RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Kinetics of soil enzyme activities under different ecosystems: An index of soil quality

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Soil microbial activity plays an important role in regulating biotransformation, nutrient cycling and hence the microbiological processes are at the center of many ecological functions. The kinetic parameters (V_{max} and K_m Michaelis constant) of different enzymes (amylase, invertase, protease, urease, and dehydrogenase) were determined in order to assess the metabolic response of soil. The maximum reaction velocity (V_{max}) represents a maximum rate of activity when all enzymes are saturated, which markedly increased in forest soil as compared to fresh mine spoil due to the gradual accumulation of soil organic matter. Smaller K_m value was estimated in forest soil (FS) as compared to fresh mine spoil (FMS), suggesting the greater affinity of soil enzymes for substrate in FS. The catalytic efficiency (V_{max}/K_m) reflects an impression on microbial community composition with a change in soil enzymes. These enzyme characters (activities and kinetic parameters) have greater significance as early and sensitive indicators of the changes in soil properties induced by different management systems. These parameters (V_{max} and K_m) can be useful markers to assess changes in microbial activity of soil, since they represent quantity and affinity of enzymes respectively. The metabolic index (dehydrogenase activity/organic carbon (OC)) was found to be correlated with V_{max} of dehydrogenase ($r = 0.953$; $p < 0.01$) and OC ($r = 0.880$; $p < 0.01$). Principal component analysis was able to discriminate seven different soil samples into seven independent clusters based on their enzyme activities and kinetic parameters. Indeed, the study revealed the importance of kinetics study of soil enzymes, which can be considered valid parameters to monitor the evolution of microbiological activity in soil, and hence an index of soil quality.

Key words: Amylase, invertase, protease, urease, dehydrogenase.

INTRODUCTION

Microbes in soil play a pivotal role by producing various enzymes (Zhang et al., 2009), which are constantly being synthesized, accumulated, inactivated and/or decomposed in soil (Tabatabai, 1994; Dick et al., 1994). Soil enzymes contribute to the total biological activities, because they are intimately involved in catalyzing reactions necessary for the stabilization of soil structure, organic matter (OM) decomposition and dissolved OM production (Allison and Vitousek, 2005), mineralization, and nutrient cycling (Tabatabai, 1994; Dick et al., 1994), energy transfer and environmental quality. Soil enzymatic activities are a useful tool for assessing the functional diversity of soil microbial communities or organic mass turnover (Kandeler et al., 1999). The activities of these enzymes in soils undergo complex biochemical processes consisting of integrated and ecologically connected synthetic processes. Soil nutrient correlate to soil productivity, while soil enzymatic characteristics can provide information about the status of key biochemical reactions involved in rate limiting steps of biotransformation of soil nutrients.

Enzyme activities may have the potential to be used as indicators of soil quality, sustainability and changes in biogeochemical function due to management or perturbations. In general, soil enzyme activities can be used to characterize abundance and metabolic activity of soil microbes, where as kinetic parameters are used to describe catalytic activity, origin, and substrate affinity of the enzymes (Zhang et al., 2009). Soil enzyme activity provides an indication of its amount and its overall contribution in soil (Farrell et al., 1994), while enzyme kinetics study can provide useful information regarding their origin, existing status and catalytic properties, state and behavior of soil enzymes.

Kinetic parameters (V_{max} and K_m) are often used to characterize free enzymes in solution, they are considered to be constant for a specific enzyme under defined experimental conditions (Marx et al., 2005), but they may vary independently. Maximum reaction velocity (V_{max}) of an enzyme catalyzed reaction imply splitting velocity or rate of dispersion of enzyme-substrate complex into enzyme and reaction products, which reflects the conjunction affinity between enzyme and substrate. The higher or lower V_{max} value can be used as an indicator to speedy or slow enzymatic process. V_{max} and K_m of an enzyme express the quantity of an enzyme and substrate affinity, respectively (Marx et al., 2005; Davidson et al.,

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Received: 6 July 2013.
Accepted: 12 December 2013.
doi:10.4067/S0718-58392014000100015

2006). However, Michaelis constant (K_m) represents the endurance of an enzyme-substrate complex, which is related with the substrate. The efficiency of the enzymes to decompose substrate at low concentration is directly related to their K_m value (Marx et al., 2005; Davidson et al., 2006). Higher is the endurance of an enzyme-substrate complex, lower will be the K_m value. Enzymes catalyzing the same reaction, but derived from different sources of soil have different K_m values (Nannipieri et al., 1990). Besides, K_m is independent of enzyme concentration and kinetically reflects the apparent affinity of enzyme for the substrate. In other words, smaller the K_m value, the greater will be the affinity for the substrate (Masciandaro et al., 2000). However, estimating K_m is challenging due to the uncertainty regarding the relative contribution of artificial and naturally occurring substrate under non-saturating conditions (Stone et al., 2011). Moreover, enzymes may operate under non-saturating conditions in soil, which supplements K_m an important parameter that merits increased attention (Davidson et al., 2006; German et al., 2011). If substrate concentration is similar to K_m, the measure of affinity for substrate/enzyme can provide information about the adsorption level or enzyme accessibility. Besides, K_m influences enzyme activity at low substrate concentration (Davidson and Janssens, 2006; Davidson et al., 2006). Many investigations have dealt with the kinetic properties of enzymes (Masciandaro et al., 2000; Zhang et al., 2009; 2010; Juan et al., 2010).

