression analysis suggested that 78.4% of the variability in dehydrogenase activity was explained by OC and an additional 21.2% of the variability (p<0.001) was explained by TN as second variable. Only a marginal effect was contributed by AP (Table-3). The clay fraction explained 60.8% and additional 28.7% of the variability (p<0.001) in dehydroganse activity was explained by TN as second variable. Soil micropores protect mineralizing microorganisms and this can be one of the reasons for higher enzyme activity in fine-textured soil (Killham, 1994). Only a marginal effect was contributed by AP and WHC. The positive effect of increasing water content and nutrient addition on soil dehydrogenase activity has been reported (Nannipieri *et al*., 1990). Further, about 61.65 of the variability in dehydrogenase activity was explained by pH and an additional 37.3% of the variability (p<0.001) was explained by TN as second variable (Table-3). The enzymes can directly be influenced in their activity by changes in the pH value (Schinner and Sonnleitner, 1997).

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 showed a positive correlation with protease activity (r = 0.994; p<0.01), which explained 98.8% of the variability in the protease activity (Table-2). Initial organic matter transformation by dehydrogenase during the microbial respiration made available substrate to protease and subsequently higher protease activity was achieved in FS. In contrast, the FMS may be lacking a high amount of proteinaceous substrates in its integral part of soil organic matter (Subrahmanyan *et al*., 2011). It can therefore be concluded that the microbial metabolic status of FS is comparatively higher as compared to FMS.

Table-3. Stepwise multiple regression analysis of soil enzymes such as amylase, invertase, protease and dehydrogenase on other soil characteristics.

Enzyme activity	Equation	R^2*
Amylase activity	= -0.6624 + 23.4 OC	0.980
	= 7.464 + 281 TN	0.885
	= -1.851 -70 TN + 28.6 OC	0.985
	= -30.03 + 6.003 Clay	0.939
	= -7.513 + 1.34 Clay + 18.5 OC	0.983
Invertase activity	= -164.56 + 478 OC	0.921
	= -65.52 + 59 OC + 5616 TN	0.989
	= -694.89 + 115.8 Clay	0.783
	= -2119.94 + 49.4 Clay + 4895 TN + 795 BD + 60.6 MC	0.999
Protease activity	= - 46.633 + 110 OC	0.784
	= -3.302 - 73 OC + 2457 TN	0.993
	= -41.276 + 0.0236 AP	0.746
	= -7.711 - 0.131 AP + 2283 TN	0.990
Dehydrogenase activity	= 0.56141 + 1.53 OC	0.784
	= 0.04163 - 1.01 OC + 34.2 TN	0.996
	= 0.02445 - 0.35 OC + 33.5 TN - 0.00134 AP	0.998
	= -2.0945 + 0.354 Clay	0.608
	= 0.7583 - 0.161 Clay + 28.7 TN	0.995
	= 0.6055 + 0.109 Clay + 33 TN - 0.00213 AP - 0.042 WHC	0.999
	= -30.476 + 4.85 pH	0.616
	= 11.787 - 1.91 pH + 27.7 TN	0.989

*All R^2- values are significant at p<0.001. BD: Bulk density; WHC: Water holding capacity: MC: Moisture content; OC: Organic Carbon; TN: Total Nitrogen; AP: Available Phosphorous.

Further, in order to view the differences among seven different soils, principle component analysis (Ludwig and Reynolds, 1988) was performed to discriminate different sites on the basis of different soil physico-chemical as well as enzyme activities. The principal component analysis indicated that components Z_1 and Z_2 explained the maximum variance and their cumulative percentage of variance was 99%, and well segregated seven different soils (Figure-1). In this study, changes in the soil enzyme activity, dehydrogenase activity in particular correlated very well with the land degradation. The dehydrogenase assay offers a continuous measure of soil microbial activity as a result of total redox sequences. Thus, it appears that the assay of soil enzyme activity can serve as an integrative measure of soil quality.

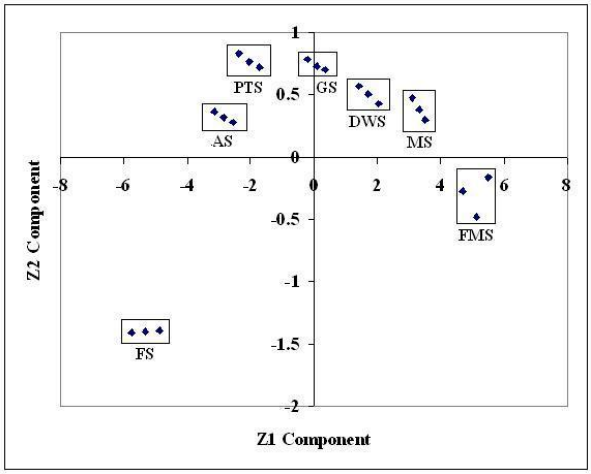

Figure-1. Principal component analysis for soil properties and different enzyme activities of different soils, Fresh mine spoil: FMS; 6yr old mine spoil: MS; Degraded wasteland soil: DWS; Grassland soil: GS; Pesticide treated soil: PTS; Agricultural soil: AS and Forest soil: FS.

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