Further, the V_{max}/K_m value represents the formation of an enzyme-substrate complex and the comparison of dispersion of this complex in soil. Higher value of V_{max}/K_m suggests that dispersion of enzyme-substrate complex occurs faster than its formation (Ekberli et al., 2006; Kizilkaya et al., 2007). The catalytic efficiency of soil enzymes *i.e.* V_{max}/K_m (Gianfreda et al., 1995) was highly affected by physico-chemical properties of soil (Dick et al., 1994), source of available substrate for the enzyme (Garcia et al., 1993; Kizilkaya and Bayrakli, 2005), soil OM content (Garcia et al., 1993), stimulation of microbial activity by compounds containing N, P, and K, and the synthesis of enzymes by increasing microbial populations.

In the present study, five enzymes (amylase, invertase, protease, urease, and dehydrogenase) representative of important nutrient cycles were selected. Amylase, invertase, and protease are hydrolytic enzymes, which control the decomposition of various biological macromolecules abundant in plant litter and soil. Amylase is a starch hydrolyzing enzyme (Ross, 1983) widely distributed in soils that hydrolyzes starch mainly to form reducing sugar an energy source for soil microbes (Eivazi and Tabatabai, 1990) and small quantity of maltose. Invertase (β-fructofuranosidase, E.C.: 3.2.1.5) hydrolyzes sucrose into glucose and fructose. The soil invertase activity is used as an index for nutrient transformation, energy metabolism, and pollutant degradation (Nannipieri et al., 1990). Soil proteases are extracellular enzymes produced mainly by bacteria, which degrade proteins and release NH_4-N important in N cycle (Sardans et al., 2008). Estimation of proteases provides information on N mediated biochemical processes in soil (Sardans and Peñuelas, 2005) and play a significant role in N mineralization regulating the amount of plant available N. Soil urease (E.C.: 3.5.1.5) is responsible for the breakdown of urea into CO_2 and ammonia. Its origin is basically microbial and its activity is extracellular (Tabatabai, 1994; Dick et al., 1994; Kizilkaya et al., 2004). It acts as an intermediary enzyme in the transformation of organic N in soil. Information regarding the nature of urease activity is beneficial for developing and employing strategies for efficient N management (Ekberli et al., 2006; Kizilkaya et al., 2007; Kizilkaya and Ekberli, 2008). Urease activity is used as a soil quality indicator (Yang et al., 2006; Makoi and Ndakidemi, 2008). Dehydrogenase activity can be used as an index of overall microbial activity (Nannipieri et al., 1990; Tabatabai, 1994), and is linked with microbial respiratory processes, which can provide information about the key reactions involved in rate limiting steps of microbial oxidoreduction processes.

Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances, and show a quick response to induced changes. The variation in soil enzyme activities is due to the variation in OM, microbial community, microbial activity associated with soil biological processes, which are affected by biotic and abiotic factors. Differential microbial community may produce distinct enzyme isoforms, which could differ in their catalytic properties and hence their V_{max} and K_m. Therefore, the knowledge about soil enzyme activities, kinetic properties, and their variation has considerable biological significance, which paves the way of greater understanding the direction of improving soil fertility. Further, the kinetic parameter is most attractive from the standpoint of the forensic comparison of soil, since it is independent of sample size and its potential usefulness is thereby enhanced. Realizing this, an attempt was made in the study with an aim to provide a comparative account on the variations in the kinetic properties of different soil enzymes, and to illustrate if kinetics of soil enzyme activities can be used as indices for soil fertility.

MATERIALS AND METHODS

Study site

The present study was carried out in sponge iron mines in Noamundi (22°9'49.96" N, 85°30'33.61" E; 540 m a.s.l.) maintained by Tata Iron Steel Corporation limited (TISCO), which is located in the revenue district of West Singhbhum, Jharkhand, India (Figure 1). The area 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

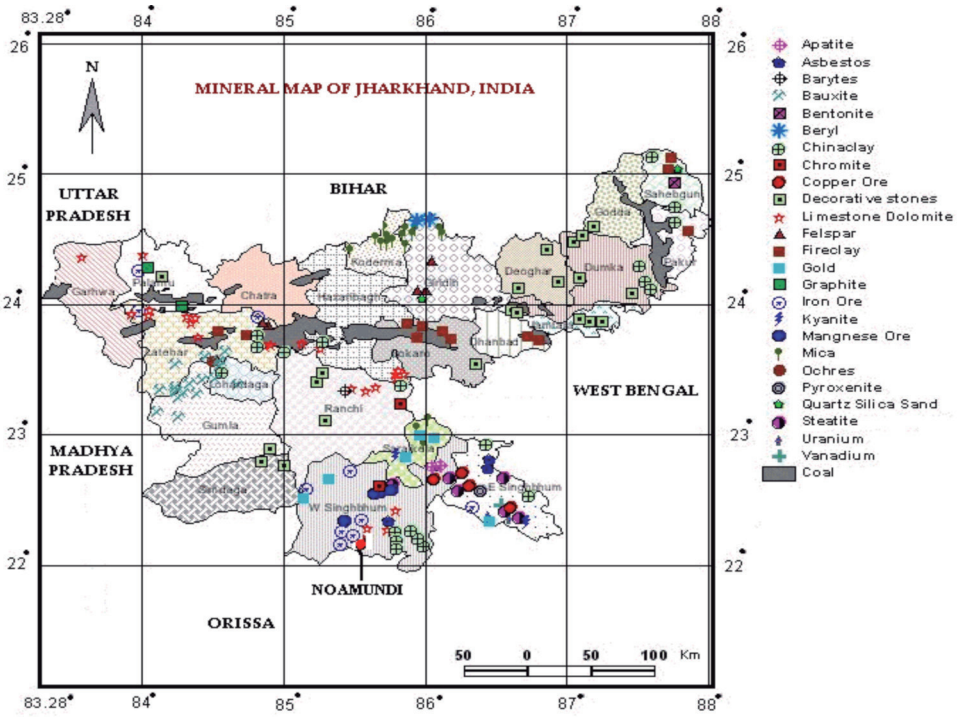


Figure 1. Geographical location and the mineral map of study site, Jharkhand, India.

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.

Soil sampling

Sampling was done in accordance with the general method of soil microbiological study. Seven different sites: fresh mine spoil (FMS); 6 yr old mine spoil (MS); degraded waste land soil (DWS); grassland soil (GS); pesticide-treated soil (PTS); agricultural soil (AS) and forest soil (FS) were selected for sampling near Noamundi within 10 km peripheral distance from the mining area. Each site was divided into three blocks, and during each sampling five soil samples were collected randomly from 0-15 cm soil depth by digging pits of 15 × 15 × 15 cm 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 (2 mm mesh size) for characterization.

Soil enzyme activities

Amylase activity of different soil samples was determined in adaptation to the procedures described by Somogyi (1952) and Roberge (1978) taking starch as substrate and incubating at 30 °C for 24 h. Invertase activity was determined by spectrophotometric method (Ross, 1983) using sucrose as substrate and incubating at 37 °C for 24 h. Protease activity was determined by spectrophotometric method (Ladd and Butler, 1972) with sodium caseinate as a substrate. Urease activity was determined by spectrophotometrically at 578 nm (Hoffmann and Teicher, 1961) using urea as substrate. Dehydrogenase activity was measured by the following reduction of 2,3,5-triphenylotetrazolium chloride (TTC) as an artificial electron acceptor to red-colored triphenyl formazon (TPF), which were determined spectrophotometrically (Nannipieri et al., 1990).

Kinetic parameters (K_m and V_{max}) of different soil enzyme activities were determined taking five different substrate concentrations individually. The substrate concentrations used for soil amylase, invertase, protease, urease, and dehydrogenase activities were ranged from 5 to 50 mM, 10 to 100 mM, 1 to 10 mM, 5 to 45 mM, and 10 to 90 mM, respectively. For every substrate concentration, triplicate analyses were carried out.

Kinetic parameters determination

The soil enzymes follow Michaelis Menten kinetics, despite soil being considered as a discontinuous, structured and heterogeneous system (Nannipieri et al., 2002). Michaelis-Menten equation linearized by Lineweaver-Burk was used to determine V_{max} (maximal velocity), K_m

(substrate concentration at ½ V_{max}) by plotting a graph i.e. 1/V against 1/[substrate concentration], and estimated by the intercept and slope respectively, and V_{max}/K_m as kinetic parameters.

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

Statistical analysis

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. Principal components analysis (PCA) was performed using Statistix PC DOS Version-2.0 (NH Analytical Software).

RESULTS AND DISCUSSION

Soil enzymes are important for their role in nutrient cycling and were considered to be early indicators of specific biochemical reactions in soil, because of their relationship to soil biology, ease of measurement (Dick et al., 1994; Kizilkaya and Bayrakli, 2005), and rapid response to changes in soil managements (Kandeler et al., 1999; Zhang et al., 2010).

Kinetics study of soil amylase activity indicated an increasing trend in V_{max} from FMS (4.3292 μg g^{-1} soil h^{-1}) to FS (66.9231 μg g^{-1} soil h^{-1}). Higher V_{max} value was estimated in AS as compared to PTS, but much lower in GS and DWS. The V_{max} of soil amylase was higher in MS (11.7646 μg g^{-1} soil h^{-1}) as compared to FMS (Table 1). Soil invertase can catalyze the rupture of β-glucose bonds resulting in sucrose hydrolysis into glucose and sucrose, and it is an important enzyme, which reflects the transformation mechanism of organic C. It not only reflects the biological properties of soil, but also serves as an evaluation indicator of soil maturity and fertility level. Comparisons of soil invertase activity showed similar trend, i.e. progressive increase in V_{max} value from FMS (10.1563 μg g^{-1} soil h^{-1}) to FS (1250.5132 μg g^{-1} soil h^{-1}). The MS (41.5135 μg g^{-1} soil h^{-1}) exhibited higher V_{max} value as compared to FMS. Similarly, V_{max} for soil invertase activity was higher in AS (787.6191 μg g^{-1} soil h^{-1}) as compared to PTS, GS, and DWS (Table 1).

It is evident from data that soil amylase activity and its V_{max} value were positively correlated with WHC, MC, organic C, total N, and available P, but negatively correlated with bulk density (Table 2). Similarly, the soil invertase activity and its V_{max} value were also positively correlated with WHC, MC, organic C, total N, and available P, but negatively correlated with bulk density (Table 3). Such variation in soil amylase as well as invertase activity with

Table 1. Michaelis constant (K_m) and maximum reaction velocity (V_{max}) (μg g^{-1} soil h^{-1}) of different soil enzymes.

Soil enzymes	Kinetic parameters	FMS	MS	DWS	GS	PTS	AS	FS
Amylase	V_{max}	4.3292	11.7646	31.2508	43.4785	52.5412	55.5555	66.9231
	K_m (mM)	47.109	35.474	29.515	17.384	14.217	13.115	11.108
	V_{max}/K_m	0.0918	0.3316	1.0588	2.5010	3.6956	4.2360	6.0247
	R^2	0.877*	0.861*	0.954**	0.949**	0.880**	0.980**	0.934**
Invertase	V_{max}	10.1563	41.5135	229.5071	325.4267	742.8251	787.6191	1250.5132
	K_m (mM)	41.398	32.818	28.554	25.609	19.995	19.631	17.365
	V_{max}/K_m	0.2453	1.2649	8.0376	12.7075	37.1505	40.1211	72.0134
	R^2	0.737*	0.797*	0.877**	0.892**	0.938**	0.839**	0.871**
Protease	V_{max}	5.6997	15.2765	52.1908	75.2672	142.8991	168.8752	320.0864
	K_m (mM)	22.157	18.013	13.116	12.083	11.985	11.651	10.183
	V_{max}/K_m	0.2572	0.8480	3.9791	6.2291	11.9231	14.4944	31.4334
	R^2	0.995**	0.847**	0.924**	0.987**	0.719*	0.774*	0.908**
Urease	V_{max}	10.1568	12.0484	27.0275	31.2518	33.3333	34.4823	37.0371
	K_m (M)	0.085	0.071	0.062	0.045	0.036	0.032	0.028
	V_{max}/K_m	119.4917	169.6957	435.9274	694.4844	925.9250	1077.5718	1322.7535
	R^2	0.921**	0.997**	0.859**	0.870**	0.947**	0.826**	0.837**
Dehydrogenase	V_{max}	0.8662	1.8772	3.2583	3.4002	4.3725	4.5614	5.6249
	K_m (M)	0.201	0.131	0.067	0.043	0.034	0.031	0.025
	V_{max}/K_m	4.3094	14.3297	48.6313	79.0744	128.6029	147.1419	224.9960
	R^2	0.976**	0.990**	0.905**	0.873**	0.935**	0.927**	0.971**

**Correlation is significant at $p < 0.01$; *correlation is significant at $p < 0.05$.
FMS: fresh mine spoil; MS: 6 yr old mine spoil; DWS: degraded waste land soil; GS: grassland soil; PTS: pesticide-treated soil; AS: agricultural soil; FS: forest soil; and V_{max}/K_m: catalytic efficiency.

Table 2. Simple correlation between soil properties and amylase activity.

Parameters	Clay	BD	WHC	MC	pH	OC	TN	AP	Amylase activity	V_{max}	K_m	V_{max}/K_m
Amylase activity	0.969**	-0.956**	0.965**	0.979**	0.965**	0.990**	0.926**	0.983**	1			
V_{max}	0.983**	-0.972**	0.976**	0.955**	0.975**	0.980**	0.911**	0.982**	0.990**	1		
K_m	-0.974**	0.998**	-0.996**	-0.928**	-0.990**	-0.934**	-0.827*	-0.928**	0.963**	-0.974**	1	
V_{max}/K_m	0.937**	-0.893**	0.919**	0.981**	0.928**	0.992**	0.974**	0.979**	0.983**	0.962**	-0.906**	1

**Correlation is significant at $p < 0.01$; *correlation is significant $p < 0.05$ (2-tailed test).
BD: Bulk density; WHC: water holding capacity; MC: moisture content; OC: organic C; TN: total N; AP: available P; V_{max}: maximum reaction velocity; K_m: Michaelis constant; and V_{max}/K_m: catalytic efficiency.

Table 3. Simple correlation between soil properties and invertase activity.

Parameters	Clay	BD	WHC	MC	pH	OC	TN	AP	Invertase activity	V_{max}	K_m	V_{max}/K_m
Invertase activity	0.885**	-0.804*	0.846*	0.943**	0.866*	0.960**	0.994**	0.950**	1			
V_{max}	0.918**	-0.844*	0.879**	0.968**	0.892**	0.972**	0.982**	0.972**	0.993**	1		
K_m	-0.994**	0.984**	-0.994**	-0.946**	-0.995**	-0.944**	-0.860*	-0.950**	-0.861*	-0.893**	1	
V_{max}/K_m	0.875**	-0.790*	0.833*	0.946**	0.851*	0.948**	0.985**	0.943**	0.995**	0.995**	-0.850*	1

**Correlation is significant $p < 0.01$; *correlation is significant $p < 0.05$ (2-tailed test).
BD: Bulk density; WHC: water holding capacity; MC: moisture content; OC: organic C; TN: total N; AP: available P; V_{max}: maximum reaction velocity; K_m: Michaelis constant; and V_{max}/K_m: catalytic efficiency.

respect to seven different soil samples may be due to the variation in available soil nutrients (Kujur et al., 2012). The organic C content was significantly correlated with soil enzyme activities (Eivazi and Tabatabai, 1990; Kujur et al., 2012). Increased soil nutrients lead to increase in microbial biomass (Singh et al., 2007; Kujur and Patel, 2012) and diversity of soil micro-biota causes increased microbial enzyme production and hence higher V_{max} (Allison and Martiny, 2008; Nemergut et al., 2008; Stone et al., 2011). The type of OM was shown to influence activities of amylase and invertase more than quantity of OM. The decrease in V_{max} in FMS is attributable mainly to the declination of enzyme synthesis due to the accumulation of heavy metals and associated toxic effects on soil microbes. The heavy metals may cause changes in the active center and structure of soil enzymes, thus making the soil 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 (Yang et al., 2006; Gao et al., 2009), thus reducing the synthesis, secretion of enzymes and finally leading to the decrease in soil amylase and invertase activity (He et al., 2002). Besides, the increase in soil amylase and invertase activity in AS as compared to PTS can be explained due to the pesticide induced changes in the soil enzymes (Achuba, 2006) as well as in microbial community composition.

The K_m value of soil amylase exhibited highest K_m value (i.e. 47.109 mM) in FMS, and then decreased with increasing soil moisture content (Kujur and Patel, 2012). The K_m value estimated in FS was minimal i.e. 11.108 mM (Table 1). Similar trend was also exhibited in soil invertase activity, where K_m value ranges from 41.398 mM (FMS) to 17.365 mM (FS) (Table 1). The K_m value of soil amylase and invertase activity showed negative correlation with all the tested soil properties except bulk density (Tables 2 and 3). The substrate diffusion rate affects K_m value due to the typical heterogenic system of soil. The stronger enzyme-substrate affinity (lower K_m value) in higher moisture content may be caused by the higher diffusion rate because of more water solubility (Zhang et al., 2009). Soil amylase K_m value as well as invertase of AS and PTS were much smaller than those in GS, DWS, MS, which may be due to the fact that AS and PTS contain much more OM and medium textured soil (Kujur and Patel, 2012) exhibit better soil structure that protect the enzyme active site, resulting lower K_m value. Secondly, the potential reason of lower K_m in FS as compared to other soils may be due to the higher water availability (water holding capacity) because of higher OM content (Zhang et al., 2009). Further, V_{max}/K_m value of soil amylase was estimated to be lowest in FMS (0.0918) as compared to FS (6.0247) (Table 1). Similar trend was also exhibited in soil invertase i.e. minimal in FMS (0.2453) and maximum in FS (72.0134) (Table 1). The lowest value of V_{max}/K_m in FMS may be attributed to the extreme soil dryness that limits solubility and restrict movement of the available organic C as energy source and thus limits the microbial respiration.

The protease activity depends upon the amount of proteinaceous substrate available in the soil OM as well as on the distribution of proteolytic bacteria. The increase in V_{max} value of soil protease showed a range from 5.6997 to 320.0864 $\mu g\ g^{-1}$ soil h^{-1} with minimum in FMS and maximum in FS (Table 1). The K_m value of soil protease varied from 22.157 mM (FMS) to 10.183 mM (FS), and V_{max}/K_m value ranged from 0.2572 (FMS) to 31.4334 (FS) (Table 1). The variation in soil protease activity between seven different soils was contributed by OC, TN, and AP (Kujur and Patel, 2012). The soil protease activity and its V_{max} value exhibited positive correlation with different soil properties such as WHC, MC, pH, OC, TN, and AP (except for BD), whereas its K_m value showed negative correlation with all the tested soil properties except BD (Table 4). The vegetation and the associated difference in litter inputs and root exudation in FS as compared to FMS may have contributed to increased V_{max} value (Stone et al., 2011). Higher V_{max} in AS (168.8752 $\mu g\ g^{-1}$ soil h^{-1}) as compared to GS and DWS is due to the progressive improvement in organic C and NH_4-N accumulation (Sardans and Peñuelas, 2005; Tischer, 2005) and the distribution of proteolyitc bacteria (Sardans et al., 2008; Anjaneyulu et al., 2011; Subrahmanyam et al., 2011). Higher V_{max} in GS (75.2672 $\mu g\ g^{-1}$ soil h^{-1}) is exhibited as compared to DWS (52.1908 $\mu g\ g^{-1}$ soil h^{-1}), which may be attributed to the gradual accumulation of proteinaceous substrate facilitated by the vegetation cover in course of time. Further, the gradual N accumulation stimulates the soil microbes for enhanced production of C-degrading enzymes.

Urease is mostly an extracellular enzyme representing up to 63% of total activity in soil. It belongs to soil hydrolases involved in urea hydrolysis (hydrolysis of soil amide N) into CO_2 and NH_3, and consequently N losses

Table 4. Simple correlation between soil properties and protease activity.

Parameters	Clay	BD	WHC	MC	pH	OC	TN	AP	Protease activity	V_{max}	K_m	V_{max}/K_m
Protease activity	0.779*	-0.703	0.755*	0.861*	0.777*	0.886**	0.981**	0.864*	1			
V_{max}	0.863*	-0.786*	0.830*	0.925**	0.849*	0.943**	0.996**	0.932**	0.986**	1		
K_m	-0.938**	0.963**	-0.955**	-0.826*	-0.949**	-0.866*	-0.777*	-0.877**	-0.672	-0.745	1	
V_{max}/K_m	0.825*	-0.745	0.794*	0.897**	0.815*	0.918**	0.992**	0.903**	0.996**	0.997**	-0.710	1

**Correlation is significant $p < 0.01$; *correlation is significant $p < 0.05$ (2-tailed test).
BD: Bulk density; WHC: water holding capacity; MC: moisture content; OC: organic C; TN: total N; AP: available P; V_{max}: maximum reaction velocity; K_m: Michaelis constant; and V_{max}/K_m: catalytic efficiency.

by NH_3 volatilization. Hence, emphasis on urease activity has been given in order to evaluate N supply to plants, because large N losses to atmosphere by volatilization mediated by these enzymes. Comparisons of V_{max} of urease activity showed similar trend like protease activity i.e. progressive increase from 10.1568 μg g^{-1} soil h^{-1} (FMS) to 37.0371 μg g^{-1} soil h^{-1} (FS) (Table 1). Similarly, the V_{max} was found to be higher in MS (12.0484 μg g^{-1} soil h^{-1}) as compared to FMS, which represents nutrient deficient situation with altered geomorphic system (Kujur and Patel, 2012). Factors contributing higher V_{max} in MS as compared to FMS may include the absence of disturbing, which mitigate the problem of runoff of the residual soil nutrients, and may due to the gradual establishment of vegetation cover in course time due to soil reclamation process. However, lower V_{max} value of soil urease was estimated in PTS (33.3333 μg g^{-1} soil h^{-1}) as compared to AS (34.4823 μg g^{-1} soil h^{-1}), due to the toxic effect of pesticides on soil microbes (Jayamadhuri and Rangaswamy, 2005). Further, less variation in V_{max} between AS and PTS was due to the buffering effects of higher OM and clay percentage (Zhang et al., 2009).

The K_m value soil urease activity varied from 0.085 M (FMS) to 0.028 M (FS) (Table 1). PTS (0.036 M) exhibited higher K_m as compared to AS (0.032 M), which may be attributed to the formation of organo-urease complex, thereby decreasing the affinity of urease to its specific substrate urea or the conformational changes in the enzyme making its active sites less accessibility to its substrate (Juan et al., 2010). The greater differences in K_m value between different soils suggested that the binding status and the origin of soil urease are dissimilar. Further, the seven different soil types undergo different pedogenic process resulted variation in physico-chemical properties and thus, soils are unlikely to have similar urease origin. The V_{max}/K_m value in FMS (119.4917) was estimated to be lowest than FS (1322.7535). Furthermore, AS (1077.5718) exhibited higher V_{max}/K_m value as compared to PTS, GS, and DWS (Table 1), which is due to the crop rotations with high input and diversity of OM containing higher concentrations of microbial biomass and enzyme activities (Klose and Tabatabai, 2000). Such variation in catalytic efficiency (V_{max}/K_m) of soil urease (Gianfreda et al., 1995) may be due to variation in soil OM and soil texture (Garcia et al., 1993).

The variances in kinetic parameters of urease as a result of an increase in V_{max}, K_m and V_{max}/K_m due to the variation in physico-chemical properties of soil (Dick et al., 1994; Sarkar et al., 2003), moisture content (Sardans and Peñuelas, 2005; Kujur et al., 2012), OM and gradual accumulation of N, which is considered to be the substrate for soil urease (Garcia et al., 1993; Kizilkaya and Bayrakli, 2005; Kizilkaya and Ekberli, 2008), and synthesis of urease enzyme by increasing microbial population. Urease activity shows a positive correlation with N availability, which indicated that this enzyme can be used to make some inferences about the nitrification process in soil and determine if N loses are due to volatilization, nitrification, or denitrification. Urease has been widely used for soil quality assessment, since its activity increases with organic fertilization and decreases with soil tillage (Saviozzi et al., 2001). Urease activity is influenced by microbial community, physico-chemical properties of soil (Corstanje et al., 2007), OM, soil depth, heavy metals, temperature, and pH (Yang et al., 2006). Further, its stability is affected by organo-mineral complexes and humic substances (Makoi and Ndakidemi, 2008).

The soil urease activity exhibited positive correlation with MC, organic C, total N and available P (except for clay percentage, WHC, and pH). Soil urease V_{max} was positively correlated with all soil properties, except for bulk density. The K_m values of soil urease had no significant correlation with urease activity, which suggested that K_m is independent of enzyme concentration (Masciandaro et al., 2000). Further, it is evident from data that V_{max}/K_m value, i.e. the catalytic efficiency of soil urease between the seven different soil samples, exhibited positive correlation with all the tested soil properties except K_m and bulk density, which were negatively correlated (Table 5).

Estimation of dehydrogenase activity is attractive due to the fact that they are an integral part of soil microorganisms and are involved in OM oxidation. Dehydrogenase is involved electron transport system of oxygen metabolism and requires an intracellular environment (viable cells) to express its activity (Kandeler and Dick, 2007). Dehydrogenase enzyme is not present in extracellular form as hydrolases (urease, phosphatase), and hence it cannot be used to evaluate the process of soil degradation. The highest V_{max} value was observed in FS (4.6249 μg g^{-1} soil h^{-1}) as compared to FMS (0.8662 μg g^{-1} soil h^{-1}) (Table 1). This is due to higher OM content in FS that support increased microbial activity

Table 5. Simple correlation between soil properties and urease activity.

Parameters	Clay	BD	WHC	MC	pH	OC	TN	AP	Urease activity	V_{max}	K_m	V_{max}/K_m
Urease activity	0.695	-0.620	0.677	0.768*	0.701	0.802*	0.939**	0.780*	1			
V_{max}	0.956**	-0.967**	0.951**	0.878**	0.943**	0.925**	0.823*	0.940**	0.946**	1		
K_m	-0.987**	0.988**	-0.993**	-0.965**	-0.992**	-0.969**	-0.876**	-0.963**	-0.692	-0.956**	1	
V_{max}/K_m	0.964**	-0.929**	0.945**	0.980**	0.951**	0.999**	0.954**	0.993**	0.804*	0.931**	-0.970**	1

**Correlation is significant $p < 0.01$; *correlation is significant $p < 0.05$ (2-tailed test).
BD: Bulk density; WHC: water holding capacity; MC: moisture content; OC: organic C; TN: total N; AP: available P; V_{max}: maximum reaction velocity; K_m: Michaelis constant; and V_{max}/K_m: catalytic efficiency.

and microbial biomass, consequently the concentration of soil dehydrogenase. Besides, less variation in V_{max} was exhibited between AS and PTS, which may be due to the toxic effect of pesticides on soil microbes in PTS (Jayamadhuri and Rangaswamy, 2005). Further, the V_{max} value in AS was found to be higher as compared to GS and DWS (Table 1). The K_m value of soil dehydrogenase ranged from 0.201 M (FMS) to 0.025 M (FS). The variation in K_m value with respect to different soils can be explained on the basis of the capability of the enzyme catalyzing the same reaction can have different sources in soil and thus different K_m values (Nannipieri et al., 1990). Value of K_m reflects the apparent affinity of the enzyme for the substrate: the smaller the K_m value, the greater the affinity ((Masciandaro et al., 2000). The catalytic efficiency (V_{max}/K_m) of soil dehydrogenase was found to be maximum in FS (224.996) and minimum in FMS (4.3094), which may be due to the change in the composition of soil microbiota with a change in the community of dehydrogenase (Masciandaro et al., 2000). Therefore, V_{max} and K_m of dehydrogenase act as useful markers to assess changes in microbial activity of soil, since they represented quality and affinity of dehydrogenase, respectively.

Several investigations have revealed correlations of soil dehydrogenase activity with soil respiration, ATP concentration, C and N turnover, OM content (Nannipieri et al., 1990). The soil dehydrogenase activity is positively correlated with all tested soil properties except bulk density, with which it had no correlation (Table 6). The V_{max} value of soil dehydrogenase is positively correlated with all the variables, whereas it is negatively correlated with bulk density. However, the K_m value of soil dehydrogenase showed negative correlation with all the tested soil properties except BD with which it is positively correlated. Similarly, catalytic efficiency of soil dehydrogenase was found to be positively correlated with the entire variables except BD (Table 6).

The kinetic parameters (V_{max} and K_m) of soil dehydrogenase activity were estimated in order to assess the metabolic response of a soil. The metabolic index is defined as the ratio between soil dehydrogenase activities and water soluble organic C, has been suggested to represent the metabolic activity of soil (Masciandaro et al., 2000). The positive correlation between metabolic index (dehydrogenase activity/organic C) and V_{max} of soil dehydrogenase ($r = 0.953$; $p < 0.01$), and the organic C ($r = 0.880$; $p < 0.01$) indicated the ecological implications related to changes in different soil profiles with respect to their soil metabolic activity need to be considered. It is evident from data that changes in kinetic parameters of dehydrogenase activity seem to indicate a change in composition and activity of soil microbes. Thus, the metabolic potential index and kinetic constants of soil dehydrogenase may be used to monitor soil fertility.

Further, in order to view differences among seven different soils, principle component analysis (Ludwig and Reynolds, 1988) was performed to discriminate different sites on the basis of soil enzyme activity including enzyme kinetic parameters (V_{max}, K_m, and V_{max}/K_m). The principal component analysis indicated that components Z1 and Z2 explained the maximum variance and their cumulative percentage of variance was 99%, and well segregated seven different soils (Figure 2). Thus, the study clearly revealed that soil enzyme activity and kinetic parameters can serve as an integrative measure of soil quality.

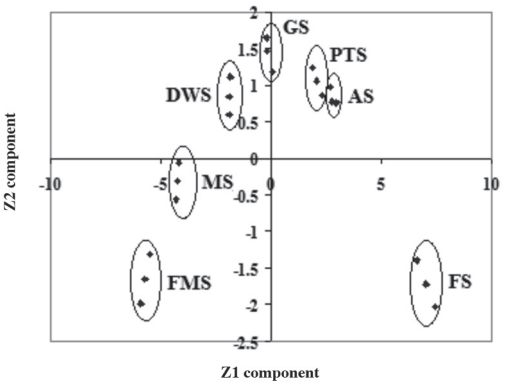
CONCLUSIONS

The study suggested that forest soil (FS) support increased microbial activity and biomass due to higher organic matter content, and consequently exhibited higher maximum reaction velocity (V_{max}) as compared to fresh mine spoil (FMS). Besides, lower K_m value in FS as compared to other soil types explained the strong

Table 6. Simple correlation between soil properties and dehydrogenase (DH-ase) activity.

Parameters	Clay	BD	WHC	MC	pH	OC	TN	AP	DH-ase activity	V_{max}	K_m	V_{max}/K_m	DH-ase /OC
DH-ase activity	0.780*	-0.700	0.755*	0.851*	0.782*	0.887**	0.983**	0.861*	1				
V_{max}	0.985**	-0.953**	0.968**	0.946**	0.974**	0.968**	0.924**	0.977**	0.849*	1			
K_m	-0.946**	0.977**	-0.966**	-0.835*	-0.957**	-0.861*	-0.748	-0.872*	-0.639	-0.935**	1		
V_{max}/K_m	0.928**	-0.870*	0.901**	0.969**	0.914**	0.984**	0.988**	0.976**	0.945**	0.954**	-0.801*	1	
DH-ase/OC	0.686	-0.676	0.725	0.583	0.756	0.880**	0.614	0.543	0.953**	0.690	-0.728	0.560	1

**Correlation is significant $p < 0.01$; *correlation is significant $p < 0.05$ (2-tailed test).
BD: bulk density; WHC: water holding capacity; MC: moisture content; OC: organic C; TN: total N; AP: available P; V_{max}: maximum reaction velocity; K_m: Michaelis constant; and V_{max}/K_m: catalytic efficiency.



FMS: Fresh mine spoil; MS: 6 yr old mine spoil; DWS: degraded waste land soil; GS: grassland soil; PTS: pesticide-treated soil; AS: agricultural soil, and FS: forest soil.

Figure 2. Principal component analysis for different soil properties including enzyme activities and kinetic parameters of different soils (FMS, MS, DWS, GS, PTS, AS, and FS).

enzyme-substrate affinity of different soil enzymes in FS. Further, higher value of catalytic efficiency (V_{max}/K_m) was exhibited by FS indicating higher rate of dispersion of enzyme-substrate complex as compared to FMS. The changes in the kinetic parameters of soil enzyme activities seem to indicate a change in composition and activity of soil microbiota, which can serve as an integrative measure of soil quality. Comparative assessment of the kinetic parameters (V_{max}, K_m, and V_{max}/K_m) can able to discriminate seven different soil types and hence can be used as an index of soil quality and sustainability. The results obtained form the studies are of sufficient significance that is presumably extendable for soil quality assessment and management practices.

ACKNOWLEDGEMENTS

The authors are thankful to the Head of School of Life Sciences, Sambalpur University, Odisha, India, for providing the necessary laboratory facilities. The investigation was made possible through the financial support provided by the University Grant Commission (UGC), Government of India in terms of Rajiv Gandhi National Fellowship (RGNF) scheme.

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